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RESEARCH ARTICLE

Growth and Characterization of Glycine Hydrobromide Crystals

T.S. Sarasvathi[1], R.Gobinath[1], S.Muruganantham[1] and S.Kumararaman[2]*

[1]Research Scholar, Department of Physics, Nehru Memorial College, Puthanampatti, (Affiliated to Bharathidasan University), Tiruchirappalli, Tamil Nadu, India.
[2]Head of Department, Department of Physics, Nehru Memorial College, Puthanampatti, (Affiliated to Bharathidasan University), Tiruchirappalli, Tamil Nadu, India.

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***Address for Correspondence**
S.Kumararaman
Head of Department,
Department of Physics,
Nehru Memorial College, Puthanampatti,
(Affiliated to Bharathidasan University),
Tiruchirappalli, Tamil Nadu, India.

ABSTRACT

Single crystals of Glycine Hydrobromide (GHB), a new semiorganic nonlinear optical material has been grown from solution by slow evaporation method at room temperature. Formation of the new crystal has been confirmed by single crystal XRD and IR spectra. GHB crystallizes in hexagonal system with cell parameters a = 7.023 Å , b = 7.024 Å, c = 5.412 Å. Optical transmission spectra revealed the optical properties of the grown crystals. The thermal stability of the crystal is investigated using thermo-gravimetric analyses studies. The NLO property of the crystal was confirmed by Kurtz SHG test.

Keywords: Characterization; Growth from solution; Nonlinear optic materials

INTRODUCTION

Complexes of amino acids with inorganic salts are promising materials for optical second harmonic generation (SHG), as they tend to combine the advantages of the organic amino acid with that of the inorganic salt. Although the salts of amino acids like, L-ariginine [1], L-histidine [2], L-alanine [3] and L-threonium [4] are reported to have high second harmonic generation efficiency. In this paper, we are presenting a preliminary report on the growth and characterization of a new complex of Glycine Hydrobromide (GHB), which is efficient in optical SHG. This is the first complex of glycine with inorganic acid, which has NLO property.

Crystal growth

Equimolar ratio of high purity Glycine salt (Loba Chemie) and analar grade hydrobromic acid (HB) were taken and dissolved in deionized water to synthesize GHB salt. The synthesized salt was purified by successive recrystallization process. A saturated solution of GHB was taken and the solution was filtered using high quality

filter paper. The filtered solution was taken in a beaker, which was tightly closed with thick filter paper so that the rate of evaporation could be minimized. After 35 days a good quality single crystals were harvested (Fig. 1).

Characterization

The grown crystals of GHB were confirmed by single crystal X-ray diffraction analysis, using ENRAF NONIUS CAD4 diffractometer. The functional groups were identified by Fourier transform infrared studies using BRUKER 66V FT-IR spec-trometer in the range of 400–4000 cm^{-1}. The optical properties of the crystals were examined between 200 and 1200 nm using Shimadzu UV-1061 UV–Vis spectrophotometer. The thermal behavior of the grown single crystal was tested by STA 1500 thermal analyzer. The NLO property of the crystal was confirmed by Nd:YAG laser. The detailed discussions of the obtained results are presented in the following sections.

RESULTS AND DISCUSSION

Single crystal XRD analysis

The structure of the grown crystal was solved by single crystal XRD analysis by direct method and retired by the full matrix-least-squares technique using SHELXL program. The calculated lattice parameters are presented in Table 1. It is observed from the XRD analysis that the GHB crystal orthorhombic structure.

FTIR analysis

The FTIR spectrum of GHB revealed at room temperature in the range of 400–4000 cm^{-1} is shown in Fig. 2. The absorption due to carboxylate group of free glycine is observed at 504.2, 892.8 and 1614 cm^{-1}, respectively. In GHB, these peaks are shifted to 506.2, 886.8, 1618 cm^{-1}, respectively. Similarly, the absorption peaks due NH^{+}_{2} group of free glycine are observed at 111, 1131 and 1505 cm^{-1}, respectively. These are shifted to 1114.7, 1138.1 and 1510.7 cm^{-1}, respectively. Other peaks at 1088, 1384 and 2837 cm^{-1} are attributed to C–C–N, COO^{-} and CH_2 groups, respectively, from a comparison of the spectra with that of glycine [11].

Optical transmittance studies

The UV–Vis spectrum gives limited information about the structure of the molecule because the absorption of UV and visible light involves promotion of the electron in σ and π orbital from the ground state to higher energy states. Transmission spectra are very important for any NLO material because a nonlinear optical material can be practical use only it has wide transparency window. To find the transmission range GHB the optical transmission spectrum of the GHF for the wavelengths between 200 and 1200 nm was recorded. A graph of transmission vs. wavelength is shown in Fig. 3. From the graph, it is evident the GHB crystal has UV cutoff below 300 nm, which is sufficiently low for SHG laser radiation at 1064 nm or other application in the blue region.

Thermal analysis

The TGA and DTA of the GHF crystal are shown in Fig. 4. From the thermogram it is observed that there is a single stage of weight loss starting at 240 °C but the range between 50 and 200 °C no loss in weight is recorded. This illustrates the absence of physically adsorbed or lattice water in the crystal. Hence, the compound is stable up to240 °C. The total weight loss corresponds to nearly 82% and the resulting residue (29%) is stable up to 500 °C.

Nonlinear optical test

Nonlinear optical property of the GHB crystal was found by carrying at Kurtz SHG test. The crystal was ground into powder and densely packed between two transparent glass slides. An Nd : YAG laser beam of wavelength 1064 nm was made to fall normally on the sample cell. The emission of green radiation from the sample confirms the second harmonic generation in the crystal.

CONCLUSION

Single crystals of GHB, a new semiorganic NLO material has been grown in solution growth technique for the first time. The lattice parameters were found by single crystal XRD technique. The FT-IR spectrum reveals that the functional groups of the grown crystals. The good optical transmittance in the visible IR region and SHG conversion efficiency makes the crystal a potential material for NLO applications. The melting point of GHB is found to be 240°C.

REFERENCES

1. D. Eimert, S. Velsko, L. Davis, F. Wang, G. Loiaceono, G. Kennedy, IEEE J. Quantum Electron. 25 (1989) 179.
2. M.D. Aggarwal, J. Choi, W.S. Wang, K. Bhat, R.B. Lal, A.D. Shield, B.G. Penn, D.O. Frazier, J. Cryst. Growth 204 (1999) 179.
3. D. Rajan Babu, D. Jayaraman, R. Mohan Kumar, R. Jayavel, J. Cryst. Growth (2002) 121.
4. G. Ramesh Kumar, S. Gokul Raj, R. Sankar, R. Mohan, S. Pandi, R. Jayavel, J. Cryst. Growth 7 (2004) 213.
5. B. Brezina, Mater. Res. Bull. 6 (1971) 401.
6. J.K. Mohan Rao, M.A. Vishwamitra, Acta Crystallogr., B 28 (1972) 1484.
7. S. Natarajan, J.K. Mohan Rao, Z. Kristallogr. 152 (1984) 179.
8. S. Natarajan, K. Ravikumar, S.S. Rajan, Z. Kristallogr. 168 (1984) 75.
9. P. Narayanan, S. Venkataraman, Z. Kristallogr. 142 (1975) 52.
10. K. Ravikumar, S.S. Rajan, Z. Kristallogr. 171 (1985) 201.
11. R.S. Krishnana, P.S. Narayanan, Crystallography and Crystal Perfection, Academic Press, London, 1963.

Table 1: Single crystal data of GHB

Single crystal data of GHB	
Cell parameters	a = 7.023 Å
	b = 7.024 Å
	c = 5.412 Å
α	90^0
β	90^0
γ	120^0
Volume	359.8706Å^3
System	Hexagonal

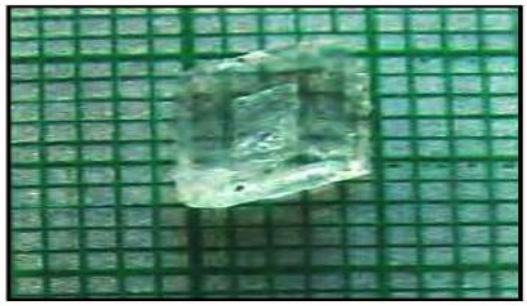

Fig. 1. Photograph of GHB crystal.

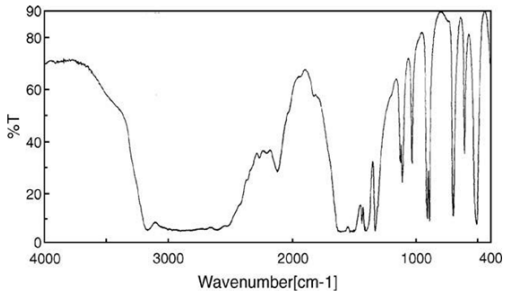

Fig. 2. FTIR spectra of GHB crystal.

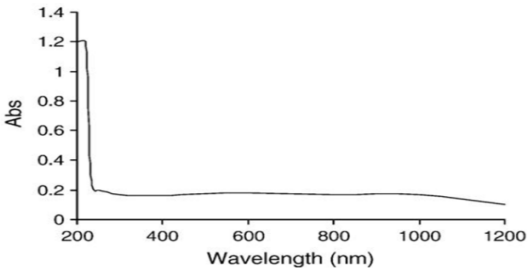

Fig. 3. Optical spectrum of GHB crystal

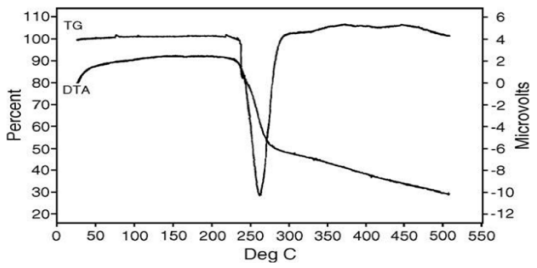

Fig. 4. TG and DTA spectrum of GHB

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RESEARCH ARTICLE

Parents Awareness on Refractive Errors and Attitude towards Vitamin A Supplementation for Children - A Cross - Sectional Study

Sindhu .V.V [1]* and G.Muthamilselvi[2]

[1]Ph.D. Scholar (N), Vinayaka Mission's College of Nursing, Puducherry, VMRF, Salem, India.
[2]Research Guide, Principal, Vinayaka Mission's College of Nursing, Puducherry, India

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***Address for Correspondence**
Sindhu .V.V
Ph.D. Scholar (N),
Vinayaka Mission's College of Nursing,
Puducherry, VMRF, Salem, India.
E.Mail: sindhuroopesh43@gmail.com

ABSTRACT

The eyes are the windows to the body and eyesight is one of the most important senses. Brain perceive up to 80 per cent of what comes through the sense of sight**.** Good Vision is an important indicator of health and quality of life. The World Health Organization estimated that19 million children aged below 15 years are visually impaired globally. Twelve millions of these are due to uncorrected refractive errors [1]. Digital learning and increased screen time triggered vide range of visual problems among school children. Vitamin A plays a crucial role in vision by maintaining a clear cornea, helps to produce pigments that make it possible to see the full spectrum of light. Vitamin A deficiency induced blindness is very predominant among under-five children in developing counties. The aim of this study was to assess parent's knowledge on refractive error and attitude towards Vitamin A supplementation for children. Descriptive cross-sectional study was conducted among the parents of children with refractive error to assess the knowledge on RE and attitudes towards vitamin A supplementation to improve vision. Study reveals that 43.33% of parents had above average knowledge, 53.33% of them had average knowledge and 3.33% of mothers were had below average knowledge regarding Refractive error. Majority of the Parents had favorable attitude towards Vitamin A supplementation to children for good vision. In India Refractive error in children is a major public health problem, it requires concerted efforts from various stakeholders including the health care workforce, education professionals and parents to manage this issue. It's critical that parents to know all about the varied eyesight problems in children, their causes, when to get eyes examined and preventive measures and care of eyes for good vision.

Keywords: Parents Knowledge, Refractive Error, Vitamin A, School children, Snellen's chart,Visual acuity.

INTRODUCTION

Based on recent figures, it is estimated that 253 million people are visually impaired worldwide, with 36 million people blind while 217 million have low vision. Globally uncorrected refractive error is the leading cause of visual impairment (43%), followed by cataract (33%) [2]. URE is responsible for 18% of blindness worldwide, second to cataract with two thirds of cases of visual impairment in children across the world. Good vision is an important part for effective communication and learning. In this era children are depending E–learning platforms for reading, writing and for entertainment. Computer and electronic gadgets are highly influenced by people especially young children who in turn affect both physical and mental issues due to excessive usage and minimal outdoor activities. When the vision suffers, pupil's routine school work and day today activities also get disrupted. But they are not mature enough to point out the deficiency at the early stage or the parents have no idea on developing vision problem [3]. Refractive error is an error in the focusing of light by the eye and a major reason for reduced visual acuity [4].In normal eyes parallel light is focused on the retina without accommodation, the emmetropia. Any optical departure from this condition is called a refractive error or ametropia. Generally there are three types of refractive errors are common among children. In Hyperopia condition parallel rays of light come to focus posterior to the retina with the eye in a state of rest i.e. non accommodating., Myopia where parallel rays of light come to focus anterior to the retina, and Astigmatism where the refractive power of the various meridians of the eye differs [5]. A refractive error can be classified as spherical or cylindrical according to the type of the lens necessary to correct it [6].

Children who have vision problems could not concentrate on studies or on any other extracurricular or recreational activities. If refractive errors are not detected early which might not only hamper children's physical, cognitive, and psychosocial development, but also future employability and earning opportunities. Moreover, the child can suffer from amblyopia (lazy eye), a condition where vision remains low even after wearing the glasses if refractive error is detected late.A systematic review conducted on prevalence of refractive errors among children in India highlighted that the overall prevalence of refractive error per 100 children was 8. The population-based prevalence of myopia, hyperopia and astigmatism was 5.3 per cent, 4.0 per cent and 5.4 per cent, respectively. The prevalence of combined refractive errors and myopia alone in schools was higher among girls and hyperopia was more prevalent among boys than girls [7].According to the World Health Organization (WHO) approximately 250,000-500,000 children in developing countries become blind each year owing to Vitamin A deficiency, with the highest prevalence in Southeast Asia and Africa[8].Poor eyesight is one of the first manifestations of Vitamin A deficiency. It leads to night-blindness, Bitot's spots, corneal xerosis and keratomalacia which is a major cause of blindness in India.

Need for the Study

It is estimated that prevalence of Childhood blindness in India is 0.8/1000 children in <16 years age group, implying a total of 300,000 blind children in our country [9]. About 60-80% of visual impairment may be due to refractive error alone. Children form one of the major age groups requiring attention to refractive errors because of the high prevalence of myopia, hypermetropia and astigmatism. A number of factors are responsible for uncorrected refractive error. Vitamin A deficiency is one among them. Parents with Myopia are more prone to have myopic offspring. Similarly, a child with high myopia is more likely to have myopic parents [10]. Research studies proved that Sustained near work, accommodative variability, accommodative lag, decreased time spent outdoors, and parental cigarette smoking are all recognized as environmental aggravating factors [11]. Poor vision and the inability to read material written on the blackboard can have a serious impact on a child's participation and learning in class and this can adversely affect a child's education, occupation and socioeconomic status for life [12]. Snellen's chart and ophthalmic missionaries and periodical eye examination are the diagnostic measures to detect refractive error. There are various treatment choices are available for correction of refractive errors like spectacles, surgical correction LAZER therapy eye exercises and yoga therapy. Prevention of refractive errors is avoiding of digital media, consuming vitamin A rich food and regular eye check-ups. Vitamin A is a key for good vision, a healthy immune system, and cell growth. Vitamin A deficiency is the second most important factor for global blindness. Every year 2,50,00 to 500,000 children become blind partially or totally due to vitamin A deficiency and it lowers the resistance

power of these children against infection. The 1995 report global prevalence of vitamin A deficiency included prevalence estimate of VAD among preschool children for two classes of indicators -clinical eye signs of disease and low serum retinol concentration. Vitamin A deficiency is one of the major deficiencies among the lower economic strata of India. In the fifties and sixties many of the states reported that blindness due to Vitamin A deficiency was one of the major causes of blindness in children below five years. A five-year long field trial conducted by NIN showed that if massive dose Vitamin A (200,000 units) was administered once in six months to children between one and three years of age, the incidence of corneal exophthalmia is reduced by about 80 percent [13]. Subclinical vitamin A deficiency may exist in developed countries and may be associated with different pathologies. Vitamin A dietary sources are cod liver oil, eggs, milk, orange and yellow vegetables and fruits, green leafy vegetables [14].Uncorrected RE's is one of the major causes of avoidable blindness and low vision. A number of factors are responsible for uncorrected RE's. They are the lack of awareness of the problem, inability to recognize the problem at personal and family level, non-availability and non-affordability of eye care services, and the cultural disincentives to compliance [15]. Uncorrected RE's can result in amblyopia and strabismus. It can restrict progress in education, limit career opportunities and restrict access to information [16].

Hence it is essential to understand the awareness among family member, school teachers and general public in the community to plan effective eye care programs to deal with this problem. Lack of awareness on visual impairment of parents delayed recognition of RE and poor attitude on eye care are the main causes for correction of RE [17]. Clinical Vitamin A deficiency often coexists with other micronutrient deficiencies and hence, there is a need for broad-based dietary diversification programs aimed at improving the overall micronutrient nutritional status of children [18]. Myopia is a common cause of visual impairment which is usually acquired and nearly progressive. New cases appear throughout the childhood, particularly between the ages of 6-15 years. A study was conducted at Maharashtra (2009) to compare the magnitude and risk factors of uncorrected refractive error in 6–15-yearold school children, study results revealed that prevalence of uncorrected refractive error especially myopia was significantly higher in school children of urban area compared to children of rural schools. The study concluded that the magnitude and causes of refractive error seem to differ in urban and rural areas of India [19]. Majority of the Parents are unaware that children are susceptible to refractive error especially due to lifestyle and environmental factors and they were vary about the potential chances of refractive error causing a debilitating condition. Though there are varied options are available for correction of RE such as spectacles, contact lenses or refractive surgical procedures [20]. But early detection and prompt management is the key for refractive error correction and food fortification with vitamin A rich micronutrients is very essential to prevent subclinical micronutrient deficiency disorders among children

Statement of the Problem

A study to assess parents' awareness on refractive errors and attitude towards Vitamin A supplementation for children

Objectives of the Study

- To assess parents knowledge on refractive errors among children
- To assess the attitude of parents towards vitamin A supplementation for children
- To find the association between the level of knowledge of Parents with the selected demographic variables

Hypothesis

H_1: There will be significant level of knowledge on refractive error among parents

H_2: There will be positive attitude towards vitamin A supplementation for eye health

H_3: There will be significant association between the levels of knowledge of parents with selected demographic variables.

METHODOLOGY

Research Design : Descriptive Cross- sectional study
Population : In this study parents of school children with refractive error residing at selected villages
Setting of the study : Selected villages at Mahe
Sampling technique : Purposive sampling technique was used
Sample size : A total number of 60 samples were selected
Study Duration : 1 month

Data collection procedure

- The investigator initially established rapport with parents explained the purpose of the study. Consent from the subjects was obtained and confidentiality was maintained
- The investigator collected the data by using structured knowledge questionnaire and attitude questions to explore the attitude towards vitamin A supplementation among60 parents.

Plan for Data Analysis Descriptive statistics:

➢Frequency, Percentage. Mean and standard deviation

Inferential statistics

➢ Chi-Square test was used to identify the association between parents knowledge on refractive errors with selected demographic variables.

Attitude

The attitude was measured by eight questions put on Likert's scale and the score point ranged from 8 to 40.The questions on Likert's scale had positive and negative responses .The scoring system used with respect to participant's responses was, strongly agree 5, agree 4, neutral 3, disagree 2, and strongly disagree 1. The responses were summed up and a total score was obtained for each respondent. The mean was calculated and those who scored above the mean value had favorable attitude and the ones who scored less than the mean value had an unfavorable attitude towards Vitamin A supplementation for children

Favorable attitude: Respondents who answered greater than or equal to the mean (31.18) attitude questions had a favorable attitude.

Unfavorable attitude: Respondents who answered below the mean (31.18) attitude questions had an unfavorable attitude.

Tool

Structured knowledge questionnaire. It contains 20 questions each correct response was given a score of 1 and a wrong response a score of 0

RESULT AND DISCUSSION

Figure 1: Bar diagram shows that majority of parents 43.33% had above average knowledge, 53.33% of them had average knowledge and 3.33% of them had below average knowledge level regarding refractive errors.

Table 1: Shows that among the total respondents, 34(56.66%) (95% CI: 51.6- 61.4) had favorable attitude towards vitamin A supplementation for good vision.

Table 2: Shows that there was a significant association between parents' knowledge on refractive errors with some of the variables like Age, educational qualification, and source of information at ($p<0.05$) level, where as religion, number of children, type of family, monthly income, dietary pattern, occupation were not significant.

DISCUSSION

In this study 43.33% of parents had above average knowledge regarding refractive error among children. This findings supported by the study conducted by Noof Ali Salim Al Ghailani.et al on 2020 to parents' awareness and perception of children's refractive error-qualitative study. Result shows that majority of the parents were well aware about refractive error[21].Majority of the Parents had favorable attitude towards Vitamin A supplementation to children .This finding is supported by the study conducted by Lubna Abdulmalek, Fatma Benkhaial on 2018 at libia shows 88% of the interviewed parents had a positive attitude of giving Vitamin A during campaign[22]. In this study the knowledge regarding refractive error among parents with 21-30 years of age is more when compared to other groups and educational qualification of parent's shows significant association. This finding is agreed with a study done by Srinivasa Reddy Pallerla, et al on awareness and knowledge about refractive errors and strabismus in South Indian population[23].A cross sectional population based survey .Study concluded that younger people and females were more aware of the refractive errors and strabismus. People with higher education had a higher awareness of both refractive errors and strabismus

CONCLUSION

The following conclusions were made based on the findings of the study

- Majority of the parents had moderately adequate knowledge regarding refractive errors among students.
- Majority of the parents had favorable attitude towards Vitamin A supplementation
- The age of parents, educational qualification and source of information shows significant association with their knowledge level at $p<0.05$* level

Nursing Implications

The scope of the study brought out the implications for nursing in the areas of practice, education, administration and research.

Nursing Practice

Public enlightenment on refractive errors in childhood as well as health education of parents and teachers is essential. Community eye health services including IEC activities should be considered as a useful tool for early detection of refractive errors as well as the enhancement of treatment compliance and follow up adherence among children with refractive errors. As per the latest report on Hindu, Vision problem on the rise among the children at kerala. There for doctors and parents should encourage children to engage in more outdoor activities to decrease the incidence of Refractive error.

Nursing Education

Children are the future of any nation. It is the responsibility of the health care providers especially nurses to prevent the children from avoidable blindness due to uncorrected refractive errors which is an important aspect of various programs at national and international level. Nursing curriculum should be such that it prepares the nursing students to assist the client and the community in all aspects of health care

Nursing Administration

Nurse administrators can take initiatives in organizing vision screening camps at community level especially hard to reach areas and conduct training programs for stakeholders how to detect refractive error using Snellen's chart.

Nursing Research

Nurses need to be engaged in multidisciplinary research so that it would help them to improve their knowledge and skills in handling various problems related to health and illness. The present study can serve as a baseline data for further nursing and other community-based research.

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Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. World Health Organization. Global data on Visual Impairments. Available at www.who.int/blin dness/GO BALDATAFINALforweb.pdf ?ua=1/fs282/en/ 2010. [accessed March 2018].
2. Bourne RRA, Flaxman SR, Braithwaite T , et al. Magnitude, temporal trends, and projections of the global prevalence of blindness and distance and near vision impairment: a systematic review and meta-analysis**.** Lancet Global Health. 2017;5(9):e888–e897. [PubMed] [Google Scholar]
3. Holden BA, Fricke TR, Wilson DA, Global prevalence of Myopia and high myopia and temporal trends, Opthalmology.2016; 123(5): 1036-1042
4. Williams KM, Bertelsen G, Cumberland P,et al. Increasing prevalence of myopia in Eurpoe and the impact of education, Ophthalmology. 2015;122(7): 1489-1497.
5. Behraman R, Kligman R, jenson H, et al. Nelson Textbook of pediatrics, 17th edition, Philadelphia: W.B.Saunders Co 2004; 2086-2087.
6. Holden B .A .SulaimanS.lmockK.the challenge of providing spectacles in the developing world .comm. eye health 2000; 13: 9-10.
7. Sethu Sheeladevi, Bharani Seelam, Phanindra ,Nukella, AditiModi.,Prevalence of refractive errors in children in India: a systematic review, ClinExpOptom. 2018 Jul;101(4):495-503.
8. K. Park.: Park's textbook of Preventive and Social Medicine. 567-569. (21st edition) Published February 2011 by BanarsidasBhanot Publishers ISBN 8190607995 (ISBN13: 978819060799
9. Shrestha GS, Digen Sujakhu, Joshi P. Refractive error among school children in Jhapa, Nepal. J Optom. 2011; 4:49-55
10. Dong LM, Everett D, Norton T, Kurtz D, et al. Comet Group. Factors associated with high myopia after 7years of follow-up in the Correction of Myopia Evaluation Trial.(COMET) Cohort. Ophthalmic Epidemiol 2007; 14:230–7.
11. Rose KA, Morgan IG, Ip J, Kifley A, Huynh S, Smith W, Mitchell P. Outdoor activity reduces the prevalence of myopia in children. Ophthalmology. 2008 Aug 1;115(8):1279-85.
12. Taylor H.R. Refractive errors: magnitude of need. J Comm. Eyehealth 2010; 13: 1-2.
13. NNMB National Nutrition Monitoring Bureau. 2002. NNMB Micronutrient Survey. National Institute of Nutrition, Hyderabad.
14. Gopalan C. Prevention of micronutrient malnutrition. NFI Bulletin.October 2001; vol 22(.4.)
15. Padhey AS, Khandekar R, Dharmadhikari.et.al. Prevalence of uncorrected refractive error and other eye problems among urban and rural school children. Middle East Afr J Ophthalmol 2009 Apr-Jun; 169 (2): 69-74
16. AbiyMaru Alemayehu, Gizchewu Tilahun Belete, et al, Knowledge, attitude and associated factors among primary school teachers regarding refractive error in school children in Gondar city, Northwest Ethiopia. 2018. https://doi.org/10.1371/journal.pone.0191199
17. Refractive error.[Online].2011 Mar 31 cited 2012 Aug 3];Available from: URL: http://www.vision2020.org/main.cfm?type=WIBREFRACTIVEERR
18. Gopalan C. Prevention of micronutrient malnutrition. NFI Bulletin. October 2001; vol 22,

19. Amruta S Padhye 1, Rajiv Khandekar.et al. Prevalence of uncorrected refractive error and other eye problems among urban and rural school children. Middle East Afr J Ophthalmol2009 Apr;16(2):69-74. doi: 10.4103/0974-9233.53864.
20. Shrestha GS, Digen Sujakhu, Joshi P. Refractive error among school children in Jhapa, Nepal. J Optom. 2011;4:49-55.
21. Noof Ali Salim Al Ghailani, et.al. parents' awareness and perception of children's refractiveerror.aqualitativestudy(2020),DOI:10.37268/MJPHM/VOL.20/NO.3/ART.656Corpus ID: 23436816
22. lubnaabdulmalek ,fatmabenkhaial et.al.Knowledge, attitude, and practice of parents regarding vitamin a supplementation to children in benghazi, Libya.ibnosina journal of medicine and biomedical sciences (2018)10(5):17
23. Srinivasa Reddy Pallerla1, Rohit C. Khanna, S. Krishnaiah et al. Awareness and knowledge about refractive errors and strabismus in South Indian population(2022) DOI: https://dx.doi.org/10.18203/2394-6040.ijcmph20220688.

Table .1 Attitudes of parents towards vitamin A supplementation for good vision

Variables	Frequency	Percentage
Favorable	34	56.66
Unfavorable	26	43.33

Table 2 :Association of parents' knowledge on refractive error with selected demographic variables

(N = 60)

Variables	Knowledge level						Chi-square	Tabulated Value
Parents	Below Average		Average		Above average		Calculated Value	
	f	%	f	%	f	%		
Age in years 20– 25 years 26– 30 years 31– 35 years 36– 40 years	2	3.3	8 2 16 2	13.3 3.3 26.6 3.3	10 4 8 8	16.67 6.66 13.33 13.33	X^2=23.422* df=6 P=0.05	**Significant**
No.of children One Two Morethan two	2	3.3	14 8 6	23.33 13.33 10.0	6 16 8	10 26.6 13.3	X^2=2.502 df=6 P=0.05	**Not significant**
Educational status Primary Secondary Gradate	2	3.3	2 16 10	3.3 26.6 16.67	0 19 11	0 31.67 18.34	X^2=18.89* df=6 P=0.05	**Significant**
Age of the child 12 years 1 3 years 14 years	2	3.3	14 10 4	23.33 16.67 6.67	14 09 07	23.33 15 11.67	X^2=3.755 df=6 P=0.05	**significant**
Religion Hindu Muslim Christian	2	3.33	14 8 6	23.33 13.33 10	18 10 2	30 16.67 3.33	X^2=9.481 df=6 P=0.05	**Not significant**
Type of family								

Joint Nuclear Extended family	2	3.33	16 10 2	26.67 16.67 3.33	11 17 2	18.33 28.33 3.33	X^2=4.847 df=6 P=0.05	**Not significant**
Dietary pattern Vegetarian Non-veg Egg- veg	2	3.33	20 6 2	33.33 10.0 3.33	08 18 04	13.33 30 6.67	X^2=5.948 df=6 P=0.05	**Not Significant**
Source of information Relatives and friends Mass media Health personal	2	3.33	6 6 16	10.0 10.0 26.67	4 10 16	6.67 16.66 26.67	X^2=21.012 df=6 P=0.05	**Significant**
Monthly income 5000-10000 10001-15000 15001-20000 >20000	2	3.33	10 12 4 2	16.66 20.0 6.67 3.33	8 12 6 4	13.33 20.0 10 6.67	X^2=4.530 df=6 P=0.05	**Not significant**
Occupation Unemployed Government job Private	2	3.33	14 2 12	23.33 3.33 20.0	13 04 13	21.67 6.67 21.67	X^2=2.042 df=6 P=0.05	**Not significant**

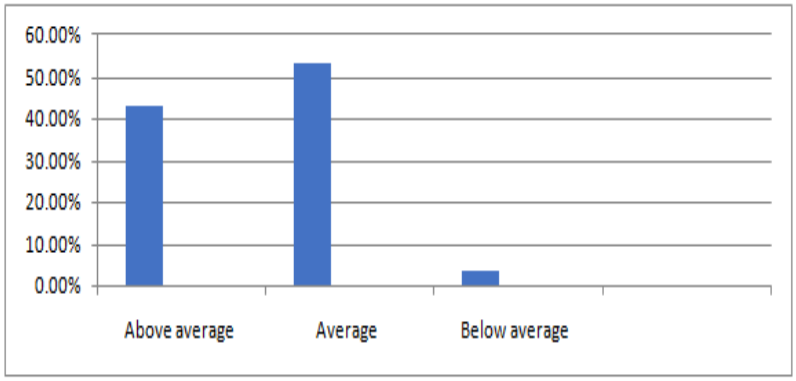

Figure 1: Frequency and percentage wise distribution of the subjects according to the level of knowledge on Refractive error (N=60)

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RESEARCH ARTICLE

Design and CFD Analysis of Double Pipe Heat Exchanger

Deepa[1] and Anand Kumar S Malipatil[2]*

[1]PG Scholar, Department of Mechanical Engineering, Visvesvaraya Technological University, CPGS, Kalaburagi, Karnataka, India
[2]Assistant Professor, Department of Mechanical Engineering, Visvesvaraya Technological University, CPGS, Kalaburagi, India

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***Address for Correspondence**
Anand Kumar S Malipatil
Assistant Professor,
Department of Mechanical Engineering,
Visvesvaraya Technological University,
CPGS, Kalaburagi, India
Email: jovemp.ask@gmail.com

ABSTRACT

Because of its simple construction, double pipe heat exchangers are extensively utilised in a extensive variety of heat transfer applications, from oil refineries to automotive radiators. It is possible to boost the heat transfer rate by applying several heat transfer augmentation methods, including dimples, which is a passive approach with the lowest pressure drop. A CFD software called Ansys Fluent has been utilize to calculate act of a twin pipe heat exchanger by then without dimples, and the layout that provides the most effective heat transfer has been discovered. Data on the tube-side heat transfer coefficient as well as pressure drop have been collected in this study. FEA-based CFD study will be used to analyse thermal properties of the exchanger model.

Keywords: Double Pipe Heat Exchanger, CFD Ansys, , Meshing, Calculation Of Heat Exchanger, conclusion

INTRODUCTION

It is possible to measure a substance's temperature by determining its energy content. Heating and cooling systems employ heat exchangers for the transmission of energy. Controlling the temperature of both the entering and departing streams is essential in process units. There are two types of streams: gases and liquids. The temperature of these streams may be raised or lowered by using heat exchangers. This kind of gadget may be utilize to transfer heat among two fluids which are detached by a solid wall. Heat is more easily transferred when there is a temperature differential. Radiation, conduction, & convection all play a part in transmission of heat. Radiation occurs when heat exchangers are utilize.

Double Pipe Heat Exchanger

In its most simple form, a twin pipe heat exchanger is nothing more than a single pipe enclosed inside a bigger pipe. One fluid travels within the pipe, while the other travels around it in an annulus. The heat transmission surface is located on the inside of the pipe. In order to save space, pipes are often doubled back numerous times as indicated in figure on left. As seen in figure, a heat exchanger with this particular design is also known as a "hairpin heat exchanger". Compared to other forms of equipment, disassembly and cleaning is prohibitively expensive and time-consuming. Double pipe exchangers are best suited for modest heat transfer surfaces, such as those between 100 and 200 square feet.

Types of Double Pipe Heat Exchangers:

1. Counter flow
2. Parallel Flow Heat Exchanger

Counter flow

When used in a proper counter flow pattern, a hairpin or double pipe heat exchanger has greatest total heat transfer coefficient of any double pipe heat exchanger design. This allows for greater efficiency.

Parallel Flow

For high pressure and temperature applications, parallel flow heat exchangers are ideal. Using this, we can also get a high Log mean Temperature. When Reynolds numbers are the same, pipes create greater turbulence than tubes because of their rougher surface. Coefficients derived from tube-data associations are smaller & safer than those derived from pipe-data correlations, & there are no pipe-data associations in literature. Organic liquids, aqueous solutions, and gases may all benefit from these equations.

OBJECTIVE

The goal of this project is to build and assess a heat exchanger that is both efficient and effective. Reliability, cost-effectiveness and practicality are the goals of the mechanism. Temperature measurements must be made possible by this heat exchanger's ability to give restricted thermodynamic optimization. In addition, this system is designed to improve the temperature and suitable circumstances for the user. Instead of creating every component from scratch, this approach relies on commonly available and already in use components. For example, you don't have to spend a lot of time and energy evaluating each part's integrity since they have previously shown their usefulness in the actual world.

Selection of Exchanger Geometry

Tube outside diameter

Most general diameter in process industry is 19.05 mm (3/4 inch).

Tube wall thickness

For this, a well-known pressure vessel code must be used as a guide.

Tube length

However, a long thin exchanger may not be possible if the surface area is equal to the tube length.

Tube layout

However, if mechanical cleaning is not necessary, then 45 or 90 degree layouts are commonly used because of the better heat transfer & hence smaller exchanger that they give.

Tube pitch

Unless a bigger pitch is required for mechanical cleaning or tube end welding, the lowest permissible pitch of 1.25 times outer diameter of tube is often employed.

Number of tube passes
This is generally one or an even number (not typically greater than 16). Increasing number of permits increases heat transfer coefficient but care must be taken to safeguard that tube side ρv^2 is not greater than about 10,000 kg/m·s^2.

Shell diameter
Shells with a diameter of up to 610 millimetres are often installed using standard pipe (24"). The shell is composed of rolled plate on top of this. As a general rule, shell diameters fall between 152 and 3000 millimetres. (6" to 120").

Baffle type
By default, single segmental baffles are employed, however if pressure drop limitations or vibration are an issue, different kinds of baffles may be considered. .

Baffle spacing
A compromise is reached between the need for higher cross flow velocity & tube support (a lower baffle pitch), pressure drop limitations. The maximum and lowest baffle pitch may be found in TEMA's guidelines. .

Baffle cut
Single segmental baffles normally have a 45 percent retention rate, whereas double segmental baffles typically have a 25 percent retention rate.

Nozzles and impingement
For shellside nozzles ρv^2 should not be greater than about 9000 in kg/m·s^2. For tubeside nozzles maximum ρv^2 should not exceed 2230 kg/m·s^2 for noncorrosive, nonabrasive single phase fluids & 740 kg/m·s^2 for other fluids

Materials of Construction
Metal is most common material utilized in shell & tube exchangers, although graphite, plastic, & glass may also be utilised for certain applications (e.g., those requiring strong acids or medicines).

Thermal Design
When designing a shell & tube exchanger, computer programmes like Heat Transfer and Fluid Flow Service (HTFS) or Heat Transfer Research Incorporated (HTRI) are often used (HTRI). The engineer must, however, comprehend the reasoning behind the computation. When calculating heat transfer coefficients &pressure drops, early judgments must be taken on fluid allocation, front & back header type, shell type, baffle type, tube diameter, tube layout

Mechanical Design
Pressure vessel design codes like ASME's Boiler and Pressure Vessel Code & the British Master Pressure Vessel Standard, BS 5500, are utilized to decide these values. 11 parts of ASME are most widely used heat exchanger code.

Double pipe Heat Exchanger
Calculation for Inner Circular Section
Diameter, d = 0.042m
Area, $A = (\pi d2)/4$
Area, $A = (3.14 \times 0.042^2)/4$
Area, $A = 0.00138 m^2$

Velocity, V = 0.125m/s
Hydraulic diameter, $H_d = (4A/P)$
Hydraulic diameter, $H_d = (4 \times 0.00138/0.15)$
Hydraulic diameter, $H_d = 0.0368m$

Mass flow rate, $m = \varrho.A.V$
Mass flow rate, m = 0.3 x.0.00138 x 0.125
Mass flow rate, m = 0.0517 kg/s

Calculation for outer section – Cylinder
Diameter, d = 0.0165m
Area, $A = (\pi d2)/4$
Area, $A = (3.14 \times 0.0165^2)/4$
Area, $A = 0.000213 m^2$

Velocity, V = 0.125m/s
Hydraulic diameter, $H_d = (4A/P)$
Hydraulic diameter, $H_d = (4 \times 0.000213/0.15)$
Hydraulic diameter, $H_d = 0.00569$m

Mass flow rate, $m = \varrho.A.V$
Mass flow rate, m = 0.3 x.0.000213 x 0.125
Mass flow rate, m = 0.00798 kg/s

Calculations required Heat Exchanger
Q = m Cp T
Q = Efficiency of Heat Exchanger
m = Mass of System Tube
Cp = Pressure coefficient
T = Temperature of the fluid
Q = m Cp T
Q = (0.0517) (4.187) (308)
Q = 66.67KJ

DESIGN PARAMETERS:
Table 2 Material Used, Table3 Geometrical Dimension's for DPHE ,Table 4: Flow rates for STHE.

Meshing
It is process of creating a polygonal or polyhedral mesh which approaches a geometric domain. Many people use word "grid generation" as a synonym for "grid power." Finite element analysis and computational fluid dynamics are two common applications. CAD, NURBS, B-rep, and STL are just a few of the most prevalent input model formats (file format). Interdisciplinary contributions may be found in mathematics, computer science and engineering. The three-dimensional meshes used in finite element investigation must be made up of tetrahedral, pyramids, prisms, or hexahedrons. The polyhedral shapes utilised in the finite volume approach are completely up to you. Multi-block structured meshes are often utilised for finite difference techniques, which need hexahedron arrays that are piecewise structured.

DISCUSSION ON CFD ANALYSYS RESULT

Figure's 2 to 7

CONCLUSION

A CFD programme called Fluent is used to study performance of a twin pipe heat exchanger. Water-to-water heat transfer properties were investigated using the simulation. In a twin pipe heat exchanger, temperature, pressure, and

velocity are all absorbed by us. That's why our outcomes were better when we were using a double-pipe heat exchanger. The following result shows that our goal of developing a heat exchanger design and conducting a CFD study utilising restricted thermodynamic optimization was achieved. As shown above figures, Contour – Static Pressure Result Max Value is 9.98 Pa, Contour – Pressure Coefficient Result Max Value is 1.04e03 Pa, Contour – Density Result Max Value is 1.23 Kg/m^3, Contour – Static Temperature Result Max Value is 3.58e2 K, Contour – Total Temperature Result Max Value is 3.58e2 K. mechanism design is meshed and solved using CFD Ansys. This is showing us that design is having better results in the case of velocity and pressure. The validation of the Double Pipe Heat Exchangers Calculation for Inner Circular Section Area, A =0.00138m^2. Velocity, V = 0.125m/s, Hydraulic diameter, H_d = 0.0368m, Mass flow rate, m = 0.0517 kg/s, Calculations required Heat Exchanger, Q = m Cp T, Q = 66.67KJ.In CFD Analysis, the heat exchanger mechanism performed successfully. A larger proportion of time spent at the pump's centre of gravity has increased its performance. All of these data indicate that our goal will be met with great regard.

REFERENCES

1. Rajeev Mukharji, "Effective design of Double Pipe Heat Exchanger", American Institute of Chemical Engineering, 1988.
2. Hari Haran, Ravindra Reddy and Sreehari, "Thermal Analysis of Double Pipe Heat Exchanger Using C and Ansys" , International Journal of Computer Trends and Technology (IJCTT) – volume 4 Issue 7–July 2013
3. Yusuf Ali Kara, OzbilenGuraras, —A computer program for designing of Double Pipe Heat Exchanger‖, Applied Thermal Engineering 24(2004) 1797-1805
4. V.K. Patel, R.V. Rao, "Design optimization of Double Pipe Heat Exchanger using particle swarm optimization technique", Applied Thermal Engineering 30 (2010) 1417-1425
5. Versteeg, H.K., Malalasekera, W., An introduction to computational fluid dynamics: the finite volume method.
6. M. M. El-Fawal, A. A. Fahmy and B. M. Taher, "Modelling of Economical Design of Double Pipe Heat Exchanger Using Specified Pressure Drop", Journal of American Science
7. JiangfengGuo, Lin Cheng, MingtianXu, "Optimization design of Double Pipe Heat Exchanger by entropy generation minimization and genetic algorithm", Applied Thermal Engineering 29 (2009) 2954–2960.
8. Pignotti, "Relation Between the Thermal Effectiveness of Overall Parallel and Counter flow Heat Exchanger Geometries", J. Heat Transfer 111(2), 294-299 (May 01, 1989), asme.org.

Table 1: Dimensions of Double Pipe Heat Exchanger

S.No	Parameter		Dimension
1	Hot Pipe	Inner Dia	16.5 mm
		Outer Dia	21.5 mm
2	Cold Pipe	Inner Dia	42 mm
		Outer Dia	48.5 mm
3	Cold Pipe – Inlet	Inner Dia	11 mm
		Outer Dia	12 mm
4	Cold Pipe – Outlet	Inner Dia	11 mm
		Outer Dia	12 mm
5	Hot Pipe Length		750 mm
6	Cold Pipe Length		450 mm

Table 2: Material Used:

S.No	Component	Material
01	Shell	SS
02	Tube	Copper

Table 3: Geometrical Dimension's for DPHE

SL NO.	PARAMETER		DIMENSION
1	Inner diameter	Hot pipe	16.5mm
	Outer diameter		21.5mm
2	Inner diameter	Cold pipe	42mm
	Outer diameter		48.5mm
3	Inner diameter	Cold pipe inlet	11mm
	Outer diameter		12mm
4	Inner diameter	Cold pipe outlet	11mm
	Outer diameter		12mm
5	Hot pipe length	750mm	
6	Cold pipe length	450mm	

Table 4: Flow rates for STHE

QUANTITIES	BOUNDARY CONDITION
Working fluid	**Water**
Inner pipe (hot fluid)	Hot inlet Mass flow rate=0.125kg/s Temperature = 82.22c
Outer pipe (cold fluid)	Cold inlet Mass flow rate=0.215kg/s Temperature = 32.22c

Figure 1 : Double Pipe Heat Exchanger

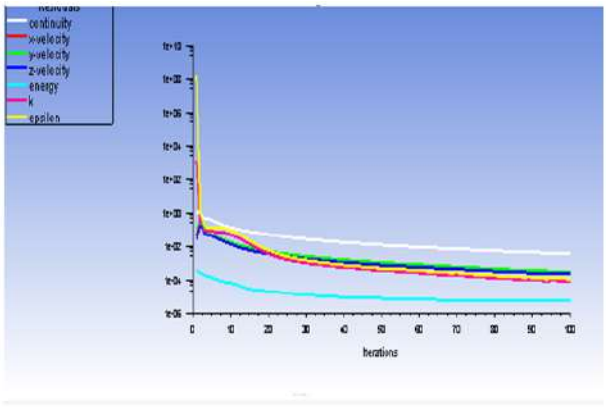

Figure 2: Residuals Vs Iterations

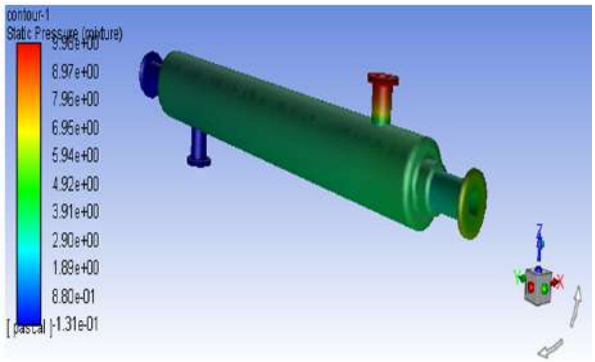

Figure 3: Contour – Static Pressure Result Max Value is 9.98 Pa

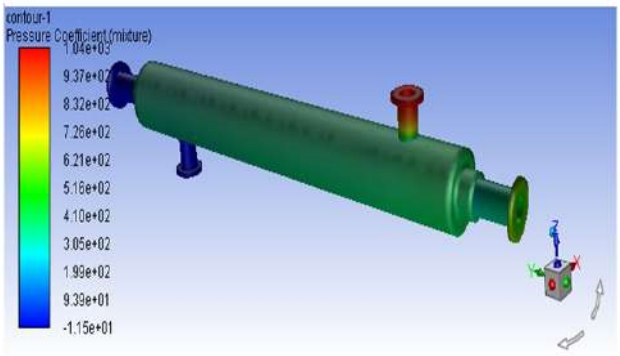

Figure 4: Contour – Pressure Coefficient Result Max Value is 1.04e03 Pa

Figure 5: Contour – Density Result Max Value is 1.23 Kg/m³

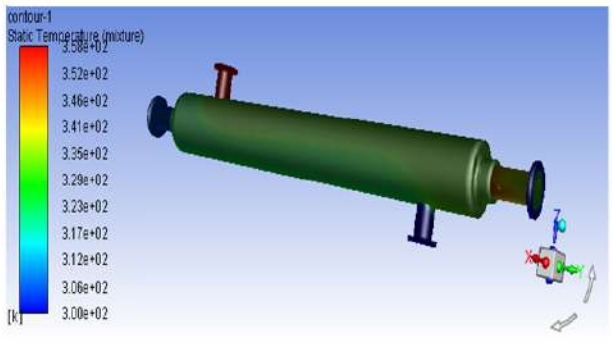

Figure 6: Contour – Static Temperature Result Max Value is 3.58e2 K

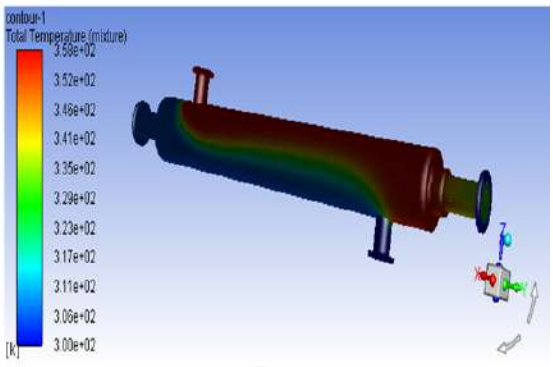

Figure 7:Contour – Total Temperature Result Max Value is 3.58e2 K

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RESEARCH ARTICLE

A Study to Assess the Effect of Video Assisted Teaching Module on Life Style Modification among Children with the Risk of Cardiovascular Diseases

S. Malairani*

Staff Nurse, Government Mohan Kumaramangalam Medical College and Hospital, Salem, Tamil Nadu, India.

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***Address for Correspondence**
S. Malairani,
Staff Nurse,
Government Mohan Kumaramangalam Medical College and Hospital,
Salem, Tamil Nadu, India.
Email: malairani83@gmail.com

ABSTRACT

A pre experimental study with pre test and post test with quantitative approach was conducted to assess the effect of Video Assisted Teaching Module on Life Style Modification among Children with the risk of Cardiovascular Diseases, in selected schools, Salem. Eight hundred and thirty children with the risk of cardio vascular diseases were selected by using physiological screening scale and data was collected from the children by using closed ended questionnaire to assess the knowledge, 5 point likert scale to assess the attitude and rating scale to assess the practice of the children. Demographic data revealed that the Highest percentage (33.7%) of children were in the age group of 12 years 1 month to 13 years. Majority (93.25%) of children were belonged to non vegetarian. Most (78.8%) of children had the habit of eating junk food. Highest percentage (71.1%) of children were not interested in playing physically active game about duration of physically active play per day majority 87 (36.2%) of children were played for 31 minutes to 60 minutes. Highest percentage (70.2%) of children were had the family history of hypertension, most (62.6%) of children were had family history of diabetes mellitus, majority (60.9%) of children did not had the family history of cardio vascular diseases. Overall pretest mean percentage of knowledge score was 28% whereas in post test it was 86% depicting a difference of 58%. Overall pretest means % of attitude score was 25%, whereas in post test it was 91% depicting a difference of 66%. Overall pre test mean percentage of PS was 42%. Whereas in post test it was 80% depicting the difference of 38%.

Keywords: Video Assisted Teaching Module, Life Style Modification, Children with the risk of Cardiovascular Diseases, Knowledge, Attitude and Practice.

INTRODUCTION

"It is easy to build healthy children than to treat a diseased adult" Children are the ones who are very vital for deciding how the world is going to be in future. So if one can do good in the life of a child then there can be change, at least a mild change, in the world to come and if most of the people thinking in same way then all can hope a best future. The main component which decides how the person is going to be in the future or the present is the amount of education related to maintenance of health he/she has within them [1]. Cardiovascular disease refers to any disease that affects the cardiovascular system, principally cardiac disease, vascular diseases and peripheral arterial disease. The causes of cardiovascular disease are diverse but atherosclerosis and hypertension are the most common, even in healthy asymptomatic individuals [2]. India has seen a speed transition in disease burden number of cases / lakh over the past twenty years. The burden of communicable and non-communicable diseases is focused to get reversed in 2020 from its distribution in 1990. This is mostly because of India's economic development and urbanization over the past one decay, the major portion of the people has moved to unhealthy lifestyles practices with increasing stress levels, physical activity, and increasing intake of saturated fats and tobacco [11]. Primary prevention of CVD beginning in early childhood includes both cardiovascular health promotion and reduction of modifiable risk factors. Primordial prevention goes beyond prevention of risk factor development in children and refers to "preserving risk-factor free societies from the penetration of risk factor epidemics". This level of prevention focused on community conditions that foster risk factor development. Two main strategies are recommended for prevention of CVD across the life course, including children and adolescents [12].

Statement of the problem

A Study to Assess the Effectiveness of Video Assisted Teaching Module on Life Style Modification among Children with the risk of Cardiovascular Diseases, Salem.

Objectives

To

- Assess the knowledge, attitude and practice of children regarding risk factors of cardiovascular diseases and life style modification to prevent cardiovascular diseases.
- Assess the effectiveness of VATM on knowledge, attitude and practice on life style modification among children with the risk of cardiovascular diseases.

METHODOLOGY

Research Design and Research Approach

Pre experimental pre test and post test without control group design with experimental approach was used to evaluate the effect of the video assisted teaching module on life style modification among children with the risk of cardiovascular diseases.

Setting of the study

The study was undertaken in private schools, out of 275 private schools in Salem district investigator selected one school for pilot study and one school for reliability and five schools for main study by simple random method.

Population

Population selected for this study was all the school children with in the age group of 12 to 17 years in selected schools, Salem. Total population of this study was 2288 school children.

Sampling

Sample

Sample selected for this study was all the school children with in the age group of 12 to 17 years in selected schools, Salem and who met with inclusion criteria.

Sampling size

The sample size of this study was 830 children with the risk of cardiovascular diseases. Power analysis was used for selecting appropriate sample size, based on power analysis required sample size was 809 but additional 43 samples were added to meet the expected attrition rate minimum 10% (809+43 = 852). So the investigator selected 852 school children with in the age group of 12 to 17 years in selected schools, Salem after the drop out of 22 children the sample size of the present study was 830 children.

Criteria for sample selection

Inclusive criteria

The study was limited to school children

- from 12 to 17 years of age.
- with the risk of cardiovascular diseases.
- who had the willing to participate in the study
- available during the time of data collection
- who can able to read and write English.

Exclusion criteria

The study was excluded the children with any other co - morbidities like hypothyroidism, cushing's syndrome, etc..

Variables

Independent variable

Independent variable of this study was Specific intervention like Video Assisted Teaching Module on life style modifications for children with the risk of cardiovascular diseases.

Dependent Variable

Dependent variable of this study was knowledge, attitude and practice of the school children with the risk of cardiovascular diseases on life style modification to prevent cardiovascular diseases.

Description of the tool

Section – 1 : Physiological checklist to screen the children with the risk of cardiovascular diseases.

Section - 2

Section – A: Demographic data to elicit baseline data of children.

Section – B: Closed ended questionnaire was used to assess the knowledge level of children regarding life style modifications.

Section – C: Likert scale was used to assess the attitude level of children regarding life style modifications to prevent cardiovascular diseases.

Section – D: Rating scale was used to assess the practice of children regarding life style modifications to prevent cardiovascular diseases.

Video Assisted Teaching Module.

Video assisted teaching module

Organization of the contents of VATM

- Meaning of cardiovascular diseases
- Anatomy and physiology of heart and blood vessels
- Structure of abnormal heart and blood vessels
- Risk factors for cardiovascular diseases like

  - Physical in activity
  - Over eating of fast food items
  - Urbanization
  - Obesity
  - Type II Diabetes and mellitus
  - Family History
  - High Blood Pressure and High Blood Cholesterol
  - Cigarette Smoking
  - Alcoholism

- Life style modifications to prevent cardiovascular diseases in children such as
  - Reemphasizing the benefits of Heart-Healthy Nutrition
  - Daily physical activity and exercises
  - Maintenance of ideal body weight
  - Smoking and Alcohol avoidance
  - Routine physical examination and Health checkup for risky adolescent and Complementary and Alternative medicine.

Validity

The video assisted teaching module (VATM) content and tool was submitted to the experts for content validity of the tools. According to the suggestions and opinion of the experts corrections and modification were made. The expert included were two pediatricians, one physician from cardiology, one statistician and four nursing experts in the field of Pediatric Nursing. The VATM and tools was in English and it was validated by language expert.

Reliability

Reliability of the tool was tested by implementing on 20 school children with the risk of cardiovascular diseases. Test-re-test method (Karl Pearson's co-efficient of correlation) was used to find out the reliability of (knowledge questionnaire) closed ended questionnaire ($r = 0.897^{**}$) correlation was significant at 0.01 level. The internal consistency coefficient correlation of the items (Cronbach's coefficient alpha) was used to find out the reliability of attitude likert scale ($r = 0.871$) and practice rating scale ($r = 0.794$). Reliability of the weighing machine, stadiometer and sphigmonanometer was tested by test re-test method.

Ethical consideration

Written permission was obtained from the management and principals of selected schools. Informed written consent (assent) of school children were taken to participate in the study. To maintain the ethical consideration informed about the data collection procedure and its importance to the sample's parents/Guardians through school circular by the principals of selected schools.

Data Collection Method

Selection of children with the risk of cardiovascular diseases

From the school children samples were selected by physiological measures such as

- Measuring height

- Weight
- Body Mass Index (BMI)
- Blood pressure
- heart rate

The children with high Body mass index, high Blood pressure and abnormal heart rate were selected as samples for this study and informed written consent was obtained from the selected school children with the risk of Cardiovascular Diseases.

Pre Test

Pre test was conducted in selected school children with the help of closed ended questionnaire to assess their knowledge on life style modification to prevent cardiovascular diseases, attitude of children on life style modification to prevent cardiovascular diseases was assessed by five point likert scale and practice of children on life style modification to prevent cardiovascular diseases was assessed by rating scale, the time taken by the children to fill the questionnaire was approximately 45 minutes.

Presentation of VATM

Immediately after the pre test video assisted teaching module on life style modification to prevent cardiovascular diseases was displayed to the selected samples in class wise each class room consists of around 50 children with the risk cardiovascular diseases before the presentation adequate explanation given by the investigator about the importance of the video, the video was displayed in the projector it was audible to all the children in that class room. The time of video presentation was 30 minutes. After the VATM investigator given adequate explanation about the risk factors of cardiovascular diseases and how to follow the life style modification to prevent cardiovascular diseases followed by that children were allowed to ask their doubts, immediately investigator cleared their doubts and appropriate explanation was given.

Post test

Evaluation was done by conducting post test after 30 days of presentation of VATM. The investigator visited the school and conducted the post test. Children's knowledge, attitude and practice was assessed by conducting the post test with same questionnaire which was used for the pre test. The duration of post test was 45 minutes.

RESULTS AND DISCUSSION

Frequency and percentage wise distribution of Children with the risk of Cardiovascular Diseases according to their demographic data.

Majority of children (33.7%) belonged to the age group of 12 years 1 month to 13 years. Majority (54.9%) were male children. Majority (47.7%) of children were 7th and 8th standard. Majority (93.25%) of children were belonged to non vegetarian. Majority (78.8%) of children had the habit of eating junk food. About interest in playing physically active game majority (71.1%) of children were not interested in playing physically active game. About duration of physically active play per day majority (58.0%) of children were played for 0 to 30 minutes. Majority (58.7%) of children were had the habit of sleeping for 5 to 7 hours. About type of family (27.7%) of children were belonged to nuclear family, about place of residence (50.4%) of children were belonged to urban area. Monthly income of the family majority (55.4%) of children were had the monthly income of more than 11,000. Majority (39.2%) of children's fathers were belonged to secondary education. Majority (38.4%) of children's mothers were belonged to secondary education. Majority (55.8%) of children were had the family history of obesity. Majority (70.2%) of children were had the family history of hypertension. Majority (62.6%) of children were had family history of diabetes mellitus. Majority (60.9%) of children did not had the family history of cardio vascular diseases.

Area – wise comparison of mean, SD and mean percentage of pre test and post test knowledge scores (KS) of children with the risk of cardiovascular diseases.

Overall comparison of Mean, SD and mean% of pre test and post test knowledge score of the children on life style modification in prevention cardiovascular diseases shows that the overall pretest mean percentage of knowledge score was (8.26 ± 2.61) which is 28% whereas in post test knowledge score was (25.67 ± 2.17) 86% depicting a difference of 58% revealing that the VATM was effective on knowledge of the children in life style modification on prevention of cardiovascular diseases.

Area – wise comparison of mean, SD and mean percentage of pre test and post test attitude scores (AS) of children with the risk of cardiovascular diseases.

Overall comparison of mean, SD and mean percentage of pre test and post test attitude scores (AS) of children with the risk of cardiovascular diseases reveals that the overall pretest means % of Attitude score was (32.65 ± 2.04) 25%, Whereas in post test it was (117.8 ± 10.7) which is 91% depicting a difference of 66% revealing that the VATM was effective in attitude of the children with the risk of cardiovascular diseases in preventing Cardiovascular Diseases.

Area – wise comparison of mean, SD and mean percentage of pre test and post test practice scores (PS) of children with the risk of cardiovascular diseases.

Overall comparison of mean, SD and mean percentage of pre test and post test practice scores (PS) of children with the risk of cardiovascular diseases reveals that the overall pre test mean percentage of PS score was (31.37± 2.06) which is 42%. Wherever in post test score was (59.86 ± 5.54) which is 80% depicting the difference of 38% revealing that the VATM was effective on practice of the children in preventing Cardiovascular disease.

Effect of VATM on knowledge, attitude and practice on life style modification among children with risk of cardiovascular diseases.

Frequency and percentage wise distribution of pre test and post test knowledge score of children with the risk of cardiovascular diseases shows that in pre test 23% had very poor knowledge, 70% had poor knowledge, 7% had average knowledge, in post test 2% had average knowledge, 6% had good knowledge and 92% had excellent knowledge. It reveals that VATM was effective in improving the knowledge level of the children with the risk of cardiovascular diseases. Frequency and percentage wise distribution of pre test and post test attitude score of children with the risk of cardiovascular diseases shows that in pre test all the children 100% had negative attitude on Life Style Modification in prevention of Cardiovascular Diseases. In post test 4% had neutral attitude and 96% had positive attitude on Life Style Modification in prevention of Cardiovascular Diseases. It reveals that VATM was effective in improving the attitude level of the children with the risk of cardiovascular diseases. Frequency and percentage wise distribution of pre test and post test practice score of children with the risk of cardiovascular diseases shows that in pre test all children 100% had poor practice on Life Style Modification to prevent Cardiovascular Diseases. In post test 0.2% had poor practice, 71.9% had average level of practice and 27.9% had good practice on LSM to prevent Cardiovascular Diseases. It reveals that VATM was effective in improving the practice level of the children with the risk of cardiovascular diseases.

REFERENCES

1. Mohamed Sanulla. “Children how important are they for the world”, July – 10, 2008, Available from: URL:http://www.Sanulla.info.com/2008/07/2008.
2. Bridget B. Kelly. “Promoting Cardiovascular Health in the Developing World: A Critical Challenge to Achieve Global Health” Washington, D.C: National Academies Press, 2010. ISBN 0-309-14774-3, Pp. 3-5.
3. A healthy lifestyle leaves a child fit, energetic and at reduced risk for getting diseases Jan 2016. Available from: URL: http://www.livestrong.com.

4. Stephen R. Daniels. "Recent Advances in Preventive Cardiology and Lifestyle Medicine Reduction of Risk for Cardiovascular Disease in Children and Adolescents", Journals of American heart association, 2011; 124; Pp. 1673-1686.
5. Davis PH, et al. "Carotid intimal-medial thickness is related to cardiovascular risk factors measured from childhood through middle age: the Muscatine Study", (2011), 104: Pp. 2815–2819. Available from: https://www.ahajournals.org/doi/full/10.1161/hc4601.099486
6. Gupta Ekta, et al. "Risk factor distribution for cardiovascular diseases among high school boys and girls of urban Dibrugarh, Assam", Journal of Family Medicine and Primary Care, 2016, Vol. 5(1); Pp. 108-113.
7. Finegold JA, Asaria P, Francis DP. "Mortality from ischaemic heart disease by country, region, and age: statistics from World Health Organisation and United Nations", International journal of cardiology, 2013, Vol. 168(2); Pp. 934-45.
8. Yusuf S, Hawken S. "Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study", Sep 2017, Vol. 364(9438); Pp. 937-52. https://www.revespcardiol.org/en-cardiovascular-risk-factors-lifestyle-associated-articulo-S1885585710000770
9. Madhab Ray, et al. "Cardiovascular health awareness and the effect of an educational intervention on school-aged children in a rural district of India", Indian Heart Journal, 2016, Vol. 68 (1); Pp. 43-47.
10. Deepa Shokeen, et al. "Risk Factors Associated with the Increasing Cardiovascular Diseases Prevalence in India", Journal of Nutrition & Food Sciences Journal of Nutrition & Food Sciences, Nov. 2015, Vol. 5(1); Pp. 1-6.
11. Hayman, et al. "CVD in Nursing Practice: Children and Youth", Journal of the American Heart Association, 2007, 116; Pp. 344-357.
12. Kavita . "Nurses role in cardiovascular risk assessment and communication: Indian nurses perspective", International Journal of Non Communicable Diseases, 2020, Vol. 5(1); Pp. 4-10.
13. World Health Report (2002), "cardiovascular diseases and its risk profile, Prevalence, Current Capacity and Epidemiological Leads in CVD Programmes"**,** World Health Organization. Geneva, Switzerland. Available from:https://www.who.int/cardiovascular diseases/media/en/635.pdf
14. Abhiruchi Galhothra Amrit Abrol Neeraja Adarwal lifestyle related risk factors for CVD in Indian adolescents, the internet journal of health 2008 : Pp. 1528-8315. Available from: http://ispub.com/IJH/9/2/4263.

Table .1 Frequency and percentage wise distribution of Children with the risk of Cardiovascular Diseases according to their demographic data. **N = 830**

Demographic data	Frequency	Percentage
Age (in years)		
12 Yrs 1 Month to 13 Yrs	280	33.7
13 Yrs 1 Month to 14 Yrs	210	25.3
14 Yrs 1 Month to 15 Yrs	180	21.7
15 Yrs 1 Month to 16 Yrs	94	11.3
16 Yrs 1 Month to 17 Yrs	66	8
Gender		
Male	456	54.9
Female	374	45.1
Class		
7^{th} and 8^{th} std	396	47.7
9^{th} and 10^{th} std	246	29.6
11^{th} and 12^{th} std	188	22.6
Food habit		
Vegetarian	56	6.7
Non vegetarian	774	93.3

Habit of eating junk food		
Yes	654	78.8
No	176	21.2
Interest in Playing games		
Yes	240	28.9
No	590	71.1
Duration of physical active play per day		
0 to 30 mts	483	58.0
31 mts to 60 mts	287	35.0
61 mts to 90 mts	34	4.0
91mts to 120 mts	26	3.0
Duration of sleeping		
5 to 7 hours	487	8.7
8 to 10 hours	270	32.5
11 to 13 hours	73	8.8
Type of family		
Joint family	230	27.7
Nuclear family	600	72.3
Place f residence		
Rural	412	49.6
Urban	418	50.4
Monthly income of the family		
<2000	0	0
2001 – 5000	10	1.3
5001 – 8000	106	12.7
8001 – 11000	254	30.6
More than 11000	460	55.4
Father's education		
No formal education	130	15.6
Primary education	62	7.5
Secondary education	325	39.2
Graduate	313	37.7
Mother's education		
No formal education	210	25.3
Primary education	112	13.5
Secondary education	319	38.4
Graduate	189	22.7
Family history of obesity		
Yes	463	55.8
No	367	44.2
Family History of hypertension		
Yes	583	70.2
No	247	29.8
Family History of Diabetes mellitus		
Yes	520	62.6
No	310	37.4

Family History of cardio vascular disease		
Yes	325	39.1
No	505	60.9

Table. 2 Area – wise comparison of mean, SD and mean percentage of pre test and post test knowledge scores (KS) of children with the risk of cardiovascular diseases. N=830

Areas of knowledge	**Max Score**	**Pre test score**			**Post test score**			**Effective-ness in mean%**
		Mean	**SD**	**Mean %**	**Mean**	**SD**	**Mean %**	
Introduction to cardiovascular disease	1	0.43	0.496	43	1	0	100	57
Risk factors								
Risk factors	3	0.94	0.72	31	2.99	0.08	100	68
Hypertension	4	1.16	0.76	29	3.92	0.35	98	69
Obesity	2	0.61	0.69	31	1.92	0.31	96	66
Alcoholism and smoking	3	0.67	0.63	22	2.75	0.53	92	69
Physical inactivity	1	0.30	0.46	30	0.91	0.28	91	61
Over eating of fast food	1	0.26	0.44	26	0.92	0.25	92	66
Type 2 diabetes mellitus	2	0.48	0.63	24	1.74	0.44	87	63
Urbanization	1	0.24	0.43	24	0.84	0.36	84	60
High cholesterol	1	0.27	0.44	27	0.72	0.44	72	45
Stress	1	0.29	0.45	29	0.72	0.44	72	43
Family History	1	0.28	0.45	28	0.70	0.45	70	42
Definition of life style modification	1	0.21	0.41	21	0.67	0.47	67	46
Life Style Modification								
Heart healthy nutrition	3	0.80	0.68	27	2.01	0.67	67	40
Introduction to cardiovascular disease	1	0.43	0.496	43	1	0	100	57
Smoking and alcohol avoidance	1	0.29	0.46	29	0.54	0.49	54	25
Physical examination and health checkup	1	0.22	0.42	22	0.83	0.37	83	61

Complementary and alternative medicine	1	0.35	0.48	35	0.99	0.09	99	64
Overall	**30**	**8.26**	**2.61**	**28**	**25.67**	**2.17**	**86**	**58**

Table.3 Area – wise comparison of mean, SD and mean percentage of pre test and post test attitude scores (AS) of children with the risk of cardiovascular diseases. N=830

Areas of Attitude	Max Score	Pre test score			Post test score			Effectiveness in mean%
		Mean	SD	Mean %	Mean	SD	Mean %	
Heart healthy nutrition	16	5	1.02	25	18.15	1.67	91	66
Physical activity and exercise	20	6.23	0.97	24	21.3	2.23	85	61
Maintenance of ideal body weight	20	6.30	1.35	25	22.95	2.29	92	67
Smoking and alcohol avoidance	16	5.08	0.78	25	18.19	1.64	91	66
Routine physical examination and health checkup	16	4.82	0.80	24	18.16	1.85	91	67
Complementary and alternative medicine	16	5.19	1.02	26	18.99	2.11	95	69
Overall	**104**	**32.65**	**2.04**	**25**	**117.8**	**10.7**	**91**	**66**

Table.4 Area – wise comparison of mean, SD and mean percentage of pre test and post test practice scores (PS) of children with the risk of cardiovascular diseases. N = 830

Areas of practice	Max Score	Pre test Score			Post test score			Effectiveness in mean%
		Mean	SD	Mean %	Mean	SD	Mean %	
Heart healthy nutrition	33	13.79	1.13	42	26.13	2.20	79	37
Physical activity and exercise	18	7.45	0.94	41	14.33	1.71	80	38
Maintenance of ideal body weight	9	3.79	0.85	42	7.25	1.06	81	38
Smoking and alcohol avoidance	3	1.24	0.48	41	2.41	0.49	80	39

Routine physical examination and health checkup	6	2.56	0.66	43	4.82	0.82	80	38
Complementary and alternative medicine	6	2.51	0.52	42	4.91	0.79	82	40
Overall	**75**	**31.37**	**2.06**	**42**	**59.86**	**5.54**	**80**	**38**

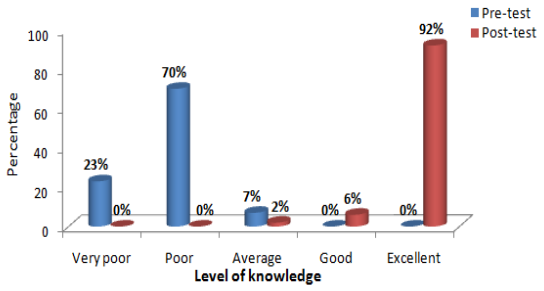

Fig. 1. To assess the effect of VATM on knowledge, attitude and practice on life style modification among children with risk of cardiovascular diseases.

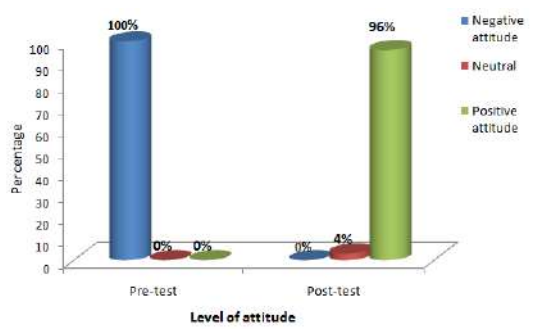

Fig. 2. To assess the effectiveness of VATM on knowledge, attitude and practice on life style modification among children with the risk of cardiovascular diseases.

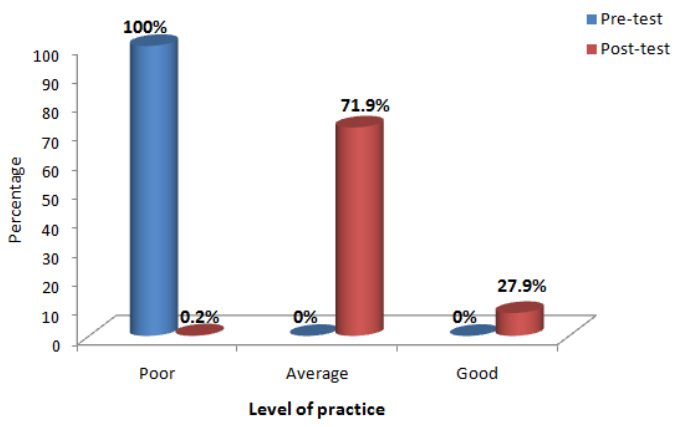

Fig. 3. To assess the effectiveness of VATM on knowledge, attitude and practice on life style modification among children with risk of cardiovascular diseases.

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RESEARCH ARTICLE

Food Safety and Food Quality Assessment by the Analytical Methods

Venkata Ravi Shankar Babu Dakshinapu[1]*, Vinod Kumar Kondreddy[2], Shaik Shakir Basha[3], Chiranjeevi Gnagarapu[4] and Talari Jayasree[4]

[1]Department of Pharmaceutical Quality Assurance, RIPER - Autonomous, Anantapur, Andhra Pradesh, India
[2]HoD and Associate Professor, Department of Pharmaceutical Quality Assurance, RIPER -Autonomous, Anantapur, Andhra Pradesh, India
[3]Associate Professor, Department of Pharmaceutical Analysis, RIPER - Autonomous, Anantapur, Andhra Pradesh, India
[4]Department of Pharmaceutical Analysis, RIPER -Autonomous, Anantapur, Andhra Pradesh, India

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***Address for Correspondence**
Venkata Ravi Shankar Babu Dakshinapu
Department of Pharmaceutical Quality Assurance,
RIPER - Autonomous,
Anantapur, Andhra Pradesh, India
Email: dakshinapuravishankkar@gmail.com

ABSTRACT

The food security is still a chief public concern, playing significant role for providing quality of life to human beings. Several food safety regulations have been amended worldwide, still the governance on food safety issues is relatively suboptimal as compared to the pharmaceutical and biological product regulations. Assessment and differential diagnosis tools among food allergy, food intolerance, food poisoning and food intoxications have scientifically evolved but still the need more precise and high throughput screening tools. This communication consolidates the various food regulations, food-illness managements, and modern analytical tools used for detecting the toxins and heavy metals. The analytical techniques used in the assessment of food toxins and heavy metals are relatively expensive and involve laborious procedure. At the end, we have postulated the key challenges and difficulties likely encountered in the regulations and monitoring food quality.

Keywords: Food safety, heavy metals, analytical methods, mycotoxins, food regulations.

INTRODUCTION

There is a gradual growth in the global food demand as the world's populace lingers to grow. Food-borne illness is a major concern to humans since the dawn of humanity. Therefore, food safety and nutritional security policies are crucial for any nation for protecting the people from food-borne illnesses. In general, food-borne sickness or illness

posed a threat to humanity in a variety of ways since the dawn of time [1]. To date, more than 250 microbes including bacteria, viruses, and parasites are known to cause food poisoning, and they contaminate the food products at any stage of food life cycle [2]. Most of the countries have their legal food regulatory bodies, and defined food safety standards to monitor the food quality. Despite the existing food regulations, globally around 600 million people fall ill after consuming tainted food, and amongst 4,20,000 people are dying every year [3]. The deaths are not only due to microbial toxin, or other poisons, but also the heavy metals levels in food also alarming the serious health issues and mortality. Accordingly, Food and Drug Administration (FDA) set the limit of heavy metals in foods, animal feed and in cosmetic. Recently, FDA announced "Closer to Zero" action plan to address toxic metals in foods eaten by children [4]. Indeed, the analytical methods playing crucial role in the detection of potential hazardous food contaminants which are likely to be present at nano- to femto- levels e.g., mycotoxin or heavy metals. Different contaminants in food products are mentioned in figure-1.

Food safety and its regulations

Following a public outcry in the twentieth century, the US Government adopted the sanitary slaughtering and butchering regulations called "Meat Inspection Act 1906". In 1947, "Mars Incorporated" turn out to be the first food company to deploy metal detectors in their plants. In 1959, Hazard analysis critical control point (HACCP) was established which has turned the food safety concepts from "reactive to proactive". Subsequently, the National Aeronautics and Space Administration (NASA) scientists designed a risk-based technique in production for evaluating finished food products. In1980s, HACCP has become the principle of food safety evaluation procedures, following this, food safety standards were enacted in every country [3] and they are presented below. The various food safety authorities are namely, Food Safety and Standards Authority of India (FSSAI; Food safety and standards Act'2006; Food safety and standards Rules'2011; India) [5], European Food Safety Authority (EFSA; General food law regulations; Europe) [6]. Department of Agriculture, Fisheries, and Forestry (DAFF), Department of Health (DOH) & Department of Trade and Industry (Agriculture Product Standard Act; 1990; South Africa) [7][8]. Food Standards Australia New Zealand (FSANZ; The Food Act 1991; Australia and New Zealand) [9]. U.S. Food Safety Inspection Service (FSIS; Federal Food, Drug, and Cosmetic Act (FD&C Act)-1938; USA) [10], Food Safety Commission of Japan (FSCJ; The Japan Agricultural Standards Law'1950; The Food Safety Basic Act'2006; Food Sanitation Act Amendment'2018; Japan) [11], State Food and Drug Administration' 2003 (SFDA; China Food and Drug Administration, or CFDA' 2018; China) [12], The Food Standards Agency (FSA; Food Safety Act'1990 And amendment 2004; United Kingdom) [13][14], The ministry of agriculture (MAPA; The Ministry of Health (MS) - through its National Agency of Sanitary Surveillance (ANVISA)' 1860; Brazil) [15].

Food safety issues

According to WHO, food safety, nutrition, and food security are interconnected. A "vicious cycle of disease and starvation" is created by a shortage of safe food, affecting public health systems and lowering quality of life [16]. Every year, millions of people get infected with food-borne illnesses, and many die as a result. Although many of these diseases can be avoided, this is an avoidable problem that hurts both people and the economy. Food safety advancements can have economic and societal benefits in addition to preventing food-borne infections. Tainted goods may enter the food chain if an effective food safety strategy is not adopted. Since they handle and carry the cost of product recalls, firms suffer major disruptions in their operations if a defective product is detected. Definitely, remarkable legal actions have been implemented on many risky food products and are summarized in Table-1 [17-28].

Bacteria

The most common food-borne infections are due to *Salmonella*, *Campylobacter*, and *Enterohaemorrhagic Escherichia coli*, Clostridium that harm millions of can be fatal. Symptoms include fever, headache, nausea, vomiting, abdominal discomfort, and diarrhea [29]. Campylobacter infections are widespread through raw milk, raw or undercooked chicken, and drinking water. *Listeria* is found in unpasteurized dairy foods and a variety of ready-to-eat foods and causes miscarriages in pregnant women [30]. *Vibrio cholera* infects humans due to contaminated water or food. Symptoms consist of abdominal pain, vomiting, and watery diarrhea, which may lead to severe dehydration and

death. Antimicrobials (antibiotics) are needed to treat bacterial infections. Antimicrobial resistance is one of pressing concerns (e.g., *Salmonella* through chickens) in the treatment [31].

Viruses

Hepatitis A, a long-term liver ailment blowout by eating raw or undercooked shellfish or infected raw foods. Nausea, vomiting, watery diarrhea, and stomach pain are all symptoms of *norovirus* infections [29].

Parasites

Fresh fruits contaminated by *Ascaris*, *Entamoeba histolytica*, or *Giardia*, *Cryptosporidium,* which can be transmitted through water or soil. Tapeworms, such as *Echinococcus* species and *Taenia solium*, can be diffused to humans by food or direct contact with animals [32].

Prions

Prions are protein-based infectious pathogens that root neurodegenerative diseases. Human Creutzfeldt-Jakob disease is allied to bovine spongiform encephalopathy (BSE, sometimes acknowledged as "mad cow disease") (vCJD). The prion agent is transmitted to humans via the ingestion of bovine products that include identified risk material, such as brain tissue [33].

Chemicals

Numerous toxins that include marine biotoxins, mycotoxins, and cyanogenic glycosides. Aflatoxin and ochratoxin are mycotoxins found in staple foods (corn and cereals). Long-term exposure to these toxins harms the immune system and lead to cancer. Dioxins and polychlorinated biphenyls (PCBs) are the by-products of industrial progressions and rubbish combustion. They get accumulated in the food chains of animals and causes hormone disruption, immune system damage, reproductive and developmental difficulties, and cancer. Pollution of the air, water, and soil is the primary source of heavy metal contamination in food. Lead, cadmium, and mercury are heavy metals that affect the brain system and kidneys [34].

Critical view on trans-fat in food

As per the traditional "diet-heart" theory, atheromatous plaques are formed due to high intake of saturated fats. WHO also encourages food manufacturers all over the world to begin enforcing their efforts to reduce the risk of heart attacks by removing industrially manufactured trans-fat or trans fatty acid (TFA) from their edible products. According to the conventional diet-heart hypothesis, there is a link between dietary lipids and blood cholesterol levels, as well as cholesterol levels and congestive heart failure (CHD) risk [35]. Effect of trans-fat mechanism in human disorders is mentioned in figure-2.

Heavy metals in food and their assessment

Metals and metalloids whose atomic number is >20 and density > 5 g cc^{-1} are known as heavy metals (HMs). HMs are of two categories namely essential and nonessential heavy metals. Essential HMs (Cu, Fe, Mn, Co, Zn, and Ni) are required by living organisms for their growth and development, and metabolism. Nonessential HMs (Cd, Pb, Hg, Cr, and Al) are those which are not essential by the body and their presence even in a trace amount also can lead to toxicity and affect the health [36]. Heavy metals should be given superior attention all over the globe because of their toxic and mutagenic effects. The World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) recommended maximum permissible level (MPL) of Cu (73.3 mg/kg), Zn (99.4 mg/kg), Ni (67.9 mg/kg), Fe (425.5 kg/g), Cd (0.2 mg/kg), and Pb (0.3 mg/kg) in vegetables for human consumption. FAO had also recommended the MPL of Fe (0.01mg/kg), Cu (0.05-0.5 mg/kg), Cd (0.5 mg/kg), Pb (0.1 mg/kg), Zn (0.3-1.0 mg/kg), Hg (0.1 mg/kg), Cr (0.05 mg/kg) in meats for human consumption [37]. So, if heavy metals concentration in food and food product exceeds the MPL then it may lead to serious health consequences. Different Analytical techniques used in detection of heavy metals in foods are mentioned in table-2 [38-47].

Analytical techniques in the assessment of food toxins

As on today, chromatography is the preferred analytical tactic among the several analytical techniques available for determining the contaminants and residues of varied types. Non-polar toxins have been identified using gas chromatography-mass spectrometry (GC-MS), while liquid chromatography-mass spectrometry (LC-MS) has been used for polar compounds. However, for thermo-labile toxins LC-MS is most recommended [48]. Hydrophilic interaction liquid chromatography (HILIC) and supercritical fluid chromatography (SFC) are other methods for identifying typical polar compounds [49]. as the alternatives. Due to the low abundance of toxin in the food product and high matrix interference, often liquid chromatography-tandem mass spectrometry (LC-MS/MS) and gas chromatography-tandem mass spectrometry (GC-MS/MS) [50] are highly recommended. The interface of electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) are used for polar and non-polar compounds, respectively. In recent time, LC-HRMS has gained much importance in screening of wide range of nano- or femto- level chemical toxins for its greater selectivity and sensitivity. The LC-HRMS has been employed for target, post-target, and non-target analysis, but not in GC [51]. The stationary phase used are C18, C8 and Phenyl columns for non-polar contaminants, whereas HILIC, cyano and amino columns used for polar compounds.

In focus, GC-MS is the widely used technology for detecting non-intentionally added substance (NIAS) like hydrocarbons, phthalates, bisphenol A (BPA) in food and food packaging products. For the analysis of numerous food contact material (FCM) and food contact article (FCA) contaminants such as perfluoroalkyl chemicals **(**PFCs), peroxyacetic acid(PAAs) etc., the LC-HRMS technique using TOF or Orbitrap mass analyzers has recently been introduced. The other routine techniques are, thin-layer chromatography (TLC), enzyme-linked immunosorbent assay (ELISA) and biosensor-based screening. The techniques mentioned above are used to determine the presence of mycotoxins, and they are remains unaffected by the sample processing and other physical, chemical, or biological procedures [52]. Raman spectroscopy and hyperspectral imaging (HSI) hyphenated with X-ray or near infra-red (NIR) techniques can be used to inspect the quality of a variety of foods [53]. The shelf life of food items can be increased through irradiation techniques. EBI is a cutting-edge food reprocessing technique that uses low-dose ionizing radiation to kill microbial contaminants in crops and food. In contrast, electron beam ionization (EBI) is a decontaminating tool, used to slow crop propagation and slows down the ripening of vegetables and fruits, extending their shelf life [54].

CONCLUSION

Though, numerous specialized food regulations at all stages of food manufacture to prevent cross-contamination, there has been still a risk of food contaminations. There is a need for continuous improvement in the monitoring and evaluation of food products for their safety in-terms of microbial contaminations and heavy metals. The accessibility of food testing with less expensive analytical technique needs to be ensured for high throughput screening. There is a need of more advanced technologies and regulatory mechanism for real-time testing of food quality especially for packed and frozen foods. This would benefit people's health and regulatory authorities for precise control of food-borne illnesses.

REFERENCES

1. Enahoro D, Bahta S, Mensah C, Oloo S, Rich K. Current and future trade in livestock products. Rev Sci Tech Off Int Epiz. 2021;40(2):2.
2. Mohammad A-M, Chowdhury T, Biswas B, Absar N. Food poisoning and intoxication: A global leading concern for human health. Food safety and preservation: Elsevier; 2018. p. 307-52.
3. Falcon WP, Naylor RL. Rethinking food security for the twenty-first century. American Journal of Agricultural Economics. 2005;87(5):1113-27.
4. Järup L. Hazards of heavy metal contamination. British medical bulletin. 2003;68(1):167-82.

5. Rajasekaran A, Kalaivani M. Designer foods and their benefits: A review. Journal of food science and technology. 2013;50(1):1-16.
6. Szajkowska A. Regulating food law: risk analysis and the precautionary principle as general principles of EU food law: Wageningen University and Research; 2012.
7. Erasmus SW, Hoffman LC. What is meat in South Africa? Animal Frontiers. 2017;7(4):71-5.
8. Molefe S, McCrindle C, Botha C, Makita K, Grace D. A critical review of food safety legislation and policy applicable to products of animal origin in South Africa. 2011.
9. Zealand AN. Food Standards Australia New Zealand. 2009.
10. Campus WO, Woodcock J, Abernethy CA, Commissioner PD. United States Food and Drug Administration.
11. Abe A, Koyama K, Uehara C, Hirakawa A, Horiguchi I. Changes in the Risk Perception of Food Safety between 2004 and 2018. Food Safety. 2020:D-20-00015.
12. Kumar NA, Dileep K, Ravindra C, Suthakaran R. An overiew of chinese drug regulatory system: Areview. International Journal of Drug Regulatory Affairs. 2014;2(3):14-8.
13. Mensah LD, Julien D. Implementation of food safety management systems in the UK. Food control. 2011;22(8):1216-25.
14. Tedstone A. Food standards agency: nutrition. Proceedings of the Nutrition Society. 2004;63(4):501-3.
15. Le Bizec B. Brazilian Ministry of Agriculture, Livestock and Food Supply (MAPA): strategies to tackle chemical food safety issues. Taylor & Francis; 2012. p. 481.
16. Organization WH, Aquaculture JFNWSGoFSIAwPf. Food safety issues associated with products from aquaculture: report of a joint FAO/NACA/WHO study group: World Health Organization; 1999.
17. Gelletly L. Somalia: Simon and Schuster; 2014.
18. Le Billon K. French kids eat everything: How our family moved to France, cured picky eating, banned snacking and discovered 10 simple rules for raising happy, healthy eaters: Hachette UK; 2012.
19. Wagner M, editor Kinder surprise's debut in discrete optimisation: a real-world toy problem that can be subadditive. Proceedings of the Genetic and Evolutionary Computation Conference Companion; 2019.
20. Rajah J. Flogging gum: Cultural imaginaries and postcoloniality in Singapore's rule of law. Law Text Culture. 2014; 18:135-65.
21. Walker MJ, Colwell P, Craston D, Axford IP, Crane J. Analytical strategy for the evaluation of a specific food choking risk, a case study on jelly mini-cups. Food Analytical Methods. 2012;5(1):54-61.
22. Weisbecker A. A legal history of raw milk in the United States. Journal of Environmental Health. 2007;69(8):62-3.
23. Pala C. Ban on Beluga caviar points to sturgeon's worldwide decline. Science. 2005;310(5745):37.
24. Dietz BM, Bolton JL. Biological reactive intermediates (BRIs) formed from botanical dietary supplements. Chemico-biological interactions. 2011;192(1-2):72-80.
25. Mishra S. The importance of "consumer type" in the attribution of crisis responsibility: The case of the Maggi Noodles crisis in India. International Journal of Strategic Communication. 2017;11(3):224-43.
26. Rana RS, Mehtani P, Sharma C. Analysis of potassium bromate in bread samples of Jaipur City, India. Annals Food Science and Technology, Mansarovar. 2020;21:109-16.
27. Moretto A, Lotti M. Poisoning by organophosphorus insecticides and sensory neuropathy. Journal of Neurology, Neurosurgery & Psychiatry. 1998 Apr 1;64(4):463-8.
28. Bobo MD L. Evidence for subpopulations of Listeria monocytogenes with enhanced invasion of cardiac cells.
29. Linscott AJ. Food-borne illnesses. Clinical microbiology newsletter. 2011 Mar 15;33(6):41-5.
30. Iwu CD, Okoh AI. Preharvest transmission routes of fresh produce associated bacterial pathogens with outbreak potentials: a review. International journal of environmental research and public health. 2019 Jan;16(22):4407.
31. Teuber M. Spread of antibiotic resistance with food-borne pathogens. Cellular and Molecular Life Sciences CMLS. 1999 Nov;56(9):755-63.
32. Bhunia AK. Foodborne microbial pathogens: mechanisms and pathogenesis. Springer; 2018 May 21.
33. Collinge J. Mammalian prions and their wider relevance in neurodegenerative diseases. Nature. 2016 Nov;539(7628):217-26.
34. Rodricks JV, Turnbull D, Chowdhury F, Wu F. Food constituents and contaminants. Environmental toxicants: human exposures and their health effects. 2020 Mar 17:149-204.

35. Werkö L. End of the road for the diet-heart theory?. Scandinavian Cardiovascular Journal. 2008 Jan 1;42(4):250-5.
36. Raychaudhuri SS, Pramanick P, Talukder P, Basak A. Polyamines, metallothioneins, and phytochelatins—Natural defense of plants to mitigate heavy metals. Studies in Natural Products Chemistry. 2021 Jan 1;69:227-61.
37. Amfo-Otu R, Agyenim JB, Adzraku S. Meat contamination through singeing with scrap tyres in Akropong-Akuapem abattoir, Ghana. Applied Research Journal. 2014 Dec 15;1(1).
38. Zhong WS, Ren T, Zhao LJ. Determination of Pb (Lead), Cd (Cadmium), Cr (Chromium), Cu (Copper), and Ni (Nickel) in Chinese tea with high-resolution continuum source graphite furnace atomic absorption spectrometry. Journal of food and drug analysis. 2016 Jan 1;24(1):46-55.
39. Gerber N, Brogioli R, Hattendorf B, Scheeder MR, Wenk C, Günther D. Variability of selected trace elements of different meat cuts determined by ICP-MS and DRC-ICPMS. Animal. 2009 Jan 1;3(1):166-72.
40. Dico GM, Galvano F, Dugo G, D'ascenzi C, Macaluso A, Vella A, Giangrosso G, Cammilleri G, Ferrantelli V. Toxic metal levels in cocoa powder and chocolate by ICP-MS method after microwave-assisted digestion. Food Chemistry. 2018 Apr 15; 245:1163-8.
41. Andriamisetra VM. Analysis of heavy metals and minerals elements in the turmeric using Total-Reflection X-ray Fluorescence analysis technique and Atomic Absorption Spectrometry; Analyses des metaux lourds et elements mineraux dans les Curcumas Longa par la technique de la fluorescence X a reflexion totale et par la spectrometrie d'absorption atomique. 2014.
42. Kusmartini I, Kurniawati S, Adventini N, Damastuti E, Prakoso D, Niken WY, Sari DK, Lestiani DD. Macro and micromineral in commercial infant formula milk in Indonesia by neutron activation analysis. InIOP Conference Series: Materials Science and Engineering 2021 (Vol. 1011, No. 1, p. 012062). IOP Publishing.
43. Pant N, Subedee A, Gharti RB, Shrestha S. Spectrometric Determination of Heavy Metals Present in Mango Fruit of Nepali Origin. Amrit Research Journal. 2020 Sep 17;1(1):72-7.
44. Romero-González R, Frenich AG, Vidal JM, Prestes OD, Grio S. Simultaneous determination of pesticides, biopesticides and mycotoxins in organic products applying a quick, easy, cheap, effective, rugged and safe extraction procedure and ultra-high performance liquid chromatography–tandem mass spectrometry. Journal of Chromatography A. 2011 Mar 18;1218(11):1477-85.
45. Marrubini G, Appelblad P, Maietta M, Papetti A. Hydrophilic interaction chromatography in food matrices analysis: An updated review. Food chemistry. 2018 Aug 15;257:53-66.
46. Wooding M, Rohwer ER, Naudé Y. Determination of endocrine disrupting chemicals and antiretroviral compounds in surface water: a disposable sorptive sampler with comprehensive gas chromatography–time-of-flight mass spectrometry and large volume injection with ultra-high performance liquid chromatography–tandem mass spectrometry. Journal of Chromatography A. 2017 May 5;1496:122-32.
47. Tsagkaris AS, Nelis JL, Ross GM, Jafari S, Guercetti J, Kopper K, Zhao Y, Rafferty K, Salvador JP, Migliorelli D, Salentijn GI. Critical assessment of recent trends related to screening and confirmatory analytical methods for selected food contaminants and allergens. TrAC Trends in Analytical Chemistry. 2019 Dec 1;121:115688.
48. Sanchis Y, Yusà V, Coscollà C. Analytical strategies for organic food packaging contaminants. Journal of Chromatography A. 2017 Mar 24;1490:22-46.
49. He HJ, Wu D, Sun DW. Nondestructive spectroscopic and imaging techniques for quality evaluation and assessment of fish and fish products. Critical Reviews in Food Science and Nutrition. 2015 May 12;55(6):864-86.
50. Grolichova M, Dvořák P, Musilova H. Employing ionizing radiation to enhance food safety–a review. Acta Veterinaria Brno. 2004;73(1):143-9.

Table 1: Selected list of prohibitions and restrictions food products at Global market [17-28].

S. NO	Banned Food product & Country	Description of prohibitions and restrictions	Year	Remarks
1.	Samosa	To the AI-Shabaab group, the snack's triangular shape appeared to be a symbol of Christianity.	2011	The form is reminiscent of the Christian Holy Trinity.

2.	Tomato Ketchup	According to recent dietary standards in schools,	2011	To encourage school-children to eat nutritious foods
3.	Kinder surprise eggs	Non-nutritive items are embedded in candies	2022	The hidden toy could be a choking hazard.
4.	Chewing gum	To keep the streets clean and stress-free	1992	To keep the streets clean and stress-free
5.	Jelly mini cups (25 Countries)	E425 – konjac gum can be a choking hazard	2020	Children may face choking hazard as a result of a food ingredient.
6.	Unpasteurized raw milk & dairy products (FDA)	Listeria, E. coli, and Campylobacter can cause food poisoning	1987	Pasteurized milk is the best alternative.
7.	Beluga Caviar (Worldwide)	Beluga sturgeon is a highly endangered fish species that needs to be protected.	2005	The Beluga sturgeon is a severely endangered fish species.
8.	Sassafras Oil	Carcinogenic agent and used as a Hallucinogen	1979	Kidney and liver damage
9.	Maggi (Product Recall)	1.Excessive lead, inaccurate labelling "No added MSG" and marketing of a non-standardized food product, such as Maggi Oats Masala Noodles with tastemaker.	2015	The product was recalled and destroyed due to excessive lead levels.
10.	Potassium Bromate in bread	This substance cause cancer as per International Agency for Research on Cancer	2016	Carcinogenic
11.	Citrus flavored drinks	Presence of Brominated Vegetable Oil (BVO)	2020	Thyroid difficulties and a rise in the risk of Schizophrenia are both linked to BVO.
12.	Raw Almonds	Considered unlawful for possible toxins and release of cyanide	2007	Raw almond skins are difficult to digest.
13.	Durian	Despite its deliciousness, the fruit stinks a lot.	2019	Stomach discomfort, diarrhea, allergy
14.	Mountain Dew	Chemicals in the drink cause serious health problems.	2020 (declared unsafe in 1970)	Birth abnormalities, schizophrenia, and deafness
15.	Junk foods in School canteen	Fat, salt, and sugar content are all high (HFSS)	2019	Obesity and cardiovascular disease.

Table 2: Different Analytical techniques used in detection of heavy metals in foods [38-43].

S.No	Food	Heavy metal	Maximum Quality detected	Analytical Technique
1	tea	Pb, Cd, Cr, Cu	0.48–10.57; 0.01 - 0.39; 0.2- 2.45; and 7.73–63.71 mg/kg, respectively	HRCS-AAS
2	Meat	Cd, Pb, Fe, Zn, Se, Mn, Cu, Mo	0.6-3.9; 1-2.1; 0.5-3.3; 0.7-5.1; 9- 44; 3.1-16.7; 0.3-132; 0.9-3.2 µg/100g	ICP-MS, ICP-DRC-MS
3	Chocolate	Pb, Cd, As, V, Cr, Sb	0.545 ± 0.070, 0.050 ± 0.006, 0.013 ±	ICP-MS

		and Se	0.002, 0.094 ± 0.013, 0.952 ± 0.097, 0.027 ± 0.003 and 0.047 ± 0.007 mg/kg, respectively	
4	Turmeric	Mn, Cu, Fe, Zn, Rb	44.7; 19.7; 53.6; 17.3; 35.2 mg/kg respectively	XRF and AAS
5	Infants' formula milk	Fe, Se, and Zn	82.7; 0.14; and 34.7 µg/g respectively	NAA
6	Mango	Fe, Zn, Co and Ni	0.570±0.48, 0.510±0.031, 0.431±0.021, 0.106±0.003 mg/kg respectively.	AAS

ppb – parts per billion, mg/kg – milligram per kilogram, ppm – parts per million, µg/L – microgram per liter, µg/kg – microgram per kilogram, mg/L – milligram per liter, µg/100g – microgram per 100 grams, ng – nano grams, µg/g – microgram per gram.

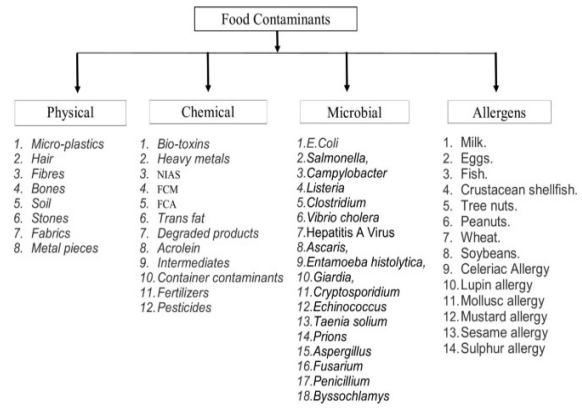

Figure 1: Different contaminants in food products

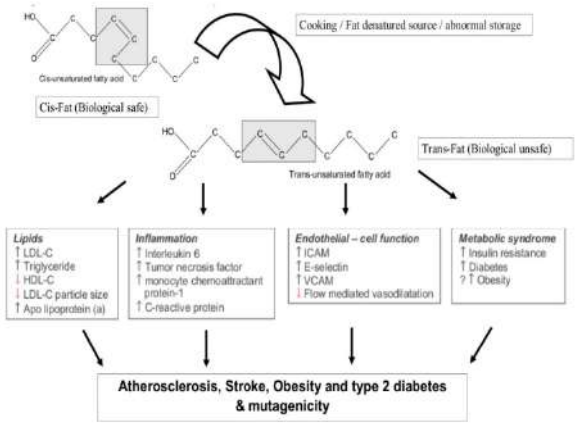

Figure 2: Effect of trans-fat mechanism in human disorders

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RESEARCH ARTICLE

Trend in Area, Production and Productivity of Sesame in India – Growth Rate Analysis

Jeevamathi.J[1] and Srinivasan. G[2]*

[1]Ph.D Scholar, Department of Agricultural Economics, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Tamil Nadu, India.
[2]Associate Professor, Department of Agricultural Economics, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Tamil Nadu, India.

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***Address for Correspondence**
Srinivasan.G,
Associate Professor,
Department of Agricultural Economics,
Faculty of Agriculture,
Annamalai University,
Annamalai Nagar, Tamil Nadu, India.

ABSTRACT

Sesame (*Sesamum indicum*) is the most important edible oilseeds in India. In recent years, the sesame area under cultivation was replaced by other oilseed crops. At this juncture, this study was carried out in India with the following specific objectives: i) to analyse the trends in the area, production and productivity of sesame in India and ii) To offer policy suggestions based on the results of the study. The Annual Report of the Ministry of Agriculture and Farmers Welfare, Government of India was used to collect secondary data and trend analysis was done using Statistical Package for Social Sciences (SPSS).The results of the study concluded that the area, production, and productivity of sesame were increased in India over the past twenty years. Even though there was an increase in the trend in the productivity of sesame, India's average sesame productivity (474kgs/ha) was lower than the world's average productivity (512 kgs/ha). As sesame is a traditional oilseed crop in India, the area and production of sesame crop (17,22,670ha and 8,16,810 tonnnes) were lower than groundnut crop (60,14,950 ha and 1,02,44,080 tonnes).The study suggested that the sesame development programme with the consideration of regional specific constraints will be carried out to attain self-sufficiency in edible oil sector.

Keywords: CAGR, Trend Analysis, Sesame Area, Production, Productivity.

INTRODUCTION

India spends a considerable amount of valued foreign exchange on the import of edible oils, to meet the demands of its growing population. In India, Sesame (*Sesamum indicum*) is the most important edible oilseeds and is frequently referred to as the "Queen of oilseeds. India's longest history of sesame farming makes it the oldest native oil plant. The crop is currently farmed in a variety of agro-ecological conditions, from temperate areas to semi-arid tropics and subtropics and also has a wide variety of cultivars and cultural techniques. At this juncture, this study was carried out with the following specific objectives: i) To analyse the trends in the area, production and productivity of sesame in India and ii) To offer policy suggestions based on the results of the study.

RESEARCH METHODOLOGY

Sampling

The present study was carried out to analyse the area, production and productivity of sesame in India. The data was collected from the Ministry of Agriculture and Farmers Welfare, Government of India. (ON2930), and an orthogonal polynomial approach was used to estimate trends in the area, production, and productivity using a twenty-year data set (2001-2002 to 2020-2021).Data analysis and processing were done using Statistical Package for Social Sciences (SPSS). The tabular values of the required number of observations (N=20) were taken from the statistical table.

Tools of Analysis

Compound Growth Rate

Growth rates were used to measure the past performance of the economic variables. The growth in the area, production and productivity of Sesame for the period 2001-2002 to 2020-21 was analysed by using the exponential growth function.

Compound growth rate analysis was done using the following formula,

$Y_t = ab^t U_t$

Where,

Y_t= Dependent variable for which growth rate wasestimated

(Area, production, yield in year "t");

a = intercept;

b = Regression coefficient;

t = Year which takes values 1, 2....n;

U_t= Distribution term in a year "t".

The equation was transformed into log-linear and written as

$\log Y_t = \log a + t \log b + \log U_t$

The equation was estimated by the using Ordinary Least Square (OLS) technique.

The compound growth rate (g) was then estimated by the identity given in the equation.

$g = (b-1) \times 100$

Where,

g = Estimated compound growth rate per annum in percent,

b= Antilog of log b.

The statistical significance was tested by using the "t" test.

RESULTS AND DISCUSSION

Estimated Area, Production, and Productivity Growth Rates for Sesame in India

The acreage, output, and productivity of sesame in India increased significantly between 2001 and 2021, as given in Table. The highest sesame area (20,83,200 hectares) occurred in 2010–2011, while the least was recorded in 2018–2019, (1419,970 in ha). The maximum output (8,93,000 tonnes) and productivity (485 kg/ha) were obtained in the years 2010–2011 and 2018–2019, respectively. There has been positive growth in the area, output, and productivity over the last 20 years.

Trends in Area

Overtwenty years, the India's compound annual growth rate has revealed adownward trend pattern (2001-2002 to 2020-2021). The compound annual growth at the 1% level of significance is -0.2 per cent (table 2), which is also statistically significant. It indicated that over the recent decades, the area of sesame has decreased.

Trends in Production

Beyond twenty years, the total compound annual growth rate of production has displayed a positive trend (2001-2002 to 2020-2021). At the 1% level of significance, the production has indicated a compound annual growth rate of 1.4per cent (table 3), which is also statistically significant. It describes that sesame production has risen over the last decades.

Trends in Productivity

Beyond twenty years, the total compound annual growth rate of productivity has shown a positive trend (2001-2002 to 2020-2021). At the 1% level of significance, the productivity growth rate over the last year was 1.3per cent (table 4), which is statistically significant. It indicates a rise in sesame productivity during the last twenty years.

CONCLUSIONS

The results of the study concluded that the area, production, and productivity of sesame were increased in India over the past twenty years. Even though there was an increase in the trend in the productivity of sesame, India's average sesame productivity (474kgs/ha) was lower than the world's average productivity (512 kgs/ha). As sesame is a traditional oilseed crop in India, the area and production of sesame crop (17,22,670 ha and 8,16,810 tonnnes) were lower than groundnut crop (60,14,950 ha and 1,02,44,080 tonnes).The sesame development programs with the consideration of regional specific constraints will be carried out to attain self-sufficiency in edible oil sector.

REFERENCES

1. Bera, R., Seal, A., Das, T.H., Sarkar, D., Chatterjee, A.K. and Roy Chowdhury, R., 2017. Production Potential, Energetics and Economic Analysis of Sesame (Sesamum indicum L.) Cultivation: A Case Study from Damodar Command Area, West Bengal. Research & Reviews: Journal of Crop Science and Technology. 6(1):17-21.
2. Bhusanar, S. B., Meena, S. S. and Aditi M., 2022. The trend in Area, Production, and Productivity of Groundnut in Rajasthan. Asian Journal of Agricultural Extension, Economics and Sociology. 40(7): 103-108.
3. Grover, D.K. and Singh, J.M., 2007. Sesamum cultivation in Punjab: Status, potential and constraints. Agricultural Economics Research Review. 20(2): 299-313.
4. Sharma, A., 2018. Current trends in oilseed crops production: An overview. International Journal of Agriculture Sciences. 10(3): 5104-5114.
5. Tegar, A., Banafar, K.N.S., Gauraha, A.K. and Chandrakar, M.R., 2016. An analysis of growth in the area, production and productivity of major vegetables in Bilaspur district of Chhattisgarh state, India. Plant Archives. 16(2): 797-800.

Table 1 Area, Production, and Productivity of Sesame in India (2001 to 2021)

Year	Area (in ha)	Production (in tonnes)	Productivity (in Kg/ha)
2001-2002	16,70,600	6,97,800	418
2002-2003	14,44,400	4,41,300	306
2003-2004	17,00,300	7,82,100	460
2004-2005	18,44,000	6,74,100	366
2005-2006	17,23,200	6,41,100	372
2006-2007	17,03,200	6,18,400	363
2007-2008	17,99,100	7,56,900	421
2008-2009	18,09,100	6,40,300	354
2009-2010	19,42,100	5,88,400	303
2010-2011	20,83,200	8,93,000	429
2011-2012	19,01,500	8,10,300	426
2012-2013	17,05,800	6,85,000	402
2013-2014	16,78,900	7,14,600	426
2014-2015	17,46,100	8,27,800	474
2015-2016	19,50,900	8,50,100	436
2016-2017	16,66,930	7,47,030	448
2017-2018	15,79,770	7,55,430	478
2018-2019	14,19,970	6,89,310	485
2019-2020	16,22,600	6,57,540	405
2020-2021	17,22,670	8,16,810	474
Mean	**1735717**	**714366**	**412.3**
SD	**161534.09**	**104921.09**	**53.85**
CV	**9.31**	**14.69**	**13.06**

Source: Ministry of Agriculture and Farmers Welfare, Government of India. (ON2930)

Table 2 Compound Growth Rate of Area of Sesame in India

'F' value	0.228
R^2	0.581
CAGR	-0.2*

(CAGR – Compound annual growth rate per cent per annum.*- Significant at 1% level of significance)

Table 3 Compound Growth Rate of Production of Sesame in India

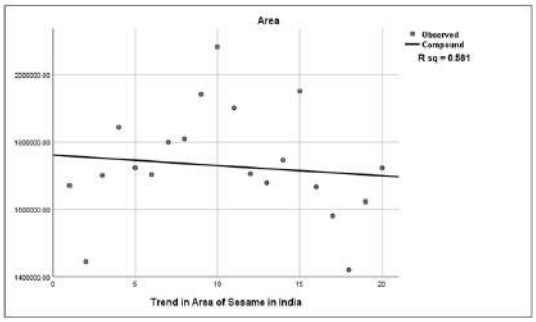
'F' value	4.156
R^2	0.628
CAGR	1.4*

(CAGR – Compound annual growth rate per cent per annum. *- Significant at 1% level of significance)

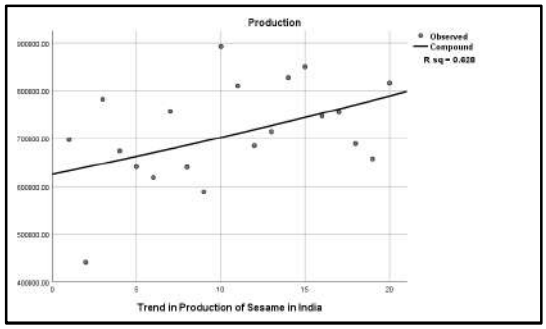
Table 4 Compound Growth Rate of Productivity of Sesame in India

'F' value	10.613
R^2	0.327
CAGR	1.3*

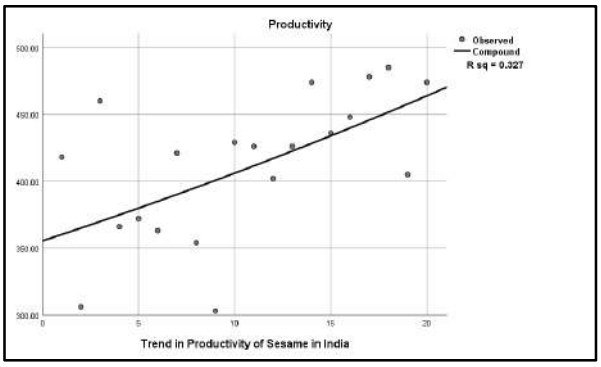
(CAGR – Compound annual growth rate per cent per annum. *- Significant at 1% level of significance)



Graph 1. Trend line of Area of Sesame in India



Graph 2. Trend line of Production of Sesame in India



Graph 3. Trend line of Productivity of Sesame in India

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RESEARCH ARTICLE

Fuzzy Ranking Game Problem Solving by using Dominance Principle with Fuzzy Trapezoidal Numbers

S.Punitha*

Associate Professor, Department of Mathematics, Vinayaka Mission's Kirupananda Variyar Engineering College, Vinayaka Mission's Research Foundation (Deemed to be University), Salem, Tamil Nadu, India.

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***Address for Correspondence**
S.Punitha,
Associate Professor,
Department of Mathematics,
Vinayaka Mission's Kirupananda Variyar Engineering College,
Vinayaka Mission's Research Foundation (Deemed to be University),
Salem, Tamil Nadu, India**.**
Email: puni.jeeju80@gmail.com

ABSTRACT

In this paper, the proposed approach provides the solution for the game matrix, and these payoffs are represented by trapezoidal fuzzy numbers. Also, we used some operations on trapezoidal fuzzy numbers to provide the fuzzy optimal solution, and the solution method is illustrated by using some numerical examples.

Keywords: Fuzzy set, Trapezoidal Fuzzy number, Fuzzy Arithmetic, Fuzzy game problem, Fuzzy ranking method, Dominance method.

INTRODUCTION

Game theory is the study of strategic decision making; it provides the mathematical process for selecting an optimal strategy. Game theory was invented by John von Neumann and Oskar Morgenstern in 1944. It has been applied to a wide variety of situations in which the choices of players. In decision making, the outcomes are controlled by the player's pure chance or mixed strategy. In real-life situations, the imprecise natures of strategies are uncertain. Sometimes these problems cannot solve by using classical mathematical techniques, in this case, fuzzy sets were introduced by Zadeh in 1965, to handle these problems in an efficient way. In this paper, we considered the game payoff matrix with imprecise values and all these imprecise values are assumed to be Trapezoidal Fuzzy numbers. An approach for solving problems by using ranking of the Fuzzy trapezoidal numbers has been considered. In this approach we converted the fuzzy valued game problem to crisp valued game problem to solve a fuzzy game problem by using the dominance method.

This paper organized as follows: In section II, recapture the some basic concepts of Fuzzy sets, fuzzy numbers and their arithmetic operations. In section III, we presented the Mathematical formulation of a Game problem. In section IV, we proposed a dominance method for the solution of fuzzy games. In section V, we illustrated the efficiency by using numerical examples. Section VI, gives the conclusion of this study.

Preliminaries

The aim of this section is to present, some important notations and results which were useful for the further discussion.

Fuzzy set

Let X is a nonempty set. A fuzzy set A in X is characterized by its membership function A→ [0, 1] and A(x) is interpreted as the degree of membership of element x in fuzzy A for each $x \in X$. The mapping A is called as the membership function of fuzzy set A. The value '0' is representing complete non-membership and the value '1' is represents complete membership. The values in between are represents intermediate degrees of membership. That is, the integration of the elements having a changing degree of membership in the set is called as fuzzy set.

Fuzzy Numbers

A Fuzzy set A defined on the set of real numbers R is said to fuzzy number if its membership function μ_A: R → [0, 1] has the following characteristics.

- A is normal that is there exist an x ∈ R such that $\mu_A(x)=1$
- A is convex that is for every $x_1, x_2 \in R$, $\mu_A(\lambda x_1+(1-\lambda)x_2) \geq \min\{\mu_A(x_1),\mu_A(x_2)\}, \lambda \in [0, 1]$
- μ_A is upper semi-continuous
- Sup (A) is bounded in R

Triangular Fuzzy number

A fuzzy number A is a trapezoidal fuzzy number denoted by $A=(a_1,a_2,a_3,a_4)$ where a_1,a_2,a_3,a_4 are real numbers and its membership function is given by

$$\mu_A = \begin{cases} \frac{x-a_1}{a_2-a_1}, & a_1 \leq x \leq a_2 \\ 1, & a_2 \leq x \leq a_3 \\ \frac{a_4-x}{a_4-a_3}, & a_3 \leq x \leq a_4 \\ 0, & \text{otherwise} \end{cases}$$

Without lost of generality we represent the trapezoidal fuzzy number $A=(a_1,a_2,a_3,a_4)=([a_2,a_3],\alpha,\beta)=(m, w, \alpha, \beta)$, where $m = \left(\frac{a_2+a_3}{2}\right)$ and $w = \left(\frac{a_3-a_2}{2}\right)$ are the midpoint and width of the $[a_2,a_3]$ respectively. Also $\alpha = (a_2 - a_1)$ denotes the left spread and $\beta = (a_4 - a_3)$ denotes the right spread of the trapezoidal fuzzy number.

Ranking of Trapezoidal Fuzzy number

There are so many ideas regarding ranking of fuzzy numbers in the literature. An effective result for comparing the fuzzy numbers is by using ranking function based on their grade means. That is, for every $A=(a_1,a_2,a_3,a_4) \in F(R)$, the ranking function $\mathbb{R} : F(R) \to R$ by graded mean is defined as $\mathbb{R}(A) = \left[\left(\frac{a_2+a_3}{2}\right) + \left(\frac{\beta-\alpha}{4}\right)\right]$. Here F(R) denotes the set of all trapezoidal fuzzy numbers defined on R. For any two arbitrary numbers $A=(a_1,a_2,a_3,a_4)$ and $B=(b_1,b_2,b_3,b_4)$ in F(R), we have the following comparison:

i) A≺B iff $R(A) \prec R(B)$
ii) A≻B iff $R(A) \succ R(B)$
iii) A≈B iff $R(A) \approx R(B)$

Arithmetic operations on Trapezoidal Fuzzy numbers

For arbitrary trapezoidal fuzzy numbers $A=(m(a),w(a),\alpha_1, \beta_1)$, $B=(m(b),w(b),\alpha_2, \beta_2)$ and $*=\{+,-,\times,\div\}$ the arithmetic operations on trapezoidal fuzzy numbers are defined by

$A*B=(m(a)*m(b), w(a) \vee w(b), \alpha_1 \vee \alpha_2, \beta_1 \vee \beta_2)$

$=(m(a)*m(b), \max\{w(a), w(b)\}, \max\{\alpha_1, \alpha_2\}, \max\{\beta_1, \beta_2\})$

Here the midpoint is taken in the ordinary arithmetic, whereas the width, left and right spread are considered to follow the lattice rule, i.e., for a, b ∈L define a ∨ b = max {a, b} and a ∧ b = min {a, b}.

In particular for any two trapezoidal fuzzy numbers $A=(m(a),w(a),\alpha_1,\beta_1)$,$B=(m(b),w(b),\alpha_2,\beta_2)$, we define

- Addition: $A+B=(m(a),w(a),\alpha_1,\beta_1)+(m(b),w(b),\alpha_2,\beta_2)$
  $=(m(a)+m(b), \max\{w(a), w(b)\}, \max\{\alpha_1, \alpha_2\}, \max\{\beta_1, \beta_2\})$
- Subtraction: $A-B=(m(a),w(a),\alpha_1,\beta_1)-(m(b),w(b),\alpha_2,\beta_2)$
  $=(m(a)+m(b), \max\{w(a), w(b)\}, \max\{\alpha_1, \alpha_2\}, \max\{\beta_1, \beta_2\})$
- Multiplication: $A\times B=(m(a),w(a),\alpha_1,\beta_1)\times(m(b),w(b),\alpha_2,\beta_2)$
  $=(m(a)\times m(b), \max\{w(a), w(b)\}, \max\{\alpha_1, \alpha_2\}, \max\{\beta_1, \beta_2\})$
- Division: $A\div B=(m(a),w(a),\alpha_1,\beta_1)\div(m(b),w(b),\alpha_2,\beta_2)$
  $=(m(a)\div m(b), \max\{w(a), w(b)\}, \max\{\alpha_1, \alpha_2\}, \max\{\beta_1, \beta_2\})$

MATHEMATICAL FORMULATION OF A GAME PROBLEM

i. **Player:** Each participant of a game.
ii. **Strategy:** The strategy of a player is the predetermined rule by which a player decides his course of action from the list of courses of action during the game, ie., pure and mixed strategy.
iii. **Optimal Strategy:** The course of action which maximizes the profit of a player or minimizes his loss.
iv. **Payoff:** The outcome of a game.
v. **Payoff Matrix:** The payoffs are represented in the form of matrix.
vi. **Saddle Point:** It is an element of the payoff matrix, in which where the maximum of row minima coincide with the minimum of the column maxima ie., the equilibrium point.
vii. **Value of the Game:** The expected outcome of the game i.e., the pay off at the saddle point.
viii. **Fair game:** The game is said to be fair; if the value of the game is zero, i.e., the gain of the first player and loss of the second player will be equal to zero.
ix. **Strictly determinable:** The game is said to be strictly determinable; if maximin=minimax=value of the game.
x. **Trapezoidal Number:** If all numbers in the sum are strictly greater than one it's called trapezoidal numbers because they represent patterns of points arranged in trapezoid.
xi. **Fuzzy Payoff Matrix:** In the game problem, let $A_1, A_2, \ldots, A_m$ be the strategies of player A and $B_1, B_2, \ldots, B_n$ be the strategies of player B. It is assumed that player A is always the gainer and Player B is always loser. Let a_{ij} be the payoff which player 'A' gains from player 'B'. Then the payoff matrix is of the form

		PLAYERB			
PLAYERA		B_1	B_2	…	B_n
	A_1	a_{11}	a_{12}	…	a_{1n}
	A_2	a_{21}	a_{22}	…	a_{2n}
	…	…		…	…
	A_m	a_{m1}	a_{m2}	…	a_{mn}

METHODOLOGY

Solution Procedure for Solving Game Problem using Principle of Dominance

Consider a solution of a Game problem involving strategies of the players using Trapezoidal fuzzy numbers.

Step-1: Check whether a saddle point exists on the problem. If it exists, the solution can be obtained directly. If the saddle point does not exist, go to the next step.

Step 2: Comparison of Column Strategies,

(i) If elements of Column A ≤ elements of Column B, Column A strategy dominates over column B strategy. Hence delete column B strategy form the payoff matrix.

(ii) Compare each column strategy with all possible column strategies and delete inferior strategies as for as possible.

Step-3: Comparison of row Strategies:

(i) If elements of Row A ≥ elements of Row B, Row A strategy dominates over Row B strategy. Hence delete Row B strategy form the pay off matrix.

(ii) Compare each row strategy with all possible row strategies and delete inferior strategies as for as possible.

(iii)The game may reduce to a single cell giving information about the value of the game and optimal strategies of players. If not go to step 4.

Step-4: Dominance need not to be based on the superiority of pure strategies only. A given strategy can be dominated if it is inferior to an average of two or more other pure strategies.

NUMERICAL ILLUSTRATION

Now consider the following game problem with payoff matrix using trapezoidal fuzzy numbers and convert the given problem with set of four integers into a game problem by using the measure.

	Player B			
Player A	(2,3,7,8)	(3,4,7,10)	(6,9,10,11)	(-1,0,2,7)
	(5,7,10,14)	(9,10,13,16)	(5,6,7,14)	(-2,5,8,13)
	(10,12,17,21)	(-1,3,4,6)	(5,9,11,15)	(2,6,7,9)

Now convert the above fuzzy problem into the form of trapezoidal fuzzy numbers, we have

	Player B			
Player A	(5,2,1,1)	(5.5,1.5,1,3)	(9.5,0.5,3,1)	(1,1,1,5)
	(8.5,1.5,2,4)	(11.5,1.5,1,3)	(6.5,0.5,1,7)	(6.5,1.5,7,5)
	(14.5,2.5,2,4)	(3.5,0.5,4,2)	(10,1,4,4)	(6.5,0.5,4,2)

Ranking of the Trapezoidal fuzzy numbers (R(A))

Trapezoidal fuzzy number	**Rank of the Trapezoidal fuzzy number**
a_{11} = (5,2,1,1)	R(a_{11}) = 5
a_{12} = (5.5,1.5,1,3)	R(a_{12}) = 6
a_{13} = (9.5,0.5,3,1)	R(a_{13}) = 9
a_{14} = (1,1,1,5)	R(a_{14}) = 2
a_{21} = (8.5,1.5,2,4)	R(a_{21}) = 9
a_{22} = (11.5,1.5,1,3)	R(a_{22}) = 12
a_{23} = (6.5,0.5,1,7)	R(a_{23}) = 8
a_{24} = (6.5,1.5,7,5)	R(a_{24}) = 6
a_{31} = (14.5,2.5,2,4)	R(a_{31}) = 15
a_{32} = (3.5,0.5,4,2)	R(a_{32}) = 3
a_{33} = (10,1,4,4)	R(a_{33}) = 10
a_{34} = (6.5,0.5,4,2)	R(a_{34}) = 6

Now we use the Maximin and minimax principle, we have

	Player B				Row minima
Player A	(5,2,1,1)	(5.5,1.5,1,3)	(9.5,0.5,3,1)	(1,1,1,5)	(1,1,1,5)
	(6.5,1.5,7,5)	(11.5,1.5,1,3)	(6.5,0.5,1,7)	(8.5,1.5,2,4)	(6.5,0.5,1,7)
Column	(14.5,2.5,2,4)	(3.5,0.5,4,2)	(10,1,4,4)	(6.5,0.5,4,2)	(3.5,0.5,4,2)
	(14.5,2.5,2,4)	(11.5,1.5,1,3)	(10,1,4,4)	(8.5,1.5,2,4)	

Maxima

Maximin (Maximum of row minima) = (6.5,0.5,1,7)
Minimax (Minimum of column maxima) = (11.5,1.5,1,3)
Here, Maximin ≠ Minimax
Therefore, it has no saddle point. Now we can apply Dominance method.
Using dominance principle between Row 1 and Row 2, Row 1 can be omitted.

	Player B			
Player A	(8.5,1.5,2,4)	(11.5,1.5,1,3)	(6.5,0.5,1,7)	(6.5,1.5,7,5)
	(14.5,2.5,2,4)	(3.5,0.5,4,2)	(10,1,4,4)	(6.5,0.5,4,2)

By dominance principle between Column 1 and column 3, column 1 can be omitted.

	Player B		
Player A	(11.5,1.5,1,3)	(6.5,0.5,1,7)	(6.5,1.5,7,5)
	(3.5,0.5,4,2)	(10,1,4,4)	(6.5,0.5,4,2)

Using by dominance principle between column 2 and column 4, column 4 can be omitted.

	Player B	
Player A	(11.5,1.5,1,3)	(6.5,0.5,1,7)
	(3.5,0.5,4,2)	(10,1,4,4)

Maximin (Maximum of row minima) = (6.5, 0.5, 1, 7)
Minimax (Minimum of column maxima) = (11.5, 1.5, 1, 3)
Here, Maximin ≠ Minimax
Therefore, it has no saddle point.
The reduced payoff matrix represent a 2x2 two person zero sum fuzzy game without saddle point. Therefore the optimum mixed fuzzy strategies are,
Value of the fuzzy game=15.7

The strategy for the Player A, ie $S_A=\left\{0,\frac{9}{11},\frac{2}{11}\right\}$
The strategy for the Player B, ie $S_B=\left\{0,\frac{4}{11},\frac{7}{11},0\right\}$

CONCLUSION

In this paper, we have obtained the optimum solution to the game problem using trapezoidal fuzzy numbers. The proposed ranking method is very easy to understand for solving fuzzy game problems in real-life scenarios. We may get different game values for different trapezoidal fuzzy numbers. A Numerical example has been considered and solved to illustrate the proposed method with its strategies and the value of the game. Also, this method may also be extended to the other type of fuzzy numbers with suitable modifications of ranking methods.

REFERENCES

1. Aubin, J. P. "Mathematical methods of game and economic theory, ser." *Studies in Mathematics and its Applications. Amsterdam: North-Holland Publishing Co* 7 (1979).
2. Aubin, Jean-Pierre. "Cooperative fuzzy games." *Mathematics of Operations Research* 6.1 (1981): 1-13.

3. Butnariu, Dan. "Fuzzy games: a description of the concept." *Fuzzy sets and systems* 1.3 (1978): 181-192.
4. C.R. Bector, S. Chandra, V. Vijay, Matrix games with fuzzy goals and fuzzy linear programming duality, Fuzzy Optimization and Decision Making 3 (2004) 255-269.
5. Campos, Lourdes. "Fuzzy linear programming models to solve fuzzy matrix games." *Fuzzy sets and systems* 32.3 (1989): 275-289.
6. Li DF. An effective methodology for solving matrix games with fuzzy payoffs. IEEE Trans Cybern. 2013;43(2):610–621.
7. Punitha, S. "An Approach for solving Game Problems Using Trapezoidal Numbers", Parishodh Journal, (2020): 3712-3715.
8. Punitha, S. "Fuzzy Ranking Game Problem solving by using Dominance Principle with Fuzzy Triangular Numbers", Indian Journal of Natural Sciences, (2021): Vol.12,Issue 69, December, 35940-35944.
9. V. Vijay, S. Chandra, C.R. Bector, Matrix games with fuzzy goals and fuzzy payoffs, Omega 33 (2005) 425-429.
10. Von Neumann, John. "Morgenstern, 0." Theory of games and economic behavior 3 (1944).
11. Zadeh, L. A. "Fuzzy sets: information and control, 1965."

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REVIEW ARTICLE

Chronic Wasting Disease and Their Potential Transmission to Cervids: an Overview

R.Saravanan[1]*, R.Srinivasan[2], A.Shanmugarathinam[3], R.Karthikeyan[4], E.Karthikeyan[1], M. Vasanth Kumar[5], N. Kishore Raghul[6], K. Vijay Aadhi Raj[6], B.Vasu raja[6] and K.Saifullaha[6]

[1]Professor, Faculty of Pharmacy, Bharath Institute of Higher Education and Research, Chennai-73, Tamil Nadu, India.
[2]Dean, Faculty of Pharmacy, Bharath Institute of Higher Education and Research, Chennai-73, Tamil Nadu, India.
[3]Assistant Professor, BIT Campus, Anna University, Tiruchirapalli, 620 024, Tamil Nadu, India.
[4]Professor, Chettinad School of Pharmaceutical Science, Chettinad Academy of Research and Education, Kelambakkam, 603 103, Tamil Nadu, India.
[5]Associate Professor, Faculty of Pharmacy, Bharath Institute of Higher Education and Research, Chennai-73, Tamil Nadu, India.
[6]Student, Faculty of Pharmacy, Bharath institute of Higher Education and Research, Chennai-73, Tamil Nadu, India.

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***Address for Correspondence**
R. Saravanan
Professor, Faculty of Pharmacy,
Bharath Institute of Higher Education and Research,
Chennai-73, Tamil Nadu, India.
Email: rssrmcp@gmail.com.

ABSTRACT

In the various parts of the world and some regions of india, the prion disease chronic wasting disease (CWD), which affects both wild and captive cervids (deer and elk), is pervasive. The effects of CWD and its spread on the environment, the economy, and public health are still unknown. We examine current understanding of CWD, its effects, and management. Newer diagnostics make it possible to properly identify CWD long before any clinical signs manifest. Although it seems unlikely, there is still widespread worry about the likelihood of natural transmission to people or conventional domestic animals; the implications on wildlife resources are still unknown. People's attitudes toward TSEs have changed significantly since the 1990s, most notably in the present debate over the eventual eradication of all prion disorders worldwide. Early intestine lymphoid tissue involvement during the CWD incubation period points to potential routes for agent escape from an infected person, such as in faces or saliva. The local upkeep of chronic wasting illness may be significantly impacted by environmental contaminants.

Because of research on the disease's epidemiology, models have been developed to assist explain the origins of CWD and forecast its consequences on deer and elk populations.

Keywords: CWD**,** Chronic wasting disease, Cervids, Prion, Dear, Elk.

INTRODUCTION

White-tailed deer, mule deer, elk, and moose are among the members of the deer family in North America that are susceptible to the fatal neurological disorder known as chronic wasting disease (CWD). Since it was originally discovered in 1967, CWD has spread over the world and increased in frequency locally. Contagious, CWD spreads quickly within and among cervid groups. There aren't any medicines or immunizations on the market right now. Chronic wasting disease is a major worry for wildlife management. It has been discovered in at least 23 states, two Canadian provinces, and South Korea. It's unclear if CWD affects humans or animals [1]. Both directly through animal-to-animal contact and indirectly through contact with objects or an environment contaminated with infectious material (such as saliva, urine, faeces, and the carcasses of CWD-infected animals) (1) are ways that CWD is disseminated. According to our findings, a group of CWD researchers had a key role in the connection of the research community. [CWD modelling] began in the early 2000s and has developed since then. The primary research areas included population-level tactics, compartment-based models for regression, and host species of game management issues. Similar to how CWD research ignored community ecology and biogeographic modelling methods in favor of focusing on single populations, species, and locations. For the majority of infection phases, conventional diagnostic methods with inadequate sensitivity were utilized to identify chronic wasting disease. The diagnosis of chronic wasting disease was made using diagnostic techniques with insufficient sensitivity.

In order to quantify uncertainty in future analytical work, more precise diagnostic methods should be used. Additionally, larger-scale studies should be conducted to clarify CWD transmission outside of population-level approaches. Assumptions used to model other infectious illnesses may not apply to CWD because infectious prions may not follow the biological rules of well-known wildlife pathogens (such as viruses, bacteria, and fungi). Chronic wasting disease has emerged as a fresh problem in wildlife epidemiology[2]. Based on in vitro and in vivo research, it is believed that human-to-human transmission of CWD is unlikely. Despite this, it's still possible for people to consume things that are contaminated with CWD, which is dangerous. According to study, environmental prions reservoirs found in soil and mud, as well as saliva, faeces, and urine, are how horizontal infection of Cervids occurs most frequently. Infected skeletal muscles have also been observed in ill deer. These findings suggest that infection can happen both directly through contact with ill animals and indirectly through contact with prion-contaminated objects [3]. However, recent study on the identification of prion infectivity during pregnancy raises the prospect of CWD transmission from mother to kid. In this work, fundamental aspects of CWD are examined.

CAUSES

Chronic wasting disease is brought on by the prion protein's flaws. Mostly the central nervous system is affected. It results in the conversion of normal protein into aberrant protein, which is not digested by enzyme activities, leading to an accumulation of abnormal protein. This results in neurological issues and the affected animal dying. Cervids are preferred by the CWD prion (deer-like animals). Animals can contract these prions from one another [3]. These are spread through the mucus, saliva, and faeces of ill animals. The decaying corpses of infected animals can potentially spread the disease. The prion can be passed on indefinitely because it is a protein. Because the prion sticks to the soil tenaciously, soil contamination contributes significantly to the development or spread of the disease [4].

SYMPTOMS

Stumbling, confusion, loss of weight, listlessness, psychological signs, excessive urine, drooping ears, and drooling insufficient coordination Having trouble moving, Lethargy, dropping of the head, a pattern of repetitive walking, Tremors [5].

DIAGNOSIS

When an affected animal dies, the brain or lymphoid tissue from that animal is examined to see if the condition is chronic and wasting. A variety of diagnostic procedures are utilized, such as assays (tests).Using procedures such as immunohistochemistry (IHC), immunoblotting, and enzyme-linked immunosorbent assays (ELISA). For white-tailed deer and mule deer, this test seems to be effective, but not for elk. Recently, a method for taking a biopsy of tonsillar tissues from live deer was developed [6]. The transmissible spongiform encephalopathies (TSE) have a disease-specific marker known as the protease-resistant prion protein (PrP-res), which may be recognized by the IHC method regardless of the species. IHC was found in ten out of the 17 elk. Solely two of the 10 animals displayed both histologic lesions and clinical signs of CWD, while the other only displayed histological lesions [4]. The most consistently IHC-positive tissue was the medulla oblongata. These results suggest that any surveillance study intended to establish the prevalence of CWD in captive ranging cervids must include the PrP-res IHC test on brain tissue, particularly the medulla oblongata at the obex [5].

CLINICAL DIAGNIOSIS

The typical incubation period is between two and four years, while the minimum incubation period is roughly 16 months [7]. CWD is fatal, and clinical diagnosis in wandering animals can be difficult, especially in the early stages of the illness. Animals may arrive suddenly and show no symptoms. Over the course of several weeks to months, cervids can lose weight and alter their behavior (lethargy, hyper excitability, low head carriage, fixed look). Even if neurological symptoms are minimal, they may nonetheless manifest as ataxia, head tremors, teeth grinding, and pacing the confines of a space. Aspiration pneumonia brought on by oesophageal dilatation and/or regurgitation can be fatal in animals. Polydipsia (excessive drinking) and polyuria (excessive urination) are typical signs in the latter stages[8].

LABORATORY DIAGNOSIS

SAMPLES: For isolation of agent

- Antemortem: Deer - palatine tonsil, Deer, elk - rectal lymphoid tissue [9].
- Post-mortem: Brain (obex), retropharyngeal lymph nodes, tonsils [10].

MECHANISM OF TRANSMISSION

The first thing that determines how the killing of antlerless deer impacts the occurrence of CWD is the mode of transfer and the level of transmission to juveniles. We made the assumption that environmental transmission occurred and that children were less likely to get sick than adults because they consumed less potentially contaminated food. We also assumed that food consumption was the primary way that the illness was spread through environmental contamination. According to recent studies, there is little evidence that CWD is transferred from plant roots to stems and leaves; instead, it is most likely that contaminated soils are consumed directly or indirectly through plant material [11]. The young have a low infection rate, which is consistent with tendencies of typically lower prevalence in fawns, but this could be because of the length of exposure. Regardless of the mechanism, our results show that a control strategy that always includes relatively more male deer is most effective because it eliminates the population segment with the highest proportion of infected people due to high food consumption, susceptibility, or other behavioral traits. Second, while harvesting only male deer rarely reduced population density sufficiently to promote maximum juvenile survival, our findings show that harvesting only male deer is rarely as effective [12].

CHRONIC WASTING DISEASE TO HUMAN TRANSMISSION

In addition to the three states of Colorado, Wyoming, and Nebraska, other CWD foci have been found in other parts of the country. CWD affects deer and elk. Although greater surveillance may be associated with identification in some areas, the introduction of CWD by animal translocation or spontaneous migration may be responsible for some new foci of infection [13]. Concerns have been raised concerning the prospect of increased human exposure to the CWD agent due to the disease's expanding prevalence. The transmission of bovine spongiform encephalopathy to humans through food raises the possibility that the species barrier does not completely shield people from animal prion diseases. A CWD-associated prion transformed human prion protein in an in vitro cell-free experiment, however there is very little conclusive proof that CWD may be transmitted from humans [14]. Additional epidemiologic and laboratory studies are needed to monitor the potential of such transmission. Prions can change, but the rate of change is governed by variation in the genes in cervid and human cells that code for the prion protein [15]. Humans can acquire prion diseases by food borne infection, genetic predisposition (gCJD and other prion diseases), or sporadic development (sCJD). The same substance that causes bovine spongiform encephalopathy (BSE) also causes variant CJD (vCJD)[16]. Only cannibalism or following medical or surgical treatment have reports of human prion disease spreading from one person to another[17-18].

TREATMENT

There is presently no known treatment for the deadly condition known as Chronic Wasting Condition. After developing the illness, a cervid will eventually pass away. Research is now being done on 2-aminothiazole-based therapies for transgenic mice inoculated with white-tailed deer CWD prions. It has been proven that IND24, or 2-aminothiazole, is effective against the CWD prions in elk [19]. When WTD CWD isolate-infected mice were administered IND24, their life span was prolonged in a manner similar to that of elk CWD prion-infected mice, however it was noticeably shorter. The WTD mice survived 200 days after inoculation, whereas the Elk animals given with IND24 daily lived 216 days after inoculation. This implies that the two strains are distinct from one another. The duration of the mice's survival following vaccination was likewise impacted by various dosage sizes [20]. The features of PrPSc in the mice's brains, as well as the infectiousness and drug susceptibility of CWD, were unaffected by the treatment with IND24 itself. Additionally, IND24 does not treat CWD, even while it prolongs the lives of mice affected with various types of the disease.

EPIDEMIOLOGICAL STUDIES

In view of the rising geographic range of CWD cases and the assumed food borne transmission of BSE to people that resulted in cases of vCJD, concerns regarding the possible zoonotic transmission of CWD have been raised. The identification of CJD in three people under the age of 30 who had either consumed deer and elk meat from family members or were deer hunters in the late 1990s sparked these concerns [21]. No convincing evidence of a connection between CWD and these case-patients' CJD state, however, was found in epidemiologic or scientific examinations of these case-patients. None of the patients admitted to hunting or consuming deer meat from Wyoming or Colorado regions where CWD is prevalent. If such a history was discovered in CJD patients who were unusually young, it would support a causal link with CWD. Furthermore, there was no evidence of CWD in the brain tissues of more than 1,000 deer and elk that were captured in the areas where the patients hunted or where they obtained their venison. Additionally, the three patients' disorders could not be accounted for by exposure to the same prion strain due to the absence of homogeneity in the clinicopathologic symptoms and codon 129 of the prion protein gene. The individuals' exposure to the same prion strain, the causative agent of BSE, was connected to the homogeneity of the genotype at codon 129 and the diagnostic and pathologic phenotype in vCJD [22].

INVESTING IN CWD RESEARCH

A crucial first step toward CWD control is investment in CWD research, which also involves support for disease surveillance and management. In 2001, CWD was declared a national emergency, and Congress immediately started allocating funds from the government to support CWD research, surveillance, and management. However, federal funding was severely curtailed after 2011, and since then, financing for CWD initiatives has primarily come from the states [23]. Between 2011 and 2012, federal funding for cervid health decreased from $14.3 million to $1.9 million.

Since that time, annual federal funding has stayed low, making it challenging for state wildlife, animal health, and public health agencies to adequately address this issue. These organizations can better create and carry out comprehensive CWD management strategies, conduct essential animal and human illness surveillance, improve hunter education, and more with the restoration of proper federal funding. Because of the lengthy testing processes and potential delays in results, there is a greater risk that hunters may be exposed [24]. Increased funding for CWD research will also help address unresolved questions about the disease's transmission risks and its origins, providing vital knowledge about the potential harm to people. Finally, research is needed to create vaccines or treatments that might effectively protect cervids and possibly lower CWD transmission.

FUTURE SURVEILLANCE

Although the exact cause of CWD is unknown, it is possible that it was caused by the interspecies spread of scrapie or another prion agent. However, because scrapie cases have been discovered all over the world in countries that breed sheep, it is possible for CWD to spread internationally. Our understanding is that the industrialized countries of Europe and Asia have been the only places where CWD monitoring has been conducted outside of the US and Canada, and that these efforts have not even come close to the size of those made in those countries [25]. Even within North America, monitoring of some cervids, like caribou, has been limited, and it is questionable if current surveillance efforts will continue to be funded and implemented. Given that current surveillance efforts are limited in relation to total cervid populations, CWD may be present at low levels in many areas that were previously believed to be CWD-free. To identify the true geographic spread of the sickness and its host range, at the absolute least, targeted surveillance of all cervids both inside and outside of North America should be conducted [25,26] This monitoring could benefit from a more practical premortem testing method [26].

CONCLUSION

The transmissible spongiform encephalopathy known as chronic wasting disease, which today affects more than half of the United States and certain European countries' wild cervid populations, was first discovered in the late 1960s. This disease should be referred to as endemic due to its resilience to multiple sterilizing techniques and environmental durability. Human health must be considered when managing CWD, and CWD prions must be kept out of human food supplies. A greater understanding of population dynamics, deer behavior that influences CWD transmission among free-ranging cervids, and environmental prions is required to facilitate CWD management. Acceptance by the public, along with continued support and dedication to intervention, are essential for the success of CWD management in free-ranging deer.

REFERENCES

1. Hopkins MC, Carlson CM, Cross PC, Johnson CJ, Richards BJ, Russell RE, Samuel MD, Sargeant GA, Walsh DP, Walter WD. Chronic wasting disease—Research by the US Geological Survey and partners. US Geological Survey; 2019.
2. Winter SN, Escobar LE. Chronic wasting disease modeling: an overview. Journal of Wildlife Diseases. 2020 Oct;56(4):741-58.
3. Onodera T, Sakudo A, Tsubone H, Itohara S. Review of studies that have used knockout mice to assess normal function of prion protein under immunological or pathophysiological stress. Microbiology and immunology. 2014 Jul;58(7):361-74.
4. Renuga G, Saravanan R, Thandapani AB, Arumugam KR. Expression of Cholera toxin B subunit in Banana callus culture. Journal of Pharmaceutical Sciences and Research. 2010;2(1):26.
5. Williams ES. Chronic wasting disease. Veterinary pathology. 2005 Sep;42(5):530-49.
6. Arifin MI, Hannaoui S, Chang SC, Thapa S, Schatzl HM, Gilch S. Cervid prion protein polymorphisms: role in chronic wasting disease pathogenesis. International Journal of Molecular Sciences. 2021 Feb 25;22(5):2271.

7. Miller MW, Wild MA, Williams ES. Epidemiology of chronic wasting disease in captive Rocky Mountain elk. Journal of wildlife diseases. 1998 Jul;34(3):532-8.
8. Prusiner SB. Prion diseases and the BSE crisis. Science. 1997 Oct 10;278(5336):245-51.
9. Baird MJ. An empirical Bayes model to assess deer-vehicle crash safety in urban areas in Iowa. Iowa State University; 2010.
10. Salzano G, Giachin G, Legname G. Structural consequences of copper binding to the prion protein. Cells. 2019 Jul 25;8(8):770.
11. John TR, Schätzl HM, Gilch S. Early detection of chronic wasting disease prions in urine of pre-symptomatic deer by real-time quaking-induced conversion assay. Prion. 2013 May 1;7(3):253-8.
12. Animal and Plant Health Agency (APHA), Biomathematics and Risk Research workgroup, United Kingdom, Martínez JM, McCarthy C, Taylor RA. Livestock Health and Food Chain Risk Assessment. EFSA Journal. 2020 Nov;18:e181111.
13. Williams ES, Miller MW, Kreeger TJ, Kahn RH, Thorne ET. Chronic wasting disease of deer and elk: a review with recommendations for management. The Journal of wildlife management. 2002 Jul 1:551-63.
14. Williams ES, Miller MW. Chronic wasting disease in deer and elk in North America. Revue scientifique et technique (International Office of Epizootics). 2002 Aug 1;21(2):305-16.
15. Belay ED. Transmissible spongiform encephalopathies in humans. Annual review of microbiology. 1999 Oct;53(1):283-314.
16. Will RG. A New Variant of Creutzfeldt-Jakob in the UK. CATTLE PRACTICE. 1996;4:347-8.
17. Belay ED, Schonberger LB. Variant Creutzfeldt-Jakob disease and bovine spongiform encephalopathy. Clinics in laboratory medicine. 2002 Dec 1;22(4):849-62.
18. Potapov A, Merrill E, Lewis MA. Wildlife disease elimination and density dependence. Proceedings of the Royal Society B: Biological Sciences. 2012 Aug 22;279(1741):3139-45.
19. Rasmussen J, Gilroyed BH, Reuter T, Dudas S, Neumann NF, Balachandran A, Kav NN, Graham C, Czub S, McAllister TA. Can plants serve as a vector for prions causing chronic wasting disease?. Prion. 2014 Jan 1;8(1):136-42.
20. Osterholm MT, Anderson CJ, Zabel MD, Scheftel JM, Moore KA, Appleby BS. Chronic wasting disease in cervids: implications for prion transmission to humans and other animal species. MBio. 2019 Jul 23;10(4):e01091-19
21. Bosque PJ. Bovine spongiform encephalopathy, chronic wasting disease, scrapie, and the threat to humans from prion disease epizootics. Current Neurology and Neuroscience Reports. 2002 Dec;2(6):488-95.
22. Belay ED, Gambetti P, Schonberger LB, Parchi P, Lyon DR, Capellari S, McQuiston JH, Bradley K, Dowdle G, Crutcher JM, Nichols CR. Creutzfeldt-Jakob disease in unusually young patients who consumed venison. Archives of Neurology. 2001 Oct 1;58(10):1673-8.
23. Geist V, Clausen D, Crichton V, Rowledge D. The challenge of CWD: Insidious and dire. Alliance for Publice Wildlife; 2017.
24. Chen D, Dougherty CA, Zhu K, Hong H. Theranostic applications of carbon nanomaterials in cancer: Focus on imaging and cargo delivery. Journal of controlled release. 2015 Jul 28;210:230-45.
25. Gough KC, Maddison BC. Prion transmission: prion excretion and occurrence in the environment. Prion. 2010 Oct 1;4(4):275-82.
26. Spraker TR, VerCauteren KC, Gidlewski T, Schneider DA, Munger R, Balachandran A, O'Rourke KI. Antemortem detection of PrPCWD in preclinical, ranch-raised rocky mountain Elk (CevvusElaphusNelsoni) by biopsy of the rectal mucosa. Journal of Veterinary Diagnostic Investigation. 2009 Jan;21(1):15-24.

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REVIEW ARTICLE

A Review on Effect of Mobile Phase on the Performance of Reverse Phase – High Performance Liquid Chromatography System

Konkala Swarupa[1]* and K .Selvakumar[2]

[1]Research Scholar, Department of Pharmaceutical Quality Assurance, ABMRCP, Bengaluru, Karnataka, India.
[2]HoD and Assistant Professor, Department of Pharmaceutical Quality Assurance, ABMRCP, Bengaluru, Karnataka, India.

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***Address for Correspondence**
Konkala Swarupa
Research Scholar,
Department of Pharmaceutical Quality Assurance,
ABMRCP, Bengaluru, Karnataka, India.
Email: swarupakonkala123@gmail.com

ABSTRACT

Nowadays, reversed-phase high-performance liquid chromatography (RP-HPLC) is a versatile and broadly using analysis method for performance standards throughout the pharmaceutical research, improvement, as well as manufacturing process. This was predicted 30 years ago that RP chromatographic mode was used for 80-90 percent of all analytical separations. Modifications of solvent composition could significantly alter the analyte's retention and selectivity. The material of the mobile phase reservoir is an important consideration depending on the type of application. Glass is typically used for small molecule analysis to prevent the leaching of materials from plastics into the eluent, which may interfere with the analysis. Modifications to the solvent composition, such as pH, type and amount of organic modifier, buffer type, and concentration, allow RP-HPLC methods to modulate analytes retention and separation.

Keywords: Reverse Phase –High performance Liquid Chromatography System (RP-HPLC), Solvent strength, Filtration, stability of mobile phase.

INTRODUCTION

The solvent that moves the analyte through the column is known as the mobile phase. After sample preparation, the analytes are dissolved in a suitable solvent and pumped into a chromatographic system this solution is not considered a separate matrix as it does not interact with the analysis. During sample preparation, the character of the major carrier (liquid solution) for such solutes could be selected to meet High performance liquid chromatography (HPLC) unique requirements such as volatility as well as miscibility with the mobile phase[1]. In HPLC, the mobile

phase is made up of high-quality or combined liquids, and also organic solvent with solid additives. Chromatography can choose from hundreds of solvents for various HPLC applications. Solvent properties such as viscosity, refractive index, non-corrosiveness, toxicity, miscibility, transparency, and so on the influence a particular selection. Another important factor is the Infomercial accessibility of sufficient pureness at an economical cost. The retention time of the analyte is controlled by the solvent strength or percent organic solvent in the mobile phase. A good rule of thumb in reverse phase liquid chromatography (RPLC) is that a 10% decrease in the organic solvent in the mobile phase results in a 3-fold increase in k or t R [2]. Solvents and toxic wastage are a global problem that then endangers not just the health of the people and also our surroundings. Faced with this issue, it is evident that the industrial sector, with all labs, must bring in new waste-reduction innovative products. This raises interest in "eco-friendly" science, such as HPLC analysis with the chemical-free sample solution. The idea of using eco-friendly water to eliminate a wide range of organic and harmful wastes generated every day is both environmentally sensitive and financially efficient [3].A 1% increase in ACN (acetonitrile) corresponds to a 5°C increase in temperature. Tran et al. obtained comparable results. Kondo and Yang carried out many studies thus demonstrated that temperature changes related to changes in the concentration of a given organic solvent depending on the conditions and column used. When using a polystyrene-divinylbenzene (Hamilton PRP-1) column, a 3.5°C temperature increase is balanced to a 1% high in methyl alcohol composition in the methanol-H_2O solutions. Temperature changes of 5°C and 8°C were comparable, whereas acetonitrile composition was increased by 1% in an acetylnitrile-H_2Omixture [4].

HPLC performance check

The effectiveness of an HPLC method can be examined to determine the important functions of the system's different modules. The pump, auto-injection system, column oven, and detector are the key parts of an HPLC method that must be tested to ensure proper operation. One of the most essential factors to recognize throughout HPLC efficiency confirmation are flow rate accuracy and gradient accuracy for the pump, precision, linearity, and carry over for the auto sampler, wavelength accuracy, and response linearity for the detector, and temperature accuracy for the column oven. And during the assessment, we would like to look for some critical characteristics of HPLC elements[5]. It must have a specific or general response to components in a mixture. It must not be affected by climatic variations or fluid properties and should be susceptible to the analyte including an over the solvent system**. (**Critical attribute of the detector as discussed in fig.1).

Overview Models Regarding Simple Mobile Phases

Even though the column is the main part of LC, the solvent is used to modify the separation's retention time and selectivity. Looking back at HPLC history, beyond visible to be new regarding the use of simple solvents for a variety of real reasons. Increased column techniques, for example, have reduced the necessity of solvent additives as well as the buffer is improved high point structures or columns' batch's-to-batch reproducibility. Furthermore, using easier numeric liquids to linear slope sections improves process reliability by reducing procedure transfer problems in regulated assays. At last, its widespread adoption of High - performance liquid chromatography-Mass spectrometry [LC-MS] like standard technologies with raising-by testing, in-processed management, life science study, and therapeutic diagnostic tools makes easier liquid chromatographic methods & organic phase more appropriate. They highlighted trends in pharmaceutical stability analysis, involves both low molecule API and biotherapeutics, enhanced ultraviolet and mass spectrometry (MS) detecting sensitively, and achieving similar high point figures for base substances [6].

RP-HPLC collection with Organic Solvent or Solvent B

In RP-HPLC, solvent B is a normal short form for effective solvents (dissolving solvents) used for pump blending and gradient elution. Acetonitrile, methanol, and tetrahydrofuran have historically been the three most popular reversed-phase LC organic solvents [7]. For purpose of photon accepting capacity, photon donating potential, and Quadra dipole interconnections, these three solvents have noticeably various parameters. The eluotropic strengths are listed in the following order: Methanol< Acetonitrile< Tetrahydrofuran. Most practitioners prefer acetonitrile because of its high eluent capacity, and low permeability (0.37 cP), which leads to better column performance, and better Ultraviolet visibility (190 nm). Acetonitrileis a polar solvent and hydrogen accepting group that can interact

with other molecules [8]. Methanol is a simple type of solvent that can behave as a proton donating or accepting group. It is less expensive than acetonitrile but produces more force (viscosity of 0.55 cP) when combined with liquid (example- a 50 percent methyl alcohol: water solution had a consistency of 1.62 cP). Methyl alcohol solvent has an ultra Violet final cut-off value to be less than 210 nm. As a result, the options for solvent B are strongly limited for Acetonitrile and Methanol [9]. Despite its high solubility and eluotropic strength, Tetrahydrofuran [THF] is hardly used in RP-HPLC. Except in gel permeation chromatography, dangerous and secure issues caused by fade format prevent its widespread use. Because of its limited miscibility with water and lack of to prevent oxidation forming, methoxytert-butyl isopropyl alcohol could be used in low concentrations replacement to THF. Many papers have recently been published that use acetonitrile in conjunction with n- or i-propyl alcohol, as well as with n-butyl alcohol, to enhance the wider regeneration of a few antibodies [10].

The work of solvent B Contain Water and Additives

HPLC methods referred for solvent B to be 95 percent Acetonitrile in water. The result is to improve merging effectively by creating the viscosity and surface tension of the two mobile phases more similar. It is normal practice to utilize similar additive sections in both solvents (a) and (b), for example, 0.1 percent THF in ethyl nitrile nitrile while using 0.1 percent trifluoroethanol acid in H_2O-like solvent (a).Other than decreasing various shifts with UV detection with fewer wavelengths, there are no discernible differences in splitting un producibility or elute order in reverse phase liquid chromatography (RP-LC) around hundred percent ethyl nitrile and 0.1 percent THF inethylnitrile [11]. The column pressure is a necessary chromatography parameter thus contributing to holding time modification, mainly while a large testing time has been utilized. That pressure adjustments in reversed section columns have been investigated on the subject of the natural modifier content material inside the cellular phase. Due to liquid gradient causing pressure, it turned into critical to determine the excellent conditions for evaluation through measuring pressures beneath gradient and isocratic situations. As a result, a variation starts 0% -100% becomes used to evaluate a rough estimate of most costs for the pressure of the column. Pursuing that, isocratic eluting changed into finished to exactly decide the mobile phase percentages as an outcome of which most less price for various solvents/columns situation (Table.1) [12].

Acetonitrile possess with a pressure gradient is excessive at an acetyl nitrile: H_2O ratio of 15:55 v/v and methanol produces mostly back-pressure at methyl alcohol: H_2O ratio of 45:55. So when ever this may reflect the interaction of the mobile phase with the solid bound segment of Reverse phase column in addition to methyl alcohol-water or acetonitrile-water interactions. Methanol accepted and donates the H- bonds, whereas acetonitrile only accepted the H- bonds. As a result, a strong bond among different solvent particles is predicted. As are different connections among methyl alcohol molecules and with silica silanol kind groups [13]. Due to Methanol interacting resistantly fluid go with the flow into the High - performance liquid chromatography columns, In comparison to acetonitrile /water systems, methyl ester structures had greater lower back extreme pressure. When Fig .2(a) and Fig. 2(b) are compared, the C18 column produces greater back strain compared to C8 columns. These will be because of greater silica groups being to be had at the surface of C18 columns as a result of constrained surface insurance throughout derivatization using large C18 molecules [14].

Mobile Phase (A): (pH Modifiers and Buffers)

In RP- LC, the weaker liquid is referred to as mobile phase A, and it contains more water and low pH modify substances, buffered solvents, as well as salted molecules to control ionic intensity but also ionic capacity. To separate neutral molecules, pure water can be used. Most drugs in chemical preparations seem to be ionized, i. e., acidified, primary, as well as zwitter ionic[15]. As a result, the pH of liquid phase A should be monitored because it has a major impact on dissolved retention.Ionized substances exist through ionized as well as non-ionized accuracy depending on the solvent ionic strength, & ionized paperwork has significantly lower retention in reversed-segment LC than non-ionized accuracy. Its additives is an acidified or basic agents, or buffers, by the addition of conjugate salt [16].

Acidic additives

Most pharmaceutical applications require an acidic pH of 2-4. The less pH inhibits the ionization with weakly acidic analytes, causes in greater retention. Trifluoroethanoic acid, formic acid, and ethanoic acid are commonly used acids atconcentrations ranging from 0.05 - 0.1 percent volume/v. Through liquids, the pH values seem to be 10 percent, 10 percent formic acid, and 0.1 percent ethanoic acid [17]. Pipette (1.0) ml acidic solution to 1000ml of purified water to make those simple liquid levels at 0.1 percentage v/v which can be used immediately without any filtration. They are often used in liquid chromatography mass spectrometry(LC-MS) packages, notwithstanding the reality that their low ionic strengths can also bring about bad peak shapes for very simple drugs. This additive is beneficial for the raw type of material or reagent purity strategies that use extra regularly occurring detecting at less UV [18].

Buffer solution

A solution of the buffer resists modifications in pH while small quantities of acid or alkali are brought to the solution. The use of buffer solution is important in HPLC separation because it can separate two closely eluting peaks or merge two separate peaks. Citrate, acetate, and phosphate buffers are the most commonly used buffers in liquid chromatography (LC). For vital assays, Buffer solutions have been required to strictly control the ionic strength of liquid segment a. According to the Henderson-Hasselbalch Equation, buffers are most effective when they are within 1.0 constant factor of their pKa values of 13. A buffer is made by combining a weak carbonic acid also with the salt of its basic solution (alternately vulnerable through salts of its conjugated acid) [19]. Phosphate has historically been the most typically used buffer in HPLC. Phosphate buffers are powerful buffered solutions at ionic strength values of rounds 2, 7, and 10 because normally phosphoric acid contains 3 ionizable hydrogens. It is Ultraviolet-visible to two hundred nms. However now not risky, making it incompatible with MS [20].It also has less soluble in acetonitrile, especially at more concentrations (like 50 mM), which causes solidification issues at the time of pump merging. This problem can be solved by switching mobile solvent b up to 85 percent Acetonitrile in water or with methanol.

Choosing the right buffer

Many factors can influence buffer selection; consider the following when selecting the right buffer:

- Mobile phase pH is required.
- The ultraviolet cut-off value.
- For Mass spectrometry-based chromatography, a volatile buffer should be used.

Buffer pH

The pH of the solution influences the retention time and peak shape. A silica-based column should not be used for buffer solutions with very low and high pH values because the stationary phase bond may be broken at low pH and silica may be dissolved at high pH [21].

Buffer Solubility and Concentration

Buffer solubility and concentration are important factors in method development. The best buffer is one that is completely soluble and produces reproducible results at low concentrations. Higher buffer concentrations may cause the buffer to become viscous and precipitate, resulting in back pressure.

New models in Mobile Phase Preparation

It includes:-

- lower composition of buffered within solvent system A section
- Removal of filtration
- pH adjustment in mobile phase A [22].

Removal of Separation Procedure

Several labs also stopped using 0.2 as well as 0.5 m membrane filters by way of the use of excessive-purity reagents (including Aldrich's 99.995 percentage ammonium salt formic acid, Cat# 516961) as well as High - performance

liquid chromatography liquids & H_2O The ability filtration is associated with mobile phase reagents section contaminations can be reduced by removing the separation step. Most HPLC pumps' inner filters (changed during preventive maintenance programs) appear like ok for ordinary HPLC operation without filtering in maximum laboratories. Similarly, fractional distillation is a residual gas decrease through sample solution that had also majorly been replaced by the suction degassed agents, that have become conventional in that vast of most HPLC systems [23].

Mobile Phase stability

There are no widely widespread hints or clinical information on the steadiness of mobile stages after guidance. Sparkling mobile stages can be organized on an everyday foundation required, however, Its time test procedure is hardly necessary nor " greencolored". The shelf existence of the liquid section is hard to generalize because it relies upon the mobile section composition, pH, storage field, garage situations, and the separation's sensitivity to small adjustments in mobile-section composition. Microorganism boom potential (microorganisms, algae, and moulds) poses the greatest risk to gadget contamination and column sturdiness. Scavenger columns (as an example, C18, 33 x four.6 mm id, 10 m) can provide excellent protection against mobile phase-born fine particles & pollutants. It has to do, however, to improve network waiting times & has been infrequently used in labs. Date all solvents and use aqueous buffers for one week, easy acidified water (consisting of 0.1 percentage formic acid or trifluoroacetic acid) for 1-2 months, and use organic solvents for at least three months. Storage of a listen (10-50X) may be useful for weakly acidic buffered mobile levels or buffers at close to neutral pH. Depending on the buffer, refrigeration (5°C) can keep the listen usable for months [24]. This technique has been validated as effective for acetate and formate buffers. Periodic pH assessments and clean injections can verify the integrity and cleanliness of the liquid phase preparations in such cases. Some laboratories may also have standard operating techniques that are based totally on preceding statistics particularly reagent shelf lifestyles depends. Labeling prepared date is supported and required in masses of regulated laboratories [25].

CONCLUSION

The entire article is a review of key concepts in the reverse phase- high performance liquid chromatography (RP – HPLC). mobile phase selection such as (buffers, pH modifiers, and mobile phase Additives,) dealing with different analytical issues by modifying the solvent system as well as using HPLC instruments. The above fashions include the use of simple acidic solvent A or reduced Mass spectrometry-compatible buffers in binary form with acetonitrile or methanol, the use of broad sequential curve or multi-segment slope methodologies for complicated dissociation, the removal of the filtering process, and the elimination of many less relating mobile phase ingredients. Latest additives (trifluoroacetic acid and 3-fluoropropionic acid) are recommended as options available for trifluoroacetic acid for enhanced MS specificity. In recent years advancements in the development of HPLC system with various features, specifications & applications. Hence it is needed to upgrade the effect of mobile phase components and the performance of the HPLC system to take forward.

CONFLICT OF INTEREST

The author has no conflict of interest.

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REFERENCES

1. E. Bulemela, P. Tremaine and S.-I. Ikawa, "Volumetric behavior of water-methanol mixtures in the vicinity of the critical region," Fluid ph.Equilibria245, (2006), 125.doi:org/10.1016/j.fluid.2010.10.005
2. K. F. ItaMcStravick, J. Lambert, N. Teahan, and W. Earle Waghorne, "Enthalpy of transfer of -CH2- between water and organic solvents or mixed aqueous organic solvents or mixed aqueous organic solvent systems," J. Mol. Liq. 94, (2001), 145- 153.
3. G. R. Behbehani, S. Ghammamy, and W. E. Waghorne, "Enthalpies of transfer of acetonitrile from water to aqueous methanol, ethanol and dimethylsulphoxide mixtures at 298.15 K," ThermochimicaActa, 448, No. 1, (2006), 37-40. doi:10.1016/j.tca.2006.06.021
4. M. Vosough and A. Salemi, "Second-Order standard Addition for deconvolution and quantification of fatty acids of fish oil using GC-MS," Talanta, 73, No. 1, (2007), 30-36. doi:10.1016/j.talanta.2007.02.025
5. M. Bechtold, A. Fellinger, M. Held, and S. Panke, "Adsorption behavior of a teicoplaninaglycone bonded stationary phase under harsh overload conditions," J. Chromatogr. A 1154, No. 1-2, (2007), 277-286. doi:10.1016/j.chroma.2007.03.103
6. D. Bylund, R. Danielsson, G. Malmquist, and K. E. Markides, "Chromatographic alignment by warping and dynamic programming as a pre-processing tool for parafac modelling of liquid chromatography-mass spectrometry Data," J. Chromatogr. A 961, No. 2, (2002), 237-244. doi:10.1016/S0021-9673(02)00588-5
7. K. M. Pierce, B. W. Wright, and R. E. Synovec, "Unsupervised parameter optimization for automated retention time alignment of severely shifted gas chromatographic data using the piecewise alignment algorithm," J. Chromatogr. A 1141, No. 1, (2007), 106-116. doi:10.1016/j.chroma.2006.11.101
8. F. Gong, Y.-Z. Liang, Y.-S. Fung and F.-T. Chau, "Correction of retention time shifts for chromatographic fingerprints of herbal medicines," J. Chromatogr.A 1029, No. 1-2, (2004), 173-183. doi:10.1016/j.chroma.2003.12.049
9. F. O. Andersson, R. Kaiser, and S. P. Jacobsson, "Data preprocessing by wavelets and genetic algorithms for enhanced multivariate analysis of LC peptide mapping," J .pharm. Biomed. 34, No. 3, (2004), 531-541. doi:10.1016/S0731-7085(03)00583-1
10. K. J. Johnson, B. W. Wright, K. H. Jarman, and R. E. Synovec, "High-speed peak matching algorithm for retention time alignment of gas chromatographic data for chemometric Analysis," J. Chromatogr. A 996, No. 1-2, (2003), 141-155. doi:10.1016/S0021-9673(03)00616-2
11. A. M. van Nederkassel, M. Daszykowski, P. H. C. Eilers and Y. V. Heyden, "A comparison of three algorithms for chromatograms alignment," J. Chromatogr. A 1118, No. 2, (2006), 199-210. doi:10.1016/j.chroma.2006.03.114
12. M.Makela and L. Pyy, "Effect of temperature on retention time reproducibility and on the use of programmable fluorescence detection of fifteen polycyclic aromatic hydrocarbons," J.Chromatogr. A, 699, No. 1-2, (1995), 49-57. doi:10.1016/0021-9673(95)00120-C
13. W. S. Gardner, H. A. Bootsma, C. Evans and P. A. S. John, "Improved chromatographic analysis of 15N:14N Ratios in ammonium or nitrate for Isotope addition experiments," Mar. Chem. 48, No. 3-4, (1995), 271-282. doi:10.1016/0304-4203(94)00060-Q
14. M. Alzweiri, J. Parkinson, D. Watson and S. Steer, "Microscopic trends in methanol/water and acetonitrile/ water Systems," Jordan J. Pharm. sci 4, (2011), 20-28.
15. Y. Satoh and K. Nakanishi, "Theoretical studies of acetonitrile-water mixtures/montecarlo simulation," Fluid Ph. Equilibria 104, (1995), 41-55. doi:10.1016/0378-3812(94)02638-H.
16. L.R. Snyder and J.J. Kirkland, Introduction to modern liquid chromatography (Wiley, Hoboken, New Jersey, 3rd Ed., j. Am. soc.mass spectrom(2010), Chapters 2, 6, and 7.
17. Y.V. Kazakevich and R. LoBrutto, Eds., HPLC for pharmaceutical scientists (Wiley, Hobo -ken, New Jersey,J.Pharma Anal(2007), Chapter 4.doi:10.1002/0470087951
18. M.W. Dong, Modern HPLC for practicing scientists (Wiley, Hoboken, New Jersey,j.modern.HPLC.p.s(2006), Chapters 1, 2, 5, 6, 8 and 10.doi:10.1002/0471973106.CH4

19. L.R. Snyder and J.W. Dolan, High-performance gradient elution: The practical application of the linear-solvent-strength model (Wiley, Hoboken, New JerseyJ. Am. Chem. Soc(2006).doi.org/10.1021/ja069848
20. W.R. Melander and C. Horváth, in HPLC, advances andperspectives 2, C. Horváth, Ed. (academic press, new york,) J. Anal. Chem. (1980). https://doi.org/10.1021/ac102570t
21. Kumar A, Jawla S, Yadav G. Recent analytical method developed by RP-HPLC.Int J Pharmacol. (2013);7(3):232-40. DOI:10.5829/idosi.gjp.2013.7.3.7579
22. M. Wong, B. Murphy, J. H. Pease, and M. W. Dong, LCGC North Am. 33(6), 40 2- 413 (2015).
23. J.L. Glajch, J.J. Kirkland, K.M. Squire, and J.M. Minor, J. Chromatogr. 199, 5 7 (19 80).doi.org/10.1016/j.jcoa.2022.100034
24. K.J. Fountain, H.B. Hewitson, P.C. Iraneta, and D. Morrison, Waters Corporation, milford, massachusetts, 720 003720EN, Sep. (2010).
25. D. Johnson, B.E. Boyes, and R.C. Orlando, J. Biomol. technology 24, 187–197 (2013).
26. M.W. Dong, G. Miller, and R. Paul, J. Chro-matogr. 987, 283–290, (2003).
27. J.J. Kirkland, M.A .van Straten, and H.A .Claessens, J. Chromatogr. A 691, 3 –19 (1995).
28. J.E.Turner, A chemistry focused approach to column screening, HPLC method development workshop, parsip-pany, new jersey, 13 June,(2018).
29. M.W. Dong, LCGC North Am. 33(10), 764–775 (2015). (20) M.W. Dong, LCGC north am. 34(6), 408 –419 (2016).
30. Snyder L.R., Kirkland J.J. and Glajch J.L. Practical HPLC method development, 2nd Edition, Wiley-interscience publisher, new york (1998) 68-70,721-728.
31. Agilent Technologies, student manual - practical High-Performance liquid chromatography, course number H5930A, USA (2001) 25-28.
32. Ornaf R.M. and Dong M.W. (2005), key concepts of HPLC in pharmaceutical analysis In Ahuja S. and Dong M.W., handbook of pharmaceutical Analysis by HPLC, Elsevier Inc, 35-38.
33. Rosario LoBrutto (2007), Reversed-phasse HPLC. In Yuri kazakevich androsarioloBrutto, HPLC for pharmaceutical Scientists (158-160). johnwiley and sons, Inc.
34. Dong M.W. (2005), How to be more successful with HPLC analysis: practical aspects in HPLC operation In S. Ahuja, M.W.Dong, handbook of pharmaceutical analysis by HPLC, Elsevier Inc, 256-258.
35. Yuri kazakevich and Rosario LoBrutto, HPLC for pharmaceutical scientists, John Wiley and Sons, Inc (2007) 375-380.
36. Prichard, E.; Barwick, V.; "Quality assurance in analytical chemistry"; Willey. (2007).
37. Sommer, K.; Chappel, S.;Kochsiek, M. "Calibration and verification: two procedures having comparable objectives and results"; OIML BULLETIN; vol. XLII, number 1, (2001)

Table 1: Solubility in water with various solvents used in HPLC system [12]

Solvent	Solvent % in water	Solvent	Solvent % in water
Water	100	n-Butanol	0.43
Formamide	100	n-Propanol	100
Dimethylsulfoxide	100	Isopropanol	100
Dimethylformamide	100	Butyl acetate	7.81
Acetic acid	100	Methylene chloride	1.6
Acetonitrile	100	Diethyl ether	6.89
Ethanol	100	Benzene	0.18
Methanol	100	Carbon tetrachloride	0.08
Acetone	100	Cyclohexane	0.01

Table 2. General HPLC Buffers and their related pKa value and UVcutoff values [28]

Buffer types	pKa value	UV threshold value (nm)
Trifluoroethanoic acid	0.3	210
Calcium Phosphate	2.1,7.2,12.3	190
Formic acid	3.8	210
Acetic acid	4.8	210
Carbonate	6.4,10.3	200
Ammonia	9.2	200

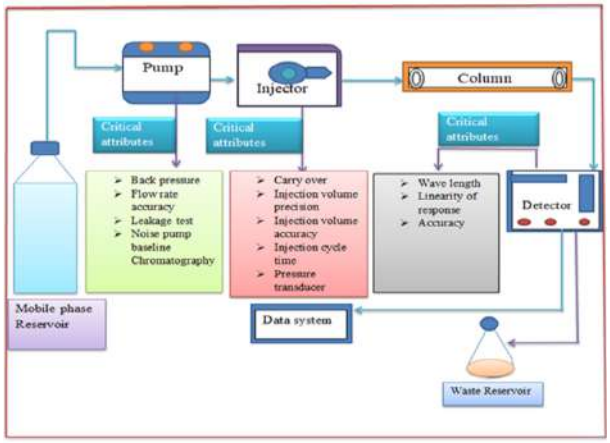

Fig.1 Critical attributes of HPLC system [5]

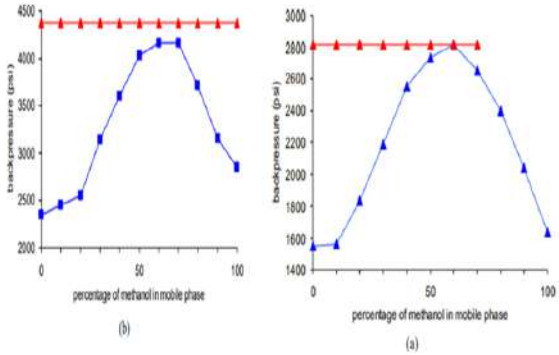

Fig.2 Shows the internal pressure of RP column exposure to Methanol (MeOH) – water (H_2O) sample mixtures during HPLC analysis. Acetonitrile C8 columns (a); Kromasil 5 ODS (b). Methanol and water gradient, whereas isocratic (45:55) MeOH: H_2O gradient [13].

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RESEARCH ARTICLE

Structure based Molecular Docking Analysis for Selected Phytochemicals from *Murraya koenigii* Targeting Aromatase Receptor against Breast Cancer

Saptarshi Samajdar*

Assistant Professor, School of Pharmacy, The Neotia University, Sarisa, West Bengal, India.

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***Address for Correspondence**
Saptarshi Samajdar,
Assistant Professor,
School of Pharmacy,
The Neotia University,
Sarisa, West Bengal, India.
Email: saptarshis.samajdar@tnu.in

ABSTRACT

Cancer can be described as the uncontrolled growth of abnormal cells. After lung cancer, breast cancer is the second most frequent type of cancer. The majority of breast cancer cells and healthy breast cells both have receptors for circulating oestrogen and progesterone. Aromatase inhibitors, all of which directly interact with the stimulation of the oestrogen signalling pathway and its production, are one of the most significant targets for treating this. Despite their success, the creation of new medicines is necessary since resistance limits clinical efficacy. As a result, due to their low cost and speedy development, in silico studies for drug discovery have grown in popularity throughout time. For docking investigations using PyRx software over the positive kind of breast cancer through aromatase receptor, 20 phytochemicals derived from *Murraya koenigii* have been chosen in this case. The usage of phytochemical substances stems from their numerous pharmacological applications, such as their anti-cancer, anti-diabetic, and antibacterial characteristics. Additionally, an ADME study and RMSD study was conducted for phytochemicals to determine the ideal drug-likeness profile. From the 20 compounds, the results showed a better dock score, hydrogen bonding, and steric interaction between compounds Germacrene, Viridifloral, α-Selenine and γ-Muurolene with aromatase receptor protein 3EQM.

Keywords: Breast cancer; aromatase inhibitor; molecular docking; *Murraya koenigii*; ADME studies

INTRODUCTION

Cancer is an uncontrollable and everlasting disease that affects aberrant cells over an extended period of time. Cancerous cells can be aggressive, invasive, and metastatic, and they frequently invade other organs. Breast cancer is quite heterogeneous in nature and interferes with the normal mammary epithelial cells' ability to function. In the entire world, breast cancer is one of the most common diseases affecting women [1]. About25% of breast cancer patients were reported from developed countries [2]. Breast cancer is one of the common types of cancer contributing more than 27% in total cancer patients in Indian population [3]. In the year 2021 alone more than 574000 breast cancer cases have been reported with 39.12%mortality. The incidence of breast cancer cases increased dramatically in patients at the age group of 50–64 [4]. One breast cancer case was reported for every 22 women in urban areas, and for every 60 women in rural areas. Males have, however, only sporadically been known to have breast cancer. Each year, more than 1500 new cases are reported in the United States [5].

This cancer is among the second most common causes of death worldwide in western nations. Although there are multiple synthetic strategies for control of breast cancer, one of the most significant clinical problems with effective drugs for the treatment of breast cancer is the occurrence of side effects and the development of therapeutic resistance. Therefore, using medicinal plant products can be a fantastic choice to manage that. It has long been common practice to treat both infectious and non-infectious diseases with medicinal herbs. Phytochemicals are defined as bioactive nutrient plant chemicals in fruits, vegetables, grains, and other plant foods that may provide desirable health benefits beyond basic nutrition to reduce the risk of major chronic diseases [6]. Numerous studies have reported the inhibitory effects of phytochemical, such as *Ginko*, goldenseal, ginseng, garlic, Echinacea, aloe vera and saw palmetto find their use against breast cancer ailments. In this study, a comprehensive report regarding the *in-silico* studies of some phytochemicals from *Murraya koenigii* and their action in breast cancer aromatase receptor.

METHODOLOGY

Ligands preparation and optimization

For this investigation, 20 ligands isolated from *Murraya koenigii* leaf drawn in Chem Draw Professional 15.0 (Fig.1.), and three-dimensional structures of the ligands were produced in Open babel and saved as SDF format for further preparation and molecular docking analysis [7,8].

Drug like properties of the ligands

Lipinski's rule of five, bioavailability score, Ghose's, and Veber's criteria were used to determine the cutoff values for the physicochemical attributes. The molecular parameters MW (molecular weight), HBD (hydrogen bond donor), HBA (hydrogen bond acceptor), log P (lipophilicity log), and log S (aqueous solubility) were assessed to determine drug likeliness[9,10]. The SWISSADME server was used to generate the parameters (www.swissadme.ch/index.php).

The breast cancer receptor targeting aromatase inhibitor protein preparation and optimization

The crystallographic structures of breast cancer targeting aromatase inhibitor protein (PDB ID: 3EQM), was obtained from the protein data bank. To prepare the protein for molecular docking, the water molecules were removed and then hydrogen atoms were supplied to the protein using the BIOVIA Discovery Studio 2021 Client programme to correct the ionization of the amino acid residues [11].

Molecular docking analyses and visualization

The proteins were saved in pdb format and then loaded into the PyRx programme for molecular docking, which was done with the Auto dock Vina tool. To find the most stable conformer, the PyPx programme was utilized. Using Discovery Studio 2021 Client software, the intermolecular interactions between the *Murraya koenigii* ligands from and residues of aromatase inhibitor protein were determined and depicted [12,13].

Molecular Dynamics Simulation

To study the stability and interactions of the ligand-receptor complexes, the top scoring natural polysaccharide was subjected to a 1500 ps MD simulation utilizing the GROMOS96 43a1 force field with GROMACS software. MD simulations were used to study structural parameters such as the Root Mean Square Deviation (RMSD) for complex stability [14, 15].

RESULTS AND DISCUSSION

PyRx docking was used to determine binding affinities and key interactions between breast cancer receptor targeting aromatase inhibitor protein (3EQM) and *Murraya koenigii* phytochemical ligands. The binding affinities were compared to those obtained with the protein inhibitors tamoxifen. Table 1. shows the binding affinity obtained from proteins bound to tamoxifen, and phytochemical ligands. The binding affinity values for *Murraya koenigii* phytochemical range from - 5.6 to –7.4 kcal/mol [16]. The molecular interactions between active site of breast cancer receptor targeting aromatase inhibitor protein and the most active ligands were visualized using the Discovery Studio 2021 Client software (Fig. 2.). These samples revealed the predicted interactions with the amino acids in the protein's active region, implying strong antagonistic capabilities against breast cancer receptor targeting aromatase inhibitor protein. For the protein 3EQM, Germacrene, Viridifloral, α-Selenineand γ-Muurolenehad the highest binding affinity with a value of -7.4 kcal/mol each, followed by Spathulenol, β-Selenine, and δ-Amorphene. The ligands with the lowest values in our investigation correspond to α-Pinene ligand with binding affinity for proteins – 5.6 kcal/mol. When compared to commonly used inhibitor Tamoxifen (-7.2 kcal/mol), the binding affinity values of natural phytochemicals derived from *Murraya koenigii* exhibit a higher binding affinity [17].

From SwissADME, the ligands' molecular weights ranged from 136.23 to 296.53 g, with LogP values ranging from 2.66 to 6.25, showing their lipophilic nature. The predictive value of all sets of ligands was in range of Lipinski's rule of five cutoff. This reflects the fact that compounds with log P values in the range are soluble in fats, oils, lipids, and nonpolar solvents, with the drug likeliness of all ligands lying within Ghosh's rule (Table 2.). The results of virtual phytochemicals from *Murraya koenigii* using molecular docking scores, and ADME studied suggest that they can potential inhibitors of the 3EQM protein [18,19]. The root mean square deviation (RMSD) values of the best four ligands from *Murraya koenigii* in association with the protein 3EQM are shown in Fig.3. Calculations of backbone atom RMSD for the ligand-protein complex, as illustrated in the figure, were used to determine the stability of each simulated model. The RMSD of A (Germacrene) rises until 200 ps, then stabilizes at 1.2Å, while C (α-Selenine), climb until 300 ps, then stabilize at 0.9-1.1 Å until 350 ps. This shows that these ligands remain stable inside the pocket and do not change their orientation in the active site of proteins. Similarly, the backbone atoms of protein B (Viridifloral) were stable about 1Å. However, D (γ-Muurolene) showed higher RMSD and maintained at 0.8 Å, which directly resembles to the charge analyses [20,21].

CONCLUSIONS

The Protein-Ligand interaction plays a significant role in structural based drug designing. In the present work we have taken the receptor Human estrogen and identified the drugs that were used against Breast Cancer. Overall, in an exploration to find phytocompounds which could act as inhibitor of breast cancer targeting aromatase inhibitor protein (PDB ID: 3EQM), our study manifested a promising anti-carcinogenic drug for breast cancer with presentation of strong binding energy activity of Germacrene, Viridifloral, α-Selenine and γ-Muurolene. Additionally, an ADME study and RMSD study was conducted for phytochemicals to determine the ideal drug-likeness profile. This information may serve as a stepping point for future research in discovery of a suitable drug in the treatment of breast cancer and salvage the humankind from the onslaught of deadly disease.

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DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

1. Jemal, F. Bray, M. M. Center, J.Ferlay, E.Ward, & D.Forman, (2011). Global cancer statistics. *CA: a cancer journal for clinicians*, 61(2), 69-90.https://doi.org/10.3322/caac.20107
2. H. Park, Z. Li, X. O. Yang, S. H. Chang, R. Nurieva, Y. H.Wang, , & Dong, C. (2005). A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nature immunology*, *6*(11), 1133-1141.https://doi.org/10.1038/ni1261
3. L. A.Torre, F.Bray, R. L.Siegel, J. Ferlay, J.Lortet-Tieulent, &A.Jemal (2015). Global cancer statistics, 2012. *CA: a cancer journal for clinicians*, 65(2), 87-108.https://doi.org/10.3322/caac.21262
4. P.Sharma, (2021). Burden of cancer: The unaddressed epidemic in India. *Cancer Research, Statistics, and Treatment*, *4*(2), 411.https://doi.org/10.4103/crst.crst_71_21
5. S. H.Giordano, A. U.Buzdar, & G. N.Hortobagyi, (2002). Breast cancer in men. *Annals of internal medicine*, *137*(8), 678-687.https://doi.org/10.7326/0003-4819-137-8-200210150-00013.
6. Liu, R. H. (2004). Potential synergy of phytochemicals in cancer prevention: mechanism of action. *The Journal of nutrition*, *134*(12), 3479S-3485S.https://doi.org/10.1093/jn/134.12.3479S
7. J. J.Sahayarayan, K. S.Rajan, R.Vidhyavathi, M.Nachiappan, D. Prabhu, S.Alfarraj,&A. N.,Daniel (2021). In-silico protein-ligand docking studies against the estrogen protein of breast cancer using pharmacophore based virtual screening approaches. *Saudi Journal of Biological Sciences*, *28*(1), 400-407.https://doi.org/10.1016/j.sjbs.2020.10.023
8. Z.Yousuf, K. Iman, N. Iftikhar, & M. U. Mirza (2017). Structure-based virtual screening and molecular docking for the identification of potential multi-targeted inhibitors against breast cancer. *Breast Cancer: Targets and Therapy*, *9*, 447.https://doi.org/10.2147/BCTT.S132074
9. A.Ayar, M.Aksahin, S.Mesci, B. Yazgan, M. Gül, & T. Yıldırım, (2022). Antioxidant, Cytotoxic Activity and Pharmacokinetic Studies by Swiss Adme, Molinspiration, Osiris and DFT of PhTAD-substituted Dihydropyrrole Derivatives. *Current Computer-aided Drug Design*, *18*(1), 52-63.https://doi.org/10.2174/1573409917666210223105722.
10. A.Yadav, & S. Mohite, (2020). Anticancer activity and In-Silico ADMET analysis of malvastrum coromandelianum. *International Journal of Pharma Sciences and Research*, *11*(5), 71-73.
11. S. V.Pattar, S. A.Adhoni, C. M. Kamanavalli, & S. S. Kumbar, (2020). In silico molecular docking studies and MM/GBSA analysis of coumarin-carbonodithioate hybrid derivatives divulge the anticancer potential against breast cancer. *Beni-Suef University Journal of Basic and Applied Sciences*, *9*(1), 1-10.https://doi.org/10.1186/s43088-020-00059-7
12. Alfaro, M., Alfaro, I., & Angel, C. (2020). Identification of potential inhibitors of SARS-CoV-2 papain-like protease from tropane alkaloids from Schizanthus porrigens: A molecular docking study. *Chemical physics letters*, *761*, 138068.https://doi.org/10.1016/j.cplett.2020.138068
13. Budiono, B., Pertami, S. B., Arifah, S. N., & Lestari, S. R. (2021, May). Molecular docking analysis of Polyscias scutellaria active compounds as inhibitor of dopamine D2 receptors to increase prolactin secretion. In *AIP Conference Proceedings* (Vol. 2353, No. 1, p. 030034). AIP Publishing LLC.
14. Dutta, M., Tareq, A. M., Rakib, A., Mahmud, S., Sami, S. A., Mallick, J., & Emran, T. B. (2021). Phytochemicals from leucas zeylanica targeting main protease of SARS-CoV-2: chemical profiles, molecular docking, and molecular dynamics simulations. *Biology*, *10*(8), 789.https://doi.org/10.3390/biology10080789.

15. Ahmed, B., Ali Ashfaq, U., & Usman Mirza, M. (2018). Medicinal plant phytochemicals and their inhibitory activities against pancreatic lipase: molecular docking combined with molecular dynamics simulation approach. *Natural product research*, *32*(10), 1123-1129.https://doi.org/10.1080/14786419.2017.1320786.
16. L. E. Mendie, & S.Hemalatha, (2022). Molecular docking of phytochemicals targeting GFRs as therapeutic sites for cancer: An in-silico study. *Applied Biochemistry and Biotechnology*, *194*(1), 215-231.https://doi.org/10.1007/s12010-021-03791-7
17. Ahmed, B., Ashfaq, U. A., ul Qamar, M. T., & Ahmad, M. (2014). Anti-cancer potential of phytochemicals against breast cancer: Molecular docking and simulation approach. *Bangladesh Journal of Pharmacology*, *9*(4), 545-50.https://doi.org/10.3329/bjp.v9i4.20412.
18. A.M.Awadelkareem, E. Al-Shammari, A. E. O. Elkhalifa, M. Adnan, A. J. Siddiqui, M.Snoussi&S. A. Ashraf, (2022). Phytochemical and In Silico ADME/Tox Analysis of Eruca sativa Extract with Antioxidant, Antibacterial and Anticancer Potential against Caco-2 and HCT-116 Colorectal Carcinoma Cell Lines. *Molecules*, *27*(4), 1409.https://doi.org/10.3390/molecules27041409.
19. M.Mahanthesh, D.Ranjith, R. Yaligar, R. Jyothi, G. Narappa, &M. Ravi, (2020). Swiss ADME prediction of phytochemicals present in *Butea monosperma* (Lam.) Taub. *Journal of Pharmacognosy and Phytochemistry*, *9*(3), 1799-1809.https://doi.org/10.1007/s00894-021-04736-x
20. S. Rampogu, S. Parameswaran, M. R. Lemuel, & K. W. Lee, (2018). Exploring the therapeutic ability of fenugreek against type 2 diabetes and breast cancer employing molecular docking and molecular dynamics simulations. *Evidence-Based Complementary and Alternative Medicine*, *2018*.https://doi.org/10.1155/2018/1943203.
21. S.Qazi, & K.Raza, (2021). Phytochemicals from Ayurvedic plants as potential medicaments for ovarian cancer: an in-silico analysis. *Journal of Molecular Modeling*, *27*(4), 1-14.https://doi.org/10.1007/s00894-021-04736-x.

Table.1: Results of compounds docking in aromatase inhibitor target

Ligands	Binding Affinity (ΔG in kcal/mol)
	3EQM
2-Carene	-5.9
Bornyl Acetate	-6.8
Caryophyllene	-5.8
D-Limonene	-5.7
Germacrene	-7.4
o- Cymene	-5.6
Phytol	-6.2
Spathulenol	-7.3
Terpeneoline	-5.6
Viridifloral	-7.4
α-Phellandrene	-5.8
α-Pinene	-5.5
α-Selenine	-7.4
β-Borbournene	-6.9
β-Linalool	-6.8
β-Pinene	-5.8
β-Selenine	-7.3
δ-Amorphene	-7.3
γ-Muurolene	-7.4
γ-Terpinene	-5.6
Tamoxifene	-7.2

Table 2:ADME parameters for each ligand from SwissADME

Inhibitors	Molecular Wt. (g)	Log P	HBD	HBA	Violation	Yes/No	Solubility	Log S
2-Carene	136.23	3.12	0	0	1	Yes	Soluble	-2.48
Bornyl Acetate	196.29	3.00	2	0	0	Yes	Soluble	-3.63
Caryophyllene	204.35	4.24	0	0	1	Yes	Soluble	-3.77
D-Limonene	136.23	3.37	0	0	0	Yes	Soluble	-2.26
Germacrene	204.35	4.46	0	0	1	Yes	Moderately soluble	-3.32
o- Cymene	134.22	3.54	0	0	1	Yes	Soluble	-3.57
Phytol	296.53	6.25	1	1	1	Yes	Moderately soluble	-5.98
Spathulenol	220.35	3.30	1	1	0	Yes	Soluble	-3.20
Terpeneoline	136.23	3.40	0	0	0	Yes	Soluble	-3.50
Viridifloral	220.35	3.44	1	0	0	Yes	Soluble	-4.07
α-Phellandrene	136.23	2.97	0	0	0	Yes	Soluble	-2.88
α-Pinene	136.23	3.32	0	0	1	Yes	Soluble	-2.48
α-Selenine	204.35	4.40	0	0	1	Yes	Soluble	-3.88
β-Borbournene	204.35	4.40	0	0	1	Yes	Soluble	-3.32
β-Linalool	154.25	2.66	1	1	0	Yes	Soluble	-3.06
β-Pinene	136.23	3.24	0	0	1	Yes	Soluble	-2.48
β-Selenine	204.35	4.50	0	0	1	Yes	Moderately soluble	-4.47
δ-Amorphene	204.35	4.12	0	0	1	Yes	Soluble	-3.43
γ-Muurolene	204.35	4.18	0	0	1	Yes	Soluble	-3.76
γ-Terpinene	136.23	3.35	0	0	0	Yes	Soluble	-3.43

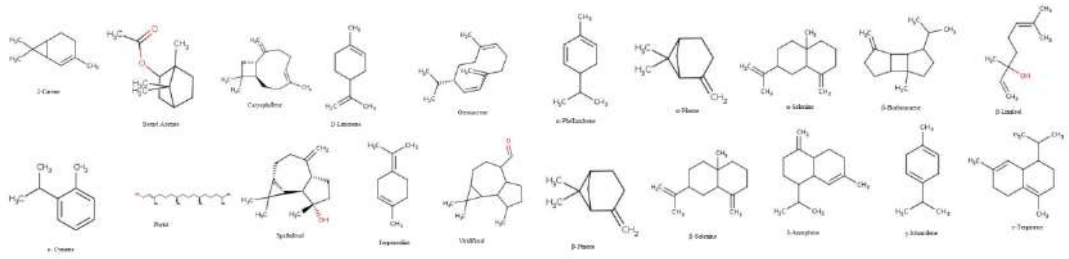

Fig.1. Different phytochemical ligands from *Murraya koenigii*

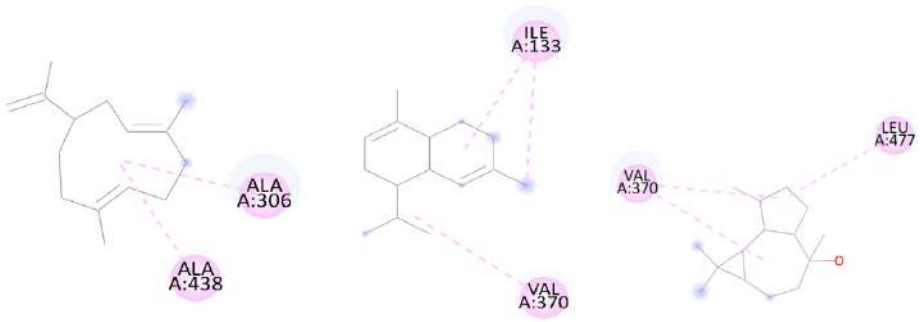

Fig. 2: Molecular interaction of 3EQM and a.Viridifloral, b. γ-Muurolene, c.δ-Amorphene.

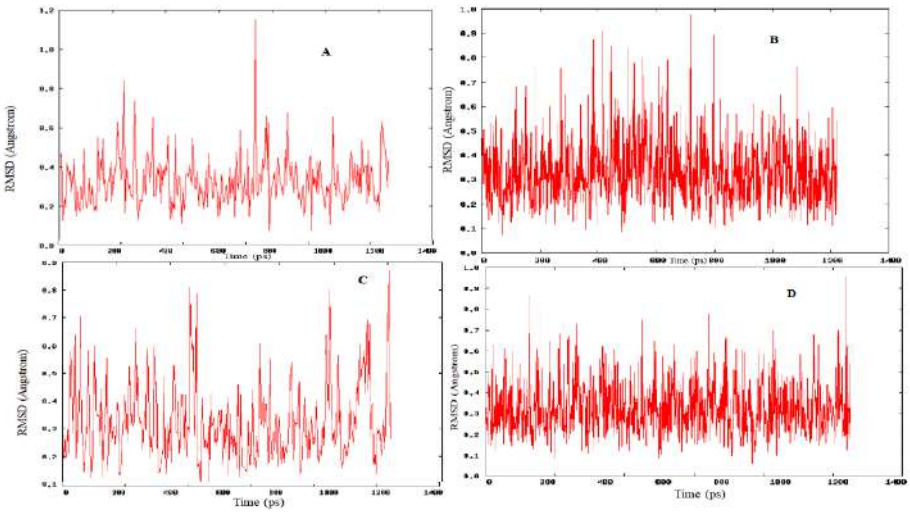

Fig.3.RMSD plot during molecular dynamics simulations.

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RESEARCH ARTICLE

Formulation Development, Evaluation and Comparative Study of Effects of Superdisintegrants on Orodispersible Tablet Containing an Antihypertensive Drug

Reshma K J[1]* and Senthila S[2]

[1]M Pharm, Department of Pharmaceutics, The Dale View College of Pharmacy and Research Centre, Punalal, Thiruvananthapuram, Kerala, India.
[2]Professor and HoD, Department of Pharmaceutics, The Dale View College of Pharmacy and Research Centre, Punalal, Thiruvananthapuram, Kerala, India.

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***Address for Correspondence**
Reshma K J,
M Pharm,
Department of Pharmaceutics,
The Dale View College of Pharmacy and Research Centre,
Punalal, Thiruvananthapuram, Kerala, India.
Email: reshmakj03@gmail.com

ABSTRACT

The oral route of drug administration is the most widely accepted route, because of its convenience of self-administration, compactness and easy manufacturing. The aim of the present work is to formulate, evaluate and to compare the effects of various superdisintegrants on orodispersible tablet containing an antihypertensive drug. Propranolol hydrochloride orodispersible tablets preparation were done by direct compression method. Twenty three formulations were prepared using different classes of superdisintegrants like natural, synthetic and co-processed in varying concentrations (2%, 4%, 6%). The drug-polymer compatibility study was done by using FTIR. All the formulations of prepared ODT were subjected to precompression evaluations such as angle of repose, bulk density, tapped density, compressibility index and Hausner's ratio. Post compression evaluations such as hardness, diameter, thickness, weight variation, friability, wetting time, water absorption ratio, uniformity of drug content, *In-vitro* dispersion time, *In-vitro* Disintegration test and dissolution study. The results of IR study showed that there is no interaction between drug, superdisintegrants and other excipients. The formulated tablet melts in mouth within fraction of seconds (within 30 sec to 3 minutes) with promising release of drug. Among the promising formulations, the formulation F18 containing 6% crospovidone and guar gum(1:1) co-processed superdisintegrant, emerged as the best formulation based on drug release characteristics. A comparison of *In-vitro* drug release of F18 was done with marketed formulation (Inderal 40), which

reveals that the ODT formulation is much better than the conventional tablets. Short term stability study was also conducted and the results shown that the formulation was stable.

Keywords: Orodispersible tablet; Propranolol Hydrochloride; Superdisintegrants; Co-processed; Natural & Synthetic.

INTRODUCTION

Oral route is the most prominent and widely accepted up to 50-60% of total dosage forms. It is the most popular route for the systemic effects due to its versatility, ease of ingestion, pain avoidance and most importantly patient compliance [1].The most popular solid dosage forms used are tablets and capsules, difficulty in swallowing, leading to patients incompliance particularly in case of pediatric and geriatric patients are the major limitation of the same[2]. For swallowing of oral dosage forms, one should drink water. Approximately 50% of population suffers with dysphagia, which is commonly found among all age groups. This difficulty in swallowing may be due to the taste, size and surface of dosage form. Elderly patients and children are the most affected population having difficulty in administration of the conventional oral dosage forms [3]. Others who may experience problems in swallowing are the mentally ill development disability, uncooperative patients with reduced liquid intake plan and nausea [4]. So the demand for solid dosage forms that can be chewed, dissolved and suspended in water or rapidly dissolved in the mouth is drastically improved in the pediatric and geriatric population.

For the ease of administration of solid dosage forms mouth dissolving drug delivery system has been introduced. So the problems related to swallowing can be reduced by Orodispersible tablets. Orodispersible tablets are also called as mouth dissolving tablets, fast dissolving tablets, melt in mouth tablets, rapimelts, porous tablets, quick dissolving etc[5].Taste masking also have critical importance in the formulation of an acceptable orodispersible tablet. One of the simplest method of taste masking is by the addition of sweeteners and flavours. The fast disintegrating tablets give out the combined benefits of a liquid formulation and a solid dosage form. Thus, it leads to an increase in bioavailability of drug by avoiding first pass metabolism. Orodispersible tablets are defined as the tablets, which are meant to disintegrate immediately upon contact with the saliva leading to very faster release of the drugs in the oral cavity and rapidly disintegrate within 15 seconds to 3 minutes.

US FDA defined ODT tablets as "A solid dosage form containing medicinal substances which disintegrates rapidly usually within a matter of seconds, when placed upon the tongue" [6,7].ODTs offers several advantages over other dosage forms like effervescent tablets, dry syrups and chewing gums or tablets, which are commonly used to enhance patient's compliance[8].Hypertension is a common problem among the population nowadays. If untreated it may lead to serious health problems also[9,10,11]. So an immediate action should require for the body. Propranolol Hydrochloride is a synthetic beta adrenergic receptor blocking agent, which is an extensively used antihypertensive. The drug undergo extensive hepatic degradation (76%) and having poor bioavailability (24%) [12,13]. If the Propranolol HCl formulated as ODT, then can improve its bioavailability and can avoid its extensive hepatic degradation by reducing the disintegration time with maximum drug release. Superdisintegrants have a major role in ODT formulation. Synthetic, natural and co-processed superdisintegrants are using in the study.

MATERIALS AND METHODS

The drug Propranolol Hydrochloride were procured from Balaji chemicals Ltd, Gujarat. Crospovidone, Croscarmellose sodium, Sodium starch glycolate, Microcrystalline cellulose were purchased from Yarrow chem products, Mumbai. Guar gum, Agar, Mannitol, Saccharin sodium were obtained from Nice chemicals, Cochin. Magnesium stearate and Talc were purchased from Vikash pharma, Mumbai.

Identification of drug

Identification of Propranolol Hydrochloride was done by using Infrared absorption spectroscopy.

Drug-excipient compatibility study

Fourier transform infrared spectrophotometer was used to perform FTIR spectroscopy. The drug-excipient compatibility study was carried out using KBr pellet method. It was done by preparing the pellets of drug and Potassium bromide which are then compressed at 20psi for 10 min on KBr press. Propranolol HCl, superdisintegrants and the physical mixture of Propranolol HCl along with superdisintegrants and excipients were scanned in the wave number range of 4000-600 cm^{-1} using FTIR.

Preparation of co-processed superdisintegrants [14,15,16]

Solvent evaporation method was used for the preparation of co-processed superdisintegrants. Chloroform was used as the volatile solvent. Superdisintegrants which has to be made as co-processed blend were taken in varying ratios (1:1 & 1:2) were mixed together by the addition of 10-15ml of chloroform. The solutions were thoroughly stirred, till almost all chloroform get evaporated. The wet coherent mass was then granulated through sieve #60. The wet granules were dried in a hot air oven at 60°C for 30 min. The dried granules were again passed through sieve # 60 in order to break lumps and then stored in an airtight container for further use.

Preparation of orodispersible tablets of Propranolol Hydrochloride [14,17]

All the ingredients were passed through #60 mesh sieves separately. An accurately weighed quantity of drug, superdisintegrants and microcrystalline cellulose were taken in a glass mortar and ground well. The other excipients like mannitol, saccharin sodium and talc were added in a geometrical order and mixed well to ensure thorough mixing of all ingredients. Lubricant was also added at the end of granulation and mixed thoroughly. The total powder blend is weighed individually for fifty tablets of each formulation. Finally, physical mixture were compressed into tablets using 10-station rotary tablet punch machine having 8mm internal diameter. The composition of formulation was shown in table 1-4.

Post compression evaluations Friability [18,19]

Roche friabilator was used to determine the friability of tablets. It is expressed in percentage (%). Twenty tablets were initially weighed (i.e. W-initial) and transferred into friabilator. The operation of friabilator was carried out at 25 rpm for 4 minutes or run up to 100 revolutions. The tablets were weighed again (i.e. W-final). Friability of the tablets less than 1% is considered acceptable.

Weight Variation [19,20]

Ten tablets were randomly selected from the lot and weighed individually to check for weight variation. Then the average weight of one tablet was determined from the collective weight. Average weight was compared with the individual weight and the percentage deviation of individual tablet was calculated.

Wetting time [21,22]

A twicely folded piece of tissue paper was placed in a small petridish containing 6 ml of water, a tablet was placed on the paper, and the time for complete wetting was measured.

Water absorption ratio [21,23]

A petridish was filled up with 6ml of distilled water. A tissue paper folded twice was placed into the petridish. After pre-weighing of a tablet, it was placed on top of the paper. Then, the tablet which got wet was weighed. Then the water absorption ratio R, was determined using following equation.

$R = \{(Wa-Wb) / Wb\} \times 100$

Where, Wb is the weight of tablet before absorption, Wa is the weight of tablet after absorption

Three tablets from each formulation were performed.

Uniformity of drug content [24,25]

Randomly five tablets were selected from each formulation and powdered them in a mortar. An accurately weighed quantity equivalent to 40mg of the drug was dissolved in 50 ml buffer solution of pH 6.8 and made up to 100ml. After filtration to remove insoluble residue if any, 1ml of the filtrate was diluted to 50 ml with the buffer. The absorbance was measured at 290nm using UV-Visible spectrophotometer. The experiments were carried out in triplicate for all formulations and average values were recorded.

***In-vitro* dispersion time [26]**

In vitro dispersion time was measured by using 10ml of phosphate buffer of pH 6.8 in 25 ml beaker at 37 ± 0.5°C temperature. Time required for the complete dispersion of the tablet was noted. Three tablets were tested from each formulation (n=3).

***In-vitro* disintegration test [27]**

The disintegration times of the manufactured tablets were evaluated in vitro using a USP disintegration apparatus operated at 37°C ± 2°C, using phosphate buffer (pH 6.8) as the disintegration medium. One tablet was introduced into each of the six tubes of the basket and one disc was added to each tube. The time required for complete tablet disintegration was recorded and the mean ± SD of six tablet was calculated.

***In-vitro* dissolution study [27,28,29]**

Dissolution studies were carried out for all the formulations employing USP - II paddle method and 900ml of pH 6.8 phosphate buffer as the dissolution medium. The medium was allowed to equilibrate to temperature of 37°C ±0.5°C. Tablet was placed in the vessel and the vessel was covered, the apparatus was operated for 30min in 6.8 pH at 50 rpm. At definite time intervals, 5 ml of the aliquot of sample was withdrawn by using pipette and filtered. The volume was then replaced with equivalent amount of the fresh dissolution medium. The samples were analysed spectrophotometrically at 290nm using UV-Visible spectrophotometer.

Comparison with Marketed Formulation[30,31,32]

The optimized formulation of Propranolol Hydrochloride orodispersible tablet was compared with commercially available tablets. Inderal 40 (Propranolol Hydrochloride tablets 40mg) was selected as a choice. Physical appearance, hardness, drug content and *In-vitro* drug release of optimized formulation of Propranolol HCl ODT was determined and compared with the marketed Propranolol Hydrochloride tablets.

Stability study [33,34,35]

Short term accelerated stability testing was carried out according to the ICH guidelines considering 40 ± 2°C/75 ± 5% relative humidity in a stability chamber for a period of 2 months. The orodispersible tablets of optimized formulation were subjected to stability studies at both initial evaluation and at the end of the 1st month of the tablets exposed to stability chamber

RESULTS

The FTIR studies reveals that all the characteristic peaks appear in the spectra of all samples were within the same wavelength number were shown in fig 1. This indicates that there were no interactions between the drug and physical mixture. The tablets of prepared formulations of Propranolol Hydrochloride ODTs (fig. 2) were found to be white, round in shape and uniform in size with flat surface without specific odour. The wetting time of all formulations (F1-F23) were found to be within 16.66±0.88 to 78.66±0.88 seconds (fig.3) and the water absorption ratio of all formulations (F1-F23) were found to be 45.19±6.9 to 95.33±4.66%. On comparison, minimum wetting time (16.66±0.88 sec) and maximum water absorption ratio (95.33%) were shown by F18 containing 6% crospovidone: guar gum(1:1) among all other formulations. The *In-vitro* dispersion time of all the formulations (F1-F23) were found to be 29±0.57 to 218±1.52 seconds (fig.4). So the results reveals that the tablets (F18) prepared with co-processed blend of crospovidone and guar gum (1:1) ratio showed less than 30sec of dispersion time. Then the order of dispersion time

according to different superdisintegrants can be shown in the order; Crospovidone (6%) <Agar(6%) <Crospovidone: Guar gum(1:1). From the results of *In-vitro* dissolution study it was clear that, maximum percentage of drug (80%) was released from all the formulations within 30 minutes. The maximum percentage of drug release within 30 minutes was achieved by F18 (98.65%) which was formulated with co-processed superdisintegrant. From comparison study shown in table 5 it was clear that, The percentage cumulative drug release of the optimized formulation was found to be 98.65% within 30 minutes and the percentage cumulative drug release of marketed formulation was 66.44% within 30 minutes and the same showed maximum drug release within 1 hour (fig.5). So the comparative drug release profile of F18 and marketed tablets indicating that the release profiles of the F18 formulation was different from that of the marketed product. And from the stability study, the parameters like physical appearance such as colour change, hardness, *In-vitro* dispersion time and drug content were determined for an interval of 60 days. The parameters were found to be satisfactory and no considerable changes were observed. So, F18 was considered as a stable formulation.

DISCUSSION

The present study is an attempt to develop and formulate orodispersible tablet of Propranolol Hydrochloride and to study the effects of different types of superdisintegrants. Hypertension is a long term medical condition which is a common problem too. So for a rapid action antihypertensive agents can be used. With better superdisintegrants, the prepared ODT disintegrate within few seconds in the oral cavity thereby reducing the time of onset of action and to prevent the first-pass metabolism of Propranolol HCl. The results of FTIR studies on drug and physical mixtures of drug, superdisintegrants and excipients confirm that both drug and excipients are compatible with each other and are devoid of interactions. Direct compression method was used for the preparation of orodispersible tablet because of the cost effectiveness and due to reduced number of manufacturing steps. The results of precompression studies like angle of repose, bulk density, tapped density, compressibility index and Hausner's ratio reveals that the prepared powder blends of all formulations possess good to passable flow properties. The prepared tablets were subjected to post compression evaluations and the results indicate that the hardness, thickness and diameters of all the tablets are uniform, which ensures that all the tablets were of uniform size and shape with good resistance against mechanical damage. The tablets of all formulations shows the drug content as per the specifications. In weight variation test, the results were within the limits which indicate that all the formulations have uniform distribution of contents of the powder blend. The friability of all the tablets were found to be < 1%, which indicates the tablets of all formulation have good mechanical resistance.

The tablets of all formulation were found to have minimum wetting time and maximum water absorption ratio which is the desired characteristic of oral dissolving tablets which enables faster disintegration of tablets. And the formulation which showed lowest wetting time and maximum water absorption ratio was selected from twenty three formulations. The *In-vitro* dispersion time and disintegration time were also carried out to ensure the faster disintegration of the tablets. The disintegration time of the formulations were found to be within 3 minutes except F6 with 6% of croscarmellose sodium. The formulation have lesser disintegration and *In-vitro* dispersion time were selected. And it was observed that the co-processed superdisintegrants showed better results than others as compared with synthetic and natural superdisintegrants. From the *In-vitro* release data, all the formulations were found to release the drug more than 80% within 30 minutes. This quality of the tablet is required for the faster absorption of the drug and leads to rapid onset of action also. And it was found to be F18 containing 6% of CP and guar gum (1:1) showed maximum drug release than other formulations within 30 minutes. The Optimized formulation of Propranolol Hydrochloride orodispersible tablet containing 6% of CP and guar gum in 1:1 ratio (F18) was compared with the commercially available conventional tablets of Propranolol HCl (Inderal 40). From the results it was clear that the orodispersible tablets of Propranolol HCl was much better than that of Inderal 40 and also it was expected to enhance a better patient compliance. The ODT was the better formulation than that of the marketed formulation, because of the rapid drug release within 30 minutes. But the marketed formulation has drug release up to 1 hour. The optimized formulation F18 was found to follow first order kinetics and the drug release occurred by

Fickian diffusion. The results of stability study revealed that the selected formulation was stable under the storage condition and showed no significant variations in all the parameters. Thus crospovidone and guar gum can be successfully used in the formulation of fast dissolving tablets.

CONCLUSION

In the present study an attempt has been made to develop, formulate and evaluate the orodispersible tablets of Propranolol HCl which is an antihypertensive agent. It was formulated with an intention to improve the disintegration, bioavailability of drug, to obtain rapid onset of action of drug, and better patient compliance. In this study, various superdisintegrants of synthetic, natural and co-processed were used for the formulation of Propranolol HCl ODTs. From this study it can be concluded that, co-processed superdisintegrant of crospovidone and guar gum (F18) could be applied effectively in the preparation of ODTs with excellent *In-vitro* dispersion time, better water absorption and maximum drug release within 30 minutes. Due to its minimum wetting time the ODT can be administered without the need of water. And the prepared ODT disintegrate within the expected time, thereby enhance the absorption leading to increased bioavailability of Propranolol HCl. Thus, the Propranolol ODT containing co-processed superdisintegrant could perform bioavailability, effectiveness and hence better patient compliance.

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REFERENCES

1. Kakar S, Singh R, Kumar S. Orodispersible tablets: an overview. *MOJ Proteomics Bio inform.* 2018;7(3):180–182.
2. Sastry SV, Nyshdham JR, Fix JA. Recent technological advances in oral drug delivery: *A review. Pharmaceutical Science and Technology Today*. 2000;3(4):138–145.
3. Ravi, Geeta Rajput, Amit Kumar. A Review Article on Orodispersible tablet formulation. *The Pharma Innovation journal*. 2013;2(2): 144-153.
4. Kuchekar B S, Bhise S B, Arumugam V. Design of fast disintegrating tablets. *Indian Journal of pharmaceutical Education and Research.* 2001; 35(4): 150-152.
5. Debjit Bhowmik, Chiranjib.B, Krishnakanth, Pankaj, R.Margret Chandira. Fast Dissolving Tablet: An Overview. *Journal of Chemical and Pharmaceutical Research.* 2009; 1(1): 163-177.
6. Amir H.shojaei. Buccal Mucosa As A Route For Systemic Drug Delivery: A Review. *Journal of Pharmacy and Pharmaceutical Sciences.* 1998; 1 (1):15-30.
7. Shivangi singh, Navneet verma. Taste masked orodispersible tablets: A highly patient complaint dosage form. *Asian Journal Pharmaceutical and Clinical Research.* 2016;9(3): 385-391.
8. Tejaswi Santosh Ubhe, Preeti Gedam. A Brief Overview on Tablet and It's Types. *Journal of Advancement in Pharmacology* 2020;1(1): 21-31.
9. Sreenivas SA. Orodispersible tablets: New- fangled drug delivery system- A Review. *Indian Journal Pharmaceutical Educational Research*. 2005;39(4):177-181.
10. Jaysukh J Hirani, Dhaval A Rathod, Kantilal R Vadalia. Orally Disintegrating Tablets: A Review. *Tropical Journal of Pharmaceutical Research,* April 2009; 8 (2): 161-172.
11. Gupta G.D et al, A Mouth dissolving Tablet: A Review. *International journal of Current Pharmaceutical Research* 2011; ISSN-0975- 7066.
12. Alok Kumar Gupta, Anuj Mittal and Prof. K. K. Jha. Fast Dissolving Tablet- A Review. *The Pharma innovation* 2012;1(1): 1-8.

13. Reddy. L.H, Gosh B, Rajneesh. Fast Dissolving Drug Delivery Systems: A Review of the literature. *Indian Journal of Pharmaceutical sciences*; 2002: 3(8): 331-336.
14. VedParkash, SaurabhMaan, Deepika, Shiv Kumar Yadav, Hemlata, and Vikas Jogpa. Fast disintegrating tablets: Opportunity in drug delivery system. *Journal of Advanced Pharmaceutical Technology and Research*. 2011 Oct-Dec; 2(4): 223–235.
15. Pavithra Putta, Saikrishna Mundra & Bhavani Boddeda. Patented Technologies in Fast Dissolving Tablets: A Review. *American Journal of Pharm Tech Research*. 2019; 9(5):244-263.
16. Pranjal Jain, Shriya Jain, Ashwani Mishra and Anupam Pathak. A Review on Orodispersible Tablet. *Current Research in Pharmaceutical Sciences*. 2014; 04 (04): 99-109.
17. Bandari S, Mittapalli RK, Gannu R, Rao YM. Orodispersible tablets: an overview. *Asian Journal of Pharmaceutics.* 2008; 2(1): 2-11.
18. Desale KY, Vidhyadhar, Bankar H, Giakwad PD, Pawar SP. Review on Fast Dissolving/ Disintegrating Tablets. *International Journal of Pharmaceutical Science Review & Research.* 2011; 11:152-158.
19. R Santosh Kumar, Kumari Annu. Superdisintegrant: crucial elements for mouth dissolving tablets. *Journal of Drug Delivery & Therapeutics*. 2019; 9(2):461-468.
20. More SA et al. Orodispersible Tablet- A Novel Drug Delivery System. *Journal of Pharmaceutical Science and Technology*. 2012; 4 (1): 798 -806.
21. Janet Roche Johnson, LI-Hua Wang, Marc S.Gordona, Zak T. Chowhan. Effect of Formulation Solubility and Hygroscopicity on Disintegrant Efficiency in Tablets Prepared by Wet Granulation, in Terms of Dissolution. *Journal of Pharmaceutical Sciences*. 1991; 80(5):469-471.
22. Digpati Roy, Debjit Bhowmik, K.P.Sampath Kumar. A comprehensive review on superdisintegrants used in orodispersible tablets. *Indian Journal of Research in Pharmacy and Biotechnology.* 2014; 2(4): 1297-1302.
23. Lavika Gandhi, MD. Semimul Akhtar. Comparative study on effect of natural and synthetic superdisintegrants in the formulation of orodispersible tablets. *Journal of Drug Delivery & Therapeutics*. 2019; 9(2):507-513.
24. Abha, Loveleen Preet Kaur. Superdisintegrants: An Arising Exemplar in Orodispersible tablets. *International Journal of Drug Research and Technology.* 2015;5 (1): 01-12.
25. Mohammad Ali Shahtalebi, Majid Tabbakhian, Sina Koosha. Formulation and evaluation of orally disintegrating tablet of ondansetron using natural superdisintegrant. *Journal of HerbMed Pharmacology*. 2015; 4(3): 102-109.
26. Nitin K Kapse, Vilas P Bharti, Arunadevi S Birajdar, Anirudha V Munde, Pranita P Panchal. Co-Processed Superdisintegrants: Novel Technique for Design Orodispersible Tablets. *Journal of Innovations in Pharmaceuticals and Biological Sciences*. 2015;2(4):541-555.
27. Pitliya Prateek, Saini Pramod, Garg Shiv K, Pareek Ajay K. Co-processed superdisintegrant a novel approach for reducing risk in dosage form formulation. *Journal of Medical Pharmaceutical and Allied Sciences*. 2014; 3:45-54.
28. Piplani Mona, Diwedi Rohini, Bhagwat Deepak Prabhakar, Pahwa Rakesh. Studies on Orally Disintegrating Tablets Prepared by Using Natural and Synthetic Superdisintegrants. *International Journal of Drug Delivery Technology*. 2018; 8(2); 60-66.
29. Sharma Shailesh, Gupta Ghanshyam Das. Preparation and evaluation of co-processed superdisintegrant in the design of prochlorperazine maleate fast dissolving tablets, Journal of Drug Delivery & Therapeutics; 2011, 1(2): 1 9.
30. Kumar R, Patil S, Patil M. B, Patil S. R, Paschapur MS. Isolation and evaluation of disintegrant properties of Fenugreek seed mucilage. *International Journal of Pharm Tech Research.* 2009; 1(4): 982-996.
31. Bhatti S, Kaushik M. Utilization of natural superdisintegrant in mouth dissolving tablet: A simplified review. *Innovations in Pharmaceuticals and Pharmacotherapy*. 2020;8(2):32-38.
32. Yonni Eshovo Apeji, Fidelis Dzarma Zechariah, Sophie Nock Anyebe, Babajide Tytler, Adeniji Kehinde Olowosulu, Avosuahi Rukayat Oyi. Effect of mode of superdisintegrant incorporation on tableting properties of metronidazole granules. *Pharmaceutical Sciences Asia* 2019; 46 (1), 25-32.
33. Siyad A R. Hypertension. *Hygeia Journal for drug and medicines.* 2011;3(1):1-16.
34. http://www.pascar.org.
35. http://www.drugbank.com

Table 1. Composition of Propranolol Hydrochloride ODTs with synthetic superdisintegrants

Ingredients (mg)	F1	F2	F3	F4	F5	F6	F7	F8	F9
Propranolol Hydrochloride	40	40	40	40	40	40	40	40	40
Crospovidone	4	8	12	-	-	-	-	-	-
Croscarmellose sodium	-	-	-	4	8	12	-	-	-
Sodium starch glycolate	-	-	-	-	-	-	4	8	12
Microcrystalline cellulose	40	40	40	40	40	40	40	40	40
Mannitol	111	107	103	111	107	103	111	107	103
Saccharin sodium	2	2	2	2	2	2	2	2	2
Magnesium stearate	2	2	2	2	2	2	2	2	2
Talc	1	1	1	1	1	1	1	1	1
Total weight (mg)	200	200	200	200	200	200	200	200	200

Table 2. Composition of Propranolol Hydrochloride ODTs with natural superdisintegrants

Ingredients(mg)	F10	F11	F12	F13	F14	F15
Propranolol Hydrochloride	40	40	40	40	40	40
Guar gum	4	8	12	-	-	-
Agar	-	-	-	4	8	12
Microcrystalline cellulose	40	40	40	40	40	40
Mannitol	111	107	103	111	107	103
Saccharin sodium	2	2	2	2	2	2
Magnesium stearate	2	2	2	2	2	2
Talc	1	1	1	1	1	1
Total weight (mg)	200	200	200	200	200	200

Table 3. Mixture code and ratio of selected co-processed blend of Superdisintegrants

Co-processed blends of superdisintegrants	CpS1	CpS2	CpS3	CpS4	CpS5	CpS6	CpS7	CpS8
Crospovidone + Sodium starch glycolate	1:1	1:2	-	-	-	-	-	-
Crospovidone + Guar gum	-	-	1:1	1:2	-	-	-	-
Sodium starch glycolate + croscarmellose sodium	-	-	-	-	1:1	1:2	-	-
Guar gum + Agar	-	-	-	-	-	-	1:1	1:2

Table 4. Composition of Propranolol Hydrochloride ODTs with co-processed superdisintegrants

Ingredients (mg)	F16	F17	F18	F19	F20	F21	F22	F23
Propranolol Hydrochloride	40	40	40	40	40	40	40	40
CpS1	12	-	-	-	-	-	-	-
CpS2	-	12	-	-	-	-	-	-
CpS3	-	-	12	-	-	-	-	-
CpS4	-	-	-	12	-	-	-	-
CpS5	-	-	-	-	12	-	-	-
CpS6	-	-	-	-	-	12	-	-
CpS7	-	-	-	-	-	-	12	-
CpS8	-	-	-	-	-	-	-	12
Microcrystalline cellulose	40	40	40	40	40	40	40	40
Mannitol	103	103	103	103	103	103	103	103

Saccharin sodium	2	2	2	2	2	2	2	2
Magnesium stearate	2	2	2	2	2	2	2	2
Talc	1	1	1	1	1	1	1	1
Total weight (mg)	200	200	200	200	200	200	200	200

Table 5. *In-vitro* drug release profile of optimized formulation and marketed formulation

Time(min)	% Cumulative drug release	
	F18	Marketed product (Inderal 40)
5	45.16	32.66
10	67.23	36.04
15	81.20	40.54
20	86.72	50.68
25	94.60	56.31
30	98.65	66.44
40	-	76.58
50	-	85.59
60	-	95.73

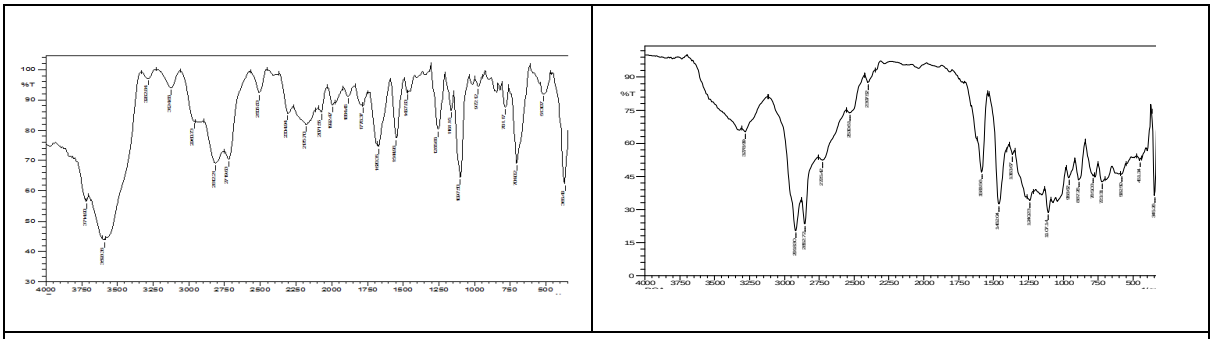
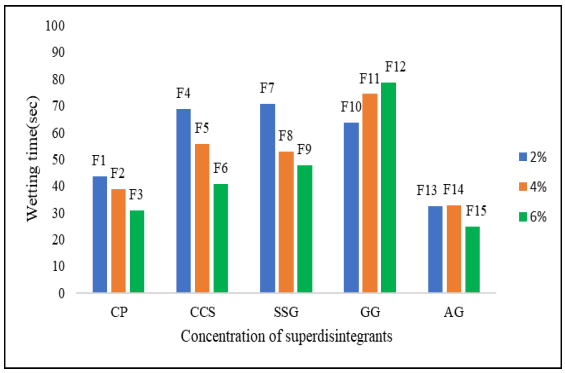


Figure 1. IR spectrum of Propranolol Hydrochloride + blend of CP+ SSG+ CCS+ Guar gum and agar + Mannitol + MCC+ Saccharin sodium+ Magnesium stearate+ Talc

Figure 2. Prepared Propranolol Hydrochloride ODTs



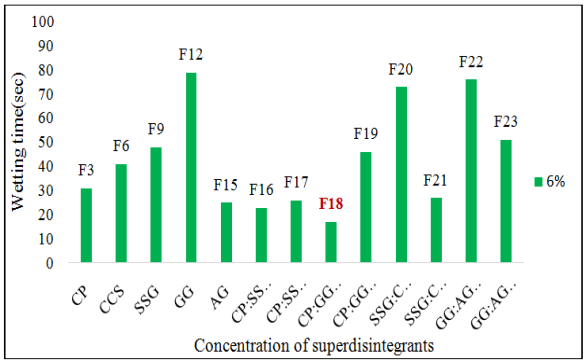
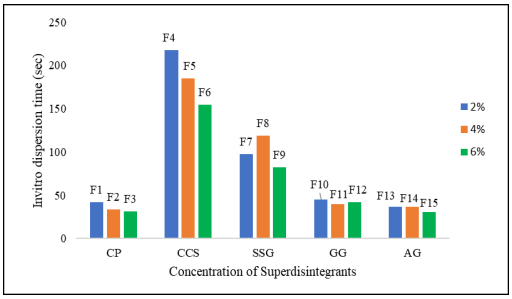


Figure 3: Wetting time of Propranolol HCl ODT with different superdisintegrants with different concentrations of synthetic and natural superdisintegrants (2%, 4%, 6%) with concentration of 6%



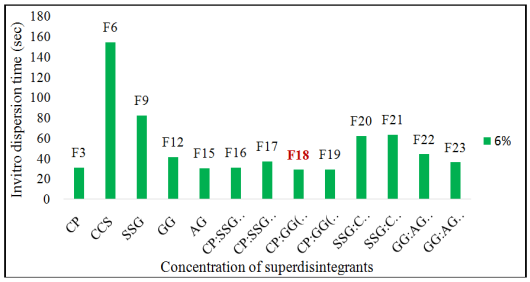


Figure 4: *In-vitro* dispersion time of Propranolol HCl ODT with different superdisintegrants with different concentrations of synthetic and natural superdisintegrants (2%, 4%, 6%) with concentration of 6%

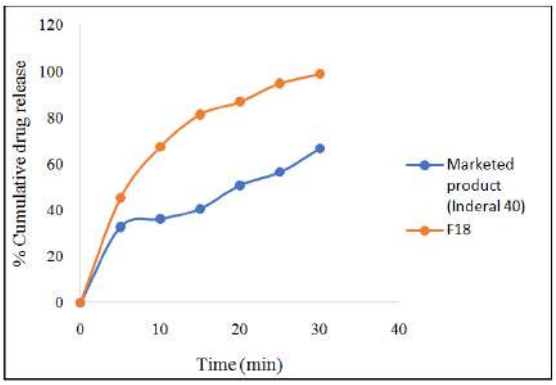


Figure 5: Dissolution profile of formulation F18 compared with marketed formulation (Inderal 40)

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RESEARCH ARTICLE

Evaluation of Different PGPR Isolates against Mulberry Root Rot Disease

N.Meena[1]* and B.Karthikeyan[2]

[1]Ph.D., Research Scholar, Department of Agricultural Microbiology, Annamalai University, Chidambaram, Tamil Nadu, India
[2]Associate Professor, Department of Agricultural Microbiology, Annamalai University, Chidambaram, Tamil Nadu, India.

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***Address for Correspondence**
N.Meena
Ph.D., Research Scholar,
Department of Agricultural Microbiology,
Annamalai University, Chidambaram,
Tamil Nadu, India
Email: meenaplantdoctor@gmail.com

ABSTRACT

Mulberry (*Morus alba* L.) is a perennial, evergreen, fast growing an invaluable tree of immense importance in the silk industry due to its foliage, which constitutes the chief food for the silkworms (*Bombyx mori* L.), the source off fabulous silk. Outbreak of mulberry root rot disease is more frequent, because of intense cultivation, loss of plant vigor and increased susceptibility to soil-borne pathogens thereby limiting leaves yield and silk production. Root rot disease of mulberry was recorded plant mortality of 30%, leaf loss 37.82 %. In the present study, the rhizosphere soil of mulberry was collected and isolated nearly ten PGPR isolates and their morphological and biochemical characteristic was studied. All the ten isolates of PGPR were tested for antagonistic property against soil borne pathogens such as *Macrophomina phaseolina, Fusarium oxysporum* and *Fusarium solani* evaluation by dual culture technique under *in vitro* condition. Mycelia growth was recorded maximum in all the treatment over control. The results of different isolates were statistically compared by Duncan's multiple range tests. The percentage mycelia growth inhibition over control was recorded in the order of T2 >T10 >T8 > T3 against the fungal pathogens tested. Among the ten PGPR isolates were tested against root rot pathogens *viz,*. The three fungal pathogens, the maximum PI%, IZ (mm) were recorded in isolates (*Pseudomonas fluorescence* - (T2) (53.33 %, 25 mm), (40%, 28mm), (32.43 %, 25mm) *Bacillus velezensis*- (T10) (51.11 %,22 mm), (28.71%, 20 mm), (65.9 %, 25mm), *Azotobacter* sp.- (T8) (44.44 %, 20 mm), (24%, 22 mm),(17.14%, 22 mm), *and Azospirillum* sp.- (T3) (42.22 %, 23 mm), (21.73 %, 22 mm) (13.15%, 23 mm). Therefore, the selected isolates were found to inhibit all the three soil borne fungal growth under *in vitro* conditions and it was used for further studies.

Keywords: PGPR, isolates, mulberry, root rot disease, antifungal activity.

INTRODUCTION

Mulberry contributed to the bulk of silk production in India with almost 24 thousand metric tonnes produced in the financial year 2021. Mulberry plants, specifically the leaves are a primary source of food for the silkworm *Bombyx mori***.** Mulberry is a perennial, evergreen, fast growing tree belonging to *Moraceae* family (Ramesh *et al.,* 2014). Root rot is the most dangerous disease due to its epidemic nature and potentiality to kill the plants completely and poses a serious problem during mulberry cultivation in almost all the sericultural cultivation countries. Various types of the mulberry root rot diseases have been reported from all over the world. They are dry root rot (*Fusarium sp*), black root rot (*botrydiploda theobromae),* charcol root rot (*Macrophomina phaseolina*) which lead to leaf yield loss of 37.82%, violet root rot (*Helicobasidium mompa*), white root rot, *Armilaria* root rot and bacterial root rot. Among them, the dry (*Fusarium*), black and charcol root rot are reported in india (Philip *et al.,* 1995; Sukumar and Padma, 1999). The disease was reported from almost all types of soil, under varied agro climatic conditions throughout the year. It initially appears in an isolated patch in few plants, Root rot disease of mulberry was recorded plant mortality of 30%, leaf yield loss of 31.5 % and reduction in cocoon production by 756 kg/ha garden (Chowdary and Govindaiah 2009, Mukunda *et al.,* 2021). Many bacteria are known to be able to stimulate plant growth through direct or indirect interactions with plant roots and these have been classified as Plant Growth Promoting Rhizobacteria. (PGPR) are root associated bacteria that colonize the rhizosphere and improve plant growth when introduced onto seeds, seed pieces, roots, or into soil. Indeed, the bacteria lodging around/in the plant roots (rhizobacteria) are more versatile in transforming, mobilizing, solubilizing the nutrients compared to those from bulk soils (Hayat *et al*., 2010). Therefore, the rhizobacteria are the dominant deriving forces in recycling the soil nutrients and consequently, they are crucial for soil fertility (Glick, 2012; Ahemad and Kibret, 2013).

PGPR improve the plant growth by one or more mechanisms: direct stimulation of plant growth; enhancement of nutrient uptake; suppression of plant pathogens; and/or induction of resistance in plant hosts against pathogens. PGPR are found in a very wide range of genera and some examples include: *Actinetobacter, Agrobacterium, Arthrobacter, Azospirillum, Bacillus, Bradyrhizobium, Burkholderia, Cellulomonas, Frankia, Pantoea, Pseudomonas, Rhizobium, Serratia, Streptomyces* and *Thiobacillus*. The use of microorganisms with the aim of improving nutrients availability for plants is an important practice and necessary for agriculture (Freitas *et al*., 2007). Significant increases in growth and yield of agronomically important crops in response to inoculation with PGPR have been repeatedly reported by many researches (Figueiredo *et al*., 2008; Arau jo 2008; Manoharm melvin *et al*.,(2009); karthikeyan *et al.,* (2010); Jay Shankar Singh, 2013; Loganathan *et al.,* 2014; Turan *et al.,* 2014); Meena and karthikeyan 2019; Karthikeyan and kasinathan (2020); Panjanthan prakash *et al*.,(2021). In the present study, an attempt was made to isolate, characterizes and as certain the antagonistic role of different PGPR strains from mulberry rhizosphere soil and tested against root rot pathogens.

MATERIAL AND METHODS

Collection and Isolation of Root Rot pathogens in Mulberry Plant:

Survey was conducted at different locations of krishnagiri district in Tamil Nadu were mulberry were grown. Nearly 15 Root rot diseases affected rhizosphere soil sample and decayed black bark samples bearing micro sclerotia of the fungus and characteristic symptoms of root rot were carefully collected from the farmer's fields. The diseased specimens were packed in paper bags and properly labeled, brought to the laboratory and soil samples were stored in refrigerator at 4ºC for further studies. Decayed roots were first washed under the tap water and then cut in to small pieces along with healthy portion. These root pieces were surface sterilized by dipping in 0.1 % sodium hyphocholride solution for 1:1 ½ minutes after three conservative washing with sterilized distilled water the pieces were transfer to autoclaved PDA medium in petriplates incubated at 25 ± 1^0 c and plates are placed in to BOD

incubator 7 days. The fungal colonies of *Macrophomina phaseolina, Fusarium oxysporum* and *Fusarium solani* enumerating from bits were examined 7 days incubation. The isolates further purified by mono - hyphal tip method, identified and maintained on PDA medium, (Raja gopal reddy *et al*., 2009).

Isolation and screening of PGPR

10 rhizosphere soil samples were collected from 10 different mulberry fields of krishnagiri area of Tamil nadu (Table 1). PGPR strains were isolated by the serial dilution method. The soil samples were serially diluted up to the dilution of 10^{-6} using Nutrient Agar and incubated at 30°C for 2 days. The further the colonies awere purified by streak plate method and colonies were identified by biochemical methods (Table 2). The PGPR isolates are *Bacillus subtilis, Pseudomonas fluorescens, Azospirillum* sp., *Pentoea dispera, Pseudomonas putida, Streptomyces rimoseus, Streptomyces monomycini*, *Azotobacter* sp, *Xandhomonas* sp, *Bacillus velezensis.* Further the PGPR isolates were analyzed for morphological and biochemical characteristics for identification, the cultures were preserved by using standard preservation methods.

Screening and *In vitro* efficacy of PGPR against root rot pathogens

Ten PGPR isolates were tested against the pathogens for their antagonistic activity by dual culture technique. The ten PGPR isolates were streaked 5 days prior to pathogens inoculation in 90 mm (O) Petri dishes and incubated at 32°C (Kunova *et al.,* 2016). Separate control plates for each pathogen without the test isolate and 3 replications were maintained. The inhibition percentage of mycelia growth (PI) was calculated using the formula (Shrivastava *et al.,* 2017), characterize and as certain the antagonistic role of PGPR strains from mulberry rhizosphere against root rot pathogens.

$$PI = \frac{\text{Radial growth in control (C)} - \text{Radial growth in treatment (T)}}{\text{Radial growth in control}} \times 100$$

Radial growth of the pathogens (cm), Radial growth in treatment (in cm) was measured and multiple comparisons were subjected of ANOVA.

***In- vitro* testing of different PGPR isolates against various mulberry root rot pathogen:**

The ten PGPR isolates were screened individually against radial mycelial growth of *Macrophomina phaseolina, Fusarium oxysporum* and *Fusarium solani* by dual culture technique (Dennis and Webster 1971). The sterilized cork borer used to disc 6 (mm) were spotted with 20 µl of bacterial suspension of are *Bacillus subtilis, Pseudomonas fluorescens, Azospirillum* sp., *Pentoea dispera, Pseudomonas putida, Streptomyces rimoseus, Streptomyces monomycini*, *Azotobacter* sp, *Xanthomonas* sp, *Bacillus velezensis.* individually and placed on one side of the Petri plate (1 cm from the edge of the plate) containing PDA medium. The bacterial suspension of 20µl added to the well one side of the Petri plate (1 cm from the edge of the plate) containing PDA medium. The mycelial disc (6 mm) of a seven-day-old culture of *Macrophomina phaseolina*, *Fusarium oxysporum* and *Fusarium solani* was placed on the opposite side of the spot inoculation. The plates were incubated at room temperature (28± 2°C) for seven days and the inhibition zone was measured shown.

RESULT

Isolation and identification of mulberry root rot pathogens

The three pathogens viz., *Macrophomina phaseolina*, *Fusarium oxysporum* and *Fusarium solani*, were isolated from diseased plants collected from different locations of krishnagiri district in Tamil nadu (Table 1). The isolated pathogens were identified according to morphological characteristics and confirmed using partial sequencing of ITS region.

Isolation of different PGPR isolates from mulberry rhizosphere soil sample

Ten PGPR isolates were obtained from mulberry rhizosphere soil were screened for various biochemical tests (Table 2) viz., Gram reaction, Motility, Cell shape, Pigmentation Production, Catalase, Oxidase, Urease, Citrate Utilization, Casein Hydrolysis, Indole Test, Starch test. The T2 isolate showed negative for gram reaction, Urease, Indole Test, Starch test and positive for catalase, oxidase, citrate utilization, casein hydrolysis and fluorescent pigment production test which helped to identify as *Pseudomonas fluorescens.* The T3 isolate shows cell shape as spiral rod and shows positive reaction for motility, gram reaction, catalase, oxidase, citrate utilization ,casein hydrolysis, urease, indole, starch and produced sub surface pellicles in nitrogen free bromothymol blue medium, which helped to identify as *Azospirillum* sp. The T8 isolate shows negative for gram reaction, Catalase, Urease, Casein Hydrolysis, Starch test and positive for pigment production, oxidase, citrate utilization and indole production test and were characterized as *Azotobacter* sp. The T10 isolates showed positive for gram reaction, motility, catalase, oxidase, urease and starch test which helped to characterize these isolates as *Bacillus velezensis.*

Efficiency of different PGPR isolates against mulberry root rot pathogen by dual culture techniques:

The ten PGPR isolates were tested against three fungal pathogens for antagonistic activity such as *Macrophomina phaseolina, Fusarium oxysporum, Fusarium solani* the PI %, RG (mm) and IZ (mm) were recorded (Table 3). Among the ten PGPR isolates were tested against three fungal pathogens, the maximum PI%, RG (mm) and IZ (mm) were recorded in the isolate *Pseudomonas fluorescens* (T2) (53.33 %, 45mm, 25 mm), (40%, 45 mm, 28mm), (32.43 %, 45mm, 25mm) followed by by *Bacillus velezensis* (T10) (51.11 %,35 mm, 22 mm), (28.71%, 48mm, 20mm), (65.9 %, 27mm, 25mm), *Azotobacter* sp. (T8) (44.44 %, 35 mm, 20 mm),(24%, 32mm, 22mm),(17.14%,32 mm,22 mm), *Azospirillum* sp. (T3) (42.22 %, 45 mm, 23 mm), (17.85%, 44mm, 22mm) (13.15%, 43mm, 23 mm) showed broad antifungal activity against in all the three fungal pathogens. However T2, T10, T8, T3 isolates exhibited significant over control. Whereas the treatment T1, T4, T5, T6, T7, T9 were recorded the minimum antifungal activity against all the three plant pathogens (Fig 1, 2, 3).

DISCUSSION

Symptoms of root rot affected mulberry plants were similar as observed by previous workers (Sharma *et al*., 2003; Saratha *et al.,* 2022) recorded progressive wilting, premature drying, loss of vigor and yield reduction as the characteristic root rot symptoms in mulberry plants soil born disease pose a serious problem in mulberry cultivation during nursery plantation and established garden, which cause severe loss in revenue generation of mulberry growers as compared to other foliar diseases. Among them, root knot and root rot affect the established plantation resulting in severe loss in leaf yield apart from deterioration in leaf quality, which is a prerequisite in successful sericulture to get the good quality of cocoons. These are dry root rot (*Fusarium*), black root rot (*Botrydiplodia theobromae*), charcoal root rot(*Macrophomia phaseolina*), violet root rot (*Helicobasidium mompa*), reported in india (Philip *et al .,* 1995; sukumar and padma, 1999). We isolated ten PGPR isolates from mulberry rhizosphere such as *Bacillus subtilis, Pseudomonas fluorescens, Azospirillum* sp., *Pentoea dispera, Pseudomonas putida, Streptomyces rimoseus, Streptomyces monomycini*, *Azotobacter* sp, *Xanthomonas* sp, *Bacillus velezensis.* Which has the capacity of plant growth promotion activity. Our results showed that PGPR isolates of *Psudomonas fluorescens* , *Bacillus velezensis* , *Azospirillum* sp and *Azotobacter* sp. could significantly decreased the root infection of mulberry plant. Plant Growth Promoting Rhizobacteria (PGPR) has the ability to colonize and suppress the soil-borne pathogens at the plant roots (Rangajaran *et al*., 2003). PGPR Mulberry rhizosphere soil was focused in this study to isolate novel and endemic antagonist as well. Antimicrobial properties of root exduates were also increasing the chances of rare and potent actinobacterial isolation (Ashokvardhan *et al.,* 2014). The application of microorganisms to control diseases, which is a form of biological control, is an environment-friendly approach (Arshad *et al*., 2007). The major indirect mechanism of plant growth promotion in rhizobacteria is through acting as biocontrol agents (Glick 2012) of plant growth-promoting traits, the ability to utilize cellulose along with N2 fixation activity and IAA production may play an important role in promoting rice growth. All the ten PGPR isolates are suppresses the three fungal isolates growth under tesded *in vitro* conditions. The antagonistic activity of PGPR isolates, against soil borne pathogens done by dual culture method under in vitro conditions was showed in Fig: 1,2 & 3. Among the tested PGPR isolates, *Pseudomonas*

fluorescens showed an maximum inhibitory effect (PI % over control). The percentage of mycelia growth inhibition compared to control was recorded in *Macrophomina phaseolina, Fusarium oxysporum* and *Fusarium solani* and was 53.33, 40 , 32.43 respectively followed by *Bacillus velezensis* (51.11, 28.71, 65.90), *Azotobacter* sp (44.44, 24,17.14), *Azospirillum* sp. (42.22, 17.85, 13.15) No growth was observed in control (Table -3). Rangeshwaram and Prasad (2000) also observed that *Pseudomonas putida* (PDB CAB 19) and *Pseudomoas fluorescens* (PDB AB2) were found to be most effective against *Fusarium oxysporum.* Srinivasan (2003) rewarded that the *Pseudomoas fluorescens* inhibited mycelia growth of *Fusarium* sp. (52%) and produced notable inhibition zone of 11.2 and 4.8 mm, respectively in petriplate, the smilar kind of activity is observed in the present experiment. Hilda sundar *et al,.*(2021) also reported the *Pseudomonas fluorescens* have antagonistic effect against *Macrophomina phaseolina, Fusarium* sp., *Rhizoctonia solani and Alternaria alternata.*

CONCLUSION AND FUTURE RECOMMENDATIONS

In this study, We isolated ten PGPR (Plant Growth-Promoting Rhizosphere) isolates from mulberry rhizosphere. The PGPR isolates have highly antagonistic activity shown inherent potential as biocontrol agents against mulberry root rot pathogens. The better disease control in the PGPR isolates may be due to the different mechanism of action isolates could significantly decreased the root infection of mulberry plant.

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REFERENCES

1. Ahemad, M. and Kibret, M. 2013. Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. Journal of King Saud University–Science. http://dx.doi.org/10.1016/j.jksus. 2013.05. 001.
2. Ashokvardhan T, Rajithasri AB, Prathyusha P, Satyaprasad K (2014) Actinomycetes from *Capsicum annuum* L. rhizosphere soil have the biocontrol potential against pathogenic fungi. Int J Curr Microbiol Appl Sci 3(4):894–903.
3. B.Karthikeyan, M. M. Joe, C A Jaleel, M. Deiveekasundram 2010. Effect of root inoculation with plant growth promoting rhizobacteria (PGPR) on plant growth alkaloid content and nutrient content control of *Catharanthus roseus* (L) , Natura cortica 19(1); 205-212.
4. B.R. Glick, 2012. Plant Growth-Promoting Bacteria: Mechanisms and Applications, Hindawi Publishing Corporation, *Scientifica,*.
5. Chowdary NB, Govindaiah (2009) Leaf yield loss assessment due to *Macrophomina* root rot disease in mulberry gardens of south India. Arch Phyto. Patho. Pf lanzenschutz 42(11):1055–1058. https:// doi. org/ 10. 1080/ 03235 40070 16218 26.
6. Figueroa-López, A. M., Cordero-Ramírez, J. D., MartínezÁlvarez, J. C., López-Meyer, M., Lizárraga-Sánchez, G. J., Félix-Gastélum, R., & Maldonado-Mendoza, I. E. (2016). Rhizospheric bacteria of maize with potential for biocontrol of Fusarium verticillioides. Springer Plus, 5, 330.
7. Freitas ADS, Vieira CL, Santos CERS, Stamford NP and Lyra MCCP. 2007.Caracterizac¸a˜o derizo´bios isolados de Jacatupe´cultivado em solosalino no Estado de Pernanbuco, Brasil. Bragantia, 66: 497-504.
8. Glick, B.R. 2012. Plant Growth-Promoting Bacteria: Mechanisms and Applications. Hindawi Publishing Corporation, Scientific.
9. Hayat, 2010 . Soil Benefical Bacteria and their role in plant Growth Promotion ;**Ann Microbiol** :60(4).

10. Hilda Sundar, Sweatha S. Mohan, Ayyappan Sornam, Geetha Sivanandam and Chandrasehar Govindan, 2021. Effects of three strains of *Pseudomonas fluorescens* to soil-borne fungal pathogens and silkworm, *Bombyx mori.* Inter. J. of Trop. Insect Sci. https://doi.org/10.1007/s42690-021-00506-7.
11. Jay Shankar Singh. 2013. Plant Growth Promoting Rhizobacteria: Potential Microbes for Sustainable Agriculture, RESONANCE. 275-281.
12. Karthikeyan .B and Kasinathan (2020). Isolation and screeing of AM fungi and PGPR of *Coleus forskholii* grown area proceedings of National conference on Horticulture invange of climate change, Department of Horticulture, Annamalai university.(1). 368 -371.
13. Kunova A, Bonaldi M, Saracchi M, Pizzatti C, Chen X, Cortesi P (2016) Selection of *Streptomyces* against soil borne fungal pathogens by a standardized dual culture assay and evaluation of their effects on seed germination and plant growth. BMC Microbiol 16(1):272.
14. Loganathan, M., R. Garg, V. Venkataravanappa, S. Saha and A.B. Rai. 2014.Plant growth promoting rhizobacteria (PGPR) induces resistance against *Fusarium* wilt and improves lycopene content and texture in tomato. African J. Microbiol. Res., 8(11): 1105-1111.
15. Arshad. M, Saleem. M, and Hussain. S, 2007. Perspectives of bacterial ACC deaminase in phytoremediation, *Trends Biotechnol*, 25, , 356–362.
16. Saratha M, Angappan .K, Karthikeyan. S, Marimuthu. S and Chozhan. K, 2022. *Actinoalloteichus cyanogriseus*: a broad spectrum bio-agent against mulberry root rot pathogens. *Egyptian J. Bio. Pest Contrl. 32:33* . https://doi.org/10.1186/s41938-022-00532-8.
17. Manoharam Melvin Joe, cheruth Abdul Jaleel Palanivel Karpagavinayaka sivakumar, Charg – zing zhao and Balathandayutham karthikeyan 2009. CO- aggregation in Azsopirillum brasiderse MTC 125 with other PGPR strains: Effect of physical and chemical factors and stress enumerate ability.
18. Meena. N and Karthikeyan. B (2019). Studies on the effects of PGPR on growth and yield of tomato PKM -1 variety. J.Pharm and phyto. Chem..8(4) 3544 -3549.
19. Mukunda K, Teligi V, Puttegowda SH, Sampangiramaiah KD (2021) Diseasenincidence, severity and phenotypic variation among the isolates of *Rhizoctonia bataticola* infected in root rot disease of mulberry in different mulberry fields of Karnataka. Biosci Biotech Res Asia 18(2):403–411.
20. Panjanthan Prakash. Balathandayudham Karthikeyan and Manoharam Melvin 2021. Chemotatic responses of sweet flag *(Acorus calamus* L.) ROOT EXUDATES and evaluation pf inoculation effects on its growth. J .Agrl Sci XXXII 117.- 126.
21. Philip, T., J. Latha, Govindhaiah, B.Mallikarajuna, K. C. Mondal and A. K. Bajpai (1995) some observations on the incidence, associated microfloa and control of root rot disease in mulberry in south India. *Indian. J. seric.*34, 137- 139.
22. Ramesh H, Sivaram V, Murthy VY (2014) Antioxidant and medicinal properties of mulberry (*Morus* sp.): a review. World J Pharm Res 3(6):320–343.p
23. Rangajaran S, Saleena LM, Vasudevan P, Nair S (2003) Biological suppression of rice diseases by *Pseudomonas* spp. under saline soil conditions. Plant and Soil 151(1):73-82.
24. Rangeshwaran R, Prasad RD (2000) Isolation and screening of rhizobacteria for control of chickpea diseases. J Biol Control 14:9–15.
25. Sharma M, Dangi P, Choudhary M (2014) Actinomycetes: source, identification, and their applications. Int J Curr Microbiol Appl Sci 3(2):801–832.
26. Shrivastava P, Kumar R, Yandigeri MS (2017) In vitro biocontrol activity of halotolerant *Streptomyces aureofaciens* K20: a potent antagonist against *Macrophomina phaseolina* (Tassi) Goid. Saudi J Biol Sci 24(1):192–199. https:// doi. org/ 10. 1016/j. sjbs. 2015. 12. 004
27. Srinivasan A, Wolfenden LL, Song X, Mackie K, Hartsell TL, Jones HD, Diette GB, Orens JB, Yung RC, Ross TL, Merz W, Scheel PJ, Haponik EF, Perl TM (2003) An outbreak of *Pseudomonas aeruginosa* infections associated with flexible bronchoscopes. N Engl J Med 348(3):221–227.
28. Sukumar , J. and S.D. Padma (1999) diseases of mulberry in india research progress and priorities; in advances in mulberry sericulture. Devaiah, M. C., K.C. Narayanaswamy and V. G. Maribashetty (eds.), pp. 152 -186, C. V. G. Publications, Bangalore, India.

Table 1. Mulberry rhizosphere soils collected from different locations of Krishnagiri district of Tamil Nadu.

S.No	Name of the location	Soil type	Mulberry variety
1.	Pollupalli	Red soil	V1
2.	Sundagiri	Red soil	V1
3.	Gangasamuthiram	Red soil	V1
4.	Melumalai	Red soil	V1
5.	Bhandharapalli	Red soil	V1
6.	Maniyandapalli	Red soil	V1
7.	Soolagiri	Red soil	V1
8.	Gumanoor	Red soil	V1
9.	Rayakottai	Red soil	V1
10.	Manavarn palli	Red soil	V1

Table 2: Morphological and biochemical characteristics of different PGPR isolates from mulberry rhizosphere soil.

S.No	PGPR isolates	Gram reaction	Motility	Cell shape	Pigmentation Production	Catalase	Oxidase	Urease	Citrate Utilization	Casein Hydrolysis	Indole Test	Starch
1	*Bacillus subtilis*	+ ve	+	Rod	-	+	+	+	+	-	+	+
2	*Pseudomonas fluorescens*	- ve	+	Rod	+	+	+	-	+	+	-	-
3	*Azospirillum* sp.	+ve	+	Rod	+	+	+	+	+	+	+	+
4	*Pentoa dispersa*	-ve	+	Rod	-	+	-	-	-	-	-	-
5	*Pseudomonas putida*	- ve	+	Rod	+	+	+	-	+	+	-	+
6	*Streptomyces rimosus*	+ ve	+	Rod	+	+	-	-	-	+	-	+
7	*Streptomyces griseus*	+ve	+	Rod	+	+	-	-	-	+	-	+
8	*Azotobacter* sp.	-ve	+	Rod	-	-	+	-	+	-	+	-
9	*Xanthomans* sp.	-ve	+	Round	+	-	+	-	+	-	+	-
10	*Bacillus velezensis*	+ve	+	Rod	+	+	+	+	-	-	-	+

(+) Showed positive growth; (-) Showed No growth.

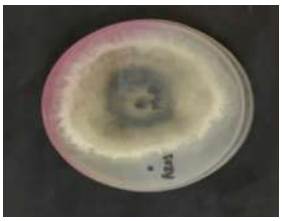
Table 3: *In - vitro* screening and efficiency of different PGPR isolates against Mulberry root rot pathogens :

S.NO	PGPR	*Macrophomina phaseolina*			*Fusarium oxysporum*			*Fusarium solani*		
		IZ (mm)	RG (mm)	PI %	IZ (mm)	RG (mm)	PI %	IZ (mm)	RG (mm)	PI %
T1	*Bacillus subtilis*	18	30	0^{e}	28	2.1	8.69^{i}	24	24	7.89^{f}
T2	*Pseudomonas fluorescens*	25	45	53.33^{a}	28	45	40^{a}	25	45	32.43^{b}
T3	*Azospirillum* sp.	23	45	42.22^{c}	22	44	21.73^{c}	23	43	13.15^{d}
T4	*Pentoa dispersa*	14	32	3.84^{e}	14	32	12^{g}	18	48	5.71^{f}
T5	*Pseudomonas putida*	16	31	9.67^{d}	14	38	17.85^{e}	19	49	14.28^{d}
T6	*Streptomyces rimosus*	17	31	9.67^{d}	11	34	20^{d}	13	43	5^{f}
T7	*Streptomyces griseus*	15	34	4.16^{e}	14	31	15.38^{f}	12	42	10.25^{e}
T8	*Azotobacter* sp.	20	35	44.44^{b}	22	32	24^{b}	22	32	17.14^{c}
T9	*Xanthomans* sp.	17	33	3.44^{e}	15	38	0^{i}	13	43	2.63^{f}
T10	*Bacillus velezensis*	22	35	51.11^{a}	20	48	28.71^{b}	25	27	65.9^{a}

T11	CONTROL	**0**	**87**	0	**0**	**87**	0	**0**	**87**	0
	F – test	*			*			*		
	S. EM	**0.058**			**0.156**			**0.256**		
	C. D.@ 5%	**0.171**			**0.464**			**0.760**		
	CV	**6.136**			**12.343**			**16.570**		

1-A

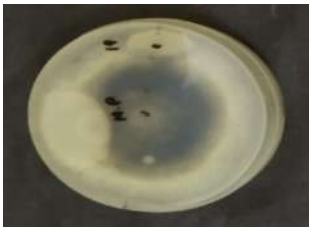
1-B



1-C

1-D

1-E



1-F

1-G

1-H

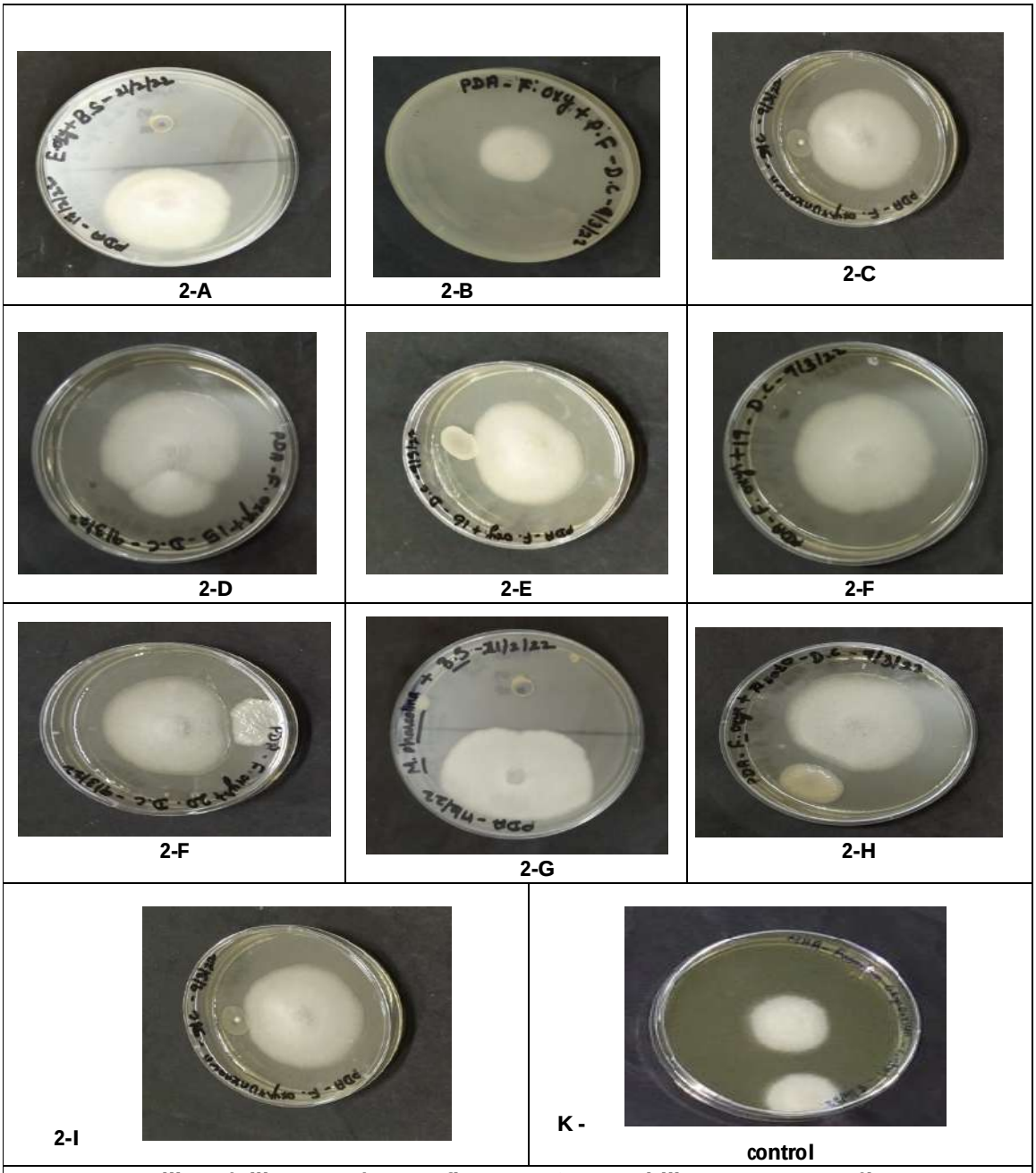
1-I

1-J

K - K-control

1. A- ***Bacillus subtilis,B- Pseudomonas fluorescens,C - Azospirillum*** **sp., D-** ***Pentoa dispersa,E- Pseudomonas putida, F- Streptomyces rimosus,G- Streptomyces griseus, H- Azotobacter*** **sp.,** ***I- Xanthomans*** **sp., J -** ***Bacillus velezensis., K*** **– control (***Macrophomina phaseolina)*

Fig. 1- Show the ***Macrophomina phaseolina*** **Root rot pathogen against different PGPR isolates**



2-A **2-B** **2-C**

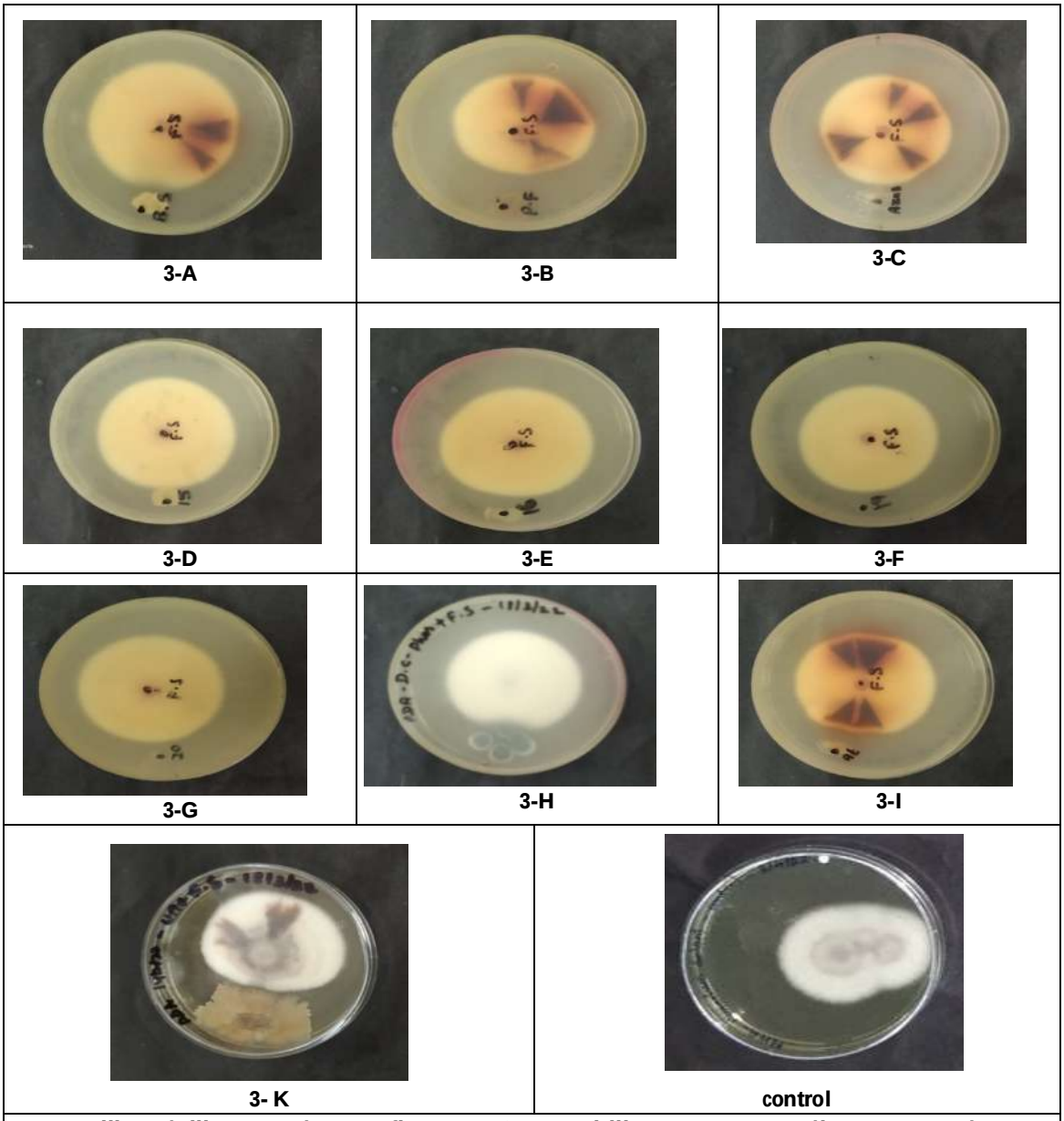
2-D **2-E** **2-F**

2-F **2-G** **2-H**

2-I **K - control**

2 A- *Bacillus subtilis*,B- *Pseudomonas fluorescens*,C - *Azospirillum* sp., D- *Pentoa dispersa*,E- *Pseudomonas putida*, F- *Streptomyces rimosus*,G- *Streptomyces griseus*, H- *Azotobacter* sp., I- *Xanthomans* sp., J - *Bacillus velezensis*., K – control *(Fusarium oxysporum)*

Fig. 2 *Fusarium oxysporum* Root rot disease pathogen against PGPR isolates



3-A **3-B** **3-C**

3-D **3-E** **3-F**

3-G **3-H** **3-I**

3- K **control**

3.A- ***Bacillus subtilis,B- Pseudomonas fluorescens,C - Azospirillum*** **sp., D-** ***Pentoa dispersa,E- Pseudomonas putida, F- Streptomyces rimosus,G- Streptomyces griseus, H- Azotobacter*** **sp.,** ***I- Xanthomans*** **sp., J -** ***Bacillus velezensis., K*** **– control (***Fusarium solani)*

Fig . 3 - ***Fusarium solani*** **Root rot disease pathogen against PGPR isolates**

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RESEARCH ARTICLE

Nanosuspension: A Special Focus on Method of Preparation

Manish Kumar Gupta[1], Sreethu.K.Sreedharan[2]* and C I Sajeeth[3]

[1]Professor, School of Pharmaceutical Sciences, Jaipur National University, Jaipur, Rajasthan, India
[2]Ph.D Research Scholar, School of Pharmaceutical Sciences, Jaipur National University, Jaipur, Rajasthan, India
[3]Professor, Grace College of Pharmacy, Kodunthirapulli po, Palakkad, Kerala, India.

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***Address for Correspondence**
Sreethu.K.Sreedharan
Ph.D Research Scholar,
School of Pharmaceutical Sciences,
Jaipur National University,
Jaipur, Rajasthan, India.
Email: sreethu09@gmail.com

ABSTRACT

Nanoparticles are particles with a characteristic dimension less than 100 nm. The properties of nanoparticles varies substantially from those of "big" colloidal particles (size bigger than 1 μm) because radius of surface forces, which is around 100 nm, is larger than or comparable the nanoparticles size. The latter means each nanoparticle can be completely covered by the surface forces of the adjacent particles at sufficiently little separation. Nanosuspension can be defined as very finely colloid, biphasic, dispersed solid drug particles in an aqueous vehicle, size below 1 μm stabilized by surfactants and polymers prepared by suitable methods for drug delivery applications. Nano suspensions have emerged as a promising strategy for the efficient delivery of hydrophobic drugs due to their versatile features and unique advantages. Techniques like media milling and high-pressure homogenization are used commercially for producing nano suspensions. The unique features of nano suspensions have enabled their use in various dosage forms, including specialized delivery systems like mucoadhesive hydrogels. Rapid strides are made within the delivery of nano suspensions by parenteral, peroral, ocular and pulmonary routes. Recently, efforts are being directed to extending their applications in site-specific drug delivery.

Keywords: Nanosuspension, solubility, homogenization, emulsion, micronization

INTRODUCTION

Nanosuspension is combination of two words i.e. Nano means very small, minute one billionth parts 10-9 and suspension means heterogenous mixture within which solute particles are suspended within the solvent floating freely within the medium. Nanosuspension is biphasic in nature during which colloidal solid fine particles are distribute in aqueous vehicle equilibrated by surfactants. quite 40% of recent chemical entities being generated through drug discovery programmes are poorly water soluble [1]. The formulation of incompetently water-soluble drugs has always been a demanding problem faced by pharmaceutical scientists. There are numerous conventional methods like micronization, solubilisation using co-solvents, surfactant dispersions and precipitation technique has been developed for improving solubility of poorly water soluble drugs. However these techniques show limitations to the drugs which aren't soluble in both aqueous and organic solvents. Nanosuspension may be processed for the API that's having either of the subsequent features: Water insoluble however which are soluble in oil (high log P) or API are insoluble in water and also in oils and drugs with reduced tendency of the crystal to dissolve, no matter the solvent API with very large dose [2].

Advantages of Nanosuspension [3,4]

1. Enhanced rate and extent of absorption
2. Enhanced dissolution rate
3. Increased physical and chemical stability of drug.
4. Suitable for hydrophobic drugs.
5. Higher drug loading will be achieved.
6. Dose reduction is feasible.
7. Provides passive drug targeting.
8. Are often delivered through various routes like oral, parenteral, ocular, and pulmonary.
9. Nanosuspensionare often formulated with compounds insoluble in water but soluble in oil.
10. Provides simplicity of producing and rescale for big scale production.
11. Oral administration of Nano-suspensions gives quick onset, improved bioavailability.
12. Rapid dissolution and tissue targeting will be accomplished by intravenous route of administration.
13. Reduction in tissue irritation just in case of subcutaneous or intramuscular administration.
14. Higher bioavailability is accomplished on account of occular administration and pulmonary delivery.
15. Drugs with higher log P worth may be planned as Nanosuspensions to reinforce bioavailability.
16. Improvement in biological performance is thanks to the high dissociation rate and saturation solubility of medication.
17. Nano-suspensions are often consolidated in tablets, pellets, hydrogels and suppositories are appropriate for various courses of organization.

FORMULATION OF NANOSUSPENSION

1. Stabilizers: Saturated the drug particles from top to bottom; put a stop to Ostwald's ripening and agglomeration of nanosuspensions, providing steric or ionic barriers. eg: Lecithin, Poloxamers, Polysorbate, Cellulosic, Povidones.
2. Co surfactants: Influence phase behaviour when micro emulsions are wont to formulate nanosuspensions. eg: Bile salts, Dipotassium Glycyrrhizinate, Transcutol,Glycofurol, Ethanol, Isopropanol.
3. Organic solvent: Pharmaceutically acceptable less hazardous solvent for preparation of formulation.eg: Methanol, Ethanol, Chloroform, Isopropanol, ester, Ethyl formate, Butyl lactate, Triacetin, Propylene carbonate, Benzyl alcohol.
4. Other additives: consistent with the necessity of the route of administration or the properties of the drug moiety. e.g: Buffers, Salts, Polyols, Osmogens, Cryoprotectant [5].

PREPARTION OF NANOSUSPENSION

Bottom up Technology

The common precipitation processes (Hydrosols) are called Bottom Up technology. By hire the precipitation method, drug is dematerialized in an organic solvent and that solution must be drawn up with an additional miscible anti solvent. As a result of less solubility in solvent-water mixture, drug precipitates. Precipitation further related to high shear processing. Nano edge method pivot on friable ingredients. Precipitation for achieve success fragmentation under circumstances of greater shear and thermal energy [6]. This is often attained by an amalgamation of fast precipitation and high- pressure homogenization. Fast adding of drug solution to any antisolvent facing abrupt super saturation of mixed solution and generation of fine amorphous or crystalline solids. Amorphous material precipitation perhaps dressing at high level super saturation during when solubility of amorphous state is surpassed [7,8].

Top down Technology

High pressure homogenization

This method is majorly employed to develop nanosuspension of various poorly aqueous soluble drugs [9]. During this method, the drug and surfactant suspension is strained under air mass by using nano sized aperture valve of a high level pressure homogenizer. The principle of this technique relies on aqueous phase cavitation. The particles with cavitations forces are adequately excessive for conversion of drug micro particles to nanoparticles. The most pander to this process is necessity for small sample particles previously loading. This kind of technology was established by R.H. Müller by employing a piston type air mass homogenizer [10].The main Principle is reduction of particle size by aqueous phase cavitation. Particle size also decreased due to high level shear forces and particles collision between each other. The dispersion present in three cm diameter of cylinder; instantaneously moves over a really tapered gap of twenty five μm. As stated by Bernoulli's Law, the liquid flow volume in an exceedingly closed system per cross section may be always constant. The decrease in diameter from 3 cm - 25 μm gives rise to growth in dynamic pressure and decreased static pressure which is below water boiling point maintained at temperature. By virtue of this, water boils at temperature and leads to formation of gas bubbles, that will burst after suspension leaves gap (called cavitations) and regular atmospheric pressure are attained. The drug nanocrystals size which might be attained chiefly looking on features like temperature, homogenization cycles number, and power density of homogenization pressure and homogenizer[11].Advantages of this method is that, it doesn't produce to abrasion of processed materials, apart to very dilute, also greatly concentrated nanosuspensions is developed by handling 1 - 400mg/ml of drug amount, and it is appropriate to all drugs which have poor solubility in aqueous and organic media. Preprocessing like drug Micronization and requirement of expensive devices are the disadvantages of this technique [12,13].

Nonaqueous Homogenisation (nanopure)

It is a method within which suspensions are homogenized in anhydrous media or aqueous mixtures. In Dissocubes methodology, the cavitation is significant determining factor of method. But then, in contrary to water, oily fatty acids and oils have almost less pressure level and greater boiling point. Thus, fall of static pressure might not be capable start cavitation. Patents veiling polymeric material disintegration using high level pressure homogenization remark that, only at temperatures of above 80^0C encouraged disintegration, which cannot use for warmth sensitive compounds. Within nanopure process, the suspensions of drug compounds which are in non- aqueous media were subjected to homogenization at 0^0 C or sometimes even but the melting point and so that they are called "deep-freeze" homogenization. The outcomes acquired to Dissocubes are also employed virtually for thermolabile compounds at warmer circumstances [14].

Nanoedge

The drug nanoparticles formed by precipitation have propensity to require up development of crystal to microcrystal size. This is required to be refined with greater level of energy forces. These are fully amorphous, partially amorphous or totally crystalline which produce difficulties in its lasting stability and also in bioavailability. Thus, the

precipitated particle suspension remains ensuing homogenization which maintain particle size acquired afterwards the precipitation [15].

Lipid emulsion/micro emulsion template

Lipid emulsions, suitable for drugs which are soluble in vaporous organic solvents and partly water miscible solvents. This method ends up in an organic solvent or mixture of solvents packed together with drug and distributed in an aqueous phase with appropriate surfactants for forming an emulsion. The organic phase was later vaporized under reduced pressure to make drug particles and precipitate briskly to make a nanosuspension that's stabilized by surfactants. Alternate approach to develop nanosuspensions are to use an emulsion which will be prepared from regular process employing a partly water miscible solvent for dispersed particles. Nanosuspensions are acquired just by thinning the emulsion. As templates, micro emulsions can develop nanosuspensions. Micro emulsions, thermodynamically firm, isotopically transparent dispersions of two immiscible liquids like oil and water which are balanced by using an interfacial film of co-surfactant and surfactant. The drug is also either loaded into inner phase or into accomplishes micro emulsion and will be soaked together with drug by dense mixing. Appropriate micro emulsion dilution gives drug nanosuspension. An exemplary of this method is that the griseofulvin nanosuspension that has been prepared by micro emulsion method employing water, lecithin, butyl lactate and taurodeoxycholate sodium [16, 17].

Media milling

This technique is foremost promoted and described by Liversidge. Nanosuspensions developed by employing high shear media mills. The milling chamber is full of water, drug, milling media, and stabilizer, and then it is subjected to rotate at an excellent shear charge under regulated temperatures for several days. The milling medium comprised of zinc oxide, glass or cross-linked polystyrene gum. As a consequence of milling media impaction, high energy shear forces are produced together with drug yielding to smashing of micro particulate drug into nanosized particles [18]. Advantages of this method includes, highly dilute and concentrated nanosuspensions could be developed through handling 1 - 400 mg/ml of drug amount and nanosized drug distribution to last nanosized product.

Dry co-grinding

Nowadays, nanosuspensions could also be acquired through dry milling processes. Developing steady nanosuspensions by employing dry-grinding of poorly soluble drugs with suitable soluble polymers and co-polymers subsequently dissolving in a very liquid media has been reported which was said to be a successful work. Itoh et al., stated that, the colloidal particles formation of various poorly aqueous soluble drugs like glibenclamide, nifedipine & griseofulvin acquired by crushing with sodium dodecyl sulfate and PVP. Numerous soluble polymers and co-polymers like polyethylene glycol, Poly vinyl pyrrolidine, cyclodextrin derivatives and hydroxypropyl methylcellulos has been utilized. Physicochemical characteristics and dissolution of poorly aqueous soluble drugs were ameliorated through co-grinding for an enhancement in transformation and surface polarity from a crystalline form to an amorphous drug [19].

Precipitation

Precipitation has been put in last decade to develop submicron particles, majorly for the aim of poorly soluble drugs. Generally, the drug is primarily dissolved in solvent and the identical solution is needed to employ a miscible anti solvent within the existence of surfactants. Fast adding of drug solution to anti solvent (i.e., water) create to unexpected drug super saturation within mixed solution and causing of ultrafine crystalline or shapeless drug solids. This method include two stages: nuclei formation and crystal growth. While making a stable suspension with least particle size, a greater nucleation rate but fewer rates is inevitable. Together rates are reliant on temperature, the foremost favourable temperature for nucleation should be below than for the sake of crystal growth [20].

Supercritical fluid method

Supercritical fluid process could also be employed to develop nanoparticles from the drug solutions. Many techniques ventured are fast supercritical solution expansion method, supercritical anti-solvent method and

precipitation - compressed anti-solvent method. RESS necessitate drug solution expansion in supercritical fluid by a nozzle that provides a solvent power loss of supercritical fluid ensuing in drug precipitation within the variety of fine particles. Young et al. developed cyclosporine nanoparticles varying a size of 400 to 700 nm employing this method. In PCA technique, the drug solution is atomized into a cabin holding compressed CO2. As solvent is removed, solution undergoes supersaturated and precipitates within the style of fine crystals. The supercritical anti-solvent technique utilizes supercritical fluid into which a poorly soluble drug and also solvent for that specific drug which too miscible within supercritical fluid is added. The drug solution is introduced into supercritical fluid so that the solvent gets removed by supercritical fluid and finally the drug solution undergoes super saturation. The drug is later precipitated within the fine crystals form. Griseofulvin nanoparticles, a poor solubility drug, were developed by Chattopadhyay et al. employing this system. The scam of above processes are utilization of dangerous solvents and utilization of high fractions of stabilizers and surfactants as compared with extra processes, particle nucleation overgrowth because of divergent great super saturation which will also ensue in improvement of an amorphous form or alternate undesired polymorph [21].

Nanojet technology

This method also called nanojet technology or opposite stream, utilizes a cabin where a suspension stream is diminished to 2 or more fractions, which is able to colloid together with each other at great pressure. The shear force developed during the strategy outcomes in reduction of particle size. Instrument with these criteria embraces M110L and M110S micro fluidizers. Dearn developed atovaquone nanosuspensions by employing micro fluidization method. The first drawback of this process is that the more number of passes within the micro fluidizer & product acquired carry a comparatively huge portion of microparticles [22].

APPLICATION OF NANOSUSPENSIONS

Oral Administration

Oral administration of nanosuspension is the first patient choice due to painless and non-invasive administration. Reduction of drug particle size to the results in an increased dissolution rate and might improve adhesion of the drug particles to the mucosa. Better contact with intestinal cells (bio adhesive phase) and a greater concentration gradient between blood and GIT increase drug intestinal absorption. Nanosuspensions are wont to control infections. Atovaquone and buparvaquone for the treatment of leishmaniasis and opportunistic Pneumocystis carinii infections in HIV patients are efficacious in high doses thanks to low bioavailability. A correspondence study of atovaquone within the kind of micronized particles and nanosuspensions showed that the latter decreased infectivity from 40% to fifteen. In another sample, buparvaquone nanosuspensions decreased infection from 2.0 to 1.02 and micronized particles only to 1.47 [23,24].

Parenteral Administration

Parenteral administration comprises administration of dosage forms like subcutaneous, intravenous, intramuscular, and intra-arterial methods. Advantages of this type of administration include avoidance of first-pass metabolism, authentic doses, and greater bioavailability. Control over the dose and rate authorize more predictable pharmacodynamic and pharmacokinetic profiles after IVadministration differentiate to oral administration. Administered drug particles are required to be less than 5 μm to blockage of capillaries. Nanosuspensions improve therapeutic efficiency and reduce the value of therapy through improved dosing efficiency and lesser injection volumes [25,26].

Pulmonary Drug Delivery

Advantages of pulmonary drug delivery over oral and parenteral drug administration consist of direct delivery to the location of action which results in decreased dosage and side effects. Conventional pulmonary delivery systems provide only rapid drug release, poor duration, and lack of selectivity. Nano suspensions can clear the problems of poor drug solubility in pulmonary secretions and lack of selectivity through direct delivery to aim on pulmonary cells. Adhesiveness of nanosuspensions to mucosal surfaces results in improved selectivity thanks to minimal drug loss and prolonged continuance at target site. Pulmonary nanosuspensions improve drug diffusion and dissolution

rate and consequently increase bio availability and forestall undesirable drug deposition within the mouth and pharynx [27].

Ocular Administration

Nanoparticle modified surface by relevant bio erodible polymer prolonged causes residual time in cul-de-sac desired for effective treatment. Commonly reported polymers in ocular nano suspensions are poly(alkyl cyanoacrylates), poly caprolactone, and poly(lactic acid)/poly(lacticco-glycolic acid) [28] . Charged nanoparticles have better adhesion to charged mucin which extends the drug release [29].

CONCLUSION

The nano suspension technology is a beneficial approach for the advancement of humans because of its simplicity, improved solubility and dissolution. In beginnings, various in vivo studies clearly demonstrate the potential of those drug delivery vehicles in parenteral, oral, ocular, and pulmonary administration, where not only a controlled release but also an appropriate bio adhesion is required. Incorporation of polymers on the particle surface and size reduction is thought to be the long term step in nano suspension research. Nano suspension is commercially possible attempt to unravel the poor solubility similarly as low bio availability problems of the drugs. For large-scale production of nano suspension formulation, high-pressure homogenization technology has been widely used. A nano suspension formulation clears the poor solubility problems, but also improves drug efficacy.

REFERENCES

1. A. Lippacher, R.H. Mu¨ller, K. Ma¨der, Liquid and semisolid SLN dispersions for topical application: rheological characterization, *Eur. J. Pharm. Biopharm.* 58 (2004) 561–567.
2. Kreuter J. In: Kreuter J, Eds. Colloidal drug delivery systems. New York: Marcel Dekker, Inc, 1994.
3. Sachin S Pawar , Bajirao R. Dahifale, Sandip P Nagargoje,Rajan S Shendge, 2017. Nano-suspensions Technologies for Delivery of Drugs. Nano sciNanotechnol, 4(2).
4. HelissaraSilveiraDiefenthaeler, Mariana DominguesBianchin, Morgana Souza Marques,JuliaLivia Non nenmacher, EmanueliTainara Bender, JúliaGabrieli Bender ,Samara FeilNery,Luiz Carlos Cichota, Irene ClemesKülkampGuerreiro, 2020. Omeprazole nanoparticles suspension: Development of a stable liquid formulation with a view to pediatric administration. Int. J. Pharm. DOI: 10.1016/j.ijpharm.119818.
5. Khandbahale SV. A Review-Nanosuspension Technology in Drug Delivery System. *Asian Journal of Pharmaceutical Research.* 2019;9(2):130-8.
6. Kesisoglou, F., S. Panmai, and Y. Wu, Nanosizing - Oral formulation development and biopharmaceutical evaluation. Adv. Drug Delivery Rev., 2007. 59(7): p. 631- 644.
7. Chaubal, M.V., Application of formulation technologies in lead candidate selection and optimization. Drug discovery today, 2004. 9(14): p. 603-609.
8. Hu, J., K.P. Johnston, and R.O. Williams III, Nanoparticle engineering processes for enhancing the dissolution rates of poorly water soluble drugs. Drug Development and Industrial Pharmacy, 2004. 30(3): p. 233-245.
9. Liversidge, G.G., et al., Surface modified drug nanoparticles. 1992, US Patents 5,145,689.
10. Chingunpituk, J., Nanosuspension technology for drug delivery. Walailak J Sci& Tech (WJST), 2007. 4(2): p. 139-153.
11. Dhiman, S., T.G. Singh, and Dharmila, Nanosuspension: a recent approach for nano drug delivery system. Int. J. Curr. Pharm. Res., 2011. 3(4): p. 96-101.
12. Bansal, S., M. Bansal, and R. Kumria, Nanocrystals: Current Strategies and Trends. Int J Res Pharm Biomed Sci, 2012. 3(1): p. 406-419.
13. Junghanns, J.U.A.H. and R.H. Muller, Nanocrystal technology, drug delivery and clinical applications. International journal of nanomedicine, 2008. 3(3): p. 295.

14. Merisko-Liversidge, E., G.G. Liversidge, and E.R. Cooper, Nanosizing: a formulation approach for poorly-water-soluble compounds. Eur. J. Pharm. Sci., 2003. 18(2): p. 113-120.
15. Chen, Y., et al., Preparation of oleanolic acid nanosuspension. ZhongguoYaoxueZazhi (Beijing, China), 2006. 41(12): p. 924-927.
16. Kocbek, P., S. Baumgartner, and J. Kristl, Preparation and evaluation of nanosuspensions for enhancing the dissolution of poorly soluble drugs. Int. J. Pharm., 2006. 312(1-2): p. 179-186.
17. Gao, L., D. Zhang, and M. Chen, Drug nanocrystals for the formulation of poorly soluble drugs and its application as a potential drug delivery system. J. Nanopart. Res., 2008. 10(5): p. 845-862.
18. Muller, R.H., C. Jacobs, and O. Kayser, Nanosuspensions for the formulation of poorly soluble drugs. Drugs Pharm. Sci., 2000. 105(Pharmaceutical Emulsions and Suspensions): p. 383-407.
19. Krause, K., et al., Heavy metal contamination of nanosuspensions produced by high-pressure homogenisation. International journal of pharmaceutics, 2000. 196(2): p. 169-172.
20. Kumar, A., et al., Review on solubility enhancement techniques for hydrophobic drugs. PharmacieGlobale, 2011. 3(3): p. 001-007.
21. Reddy, G.A., Nanosuspension Technology-A Review. IJPI's Journal of Pharmaceutics and Cosmetology, 2012. 2(8): p. 47-52.
22. Keck, C.M. and R.H. Muller, Drug nanocrystals of poorly soluble drugs produced by high pressure homogenisation. European journal of pharmaceutics and biopharmaceutics, 2006. 62(1): p. 3-16.
23. Jain D, Thakur PS, Thakore SD, Samal SK, Bansal AK. Impact of differential particle size of fenofibratenanosuspensions on biopharmaceutical performance using physiologically based absorption modeling in rats. *Journal of Drug Delivery Science andTechnology*. 2020;60(2020):1-12.
24. Jacob S, Nair AB, Shah J. Emerging role of nanosuspension in drug delivery system.
Biomaterials Research, 2020; 24: 3.
25. Goel S, Sachdeva M, Aggarwal V. Nanosuspension Technology: Recent Patents on Drug Delivery and their Characterizations. Recent Patents on Drug Delivery & Formulation, 2019; 13: 91-104.
26. Duan Y, Dhar A, Patel C, Khimani M, Neogi S, Sharma P, et al. A brief review on solid lipid nanoparticles: part and parcel of contemporary drug delivery systems. RSC Advances. 2020;10(45):26777-91.
27. Praveen Kumar G, Krishna KG; Nanosuspensions: The Solution to Deliver Hydrophobic Drugs. *International Journal of Drug Delivery,* 2011; 3: 546-557.
28. Kuno J, Miyake K, Katao S, Kawai T, Nakashima T. Enhanced enantioselectivity in the synthesis of mercury sulfide nanoparticles through ostwald ripening. Chemistry of Materials. 2020;32(19):8412
29. Dhiman, S Dharmila and Thakur, GS "Nanosuspension: A recent approach for nano drug delivery system", *Int J Curr Pharm Res*, Vol 3, Issue 4, 96-101.

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RESEARCH ARTICLE

Identification and Analysis of Gait using Neural Network Pattern Recognition System

A. Stephen Sagayaraj[1]*, S. Elango[2], S. Srinivasan[3], M. Aravinth[3] and G Monish[3]

[1]Assistant Professor, Department of ECE, Bannari Amman Institute of Technology, Sathyamangalam, Erode, Tamil Nadu, India
[2]Assistant Professor-III, Department of ECE, Bannari Amman Institute of Technology, Sathyamangalam, Erode, Tamil Nadu, India
[3]III Year ECE Student, Department of ECE, Bannari Amman Institute of Technology, Sathyamangalam, Erode, Tamil Nadu, India.

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***Address for Correspondence**
A. Stephen Sagayaraj
Assistant Professor,
Department of ECE,
Bannari Amman Institute of Technology,
Sathyamangalam, Erode, Tamil Nadu, India
Email: stephensagayaraja@bitsathy.ac.in

ABSTRACT

Gait is the systematic study of any human being or animal by extracting reliable gait features. Gait data is acquired and analyzed by using image processing and machine learning techniques. The proposed methodology is to identify the illegal intruders in military base by using machine learning techniques. The image is capture by using CCTV cameras from which the features are extracted and classified. The extracted features are analyzed in the machine – learning – neural networks pattern recognition. The features of military people in the base are extracted and saved in the database. The intruders with different gait features entering the military base is been detected by comparing the gait data base of military people. The Neural Network pattern recognition identifies the intruders with good percentage of accuracy. It is used to clearly classify the gait database and newly entering intruders in military base.

Keywords: saved in the database, gait features entering, recognition identifies

INTRODUCTION

Most gait recognition are extracted from windowed silhouette images since they need to be invariant to features which are easy to concealed [2,3,4]. To avoid shape information a five linked biped human locomotion is used to extract the gait features. Jeff P. Fosterin his paper proposed that more vital information is obtained in the set of sequential silhouette images than single silhouette image. Holistic approaches were used to extract features from

silhouette images in previous works but the temporal component of gait was ignored. Computational complexity was the disadvantage of model based approach. Jeff P. Foster in his paper proposed a new technique which aims to consider temporal information by overcoming the above mentioned disadvantages [2].For most of the recognition achieved silhouette accounted for lower 20% (approx.) [3]. Gait recognition can also be used to detect the symptoms of cerebral palsy and parkinson's disease [4]. Bing Sun on his paper proposed two different approaches such as non wearable and wearable sensors based gait recognition [4]. Gait recognition is done through Optical sensors and Pressure sensors placed on floor in non wearable sensor based recognition. Sensors like accelerometers, gyroscope [4], are attached to the target in Wearable sensor gait recognition method. Gait kinematics, Gait kinetics , electromyography are the major divisions of Wearble sensor method which is widely used in medical and sports applications [11]. Robert T. Collins, proposed the idea of using 3X3 median filter operator to supress isolatedpixels [7]. Xi Chen in his paper developed a new methodology called fuzzy logic inference system to avoid the disturbance of moving object while extracting human silhouette [10]. According to Jiande Sun Gait recognition is non-contactable and non invasive biometric identification which is non imitate able.

Literature Survey

The idea of Gait analysis was first proposed by Aristotle in 350 BCE. Borelli, well known mathematician who is also known as Father of Biomechanics [17], on 1679 extended Aristotle's idea in work 'On the Gait of Animals' as 'The study of animals locomotion' . Forward leg motion in human locomotion resembles like pendulum proposed by Wilhelm and Eudard [18].Liang Wang et al, approached automatic gait recognition method by spatiotemporal silhouette analysis walking [12]. Ali et al, in his paper applied Principal Component Analysis(PCA) with and without Radon transform which is used to reduce dimension of images without loss of actual information in less duration of time [13]. Eigenspace transformation which is developed from PCA is more accurate. A silhouette analysis based gait recognition algorithm using traditional PCA is developed by Liang Wang et al [12]. Benbakreti et al in his paper classified the silhouette into three parameters which includes angle between the right and left leg, perimeter and area of contour. Dynamic time wrapping technique is being approached to distinguish silhouette images[14].A gait verification method was developed on basis of static body and stride parameter (Aaron F. Bobick.). Table : 1.Literature survey.

METHODOLOGY PROPOSED

The method of recognizing intruders in military camp using Gait Recognition technique is shown in Figure 1. Input image is collected from CCTV camera. The images collected thereby are converted into silhouette images, in order to extract the Gait feature. The extracted gait features are compared with database which is stored earlier. The accuracy of prediction is done by using Neural Network. The silhouette images are used to extract the behavior of the Human Beings. The Silhouette extracts the maximum competency of human behavior activities and to maintain the Human stability in cameras. The Silhouette of the images are taken and features are extracted which differs for every Human. The Human activities are measured by feature extraction process and the extracted features are meant for the comparison with the existing database. The comparison made are verified and checks for the classification accuracy using the neural network pattern recognition. By using various parameters and generalized epochs the Pattern recognition differentiate the accuracy and sensitivity. The terms are defined by using obtained confusion matrix.

Acquision of silhouette images

The images are acquired from CCTV footage in order to recognize the Gait human identification. The acquired images are converted into silhouette images. The purpose of silhouette images is to separate background and target images. In this research work, the silhouette images are taken from SACV GAIT database which is shown in Figure 2. The silhouette images are used to extract the gait features.

Gait Recognition

Gait recognition is the systematic study of analysing a human being or animal by extracting reliable gait features. Silhouette images converted from CCTV sequential images is the main source for extracting Gait features of the target. The proposed methodology is to identify the illegal intruders in the military base. This is done by extracting and storing the gait features such as foot length, hand swing, gait cycle, heal strike, stance phase of the military men in a database. The data base is compared then and there. The data base is compared with already stored data base of the military man. If the data base doesn't match the input data, then it is classified using neural network technology.

Foot length

Foot length is the structural approach of gait feature recognition. Foot length is defined as distance between the right and left feet of a person while walking. Foot length of target person is measured using sequential silhouette images and tabulated for observation.

Hand Swing

Hand swing is defined as the distance between the right and left hand of the target image. The hand swing The sequential silhouette image is the source for measuring the hand swing.

Stance phase:

Stance phase is the entire process starting right from heel strike till preparation for oscillation. It comprises of loading response, mid stance, terminal stance.

Gait Cycle

The combination of swing phase and stance phase is called as gait cycle. The swing phase consists of initial swing, mid swing and terminal swing.

Heel strike

Heel strike is the minimum distance between the toe and the ground when the target makes initial contact with the ground. In this method ground is taken as reference for taken for measurement.

Knee distance

Knee distance is defined as the distance between the two knees of the target person while walking.

Feed Forward Network

Classification of inputs according to specified target is done by pattern recognition. The value '1' is assigned for target and '0' for the rest. Random division is done for samples representing training, testing and validation. The performance of the network is measured on the basis of Cross entropy and Error percentage. Small value of cross entropy gives better performance while classification. Misclassified data is decided by the error percentage. For a good classification error percentage should be minimum. All confusion matrix is plotted to detect TP,FP,TN,FN. The sensitivity value is 97, Accuracy is 82 and precision value is 83. Training record of the data is provided by the training state is shown in the Fig 5. The gradient is found to be 0.025257 at epoch 21. The accuracy, sensitivity, precision is described in table 2.

CONCLUSION

Gait recognition is made to identify intruders in military base. The gait features are extracted to precede classification. Feed Forward Neural network is identified to classify the gait extracted. The accuracy of predicting intruders is 82% The future scope is to increase the prediction by implementing different machine learning algorithms.

REFERENCES

1. Britto, A.V. and Ansari, V., 2018, December. Human Gait Analysis using Artificial Neural Networks. In *2018 IEEE International Conference on Computational Intelligence and Computing Research (ICCIC)* (pp. 1-6). IEEE.
2. Foster, J.P., Nixon, M.S. and Prügel-Bennett, A., 2003. Automatic gait recognition using area-based metrics. *Pattern Recognition Letters*, *24*(14), pp.2489-2497.
3. Phillips, P.J., Sarkar, S., Robledo, I., Grother, P. and Bowyer, K., 2002, August. The gait identification challenge problem: Data sets and baseline algorithm. In *Object recognition supported by user interaction for service robots* (Vol. 1, pp. 385-388). IEEE.
4. Kale, A., Cuntoor, N., Yegnanarayana, B., Rajagopalan, A.N. and Chellappa, R., 2003, June. Gait analysis for human identification. In *International Conference on Audio-and Video-Based Biometric Person Authentication* (pp. 706-714). Springer, Berlin, Heidelberg.
5. Sun, B., Wang, Y. and Banda, J., 2014. Gait characteristic analysis and identification based on the iPhone's accelerometer and gyrometer. *Sensors*, *14*(9), pp.17037-17054.
6. Collins, R.T., Gross, R. and Shi, J., 2002, May. Silhouette-based human identification from body shape and gait. In *Proceedings of fifth IEEE international conference on automatic face gesture recognition* (pp. 366-371). IEEE.
7. Tao, D., Li, X., Wu, X. and Maybank, S.J., 2007. General tensor discriminant analysis and gabor features for gait recognition. *IEEE transactions on pattern analysis and machine intelligence*, *29*(10), pp.1700-1715.
8. Britto, A.V. and Ansari, V., 2018, December. Human Gait Analysis using Artificial Neural Networks. In *2018 IEEE International Conference on Computational Intelligence and Computing Research (ICCIC)* (pp. 1-6). IEEE.
9. Sundaresan, A., RoyChowdhury, A. and Chellappa, R., 2003, September. A hidden markov model based framework for recognition of humans from gait sequences. In *Proceedings 2003 International Conference on Image Processing (Cat. No. 03CH37429)* (Vol. 2, pp. II-93). IEEE.
10. Chen, X., He, Z., Anderson, D., Keller, J. and Skubic, M., 2006, October. Adaptive silouette extraction and human tracking in complex and dynamic environments. In *2006 international conference on image processing* (pp. 561-564). IEEE.
11. Tao, W., Liu, T., Zheng, R. and Feng, H., 2012. Gait analysis using wearable sensors. *Sensors*, *12*(2), pp.2255-2283. Tao, W., Liu, T., Zheng, R. and Feng, H., 2012. Gait analysis using wearable sensors. *Sensors*, *12*(2), pp.2255-2283.
12. Wang, L., Tan, T., Ning, H. and Hu, W., 2003. Silhouette analysis-based gait recognition for human identification. *IEEE transactions on pattern analysis and machine intelligence*, *25*(12), pp.1505-1518.
13. Hayder, A., Dargham, J., Chekima, A. and Ervin, G.M., 2011. Person identification using gait. *International Journal of Computer and Electrical Engineering*, *3*(4), p.477.
14. Benbakreti, S. and Benyettou, M., 2012, March. Gait recognition based on leg motion and contour of silhouette. In *2012 International Conference on Information Technology and e-Services* (pp. 1-5). IEEE
15. Bobick, A.F. and Johnson, A.Y., 2001, December. Gait recognition using static, activity-specific parameters. In *Proceedings of the 2001 IEEE Computer Society Conference on Computer Vision and Pattern Recognition. CVPR 2001* (Vol. 1, pp. I-I). IEEE.
16. Saadoon, A. and Nordin, M.J., 2015. An automatic human gait recognition system based on joint angle estimation on silhouette images. *Journal of Theoretical and Applied Information Technology*, *81*(2), p.277.
17. Khan, M.H., Farid, M.S. and Grzegorzek, M., 2021. Vision-based *approaches towards person* identification using gait. *Computer Science Review*, *42*, p.100432
18. Weber, W., Weber, E. and Lawrence, C., 1994. Mechanics of the Human Walking Apparatus. *Annals of Science*, *51*(2).
19. Kale, A., Sundaresan, A., Rajagopalan, A.N., Cuntoor, N.P., Roy-Chowdhury, A.K., Kruger, V. and Chellappa, R., 2004. Identification of humans using gait. IEEE Transactions on image processing, 13(9), pp.1163-1173.
20. Begg, R.K., Palaniswami, M. and Owen, B., 2005. Support vector machines for automated gait classification. IEEE transactions on Biomedical Engineering, 52(5), pp.828-838.

21. Ioannidis, D., Tzovaras, D., Damousis, I.G., Argyropoulos, S. and Moustakas, K., 2007. Gait recognition using compact feature extraction transforms and depth information. IEEE Transactions on Information Forensics and security, 2(3), pp.623-630.
22. Moustakas, K., Tzovaras, D. and Stavropoulos, G., 2010. Gait recognition using geometric features and soft biometrics. IEEE Signal processing letters, 17(4), pp.367-370.
23. Kim, D. and Paik, J., 2010. Gait recognition using active shape model and motion prediction. IET Computer Vision, 4(1), pp.25-36.
24. Wang, C., Zhang, J., Wang, L., Pu, J. and Yuan, X., 2011. Human identification using temporal information preserving gait template. IEEE transactions on pattern analysis and machine intelligence, 34(11), pp.2164-2176.
25. Kusakunniran, W., Wu, Q., Zhang, J. and Li, H., 2012. Gait recognition across various walking speeds using higher order shape configuration based on a differential composition model. IEEE Transactions on Systems, Man, and Cybernetics, Part B (Cybernetics), 42(6), pp.1654-1668.
26. Hu, M., Wang, Y., Zhang, Z., Little, J.J. and Huang, D., 2013. View-invariant discriminative projection for multi-view gait-based human identification. IEEE Transactions on Information Forensics and Security, 8(12), pp.2034-2045.
27. Guan, Y., Sun, Y., Li, C.T. and Tistarelli, M., 2014. Human gait identification from extremely low-quality videos: an enhanced classifier ensemble method. IET biometrics, 3(2), pp.84-93.
28. Hu, M., Wang, Y., Zhang, Z., Zhang, D. and Little, J.J., 2012. Incremental learning for video-based gait recognition with LBP flow. IEEE transactions on cybernetics, 43(1), pp.77-89.
29. Wu, Z., Huang, Y., Wang, L., Wang, X. and Tan, T., 2016. A comprehensive study on cross-view gait based human identification with deep cnns. IEEE transactions on pattern analysis and machine intelligence, 39(2), pp.209-226.
30. Khamsemanan, N., Nattee, C. and Jianwattanapaisarn, N., 2017. Human identification from freestyle walks using posture-based gait feature. IEEE Transactions on Information Forensics and Security, 13(1), pp.119-128.
31. Chaurasia, P., Yogarajah, P., Condell, J. and Prasad, G., 2017. Fusion of random walk and discrete fourier spectrum methods for gait recognition. IEEE Transactions on Human-Machine Systems, 47(6), pp.751-762.

Table 1: Literature Survey

S.NO	AUTHOR	PAPER DESCRIPTION
1.	Liang Wang ; Tieniu Tan ; Huazhong Ning ; Weiming Hu	• Methodology: spatial temporal silhoutte analysis • PCA- reduce the dimensionality • Advantage: low computational cost • Demerits: simplified assumed work
2.	A. Kale et.al	• To recognize human from gait • Methodology: Hidden markov model • Lends overall robustness
3.	R.K. Begg	• Automatic gait recognition-early identification of risk gait • Methodology: SVM • To differentiate young and old gaits • Risk minimization in elderly
	• Dimosthenis Ioannidis	• 2D and 3D features • Genetic algorithm for feature fusions
	• konstantinos Moustakas	• Soft biometrics features is used (User's height and stride length information) • Drawback: Directly analyzing the gait pattern

	• D. Kim ; J. Paik	• Human identification from noisy images • Methodology: Active Shape Model • Advantage: Not directly analyzed the gait pattern
	• Chen Wang	• Methodology: CGI(Chronic Gait Images) • ADVANTAGE :Robustness and efficiency
	• Worapan Kusakunniran	• For variation of walking speeds • Methodology: DCM (Differentiate composite Model) • Which measures speed changes in human body
	• Maodi Hu	• Methodology: (ViDP)-View Invariant Discriinative Projection • Multi view Gait Features
	• Yu Guan	• Gait recognition for low quality images • Average Gait image • Appearance based feature
	• Maodi Hu	• LBP is employed to get texture information • HMM Hidden Markov Model is used
	• Zifeng Wu	• Methodology: CNN (Convolution Neural Network) • To measure Discriminative Changes
	• Nirattaya Khamsemanan	• Poster based feature extraction. • Poster based classification
	• Priyanka Chaurasia	• To separate different body parts efficiently

Table 2. Feature extracted for gait recognition – a sample

Gait Features/Sample	1	2	3	4	5	6	7	8	9	10
Foot length (distance between the foots)	42.72	39.92	42.97	39.77	37.99	36.62	36.53	38.01	42.95	38.28
Hand swing	28.08	43.38	35.76	34.52	30.92	47.56	42.89	32.73	40.93	37.18
Heel strike	9.06	8.81	8.57	7.82	7.99	7.13	10.84	9.82	10.51	9.49
Knee distance	26.43	20.34	25.37	16.95	22.32	19.74	22.47	24.02	23.68	21.42
Stance phase	84.44	78.83	84.93	80.03	71.17	73.94	70.9	78.61	84.56	78.07
Gait cycle	128.17	120.11	124.91	121.01	107.89	110.88	107.99	117.21	123.86	116.64

Table 3: Accuracy Measures of NN Pattern Recognition

Classification Parameter	TP	FP	TN	FN	SENSITIVITY	ACCURACY	PRECISION
Test Images	39	8	2	1	97	82	83

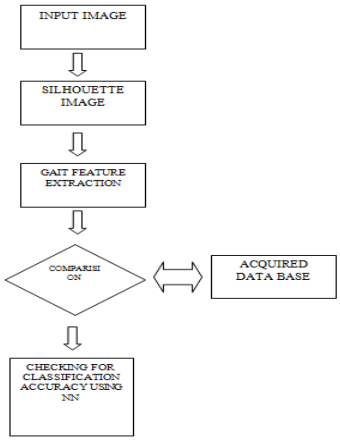

Figure 1 : Flow of Gait Recognition Technique

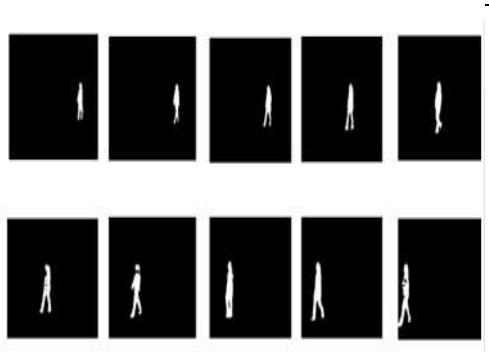

Figure 2: Extracted silhouette images from SACV GAIT

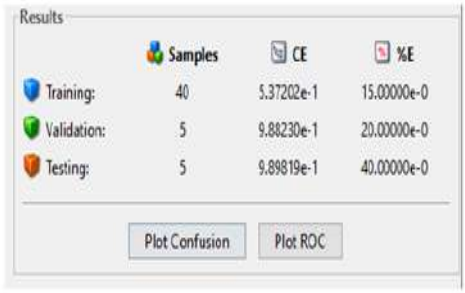

Figure 3: Cross Entropy and Error percentage

Figure 4: All Confusion Matrix

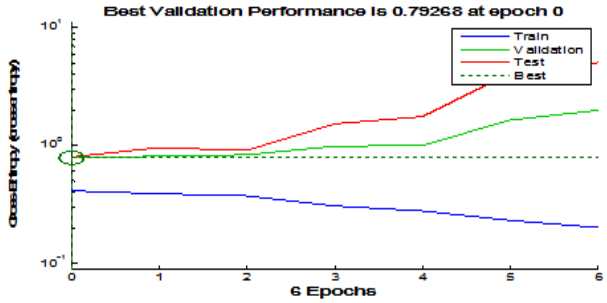

Figure 5: Cross Entropy plot

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RESEARCH ARTICLE

Factors Influencing the Mental Health of Women during COVID-19

Jeevan Swetha[1], Suresh Monica[1], M. D Pooja[1], Jhanani Gopalraaj[1], K Dharani Shree[1] and P.Manikantan[2]*

[1]Ph.D Scholar, Department of Life Science, Christ University, Bangalore, Karnataka, India.
[2]Assistant Professor, Department of Life Science, Christ University, Bangalore, Karnataka, India.

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***Address for Correspondence**
P.Manikantan,
Assistant Professor,
Department of Life Science,
Christ University, Bangalore,
Karnataka, India.
E.Mail: manikantan.p@christuniversity.in

ABSTRACT

The recent pandemic, COVID -19, has so far proven to have had a diverse impact on the social well-being of human beings, especially the mental stability of women. Onset of COVID-19 during the year 2020 has caused a dramatic effect on the lives of many due to economic disruption, isolation from social life, and social beings during the extended periods of social distancing and lockdown across the globe. Looking into data across the world indicates that a large portion of the population is undergoing depression and anxiety more now than before the pandemic. Women are mostly affected mentally, mainly homemakers, working women, and children compared to men. This systematic review article looks at all the papers since the beginning of the pandemic from the year 2020 to 2021 especially concentrating on the mental stability of women and children. Several factors affect the level of psychological stress faced by women, some of them being age, physical health, socioeconomic status, workplace stress, and unemployment.

Keywords: COVID-19, pandemic, mental health, women, domestic violence, children

INTRODUCTION

COVID-19 has been one of the most dangerous pandemics affecting the lives of many resulting in several deaths, decrements in socio-demographic status, and unemployment. Across the Globe, 70% of the women are known to work as primary health workers i.e., Doctors, nurses, at tenders, and hospital service staff [1]. There has been a widespread increase in stress, depression and anxiety ever since COVID-19 pandemic started [2]. Women especially are facing a lot of challenges [3,4]. Moreover, this rise in challenges and stress in women has not just commenced during post-pandemic and pre- covid pandemic period but researchers prove that, in general, women face more challenges and are victims of depression and various anxiety disorders when compared to men all over the world [5,6]; even in a dual-earner households women handle more childcare responsibilities when compared to men [7].

Women are victimized and injured by IPV(intimate partner violence) [8]. It is also noted that women especially suffer from an increased risk of drug misuse [9]; abuse of the substance is more likely among women due to the various interpersonal reasons [10]. According to UN, Violence against Women (VAW) has been often experienced by women around the world but the pandemic has known to have locked these women with the potential abuser being their partner, parent, or any such person due to restrictions upon traveling and lockdown resulting in an increasing percentage of VAW. Hence this isolation, enforcement to undertake activities against one's will, and lack of enthusiasm have led to increased cases of depression amongst women [11].

The initial inquiry has proven that women were subjected to more unemployment than men, during COVID -19 [12] either due to severe health issues or responsibilities of taking care of family and children [13]. Most likely 70% of the health sector staff are made up of women leading to an increased chance of exposure to the virus as well as prolonged hours of work shift without proper rest or breaks in between duty leading to a higher risk of mental pressure [1]. The responsibility of women in the family has increased during the pandemic due to the shutdown of schools, telecommuting, and other commitments [14]. However, women in the family are bound to various responsibilities, one of which is childcare. Lack of social services like day-care and adult care made the women prone to more stress. Previous studies showed that women and children in nuclear families are highly vulnerable. Hence the impact of COVID-19 on women and men is different. A prediction model by [15] on life lost due to COVID-19, Socioeconomic Status, and suggests that the social class distress outweighs the disease burden due to COVID-19 rapidly in poorer and uneven societies.Therefore this pandemic has certainly had a tougher mental impact on women more extremely in some fields than on men. Women are prone to emotional and mental distress upon a lack of sufficient support leading to anxiety, depression, and Post Traumatic Stress Disorder [PTSD] [16].

Factors affecting the mental health of women during COVID-19

Socioeconomic Status

According to the latest study, the epidemic would put millions of people into extremely poor socioeconomic status in society by 2021, with 47 million of them being girls and women. COVID-19 made a poverty spike that would further increase the gender poverty divide, with a greater number of women than men being forced into dire poverty [32]. Poverty in women occurs when they are limited to any external activities in society that decreases their chance to earn their income during the COVID-19 pandemic. Women are particularly vulnerable, struggling out of a variety of potential drawbacks due to their poverty and status as workers. Women risk losing their jobs, facing human rights violations, and being exposed to COVID-19 infections [33]. Women are far more affected because they are often thought to be less productive and thus have a lower social status [34]. COVID-19 has a particularly negative impact on women's households, which cannot meet basic needs caused due to a lack of financial resources [35]. COVID-19 largely cased discrimination and vulnerabilities, also termed a gender-specific pandemic with racialized and classed dimensions [20]. In public health, education, and the economy, COVID-19 has seen an uneven impact on women and girls [34]. According to UN Women Executive Director, women's poverty rate was predicted to decline by 2.7 percent through 2019 and 2021, but forecasts instead show a 9.1 percent increase owing to the Covid and its aftermath and the truth could be even worse, because these forecasts of decreased economic status rates for women and girls that consider the relative decline of GDP, leaving out other variables like women prone to quit the profession due to family responsibilities which could affect the gender distribution of poverty. Also, Achim Steiner, UNDP Administrator has addressed that a comprehensive approach aiming at boosting the right to education and sex education, equitable and equivalent salaries, and widening social assistance could lift more women and girls out of poverty (UNDP.org, 2020. Women find it challenging to establish and produce economic growth without the help of suitable facilities and an inability in understanding information technology. Women are more vulnerable to poverty. These women must make deliberate attempts to climb up and add abilities in understanding technology in order to establish economic growth [26].

Domestic violence and substance use

Different forms of abuse like child abuse, elder abuse, intimate partner violence, pet abuse, etc., are examples of domestic violence [36]. People of all ages are affected, causing feminicide majorly. Pandemic forced family members

to have prolonged contact, restrictions on any source of entertainment, economic difficulties, loss of social life, and boredom has played havoc on the relationships [37]. Reports on domestic violence show that they have a variety of long-term implications, including being at risk for several mental health issues such as anxiety disorders, mood disorders, posttraumatic stress disorder, eating disorders, and alcohol or substance abuse [21], this leads to developing health conditions such as cardiovascular disease, chronic pain, sleep disturbances, gastrointestinal problems, brain trauma, and sexually transmitted diseases [38]. According to the survey done in 2020, due to Intimate Partner Violence (IPV) is led to women assassination at home. IPV is considered sexual violence or any sort of physical abuse. It is proved that 30% of women in America suffered from IPV and in 25% of women it has led to severe conditions like cardiovascular disease, and mental stress they also experienced battle fatigue or delayed stress disorder [21]. Even though diseases linked with physiological imbalance is not yet clearly known, it is thought that the severe and continuous stress due to domestic violence would lead to a high risk of mental and physical disorders and also it is said that abused girl child and women survivors of domestic violence have developed the cardio metabolic disease [39] camp still further studies are required to strongly prove this.

Activists have raised concerns over the increase in violence against women during this COVID- 19 [40]. Also, it has been found that depression, anxiety, and stress were prevalent more amongst abused women. Reports from OECD (Organization for Economic Co-operation and Development) countries shows that there has been a steady increase in domestic violence against women, especially during the pandemic. This is because many women and their abusers are stuck together at home [25, 41]. Due to the imposition of the lockdown, the victims of violence have a limited access to support systems Due to this limited availability of assistance, a sense of abandonment spread across the victims of violence, forcing them to be together with their abusers quarantined. Home, a protective "shelter" from the pandemic, was nothing more than a source of insecurity and distress to women. Psychological distress related to COVID is connected with a rise in alcohol consumption, especially in women population[42].Substance abuse is a neuropsychiatric disorder marked by a continuous desire to use the drug, despite being self-injurious [43]. Substance abuse and its consequences are well-known, affecting young adults and society [44, 45, 46]. It is a significant global public health concern, with a prevalence rate ranging from 20% to 25% of people aged 12 and above [47]. Substance use in women had increased during the pandemic to suppress the increased stress, anger, and depression idea of suicide, due to several reasons such as unemployment, no immediate access for medical facilities, finance-related problems, taking care of children and people at home [48].Substance usage to confront the deteriorating mental health has physical as well as psychological impacts[49]. Though earlier men were at much higher risk for substance use disorders, now the gap is gradually narrowing [50] , also studies have found that alcohol usage to cope up with stress is more likely amongst women[51, 52, 48]. Increased alcohol usage was linked with high scores of depression and anxiety measures, showing that deteriorating mental health problems due to the pandemic, could be linked to an increased alcohol consumption amongst women. Furthermore, concerns about the pandemic daily effect and low self-efficacy were also predictors of COVID-19 anxiety in a study conducted on adult women. They concluded that high-risk alcohol intake significantly leads to anxiety levels and depression than low-risk drinking habits [53].

Workplace stress and unemployment

Psychological trauma and suicide among healthcare personnel have grown as a result of the COVID-19 pandemic [54]. Furthermore, a poll of health care workers performed by the British Medical Association in April 2020 revealed that 44% of participants said COVID-19-related factors were causing stress, depression, panic, or other mental health conditions[55]. Women Health Care Workers who have medical or psychiatric problems or who drink excessively are more likely to develop mental health problems. Women Health Care Workers with more than two children have a higher incidence of psychological well-being[56]. Therefore financial assistance for Health Care Workers [57],rest areas for nap and retrieval[58], basic physical necessitates such as food [59], resiliency training programs [60], information on safety precautions and accessibility to recreational activities, and therapists[61] are all considered a promising approaches to sustain Health Care Workers during this pandemic. The covid 19 pandemic impacted the health sector and led to the labour market crisis, leading to unemployment. Studies showed a direct relationship between employment status and the health status of individuals [14]. According to the International Labour Organization 8th edition report, in 2020, women were disproportionately employed, accounting for 38.9 percent of

total employment before the COVID-19 crisis but making up 47.6 percent of employment losses in 2020. Global employment declined for youth, medium, and low-skilled workers [62]. There is a large working population in India, of which 450 million people are employed in the informal sector [22]. The research estimated that 90% of women work in the informal sector, with 20% working in cities [63].In India, the informal sector is highly uncertain and poorly regulated, with few or no regulations for social security. Because informal sector workers are the most vulnerable populations and, therefore, more exposed to the present worldwide pandemic, the COVID-19 will have long-term implications [64, 65]. Women, in general, are affected more when compared to men by any crisis-driven loss of income. On average, women's income is much lower than men's across the OECD [66], with a higher rate of poverty [67]. Possession of wealth is also more amongst men than women [68, 69]. Due to higher caring responsibilities, it becomes more difficult in finding another employment opportunity and income sources. Single parenting which comprises mostly women, are especially in a susceptible position. Dependence on a single income makes them more vulnerable since job loss can be disastrous for single-parent families [70].

Health Conditions

The women represented in health care sectors are about 70%, particularly in high wage professions like physicians, nurses, and pharmacists, whose role during covid are immense [71]. Indeed, these doctors and nurses are more vulnerable to infections as they serve at the forefront. A report on the occurrence of symptomatic COVID-19 disease among health care workers between February 21 and May 19, 2021, shows that among the 1350 staff who tested positive for COVID-19 on reverse transcriptase-polymerase chain reaction, the female to male ratio was 3:2 [72]. In a study conducted by [73], pregnant women and the one who have just given birth are prone to get infected more during this pandemic. During the pandemic, couples were in the dilemma of whether to have a child or not because of the financial crisis. They didn't have a clear idea whether SARS-CoV-2 would affect the healthy development of a child or not. The postponement of having children among couples created lots of mental stress, especially for women [74]. Young women of economically low background who gave birth during the pandemic could not feed their children because they were undernourished and they struggled to buy the packed milk too to feed their children. This kind of situation made it more stressful for women which had drastic effects on their health. Since women play a major role in cooking and feeding children, they underwent a more stressful situation when a ration from the government was not sufficient [75].

Studies proved that women underwent depression, anxiety, and mental stress during COVID 19 and post COVID 19 due to the availability of the information on the transmission of COVID 19 on the social media [76], but again as a contradiction, the same studies even discuss that viral infections leave the fatigue and mental stress after the infection .So, this topic requires further studies to understand the true reasons for the condition of mental stress and tiredness. Compared to men, women underwent high levels of mental stress, this condition was seen in the women, especially those who had intermediate jobs and even women who were at home. Women in developing countries are more vulnerable to risks associated with health, especially where health care facilities are not well developed. Women caretakers working in such poorly developed places, have an increased risk of infection, since their roles include taking care of the diseased and elderly. Evidences from past pandemics have shown that, one of the biggest contributor for the increased rate of infection in female is their care responsibilities [88,89, 90]. The effects on women doesn't limit itself to just infection, but has impacted women's health significantly, especially pregnant women. Reports from crisis faced in the past have clearly shown that health systems are a huge failure at delivering timely and proper maternal health services, which includes prenatal care as well as delivery [91, 92]. Additionally, women in the first trimester of pregnancy was reported to be more anxious and had psychological impact than the other two trimesters during covid 19 [86]. The multiple measures taken to combat the spread of the disease, and the fear of infection in the health care facilities in turn reduces the chances of women visiting the maternal health services, and therefore increases the possibility of unattended birth [91].

CONCLUSION

COVID-19 has proven to have caused huge havoc in the lives of people majorly among women. This review has given sufficient evidence and facts suggesting the miseries and hardships women had to undergo such as in the field of health caretakers, as domestic helpers in households, physical and mental distress and abuse, losing jobs, and leading a life of poverty due to unemployment. The socioeconomic status of women, when compared to men, has shown that women are in a very low economic independent status in this society before the pre-pandemic time but has fallen even more severely during this current pandemic making them dependent on their family members which in turn later during time may subject them towards domestic violence and even suicide due to depression affecting the mental health of women. Therefore it is suggested that women try to make themselves more independent socially and economically so as to cope with the uncertainties that this pandemic brings and make themselves stronger both mentally and physically.

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REFERENCES

1. F. Thibaut and P. J. M. van Wijngaarden-Cremers, "Women's mental health in the time of Covid-19 pandemic," *Front Glob Womens Health*, vol. 1, p. 588372, Dec. 2020.
2. N. Salari *et al.*, "Prevalence of stress, anxiety, depression among the general population during the COVID-19 pandemic: a systematic review and meta-analysis," *Global. Health*, vol. 16, no. 1, p. 57, Jul. 2020.
3. K. M. Osland, M. G. Røysamb, and J. Nortvedt, "The impact of covid-19 on the women, peace and security agenda," Norwegian Institute of International Affairs (NUPI), 2020.
4. D. Lefkowitz and J. S. Armin, "Why employment during and after COVID-19 is a critical women's health issue," *Womens. Health Issues*, vol. 31, no. 3, pp. 190–194, May 2021.
5. T. A. Pigott, "Anxiety disorders in women," *Psychiatr. Clin. North Am.*, vol. 26, no. 3, pp. 621–672, Sep. 2003.
6. P. Ferrara *et al.*, "Femicide and murdered women's children: which future for these children orphans of a living parent?," *Ital. J. Pediatr.*, vol. 41, no. 1, p. 68, Sep. 2015.
7. J. E. Yavorsky, C. M. K. Dush, and S. J. Schoppe-Sullivan, "The production of inequality: The gender division of labor across the transition to parenthood," *J. Marriage Fam.*, vol. 77, no. 3, pp. 662–679, Jun. 2015.
8. L. K. Hamberger and S. E. Larsen, "Men's and women's experience of intimate partner violence: A review of ten years of comparative studies in clinical samples; Part I," *J. Fam. Violence*, vol. 30, no. 6, pp. 699–717, Aug. 2015.
9. B. W. Lex, "Alcohol and other drug abuse among women," *Alcohol Health Res. World*, vol. 18, no. 3, pp. 212–219, 1994.
10. E. Tuchman, "Women and addiction: the importance of gender issues in substance abuse research," *J. Addict. Dis.*, vol. 29, no. 2, pp. 127–138, Apr. 2010.
11. B. Javed, A. Sarwer, E. B. Soto, and Z.-U.-R. Mashwani, "The coronavirus (COVID-19) pandemic's impact on mental health," *Int. J. Health Plann. Manage.*, vol. 35, no. 5, pp. 993–996, Sep. 2020.
12. K. J. Golden L., "The involuntary part-time work and underemployment problem in the U.S," 2020.
13. J. Connor *et al.*, "Health risks and outcomes that disproportionately affect women during the Covid-19 pandemic: A review," *Soc. Sci. Med.*, vol. 266, no. 113364, p. 113364, Dec. 2020.

14. I. Escudero-Castillo, F. J. Mato-Díaz, and A. Rodriguez-Alvarez, "Furloughs, teleworking and other work situations during the COVID-19 lockdown: Impact on mental well-being," *Int. J. Environ. Res. Public Health*, vol. 18, no. 6, p. 2898, Mar. 2021.
15. J. John, "Modeling years of life lost due to COVID-19, socioeconomic status, and nonpharmaceutical interventions: Development of a prediction model," *JMIRx Med*, vol. 3, no. 2, p. e30144, Apr. 2022.
16. I. Jalnapurkar and University of Texas Health Science Center at Houston, "Sex differences in anxiety disorders: A review," *Psychiatry Depress. Anxiety*, vol. 4, pp. 1–9, Jul. 2018.
17. P. Dashraath *et al.*, "Coronavirus disease 2019 (COVID-19) pandemic and pregnancy," *Obstet. Anesth. Dig.*, vol. 41, no. 1, pp. 7–7, Mar. 2021.
18. S. Awan *et al.*, "Suicide in healthcare workers: Determinants, challenges, and the impact of COVID-19," *Front. Psychiatry*, vol. 12, p. 792925, 2021.
19. V. K.-N. &. Tajik, Ed., "Female-headed households bear the brunt of Covid-19 as livelihood gaps increase," in *UNICEF*, 2020.
20. D. Harvey, "Anti-Capitalist Politics in the Time of COVID-19," 20-Mar-2020. [Online]. Available: https://jacobin.com/2020/03/david-harvey-coronavirus-political-economy-disruptions.
21. R. El-Serag and R. C. Thurston, "Matters of the heart and mind: Interpersonal violence and cardiovascular disease in women," *Journal of the American Heart Association*, vol. 9, no. 4. Ovid Technologies (Wolters Kluwer Health), p. e015479, 18-Feb-2020.
22. Y. S. Sharma, "National database of workers in informal sector in the works," *The Economic Times*, 19-Jan-2020.
23. S. Malik and K. Naeem, "Impact of COVID-19 Pandemic on Women: Health, livelihoods & domestic violence," May-2020. [Online]. Available: https://think-asia.org/handle/11540/11907.
24. A. P. Miller and E. al., "Exploring the impact of COVID-19 on women's alcohol use, mental health, and experiences of intimate partner violence in Wakiso, Uganda," *CrimRxiv*, Feb. 2022.
25. D. Qiongfang, "Chinese families see surge in domestic violence amid COVID-19 lockdown," 12-Mar-2020. [Online]. Available: https://www.globaltimes.cn/page/202003/1182484.shtml.
26. I. Gani, "Poverty of Women and the Covid-19 Pandemic in Indonesia," *Budapest International Research and Critics Institute (BIRCI-Journal): Humanities and Social Sciences*, vol. 4, no. 1, pp. 1034–1041, Feb. 2021.
27. "How COVID-19 may increase domestic violence and child," *https://www.apa.org › topics › domestic-violence-child-...https://www.apa.org › topics › domestic-violence-child-...*
28. K. Shah, D. Kamrai, H. Mekala, B. Mann, K. Desai, and R. S. Patel, "Focus on Mental Health During the Coronavirus (COVID-19) Pandemic: Applying Learnings from the Past Outbreaks," *Cureus*, vol. 12, no. 3, p. e7405, Mar. 2020.
29. Y. Broche-Pérez, Z. Fernández-Fleites, E. Jiménez-Puig, E. Fernández-Castillo, and B. C. Rodríguez-Martin, "Gender and Fear of COVID-19 in a Cuban Population Sample," *Int. J. Ment. Health Addict.*, pp. 1–9, Jun. 2020.
30. S. Germain and A. Yong, "COVID-19 highlighting inequalities in access to healthcare in England: A case study of ethnic minority and migrant women," *Fem. Leg. Stud.*, vol. 28, no. 3, pp. 301–310, Oct. 2020.
31. M. Nyashanu, R. Chireshe, F. Mushawa, and M. S. Ekpenyong, "Exploring the challenges of women taking antiretroviral treatment during the COVID-19 pandemic lockdown in peri-urban Harare, Zimbabwe," *Int. J. Gynaecol. Obstet.*, vol. 154, no. 2, pp. 220–226, Aug. 2021.
32. COVID-19 will widen poverty gap between women and men, new UN Women and UNDP data shows," *UNDP*. [Online]. Available: https://www.undp.org/press-releases/covid-19-will-widen-poverty-gap-between-women-and-men-new-un-women-and-undp-data. [Accessed: 01-Jun-2022].
33. From insights to action: Gender equality in the wake of COVID-19," *UN Women – Headquarters*. [Online]. Available: https://www.unwomen.org/en/digital-library/publications/2020/09/gender-equality-in-the-wake-of-covid-19. [Accessed: 01-Jun-2022].
34. S. Chakraborty, "Increased Care Work, Reduced Wages: Informal Women Workers Are Barely Getting By," *The Wire*. [Online]. Available: https://thewire.in/women/women-informal-workers-lockdown. [Accessed: 01-Jun-2022].

35. "Female-headed households bear the brunt of Covid-19 as livelihood gaps increase." [Online]. Available: https://www.unicef.org/rosa/stories/female-headed-households-bear-brunt-covid-19-livelihood-gaps-increase. [Accessed: 01-Jun-2022].
36. M. Mazza, G. Marano, C. Lai, L. Janiri, and G. Sani, "Danger in danger: Interpersonal violence during COVID-19 quarantine," *Psychiatry Res.*, vol. 289, p. 113046, Jul. 2020.
37. E. S. Marques, C. L. de Moraes, M. H. Hasselmann, S. F. Deslandes, and M. E. Reichenheim, "Violence against women, children, and adolescents during the COVID-19 pandemic: overview, contributing factors, and mitigating measures," *Cad. Saude Publica*, vol. 36, no. 4, p. e00074420, Apr. 2020.
38. A. Clarke, P. Olive, N. Akooji, and K. Whittaker, "Violence exposure and young people's vulnerability, mental and physical health," *Int. J. Public Health*, vol. 65, no. 3, pp. 357–366, Apr. 2020.
39. J. S. Chandan, T. Thomas, C. Bradbury-Jones, J. Taylor, S. Bandyopadhyay, and K. Nirantharakumar, "Risk of Cardiometabolic Disease and All-Cause Mortality in Female Survivors of Domestic Abuse," *J. Am. Heart Assoc.*, vol. 9, no. 4, p. e014580, Feb. 2020.
40. A. M. Campbell, "An increasing risk of family violence during the Covid-19 pandemic: Strengthening community collaborations to save lives," *Forensic Science International. Reports*, vol. 2, p. 100089, Dec. 2020.
41. L. M. A. Reuters, "Confinement : les violences conjugales en hausse, un dispositif d'alerte mis en place dans les pharmacies," *Le Monde*, Le Monde, 27-Mar-2020.
42. L. M. Rodriguez, D. M. Litt, and S. H. Stewart, "Drinking to cope with the pandemic: The unique associations of COVID-19-related perceived threat and psychological distress to drinking behaviors in American men and women," *Addict. Behav.*, vol. 110, p. 106532, Nov. 2020.
43. Z. Zou, H. Wang, F. d'Oleire Uquillas, X. Wang, J. Ding, and H. Chen, "Definition of Substance and Non-substance Addiction," *Adv. Exp. Med. Biol.*, vol. 1010, pp. 21–41, 2017.
44. A. M. Shillington, S. I. Woodruff, J. D. Clapp, M. B. Reed, and H. Lemus, "Self-Reported Age of Onset and Telescoping for Cigarettes, Alcohol, and Marijuana Across Eight Years of the National Longitudinal Survey of Youth," *J. Child Adolesc. Subst. Abuse*, vol. 21, no. 4, pp. 333–348, Sep. 2012.
45. D. M. Kazemi, B. Borsari, M. J. Levine, S. Li, K. A. Lamberson, and L. A. Matta, "A Systematic Review of the mHealth Interventions to Prevent Alcohol and Substance Abuse," *J. Health Commun.*, vol. 22, no. 5, pp. 413–432, May 2017.
46. D. Jutras-Aswad, "Substance Use and Misuse: Emerging Epidemiological Trends, New Definitions, and Innovative Treatment Targets," *Can. J. Psychiatry*, vol. 61, no. 3, pp. 134–135, Mar. 2016.
47. W. Li, M. O. Howard, E. L. Garland, P. McGovern, and M. Lazar, "Mindfulness treatment for substance misuse: A systematic review and meta-analysis," *J. Subst. Abuse Treat.*, vol. 75, pp. 62–96, Apr. 2017.
48. T. MacMillan, M. J. Corrigan, K. Coffey, C. D. Tronnier, D. Wang, and K. Krase, "Exploring Factors Associated with Alcohol and/or Substance Use During the COVID-19 Pandemic," *Int. J. Ment. Health Addict.*, Jan. 2021.
49. P. M. Mauro, S. L. Canham, S. S. Martins, and A. P. Spira, "Substance-use coping and self-rated health among US middle-aged and older adults," *Addict. Behav.*, vol. 42, pp. 96–100, Mar. 2015.
50. R. K. McHugh, V. R. Votaw, D. E. Sugarman, and S. F. Greenfield, "Sex and gender differences in substance use disorders," *Clin. Psychol. Rev.*, vol. 66, pp. 12–23, Dec. 2018.
51. K. G. Rice and A. C. Van Arsdale, "Perfectionism, perceived stress, drinking to cope, and alcohol-related problems among college students," *J. Couns. Psychol.*, vol. 57, no. 4, pp. 439–450, Oct. 2010.
52. S. Nolen-Hoeksema and Z. A. Harrell, "Rumination, depression, and alcohol use: Tests of gender differences," *J. Cogn. Psychother.*, vol. 16, no. 2002, pp. 391–403, 2002.
53. A. Devoto, M. Himelein-Wachowiak, T. Liu, and B. Curtis, "Women's Substance Use and Mental Health During the COVID-19 Pandemic," *Womens. Health Issues*, vol. 32, no. 3, pp. 235–240, May 2022.
54. A. Sriharan *et al.*, "Occupational Stress, Burnout, and Depression in Women in Healthcare During COVID-19 Pandemic: Rapid Scoping Review," *Front Glob Womens Health*, vol. 1, p. 596690, Nov. 2020.
55. J. McArdle, "Stress and burnout warning over COVID-19," *British Medical Association*, 19-Apr-2020. [Online]. Available: https://www.bma.org.uk/news-and-opinion/stress-and-burnout-warning-over-covid-19. [Accessed: 01-Jun-2022].

56. R. Y. Elbay, A. Kurtulmuş, S. Arpacıoğlu, and E. Karadere, "Depression, anxiety, stress levels of physicians and associated factors in Covid-19 pandemics," *Psychiatry Res.*, vol. 290, p. 113130, Aug. 2020.
57. R. H. Almaghrabi, H. A. Alfaraidi, W. A. Al Hebshi, and M. M. Albaadani, "Healthcare workers experience in dealing with Coronavirus (COVID-19) pandemic," *Saudi Med. J.*, vol. 41, no. 6, pp. 657–660, Jun. 2020.
58. Q. Yin *et al.*, "Posttraumatic stress symptoms of health care workers during the corona virus disease 2019," *Clin. Psychol. Psychother.*, vol. 27, no. 3, pp. 384–395, May 2020.
59. L. Kang *et al.*, "Impact on mental health and perceptions of psychological care among medical and nursing staff in Wuhan during the 2019 novel coronavirus disease outbreak: A cross-sectional study," *Brain Behav. Immun.*, vol. 87, pp. 11–17, Jul. 2020.
60. J. Zhu *et al.*, "Prevalence and Influencing Factors of Anxiety and Depression Symptoms in the First-Line Medical Staff Fighting Against COVID-19 in Gansu," *Front. Psychiatry*, vol. 11, p. 386, Apr. 2020.
61. C.-Y. Liu *et al.*, "The prevalence and influencing factors in anxiety in medical workers fighting COVID-19 in China: a cross-sectional survey," *Epidemiol. Infect.*, vol. 148, p. e98, May 2020.
62. "ILO Monitor: COVID-19 and the world of work. Eighth edition Updated estimates and analysis," 27-Oct-2021. [Online]. Available: https://www.ilo.org/wcmsp5/groups/public/---dgreports/---dcomm/documents/briefingnote/wcms_824092.pdf.
63. Women Working in Informal Sector in India : A saga of ...WOMEN WORKING IN INFORMAL SECTOR IN INDIA: A SAGA OF," *https://www.semanticscholar.org › paper*
64. S. Sengupta and M. K. Jha, "Social Policy, COVID-19 and Impoverished Migrants: Challenges and Prospects in Locked Down India," *The International Journal of Community and Social Development*, vol. 2, no. 2, pp. 152–172, Jun. 2020.
65. S. Pachauri, "COVID-19 outbreak brings attention back to informal sector." [Online]. Available: https://www.downtoearth.org.in/blog/urbanisation/covid-19-outbreak-brings-attention-back-to-informal-sector-69947. [Accessed: 01-Jun-2022].
66. "OECD Income (IDD) and Wealth (WDD) Distribution Databases - OECD." [Online]. Available: https://www.oecd.org/social/income-distribution-database.htm. [Accessed: 01-Jun-2022].
67. "Gender Differences in Poverty and Household Composition ...Gender Differences in Poverty and ... - World Bank eLibrary," *https://openknowledge.worldbank.org › handlehttps://openknowledge.worldbank.org › handlehttps://elibrary.worldbank.org › doi › abshttps://elibrary.worldbank.org › doi › abs*.
68. A. Schneebaum, M. Rehm, K. Mader, and K. Hollan, "The gender wealth gap across European countries," *Rev. Income Wealth*, vol. 64, no. 2, pp. 295–331, Jun. 2018.
69. E. M. Sierminska, J. R. Frick, and M. M. Grabka, "Examining the gender wealth gap," *Oxf. Econ. Pap.*, vol. 62, no. 4, pp. 669–690, 2010.
70. Y. Chzhen, "Child Poverty and Material Deprivation in the European Union during the Great Recession," *Papers*, 2014.
71. M. Boniol, M. McIsaac, L. Xu, T. Wuliji, K. Diallo, and J. Campbell, "Gender equity in the health workforce: analysis of 104 countries," World Health Organization, WHO/HIS/HWF/Gender/WP1/2019.1, 2019.
72. P. J. Victor, K. P. Mathews, H. Paul, J. J. Mammen, and M. Murugesan, "Protective Effect of COVID-19 Vaccine Among Health Care Workers During the Second Wave of the Pandemic in India," *Mayo Clin. Proc.*, vol. 96, no. 9, pp. 2493–2494, Sep. 2021.
73. B. Kotlar, E. Gerson, S. Petrillo, A. Langer, and H. Tiemeier, "The impact of the COVID-19 pandemic on maternal and perinatal health: a scoping review," *Reprod. Health*, vol. 18, no. 1, p. 10, Jan. 2021.
74. S. Upadhaya, J. X. Yu, C. Oliva, M. Hooton, J. Hodge, and V. M. Hubbard-Lucey, "Impact of COVID-19 on oncology clinical trials," *Nat. Rev. Drug Discov.*, vol. 19, no. 6, pp. 376–377, May 2020.
75. S. Napier-Raman, A. Rattani, Y. Qaiyum, V. Bose, R. Seth, and S. Raman, "Impact of COVID-19 on the lives of vulnerable young people in New Delhi, India: a mixed method study," *BMJ Paediatr Open*, vol. 5, no. 1, p. e001171, Jul. 2021.
76. S. K. Sagar *et al.*, "Mental health status of married women during COVID-19 pandemic in Bangladesh: A cross-sectional study," *Heliyon*, vol. 8, no. 1, p. e08785, Jan. 2022.

77. C. L. Scherr *et al.*, "How patients deal with an ambiguous medical test: Decision-making after genetic testing," *Patient Educ. Couns.*, vol. 104, no. 5, pp. 953–959, May 2021.
78. O. Alagoz *et al.*, "Impact of the COVID-19 Pandemic on Breast Cancer Mortality in the US: Estimates From Collaborative Simulation Modeling," *J. Natl. Cancer Inst.*, vol. 113, no. 11, pp. 1484–1494, Nov. 2021.
79. A. Edelman *et al.*, "Association Between Menstrual Cycle Length and Coronavirus Disease 2019 (COVID-19) Vaccination: A U.S. Cohort," *Obstetrics & Gynecology*, p. 10.1097/AOG.0000000000004695, May 2022.
80. A. Popescu *et al.*, "COVID-19 Pandemic Effects on Cervical Cancer Diagnosis and Management: A Population-Based Study in Romania," *Diagnostics (Basel)*, vol. 12, no. 4, Apr. 2022.
81. "Breastfeeding and COVID-19." [Online]. Available: https://www.who.int/news-room/commentaries/detail/breastfeeding-and-covid-19. [Accessed: 01-Jun-2022].
82. L. Dong *et al.*, "Possible Vertical Transmission of SARS-CoV-2 From an Infected Mother to Her Newborn," *JAMA*, vol. 323, no. 18, pp. 1846–1848, May 2020.
83. C. Wenham, J. Smith, R. Morgan, and Gender and COVID-19 Working Group, "COVID-19: the gendered impacts of the outbreak," *Lancet*, vol. 395, no. 10227, pp. 846–848, Mar. 2020.
84. M. Olff, "Sex and gender differences in post-traumatic stress disorder: an update," *Eur. J. Psychotraumatol.*, vol. 8, no. sup4, 2017.
85. A. Biaggi, S. Conroy, S. Pawlby, and C. M. Pariante, "Identifying the women at risk of antenatal anxiety and depression: A systematic review," *J. Affect. Disord.*, vol. 191, pp. 62–77, Feb. 2016.
86. G. Saccone *et al.*, "Psychological impact of coronavirus disease 2019 in pregnant women," *Am. J. Obstet. Gynecol.*, vol. 223, no. 2, pp. 293–295, Aug. 2020.
87. V. Beri, "Impact of COVID-19 on mental health of employed women residing in Delhi-NCR, India: Mediating role of working from-for home," *Health Care Women Int.*, vol. 42, no. 3, pp. 323–334, Mar. 2021.
88. S. E. Davies and B. Bennett, "A gendered human rights analysis of Ebola and Zika: locating gender in global health emergencies," *Int. Aff.*, vol. 92, no. 5, pp. 1041–1060, Sep. 2016.
89. C. Menéndez, A. Lucas, K. Munguambe, and A. Langer, "Ebola crisis: the unequal impact on women and children's health," *Lancet Glob Health*, vol. 3, no. 3, p. e130, Mar. 2015.
90. M. N. Nkangu, O. A. Olatunde, and S. Yaya, "The perspective of gender on the Ebola virus using a risk management and population health framework: a scoping review," *Infect Dis Poverty*, vol. 6, no. 1, p. 135, Oct. 2017.
91. K. J. Brolin Ribacke, D. D. Saulnier, A. Eriksson, and J. von Schreeb, "Effects of the West Africa Ebola Virus Disease on Health-Care Utilization - A Systematic Review," *Front Public Health*, vol. 4, p. 222, Oct. 2016.
92. L. Sochas, A. A. Channon, and S. Nam, "Counting indirect crisis-related deaths in the context of a low-resilience health system: the case of maternal and neonatal health during the Ebola epidemic in Sierra Leone," *Health Policy Plan.*, vol. 32, no. suppl_3, pp. iii32–iii39, Nov. 2017.

Table 1.Challenges Among Women's Population During Pandemic

S. No	Type of factors	Month /year	Reference
1	Corona virus disease 2019 (COVID-19) pandemic and pregnancy	March 2020	[17]
2	Nurses and staff in the emergency room and idea of suicide	February2022	[18]
3	Responsibilities in the family including childcare, telecommuting	March 2021	[14]
4	Lack of financial resources: a negative impact on women's households	April 2020	[19]
5.	Women facing more unemployment due to COVID-19	May 2020	[12]
6	Gender-specific discrimination and vulnerabilities among women population	March 2020	[20]
7	High rates of feminised due to domestic violence	February2020	[21]
8	Womens employed under informal sector	June 2020	[22]
9	Widespread increase in anxiety, stress and depression among women.	May 2020	[23]
10	The Impact of COVID19 on the Women, Peace and Security Agenda	May 2020	[3]
11	Alcohol use, mental health, and experiences of intimate partner	February,	[24]

	violence	2022	
12	Girl child was trapped with the abusers at home	March, 2020	[25]
13	Women are more vulnerable to poverty	February, 2021	[26]
14	COVID-19 may increase domestic violence and child abuse.	April,2020	[27]
15	How Covid-19 is amplifying gender inequality in India	May, 2020	[28]
16	Women and children in nuclear families are highly vulnerable to domestic violence	April , 2020	[15]
17	women leading and prolonged hours of work shift leading to a higher risk of mental pressure : COVID -19	December, 2020	[1]
18	High levels of fear of COVID-19 in women compared to men	June,2020	[29]
19	Treating women as homogeneous entity; resulted in ignorance of individual needs among several women groups	October, 2020	[30]
20	Women exposed to stress with managing children indoors	August, 2021	[31]

Table 2.Last three years' data of extrinsic factors affecting women during the COVID-19

S. No	Factors affecting to women's health/type of factors	Year /month	References
1	How patients deal with an ambiguous medical test: Decision-making after genetic testing	2021, May	[77]
2.	Pregnant women and stress: COVID-2019	2021, January	[73]
3.	The incidence of symptomatic COVID-19 disease among health care workers female to male	2021, May	[72]
4	Cardiovascular disease, sleep disturbance, high anxiety levels due to high substance usage	September to November 2020	[53]
5	Domestic violence among adult women cause long term mental disorders	February 2020	[21]
6	Impact of the COVID-19 Pandemic on Breast Cancer Mortality	November 2021	[78]
7	COVID -19 effect on menstrual cycle	April 2022	[79]
8	COVID-19 effect on cervical cancer diagnosis	April 2022	[80]
9	The postponement of having children among couples created lots of mental stress	November 2020	[74]
10	Unwanted pregnancy among economically low background women suffered even to feed their children.	July 2021	[75]
11	This pandemic having tougher mental impact on women	December, 2018	[16]
12	COVID19 and breastfeeding: not that simple	May,2020	[81]
13	Possible vertical transmission of SARS-CoV-2 from an infected mother to her newborn	May,2020	[82]
14	Failure of proper maternal health services: COVID-19	March,2020	[83]
15	Higher levels of Post-traumatic stress disorder symptoms were observed in women compared to men	November, 2020	[84]
16	Improper response of health care to the needs of pregnant womens affected their physical, mental and spiritual needs.	Pandemic lockdown, 2020	[85]
17	High psychological impact and anxiety of women in the first trimester of pregnancy	August, 2020	[86]
18	Notable interaction between the marital status and working from home on depressive tendencies	January 2021	[87]

Figure 1: Factors influencing women's mental health

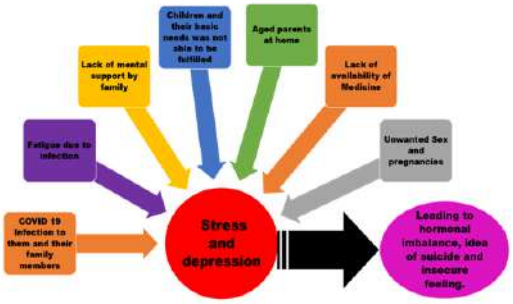

Figure 2: Extrinsic factors affecting the mental health of women.

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REVIEW ARTICLE

Maternal Postpartum Depression: The Stepping Stone for Inducing Depressive Symptoms in Fathers

Divya Dhull[1]*, Charu Dhankar[2] and Gargi Sharma[2]

[1]Research Scholar, Department of Psychology, Manipal University Jaipur, Rajasthan, India.
[2]Assistant Professor, Department of Psychology, Manipal University Jaipur, Rajasthan, India.

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***Address for Correspondence**
Divya Dhull,
Research Scholar,
Department of Psychology,
Manipal University Jaipur,
Rajasthan, India.
Email: 27dhull@gmail.com

ABSTRACT

Parenthood, which is considered as the biggest pleasure, does come with its own struggles resulting in the symptoms of depression. As the joy of becoming parent is not limited to mother only, so is the struggling side. The purpose of this investigation is to study a) the relationship between maternal postpartum depression and paternal postpartum depression b) relationship between maternal postpartum depression and paternal perceived stress c) relationship between maternal postpartum depression and paternal sleep d) Relationship between personality of father and maternal postpartum depression. In order to achieve the desired objective numerous research studies were reviewed from various databases like EBSCO, EMERALD, PUB MED, MED LINE, SCIENCE DIRECT and GOOGLE SCHOLAR.

Keywords: maternal postpartum depression, paternal postpartum depression, perceived stress, sleep, marital relation

INTRODUCTION

Feminism is on its peak nowadays and so are the issues related to women. But in this we likely to forget that men too can be a victim of similar issue. Through pre and post natal visits women are given support and education about the probable postnatal disorders waiting for them whereas there is a lot of ignorance on creating awareness for similar issue among other members of family and even to the fathers (Murray & McKinney, 2013). It was also indicated in research about the ignored treatment regarding the awareness of postnatal issues for the new father and rest of the family members during the postpartum period (Camp, 2013). Their role is least considered which is a matter of

concern. APA (2015) stated that due to mood disorders it becomes difficult for a new mother to handle the things and baby, due to which more responsibility comes on father's shoulders. In ICD 10, Coding for the Post partum Depression in ICD 10 is ICD-10-CM 090.6. In DSM 5, it is classified in **Bipolar Disorder or Depression with Peripartum onset. The reason defined in DSM 5 for such classification is that** fifty percent of "postpartum" major depressive episodes actually begin prior to delivery. "The overall pooled estimate of the prevalence of postpartum depression in Indian mothers was 22%." (Upadhyay *et al*., 2017). "Studies suggest anywhere from 4 to 25 percent of fathers experience paternal PPD, 2, 3 rates that are not dissimilar to mothers". (Wee *et al.,* 2011).

Men concerned with their image of masculanity, stigmatize on being vocal on depressive symptoms and hence under report their symptoms (Fisher, 2016)and doesn't accept the reality of suffering from postpartum depression. "Maternal depression was identified as the strongest predictor of paternal depression during the postpartum period". (Goodman, 2004). The stress faced by mother during post natal symptoms emphasize the father to take care of infant. "Maternal PND affects fathers in negative ways, as evidenced by higher levels of depression and parent stress" (Goodman, 2008). Several studies have found that the main factors associated with depression among fathers during the postnatal period are: reduced satisfaction with the relationship to the mother and the mother being depressed or reporting a high degree of depressive symptoms. Other risk factors which were found associated with maternal postpartum depression were father's aggressive behavior, domestic violence, substance abuse and the financial stress, which are also associated with paternal depression too (Letourneau et. al, 2012). Several studies have found the detrimental effects of paternal postpartum depression on emotional, behavioral and cognitive development of child. Gutierrez-Galve, Heron, & Ramchandani, 2015; Ramchandani *et al.,* 2008; Wilson & Durbin, 2010; Edward *et al*., 2015). There are plenty of consequences which mother, father, family or the infant can face if the parents both or any of the parent is suffering from post natal mood disorders. Numerous researches is done over the topic. Here in the study, the idea is to explore about the individual risk factors of father which can further aggravate the symptoms of depression in mothers. The current research study has the goal to identify the relationship of depression in mothers with that of stress perceived by father, paternal sleep, postpartum depression in fathers and the personality of fathers during the postnatal period.

METHODS

This research paper utilized appropriate sources of texts for review. Papers chosen for study are by screening titles and reading of abstracts. The research studies were made to be part of the current review study based on the following criteria: (1) Research on correlates of maternal postpartum depression and paternal postpartum depression, (2) Studies on relationship of paternal sleep with depression in mothers post child birth, (3) research on relationship of postnatal depression of mothers with paternal perceived stress (4) research on relationship of paternal postpartum depression with paternal sleep and paternal perceived stress (5) research on relationship of mother's postnatal depression with the personality of father (6) research based on analyzing the relationship between maternal postpartum depression and the marital relationship of couple. Papers included for the review are only those papers which contained the relevant content for the study. Some papers are exclusively used as source for writing about the background information whereas maximum remaining research papers played their part for the systematic review of the factors.

REVIEW OF LITERATURE

Rigorous search for the literature was carried out to find out the relevant studies which helped to explore the relationship of postnatal depression related to mothers and fathers and the other associated factors. Kamalifard *et al.,* (2018) The study described the direct and strong association in the scores of mothers and fathers on the scale of EPDS. Anding *et al.,* (2016) In the research paper the depressive symptoms in the mothers and fathers was analyzed with help of EPDS to find out the prevalence of postpartum depression among them. The cut off score taken for mother was more than 12 and for father it was 10.The prevalence rate thus found was 15.9% for mothers and 5.4% for

fathers. Also a positive association in the scores of mothers and fathers was reported. Further parental stress was found as the greatest predictor of depressive symptoms in both mothers and fathers. Nishimura *et al.,* (2015) The research concluded that depressive symptoms in fathers are positively correlated with the depression among their spouse. In Japan the prevalence rate of depression in fathers during postpartum period was reported as 13.6%, which is not less. Another factor which was found highly correlated with paternal postpartrum depression was the disrupted marital relations among the parent couple. Mao *et al*.,(2009) The study claimed that the similar level of stress is experienced by fathers as it is experienced by mothers during the post partum period but the support received is negligible in comparison to mothers. Perceived stress by both mothers and fathers, the social support received and the respective partner's scores on the scale of EPDS were made to analyze to find out the association of mentioned factors with the depressive symptoms in mothers or fathers as individuals. Kamalifard *et al.,(*2014) The postpartum depression in fathers and the relationship of it with perceived stress and components of social stress was assessed in the study. Results indicated the positive correlation among the analyzed factors.

Since paternal postpartum depression has a relationship with perceived stress and from the studies it is also evident that maternal postpartum depression has a relationship with paternal post partum depression. Hence it can be concluded that maternal postpartum depression shares a relationship with paternal perceived stress. Costa et.al (2019) At 2 months of post partum period, the prevalence rate of depressive symptoms in father was 13.76% while at 6 months it was 13.6%. Sleep of fathers was assessed using Pitsburgh Sleep Quality Index, which included 19 items and generated scores in seven elements i.e : sleep latency, duration of sleep, persistent sleep efficiency, disturbances in sleep, subjective sleep quality, daytime dysfunction and intake of sleeping pills.. "The risk of paternal depressive symptoms at 2 months postpartum increased for men who concurrently had worse sleep quality (OR,1.25; CI:1.10,1.42), poorer couple relationship adjustment (OR,0.97; CI:0.94,0.99), and higher parenting stress (OR,1.07; CI:1.02,1.11). Unemployment (OR,3.75; CI:1.00,13.72), poorer sleep quality (OR,1.37; CI:1.16,1.65), lower social support (OR,0.92; CI;0.84,1.00), poorer couple relationship adjustment (OR,0.95, CI:0.92,0.98) and higher financial stress (OR,1.21, CI:1.04,1.42), assessed concurrently were all significantly associated with paternal depressive symptoms at 6 months postpartum."

Dana, Inai, and Iat (2012). Sleep of infants was correlated with that of fathers and mothers sleep to identify the correlation. Results confirmed that the frequency and duration of walking up at night of mothers and fathers were positively correlated with the infants."Using paired sample t-tests, a significant difference was found between infants' and fathers' number of night time waking, $t(49) = 7.282$, $p < .0001$, infants' and father's amount of night time wakefulness, $t(49) = 9.668$, $p < .0001$." In the fathers who complaint infant sleep as problem causing, the parenting stress was reported higher in those fathers. *Et al.,(*2017) Research depicted the prevalence of insomnia of sleep deficiency among parents during the gestation period. It was further emphasized over the required intervention or preventive measure during pregnancy to avoid the consequences. Maghaireh *et al*.,(2017) The study investigated The Jordian parents with their infants in NICU for their stress level and the stress inducing factors. The relationship of those stressors was further investigated with the factors like anxiety, sleep and depression. It was a cross sectional survey on 310 parents from two hospitals in Jordan. Results indicated the presence of high level of all the mentioned three factors. Hall, Moynihan, Bhagat, and Wooldridge (2017) In their study on sleep, the sleep of mothers and fathers was linked to that of infant. Results demonstrated that there lies a complex relation between depression scores and the parents cognition regarding infant's sleep.

Montgomery , Stremler and Insana (2013) The review study explained that after child birth there is fatigue which is universal and even the sleep is not proper and highly disturbed. It further stated about the contribution of high fatigue and sleeplessness in the onset of agitation. The disturbed moods are highly correlated with bad interaction of parents with child which is not at all good for the emotional and cognitive development of infant. The research through numerous articles also emphasized about the sleep disturbances faced by fathers during postpartum period. Dhull, Dhankar & Sharma (2019). The study was conducted on 392 new fathers from India where they were analyzed using Perceived stress scale, EPDS and the big 5 inventory. There found a strong positive association between the EPDS score and the perceived stress ($r= 0.517$, $p= 0.000$). On the scale of personality assessment, neuroticism($r=0.381$,

p value= 0.000) and agreeableness(r=0.240, p value= 0.012) was found to share positive correlation with the paternal postpartum depression. Here openness to experience(r= -0.398, p value= 0.000) had inverse correlation with EPDS (Edinburgh Postnatal Depression Scale) scores. There are a few studies which have been conducted on the relationship of maternal postpartum depression with paternal perceived stress and paternal sleep, the investigation is therefore focused on these variables.

DISCUSSION AND CONCLUSION

Usually it is the belief that postpartum depression is the term limited for women as they are birth givers. Detailed investigation in existing research confirmed that it is not only mother who is victim but also the father who get victimized with depression post child birth. The entire articles presented in the current literature review study suggest maternal depression as the strongest predictor of the onset of signs of depression in father after the arrival of child in their life. It was the objective of the research to find out the association of mother's postnatal depression with paternal postpartum depression, paternal sleep and paternal perceived stress and father personality. For the similar reason 20 studies were taken into consideration for minute details. The studies selected for review were mostly related to analysis of the association of depression in the mother's and father's during post natal period. Very few studies were found on the relationship of maternal postpartum depression with paternal sleep and paternal perceived stress. There were sufficient research studies found stating the connection paternal sleep and paternal perceived stress with the depression in fathers. Taken into consideration the mentioned studies and using the Transitive law, it could be concluded that there is a relationship of maternal postpartum depression with paternal postpartum depression, paternal sleep and paternal perceived stress. Studies stating the effect of maternal postpartum depression on that of the marital relation of the couple parent were also found sufficient in number whereas the research exploring the maternal depression and its relationship with the personality of father were really scarce. The limitation of this research is the finite number of research studies evaluating the association of postpartum depression in mothers with paternal sleep and paternal perceived stress. Taken into consideration future research studies can be oriented towards the topic to explore it deeply and minutely.

REFERENCES

1. Anding, J. E., Rohrle, B., Grieshop, M., Schucking, B., Christiansen, H. (2016). Couple comorbidity and correlates of postnatal depressive symptoms in mothers and fathers in the first two weeks following delivery. Doi: 10.1016/j.jad.2015.10.033.
2. Bergstrom, M. (2013). Depressive symptoms in new first-time fathers: Associations with age, sociodemographic characteristics, and antenatal psychological well-being. Birth, 40(1), 32–38.
3. Biebel, K., Alikhan, S (2016). Paternal Postpartum Depression . Journal of Parent and Family Mental Health Volume 1 Issue 1
4. Dana, S., Inai, L., Iat, T., Ikotzky (2012) Infant sleep, parental sleep and parenting stress in families of mothers on maternity leave and in families of working mothers. Infant Behavior and Development Volume 35, Issue 2, Pages 179-186
5. Deborah Da Costa , Coraline Danieli , Michal Abrahamowicz ,Kaberi Dasgupta , Maida Sewitch , Ilka Lowensteyn , Phyllis Zelkowitz , A Prospective Study of Postnatal Depressive Symptoms and Associated Risk Factors in First-Time Fathers, Journal of AffectiveDisorders (2019), doi: https://doi.org/10.1016/j.jad.2019.02.033
6. Dhull, D., Dhankar, C., & Sharma, G. (2020). Paternal Postpartum Depression: An Inaudible Cry.Test Engineering and management. Vol. 82 ISSN: 0193-4120 Page No. 16364-16371
7. Don, B. P., Mickelson, K. D. (2012). Paternal postpartum depression: The role of maternal postpartum depression, spousal support, and relationship satisfaction. Couple and Family Psychology: Research and Practice, 1(4), 323-334. Doi: http://dx.doi.org/10.1037/a0029148
8. Downs, H., E., M., Stremler, R. and Insana, S. P. (2013) Postpartum Sleep in New Mothers and Fathers . The Open Sleep Journal, 6, (Suppl 1: M11) 87-97 87 1874-6209/13

9. Fisher, S.D. (2016). Paternal mental health why is it relevant? American Journal of Lifestyle Medicine. Advance online publication. doi: 10.1177/1559827616629895
10. Gagnon, M., & Oliffe, J. L. (2015). Male depression and suicide: What NPs should know. The Nurse Practitioner, 40(11), 50–55.
11. Gao, L., Chan, S.W., Mao, Q. (2009). Depression, perceived stress, and social support among first-time Chinese mothers and fathers in the postpartum period. Research in Nursing and Health 32, 50–58.
12. Garfield, C.F., Isacco, A., Bartlo, W.D. (2010). Men's health and fatherhood in the urban Midwestern United States. International Journal of Men's Health 9, 161–174.
13. Gawlik, S., Muller, M., Hoffman, L., Dienes, A., Wallwiener, M., Sohn, C., Schlehe, B., Reck, C. (2013). Prevalence of paternal perinatal depressiveness and its link to partnership satisfaction and birth concerns. Archives of Women's Mental Health 17,49– 56.
14. Goodman, J.H., (2004). Paternal postpartum depression, its relationship to maternal postpartum depression, and implications for family health. Journal of Advanced Nursing 45, 26–35.
15. Hall, W. A., Moynihan, M., Bhagat, R. and Wooldridge, J. (2017). Relationships between parental sleep quality, fatigue, cognitions about infant sleep, and parental depression pre and post-intervention for infant behavioral sleep problems. BMC Pregnancy and Childbirth. 17:104 DOI 10.1186/s12884-017-1284x
16. Kumar, S. V., Oliffe, J. L., and Kelly, M. T. Promoting Postpartum Mental Health in Fathers: Recommendations for Nurse Practitioners (2017). American Journal of Men's Health 2018, Vol. 12(2) 221–228. DOI: 10.1177/1557988317744712
17. Kamalifard, M., Hasanpoor, S., Kheiroddin, J., B., Panahi, S., Payan , S., B. (2018)Paternal Postpartum Depression and Its Relationship With Maternal Postpartum Depression. J Holist Nurs Midwifery. 28(2):115-120. https://doi.org/10.29252/HNMJ.28.2.115
18. Kamalifard, M., Hasanpoor, S., Babapour, J., Kheiroddin, S., Panahi, E., Payan, S.,Bayati, A. (2014). Relationship between fathers' depression and perceived social support and stress in postpartum period. Journal of Caring Sciences 3, 57–66.doi: 10.5681/jcs.2014.007
19. Letourneau, N., Duffett-Leger, L., Dennis, C. L., Stewart, M.& Tryphonopoulos, P. D. (2011). Identifying the support needs of fathers affected by post-partum depression: A pilot study. Journal of Psychiatric and Mental Health Nursing, 18(1), 41–47.
20. Maghaireh, D. F. A., Abdullah, K. L., Chong, M. C., Chua, Y. P. and Kawafha, M. M. A. Stress, Anxiety, Depression and Sleep Disturbance among Jordanian Mothers and Fathers of Infants Admitted to Neonatal Intensive Care Unit: A Preliminary Study (2017). Journal of Pediatric Nursing 36 , 132–140
21. Matthey, S., Barnett, B., Howie, P., Kavanagh, D.J. (2003). Diagnosing postpartum depression in mothers and fathers: whatever happened to anxiety? Journal of Affective Disorders 74, 139–147.
22. Musser, A.K., Foli, K.J. (2013). Paternal Postpartum Depression: what Health Care Providers Should Know. Journal of Pediatric Health Care 27, 479–485.
23. Nishimura, A., Fujita, Y., Katsuta, M., Ishihara, A., & Ohashi, K. (2015). Paternal postnatal depression in Japan: An investigation of correlated factors including relationship with a partner. BMC Pregnancy and Childbirth, 15, 128.
24. Nishimura, A., Ohashi, K. (2010). Risk factors of paternal depression in the early postnatal period in Japan. Nursing and Health Sciences 12, 170–176.doi: 10.1111/j.1442- 2018.2010.00513.x)
25. Oliffe, J. L., Bottorff, J. L., & Sarbit, G. (2012). Supporting fathers' efforts to be smoke-free: Program principles. Canadian Journal of Nursing Research, 44(3), 64–82.
26. Paul G. Ramchandani, P. G., Psychogiou, L., Vlachos, H., Iles, J., Sethna, V., Netsi, V., Lodder, V. (2011) Paternal depression: an examination of its links with father, child and family functioning in the postnatal period. Doi: https://doi.org/10.1002/da.20814
27. Paulson, J.F., Bazemore, S.D. (2010). Prenatal and postpartum depression in fathers and its association with maternal depression: a meta-analysis. The Journal of the American Medical Association 303, 1961–1969.
28. Quevedo, L., Silva, R.A., Coelho, F., Pinheiro, K.A., Horta, B.L., Kapczinski, F., Pinheiro, R.T. (2011). Risk of suicide and mixed episode in men in the postpartum period. Journal of Affective Disorders 132, 243–246.

29. Qing, M., Zhu, L. X., Su, X. Y. (2011). A comparison of postnatal depression and related factors between Chinese new mothers and fathers, Journal of Clinical Nursing, 20, 5-6, 645-652.
30. Ramchandani, P.G., Stein, A., O'Connor, T.G., Heron, J., Murray, L., Evans, J. (2008). Depression in men in the postnatal period and later child psychopathology: a population cohort study. Journal of the American Academy of Child and Adolescent Psychiatry 47, 390–398.
31. Ramchandani, P.G., Domoney, J., Sethna, V., Psychogiou, L., Vlachos, H., Murray, L. (2013). Do early father-infant interactions predict the onset of externalizing behaviours in young children? Findings from a longitudinal cohort study. Journal of the American Academy of Child and Adolescent Psychiatry 54, 56–64.
32. Ramchandani, P. G., Psychogiou, L., Vlachos, H., Iles, J., Sethna, V., Netsi, E., & Lodder, A. (2011). Paternal depression:An examination of its links with father, child and family functioning in the postnatal period. Depression and Anxiety, 28(6), 471–477.
33. Upadhyay, R. P., Chowdhury, R., Salehi, A., Sarkar, K., Singh, S. K., Sinha, B., Pawar, A., Rajalakshmi, A. K. and Kumar, A.(2017) Postpartum depression in India: a systematic review and meta-analysis. Bulletin of the World Health Organization 2017;95:706-717C. doi: http://dx.doi.org/10.2471/BLT.17.192237
34. Veskrna, L. (2010). Peripartum depression - does it occur in fathers and does it matter? Journal of Men's Health 4, 420–430.
35. Wee, K.Y., Skouteris, H., Pier, C., Richardson, B., Milgrom, J. (2011). Correlates of prenatal and postnatal depression in fathers: a systematic review. Journal of Affective Disorders 130, 358–377. doi:10.1016/j.jad.2010.06.019.
36. Zhang, Y.P., Zhang, L.L., Wei, H.H., Zhang, Y., Zhang, C.L., Porr, C. (2016). Postpartum depression and the psychosocial predictors in first-time fathers from north western China. Midwifery 35, 47–52.

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RESEARCH ARTICLE

Optimal Battery Selection for Electric Vehicles using ELECTRE

F.X.Edwin Deepak[1], Nivetha Martin[2]* and P.Pandiammal[3]

[1]Associate Professor, Department of Electrical and Electronics Engineering, PSNA College of Engineering and Technology, Dindigul, India.
[2]Assistant Professor, Department of Mathematics, Arul Anandar College (Autonomous), Karumathur, Madurai, Tamil Nadu, India.
[3]Assistant Professor, Department of Mathematics, GTN Arts College (Autonomous), Dindigul, Tamil Nadu, India.

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***Address for Correspondence**
Nivetha Martin,
Assistant Professor,
Department of Mathematics,
Arul Anandar College (Autonomous),
Karumathur, Madurai, Tamil Nadu, India.
Email: nivetha.martin710@gmail.com

ABSTRACT

Due to rising fuel prices and global environmental concerns, the market for the electric vehicle industry has boomed recently. The choice of electric vehicle is determined by the type and characteristics of the batteries used. Several extensive studies have been conducted on the four main types of batteries, but to our knowledge, the battery types have not been classified using multi-criteria decision-making (MCDM) methods. In this paper, the four types of battery namely Lithium-Ion (Li-Ion), Molten Salt (Na-NiCl2), Nickel Metal Hydride (Ni-MH) and Lithium Sulphur (Li-S) are ranked based on the seven prime criteria using the method of ELECTRE I. This paper aims in testing whether the theoretical findings based on simulations agree with the numerical rankings obtained by the MCDM approach. It was observed that the optimal rankings obtained are in alignment with the theoretical perspectives of the researchers. As future work of extension, the same decision making problem shall be dealt with other MCDM methods to determine the consistency of the results obtained in this research work.

Keywords: ELECTRE, battery types, electric vehicles

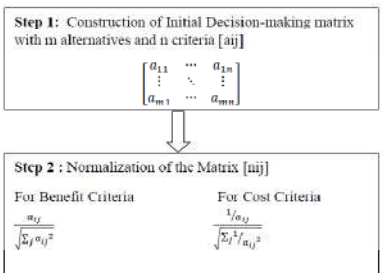
INTRODUCTION

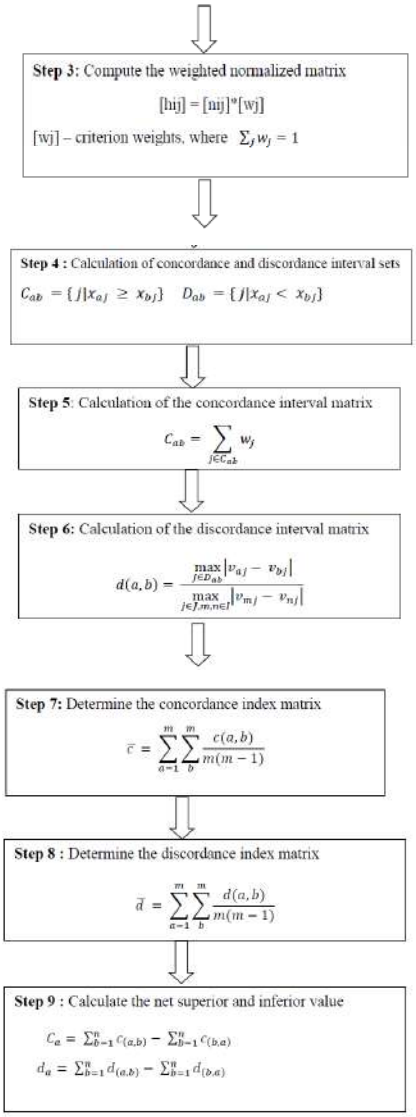
Bernard Roy developed the method of ELECTRE (Elimination and Et Choice Translating Reality) , a multi-criteria decision-making method. The ELECTRE method has several variants and some of the core types are ELECTRE I, II, III, IV, TRI. Researchers like Tiwari *et al.,* [1], Chatterjee *et al.,* [2] have presented the classifications and applications of different ELECTRE methods. From the literature of ELECTRE methods, it is vivid that the ELECTRE method I is the ground for other classifications and the methodology of other ELECTRE types slightly gets deviated from ELECTRE I. It is also found that the ELECTRE method I has extensive applications and it is one of the most preferred methods in comparison to all other ELECTRE types. ELECTRE I has been widely applied in several decision-making scenarios such as natural resources management (David and Duckstein [3], Duckstein and Opricovic [4],Bender and Simonovic [5], Gershon, Duckstein, and McAniff [6], Roy [7], Slowinski [8], Tecle [9], Fogel, and Duckstein[10]), environmental management (Cheng, Chan, and Huang [11], Nijkamp [12], Infante , Mendonca, Purcidonio, and Valle [13], Wu and Chen [14], Banias [15], Augusto [16]), engineering(Thiel [17], Chatterjee, Athawale, and Chakraborty [18], Ulubeyli and Kazaz [19], Bojkovic, Anic, and Tarle [20]), financial management (Zielina [21], Sawicka, Weglinski, and Witort [22], Bergeron, Martel, and Twarabimenye [23]), agriculture (Van Huylenbroeck [24] ,Ahrens and Kantelhardt [25] Arondel and Girardin [26] ,Blanquart [27]) health and safety (Marbini, Tavana, Moradi, and Kangi [28],Martel and D'Avignon [29]) and its related domains.

The literature on the applications of ELECTRE I has shreds of evidence of around 189 research works and this gives a clear picture of the robust nature of ELECTRE I. This has motivated the authors to apply the method of ELECTRE I to make optimal ranking on the electric vehicle battery types. The automobile industry is witnessing a paradigm shift from fuel engines to electric engine systems. The demand for fuel, escalating prices of fuel, environmental hazards of fuel-powered vehicles are some of the major causes for drifting towards electric vehicles and the advent of these new types of vehicles in Indian markets is gaining many openings. Researchers have investigated the need and the prevailing opportunities for such kind of energy-efficient electric automobiles. The choice of electric vehicles is based on their efficiency and it is decided by the type of battery installed. Four major types of batteries are predominantly used in electric vehicles such as Lithium Ion (Li-Ion), Molten Salt (Na-NiCl2), Nickel Metal Hydride (Ni-MH) and Lithium Sulphur (Li-S). Iclodean *et al* [30] compared these four batteries based on various attributes and presented the merits and demerits but not have stated the optimum choice of battery. This is one of the limitations of this comparative research work and this has laid the basis of this research work. This paper intends to check whether the earlier theoretical findings agree with the MCDM results for which a decision making problem with four alternatives and seven criteria is formulated. The alternatives are the four battery types and the seven criteria are charging capacity, voltage, cell rows, the mass of battery, operating temperature, specific heat capacity and cost. The alternatives are ranked using the method of ELECTRE I. The remaining contents are fragmented into few divisions, the following division presents the methodology of ELECTRE I, followed by the decision-making problem, discussion and finally the conclusion part.

METHODOLOGY

This section presents the diagrammatic representation of the algorithmic procedure of the method ELECTRE I





OPTIMAL RANKING OF ELECTRIC VEHICLES BATTERIES

This section presents the decision-making problem on ranking the batteries of electric vehicles. The initial decision-making matrix with four alternatives and seven criteria (B-Benefit & C- Cost criteria) is presented in Table.1. The normalized weighted matrix presented in Table.2 is obtained using step 2 & 3 by considering equal weight age to all criteria. The concordance and discordance interval sets are presented in Table.3 using step 4. The concordance interval matrix is obtained using step 7 and the discordance interval matrixis obtained using step 8.The net superior and inferior values are obtained using step 9. Table.7 presents the optimal ranking of the alternatives.

DISCUSSIONS

From the ranking of the batteries of electric vehicles, it is found that Li-S battery is placed in the first position followed by Li-Ion, Na-NiCl2 and Ni-MH. The optimal ranking obtained by the method of ELECTRE I highly synchronizes with the simulation results obtained by (). The choice of Li-S battery was addressed as the optimum,

choice of Li-Ion was described as the best, choice of Na-NiCl2 was also addressed as the ideal and the choice of Ni-MH was addressed as least. Thus the MCDM results are in accord with the simulation results with respect to the energy efficiency of the batteries.

CONCLUSION

This paper has attempted to test the theoretical findings on the attributes of electric vehicle batteries using the MCDM approach. In this research work, the alternatives i.e the four batteries are ranked using the method of ELECTRE I and the optimal ranking obtained is in alignment with the simulation results on energy efficacy. The MCDM results appear to be as promising as it has given the decision-makers the choice of choosing the suitable battery and it has rectified the shortcomings of the comparative research work of electric vehicle batteries.

REFERENCES

1. R. Tiwari, S. Agrawal and D.K. Kasdekar, "Application of ELECTRE-I, II methods for EDM performance measures in manufacturing decision making", *In IOP Conference Series: Materials Science and Engineering*, vol. 748, no.1, pp. 012015, 2020.
2. P. Chatterjee, S. Mondal andS. Chakraborty, "A comprehensive solution to automated inspection device selection problems using ELECTRE methods", *International Journal of Technology*, vol. 2, pp.193-208,2014.
3. L. David and L. Duckstein, "Multi-criterion ranking of alternative long-range water resource systems",*Water Resources Bulletin*, vol. 12, no. 4, pp. 731–754, 1976.
4. L. Duckstein, M. Gershon andR. McAniff, "Model selection in multiobjective decision making for river basin planning", *Advances in Water Resources*,vol. 5, no. 3, pp.178-184,1982.
5. M.J. Bender andS.P. Simonovic, "A fuzzy compromise approach to water resource systems planning under uncertainty", *Fuzzy Sets and Systems*, vol. 115, no. 1, pp.35-44, 2000.
6. M. Gershon andL.A. Duckstein, "Procedure for selection of a multiobjective technique with application to water and mineral resources", *Applied Mathematics and Computation*,vol. 14, no. 3, pp.245-271, 1984.
7. Roy, "Classement et choix en présence de points de vue multiples, *Revue francaise d'informatique et de recherche opérationnelle*", vol. 2, no. 8,pp.57-75, 1968.
8. R. Slowinski, "Interactive multiobjective optimization based on outranking and ordinal regression", *Found. Control Eng*, vol. 14, no.3, pp. 127- 134, 1989.
9. A. Tecle, "Selecting a multicriterion decision making technique for watershed resources management", *JAWRA Journal of the American Water Resources Association*, vol. 28, no. 1, pp.129-140, 1992.
10. L. Duckstein, W. Treichel W andS.E. Magnouni, "Ranking ground-water management alternatives by multicriterion analysis", *Journal of Water Resources Planning and Management*, vol. 120, no. 4,pp.546-565, 1994.
11. S. Cheng, C.W.Chan and G.H. Huang,"An integrated multi-criteria decision analysis and inexact mixed integer linear programming approach for solid waste management", *Engineering Applications of Artificial Intelligence*,vol.16 no.5-6, pp.543-554, 2003.
12. P. Nijkamp and J.B. Vos, "A multicriteria analysis for water resource and land use development", *Water Resources Research*, vol. 13, no. 3, pp.513-518, 1977.
13. C.E.D.D.C. Infante, F.M. de Mendonca, P.M. Purcidonio and R. Valle, "Triple bottom line analysis of oil and gas industry with multicriteria decision making", *Journal of Cleaner Production*, vol. 52, pp.289-300, 2013.
14. M.C. Wu and T.Y. Chen, "The ELECTRE multicriteria analysis approach based on Atanassov's intuitionistic fuzzy sets", *Expert Systems with Applications*, vol. 38, no. 10, pp.12318-12327, 2011.
15. G. Banias, C. Achillas, C. Vlachokostas, N. Moussiopoulos andS. Tarsenis, "Assessing multiple criteria for the optimal location of a construction and demolition waste management facility", *Building and Environment*, vol. 45, no. 10, pp.2317-2326,2010.
16. M. Augusto,J. Lisboa, M. Yasin and J.R. Figueira,"Benchmarking in a multiple criteria performance context: An application and a conceptual framework", *European Journal of Operational Research*, vol.184, no. 1, pp.244-254, 2008.

17. T. Thiel, "Application of multicriteria decision-aid methodology in building production engineering", *Statyba*, vol. 6, no. 6, pp.420-430,2000.
18. P. Chatterjee, V.M. Athawale and S. Chakraborty, "Selection of industrial robots using compromise ranking and outranking methods", *Robotics and Computer-Integrated Manufacturing*, vol. 26, no. 5, pp.483-489,2010.
19. S. Ulubeyli and A. Kazaz, "A multiple criteria decisionmaking approach to the selection of concrete pumps. Journal of Civil Engineering and Management",vol.15, no. 4, pp.369-376, 2009.
20. N. Bojkovic, I. Anic and S. Pejcic-Tarle, "One solution for cross-country transport-sustainability evaluation using a modified ELECTRE method", *Ecological Economics*, vol. 69, no. 5, pp.1176-1186,2010.
21. E. Radziszewska Zielina, "Methods for selecting the best partner construction enterprise in terms of partnering relations", *Journal of Civil Engineering and Management*, vol. 16, no. 4, pp.510-520,2010.
22. H. Sawicka, S. Weglinski andP. Witort, "Application of multiple criteria decision aid methods in logistic systems",*LogForum*, vol. 6, no.3,pp.99-110, 2010.
23. M. Bergeron, J.M. Martel and P. Twarabimenye, "The evaluation of corporate loan applications based on the MCDA"*Journal of Euro-Asian Management*,vol. 2, no. 2, pp.16 – 46, 1996.
24. G. Van Huylenbroeck and D.D. Tagarino, "Analysing crop choice of Philippine vegetable farmers with multicriteria analysis",*Journal of Multi-Criteria Decision Analysis*,vol. 7, no. 3, pp.160-168, 1998.
25. H. Ahrens and J. Kantelhardt, "Accounting for farmers' production responses to environmental restrictions within landscape planning",*Land Use Policy*,vol. 26, no. 4, pp.925-934,2009.
26. C. Arondel and P. Girardin, "Sorting cropping systems on the basis of their impact on groundwater quality",*European Journal of Operational Research*, vol. 127, no. 3, pp.467-482,2000.
27. S. Blanquart, "Role of multicriteria decision-aid (MCDA) to promote sustainable agriculture: heterogeneous data and different kinds of actors in a decision process", *International journal of agricultural resources, governance and ecology*, vol. 8, no. 2-4, pp.258-281, 2009.
28. A. Hatami-Marbini , M. Tavana ,M. Moradi and F. Kangi ,"A fuzzy group Electre method for safety and health assessment in hazardous waste recycling facilities", *Safety science*, vol. 51, no. 1, pp.414-426,2013.
29. J.M. Martel andG.R. D'Avignon, "Projects ordering with multicriteria analysis",*European Journal of Operational Research*, vol. 10, no. 1, pp.56–69, 1982.
30. C. Iclodean, B.Varga, N. Burnete, D. Cimerdean and B. Jurchis, "Comparison of different battery types for electric vehicles",*In IOP conference series: materials science and engineering, IOP* Publishing, vol 252, no. 1, pp. 012058, 2017.

Table.1: Initial Decision-making matrix

Alternatives/Criteria	Charging Capacity (C1)	Voltage (C2)	Cell rows (C3)	Mass of Battery (C4)	Operating Temperature (C5)	Specific Heat Capacity (C6)	Costs (C7)
Li-Ion	75	323	17	318	33	795	300
Na-NiCl2	84	289	30	457	270	950	500
Ni-MH	85	288	20	534	36	677	400
Li-S	80	305	1	173	30	1650	250
Benefit/Cost	B	B	B	B	C	C	C

Table.2: Normalized Matrix

	C1	C2	C3	C4	C5	C6	C7
Li-Ion (B1)	4.9594230	24.734229	1.0364188	18.290691	0.0140504	0.0027136	0.0043818
Na-NiCl2 (B2)	6.2211002	19.801087	3.2276020	37.775331	0.0017172	0.0022709	0.0026291
Ni-MH (B3)	6.3701033	19.664292	1.4344898	51.577274	0.0128796	0.0031866	0.0032864
Li-S (B4)	5.642721339	22.05428732	0.003586225	5.4133746	0.015455	0.001307	0.005258

Table.3: concordance and discordance interval sets

C12 –{2,5,6,7}	D12 – {1,3,4}
C13 = {2,5,7}	D13={1,3,4,6}
C14 – {2,3,4,6}	D14–{1,5,7}
C21={1,3,4}	D21={2,5,6,7}
C23 {2,3}	D23 {1,4,5,6,7}
C24={1,3,4,6}	D24={2,5,7}
C31–{1,3,4,6}	D31–{2,5,7}
C32={1,4,5,6,7}	D32={2,3}
C34–{1,3,4,6}	D34–{2,5,7}
C41={1,5,7}	D41={2,3,4,6}
C42–{2,5,7}	D42–{13,4,6}
C43={2,5,7}	D43={1,3,4,6}

Table.4: concordance interval matrix

	B1	B2	B3	B4
B1	0	0.572	0.429	0.572
B2	0.429	0	0.286	0.572
B3	0.572	0.715	0	0.572
B4	0.429	0.429	0.429	0

Table.5: discordance interval matrix

	B1	B2	B3	B4
B1	0	1	1	0.053062164
B2	0.253181115	0	1	0.069624968
B3	0.15231174	0.12991737	0	0.051771944
B4	1	1	1	0

Table.6: Net Superior and Inferior values

Alternatives	Net Superior Value	Net Inferior Value
B1	0.143	0.647569309
B2	-0.429	-0.807111288
B3	0.715	-2.665998946
B4	-0.429	2.825540924

Table.7: Ranking of the Alternatives

Alternatives	**Rank**
Li-Ion	2
Na-NiCl2	3
Ni-MH	4
Li-S	1

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RESEARCH ARTICLE

Evaluating the Therapeutic Potentials of Golden Milk *In vitro*

Ganga U[1], Asiya S[1], Sreerakhi R S[1], Tigna Paul P B[1], Shamsiya Mol R[1] and Merlin NJ[2]*

[1]IV B.Pharm Student, Department of Pharmacology, Ezhuthachan College of Pharmaceutical Sciences, Marayamuttom, Trivandrum, Kerala, India.
[2]Professor and Head, Department of Pharmacology, Ezhuthachan College of Pharmaceutical Sciences, Kerala, India.

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***Address for Correspondence**
Merlin NJ
Professor and Head,
Department of Pharmacology,
Ezhuthachan College of Pharmaceutical Sciences,
Kerala, India.
Email: merlinbinu76@gmail.com

ABSTRACT

The study's primary objectives are the preparation, standardization, investigation of the proximate composition, and *In vitro* pharmacological evaluation of golden milk for antidiabetic, anti antioxidant, anti-inflammatory, anti-mitotic, and anti-proliferative properties. *Curcuma longa* (turmeric), *Cinnamon verum* (cinnamon), *Zingiber officianale* (ginger), *Piper nigrum* (pepper), and honey were used in the preparation of golden milk. Standard operating procedures were used for standardisation and proximate composition analysis. Golden milk's antioxidant activity was tested using an in-vitro hydroxyl radical scavenging assay. An *In-vitro* alpha amylase inhibitory assay was used to measure the anti-diabetic efficacy. For anti-inflammatory efficacy, albumin denaturation inhibition was used *In vitro*. *Allium cepa* roots were used to evaluate the antimitotic activity *In vitro*. The assessment of cell viability count was used to measure *In-vitro* anti proliferative activity. Standardization demonstrates that golden milk is acceptable to patients. The Golden milk powder has substantial anti-diabetic, anti-oxidant, anti-inflammatory, anti-mitotic, and anti-proliferative potential due to the presence of phytoconstituents. A stimulating beverage that raises immunity is golden milk. Anti-diabetic, anti-oxidant, anti-inflammatory, anti-mitotic, and anti-proliferative are just a few of its outstanding qualities. Golden milk contains antioxidants that have anti-aging properties. Therefore, it may have health advantages to ward off various diseases.

Keywords: Golden milk, anti-diabetic, anti-oxidant-, anti- inflammatory,anti- mitotic and anti-proliferative.

INTRODUCTION

Golden milk, also known as Haldi dhooth, is a traditional Ayurvedic beverage made with turmeric, ginger, cinnamon, black pepper, and honey, all of which have a variety of health advantages. It is an alternate treatment for several diseases and for boosting immunity [1]. Antioxidant-rich golden milk also contains antibacterial, anti-diabetic, and anti-inflammatory properties. Golden milk's main ingredient, turmeric, is known as "turmeric milk" in popular culture. The potential health advantages include lowering blood sugar levels, preventing heart disease, preventing cell damage, enhancing mood, lowering cancer risk, increasing the immune system, enhancing bone health, etc. There are numerous ways to transform vegan milk or regular non-vegan milk into Golden milk [2]. Milk, turmeric, ginger, pepper, cinnamon, and honey make up the majority of the ingredients. In general, calcium and vitamin D are abundant in cow milk and fortified plant milks, which helps the body, especially the bones, by strengthening them.

METHODOLOGY

Collection of ingredients

Dried plant parts of pepper, ginger, cinnamon, and turmeric. were purchased from the local market. The plant components were then finely ground and kept in an airtight container. Table 1 lists the ingredients in TCGP powder for the 120 ml golden milk formulation.

Preparation of golden milk

120 ml of milk have been taken. The TCGP powder was added followed by ten minutes of boiling. After that, strain the mixture using a muslin towel. Then, to make it sweeter, 4ml of honey was added.

Standardization

Organoleptic parameters

The prepared golden milk's appearance, flavour, texture, and smell were all manually assessed.

Proximate composition analysis

Following the preparation of the golden milk, the formulation was tested for pH and viscosity using the 2003 Association of Official Analytical Collaboration techniques

In vitro studies

In-vitro anti diabetic activity evaluation

The screening of *In vitro* anti diabetic activity was done by alpha amylase inhibition assay [3] .

In-vitro antioxidant activity evaluation

The screening of *In vitro* antioxidant activity was done by hydroxyl radical scavenging assay [4,5,6] .

In-vitro anti-inflammatory activity evaluation

The screening of *In vitro* anti inflammatory activity was performed by Egg Albumin denaturation method [7] .

In-vitro antimitotic activity evaluation

The screening of antimitotic activity was done by using *Allium cepa* root meristematic cells [8].

In-vitro anti-proliferative activity evaluation

Cell viability count method

In vitro anti-proliferative activity was evaluated using cell viability count method[9] .

RESULTS AND DISCUSSION

Standardization of golden milk

Organoleptic parameters

Sensory analysis, or the evaluation of the impression on the sense organs, was used to examine organoleptic qualities such as colour, odour, taste, and texture. Table 2 provides a summary of the findings from the organoleptic evaluation of golden milk.

Proximate composition analysis

Viscosity and pH

Golden milk has a viscosity of 1.4364 and a pH of 6.

***In-vitro* studies**

***In-vitro* antidiabetic activity by α-Amylase method**

Inhibiting the enzyme -amylase to reduce the absorption of glucose in the intestine is the treatment for type 2 diabetes. The main cause of diabetes mellitus is hyperglycemia. The hydrolysis of starch into a variety of smaller oligosaccharides is catalysed by alpha amylase. Golden milk inhibits the enzyme -amylase, and the results are summarised in Table 3.

***In-vitro* anti-oxidant activity by hydroxyl radical scavenging assay**

Free radicals are compounds with an unpaired electron in the atomic orbital. They are highly reactive and unstable due to this characteristic. They can damage cells through oxidative oxidation and can also act as reductants. A chemical that can give an electron to a reactive free radical and neutralize it is an antioxidant. As a result, an antioxidant's ability to scavenge free radicals can lessen their ability to harm cells. Golden milk has the ability to scavenge hydroxyl radicals, and Table 4 summarizes the findings.

***In vitro* anti-inflammatory activity using egg albumin denaturation method**

Protein denaturation causes the molecules' biological properties to be lost. It causes antigens linked to type-3 hypersensitivity reactions to develop, which triggers an inflammatory response in the tissues. Golden milk has anti-inflammatory characteristics, and Table 5 summarizes the findings.

***In-vitro* antimitotic activity using *Allium cepa* roots**

Drugs with antimitotic action have been extensively screened using meristematic cells. All plants have distinct regions in their roots, and one of these regions has a cell division that extends beyond the root cap and a few millimetres after the region's cells repeatedly divide. Meristematic region refers to the area of a tissue where cell division is higher than in other tissues. Golden milk has antimitotic properties, as shown by the findings in Table 6.

***In-vitro* anti-proliferative activity by cell viability count method**

The cell viability count method can be used to assess the anti-proliferative activity. By counting the viable cells, we can determine the percentage of viable cells. Methylene blue was employed as an indication to determine which cells were still alive. Golden milk has anti-proliferative properties, and Table 7 summarises the findings.

DISCUSSION

Organoleptic characteristics aid in assessing the formulation's acceptability. Golden milk has a pleasant appearance, flavour, aroma, and texture that makes it suitable for regular usage. According to proximate composition analyses, the viscosity and Ph were within normal bounds. The crucial carbohydrate hydrolyzing enzyme alpha amylase is in charge of rupturing the α-1-H bonds in disaccharides and polysaccharides. It can be kept in check by inhibiting enzymes like glycosidase and -amylase, among others. Due to the presence of phenolic compounds, alkaloids, and

other substances, Golden milk has antidiabetic properties. Reactive oxygen species called hydroxyl radicals can damage proteins, peroxide lipids, and affect membrane permeability. Antioxidants and free radical scavengers can stop it from causing cancer, heart disease, and degenerative conditions. The Golden Milk composition has strong antioxidant properties. *Curcumin* in turmeric, 6-Gingerol, 6-Shogaol, and oleoresins in ginger, Cinnamaldehyde, procyanidins, and catechins in cinnamon, and phenolic amides in black pepper are the major phytochemicals responsible for antioxidant action. Albumin protein denaturation results in the development of type III hypersensitivity reaction, which causes inflammation. An agent's ability to inhibit denaturation is a sign of its anti-inflammatory properties. Increased inhibition would result in increased anti-inflammatory action. Alkaloids found in golden milk have considerable anti-inflammatory properties. The reported mechanism of action for anticancer medications is the suppression of DNA synthesis and the prevention of cell division. The creation of terahydrofolic acid depends on the folic acid in our diet. Folic acid is transformed into terahydrofolic acid in the presence of the enzyme folate reductase. Folic acid should compete with anticancer medications for the enzyme while limiting terahydrofolic acid synthesis, which is necessary for DNA replication. *Allium cepa* root meristamatic cells can be used to screen for antimitotic activity. The growth of *Allium cepa* root tips is halted or reduced. The root's length shrank after being dipped in golden milk, and it does not now. A yeast model was used to study in-vitro anti-proliferative efficacy. This counts the number of viable cells and the number of dead cells. Methylene blue, an indicator, was used to distinguish it. While the dead cells had stain and were blue, the live cells did not have stain. The outcome demonstrates that the Golden milk had antiproliferative properties.

CONCLUSION

A immune boosting health drink is golden milk. A mixture of milk, turmeric, ginger, cinnamon, black pepper, and honey is used to make golden milk. Lowering blood sugar levels, lowering the risk of cancer, boosting the immune system, improving digestive health, and reducing inflammation are few of the potential health advantages of golden milk. These benefits may help prevent heart disease, diabetes, cancer, arthritis, Alzheimer's disease, metabolic syndromes, and other inflammatory conditions. From this, we can infer that Golden milk is a magnificient drink with numerous health advantages. One can consume it frequently to increase the immunity and can prevent many illnesses.

CONFLICTS OF INTEREST

The authors have no conflicts of interest regarding this investigation

REFERENCES

1. https://theindianmed.com/benefits-of-turmeric-milk/
2. https://www.medicalnewstoday.com/articles/324181
3. T. Sundarrajan, V. Velmurugan, R. Srimathi. Phytochemical Evaluation and *In-vitro* Anti-diabeticActivityofEthanolicExtractof*AlternantheraficodiaLinn*.ResearchJ.Pharmand Tech.2017;10(9):2981-2983.
4. Sharma SK, Singh AP. In vitro antioxidant and free radical scavenging activity of NardostachysjatamansiDC.JournalofAcupunctureandMeridianStudies.2012;5(3):112-8.
5. Wang M, Li C, Li H, Wu Z, Chen B, Lei Y, Shen Y. In Vitro and in silico antioxidant activity of novel peptides prepared from *Paeonia ostii* 'Feng dan'hydrolysate. Antioxidants.
6. Geng M, Ren M, Liu Z, Shang X. Free radical scavenging activities of pigment extract from *Hibiscus syriacus* L. petals in vitro. African journal of biotechnology. 2012; 11(2):429-
7. 7.Sharmila Dharmadev, Lahira Sandharuwan Galgamuwa. *In-vitro* anti-inflammatory activity of *Ficus racemose* L. bark using albumin denaturation method. An International Quarterly Journal of Research in Ayurveda. 2018;39(4):239-242.

8. Chandrashekhar Naik, Shivasharanappa Chandrappa Gadal, Aparna R. Isolation, identification and evaluation of antioxidant, anti-inflammatory and antimitotic properties of bio-active pigment from Rhodococcus corynebacteriodies SCG11. – IJAMBR 2020; (8):1-4.
9. Gaurav M. Doshi, Preeti P. Kanade . Evaluation of bioactivity of *Cucurbita pepo* L; *Cucumis melo* L. and *Cucumis sativas* L seed extracts. Indian journal of Experimental Biology 2019; (57):269-273.

Table 1. Composition of TCGP powder

Ingredients	Quantity
Turmeric	2.56g
Cinnamon	1.12g
Ginger	1.12g
Pepper	0.35g

Table 2: Organoleptic evaluation of golden milk

Parameters	Observations
Colour	Yellow
Odour	Aromatic
Texture	Smooth
Taste	Sweet

Table 3: Percentage inhibition of α-amylase by Golden milk .

Sl no.	Concentration of sample (µg/ml)	Percentage inhibition (%)	
		Golden milk	Standard
1	100	21.62±1.252	53.8±5.376
2	200	24.18±0.513	68.4±1.161
3	300	25.08±0.254	79.7±2.100
4	400	32.96±0.638	87.8±4.438

The values are expressed as mean S.E.M, n = 3.

Table 4: Percentage inhibition of hydroxyl radical by Golden milk

S.No	Concentration of sample(µg/mL)	Percentage inhibition(%)	
		Golden milk	Standard
1	100	15.9183±1.095	25.189±2.173
2	200	18.4638±0.3602	30.088±0.759
3	300	21.6149±0.5493	33.781±0.306
4	400	22.8539±0.906	41.820±2.627

The values are expressed as mean S.E.M, n = .3

Table 5: Percentage inhibition of protein denaturation by Golden milk

S.No	Concentration of sample(µg/ml)	Percentage inhibition(%)	
		Golden milk	standard
1	100	48.19±2.472	63.77±4.294
2	200	55.08±0.483	73.83±1.389
3	300	61.36±1.329	86.17±2.172
4	400	62.39±1.626	90.81±3.511

The values are expressed as mean S.E.M, n = .3

Table 6: **Antimitotic activity of Golden milk.**

S.no	Groups	Root length (cm)	% inhibition of root length
1	Control	12.5±2.72	0
2	Golden Milk (100 µg/ml)	3.5±0.952	72±7.620
3	Methotrexate (100 µg/ml)	1.5±1.768	88±14.153

The values are expressed as mean S.E.M, n = 3.

Table 7: Anti-proliferative activity of golden milk.

S. no	Groups	% cell viability
1	Methotrexate (100 µg/ml)	48.9±4.547
2	Golden milk(100 µg/ml)	51.72±3.733
4	Control	100±0.01

The values are expressed as mean S.E.M, n = 3.

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RESEARCH ARTICLE

A Retrospective Study on Poisoning Cases in a Tertiary Care Hospital in Dharmapuri District, Tamilnadu

Anandharaj G[1]*, Senthil Kumar K L[2], Sriram K[3], Rajamanickam P[4], Sowparnika M[5], Srisakthi S[5] and Subash Chandra Bose G[5]

[1]Assistant Professor, Department of Pharmacy Practice, Sri Vijay Vidyalaya College of Pharmacy, Dharmapuri, Tamil Nadu, India.
[2]Principal, Sri Vijay Vidyalaya College of Pharmacy, Dharmapuri, Tamil Nadu, India.
[3]Sivaraj Homeopathic Medical College and Research Institute, Salem, Tamil Nadu, India
[4]Assistant Professor, Vellalar College of Pharmacy, Erode, Tamil Nadu, India.
[5]B. Pharm Final Year Student, Sri Vijay Vidyalaya College of Pharmacy, Dharmapuri, Tamil Nadu, India.

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***Address for Correspondence**
Anandharaj G
Assistant Professor,
Department of Pharmacy Practice,
Sri Vijay Vidyalaya College of Pharmacy,
Dharmapuri, Tamil Nadu, India.
Email: Anandpharmacist3@gmail.com

ABSTRACT

Around the world, poisoning significantly increases patient death and morbidity. To prevent death, poisoning must be managed immediately. The goal of this study was to identify the patterns of poisoning cases and their treatment at emergency department of the Tertiary care hospitals in Dharmapuri district, Tamil Nadu, India. The present study was a retrospective study conducted over a period of six months. Case records of poisoning cases from January 2021 to July 2022**.** Total 200 patients of poisoning cases included in this study, 63 % were male and 37 % were females with a large portion of poison patients 38% in the age group of 40-49 years. Out of the total poisoning cases, 69 % were from a suicidal cases and 31 % were from accidental cases, majority of the cases 78% were from a rural area and 22 % were from an urban area, In the study population, 46% of the patients were with unmarried in which 42% patients were married and 12% patients divorced. The causative poison was recorded for 200 cases. The most common poisoning agents were organophosphate (30%). In all cases of poisoning, both non-pharmacological and pharmacological approaches were used as a treatment for poisoning cases. The most widely used Pharmacological treatments were Cimetidine (89%). Among the poisoning related admissions 79 % of patients recovered and remaining 21 % of cases died due to various poisoning agents. In the study total 200 poisoning cases, 30 % organophosphate were the common poison used of the total poisoning cases. Cimetidine 89 % was the most commonly used treatment of the total poisoning cases.

Keywords: Poison, mortality and morbidity, organophosphate.

INTRODUCTION

Poisoning is a significant issue across the nation, albeit the type and accompanying mortality and morbidity differ from country to country. All poisoning deaths are classified as unnatural deaths under our country's legal system, and medical-legal autopsies are commonplace. In order to determine whether any exogenous chemical agent is present in biological specimens made available in connection with medicolegal investigations, modern toxicology needs to be an integrated science and forensic science [1]. Due to changes in human social behaviour and lifestyle, poisoning incidents are becoming more frequent. According to a WHO estimate, unintentional poisoning caused 346,000 fatalities globally in 2004 (3.5 per 100,000). 91 percent of these deaths occurred in low-income countries [2-3]. Cross-contamination prevention, toxin detection through physical or medical examinations, supportive and palliative care, elimination, and antidote therapy are the main components of poisoning management [4]. The current study sought to identify the pattern of poisoning cases admitted to the Tertiary care hospitals in Dharmapuri district, Tamil Nadu, India.

MATERIALS AND METHODS

The present study was a retrospective study conducted over a period of six months. Case records of poisoning cases from January 2021 to July 2022 poisoning cases reported to the emergency department and intensive care unit Patients with poisoning were treated by the doctors. The study procedures did not interfere with the treatment of the poisoning patients. A specially designed data collection form was used to collect the details of patients' demography, residence, name of poisoning agents consumed, and mode of poisoning cases, treatment outcomes, and causes of mortality. The study was approved by the institutional ethical review committee.

Statistical analysis

The computer's database was filled with the data that were obtained, and Ms. Excel software was used to perform a frequency analysis.

RESULT AND DISCUSSION

Patient Demographic Characteristics

Total 200 patients of poisoning cases included in this study, 63 % were male and 37 % were females with a large portion of poison patients 38% in the age group of 40-49 years. Out of the total poisoning cases, 69 % were from a suicidal cases and 31 % were from accidental cases, majority of the cases 78% were from a rural area and 22 % were from an urban area, In the study population, 46% of the patients were with unmarried in which 42% patients were married and 12% patients divorced (Table 1).

Nature of Poisoning Agents

Out of 200 patients, the most common poisoning agents were organophosphate (30%), rat killer (22%), cow dunk (14%), snake bite (11%), Tablet poisoning (8%), Insecticide (8%), Mortein coil (4%), Unknown bite (2%), Dettol + Kerosene (2%), Alcohol + Orgno phosphorous (2%) and rest 1% were poisoned with an Unknown agents (Table 2).

Treatment approaches of poisoning cases

In this study, both pharmacological and non-pharmacological approaches were used as a treatment for poisoning cases. The most widely used Pharmacological treatments were Cimetidine (89%), proceeding by Metoclopramide (69%), Atropine (31%), Antibiotics (24%). Additionally, non-Pharmacological approaches were used including IV fluid (68%), NG feeding tube (45%), Gastric lavage (34%), milk (10%) and water (9%) (Table 3).

Treatment outcome of the Poisoning cases

Among the poisoning related admissions 79 % of patients recovered and remaining 21 % of cases died due to various poisoning agents (Table 4).

CONCLUSION

The Tertiary care hospitals of the Dharmapuri district, Tamil Nadu, indicate that significant chance for decrease mortality rate exist by greater medical management and further limitation on the most toxic agents. This study highlighted the need to centralised poison information centre for the better treatment and prevention of poisoning cases.

REFERENCES

1. Muhammad NI, Nasimul I. Retrospective study of 273 death due to poisoning at Sri Salimullah Medical College from 1988 to 19787. *Leg Med* 2003; 5:S129-31.
2. Teklemariam E, Tesema S, Jemal A, Pattern of acute poisoning in Jimma University Specialized Hospital, South West Ethiopia. *World J Emerg Surg*.2016;7(4):290.
3. Kaale E, Mori A, Risha P, Hasham S, Mdambete K, A Retrospective study of poisoning at Muhimbili National Hospital in Dar-Es Salaam, Tanzania. *J Public Health Front*.2013;2(1):21-26.
4. Kaale E, Mori A, Risha P, Hasham S, Mdambete K, A Retrospective study of poisoning cases and their management in emergency centres of government hospitals in northwest Ethiopia. *Afr J Emerg Med*.2017;7(2):74-78.
5. Subash Vijay kumar, B.Venkateshdarlu, M.Sasikala, G.Vijaykumar, A study on poisoning cases in a tertiary care hospital. *Journal of natural science, biology and medicine*.2010;1(1):35-39.
6. J.Jesslin, R.Adepu and S.Churi, Assessment of prevalence and mortality incidence due to poisoning in a south Indian tertiary care teaching hospital, Indian journal of pharmaceutical sciences.2010;587-591.
7. AbebeGetieYaschilalMucheBelayneh, A retrospective study on acute poisoning cases and their management at emergency department of dessie referral hospital, northeast Ethiopia, *Drug, healthcare and patient safety* 2020:41-48.
8. Dhanya S.P, Dhanya T.H, BinduLatha R Nair, Hema C.G, A Retrospective analysis of the pattern of poisoning in patient admitted to medical college hospital. Calicut medical journal 2009: 7(2):e3.
9. Anandharaj G, Senthilkumar K L, Sheik Nasar I, Rajamanickam P, Gokul P, Kaviyarasu A, Mahendiran N, Study on Assessment of Drug Compliance in Diabetic Patients in Rural Area of Dharmapuri District Tamilnadu. *International Journal of Pharmacy and Pharmaceutical Research*, 2022,23(3), 150-155.
10. Azazh A. Severe organophosphate poisoning with deleyed cholinergic crisis, intermediate syndrome and organophosphate induced delayed polyneuropathy on succession. *Ethiop J Health Sci*2011:21(3).
11. Meheswari E, Abraham L, Chacko CS, Saraswathy GR, Ramesh AC, Assessment of pattern,severity and outcome of poisoning in emergency cared unit, *J Appl Pharm Sci*.2016:6(12):178-183.
12. Sriram k, Tharun Krishnan, Nancy JeevaPriya N, MariyaMol and Lavanya K. Prevalence and association of sexual dysfunction in female patients taking Psychotropic drugs in A Tertiary care Hospital: Indian Journal of Natural Sciences, 2021;12(67), 32858-32862.

Table 1 : Patient Demographic Characteristics

Characteristic	No.(%) of Patients`
Sex	
Male	63
Female	37
Age	
20-39	31
40-49	38
Above 70	32

Mode of Poison cases	
Suicidal cases	69
Accidental cases	31
Area wish poisoning cases	
Rural area	78
Urban area	22
marital status	
Married	42
Unmarried	46
Divorced	12

Table 2: Nature of Poisoning Agents

Category of Poisoning	Number of patients (n=200)	Percentage (%)
Cow dunk	27	14
Tablet poisoning	16	8
Orgno phosphorous	59	30
Mortein coil	07	4
Rat Killer	43	22
Insecticide	15	8
Unknown bite	04	2
Snake Bite	21	11
Dettol + Kerosene	3	2
Alcohol + Orgno phosphorous	3	2
Unknown	2	1

Table 3: Treatment approaches of poisoning cases

Treatment		Frequency (n=200)	Percentage (%)
Non-Pharmacologic Treatment	Gastric lavage	68	34
	Water	17	9
	Milk	20	10
	NG Feeding tube	89	45
	IV Fluid	136	68
Pharmacologic Treatment	Cimetidine	178	89
	Metoclopramide	138	69
	Atropine	62	31
	Antibiotics	47	24

Table 4 : Treatment outcome of the Poisoning cases

Outcome	Number of patients (n=200)	Percentage (%)
Recovered	158	79
Death	42	21

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RESEARCH ARTICLE

Design, Synthesis and Antitubercular Activity of Sulphur Substituted Chalcones and *In silico* Docking Studies

Mejo Joseph[1]*, S.Alaxander[2], Anandkumar[3], J.Banurekha[4] and G.Geetha[5]

[1]Assistant Professor, Nehru College of Pharmacy, Pampady, Kerala ,India.
[2]Assistant Professor, Vinayaka Mission College of Pharmacy, Salem, Tamil Nadu, India.
[3]Professor, Swamy Vivekanandha College of Pharmacy, Thiruchengode, Namakkal, Tamil Nadu, India.
[4]Department of Pharmaceutical Chemistry, Vinayaka Mission College of Pharmacy, Salem, Tamil Nadu, India.
[5]Professor, Department of Pharmaceutical Chemistry, Nehru College of Pharmacy, Pampady, Kerala, India.

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***Address for Correspondence**
Mejo Joseph,
Assistant Professor,
Nehru College of Pharmacy,
Pampady, Kerala ,India.
Email: mejojoseph000@gmail.com

ABSTRACT

A novel series of methoxy linked benzothiophene derivatives have been linked to para-amino acetophenone and different aldehydes were successfully synthesized and biological activity was predicted using various computational software's such as Molinspiration, Chemsketch and admetSAR synthesized and confirmed by IR,^{1}HNMR,^{13}CNMR,and mass spectra. The synthesized compounds were screened for their antitubercular activity using microplate Alamar blue assay method. Among the tested compounds BT-IV-A, BT-IV-B, BT-IV-E,BT-IV-G, and BT-IV-H showed potent antitubercular activity in comparison with standard drug INH. In addition, the synthesized compounds were docked into Enoyil reductase complex GSK 625 (PDB ID-5FJO) to explore their binding interactions at the active site. The compounds exhibited essential key interactions at that of reported GSK 625 inhibitors and hence the synthesized compounds may be considered as molecular scaffolds for antitubercular activity.

Keywords: Benzothiophene, Antitubercular, Docking methods, MABA**,**

INTRODUCTION

Tuberculosis is one of the wide spread infections which caused by *Mycobacterium tuberculosis* [1]. It is important to note that despite having an effective treatment about 1.5 million deaths and 11.2 million hospital cases during 2021[2]. However long duration of therapy and the need of multidrug resistant tuberculosis have generated an outmost requirement to identify more selective newer antitubercular drugs for effective treatment [3]. TB is most generally occurring in lungs but affect other organs including skin, bones, digestive tracts, CNS, lymphatic nodes and liver[4]. In the recent years large number of synthetic sulphur substituted hectocycles and its derivatives have been paid more attention because of their diverse biological activities such as antibacterial, antitubercular, antioxidant and anticancer activities[5]. In view of present study, it is planned to design target compounds by incorporating methoxy substituted benzothiophene molecules with different substituted chalcones to produce hybrid molecules. Antitubercular activity of synthesized compounds were evaluated against *Mycobacterium tuberculosis* using a microplate alamar blue assay[6]method and performed molecular docking studies against Enoyil reductase inhibitors. Chemsketch is used for the prediction of physiochemical properties which includes bioavailability, lipophilicity, druglikeness, AdmetSAR, and logP of novel sulphur substituted chalcones.

MATERIALS AND METHODS

The present scheme 1 describes about synthesis of novel substituted methoxy derivatives of benzothiophene and their different chalcone derivatives. which on further refluxed with para amino acetophenone in presence of dry acetone to form 4-(acetyl phenyl)-3-chloro-methoxy-1-benzothiophene-2-carboxamide (IV) which further on by Claisen Schmidt condensation[7]which involves crossed aldol condensation appropriate aldehyde in presence of base catalyzed reactions followed by dehydration to form different derivatives of Meta-chloro-meta-methoxy-N-[4-(2E)-3-phenylprop-2-enyl]phenyl-1-benzothiophene-2-Carboxamide in respectively. TLC by n-hexane and chloroform (9:1) as an eluent. General scheme (I) for the preparation of compounds.

Meta chloro-N (4-cinnamoylphenyl)-6-methoxybenzo[b]thiophene-2-carboxamide. **(BT IV-A**)
Yellowish colour. Yield was 74 %. IR vales: (cm^{-1}) 3342(N-H, Stretching),1 710(C=O Stretching, Ketone), 1674 (C=O, Stretching, Amide),2825(OCH_3), 1587(C=C Aromatic Stretch), 1060(=C-Cl, Stretch) 825 (C-H Aromatic), 738(CH=CH, Stretching), 674(C-S-C, Stretch). ^{1}H-NMR-δ ppm: 9.15(s, 1H, CONH),8.09-7.01(m.12H.Ar-H),8.07-7.59 (d,2H,CH=CH),3.87(s,3H, OCH_3). ^{13}C-NMR-δppm:145.1,121.3Ar-CEthylene, 55.8-CH3, Aliphatic, 189.7-Ar-C(C=C) Carbonyl,135.2,128.6,128.5, 127.9-Ar-C-(C=C) Benzene, 143.7,133.5,131.4,122.1-Ar-C(N-C=O) Benzene, 159.4,152.8,143.7, 141.6,129.9, 125.7-Ar-C(C=O) Cl Benzothiophene. M^+(m/z): 416.

Meta chloro-N (4-(3-(2-chlorophenyl) acryloyl) phenyl)6-methoxybenzo[b]thiophene-2- carboxamide (BT IV-B)
Brownish power. Yield was 78%. IR vales. (cm^{-1}) 3340(N-H Stretching), 2833(OCH3 Stretch), 1710(C=O, Stretch Ketone), 1674(C=O, Stretch Amide), 1482(C=C, Aromatic stretch), 1060(=C-Cl, Stretch) 740(CH=CH, Stretch), 607(C-S-C Stretch). ^{1}H-NMR-δ ppm: 9.18 (s, 1H, CONH), 8.48-7.07(m, 14H, Ar-H), 8.30-7.48, (d, 2H, CH=CH), 3.83(s, 3H, OCH_3). ^{13}C-NMR-δ ppm: 145.1,121.3-ArC- of ethylene(C-H), 55.8- Aliphatic (CH3),189.7-(ArC) of carbonyl, 161.8-ArC of N- Amide,126.3,129.3,127.3,129.9,131.4,134.7-ArC-Benzene(C=C) Cl, 131.4,122.1, 133.5, 143.7-ArCBenzene(N-C=O),129.9,141.6, 152.8, 125.7-ArC-Benzothiophene(C=O) Cl, 124.2ArC-Benzothiophene(O-C). M^+(m/z):483.

Metachloro-6-methoxy-N-(4-(3-(2-nitrophenyl) acryloyl) phenyl) benzo[b]thiophene-2-carboxamide (BT IV-C)
Pale yellowish power. Yield was 85%. IR vales. (cm^{-1})3387 (N-H Stretching), 2829 (OCH_3 Stretching), 1713(C=O, Stretching, Ketone), 1691(C=O, Amide), 1547(C=C, Stretch, Aromatic), 1381(NO_2 Stretch), 1063(=C-Cl, Stretching), 713(C-S-C Stretching). ^{1}H-NMR-δ ppm: 9.18(s, 1H, CONH), 8.48-7.07(m, 14H, Ar-H), 8.30-7.48, (d, 2H, CH=CH), 3.83(s, 3H, OCH_3). ^{13}C-NMR-δ ppm: 145.1,121.3-ArC of ethylene(C-H),55.8-C, Aliphatic-CH3,189.7-ArC of carbonyl,161.8-ArC-N-amide,126.3,129.3,127. 3,129.9,131.4,134.7-ArC-Benzene (C=C) Cl, 131.4,122.1,133.5,143.7-ArC-

Benzene (NC=O)129.9,141.6,152.8,125.7-ArC-of Benzothiophene (C=O) Cl,124.2-ArCBenzothiophene (O-C). M^+(m/z): 459.

Metachloro-N-(4-(3-(furan-3-yl)acryloyl)phenyl)-6-methoxybenzo[b]thiophene-2-carboxamide. (BT IV-E)
Yellowish brown powder. Yield was 67%. IR vales. (cm^{-1})3391(NH, Stretching), 2817 (OCH_3,Stretching),1718(C=O, Stretching, Ketone,),1658(C=O, Amide), 1171(C-O-C, Stretching),1062(=C-Cl, Stretch)756(CH=CH, Stretching), 682(C-S-C, Stretching.). ^{1}H-NMR-δppm:9.18(s,1H, CONH),8.18-6.89(m,10H, ArH),7.12-7.09(d,2H, CH=CH), 3.87-(s,3H, OCH_3).^{13}C-NMRδppm:129.9,141.6ArC(C=O)Cl of Benzothiophene,152.8, 125.7 ArC(C=O)Cl, (OC)Benzothiophene, 143.0,139.0,124.4, ArC(C=C) Furan, 159.4, 115.8, 126.3, 124.2Ar(C-H) Benzothiophene, 143.7,133.5,122.1,131.4-ArC(CH)Benzene.161.8-ArC-(N-Amide), 189.7-Carbonyl,55.8-9CH_3),145.1,127.3-ArC(CH)-C=O ethyle- ne. M^+(m/z): 438.

Meta chloro-N-(4-(3-(4-chlorophenyl) acryloyl) phenyl)-6-methoxybenzo[b]thiophene-2-carboxamide (BT-IV-G)
Dark yellowish powder. Yield was 69%. IR vales (cm^{-1}) 3336(N-H Stretching), 2823 (OCH_3 Stretching),1715 (C=O Stretch Ketone),1659(C=O, Stretching Amide), 1596 (C=C, Stretch),1096(=C-Cl, Stretch),976(C=C Bending), 661(C-S-C Stretching). ^{1}H-NMR-δppm:9.15(s1H,CONH),8.09-7.60(m,11H,Ar-H),7.49-7.48(d,2H,CH=CH), 3.87(s,3H,OCH_3).^{13}C-NMR-δppm:129.9,152.8,125.7-ArC-(C=O)Cl-Benzothiophene, 133.5,133.3-ArC-Cl(C=C)Benzene.28.7,129.0-ArC-(C=C)ClBenzene.115.8,124.2,126.3 (CH)Ar C(O-C) Benzothiophene of benzene,133.5, ,131.4-(N-C=O)-C=O Benzene, 161.8-ArC-(N-Amide), 55.8-ArC-(CH3)Aliphatic,121.3,145.1-ArC-(C(=O)Ethylene. M^+(m/z): 448.

Meta chloro-N-(4-(3-(4-hydroxyphenyl) acryloyl) phenyl)-6-Methoxybenzo[b] thiophene-2-carboxamide (BT-IVH)
Brick red colour. Yield was 66%.IRvales: (cm^{-1})3566(O-H, Stretching),3283(N-H Stretching),1715(C=O Stretching Ketone),1693(C=O, Stretching Amide),1484(C=C Stretching),1063(=C-Cl,Stretching), 837 (C=CStretching), 682 (CH=CHStretching), 795 (C-S-C, Stretching). ^{1}H-NMR-δppm:9.18-(s,1H,CONH), 8.06-6.65(m,11H,ArH), 7.54-7.53 (d,2H, CH= CH), 5.35 (S, 1H,OH), 3.87-3.83 (s,3H,OCH3). ^{13}C NMR-δ ppm: 129.9, 141.6,152.8,125.7-ArC-(C=O) Cl-Benzothiophene,159.4-ArC,115.8(CH),124.2,(CH)126.3(CH)-ArC-(O-C)Benzothiophene,157.7-ArC Benzene (=O(C=C), 127.8ArC, 115.8(CH), 130.6(CH), Benzene(=O(C=C),143.7,133.5ArC,122.1,131.4,ArC-(N-C=O) Benzene.189.7,161.8-ArC-(N-Amide),55.8-ArC-(CH3)Aliphatic,121.3,145.1-ArC-(C(=O)Ethylene.M^+(m/z): 430.

RESULT AND DISCUSSION

Docking Methodology
Docking in Discovery Studio, there are some pre-docking steps to perform docking.

Protein Preparation
Prepare the protein structure before docking because, in general PDB structures contain water molecules, all water molecules are removed except the important ones in protein preparation [8]. Hydrogen atoms will be missing in PDB structure; many docking programs need the protein to have explicit hydrogen. Hydrogen can be added unambiguously except in the case of acid/ basic side chains through protein preparation. The PDB structure can be incorrect in some protein side chains. The crystallographic structure gives electron density, not molecular structure. Click on, Macromolecule, prepare protein, automatic preparation followed by prepare protein add Input protein (select the saved protein structure) then Run. Then save the resultant prepared structure in a new file form.

Ligand Preparation
Preparation of ligand is also done because of some reasons; A reasonable 3D structure is needed as starting point [9]. Protonation state and tautomeric form of a particular ligand could influence its hydrogen bonding ability. Small molecule ligand Prepared Input ligand and saved ligand structure then Run. The resultant prepared structures of ligands are saved in new file format.

Define Binding Site

After the protein and ligand preparation, next step is to define binding site for docking by receptor ligand interaction then define & Edit binding site Selected the residues then click on and select from Current Selection.

Docking

Click on Receptor ligand interactions, dock Ligands click LibDock which was used for docking, because a target needs to dock with multiple ligands. After docking all poses of dock result are evaluated in detail. Then the result was screened based on presence of H-bond interaction and Libdock score, and are listed out [10]. The listed ligand poses are screened based on the presence of H-bond interaction at GLU230 residue and the molecular properties of these ligands are calculated in docking score by ADMET descriptors and toxicity prediction. The binding energy of the ligands was also calculated.

Antitubercular Activity

The selected synthesized thianaphthene derivatives of the present investigation were screened for their antitubercular activity by subjecting the compounds to standard procedures. The antitubercular activity was evaluated against bacterial strain *M. tuberculosis* H37Rv (ATCC 27294) by Micro Plate Alamar Blue assay method[11]. Plain, clear bottomed, ninety-six well micro plates were utilized to conduct anti TB susceptibility test. Synthesized compounds to be analysed were initially diluted with DMSO followed by two-fold dilution with 7H12media. 7H9 media was used to dilute H37RV to obtain about 2 $X10^5$cfu/ml, and0.1ml was added to well micro plates, plates which contain only compounds were employed to determine auto fluorescence [12]. Plates were incubated at 37°C on 7^{th} day 20 µl of Alamar blue solution, 12.5ml of 20% Tween were added and incubation in continued for another twenty-four hours. Development of blue colour in the well was interpreted as no bacterial growth and pink color was scored as growth. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink[13]

CONCLUSION

The present study can be summarized as the designing of novel Enoyil reductase (PDB ID: 5JFO) selective inhibitors and analysis of the compounds through ADMET filters and molecular docking studies. From a library of designed compounds 10 compounds which had binding energy more could serve as lead compound for the development of newer potent anti-tubercular agents. Result obtained from the *in vitro* study, we can conclude that compounds BT-IV-A, BT-IV-B, BT-IV-E, BT-IV-G and BT-IV-H exhibits significant antitubercular activity and SAR also reveals that presence of Sulphur and other electronegative substituents may provide better activity.

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REFERENCES

1. LoBue PA, Enarson DA, Thoen CO. Tuberculosis in humans and animals: an overview [Serialised article. Tuberculosis: a re-emerging disease in animals and humans. Number 1 in the series]. The International Journal of Tuberculosis and Lung Disease. 2010 Sep 1;14(9):1075-8.
2. Premkumar A, Kolin DA, Farley KX, Wilson JM, McLawhorn AS, Cross MB, Sculco PK. Projected economic burden of periprosthetic joint infection of the hip and knee in the United States. The Journal of Arthroplasty. 2021 May 1;36(5):1484-9.

3. Matteelli A, Migliori GB, Cirillo D, Centis R, Girard E, Raviglione M. Multidrug-resistant and extensively drug-resistant Mycobacterium tuberculosis: epidemiology and control. Expert review of anti-infective therapy. 2007 Oct 1;5(5):857-71.
4. Ebert EC, Kierson M, Hagspiel KD. Gastrointestinal and hepatic manifestations of sarcoidosis. Official journal of the American College of Gastroenterology| ACG. 2008 Dec 1;103(12):3184-92.
5. Yadav S, Narasimhan B, Lim SM, Ramasamy K, Vasudevan M, Shah SA, Mathur A. Synthesis and evaluation of antimicrobial, antitubercular and anticancer activities of benzimidazole derivatives. Egyptian Journal of Basic and Applied Sciences. 2018 Mar 1;5(1):100-9.
6. Laxmi SV, Reddy YT, Kuarm BS, Reddy PN, Crooks PA, Rajitha B. Synthesis and evaluation of chromenyl barbiturates and thiobarbiturates as potential antitubercular agents. Bioorganic & medicinal chemistry letters. 2011 Jul 15;21(14):4329-31.
7. Guida A, Lhouty MH, Tichit D, Figueras F, Geneste P. Hydrotalcites as base catalysts. Kinetics of Claisen-Schmidt condensation, intramolecular condensation of acetonylacetone and synthesis of chalcone. Applied Catalysis A: General. 1997 Dec 23;164(1-2):251-64.
8. Madhavi Sastry G, Adzhigirey M, Day T, Annabhimoju R, Sherman W. Protein and ligand preparation: parameters, protocols, and influence on virtual screening enrichments. Journal of computer-aided molecular design. 2013 Mar;27(3):221-34.
9. Cole JC, Murray CW, Nissink JW, Taylor RD, Taylor R. Comparing protein–ligand docking programs is difficult. Proteins: Structure, Function, and Bioinformatics. 2005 Aug 15;60(3):325-32.
10. Joseph M, Alaxander S. Design, Synthesis and Characterization of Thiophene Substituted Chalcones for Possible Biological Evaluation.2021JPRI9(6).76-87
11. Collins LA, Franzblau SG. Microplate alamar blue assay versus BACTEC 460 system for high-throughput screening of compounds against Mycobacterium tuberculosis and Mycobacterium avium. Antimicrobial agents and chemotherapy. 1997 May;41(5):1004-9.
12. Sam IK, Chen YY, Ma J, Li SY, Ying RY, Li LX, Ji P, Wang SJ, Xu J, Bao YJ, Zhao GP. TB-QUICK: CRISPR-Cas12b-assisted rapid and sensitive detection of Mycobacterium tuberculosis. Journal of Infection. 2021 Jul 1;83(1):54-60.
13. Franzblau SG, Witzig RS, McLaughlin JC, Torres P, Madico G, Hernandez A, Degnan MT, Cook MB, Quenzer VK, Ferguson RM, Gilman RH. Rapid, low-technology MIC determination with clinical Mycobacterium tuberculosis isolates by using the microplate Alamar Blue assay. Journal of clinical microbiology. 1998 Feb 1;36(2):362-6.

Table No: 1. Docking score of different synthesized compounds

LIGANDS	DOCKING SCORE
BT-IV-A	134.504
BT-IV-B	134.15
BT-IV-C	131.461
BT-IV-E	124.732
BT-IV-G	136.961
BT-IV-H	137.55

Table: 2. Molecular descriptors of synthesized derivatives

Compound code	MR, cm^3	MV, cm^3	Parachor, cm^3	Surface tension, dynes/cm	Polarizability, cm^3
BT-IV-A	124.58±0.3	316.2±3.0	874.2±4.0	58.3±3.0	49..39±0.510-24
BT-IV-B	134.37±0.3	340.1±3.0	945.9±4.0	59.7±3.0	53.27±0.510-24
BT-IV-C	131.13±0.3	321.1±3.0	929.7±4.0	64.4±3.0	51.98±0.510-24
BT-IV-E	121.78±0.3	311.0±3.0	986.4±4.0	59.5±3.0	48.27±0.510-24
BT-IV-G	129.41±0.3	332.5±3.0	911.8±4.0	56.5±3.0	51.30±0.510-24
BT-IV-H	126.47±0.3	314.7±3.0	889.2±4.0	63.7±3.0	50.13±0.510-24

Table: 3. Details of Lipinski's Rule of Five

Compound	LogP	MW	HBD	HBA	Lipinski's rule
BT-IV-A	5.94	413.50	1	4	1
BT-IV-B	7.00	482.39	1	4	1
BT-IV-C	5.67	458.50	1	7	1
BT-IV-E	5.31	437.90	1	5	1
BT-IV-G	6.62	447.94	1	4	1
BT-IV-H	5.46	429.50	2	5	1

Table: 4. Drug likeness analysis of novel analogues

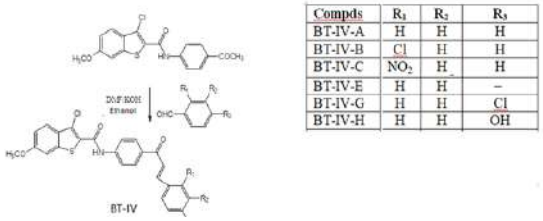
Compound	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitors	Enzyme inhibitors
BT-IV-A	0.14	0.34	0.09	0.18	0.11	0.03
BT-IV-B	0.34	0.60	0.49	0.43	0.23	0.23
BT-IV-C	0.25	0.35	0.24	0.26	0.28	0.14
BT-IV-E	0,28	0.62	0.46	0.52	0.28	0.19
BT-IV-G	0.14	0.34	0.10	0.19	0.15	0.06
BT-IV-H	0.10	0.28	0.05	0.07	0.09	0.02

Table: 5.ADMET prediction of the ligands

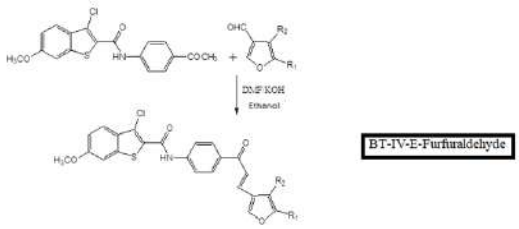
Sl. No	PubChem ID	Solubility	BBB	CYP2D6	Hepatotoxic	Absorption	PBB	A Log P	PSA	Ames Mutagenicity
1	BT-IV-A	1	4	false	true	1	true	6.197	56.341	Non-mutagen
2	BT-IV-B	1	4	false	true	2	true	6.861	56.341	Non-mutagen
3	BT-IV-C	1	4	false	true	2	true	6.091	99.165	Non-mutagen
5	BT-IV-E	1	1	false	true	0	true	5.301	68.896	Non-mutagen
7	BT-IV-G	1	4	false	true	2	true	6.861	56.341	Non-mutagen
8	BT-IV-H	1	4	false	true	1	true	5.955	77.157	Non-mutagen

Table: 6. *In-vitro* anti-tubercular activity of synthesized compound in different concentration against *Mycobacterium tuberculosis*

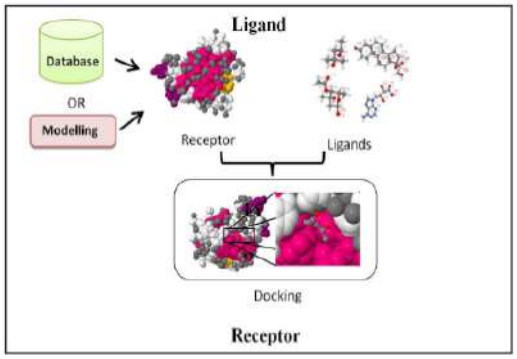
Compd. Code	**Concentration (µg\ml)**, (**S**- *Sensitive*, **R**- *Resistant)*							
	100	50	25	12.5	6.25	3.125	1.6	0.8
BT-IV-A	S	S	S	S	S	S	S	R
BT-IV-B	S	S	S	S	S	S	S	R
BT-IV-C	S	S	S	S	R	S	R	R
BT-IV-E	S	S	S	S	R	S	S	R
BT-IV-G	S	S	S	S	S	S	S	R
BT-IV-H	S	S	S	S	S	S	S	R
INH (control)	S	S	S	S	S	S	S	S



Compds	R_1	R_2	R_3
BT-IV-A	H	H	H
BT-IV-B	Cl	H	H
BT-IV-C	NO_2	H	H
BT-IV-E	H	H	–
BT-IV-G	H	H	Cl
BT-IV-H	H	H	OH



Scheme - I . General scheme (I) for the preparation of compounds



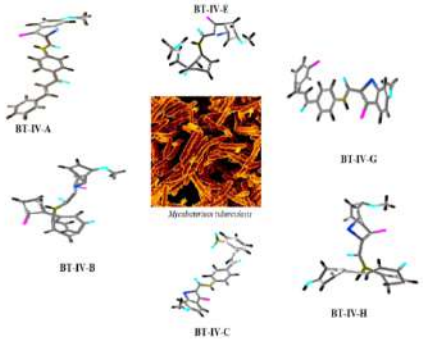


Fig . 1. General method of docking and graphical representation of SAR

Fig. 2. Crystallographic structure of target from PDB database

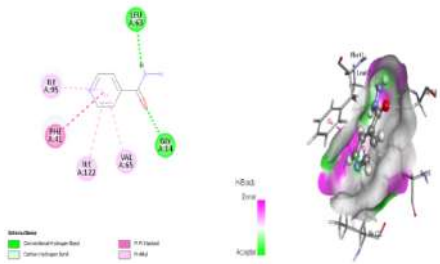
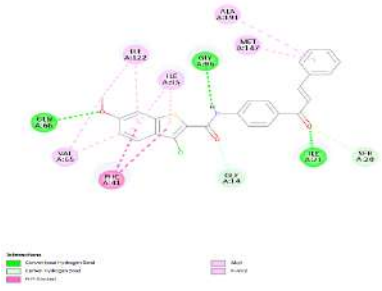


Fig. 3. Interaction of standard drug (Isoniazid) with5JFO



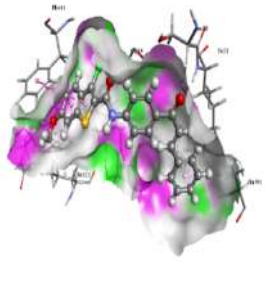


Fig. 4. Interactions between target and ligand BT-IV-A

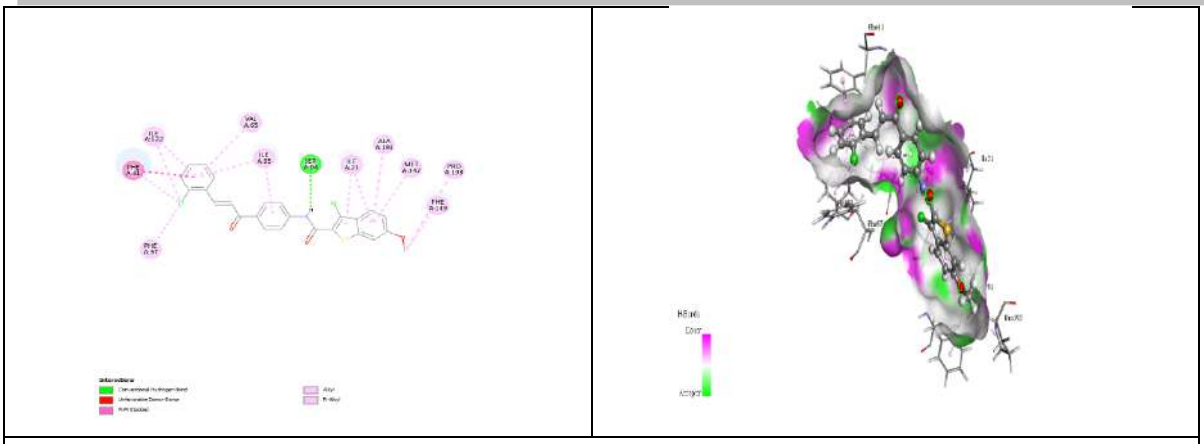


Fig. 5. Interactions between target and ligand BT-IV-B

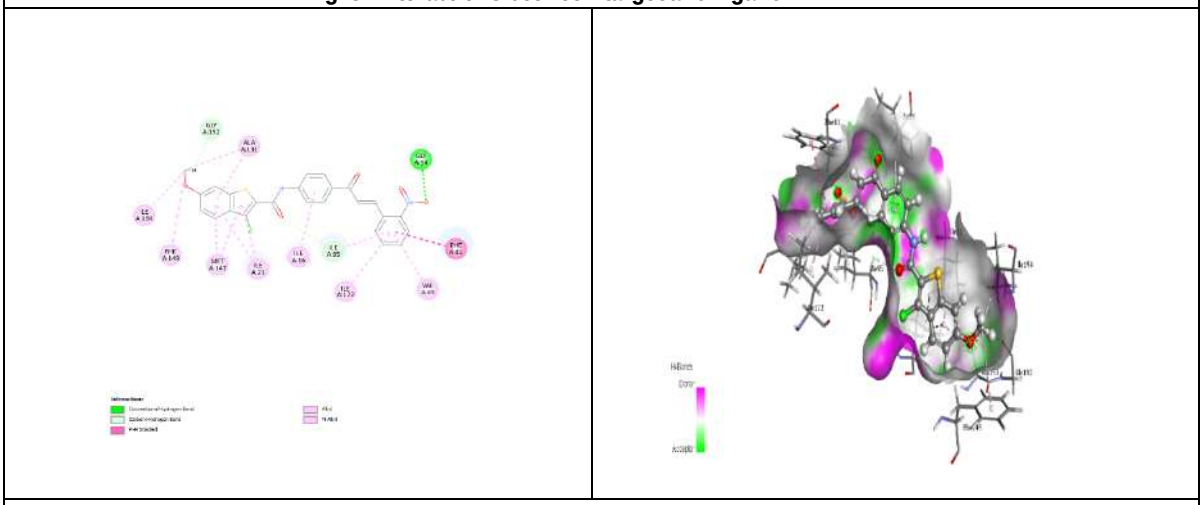


Fig. 6. Interactions between target and ligand BT-IV-C

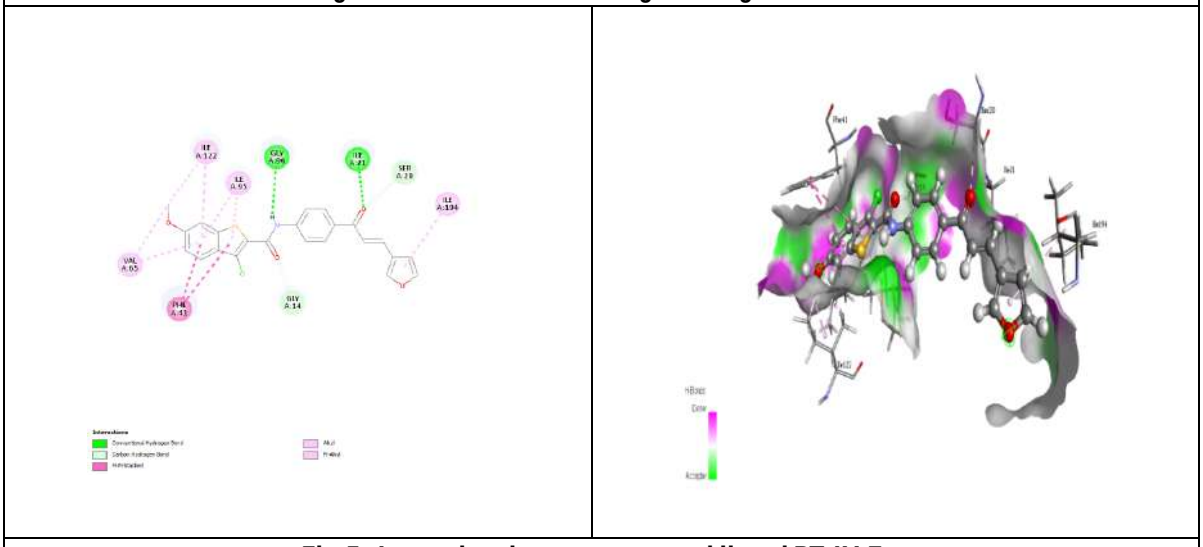


Fig. 7. Interactions between target and ligand BT-IV-E

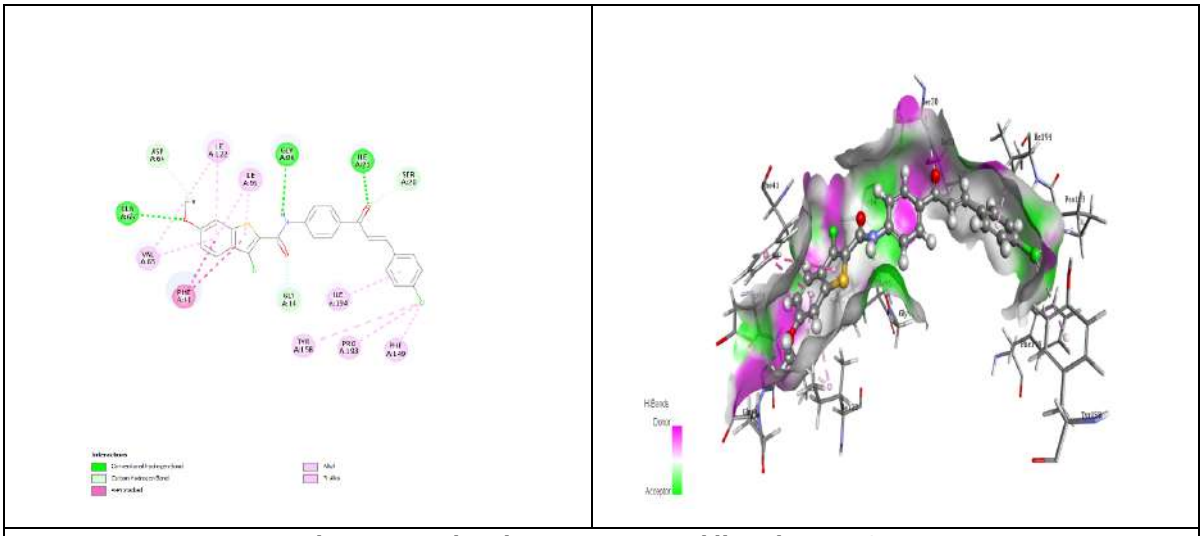


Fig. 8. Interactions between target and ligand BT-IV-G

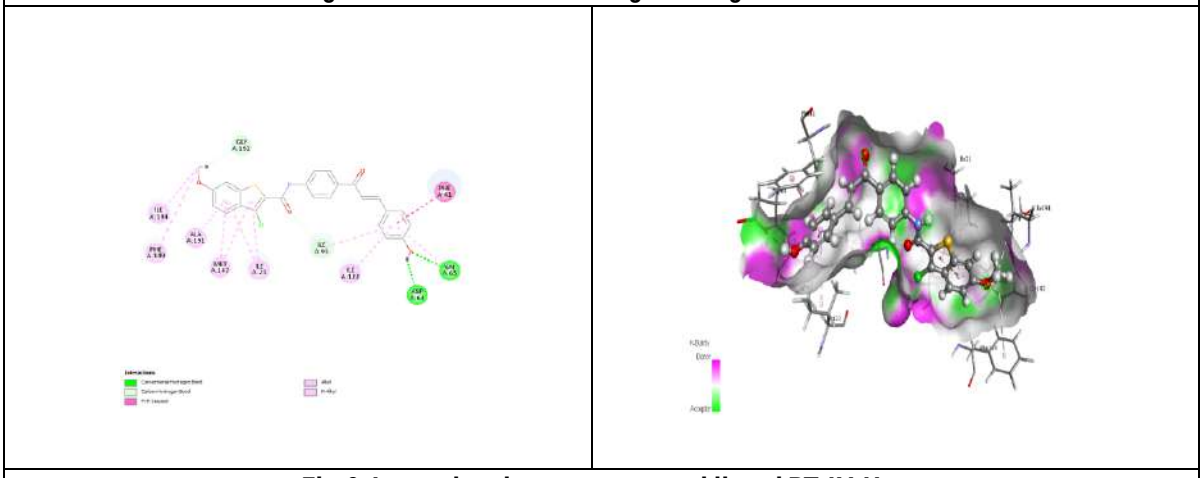


Fig. 9. Interactions between target and ligand BT-IV-H

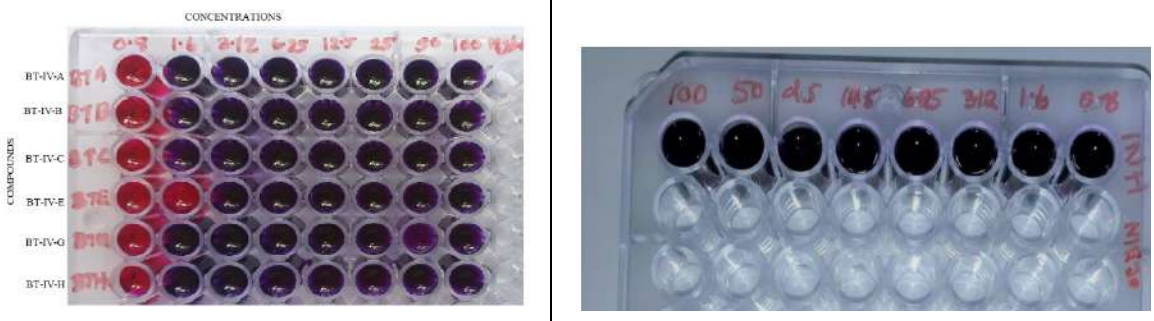


Fig. 10. Representative photograph for anti-tubercular activity performed against H37Rvs train using96- well microplate by MABA (Microplate Alamar Blue Assay) method for the compounds from benzothiophene series with standard INH serial dilutions for test compounds between 100 to 0.8µg/ml.

Spectral images of all synthesized compounds confirmed by its synthesis

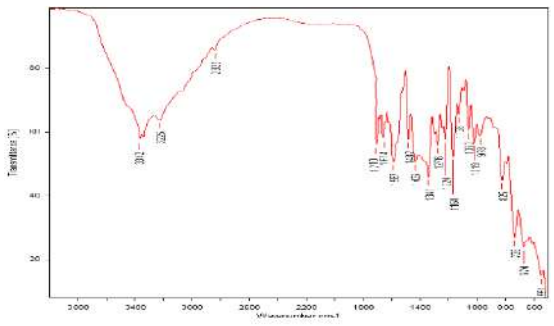


Fig. 11. IR Spectra of BT-IV-A

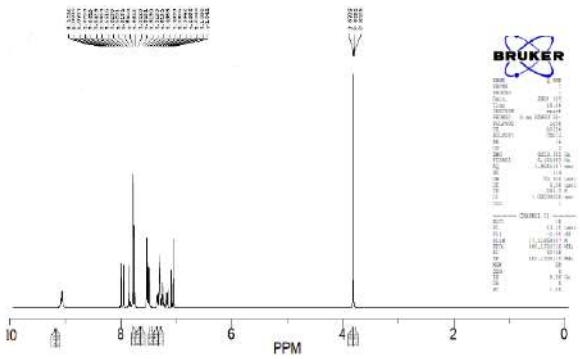


Fig.12. ^{1}H NMR Spectra of BT-IV-A

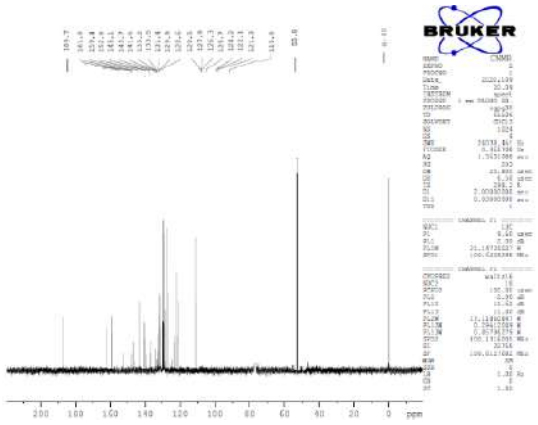


Fig. 13. ^{13}C NMR Spectra of BT-IV-A

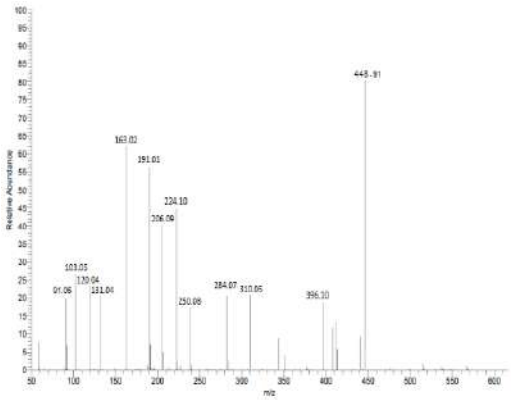


Fig.14. Mass spectra of BT-IV-A

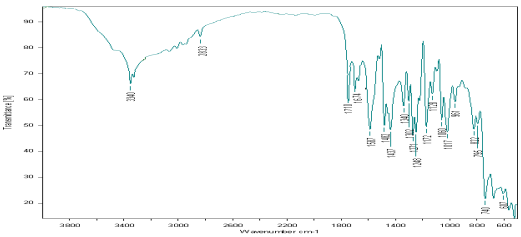


Fig. 15. IR Spectra of BT-IV-B

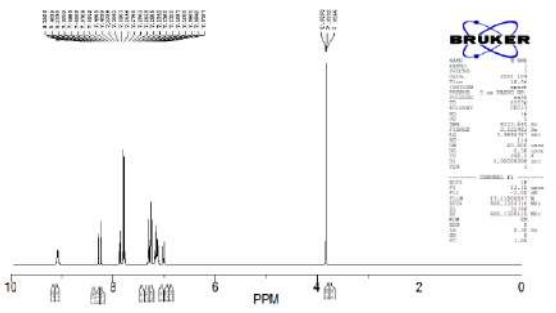


Fig.16. ^{1}H NMR Spectra of BT-IV-B

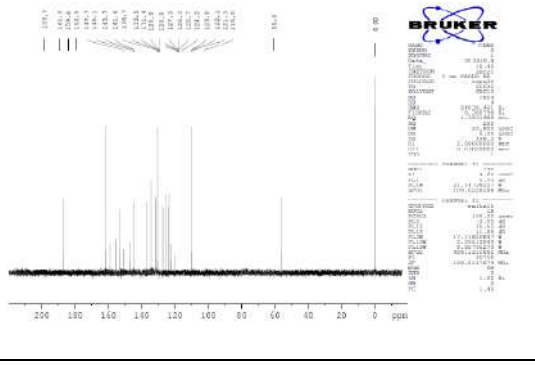


Fig.17. ^{13}CNMR Spectra of BT-IV-B

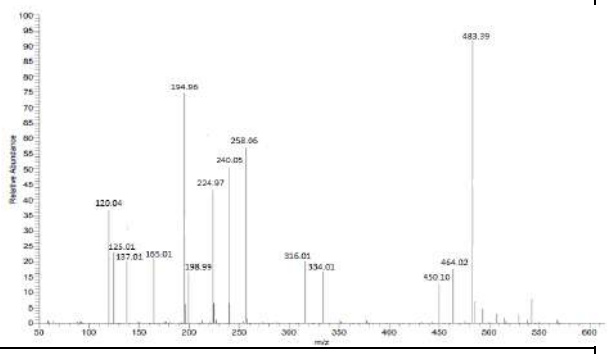


Fig. 18. Mass spectra of BT-IV-B

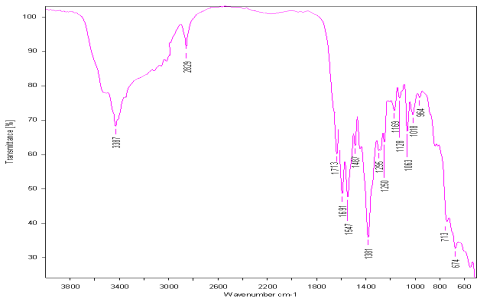


Fig. 19. IR Spectra of BT-IV-C

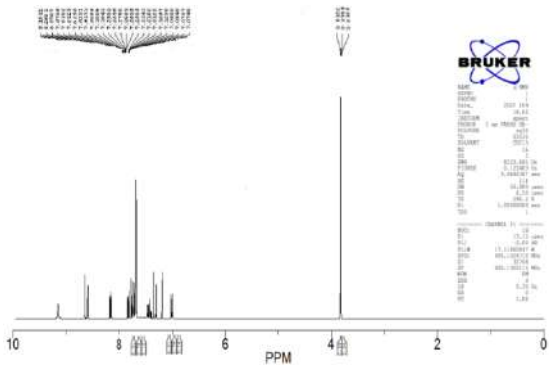


Fig. 20. NMR Spectra of BT-IV-C

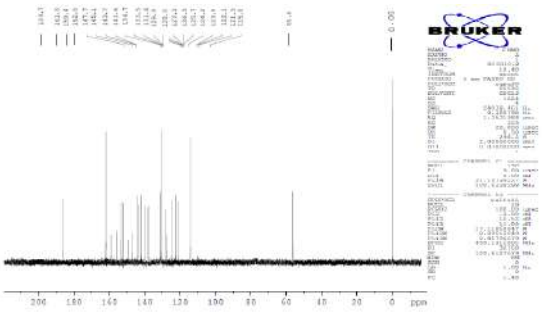


Fig. 21. ^{13}CNMR Spectra of BT-IV-C

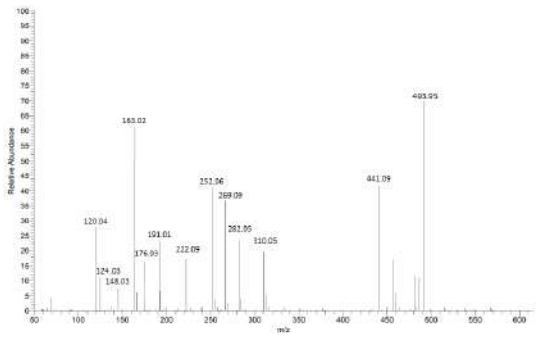


Fig. 22. Mass spectra of BT-IV-C

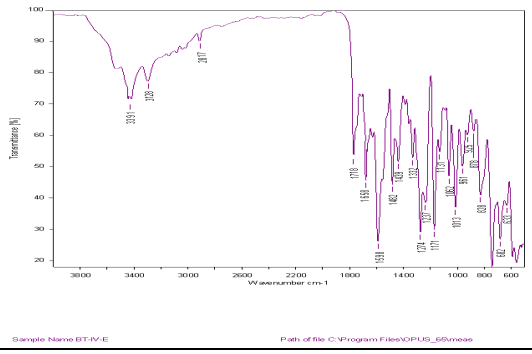


Fig. 23. IR Spectra of BT-IV-E

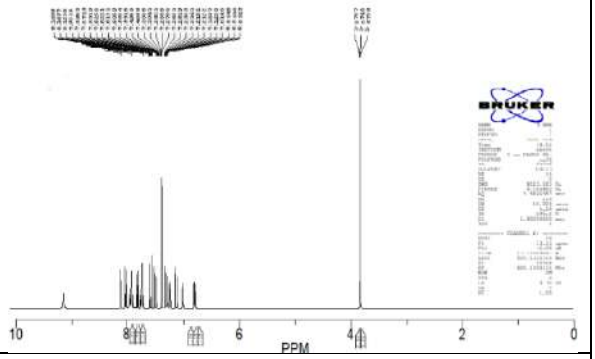


Fig. 24. NMR Spectra of BT-IV-E

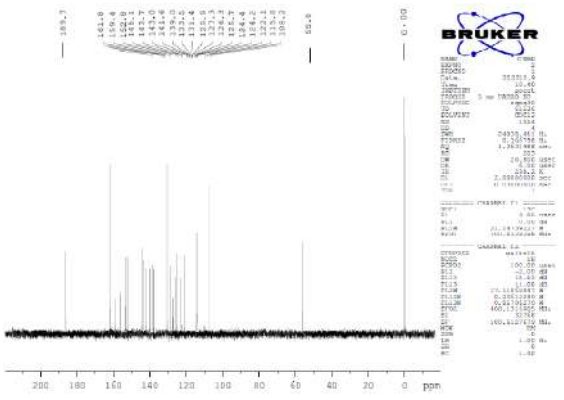


Fig. 25. ^{13}CNMR Spectra of BT-IV-E

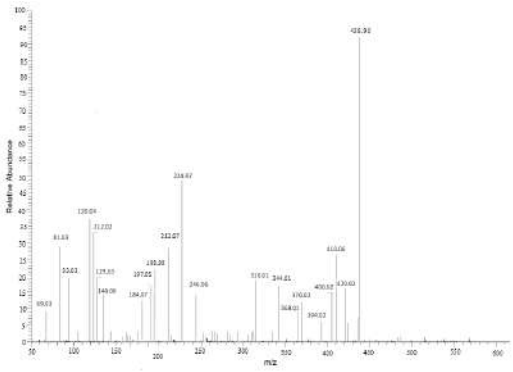


Fig. 26. Mass spectra of BT-IV-E

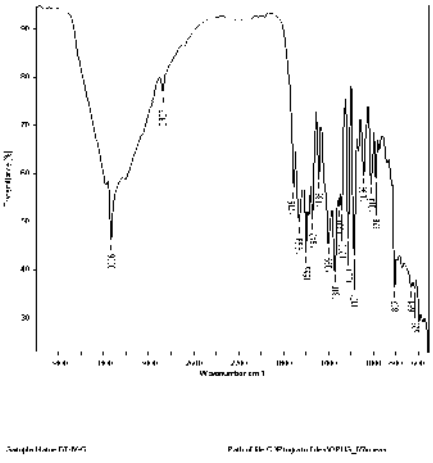


Fig. 27. IR Spectra of BT-IV-G

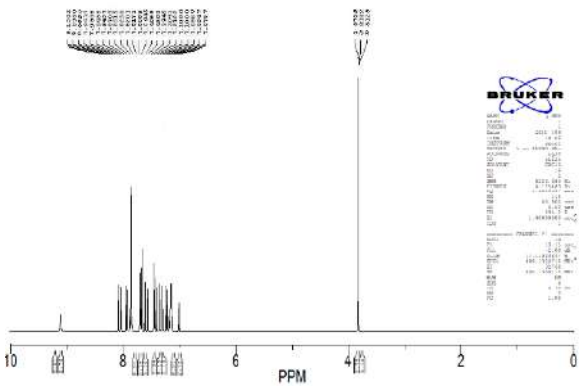


Fig.28. NMR Spectra of BT-IV-G

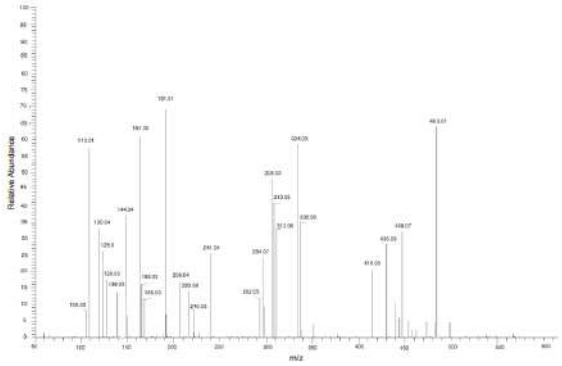


Fig.29. Mass spectra of BT-IV-G

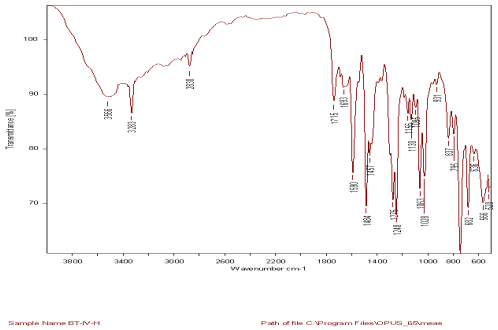


Fig. 30. IR Spectra of BT-IV- H

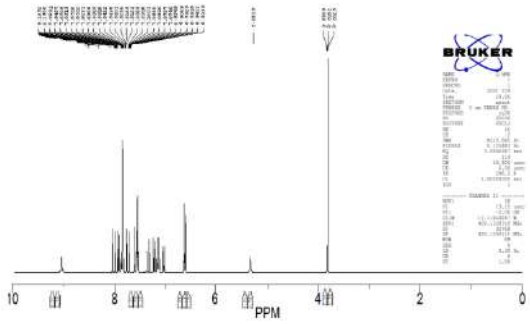


Fig. 31. ^{1}H NMR Spectra of BT-IV-H

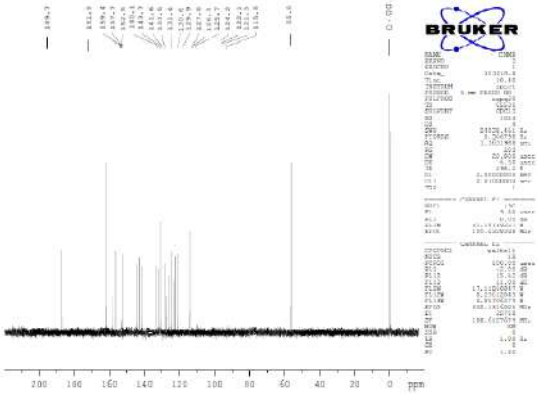


Fig. 32. ^{13}CNMR Spectra of BT-IV-H

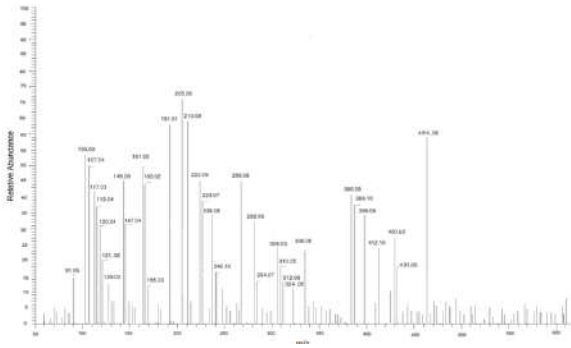


Fig. 33. Mass spectra of BT-IV-H

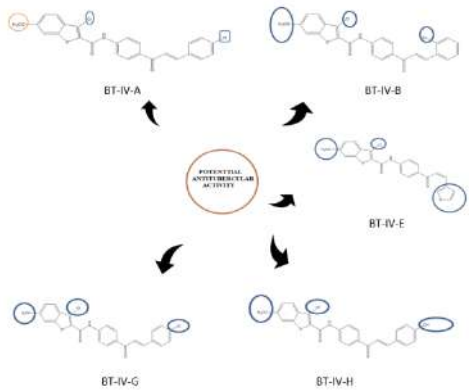


Fig . 34. SAR of synthesized compounds

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RESEARCH ARTICLE

Anti Microbial and Diabetic Wound Healing Activity of Poly-Herbal Formulation on Streptozotocin - Nicotinamide Induced Diabetic *Albino wistar* Rats

V. Jhansi Lakshmi[1]* and Suhasini.G[2]

[1]Assistant Professor, Department of Pharmacology, Vignan Institute of Pharmaceutical Technology, Duvvada, Visakhapatnam, Andhra Pradesh, India.
[2]Associate Professor, Department of Pharmacology, Gitam institute of Pharmacy, Rushikonda Visakhapatnam, Andhra Pradesh, India.

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***Address for Correspondence**
V. Jhansi Lakshmi
Assistant Professor,
Department of Pharmacology,
Vignan Institute of Pharmaceutical Technology,
Duvvada, Visakhapatnam, Andhra Pradesh, India.
Email: jhanu.18@gmail.com

ABSTRACT

To investigate diabetic wound healing activity of poly-herbal formulation prepared from hydro-alcoholic extracts on Nicotinamide-Streptozotocin induced diabetic Albino Wistarrats. *Ocimum sanctum, Aegle marmelos & Syzygium cumini* leaves were dried and made into hydro-alcoholic extracts. Extraction was done by cold maceration method. Poly-herbal formulation (ointment and mixture) was prepared using the extracts prepared. Three formulations were prepared in combinations of three mixtures of extracts consisting of *Ocimum sanctum, Aegle marmelos* and *Syzygium cumini* respectively in the ratio of 1:2:3, 2:3:1 and 3:1:2.The rats were made diabetic (type-2) by a single dose of NA-STZ Nicotinamide (NA) (100mg/kg)i.p and Streptozotocin (STZ) (45 mg/kg) i.p. Excision wound creation done on all groups. Poly-herbal formulations (PHF) were used upon PHF groups (3) in a concentration of (200 mg/kg) once daily for 21 days. 36 rats (150-200 gms) were placed in 6 groups were used for the study. Wound area of animals were measured on 0th, 14th and 21st day and blood glucose on 0th and 21st days. Soframycin and metformin were used as standard treatment. PHF-3 (consisting of 3 parts of *Ocimum sanctum,*1 parts of *Aegle marmelos &* 2 part of *Syzygium cumini* leaves showed significant (P***<0.0001) wound closure compared to PHF-1&2. By the results obtained, it's evident that the P.HF-3 have better diabetic wound healing ability. So, can be further investigated for the development of the formulation.

Keywords: *Ocimum sanctum, Aegle marmelos, Syzygium cumini*, Streptozotocin, Nicotinamide etc.

INTRODUCTION

Diabetes mellitus (DM) is a progressive metabolic disease marked by insulin deficiency and insulin resistance. Type 2 Diabetes Mellitus (T2DM) is the most common type of diabetes and is associated with a variety of disorders such as microvascular and macrovascular disorders, which may be the leading cause of illness and death in the world population. Diabetes is expected to affect more than 150 million people worldwide. It is expected that the presence of DM in world population is greater than 150 million. T2DM patients are more vulnerable to serious infections due to a variety of factors, including decreased chemotaxis and phagocytic activity by neutrophils. These patients are more susceptible to bacterial infections and have slower wound healing, which is a repair mechanism involving the interactions of various cells and extracellular molecules[1]. It has become an expensive stint for diabetes patients to get better health care. In elderly diabetic patients the diabetic foot ulcer or wound is a common problem. Management of diabetic wounds costs more than 20 billion dollars and a loss of more than two million workdays. Comparatively diabetic foot ulcers are difficult to treat, expenses range between $7,000 and $10,000 per ulcer. Most of the ulcers lead to limb amputation, which may cost way more than the treatment.[2] Complex biological events lead to healing process in order to repair damaged tissue.[3]Herbal drugs are gaining momentum when compared to synthetic drugs. Plants species like *Aloe-vera, Momordica charantia, Calotropis procera, Portulaca oleracea, Acalypha langiana* and *Plagiochasma appendiculatum* were reported to have wound healing properties in traditional medicine systems like Ayurveda[4]. There are multiple reasons for the rise in the use of herbal medicines for a variety of ailments [5].

MATERIALS AND METHODS

After getting approval from IAEC (Institutional Animal Ethics Committee), we started the study. Streptozotocin, Nicotinamide and Metformin, Ofloxacin was procured from Sai chemicals, Visakhapatnam.

Plants collection, authentication and extraction

Early leaf twigs of plants (*Ocimum sanctum, Aegle marmelos and Syzygium cumini*) were gathered from the local regions of Visakhapatnam. The collected plants were washed carefully under low pressure tap water and shade dried for 11 days. Once dried the leaves were ground into powder by using grinder.

Preparation of extract

Powders of *Ocimum sanctum, Aegle marmelos,* and *Syzygium cumini* leaves were made into hydro-alcoholic extracts individually, by adding ethanol and water in a ratio of 70:30. These extracts were then kept under cold maceration process. These hydro-alcoholic solutions are filtered with filter paper followed by Vacuum filtration and Steam distillation, Rota-rod evaporation and the final extract is used for Anti-Microbial test and then for ointment preparation.

PHYTO CHEMICAL SCREENING

Powders of *Ocimum sanctum, Aegle marmelos ,* and *Syzygium cumini* leaves are subjected to flavanoid test [6].

ANTI-MICROBIAL ACTIVITY OF PHF

Agar well diffusion method

Test microbial strains: By cup plate method, the test extracts were tested against gramme positive strain (*Bacillus subtilis*) and gramme negative strain (*Pseudomonas aeruginosa*).

Preparation of agar plates for anti-microbial testing

The agar plates were inoculated by streaking a swab of bacterial strains across the completely sterile agar surface for 2-3 times before rotating the sterile agar plate at a 60^{o} angle to ensure uniform distribution of the inoculum. Boring of 9mm diameter wells into the agar was done once they were dried under sterile conditions at room temperature. Test extracts (*Ocimum sanctum, Aegle marmelos, and Syzygium cumini*) were made into solutions using Dimethyl

Sulphoxide (DMSO) solvent. Ofloxacin was used as the standard anti-microbial agent. These test extract solutions were added into wells using aseptic micropipette. The plates were incubated in a bio-Oxygen Demand for 24 hours at 37° C. The zone of inhibition of bacterial strains was measured in triplicate using a calibrated digital Vernier calliper.

Establishment of minimum inhibitory concentration (mic)

Broth dilution method

100µl of 105 Colony Forming Unit per ml of test strains (*Bacillus subtilis* and *Pseudomonas aeruginos*a) was inoculated in each test tube with uniform volume of Nutrient Broth Medium. At 37ºC Aerobically the test tubes were incubated for 24 to 48 hrs. For each strain three control tubes were used containing (media, organism and extract controls) respectively. If an extract produces no visible microbial growth at the lowest concentration when compared to standard in 24hr, the concentration is declared as MIC. Determination of MIC for different microbial strains was done separately.

Polyherbal Mixture Preparation

The extracts of *Ocimum sanctum, Aegle marmelos, Syzygium cumini* were mixed in the ratios earlier mentioned respectively to form 3 mixtures. The 3 mixtures formed are PHM-1 (1:2:3), PHM-2 (2:3:1), PHM-3 (3:1:2).

Acute Toxicity Studies

Acute toxicity study was done to select dose in accordance to OECD guidelines 423[7]. At doses of 0, 100, 200, 400, 800… the test drug was given to the rats in increasing order of the dose. For the next 48hrs the rats were under observation to notice possible signs of toxicity. If one or more doses were given within a period of 24hrs then the study is acute toxicity study. Toxicity was observed at 2000mg/kg. The standard anti-diabetic drug Metformin was used at a dose of 200mg/kg.

Dose Selection

The dose selection was done by observing the results obtained in acute toxicity studies. Based on the acute toxicity results 200mg/kg was used in the study.

Ointment Preparation

Basing on the already reported anti-microbial efficacy (*Ocimum sanctum, Aegle marmelos, Syzygium cumini*) in ratios 1:2:3, 2:3:1, 3:1:2 respectively were [8,9,10]. Grated hard paraffin was weighed and placed in evaporating dish on water bath. Upon it's melting by fusion method all the other ingredients were weighed and added depending upon formulation ratios, gradually adding bases and stirred to melt and mix homogenously, finally the ointment was prepared which was then transferred to suitable containers then cooled and stored at 4 ºC.

Evaluation of Poly-Herbal Formulation for Wound Healing Activity in Male *Albino wistar* Rats

Animals

Inbred male *Albino wistar* rats (150-250gm), were acquired and used in the study. Propylene cages were used to house the animals, under standard temperature (24±2ºC temperature), standard humidity (Relative humidity of 45-55%) and 12hr light cycle and 12hr dark cycle respectively. Standard pellet diet was fed to animals along with water ad libitum. The animals were being starved for 16-18hr before the commencement of the experiment but water is made available ad libitum. Approval from the institutional animal Ethical committee (IAEC/VIPT/2021/02) were was obtained and the study done under strict compliance of committee for the purpose of control and supervision of experiments on animals (CPCSEA) guidelines (1275/ac/09/CPCSEA).

Ointment formulation evaluation

Colour and Odour

Visual examination is performed; if the ointment colour and/or odour are objectionable, the formulation is rejected [11].

pH

Digital pH meter was used to measure pH of the formulation. By dissolving a gram of formulation in 100 ml of distilled water the pH of the formulation is measured. PH measurement was done in triplicate manner for each formulation and average values were noted [11].

Spread ability test

For any topical formulation spreadability should be good so that uniform distribution is ensured. Too much dragging should not be needed and also friction produced should be minimum during the application. The sample was sandwiched between two glass slides, and weight was applied to the slide for 5 minutes to press it to a uniform thickness. The spread ability of the ointment is measured in seconds by the time it takes to separate the two sides [12]. Formula to calculate spread ability:

$S = M \times L/T$

here, S = Spread ability of formulation,
M = Weight placed on upper slide,
L = Length of Glass Slides,
T = Time taken to separate the slides in seconds.

Consistency

Smooth as expected.

Skin irritability test:

The prepared Poly-herbal formulations were tested for skin irritability by simply applying the formulation on healthy animal skin 24hrs before wound creation. This test is to know the irritability and safety of the formulation. The animals were observed for any rashes or skin damage.

Streptozotocin induced diabetic wound model

Induction of diabetes

The diabetes was induced in 5 groups (disease control group, standard treatment group, 3 test formulation treatment groups) by a single I.P injection of newly prepared Nicotinamide (100mg/kg) and Streptozotocin (45mg/kg bd. wt.) mixed in citrate buffer (pH 4.00) to overnight fasted rats [13]. 20% glucose solution was given for the Nicotinamide-Streptozotocin treated rats for 24hr to avoid drug induced hypoglycemic mortality. Measurement of blood glucose was done using diabetes testing kit (GOD/POD method)[14] and confirmation of diabetes was done on 4^{th} day of Streptozotocin induction. Fasting blood glucose value of rats above 200 mg/dl were considered as diabetic and were included in the study. Presence of diabetes was confirmed in all rats, the confirmation was done as their blood glucose level was above 200 mg/dl (mg per deciliter of blood).

Induction of excision wound

After induction of diabetes on 4^{th} day diabetes is confirmed and wound is created Six groups of animals(one normal control, one disease control, one standard treated and 3 test drug treated group) six rats per group. After initially shaving the dorsal portion using depilatory cream and then excision wound was made by removing epidermal layer of 500mm^2 area and left open to the environment. Wound healing was measured as percentage wound contraction. The test formulations with spatula full and standard drug were applied twice a day to the wound area of the grouped animals according to their grouping and treatment formulation allotted. The wound area was measured on the 0th, 14th, and 21st days by tracing the wound with a tracing paper. The traced area on the paper was later measured by a scale to calculate the wound area. The wound areas on different days were compared to the wound area on day 0 and thereby percentage wound closure was calculated. Percentage wound closures of different groups were compared so their wound healing efficacy.

$$\%wound\ closure = \frac{Initial\ wound\ area - wound\ area\ on\ the\ particular\ day}{Initial\ wound\ area} \times 100$$

Plan of work

All the animals except group 1&2 were provided with respective topical treatment twice daily for 21 days. Poly herbal mixtures were administered orally to the animals twice daily for 21 days.

RESULTS

Table 5 and figure 1, 2 represent zone of inhibition of poly-herbal extracts anti-microbial assay. Table 6and figure 4 represent fasting blood glucose levels on 0^{th} and 21^{st} day. Wound areas (mm^2) on 0^{th}, 14^{th}, 21^{st} day and percentage of wound contraction were depicted in Tables 7, 8. PHF-3 have better wound closure effect and exhibited comparatively good wound healing properties when compared to PHF-1, PHF-2 and disease control with same ingredients but in different ratio of quantities. The standard Soframycin showed fastest wound healing.

Wound area measurement

Wound area measurement is done using a tracing paper, by placing it upon the wound and marking the wound area. The Marked portion on the tracing paper is then measured using a scale.

Statistical analysis

Graph pad prism version 5.2 was used to perform statistical analysis. Results were depicted as the mean ± SEM n=36 for all treatment groups. The data recorded was analyzed by two way and one way ANOVA.

DISCUSSION

Diabetes is one of the most prevalent disease observed among majority of population. Physiological changes that occur due to chronic diabetic condition lead to decreased wound healing ability. So, wound healing in diabetic condition is a complex process. In spite of regular usage of proved anti-diabetic medication, deprived wound healing ability is still posing a great risk which may lead to chronic foot ulcers, leg amputation etc., this necessitates usage of topical medication along with oral anti-diabetic medication. Topical medications like ointments containing anti-microbial, anti-inflammatory activities are used to treat normal and diabetic wounds. Using herbal formulations for treating and healing process is the need of the hour to encourage natural treatment process. According to literature, leaves of *Ocimum sanctum* contain properties like anti-inflammatory, anti-bacterial, anti-oxidant, anti-stress etc.,[9] and *Syzygium cumini* contain anti-inflammatory, anti-septic, enhance wound healing, anti-diabetic, cytoprotective, anti-bacterial, anti-oxidant etc.,[15, 8] and *Aegle marmelos* contain anti-microbial, ulcer healing, anti-inflammatory activity etc.,[16] which enhance and aid in wound healing process. In this study the poly-herbal formulations were made of extracts of plants leaves of *Ocimum sanctum, Syzygium cumini* and *Aegle marmelos.* Oral anti-diabetic therapy was performed by using the poly-herbal extracts , this therapy in combination with topical therapy by poly-herbal ointment prepared using the same poly-herbal extracts yielded good results. Comparatively poly-herbal mixture-3 and ointment-3 combination exhibited better wound healing activity than other poly-herbal combinations. The order of the wound healing among all test groups is as follows: PHF-1 + PHM-1 < PHF-2 + PHM-2 < PHF-3 + PHM-3 .

CONCLUSION

Since, the above formulations have yielded better results when tested on animals, they can also be studied on humans for development of newer drugs for the betterment of life standards of diabetic patients.

CONFLICT OF INTEREST

There is no conflict of interest.

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REFERENCES

1. Carvalho PD, Mazzer N, Reis FA, Belchior AC, Silva IS. Analysis of the influence of low-power HeNe laser on the healing of skin wounds in diabetic and non-diabetic rats. ActaCirúrgicaBrasileira. 2006; 21:177-83.
2. Sherman RA. Maggot therapy takes us back to the future of wound care: new and improved maggot therapy for the 21st century. Journal of diabetes science and technology. 2009 Mar; 3(2):336-44.
3. Modolin M, Bevilacqua RG. Cicatrização das feridas: síntese das aquisiçöesrecentes. Rev. Bras. Clín. ter. 1985:208-13.
4. A.K. Nadkarni. Indian Materia Medica. 2000; 779-780.
5. Heinrich M, Williamson EM, Gibbons S, Barnes J, Prieto-Garcia J. Fundamentals of pharmacognosy and phytotherapy E-BOOK. Elsevier Health Sciences; 2017 Nov 24.
6. Senthilkumar M, Gowri M, Vadivel V. PHYTOCHEMICAL ANALYSIS OF BIOACTIVE COMPOUNDS OF SYZYGIUM CUMINI (L.) SKEELS. World Journal of Pharmaceutical Research. 2017 May 9;6(7).
7. Briggs ON, Nwachuku EO, Tamuno-Emine D, Nsirim N, Elechi-Amadi KN. Biochemical and oxidative changes in high fat diet/streptozotocin-induced diabetic rats treated with metformin and the polyherbaldiawell. Journal of Complementary and Alternative Medical Research. 2019 Aug 26:1-1.
8. Lakshmi VJ, Manasa K. Various Phytochemical Constituents and Their Potential Pharmacological Activities of Plants of the Genus Syzygium.
9. Singh D, Chaudhuri PK. A review on phytochemical and pharmacological properties of Holy basil (*Ocimum sanctum* L.). Industrial Crops and Products. 2018 Aug 1; 118:367-82.
10. Sekar DK, Kumar G, Karthik L, Rao KB. A review on pharmacological and phytochemical properties of *Aegle marmelos* (L.) Corr. Serr. (Rutaceae). Asian Journal of Plant Science and Research. 2011; 1(2):8-17.
11. Roesch M, Perreten V, Doher MG, Schaeren W, Schallibaum M, Blum JW. Comparison of antibiotic resistance of udder pathogens in dairy cows kept on organic and on conventional farms. J Dairy Sci. 2006; 89:989–97.
12. Schwarz S, Chaslus-Dancla E. Use of antimicrobials in veterinary medicine and mechanisms of resistance. Vet Res. 2001; 32:201–25.
13. Pari L, Murugan P. Tetrahydrocurcumin prevents brain lipid peroxidation in streptozotocin-induced diabetic rats. Journal of medicinal food. 2007 Jun 1; 10(2):323-9.
14. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Annals of clinical Biochemistry. 1969 Jan; 6(1):24-7.
15. Ramya S, Neethirajan K, Jayakumararaj R. Profile of bioactive compounds in *Syzygium cumini*–a review. Journal of Pharmacy research. 2012 Aug; 5(8):4548-53.
16. Rahman S, Parvin R. Therapeutic potential of *Aegle marmelos* (L.)-An overview. Asian Pacific journal of tropical disease. 2014 Feb 1; 4(1):71-7.

Table 1: Formulation of poly-herbal ointment: PHO1

S. No	Ingredients	Quantity
1	Prepared extract of sample 1	0.1 gm
2	Prepared extract of sample 2	0.2 gm
3	Prepare extract of sample 3	0.3 gm
4	Ointment base	10 gm

Table 2: Formulation of poly-herbal ointment: PHO2

S. No	Ingredients	Quantity
1	Prepared extract of sample 1	0.2 gm
2	Prepared extract of sample 2	0.3 gm
3	Prepared extract of sample 3	0.1 gm
4	Ointment base	10 gm

Table 3: Formulation of poly-herbal ointment: PHO3

S. No	Ingredients	Quantity
1	Prepared extract of sample 1	0.3 gm
2	Prepared extract of sample 2	0.1 gm
3	Prepared extract of sample 3	0.2 gm
4	Ointment base	10 gm

Table 4: Grouping of animals

Groups	Treatment
Group -1 Normal control	Vehicle
Group -2 Diabetic control	Nicotinamide - Streptozotocin
Group-3 standard	Nicotinamide - Streptozotocin + Metformin(200mg/kg bodyweight)
Group-4 PHF-1	Nicotinamide- Streptozotocin + PHM-1+ PHF-1
Group-5 PHF-2	Nicotinamide – Streptozotocin + PHM-2 + PHF-2
Group-6 PHF-3	Nicotinamide – Streptozotocin + PHM-3 + PHF-3

Table 5: Zone of Inhibition (mm) of Poly Herbal Ethanolic Extracts against *Bacillus subtilis* and *Pseudomonas aeruginosa*.

S.No	*Bacillus subtilis* (mm)	*Pseudomonas aeruginosa* (mm)
Ofloxacin	30.21±1.11	35.34±1.78
PHM1	14.70±1.16*	11.00±1.69*
PHM2	17.00±1.18**	14.10±1.08**
PHM3	25.70±1.10***	21.00±1.15***

n=3, *p<0.05,**p<0.01, ***p<0.001 PHM1,PHM,PHM3vsOfloxacin(Two way ANOVA) and then Bonferroni post hoc tests. Values are expressed as (MEAN±SEM)

Table 6: Fasting blood glucose level of different treatment groups

Groups	0th Day	21st day
Normal Control	99.13±1.02	90.06±1.12
Disease Control	205±1.91	361±2.01
Soframycin + metformin	201±1.05	110±1.1
PHF-1+ PHM-1	200±2.23	135±2.14*
PHF-2+ PHM-2	203±1.02	130±1.24**
PHF-3+ PHM-3	201±1.29	125±1.32***

n=6 *p<0.05,**p<0.01, ***p<0.001 PHM1,PHM,PHM3 vs disease control (Two way ANOVA) f and then Bonferroni post hoc tests. Values are expressed as (MEAN ± SEM)

Table 7: Determination of wound area (mm²) on 0th day and 21st day in *Albino wistar* Rats

Groups	wound area (mm²) on 0th day	wound area (mm²) on21st day
Normal control	504±0.39	24.73±0.23
Disease control	508.66±4.45	292±6.44*
Standard treatment	504±2.28	26.5±2.29**
PMF-1	507.17±3.14	144.5±7.08***
PMF-2	506.5±2.49	121.5±7.56
PMF-3	504.67±2.01	63±5.75

n=6 *p<0.05,**p<0.01, p***<0.001 PHM1,PHM2,PHM3 vs disease control (two way ANOVA followed by Bonferroni post hoc test. Values are expressed as (MEAN ± SEM). n=36

Table 8: Comparison of percentage of wound closure (Mean ± SEM) by all treatment groups on 0th and 21st day in *Albino wistar* rats.

Groups	0th day	21st day
Normal control	11.12±2.13	97.09±0.05
Disease control	12.70±5.32	39.22±3.14
Standard treatment	7.60±3.13	94.73±0.48
PHF-1	11.73±4.75	71.49±1.47*
PHF-2	9.50±3.90	76.0±1.51**
PHF-3	11.21±4.34	87.53±1.12***

n=6 **, p<0.05*,p<0.01**, ***p<0.001 ,PHF1,PHF2,PHF3 vs disease control respectively (Two way ANOVA) followed by Bonferroni post hoc tests.

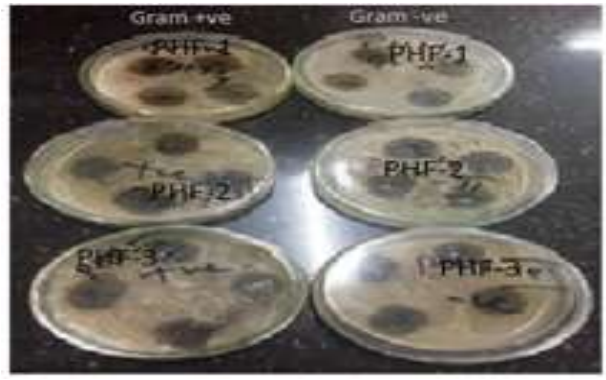

Figure 1: Zone of inhibition of poly-herbal formulations 1, 2, 3 against gram +ve and gram –ve bacterial strains

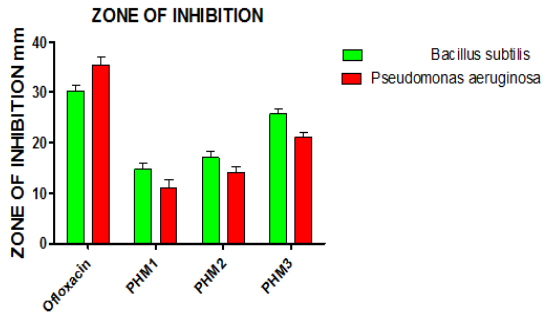

Figure 2: Zone of inhibition (mm) of poly herbal hydro-alcoholic extracts against *Bacillus subtilis* and *Pseudomonas aeruginosa*

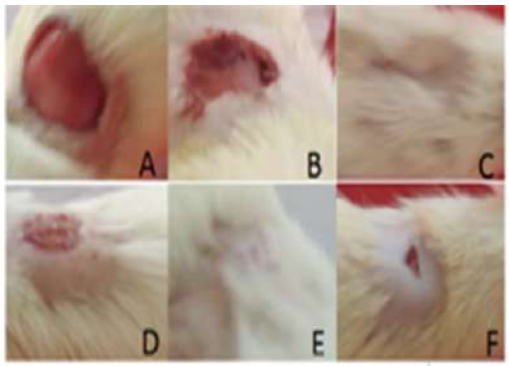

Figure 3: Pictures of excision wound on 0th day and 21st day

A- Wound area of disease control rat on 0th day, B- wound area of disease control rat on 21st day, C-Wound area of standard on 21st day, D- wound area of PHF-1 ON 21ST day, E- wound area of PHF-3, F- wound area of PHF-2 on 21st day.

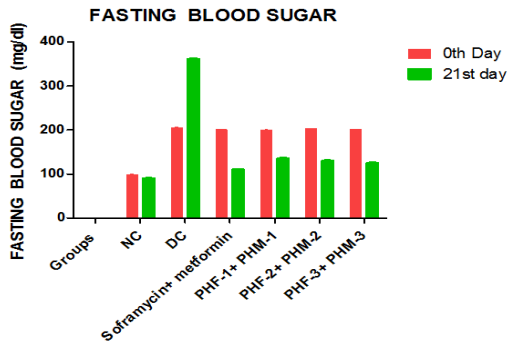

Figure 4: Fasting blood glucose level of different treatment groups on 0th day and 21st day

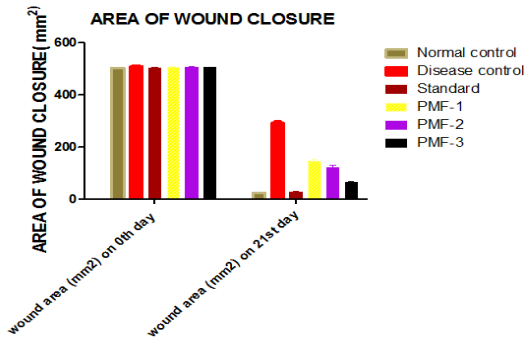

Figure 5: Comparison of area wound closure (Mean ± SEM) by all treatment groups on 0th and 21st day in *Albino wistar* rats.

Figure 6: Comparison of percentage wound closure (Mean ± SEM) by all treatment groups on 0th and 21st day in *Albino wistar* rats

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RESEARCH ARTICLE

An Experimental Investigation on Thermal Performance of Concentric Tube Heat Exchanger Integrated with Diesel Engine Exhaust

Raja T[1]*, Chandrasekar R[2], Venkatesh R[2] and Senthil Kumar M[2]

[1]Associate Professor, Department of Mechanical Engineering, Vinayaka Mission Kirupananda Variyar Engineering College, Periyaseeragapadi, Salem, Tamil Nadu, India.
[2]Assistant Professor, Department of Mechanical Engineering, Vinayaka Mission Kirupananda Variyar Engineering College, Periyaseeragapadi, Salem, Tamil Nadu, India.

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***Address for Correspondence**
Raja T,
Associate Professor,
Department of Mechanical Engineering,
Vinayaka Mission Kirupananda Variyar Engineering College,
Periyaseeragapadi, Salem, Tamil Nadu, India.
Email: rajat@vmkvec.edu.in

ABSTRACT

Recovery of Waste heat from internal combustion engine has a lot of encouraging research potential as far as energy conservation is concerned. This paper deals with an experimental investigation on Thermal Performance of Diesel engine exhaust gas heat recovery using a concentric tube heat exchanger and transitory thermal storage. The energy available in this exhaust stream of gases goes as waste and not utilized properly. Due to mismatched demand and availability of energy, it is not possible to successfully implement waste heat recovery. In this work, a concentric tube heat exchanger is integrated with exhaust port of a diesel engine setup to extract the waste heat of the exhaust gas and a transitory thermal energy (TTE) vessel is used to store the surplus energy obtainable is analysis. The performance parameter of the engine with and without heat exchanger is evaluated and it is found that approximately 9 - 20 % of fuel power is stored as the heat in the TTE which is available at reasonably higher temperature for process applications. The performance parameter of the heat exchanger and TTE tank such as the amount of waste heat recovered, heat lost and energy saved are evaluated and also reported in this research. Through this work, the developed concentric tube geometry and the experimental study has established the waste heat recovery system, computation of overall heat transfer co- efficient and performance of the heat exchanger using Wilson plot method and the adoption of Transitory Thermal Energy Storage tank store excess energy was conducted.

Keywords: Internal combustion engine, heat exchanger, Heat recovery, boiler, Transit nary Thermal Energy storage tank.

INTRODUCTION

Nearly two-third of input energy is wasted through exhaust gas and cooling water of these engines. It is imperative that a serious and concrete effort should be launched for conserving this energy through waste heat recovery techniques. Such a waste heat recovery would ultimately reduce the overall energy requirements and also the impact on global warming. Waste heat is generated in a process by the way of fuel combustion or chemical reaction, and then dumped into the environment even though it could still be reused for some and economic purpose. In order to minimize the size and weight of a gas to liquid heat exchanger, thermal conductance (ha) on both sides of the exchanger should be approximately the same. Desai and bannur (2001) performed experiments in a twin cylinder diesel engine, to recover heat from engine exhaust gas using a shell and tube heat exchanger. Morcos (1998) studied the performance of shell and dimpled tube exchangers for waste heat recovery. Talbi and Agnew (2002) interfacing of turbocharged diesel engine with an absorption refrigeration unit and estimated the performance enhancement due to the energy recovery from the engine exhaust gas. Zhang (2000) has made a prototype which can be successfully used for waste heat driven air- conditioning for an automobile. Pandiyarajan *et.al* (2011) made an experimental investigation on heat recovery from diesel engine exhaust using finned shell and tube heat exchanger and thermal storage system. Smith et. Al (2008) has performed heat transfer and friction characteristics were experimentally investigated lowered strips inserted in a concentric tube heat exchanger. Mavridou *et.al* (2010) made a comparative design study of diesel exhaust gas heat exchanger for truck applications.

Kauranen *et.al* (2010) made a performance study the temperature optimization of a diesel engine using exhaust gas heat recovery and thermal energy storage. Modern industrial has created new problem for waste heat recovery of any energy and reuse. The Waste heat recovery plays an active role in the preservation of the substantial economically and socially as well as in term of environmental impacts. Energy is a basic need for a any country. Waste heat recovery is a technologically and economically viable solution for industrial growth and enhancing energy efficiency in industrial processes. As a global competition, the need for energy rapidly increased in the past years where as 1/3 of population in a world. Every industry is responsibility for 32% of worldwide green house gas pollution system. However, the challenges of reinventing industries are more substantial economically and socially as well as in term of environmental impacts. Because environmental impacts is a crucial factor of performance in the industrial aspects. Under such a condition, utilizes more energy efficiency and environmental performance for all of its products at a lower emission condition. Exhaust gas carries about 30% to 35% of the heat of energy from an internal combustion engine in order to substantial effort have been made to recovery the excesses waste heat in the exhaust gas and convert into a useful work. The energy losses in a waste heat cannot be fully recovered but by using certain devices (heat exchanger) maximum possible heat can be recovery in an exhaust gas in turn minimizing the energy and improving environmental impacts.

High-capacity diesel engine is mostly used for power generation and transportations. Recovery of waste energy can be achieved by using energy conversation technologies i.e., Recovery of waste heat is an ultimately reduced the overall energy y requirement and environmental impacts in a global warming. the great care must be exercised by the heat recovery in diesel engine from the exhaust gas under consideration of pollution by adopting better design method for heat exchanger and recovery heat. The heat recovery in diesel engine is an attractive research topic and suitable for industries also Energy conservation plays are an important role for economic development of any Nation. In the recent years, fast manufacture in India and China, where ever requirement of the residents of globe is a gift has raised the requirement for energy. Consider the environmental protection and environment of great uncertainty over future energy supply, attention is rigorous on the use of sustainable energy sources and energy conservation methodologies. There are many researches works which have been carried out on exhaust gas waste heat recovery system to improve its thermal efficiency and economic performance. Materials selection, adoption of improved design and thermodynamic analysis were performed.

Literature Review

The literature survey pertaining to this research work is highlighted in this section. Hatami *et al*,*[*1], studied optimal design of finned-tube heat exchangers for diesel engine exhaust waste heat recovery using CFD and response surface methodology based on central composite design. Hatami *et al*,[2], to enhance the thermal performance of a concentric tube heat exchanger using porous material inserts. The porous particle improves the convective heat transfer co-efficient between the tube wall and fluid flow surface. Hatami *et al*, [3], experimentally studied the Turbocharged OM314 DIMLER diesel engine was tested at various engine speeds (1200, 1400, 1600, 1800and 2000 rpm) and torques (20, 40, 60, 80 and 100 N m). The reduction of brake specific fuel consumption due to the use of recovered energy from exhaust. Mohsen Ghazikhani *et al,* [4], had studied experimentally and thermo dynamical analysis of the diesel exhaust vortex generator heat exchanger is used to recover energy from the exhaust of an OM314 diesel engine. Hatami e*t al*,[5], experiment conducted on concentric with helical finned tube heat exchanger integrated with a diesel engine setup to extract the waste heat from the exhaust and stored the energy in TTE tank. Senthil Kumar and Palanisamy,[6]. Numerically studied concentric tube heat exchanger using dimpled tubes with Nano fluid and improved thermal performance. Senthil Kumar *et al, [*7], studied and better performance of secondary heat exchanger for latent heat recovery from flue gas using mini-tubes. Junpei Yamashita and Yoshio Utaka ,[8], better heat storage rate for an automobile coolant waste heat recovery system and using phase-change material in a fin- tube heat exchanger. Jungwook Shon *et al*,*[*9], proposed dissimilar heat exchanger design for increasing the diesel exhaust waste heat recovery. Hatami *et al,* [10], studied the combustion chamber of a single cylinder diesel was coated with aluminum titanate and waste heat recovery system was developed using a thermoelectric power generation module.

Ming Pan *et al,* [11], implemented the heat transfer intensification for shell and tube heat exchangers as an efficient technique to increase energy saving when retrofitting heat exchanger networks. Mariusz Markowski, [12], introduced the novel method for determination of the thermal resistance of fouling in shell and tube heat exchangers. Saiful Bari and Hossain, [13], had improved the total performance of the exhaust waste heat recovery system by optimizing the design of the heat exchangers. Gulsah Cakmak, [14], studied experimental investigation of thermal storage in U-tube heat exchanger. Ozden Agra, [15] developed new model to determine the sizing and selection of heat exchanger at defined saving- investment ratio. Pandiyarajan *et al,* [16] studied waste heat recovery from diesel engine exhaust using finned shell and tube heat exchanger and thermal storage system. Pethkool *et al,* [17], studied experimental investigation of convective heat transfer and thermal performance of a heat exchanger with the helically corrugated tube. Nasser Ghorbani ,[18] studied an experimental investigation of mixed convection heat transfer in the tube diameter, coil pitch, shell-side and tube-side mass flow rate over the performance coefficient and modified effectiveness of vertical helical coiled tube heat exchangers. In the present work, a concentric-wavy tube heat exchanger is preferred to extract heat from the exhaust gas and separate TTE tank connected to the mini boiler to utilize the heat energy.

MATERIALS AND METHODS

Development of Inner Tube Geometry

The tube is made of copper. The dimensions of outer diameter are 11mm and the length of the tube is 3000 mm, shown in the figure.1 Concentric tube configurations.

Experimental Setup

The experimental setup consists of a diesel engine, 4-stroke, water cooled, Kirloskar diesel engine (bore diameter 87.5 *mm*, stroke 110 *mm*, rated power 7.4 *kW* at 1500 *rpm*) coupled to a mechanical load, integrated with waste heat recovery heat exchanger and TTE tank, figure 2 schematic diagram of arrangement of experimental setup and its photographic view shown in figure 3. The waste heat recovery system is made up of galvanized iron and copper tube, annular aspect fluid as exhaust gas and tube aspect fluid as water. The total surface area of concentric tube surface area is 0.0691 m^2. Table.1 shows the specifications of concentric tube heat exchanger.

The waste heat recovery system is set into the exhaust port of the engine. The exhaust gas is allowed to flow either through the waste heat recovery system or to the atmosphere by using valves. Water flows through tube aspect of the warmth exchanger using pump and is passed through TTE tank embedded with boiler. The tank is made-up of stainless-steel cylindrical vessel of inner diameter 350 mm and height 550 mm with the capacity of the tank 50 liters, and mini boiler capacity is 15 liters. Data logger is used to record the temperatures at various locations, model CT708U; resolution is 1°C for thermocouples, and accuracy is ±0.1°C least significant digital for thermocouples (type K). Thermocouples are placed at the inlet and outlet of the heat exchanger. A pump maintains the circulation of water in this setup. The mass flow rate of the fluid is measured by Rota meter.

Experimental Technique

The diesel engine is operated at various load conditions. Readings are initially taken at 25% load condition. The exhaust gas is not allowed to flow through the heat exchanger to avoid carbon deposition on the tube surface. After a small time, lag from the start of the engine, the exhaust gas is allowed to pass through the annular side of the heat exchanger while ensuring the water circulation through the inner tube. A tachometer is used to measure the speed of the diesel engine, and to make sure the rated speed of 1500 *rpm*. The torque and speed of the engine are taken for the evaluation of the brake power. The pressure difference in the orifice meter is observed from the U-tube manometer which is used to measure the mass flow rate of air entering into the engine. The fuel consumption of the engine is measured by noting the time required to consume a constant mass of diesel fuel equal to 0.042 *kg*. Temperatures for Parallel flow and counter flow mode are continuously monitored. The above said readings are used to evaluate the heat recovered. Several experiments are repeated to check the repeatability of the results. The experiments were conducted for 25%, 50%, 75% and 100% load conditions. The results along with the evaluated parameters were analyzed and discussed in the following section.

RESULTS AND DISCUSSION

The results obtained from the experiments conducted at a range of load conditions are studied in detail and presented. In the current work, attempts have been made to recover the maximum possible heat energy from the exhaust gas through a concentric tube heat exchanger and to store it in a transitory thermal energy storage tank.

Performance of waste Heat Recovery from Counter flow Heat Exchanger with Concentric Tubes

The temperature variation of the exhaust gas and the water at the inlet and outlet of the HRCPHE with respect to time for various engine load conditions (25%, 50%, 75% and full load condition) are shown in Fig. 3a and Fig.7a. In a diesel engine, normally, the temperature of exhaust gas will attain steady state within a period of 5minutes for a given load. However it is observed in the present work that at all loads, the temperature of the gas at the inlet of the heat exchanger attains a steady state after a time interval of 30minutes. It is due to the thermal inertia of the exhaust gas pipe along with insulation material from exhaust manifold to the HRCPHE. As the engine load increases the exhaust gas temperature also increases due to its higher heat release from the engine, at all loads it is observed from the water and the gas outlet temperature variation that the temperature increases at the beginning and the slope decreases when the temperature of the water attains approximately 60°C and further increases at a higher rate after a certain interval of time, at 25% load a flue gas temperature raise from 300°C to 353°C is observed for a longer duration and cold water temperature raise from 26°C to 53°C is observed, at 50% load a flue gas temperature raise from 353°C to 410°C is observed for a longer duration and cold water raise from 26°C to 56°C is observed , at 75% load a flue gas temperature raise from 410°C to 460°C is observed for a longer duration and cold water temperature raise from 26°C to 59°C is observed, at full load a flue gas temperature raise from 460°C to 490°C is observed for a longer duration and cold water temperature raise from 26°C to 62°C is observed, It is also observed from the s that there is a large temperature drop in the exhaust gas at all times and the increase in temperature of the water is very low since the heat capacity of the water (m^3 c c p,c) is much higher than the heat capacity of the exhaust gas (m^3 g C p , g).

In all the four loads the exit temperature of the exhaust gas from HRCPHE approaches the inlet temperature of the water and they are almost equal at the end of the experiment. The variation of diesel engine flue gas temperature, and water at the inlet, and outlet of the heat recovery concentric plain and wavy tube heat exchangers, (HRCPTHE & HRCWTHE) with respect to time for a range of engine load conditions (25%, 50%, 75% and 100% load condition) are shown in Figs. 4a–4d Concentric tube.

In the diesel engine, exhaust gas temperature will attain steady state within duration of 5 minutes at a given load. However, it is observed the engine exhaust temperature at all loads of the flue gas at the inlet attain a steady state after 30 minutes time interval. It is as a result of the thermal inertia of the diesel engine exhaust gas pipe to insulate from manifold to the HRHE. The higher heat release from the engine increases engine load. The exhaust gas temperature also increases. It is observed from all loads, the temperature difference of water and the gases, that the temperature increases at the starting and the slope decreases when the temperature of the water attains approximately 50°C and further increases at the higher rate after a certain interval of time at 25% load a near constant temperature around 50°C is observed for a longer duration for a From the experimental results with different load condition on temperature difference recorded and used to calculate the effectiveness and heat extraction rate. The effectiveness can be written as

$$\varepsilon = \frac{actual\,heat\,transfer}{\max imum\,possible heat\,transfer} = \frac{T_{h,1} - T_{h,2}}{T_{h,1} - T_{c,2}} \qquad \text{---------(1)}$$

It is also observed from the fig. 5, that there is a large temperature drop in the exhaust gas at all times and the increase in temperature of the water is very low since the heat capacity of the water ($\dot{m}_c C_{p,c}$) is much higher than the heat capacity of the exhaust gas ($\dot{m}_h C_{p,h}$). In all the four loads the exit temperature of the exhaust gas from Heat Recovery Heat Exchanger (HRHE) approaches the inlet temperature of the water and they are almost equal at the end of the experiment. This shows the effectiveness of the Heat exchanger (plain, wavy) approaches 76% and 81% at the end of the experiment in all the cases. Fig. 6(plain) and Fig. 7(wavy) shows the variation of the heat extraction rate from the exhaust gas through the waste heat recovery (WHR) heat exchanger evaluated at different loads and calculated. Heat extraction rate (*kW*) can be written as Yunus Cengel, [19].

$$Q_{ext} = \dot{m}_c C_{p,c}(T_{c,2} - T_{c,1}) \qquad (2)$$

The two different tubes geometry shows the heat extraction rate with a different load condition, the maximum heat extraction rate for Concentric tube is 0.93 *kW*. Engine Exhaust Heat (*kW*) can written as Yunus Cengel,[19].

$$Qg = \dot{m}_g C_{p,g}(T_{g,1} - T_{g,2}) \qquad (3)$$

Where $\dot{m}_g$ – mass flow rate of the exhaust gas $T_{g,1}, T_{g,2}$ – exhaust gas temperature at the inlet and outlet of HRHE. The maximum heat is extracted at full load condition of 4.71 *kW* .It is very high when compared to all other engine load conditions due to very high rate of heat release from the engine at maximum load. The variation of heat extraction rate and LMTD with time for 25%, 50%, 75%, and 100% load conditions are shown in Fig. 6a–6d Concentric tube. It is observed from the Fig. 6c and 6d (75% load and 100% load) that the decrease in heat extraction rate and LMTD has a similar trend. However, at 25% load Fig. 6a the decrease in heat extraction rate is much smaller whereas the decrease in LMTD with respect to time is appreciable. This trend is also seen at 50% load Fig. 6b with variation marginally lesser than 25% load. The near uniform heat extraction rate with higher LMTD with respect to time at 25% load reveals that the overall heat transfer coefficient is increasing with respect to time. It could be due to condensation of water vapor from the exhaust gas in most part of the heat exchanger at 25% and 50% loads.

The variation in overall heat transfer coefficient with respect to time at all loads is shown in the Fig. 7 Concentric tube. The experimental results were compared with the Dittus Boelter equation was formulated for smooth-surface Concentric tube. The variation in Nusselt and Reynolds Number with respect to time at different loads is shown in the Figures 8a to8d. The comparison between the Nusselt number and Reynolds number from the present work and the Dittus Boelter Correlation shows the better heat transfer in the Concentrictube heat exchanger at full load conditions.

CONCLUSIONS

In the present work, a concentric tube heat exchanger and TTES tank were developed and attached to exhaust port of Diesel engine having a capacity of 7.4*kW*. The experimental investigation has given the following conclusions:

1.The effectiveness of the HRHE approaches nearly 76% concentric tube and 81% wave tube at full load conditions.

2. Nearly 8–17% of total heat (that would otherwise be gone as waste) is recovered from the system, and at full load condition the maximum heat extracted is around 0.93*Kw concentric tube.*

3. By decreasing the exhaust gas temperature, it is reaches below 110˚C; the heat liberated from the fuel along with the exhaust gas during the burning is possible to recover the heat (HCV– LCV).

4.The life of the engine is assumed as 100000 engine hours, and the experimental set-up can save up to 1,18,611.11 kW-hr of electricity.

REFERENCES

1. Hatami, M., Jafaryar, M., Ganji, D.D., Gorji-Bandpy, M., Optimization of finned-tube heat exchangers for diesel exhaust waste heat recovery using CFD and CCD techniques, International Communications in Heat and Mass Transfer, Vol.no.57, pp.254-263, 2014.
2. Hatami, M., Ganji, D.D., and Gorji-Bandpy, M., Numerical study of finned type heat exchangers for ICEs exhaust waste heat recovery, Case Studies in Thermal Engineering, Vol.no.4, pp. 53–64, 2014.
3. Hatami, M., Ganji, D.D., and Gorji-Bandpy, M.,CFD simulation and optimization of ICEs exhaust heat recovery using different coolants and fin dimensions in heat exchanger, Neural Computing & Applications, Vol. no.25, Issue 7-8, pp. 2079, 2014.
4. Mohsen Ghazikhani ., Mohammad Hatami ., Davood Domiri Ganji., Mofid Gorji-Bandpy., and Ali Behravan, Gholamreza Shahi ., Exergy recovery from the exhaust cooling in a DI diesel engine for BSFC reduction purposes, Energy, Vol.no.65 , pp.44-51,2014.
5. Hatami, M., Ganji, D.D., and Gorji-Bandpy,M., Experimental and thermo dynamical analyses of the diesel exhaust vortex generator heat exchanger for optimizing its operating condition, Applied Thermal Engineering, Vol.no.75, pp. 580–591,2015.
6. Senthil Kumar, K., and Palanisamy, P., Experimental investigation on diesel engine exhaust gas heat recovery using a concentric tube heat exchanger with TTE, Australian Journal of Basic and Applied Sciences, vol.8, no.2, pp. 194-206, 2014.
7. Senthil Kumar K., Palanisamy, P., Vinoth, R., and Perumal, S., Numerical study of a concentric tube heat exchanger using dimpled tubes with Al_2O_3 Nanofluid. Australian Journal of Basic and Applied Sciences, vol. 8,no.1,pp. 185-193, 2014.
8. Junpei Yamashita., and Yoshio Utaka., Improved performance of secondary heat exchanger for latent heat recovery from flue gas using mini-tubes, Applied Thermal Engineering,vol.67,pp. 230–239, 2014.
9. Jungwook Shon., Hyungik Kim., and Kihyung Lee., Improved heat storage rate for an automobile coolant waste heat recovery system using phase-change material in a fin–tube heat exchanger, Applied Energy,vol.no.113, pp. 680–689, 2014.
10. Hatami, M., Ganji, D.D and Gorji-Bandpy M., A review of different heat exchanger designs for increasing the diesel exhaust waste heat recovery, Renewable and Sustainable Energy Reviews, vol. 37, pp. 168–181, 2014.

11. Ming Pan., Robin Smith.,and Igor Bulatov., A novel optimization approach of improving energy recovery in retrofitting heat exchanger network with exchanger details. Energy, vol. 57, pp. 188–200, 2013.
12. Mariusz Markowski, Marian Trafczynski and Krzysztof Urbaniec., Validation of the method for determination of the thermal resistance of fouling in shell and tube heat exchangers, Energy Conversion and Management, vol.76, pp.307–313, 2013.
13. Saiful Bari., and Hossain., S.N., Waste heat recovery from a diesel engine using shell and tube heat exchanger. Applied Thermal Engineering, vol. 61, pp. 355–363, 2013.
14. Gulsah Cakmak., Experimental investigation of thermal storage in U-tube heat exchanger. International Communications in Heat and Mass Transfer,Vol.no.44, pp. 83–86, 2013.
15. Ozden Agra., Sizing and selection of heat exchanger at defined saving investment ratio. Applied Thermal Engineering, vol. 31, pp. 727-734, 2011.
16. Pandiyarajan, V., Chinna Pandian, M., Malan, E., Velraj, R and Seeniraj R.V. Experimental investigation on heat recovery from diesel engine exhausts using finned shell and tube heat exchanger and thermal storage system. Applied Energy, vol. 88, pp.77-87, 2011.
17. Pethkool S., Eiamsa-ard, S., Kwankaomeng, S., and Promvonge, P., Turbulent heat transfer enhancement in a heat exchanger using helically corrugated tube. International Communications in Heat and Mass Transfer, vol. 38, pp. 340–347, 2011.
18. Nasser Ghorbani., Hessam Taherian., Mofid Gorji., and Hessam Mirgolbabaei., An experimental study of thermal performance of shell-and-coil heat exchangers. International Communications in Heat and Mass Transfer, vol. 37, pp.775–781, 2010.
19. Yunus Cengel, Heat and Mass Transfer, Tata Mcgraw Hill Education Private Limited, 2010

Table 1 Specification of the heat exchanger

MATERIALS	SPECIFICATIONS
Annulus material	Galvanized iron
Outer diameter	76.2mm
Inner diameter	72.2mm
Annulus thickness	4mm
Thermal conductivity of galvanized iron	72.7 W/mK
Tube material	Copper
Inner diameter	10mm
Outer diameter	11mm
Tube thickness	1mm
Length of the tube(l)	3000mm
Thermal conductivity of copper	386 W/mK
Spacer length(L)	26 mm
Total Spacer length(Lts = (N-1) x L,m)	1014 mm
Length of straight portion of the test section(Ls =Lts+ Li(50mm)+ Lo(51mm)	1115 mm
Length of bent portion of the test section(Lc = $N\pi R$,m)	1884 mm
Total length of the test section Lt = Lc+Ls,m (copper tube)	$2999 \approx 3000$ mm
Curvature ratio ($2R/d_i$)	3
Dimensionless spacer length(L/d_i)	2.5mm
Type of insulation used	Glass wool, aluminum cladding sheet

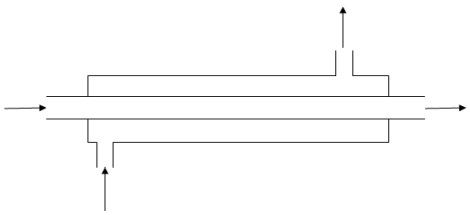

Figure 1. Concentric tube configurations

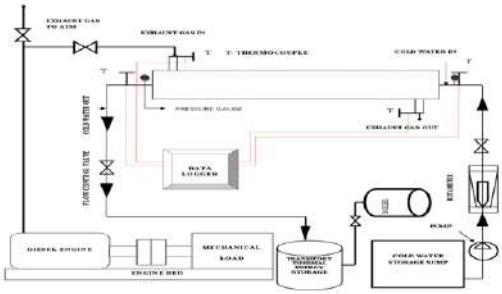

Figure 2 .The Experimental setup flow diagram

Figure 3 .Photographic view experimental setup

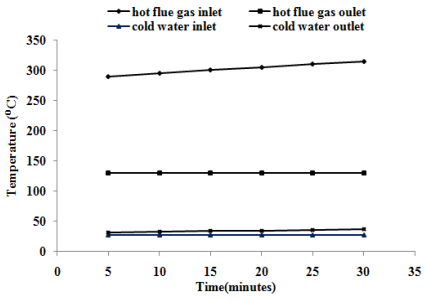

Figure 4a. Temperature variation of the exhaust gas and water at 25% Load Concentric tube

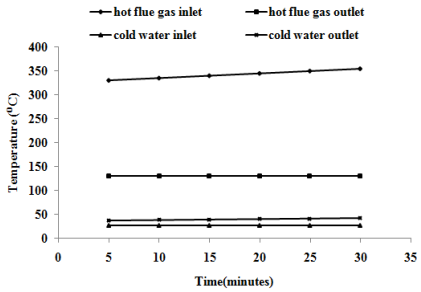

Figure 4b. Temperature variation of the exhaust gas and water at 50% Load Concentric tube

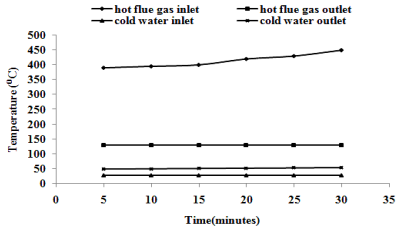

Figure 4c. Temperature variation of the exhaust gas and water at 75% Load Concentric tube

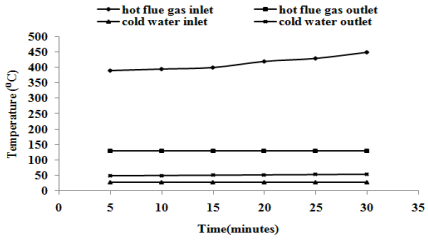

Figure 4d. Temperature variation of the exhaust gas and water at 100% Load Concentric tube

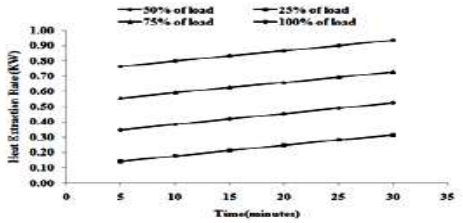

Figure 5. Heat extraction rate from exhaust gas at different loads conditions Concentric

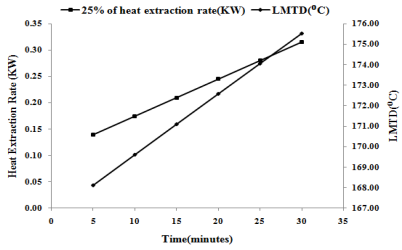

Figure 6a. Heat extraction rate and LMTD 25% Load Concentric tube

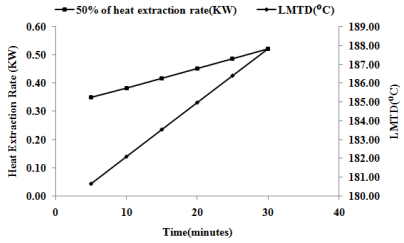

Figure 6b. Heat extraction rate and LMTD at 50% Load Concentric tube

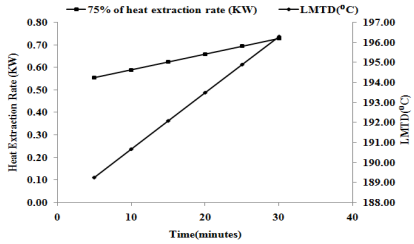

Figure 6c. Heat extraction rate and LMTD 75% Load Concentric tube

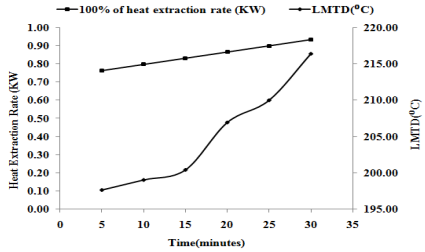

Figure 6d. Heat extraction rate and LMTD at 100% Load Concentric tube

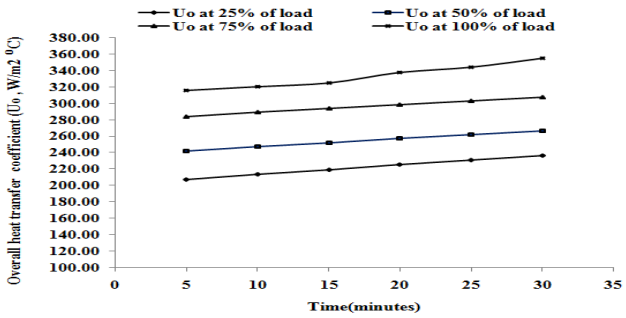

Figure 7. Variation of overall heat transfer coefficient of the HRHE at various Load Concentric tube

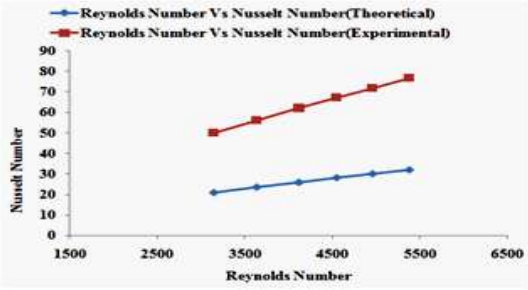

Figure 8a. Variation of Reynolds and Nusselt Number of the HRHE at 25% loads Concentric tube

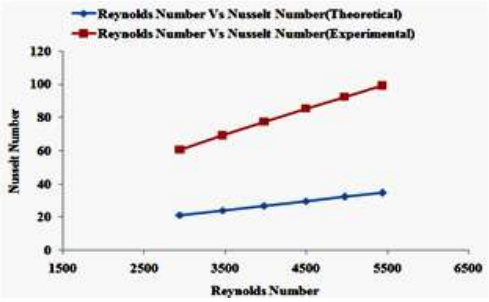

Figure 8b. Variation of Reynolds and Nusselt Number of the HRHE at 50% loads Concentric tube

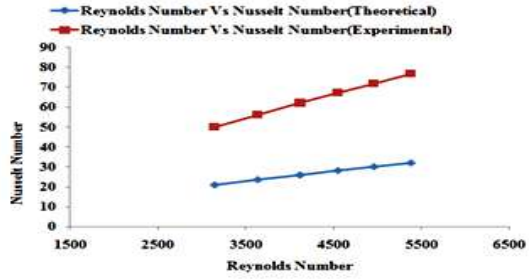

Figure 8c. Variation of Reynolds and Nusselt Number of the HRHE at 75% loads Concentric tube

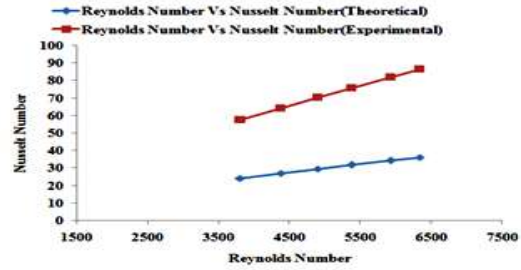

Figure 8d. Variation of Reynolds and Nusselt Number of the HRHE at 100% loads Concentric tube

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RESEARCH ARTICLE

Design of Synthetic Route, Characterization and Biological Evaluation of Novel 9 - Substituted Acridine Derivatives

Kyathi N Bhojak[1], Shadiya C K[2], Amrutha P.G[3], Anilkumar.V[3], Cyril Mathew Jacob[3] and Akash Marathakam[4]*

[1]M.Pharm Student, Department of Pharmaceutical Chemistry, National College of Pharmacy, Kozhikode, Kerala, India
[2]Associate Professor, Department of Pharmaceutical Chemistry, National College of Pharmacy, Kozhikode, Kerala, India
[3]Assistant Professor, Department of Pharmaceutical Chemistry, National College of Pharmacy, Kozhikode, Kerala, India
[4]Professor and Head, Department of Pharmaceutical Chemistry, National College of Pharmacy, Kozhikode, Kerala, India

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***Address for Correspondence**
Akash Marathakam
Professor and Head,
Department of Pharmaceutical Chemistry,
National College of Pharmacy, Kozhikode, Kerala, India
Email: akashmarathakam@gmail.com

ABSTRACT

The current project involves designing, synthesizing, and biologically evaluating a variety of new compounds that have the potential to exhibit pertinent biological activity. Anticancer, anti-inflammatory, antioxidant, anti-microbial, analgesic, acetyl cholinesterase inhibitory, and anti-herpes ect properties are all exhibited by 9-substituted acridine derivatives. Using a variety of techniques, a number of 9-substituted acridine derivatives were created, and IR, NMR, and MASS spectroscopy validated the structure. The anticancer, anti-inflammatory, and antioxidant activity of derivatives was then assessed. The 9- substituted acridine was created through the Ullmann condensation procedure. 9- chloro acridine have been synthesized by Ullmann condensation reaction employing 2- chlorobenzoic acid and aniline reacts to form N-Phenyl anthranilic acid in the presence of copper oxide and anhydrous potassium carbonate to form N-Phenyl anthranilic acid which is then made to react with phosphorous oxy chloride to generate 9-chloro acridine. Synthesized parent molecule will undergo different reactions with various aldehydes and other reagents to form 9-substituted acridine derivatives. Among the synthesized compounds, 9SAD-A and 9SAD-C show significant anticancer activity based in-vitro cytotoxicity study on DLA cell lines. 9SAD-B show prominent anti-inflammatory activity on protease inhibition assay and the compound 9SAD-A exhibit greatest antioxidant activity by hydrogen peroxide scavenging assay.

Keywords: 9- chloro acridine; Ullmann condensation reaction; Anti-inflammatory; Anti-oxidant; Cytotoxicity; Molecular Docking

INTRODUCTION

Drug discovery and development is a profound and extensive inter-disciplinary endeavor. In recent times, an inclination towards the use of *in-silico* chemistry and molecular modelling for computer-aided drug design has expanded significantly [3]. Discovery may involve screening of chemical libraries, identification of the active ingredient after natural remedy or design resultant from an understanding of the target.Development includes study on microorganisms and animal, clinical trials and ultimately regulatory approval [4]. Acridine, a heterocyclic compound was first isolated from anthracene fraction of coal tar in 1871 by Carl Grabe and Henrich Caro. It may also be known as benzoquinoline or dibenzopyridine. Since 19th century they were first used as a raw material for the production of dyes and some valuable drugs [28]. At present a wide range of acridine derivatives are used for the treatment of acute leukemia (amsacrine), as anticancer agents (ledacrine), as antibacterial agent (acriflavine and ethacridine), for action againstparasites in the treatment of malaria, trypanosomiasis and leishmaniasis (quinacrine, acranil) and for treatment of Alzheimer's disease (tacrine).

MATERIALS AND METHODS

All the chemicals and solvents are procured from Sigma-Aldrich and Merck, India. The melting points of the synthesized compounds have been determined in open capillary tubes and are uncorrected. The infra-red (IR) spectra of the compounds have been recorded on Perkin Elmer Fourier Transform infrared (FTIR) spectrometer (Model Shimadzu 8700) in KBr discs method. 1H and 13C nuclear magnetic resonance (NMR) spectra have been recorded on MHz^{1}HNMR (400MHz in CD_3OD). The chemical shifts (δ) have been reported in parts per million (ppm) downfield from internal reference Tetramethylsilane (TMS). Mass spectra have been recorded on Mass spectrophotometer.

Synthesis of 9- Chloro Acridine

A mixture of 18.88ml of aniline, 5.08g of o-chloro benzoic acid, 5.18g anhydrous potassium carbonate and 0.25g of copper oxide were refluxed for 2 hours. After completion of the reaction, the reaction mixture was poured with stirring, to a mixture of 25ml of concentrated hydrochloric acid and 100ml of water. The precipitated acid was filtered with suction when cold and product (N-phenyl anthranilic acid) obtained was dried. Take 1.278g of N-phenyl anthranilic acid, 11.80ml of phosphorous oxy chloride were taken and heated slowly in a water bath at 85-90°C for 3hrs. Further the reaction mixture was poured into a well-stirred mixture of 5.21ml concentrated ammonia, 15g of crushed ice. Then it was allowed to stand for 30mins to precipitate the solid. Filtered and dried and recrystallised using ethanol.

Synthesis of 9-Substituted Acridine Derivative A

About 0.01 mole 9- chloro acridine, 0.01 mole salicylaldehyde and 0.01 mole of anhydrous potassium carbonate dissolve in tetra hydro furan and reflux it for about 3 -4 hrs on water bath. The reaction mixture was then poured into cold water, appearanceof dark green colored product will be observed, filter the precipitate, dry and recrystallize it from ethanol.

Synthesis of 9- Substituted Acridine Derivative B

About 0.01 mole 9- chloro acridine, 0.01 mole p-dimethylamino benzaldehyde and 0.01mole of anhydrous potassium carbonate dissolve in tetra hydro furan and reflux it for about 3 -4 hrs on water bath. The reaction mixture was then poured into cold water, dark brown colored product precipitated out from mixture, filter it, dry and recrystallize it from ethanol.

Synthesis of 9 - Substituted Acridine Derivative C

About 0.01 mole 9- chloro acridine, 0.01 mole p-dimethylamino cinnamaldehyde and 0.01 mole of anhydrous potassium carbonate dissolve in tetra hydro furan and reflux itfor about 3-4 hrs on water bath. The reaction mixture was then poured into cold water,dark yellow to orange colored product will be perceived, filter the precipitate, dry and recrystallize it from ethanol.

Spectroscopic Analysis

The structural characterization is done by IR, NMR and MASS spectroscopy. IR spectroscopy was carried out from Central Laboratory for Instrumentation and Facilitation (CLIF), University of Kerala, Thiruvananthapuram. NMR and mass spectroscopy were performed from Sophisticated Test and Instrumentation Centre, Cochin University of Science & Technology Campus, Kochi, Kerala.

IR spectroscopy

Is the analysis of infrared light interacting with a molecule. This can be analysed in three ways by measuring absorption, emission and reflection. The mainuse of this technique is in organic and inorganic chemistry. It is used by chemists to determine functional groups in molecules. IR Spectroscopy measures the vibrations of atoms, and based on this it is possible to determine the functional groups.

H^1 NMR spectroscopy

It gives an idea about number of protons present in the given structure. Principle behind NMR is that many nuclei have spin and all nuclei are electrically charged.

Mass spectroscopy (MS)

Is a technique that ionizes chemical species and sorts the ions based on their mass to charge ratio, a mass spectrum measures the masses within a sample. Mass spectrometry is used in different fields and is applied to pure samples as well as complex mixtures.

BIOLOGICAL EVALUATION

Anticancer activity

Trypan blue exclusion method

The test compounds were studied for short term *in vitro* cytotoxicity using Dalton's lymphoma as cites cells (DLA). The tumour cells aspired from the peritoneal cavity of tumour bearing mice were washed thrice with PBS or normal saline. Viable cell suspension (1x 10^6 cells in 0.1 ml) was added to tubes containing various concentration of test compounds and the volume was madeupto1mlusing phosphate buffered saline (PBS). Control tube contains only cell suspension. These assay mixtures were incubated for 3 hrs at 37°C. Further cell suspension was mixed with 0.1ml of 1% trypan blue and kept for 2-3 min and loaded on haemocytometer. Dead cells taken up the blue colour of trypan blue but live cells not take. The number of stained and unstained cells was counted separately.

$$\%\ \text{cytotoxicity} = \frac{\text{No. Of dead cell}}{\text{No. of dead cell + No. of live cell}} \text{ X } 100$$

Anti-inflammatory Activity

Protease Inhibition Assay

100 ml of bovine albumin was added to 0.1 ml of sample. This was incubated at room temperature for 5 minutes. Reaction was inhibited by the addition of 0.25ml of trypsin followed by centrifugation. The supernatant was collected, and absorbance was observed at 210 nm. Naproxen was used as a control. The experiment was carried out and percent inhibition of protease inhibition was estimated [71].

% Inhibition=100– $((A_1-A_2)/A_0)*100)$

Where,
A_1 = absorbance of the sample
A_2 = absorbance of the product control
A_0 = absorbance of the positive control.

Anti-oxidant Activity

Hydrogen Peroxide Scavenging Activity

The H_2O_2 scavenging potential of the test compounds were detected according to the method of Ruch et al. Solution of H_2O_2 (40mM) was assembled in phosphate buffer (pH 7.4). 20, 40, 60, 80 and 100 mg/ml concentrations of the test compounds in 3.4 ml phosphate buffer were added to H_2O_2 solution (0.6 ml, 40 mM). The absorbance value was recorded at 230 nm. The percentage scavenging of H_2O_2 was calculated as: % of scavenging = [(Abs control - Abs sample)/ Abs control] x 100

RESULT

***In silico* Molecular Modelling**

In silico molecular analysis of diverse 9 substituted acridine derivatives have been evaluated. And all the compounds were checked by 'Lipinski rule of five'. None of thecompound violates the rule. Table No: 2 Lipinski rule analysis of proposed 9 substituted acridine derivatives by SCFBio online software.

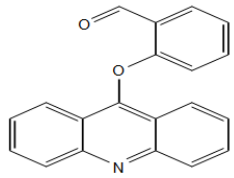
Molecular Docking

The proposed derivatives were docked with various cancer targets and inflammatory targets and the docking scores obtained are as follows. Table No: 3 Docking scores for synthesized compounds against 2M59. Fig No: 2 Docking images of synthesized compounds in 2M59 targets. Table No: 4 Docking scores for synthesized compounds against 6N2W. Fig No: 3 Docking images of synthesized compounds in 6N2W targets.

CHARACTERISATION

9SAD-A

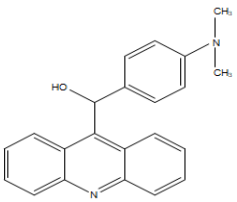
2-(acridin-9-yloxy)benzaldehyde



Dark green crystal, (yield 76%); Melting point 333.08^{0}C, Molecular formula $C_{20}H_{13}NO_2$; Molecular weight (gm/mol)-299.32. ^{1}HNMR ppm (400MHz in CD_3OD) data; this synthesized compound complies with standard protocol [Table no:5]. Table No: 5 NMR spectra of 9SAD-A. Table No: 6 IR interpretation of 9SAD-A

9SAD-B

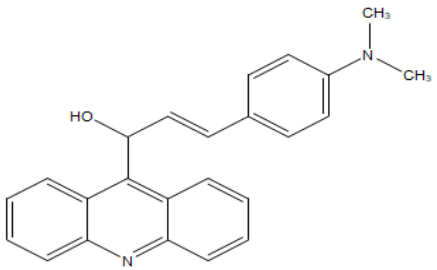
Acridin-9-yl-(4-dimethylamino-phenyl)-methanol,



Dark brown crystal, (yield 80%); Melting point 369.68^{0}C, Molecular formula $C_{22}H_{20}N_2O$; Molecular weight (gm/mol)-328.41. ^{1}HNMR ppm (400MHz in CD_3OD) data; this synthesized compound complies with standard protocol [Table no: 7]. Table No: 7 NMR spectra of 9SAD-B. Table No: 8 IR interpretation of 9SAD-B.

9SAD-C

1-(acridin-9-yl)-3-(4-dimethylamino-phenyl)prop-2-en-1-ol.



Dark yellow to orange crystal, (yield 77%); Melting point 387.14^{0}C, Molecular formula $C_{24}H_{22}N_2O$; Molecular weight (gm/mol)-354.44, ^{1}HNMR ppm (400MHz in CD_3OD) data; this synthesized compound complies with standard protocol [Table no:9]. Table No: 9 NMR spectra of 9SAD-C. Table No: 10 IR interpretation of 9SAD-C

Biological Activity

Antioxidant activity

The percentage inhibition of synthesized compounds was found out by hydrogen peroxide free radicle scavenging activity technique. The concentrations of synthesized compounds were compared with the standard ascorbic acid at 440nm and is shown in (Table No:11) and (Fig No:4). IC_{50} values of standard ascorbic acid and synthesized compounds were Calculated. Table No: 11 *In-vitro* antioxidant value with standard deviation. Fig No: 4 Anti-oxidant activities of derivatives. Fig No: 5 Hydrogen peroxide scavenging assay of 9SAD-A. Fig No: 6 Hydrogen peroxide scavenging assay of 9SAD-B. Fig No: 7 Hydrogen peroxide scavenging assay of 9SAD-C.

***In- vitro* Cytotoxicity Study**

The anticancer activity of the novel analogues was screened by determining the percentage growth inhibition of Dalton's Lymphoma Ascites (DLA) cell by Trypan blue exclusion method. The calculation of IC_{50} values of standard 5- flurouracil and synthesized compounds were perfomed. Here 9SAD-A & 9SAD-C shows significant anticancer activity. Table No: 12 *In-vitro* cytotoxicity study of synthesized compounds against DLA.

Anti-inflammatory Activity

Inhibition of Protein Denaturation

The anti-inflammatory activity of selected novel analogues was screened by the method of inhibition of protein denaturation. Denaturation of proteins is the main cause of inflammation. As part of the investigation on the mechanism of the anti-inflammatory activity, ability of the synthesized derivative to inhibit protein denaturation was studied. Selected synthesized derivative were effective in inhibiting heat induced albumin denaturation. Diclofenac was used as a standard anti-inflammation drug. IC_{50} values of standard Diclofenac and synthesized compounds were calculated. Table No: 13 *In-vitro* anti-inflammatory activity of synthesized compounds. Fig No: 9 Comparison of IC_{50} of synthesized compounds on protein denaturation.

DISCUSSION

The present study involves synthesis of 9 substituted acridine derivatives and evaluation of anticancer, anti-inflammatory and antioxidant activity. The preliminary *in silico* design of different analogues of 9 substituted acridine was performed. Molecular structure was drawn by using Chem Draw software and various properties were

determined. Docking studies of three different analogues were carried out against the cancer target 2M59 and also anti-inflammatory against 6N2W target. Docking scores were tabulated.

The Synthetic Method Includes Two Step Processes

First step including Jourdan-Ullmann condensation reaction mechanism which ultimately results in the synthesis of 9-chloro acridine. In second step various aldehydes were made to react with 9-chloro acridine to form 9 substituted acridine derivatives by a simple dehydrohalogenation mechanism. The structure of 9SAD-A, 9SAD-B and 9SAD-C were established by performing NMR, IR and MASS spectroscopic study.

Biological Assessment Comprises

In- vitro cytotoxicity studies of synthesized compounds were carried out by Trypan blue dye exclusion method on DLA cell lines and the result revealed that all the test compounds had anticancer activity against DLA cell lines. It seems that 9SAD-A containing salicylaldehyde and 9SAD-C containing p-dimethylamino cinnamaldehyde shows significant anticancer activity. The trypan blue exclusion method is used to establish the number of viable cells present in a cell suspension. The principle is based on the live cells possess whole cell membranes that exclude certain dyes, such as trypan blue, whereas dead cells do not. A viable cell will have a clear cytoplasm whereas a nonviable cell will have a blue cytoplasm. In-vitro anti-inflammatory activity of synthesized compounds was carried out using protease inhibition assay. 9SAD-B shows great anti-inflammatory activity. Trypsin inhibiting assay is a method used to investigate the anti-inflammatory activity of synthesized compounds. Trypsin cleaves peptides on the C terminal side of lysine and arginine amino acid residues. Bovine albumin was used as the substrate for trypsin. Antioxidant activity was evaluated by hydrogen peroxide scavenging activity. H_2O_2 is an important molecule as although it is not toxic by itself, but can be converted to other even more toxic radicals such as OH by Fenton reaction or hypochlorous acid by the enzyme myeloperoxidase. The generation of H_2O_2 by activated phagocytes is known to play an important role as bactericidal and antifungal since it also acts as mediators of inflammation by activation of signal transduction pathways. 9SAD-A, 9SAD-B and 9SAD-C undergoes antioxidant evaluation. However, 9SAD-A and 9SAD-C showed good activity.

CONCLUSION

The present study reports the successful synthesis of various 9-substituted acridine derivatives and assessment of their anticancer, anti-inflammatory, antioxidant activity. First the proposed derivatives were screened on the basis of lipinski rule of five using SCF Bio online software. After the screening, three derivatives were selected namely, 9SAD-A, 9SAD-B and 9SAD-C. Then it was made to dock with receptor selected from PDB. Here for anticancer activity docking was done on tyrosine kinase (PDB ID: 2M59), and for anti-inflammatory activity, on ALOX5 (PDB ID:6N2W). It showed good docking score compared to the standard drugs. The 9-chloro acridine was synthesized by Jourdan-Ullmann Condensation reaction from this using different aldehydes the 3 derivatives were synthesized. After that, characterization of the derivatives was performed by IR, NMR and MASS spectroscopy. From the synthesized derivatives, 9SAD-A and 9SAD-C showed good anticancer activity, whereas 9SAD-B and 9SAD-A showed good anti-inflammatory activity, and 9SAD-A and 9SAD-C showed good antioxidant activity. These results make these synthesized 9 substituted acridine derivatives an interesting lead molecule for more synthetic and biological evaluation. These compounds certainly hold great promise towards pursuit to discover novel class of anticancer, anti-inflammatory, antioxidant agents in order to further improve these activities in future.

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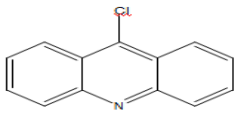
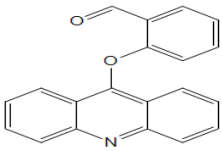
REFERENCES

1. Khan MOF, Deimling MJ, Philip A. Medicinal chemistry and the pharmacy curriculum. Am J Pharm Educ. 2011;75(8).
2. Lombardino JG, Lowe JA. The role of the medicinal chemist in drug discovery—then and now. Nat Rev Drug Discov. 2004;3(10):853–62.
3. Wadood A, Ahmed N, Shah L, Ahmad A, Hassan H, Shams S. In-silico drug design: An approach which revolutionarised the drug discovery process. OA drug Des Deliv. 2013;1(1):3–7.
4. Schreiber SL. Target-oriented and diversity-oriented organic synthesis in drug discovery. Science (80-). 2000;287(5460):1964–9.
5. Baranczewski P, Stanczak A, Sundberg K, Svensson R, Wallin A, Jansson J, et al. Introduction to in vitro estimation of metabolic stability and drug interactions of new chemical entities in drug discovery and development. Pharmacol reports.2006;58(4):453.
6. Kalyani G, Sharma D, Vaishnav Y, Deshmukh VS. A review on drug designing,methods, its applications and prospects. Int J Pharm Res Dev. 2013;5:15–30.
7. Kopec KK, Bozyczko-Coyne D, Williams M. Target identification and validation in drug discovery: the role of proteomics. Biochem Pharmacol. 2005;69(8):1133–9.
8. Bleicher KH, Böhm H-J, Müller K, Alanine AI. Hit and lead generation: beyondhigh- throughput screening. Nat Rev Drug Discov. 2003;2(5):369–78.
9. Singh J, Ator MA, Jaeger EP, Allen MP, Whipple DA, Soloweij JE, et al. Application of genetic algorithms to combinatorial synthesis: A computational approach to lead identification and lead optimization. J Am Chem Soc. 1996;118(7):1669–76.
10. Rester U. From virtuality to reality-Virtual screening in lead discovery and lead optimization: a medicinal chemistry perspective. Curr Opin Drug Discov Devel.2008;11(4):559–68.
11. Phoebe Chen Y-P, Chen F. Identifying targets for drug discovery using bioinformatics. Expert Opin Ther Targets. 2008;12(4):383–9.
12. Drews J. Drug discovery: a historical perspective. Science (80-). 2000;287(5460):1960–4.
13. Caldwell GW, Ritchie DM, Masucci JA, Hageman W, Yan Z. The new pre- preclinical paradigm: compound optimization in early and late phase drugdiscovery. Curr Top Med Chem. 2001;1(5):353–66.
14. Ooms F. Molecular modeling and computer aided drug design. Examples of their applications in medicinal chemistry. Curr Med Chem. 2000;7(2):141–58.
15. Veselovsky A V, Ivanov AS. Strategy of computer-aided drug design. Curr Drug Targets-Infectious Disord. 2003;3(1):33–40.
16. Schlick T. Molecular modeling and simulation: an interdisciplinary guide: an interdisciplinary guide. Vol. 21. Springer Science & Business Media; 2010.
17. Nadendla RR. Molecular modeling: A powerful tool for drug design and molecular docking. Resonance. 2004;9(5):51–60.
18. Zhou T, Huang D, Caflisch A. Quantum mechanical methods for drug design. Curr Top Med Chem. 2010;10(1):33–45.
19. Ricci A, Anthopoulos A, Massarotti A, Grimstead I, Brancale A. Haptic-driven applications to molecular modeling: state-of-the-art and perspectives. Future Med Chem. 2012;4(10):1219–28.
20. Mehra RK, Winge DR. Metal ion resistance in fungi: molecular mechanisms and their regulated expression. J Cell Biochem. 1991;45(1):30–40.
21. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, et al. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J Comput Chem. 2009;30(16):2785–91.
22. Friesner RA, Banks JL, Murphy RB, Halgren TA, Klicic JJ, Mainz DT, et al. Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. J Med Chem. 2004;47(7):1739–49.
23. Bursulaya BD, Totrov M, Abagyan R, Brooks CL. Comparative study of several algorithms for flexible ligand docking. J Comput Aided Mol Des. 2003;17(11):755–63.

24. Dar AM, Mir S. Molecular docking: approaches, types, applications and basic challenges. J Anal Bioanal Tech. 2017;8(2):1–3.
25. Supriya T, Shankar M, Kavya S, Lalitha J, Dastgiri M, Babu N. An overview on molecular docking. Am J Biol Pharm Res. 2016;3:83–9.
26. Ekins S, Mestres J, Testa B. In silico pharmacology for drug discovery: methods for virtual ligand screening and profiling. Br J Pharmacol. 2007;152(1):9–20.
27. Stroganov O V, Novikov FN, Stroylov VS, Kulkov V, Chilov GG. Lead finder: an approach to improve accuracy of protein– ligand docking, binding energy estimation, and virtual screening. J Chem Inf Model. 2008;48(12):2371–85.
28. Belmont P, Andrez J-C, Allan CSM. New methodology for acridine synthesis using a rhodium-catalyzed benzannulation. Tetrahedron Lett. 2004;45(13):2783–6.
29. Ziegler DT, Choi J, Muñoz-Molina JM, Bissember AC, Peters JC, Fu GC. A versatile approach to Ullmann C–N couplings at room temperature: New families of nucleophiles and electrophiles for photoinduced, copper-catalyzed processes. J Am Chem Soc. 2013;135(35):13107–12.
30. Cheng X-M, Lee C, Klutchko S, Winters T, Reynolds EE, Welch KM, et al. Synthesis and structure-activity relationships of 9-substituted acridines as endothelin-A receptor antagonists. Bioorg Med Chem Lett. 1996;6(24):2999– 3002.
31. Patel BB, Patel RG, Patel MP. Synthesis and studies of the biological activity of novel pyrimido fused acridine derivatives. J Serbian Chem Soc. 2006;71(10).
32. Anderson MO, Sherrill J, Madrid PB, Liou AP, Weisman JL, DeRisi JL, et al. Parallel synthesis of 9-aminoacridines and their evaluation against chloroquine- resistant Plasmodium falciparum. Bioorg Med Chem. 2006;14 (2):334–43.
33. Sondhi SM, Singh N, Lahoti AM, Bajaj K, Kumar A, Lozach O, et al. Synthesis of acridinyl-thiazolino derivatives and their evaluation for anti-inflammatory, analgesic and kinase inhibition activities. Bioorg Med Chem. 2005;13(13):4291–9.
34. Guetzoyan L, Yu X-M, Ramiandrasoa F, Pethe S, Rogier C, Pradines B, et al. Antimalarial acridines: synthesis, in vitro activity against P. falciparum and interaction with hematin. Bioorg Med Chem. 2009;17(23):8032–9.
35. Di Giorgio C, Shimi K, Boyer G, Delmas F, Galy J-P. Synthesis and antileishmanial activity of 6-mono-substituted and 3, 6-di-substituted acridines obtained by acylation of proflavine. Eur J Med Chem. 2007;42(10):1277–84.

Table : 1 Compound with Structure

Compound Code	Structure	IUPAC name of the compound
9-chloro acridine		9-chloro acridine
9-substituted acridine derivative A (9SAD- A)		2-(acridin-9-yloxy)benzaldehyde

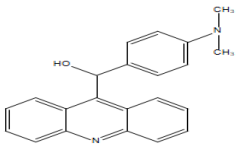
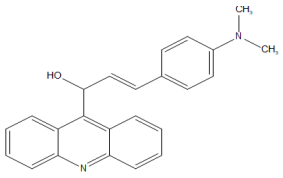
9-substituted acridine derivative B (9SAD- B)		Acridin-9-yl-(4-dimethylamino-phenyl)-methanol
9-substituted acridine derivative C (9SAD- C)		1-(acridin-9-yl)-3-(4-dimethylamino-phenyl)prop-2-en-1-ol

Table No. 2: Lipinski rule analysis of proposed 9 substituted acridine derivatives by SCFBio online software

Compound code	Molecular mass	Hydrogen bond donor	Hydrogenbond acceptor	LogP	Molar refractivity
9SAD-A	299.32	0	2	0.45	63.86
9SAD-B	328.16	0	1	0.97	72.25
9 SAD-C	354.44	0	1	0.98	78.07

Table No.3: Docking scores for synthesized compounds against 2M59

COMPOUND CODE	DOCKING SCORE AGAINST 2M59
9SAD-A	-8.545
9SAD-B	-6.683
9SAD-C	-7.799

Table No.4: Docking scores for synthesized compounds against 6N2W

COMPOUND CODE	DOCKING SCORE AGAINST 6N2W
9SAD-A	-6.969
9SAD-B	-7.990
9SAD-C	-5.893

Table No. 5 : NMR spectra of 9SAD-A

Node	shift obtain NMR spectra	base value	remarks (ppm rel. to TMS)
CH	9.605	9.6	CHO
CH	7.704	7.26	C=C(benzene)
CH	6.97	7.26	C=C(benzene)
CH	7.73	7.26	C=C(benzene)
CH	7.32	7.26	C=C(benzene)
CH	7.72	7.61	H on quinoline Ring
CH	8.28	7.69	H on quinoline Ring
CH	7.85	7.61	H on quinoline Ring
CH	7.97	8.05	H on quinoline Ring

Table No. 6 : IR interpretation of 9SAD-A

Functional group	Mode of vibration	Wavelength (cm^{-1})
CH on Ar	Bending	748.40
C=N	Stretching	1643.90
CH on Ar	Stretching	3180.38
C=C on Ar	Stretching	1483.91
C-C on Ar	Stretching	1571.12
C-N	Stretching	1232.05
C-O	Stretching	1343.90
C=O in aldehyde	Stretching	1732.05

Mass spectrum: molecular ion peak at m/z: 300.16Da

Table No.7 : NMR spectra of 9SAD-B

Node	Shift obtain NMR spectra	Base value	Remarks (ppm rel. to TMS)
CH	3.327	0.86	Methyl-CH_3
OH	6.43	4.20	Alcohol-OH
CH	6.64	7.26	C=C(benzene)
CH	7.23	7.26	C=C(benzene)
CH	7.69	7.61	H on quinoline Ring
CH	7.82	7.61	H on quinoline Ring
CH	7.95	8.05	H on quinoline Ring
CH	8.534	7.68	H on quinoline Ring
CH	6.157	1.50	Methine-alpha

Table No. 8 : IR interpretation of 9SAD-B

Functional group	Mode of vibration	Wavelength (cm^{-1})
CH on Ar	Bending	810.10
C=N	Stretching	1654.90
CH on Ar	Stretching	3180.48
C=C on Ar	Stretching	1546.66
C-C on Ar	Stretching	1585.41
C-N	Stretching	1368.22
C-O	Stretching	1311.76
CH on CH_3	Stretching	2902.28
CH on CH_3	Bending	1430.97
CH on CH_3	Rocking	1392.29
OH	Stretching	3380.48
OH (alcohol)	Bending	1064.33

Mass spectrum molecular ion peak at m/z: 327.69Da

Table No. 9: NMR spectra of 9SAD-C

Node	shift obtain NMR spectra	Base value	Remarks (ppm rel. to TMS)
CH	3.04	0.86	CH_3
OH	6.43	4.20	OH (alcohol)
CH	6.76	7.26	C=C(benzene)

CH	7.56	7.26	C=C(benzene)
CH	7.67	7.61	H on quinoline Ring
CH	7.82	7.61	H on quinoline Ring
CH	7.95	8.05	H on quinoline Ring
CH	8.13	7.68	H on quinoline Ring
CH	6.61	5.25	Ethylene H-C=C-H
CH	5.794	5.25	Ethylene H-C=C-H
CH	5.56	1.50	Methine-alpha

Table No. 10: IR interpretation of 9SAD-C

Functional group	Mode of vibration	Wavelength (cm^{-1})
CH on Ar	Bending	806.84
C=N	Stretching	1689.31
CH on Ar	Stretching	3211.87
C=C on Ar	Stretching	1471.46
C-C on Ar	Stretching	1524.29
C-N	Stretching	1233.35
=CH	Stretching	3394.17
=CH	Bending	970.77
C-O	Stretching	1211.87
CH on CH_3	Stretching	2902.04
CH on CH_3	Bending	1430.64
CH on CH_3	Rocking	1367.96
OH (alcohol)	Bending	1119.20
C=C on alkenes	Stretching	1653.27

Mass spectrum: molecular ion peak at m/z: 354.86Da

Table No.11: *In-vitro* antioxidant value with standard deviation

Compound Code	Percentage inhibition					IC_{50}
	10 (mcg/ml)	20 (mcg/ml)	30 (mcg/ml)	40 (mcg/ml)	50 (mcg/ml)	
9SAD-A	26.8	43.3	58	63.7	74.4	28.17
9SAD-B	18.8	20.3	34.2	39.2	40.1	49.14
9SAD-C	25.3	38.7	46.6	58.2	64.6	32.13
Ascorbic acid	32.6	48.4	59.5	74.2	81.5	25.32

Each value expressed as percentage of activity mean ± standard deviation

Table No. 12 : *In-vitro* cytotoxicity study of synthesized compounds against DLA

Derivatives	Percentage growth inhibition (%) (concentration in μg/ml)					IC_{50}
	10	20	50	100	200	
9SAD-A	10.4±1.5	15.0±1.3	21.3±2.7	46.3±1.8	86.7±2.6	105.73
9SAD-B	6.8±0.06	9.82±2.35	10.3±1.83	17.1±1.96	23.9±2.01	279.70
9SAD-C	6.88±1.3	9.24±1.2	10.4±1.4	18.7±0.7	49.1±0.3	201.44
5-flurouracil	20	32	50	58	70	82.60

Table No. 13: *In-vitro* anti-inflammatory activity of synthesized compounds

Compound code	percentage inhibition± standard deviation			
	25mcg /ml	50mcg/ml	100mcg/ml	IC_{50}
9SAD-A	29.2341±0.1329	33.7891±0.1267	37.9753±0.1521	86.00
9SAD-B	34.1456±0.1201	39.4356±0.1034	46.4765±0.0863	72.66
9SAD-C	15.3513±0.1217	31.13063±0.1167	36.8588±0.0974	105.26
Diclofenac	78.4924±0.0973	88.4861±0.0521	93.2154±0.0307	34.590±1.090

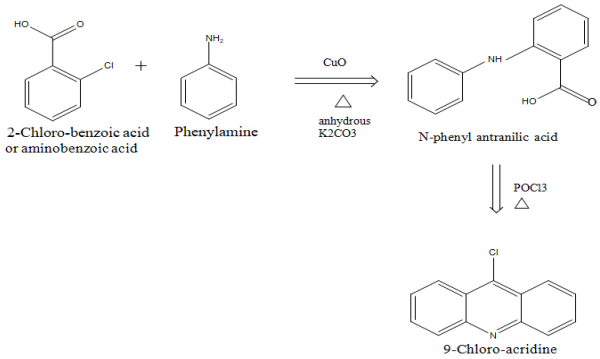
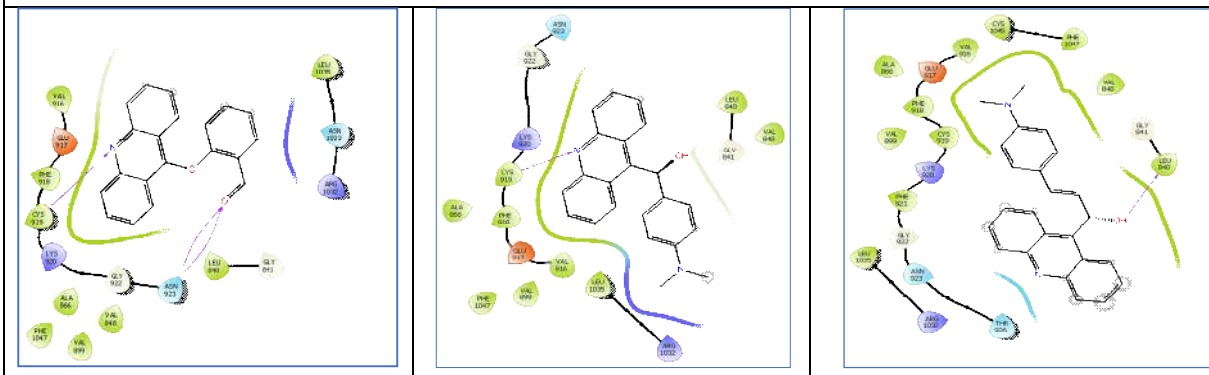
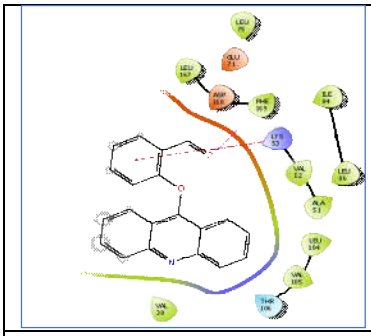


Fig.1: Methods employed

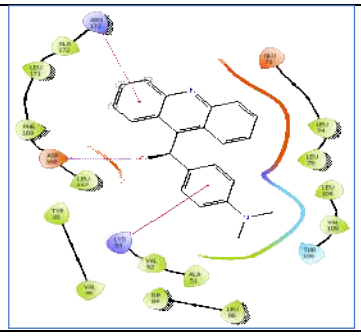


9SAD-A | **9SAD-B** | **9SAD-C**

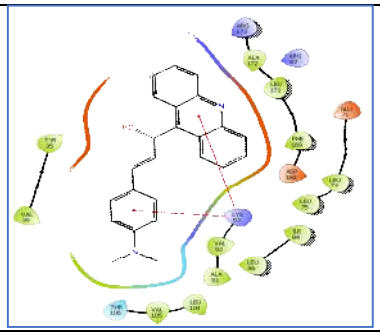
Fig. 2. Docking images of synthesized compounds in 2M59 targets



9SAD-A



9SAD-B



9SAD-C

Fig. 3. Docking images of synthesized compounds in 6N2W targets

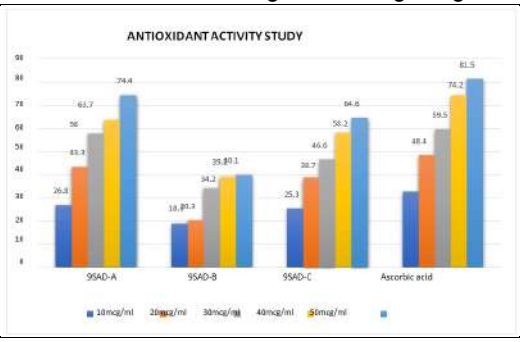


Fig. 4. Anti-oxidant activities of derivatives

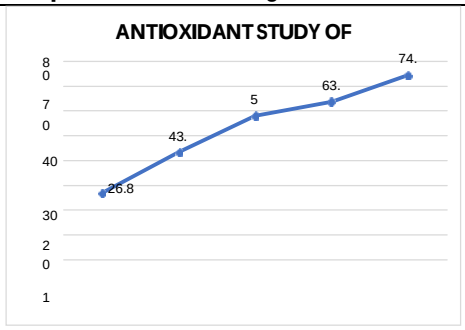


Fig.5. Hydrogen peroxide scavenging assay of 9SAD-A

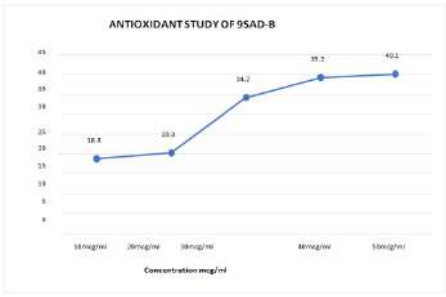


Fig. 6. Hydrogen peroxide scavenging assay of 9SAD-B

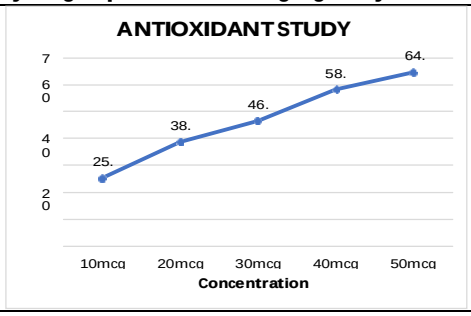


Fig. 7. Hydrogen peroxide scavenging assay of 9SAD-C

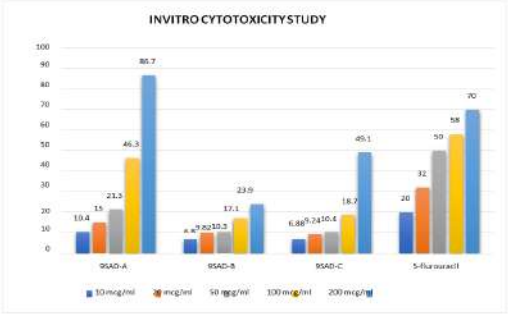


Fig. 8. *In vitro* Cytotoxicity Study

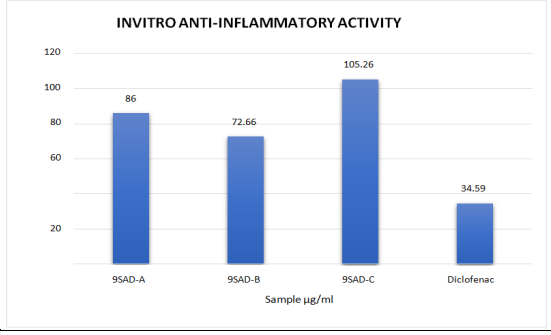


Fig. 9. Comparison of IC_{50} of synthesized compounds on protein denaturation

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RESEARCH ARTICLE

Assess Variation of Blood Pressure during Intravenous Administration of Contrast Media in Computed Tomography

Vashist Baburao Mhalsekar[1]* and Sashisaroj T Tiwari[2]

[1]Assistant Professor, Yenepoya School of Allied Health Sciences, Yenepoya (Deemed to be University), Mangalore, Karnataka, India
[2]Assistant Professor, Department of Radiology, School of Paramedics and Allied Health Sciences, Centurion University of Technology and Management, Bhubaneshwar, India

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***Address for Correspondence**
Vashist Baburao Mhalsekar
Assistant Professor,
Yenepoya School of Allied Health Sciences,
Yenepoya (Deemed to be University),
Mangalore, Karnataka, India
Email: Vashistmhalsekar@gmail.com

ABSTRACT

The advancement in Computed Tomography(CT) have provided an various opportunities to upgrade an new clinical applications, new protocols also good image quality. Every medication might have adverse reactions at the time of injecting the dose or some of the medications will have late or minor effects after administration. It shows in various literatures that Computed Tomography contrast media have been injected and assessment of blood pressure have been done in Contrast Enhanced Computed Tomography (CECT) protocols. The blood pressure have been measured before the Computed Tomography scan without injecting contrast and after injecting blood pressure have been measured. With respect to various literatures have been published it seems that there is an slight increase in blood pressure seen in maximum number of cases in results of the different literatures after injecting contrast media.

Keywords: CECT Scan, Contrast media, Intravenous Injection, Systolic Blood Pressure, Diastolic Blood Pressure

INTRODUCTION

As the advancement in CT Technology is getting developed Intravenous administration of contrast media practice at the time of CT scan is also getting updated [1]**.** With every medication there will be potential adverse reactions or side effects [2]**.** Administration of iodinated contrast media may get some reactions or side effects depending upon characteristics of contrast media and its phenomenon or incident of reaction

ranges from 0.2% to 12.7% of contrast injections [3]**.** Depending upon the osmolality of contrast media hemodynamic changes observed rapid intravenous injection of contrast media [4]**.** In 1960s and 1970s patients with history of pheochromocytoma, Hypertensive crisis were noted in CT protocols of intravenous examinations of contrast media [5,6]. In Interventional procedures decrease in blood pressure were noted with non-ionic contrast media administration [7]**.** Currently, non-ionic low osmolar contrast media were used while performing CECT protocols in CT [8]. As a result of high-osmolar contrast media Hypertensive crisis were reported, low osmolar contrast media need to assess for different variations in blood pressure.

BLOOD PRESSURE

Blood pressure is defined as force is applied on the walls of the blood vessels by circulating blood. Basically the blood pressure depends upon three types of interaction namely as compliance of arterial walls, blood volume and cardiac contractility. Previoulsy blood pressure is measured in Millimeters of mercury (mmHg). As heart contracts maximum pressure in main circulation of blood vessel is known as systolic blood pressure and minimal pressure at the time of diastole just before next to systole known as Diastolic blood pressure. The difference in systolic and diastolic pressures known as Pulse pressure [9].

DISCUSSION

BALD et al. have done study in patients with pheochromocytoma after contrast injection they concluded that there is an increase in blood pressure [10]. Lin et al. has done the study on blood pressure changes during retrograde brachial angiography in which they had concluded that rise in carotid artery blood pressure at the time of angiography [11] which is similar to Stoeter et al., [12] study. Morris et al. had done study on rabbit in which contrast media was injected by hand push method and they found an decrease in blood pressure [4]. In counter to this study Saithoh et al. had done study on administration of contrast media through catheter into carotid artery in which they observed that there is an increase in intracarotid systolic blood pressure in Dogs [13].The study was done by Arathy and Sushil yadav on variation in blood pressure during intravenous injection of contrast media for CECT abdomen scan were concluded that intravenous injection of non-ionic low-osmolar contrast media there will be increase in both systolic and diastolic blood pressure [14]. With respect to the results of various literatures of blood pressure variation after contrast media administration shows that there is an increase in blood pressure values.

CONCLUSION

Depending upon subject to subject the values of systolic and diastolic blood pressure might be change. The findings in various literatures suggested that there will be an some amount of elevation in blood pressure after administration of contrast media.

REFERENCES

1. Bae KT. Intravenous contrast medium administration and scan timing at CT: Considerations and appoaches. Radiology 2010;256:32-61.
2. Kumar L. Harmacovigilance/reporting adverse drug reactions: An approach to enhance health surveillance and extending market share by minimizing the chances of drug withdrawals. Int J Pharm PharmSci 2015;7:1-7.
3. Juchem BC, Dall'Agnol CM. Immidiate Adverse reactions to intravenous iodinated contrast median in computed tomography. Rev Latinoam Enfermagem 2007;15:78-83.
4. Morris TW, Prentice L, Ventura J.A comparison of the systemic responses to rapid intravenous injections of ioxilan, iohexol, and diatrizoate in rabbits. Invest Radiol 1989;24:294-7.

5. Rossi P, Young IS, Panke WF. Techniques, usefulness, and hazards of arteriography of pheochromocytoma: Review of 99 cases. JAMA 1968;205:547-53.
6. Gold RE, Wisinger BM, Geraci AR, Heinz LM. Hypertensive crisis as a result of adrenal venography in a patient with pheochromocytoma. Radiology 1972;102:579-80.
7. Cooper MW, Reed P J. Comparison of ionic and non-ionic contrast agents in cardiac catheterization: The effects of ventriculography and coronary arteriography on hemodynamics, electrocardiography, and serum creatinine. CathetCardiovascDiagn 1991;22:267-77.
8. Sachiko N, Yoshito T, Ayako TT, Tetsuya H, Makato A, Keigo E, *et al.* Hypertensive crisis due to contrast-enhanced computed tomography in a patient with malignant pheochromocytoma. Jpn J Radiol 2011;29:449-51.
9. Bell, D. Blood pressure. Reference article, Radiopaedia.org. (accessed on 14 Jul 2022) https://doi.org/10.53347/rID-69299
10. Bald KS, Edwln LW, Robert WA, Henrl LA. Brief communication: Radiographic contrast infusion and catecholamine release in patients with pheochromocytoma. Ann Intern Med 2009;150:27-33.
11. Lin JP, Kricheff II, Chase NE. Blood pressure changes during retrograde brachial angiography. Radiology. 1964 Oct;83(4):640-6.
12. Stoeter P, Prey N, Hoffmann C, Büdingen HJ, Bergleiter R. Doppler-sonographic examination of the arterial flow in the carotid and supratrochlear arteries during carotid angiography. Neuroradiology. 1984 May;26(3):199-207.
13. Saithoh H, Hayakawa K, Nishimura K, Okuno Y, Murayama C, Miyazawa T, *et al.* Intracarotidnlood pressure changes during contrast medium injection. AJNR Am J Neuroradiol 1996;17:51-4.
14. John AM, Yadav S. Evaluation of blood pressure variations during the administration of intravascular contrast media in CECT abdomen. EVALUATION. 2018;11(6).

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RESEARCH ARTICLE

Effect of Mulching on Growth of Dragon Fruit [*Hyloceruscostaricensis* (Web.) Briton and Rose]

S. Maji[1]*, Pradhan Bairwa[2] and Maya Ram[3]

[1]Assistant Professor, Department of Horticulture, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh, India.
[2]M.Sc Student, Department of Horticulture, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh, India.
[3]Research Scholar, Department of Horticulture, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh, India.

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***Address for Correspondence**
S. Maji,
Assistant Professor,
Department of Horticulture,
Babasaheb Bhimrao Ambedkar University,
Lucknow, Uttar Pradesh, India.
Email: majisutanu@gmail.com

ABSTRACT

Dragon fruit is now getting popularity across the world including Indian market due to its nutraceutical values. It has potential production under less water available zone and thus, present experiment was done to see its growth performance under enhanced water use efficiency through mulching. There were 8 mulching treatment condition including control having three replication was carried out following RBD statistical design. Results showed that a significant improvement in water use efficiency with higher vegetative growth under organic mulching systems. Among them, wheat straw mulch and news paper mulch showed a significant increase in plant growth that may influence the crop yield parameters in future.

Keywords: dragon fruit, mulching, growth, *Hylocerus* sp.

INTRODUCTION

Dragon fruit, member of Cactaceae family, is a herbaceous perennial climbing cactus with triangular green stem found especially in the semi-desert, hot/summer tropical regions of Latin America (Spichiger *et al.,* 2000), however, due to its nutraceutical properties, it is being cultivated commercially as fruit crop in 22 countries, such as Australia, Columbia, China, Cambodia, Hawaii, Israel, Japan, Mexico, New Zealand, Taiwan, Srilanka, Spain etc. It is one of the newly introduced exotic fruit crops in India (Maji, 2019; Maji *et al*., 2021). It is also known as pitaya, Strawberry pear,

Queen of night, Honorable queen etc. Dragon fruit has broad medicinal and nutraceutical properties. It is widely used as juice and in fruit salads and mostly consumed as fresh fruit as well as in form of many processed foods. Regular consumption of Dragon fruit helps in fighting against cough and asthma, helps for healing wounds and cut quickly by enhancing immune system. Dragon fruit is also rich in flavonoids, fibers and B group vitamins (B1, B2 and B3) which possess an important role to improve eye sight and prevent hypertension. Dragon fruit is also helpful in reducing blood sugar levels. It contains high level of phosphorus and calcium that helps to reinforce bones and play an important role in tissue formation and forms healthy teeth. For the many health benefits and in point of crop diversification to increase farmers' income proper cultivation of dragon fruit can help a lot. Although, dragon fruit has xerophytic tendency, judicial water management can play important role for its better growth and development. In this context, mulching is one of the practices that can conserve soil moisture and help for the plant growth under less water availability period. Mulch insulated the soil helping to provide a buffer from heat and cold temperature. Mulch keep weeds out to help prevent root competition. Mulch prevents soil compaction.

Mulching is a method to cover the soil while growing the crop. Apart from soil moisture conservation, it also protects soil erosion, weed infestation, reduce evaporation etc. Various materials are used as mulch to cover the soil like straw, plastic, leaves, wood chips, sawdust, newspaper, cardboard etc. Mulching is done in a wide range of vegetables and fruit crops show better results with, mulching. With the extensive spread of technology and the adoption of various methods, a farmer is becoming progressive and is getting maximum yield in a minimum area. Mulching is one of the techniques that help to get maximum yield and quality produce in a minimum area. Mulches preventing the contact between plants and soil, thus reducing the risk of disease (FAO, 2014), had significant effects on micro-meteorological factors and individual growth of rice, as shown by an increase of relative humidity (Zhang *et al.*, 1994 and 2008). Furthermore, organic mulches can reduce the effect of salt toxicity on plant growth (Ansari *et al.,* 2001, Landis, 1988, Yobterik and Timmer, 1994) or actively accelerate soil desalinization (Dong *et al*., 1996). Organic mulches can also help degrade pesticides and other contaminants (Smith and Skroch, (1995), presumably by providing increasing microbial populations that degrade pesticides.

Living and organic mulches can increase, decrease, or have no effect upon nutrient levels depending upon mulch type, soil chemistry, and particular nutrients of interest. As living and organic mulches decompose under appropriate water and temperature levels, nutrients are released into the soil and become available for root uptake or microbial use. Plastic mulching has the capacity to trap heat, which causes the soil temperature to increase. Light-colored mulches reflect sunlight, whereas dark mulch absorbs it. Soil temperature can be lowered under light-colored mulch. It is also beneficial to early crop production as well as increased production and productivity. Organic mulch plays very important role to reflect solar radiation. This improves root growth increases the infiltration of water, and also improves the water-holding capacity of the soil Newspaper mulching helps to control weeds and also add little organic matter in soil. 3-4 cm thick sheet of newspaper should be used and edges should be fastened with materials like pebbles gravels etc. keep wet or cover with bark or other mulch. The availability of scientific information on dragon fruit cultivation is very limited because of low availability of quality planting material, technical know how about proper cultivation with low cost technology having high return. Therefore, the present experiment was conducted to standardize low cost mulching technology for better water efficiency and its effect on crop growth.

MATERIALS AND METHODS

The present experiment entitled was carried out at the Horticulture Research Farm of the Department of Horticulture, Babasaheb Bhimrao Ambedkar University, Lucknow, U.P., India during *Rabi* season of 2020 and 2021. The geographically Lucknow is situated at 26^0 50' N latitude, 80^0 5' E longitude and the altitude of 111 meter above mean sea level (MSL). Lucknow is fall under the humid subtropical climate with the average rainfall of about 110 cm and relative humidity ranged during these condition approximately ranging 60-90% depending upon the weather and the climatic factor. During the winter condition (December-January) the temperature may be fall up to

the 2^{0}C and during summer (May-June), the temperature may go beyond the 43^{0}C. The Lucknow condition received major form of the rainfall only by south-west monsoon, which received during the generally third week of June and precedes by the end of September with heavy rainfall during the monsoon season. Mulch materials Black polythene, White polythene, News paper, Wheat straw, Rice straw, Wood husk, Grass mulch were used and a treatment with no mulch was considered as control.

Mulch covers was spread over 45 - 60 cm from base and about 1.5-2 inch thick near the base. There were eight treatments randomly laid out following RBD design having three replications. The observations were taken for vegetative growth characters as the plants were 2 years old and do not have flowering period during winter months. The pinkish red fleshed dragon fruit plants [*Hylocerus costaricensis* (Web.) Briton and Rose] with uniform growth were selected for the study. The plants were raised from stem cutting done in the institute and maintained organic nutrition along with other cultural operations. Field soilwas sandy loam and slightly alkaline in nature with soil pH 8.2, with uniform topography. Vegetative growth observations were taken for consideration namely- height of the plant was recorded from surface of soil to the tip of the longest stem of the plant with the help of meter scale; Number of branch per plant was counted as primary branches at 30, 60 and 90 days after treatment (DAT). Stem circumference was measured with help of vernier caliper at central portion of a middle cladode. Base cladode length of 70-75 cm length was chose for taking the reading increase in length was measured with the help of measuring tape/scale and expressed in cm. Number of spines counted in each areole manually of randomly selected cladode and average number was calculated by dividing by total number of areoles. Similarly, average length of spine was measured with the help of vernier caliper. Statistical analysis was done as per standard method (Sahu and Das, 2014) and treatment means were compared at 5% level of significance.

RESULTS AND DISCUSSION

It was observed that mulching had a significant effect of increase in plant height. It was seen that from 30 to 60 days the rate of increase in plant height was higher under news paper mulching followed by black polythene mulch and wheat straw mulch. Similar pattern of increase was observed at 90 days but was statistically at par with grass mulch and control, however, reason behind that was unclear. From 30 to 90 days, it was clear that newspaper mulch caused maximum increase of plant height (5 cm) which was significantly higher as compared to other mulching. The current finding is in harmony with those of Verma *et al.* (2005), who reported that mulching improved vegetative growth of apple trees and distribution of shoots as well as absorption of nutrients. The distance between areoles found higher under news paper mulch and rice straw mulch at 30 and 60 days after treatment (DAT). However, the increase in areole distance was maximum under grass mulch followed by white polythene mulch. The rate of increase in areole distance was found higher with wheat straw mulch followed by white polythene and news paper mulch. But, the total increase from 30 to 90 DAT was recorded highest with white polythene mulch and wheat straw mulch.

The maximum increase rate in areole distance also increase the total plant height since there two are interlinked. Number of spine per areole was non-significantly influenced by different application of mulching. Number of spine per areole was varied from 4.19 at 30 DAT to 4.83 at 90 DAT. Similar pattern in number of spine in different treatment might be due to the fact that it might be a genetically governed character. Mauseth (2006 also found that there was no significant variation in spine number per areole even studied among different clones. Similar to number of number of spine, the length of spine also was not significantly influenced by different mulching treatments. It was varied from 0.43 cm at 30 DAT to 0.58cm at 90 DAT. It was observed that maximum of spine length (0.58 cm) was recorded under treatment T_2 i.e. wheat straw mulch (0.58 cm) followed by control treatment (0.52 cm) at 90 DAT. Similar kind of result was also observed by Mosco (2009) and it might be due to genotypic effect. It was observed that maximum increase of cladode length (2.17 cm) in dragon fruit plant was recorded in rice straw mulch after 60 days of treatment followed by black polythene. At 90 DAT, the highest increase was observed under white polythene (3.33 cm). However, total increase from 30 to 90 DAT was found maximum in rice straw mulch followed by grass

and wheat straw mulch. That may be due to organic nature of that mulch which may facilitate favourable condition for better growth. Zhang et al. (2010, 2014) reported that soil moisture content in apple trees zone was the highest with gravel mulch treatment. This could be possible due to increase intensive metabolic processes (Lei et al., 2012; Liang et al., 2002; Liu et al., 2014). Higher areole distance due to white polythene and rice straw might increase overall cladode length. It was observed that mulching also had a significant influence on number of branch and stem circumference at central cladode position.

The maximum number of branch per plant at 90 DAT was counted with news paper mulching. The increase rate was higher during early growth from 30 to 60 DAT as compared to second growth period from 60 to 90 DAT. Maximum 5.33 branches increase was found in saw dust mulch from 30 to 90 DAT but statistically at par with news paper mulch (5.0). Pang *et al.* (2012) showed that the soil moisture and microbial quantity increased when mulched by gravel and sand. Hence, this could increase nutrients uptake and translocation of nutrients. Many investigators supported these findings (Belatus, 2002). In addition to that, Moslem *et al.* (2012) reported that, all treatments of mulching significantlyincreased the number of leaves of fig trees compared with control.

CONCLUSION

From the investigation with mulching on dragon fruit it can be concluded wheat straw mulching and newspaper mulching significantly increase the height of plant, circumference (central portion of areole), number of branch or cladode per plant, cladode length (1stcladode) and number of spine (12 central areole), spine length followed by saw dust mulching. Thus, organic mulching was best and rice straw, wheat straw and news paper mulch could be used for dragon fruit cultivation for better growth.

REFERENCES

1. Ansari, R., Marcar, N.E., Khanzada, A.N., Shirazi, M.U., Crawford, D.F. (2001). Mulch application improves survival but not growth of Acacia ampliceps Maslin, Acacia niloticaL. and *Conocarpus lancifolius* L. on a saline site in southern Pakistan. International Journal Review, 3:158–163
2. Belatus, E.L. (2002). Em Technology. Regional Workshop on usage of EM Technology and agriculture waste Recyling. 26-28. March. Ministry of Agriculture . Affairs. Cairo, Egypt.
3. Dong, B. B., Zhu, H. T., Zhong, Z.K., Ye, G.F. (1996). Study on ecological effect of the forest land under crop sowing and mulching of coastland soil by newly planted. Acta Agriculurae Zhejiangensis, 8: 154-157.
4. FAO 2014. Mulching to control soil erosion. Strategic Objective 5 – Resilience, in FAO.
5. Landis, T.D. (1988). Management of forest nursery soils dominated by calcium salts. New. Forests, 2:173–193
6. Lei, Pang, Xiao, Hong-lang, Xie, Zhong, Kui, Chai, Shou-Xi, Lu Jian- Long, Zhou, Mao-Xian, Change, Lei, Wang, Ya-Jun (2012). Effect of gravel–sand Microbial Composition. Journal of Desert Research, 32 (2), 351-358.
7. Liang, Y.l., Zhang, C.E., Guo, D.W. (2002). Mulch types and their benefit in cropland ecosystems on the loess plateau in China. Journal of Plant Nutrition, 25 (5): 945-955.
8. Liu,Yi, Wang, Jing, Liu, Dongbi, Li, Zhiquo, Zhang, Guoshi, Zhang, Tao, Yong, Xie, Juan, Pan, Junfeng, Chen, F. (2014). Straw mulching reduces the harmful effects of extreme hydrological and temperature conditions in Citrus Orchards. Plos One. 9 (1): e87094:1-9.
9. Maji, S. (2019). Dragon Fruit: The Super Fruit. Book Publisher International. DOI- 10.9734/bpi/mono/978-93-89562-60-6.
10. Maji, S., Meena, K., Kumar, S. (2021). Influence of various length of stem cutting for successful propagation of Dragon fruit [*Hylocerus costaricensis* (Web.) Briton and Rose]. Crop Research, 56 (6) : 313-316. DOI : 10.31830/2454-1761.2021.050
11. Mauseth, J.D. (2006). Structure-function relationships in highly modified shoots of Cactaceae. Annals of Botany, 98: 901–926. doi: 10.1093/aob/mcl133
12. Mosco, A. (2009). Micro-morphology and anatomy of Turbinicarpus (Cactaceae) spines.: 119–128.

13. Moslem, J., Haghighi, J.A.P., Hamid, Z. (2012). Mulching impact on plant growth and production of raifed fig orchards under drought conditiond. Jounal of Food Agriulture Environment, 10 (1): 428-433
14. Pang, Lei, Xiao, Hong-Lang Xie, Zhong- Kui, Chai, Shou-xi., Lu, Jian-Long, Zhou, Mao- Xian, Chang, Lei and Wang, ya-Jun (2012) Effect of gravel-sand mulching on soil microbial composition. Journal of Desert Research, 32 (2): 351-358.
15. Sahu, P.K., Das, A K (2010). Agriculture and Applied Statistics-I, 2nd edition, Kalyani Publishers, New Delhi.
16. Smith, L.J., Skroch, W.A. (1995). Turf herbicide injury to landscape trees as influenced by mulch. Journal of Environmental Horticulture, 13:60–63
17. Spichiger, R.E., Savolainen, V.V., Figeat, M. (2000). Botanique systématique des plantes à fleurs – une approche phylogénétique nouvelle des angiospermes des regions tempérées et tropicales, Presses Polytech. Univ. Romand, Lausanne, Suisse 2000, 372.
18. Verma, M.L., Bhardwaj, S.P., Thakur, B.C., Bhandria, A.R. (2005). Nutritional and mulching studies in apple. Indian. Journa of Horticulture, 62 (4): 721-730.
19. Yobterik, A.C., Timmer, V.R. (1994). Nitrogen mineralization of agro-forestry tree mulches under saline soil conditions. In: R.B. Bryan (ed.) Adv. Geo-eco. 27:181–194.
20. Zhang, S. F., and Ma, T. L. (1994). Yield components and cultivation technology of corn with high grain yield through plastic film cover in West Yellow River area. Gansu Agricultural Science and Technology, 1: 16–17.
21. Zhang, X., Yu, G. Q., Li, Z. B., Li, P. (2014). Experimental Study on Slope Runoff, Erosion and Sediment under Different Vegetation Types. Water Resource and Management, 28: 2415–2433
22. Zhang, Y., Xie, Y.S., Hao, M.D., She, X.Y. (2010). Effects of different patterns surface mulching on soil properties and fruit trees growth and yield in an apple orchard. Journal Applied ecology. 21 (2): 279 -286.
23. Zhang, Z., Zhang, S., Yang, J., Zhang, J., (2008). Yield, grain quality and water use efficiency of rice under non-flooded mulching cultivation. Field Crops Research, 108: 71–81.

Table 1: Effect of mulching on plant height of dragon fruit at 30, 60 and 90 DAT

Treatment	30 DAT (cm)	60 DAT (cm)	Increase at60 DAT (cm)	90 DAT (cm)	Increase at 90 DAT (cm)	Total increase from 30 to 90 DAT (cm)
T_0 - Control	131.33	132.33	1.00	134.33	2.00	3.00
T_1- Grass mulch	164.66	165.66	1.00	167.66	2.00	3.00
T_2 - Wheat straw mulch	164.66	166.66	2.00	167.66	1.00	3.00
T_3 - Saw dust mulch	142.33	143.66	1.33	145.33	1.67	3.00
T_4 - Black polythene mulch	101.66	104	2.34	105.33	1.33	3.67
T_5 - White polythene mulch	144.33	146	1.67	147.66	1.46	3.33
T_6 - News paper mulch	180.66	183.66	3.00	185.66	2.00	5.00
T_7 - Rice straw mulch	155.33	157	1.67	158.50	1.50	3.17
SEm (±)	5.591	6.801	0.097	6.896	0.102	
CD (P = 0.05)	17.122	20.829	0.297	21.12	0.312	

Table 2: Effect of on average areole distance at 30, 60, and 90 DAT

	30 DAT (cm)	60 DAT (cm)	Increase at 60 DAT (cm)	90 DAT (cm)	Increase at 90 DAT (cm)	Total increase from 30 to 90 DAT (cm)
T_0 - Control	16.29	17.82	0.93	19.82	2.00	2.93
T_1- Grass mulch	16.60	19.01	2.41	20.42	1.41	3.82
T_2 - Wheat straw mulch	16.30	17.83	1.53	21.81	3.98	5.43
T_3 - Saw dust mulch	19.27	19.52	0.27	22.84	3.32	3.59

T_4 - Black polythene mulch	18.94	19.46	0.52	21. 76	2.80	3.32
T_5 - White polythene mulch	19.94	21.75	1.81	23.19	3.82	5.63
T_6 - News paper mulch	21.70	22.67	0.97	26.49	3.82	4.69
T_7 - Rice straw mulch	21.69	22.65	0.96	25.47	1.44	2.40
SEm (±)	0.892	0.946	0.051	1.073	0.137	
CD (P = 0.05)	2.732	2.897	0.156	3.285	0.420	

Table 3: Effect of number of spine at 30, 60, and 90 DAT

Treatment	30 DAT	60 DAT	90 DAT
T_0 - Control	4.19	4.33	4.42
T_1- Grass mulch	4.33	4.39	4.47
T_2 - Wheat straw mulch	4.31	4.33	4.50
T_3 - Saw dust mulch	4.44	4.72	4.78
T_4 - Black polythene mulch	4.53	4.56	4.61
T_5 - White polythene mulch	4.56	4.64	4.83
T_6 - News paper mulch	4.44	4.47	4.50
T_7 - Rice straw mulch	4.47	4.67	4.75
SEm (±)	0.94	0.59	0.64
CD (P =0.05)	NS	NS	NS

Table 4: Effect of mulching on length of spine at 30, 60, and 90 DAT

Treatment	Length of spine (cm)		
	30 DAT	60 DAT	90 DAT
T_0 - Control	0.50	0.50	0.52
T_1- Grass mulch	0.46	0.46	0.46
T_2 - Wheat straw mulch	0.56	0.56	0.58
T_3 - Saw dust mulch	0.40	0.40	0.42
T_4 - Black polythene mulch	0.43	0.43	0.44
T_5 - White polythene mulch	0.46	0.46	0.47
T_6 - News paper mulch	0.43	0.43	0.44
T_7 - Rice straw mulch	0.43	0.43	0.45
SEm (±)	0.023	0.023	0.024
SD (P = 0.05)	0.072	0.072	0.073

Table 5: Effect of mulching on cladode length (1st cladode) at 30, 60,and 90 DAT

Treatment	Cladode length (cm) 1st cladode					
	30 DAT (cm)	60 DAT (cm)	Increaseat 60 days (cm)	90 DAT (cm)	Increase at 90 DAT (cm)	Total increase from 30 to 90DAT (cm)
T_0 - Control	69.00	70.00	1.00	72.00	2.00	3.00
T_1- Grass mulch	94.33	96.00	1.67	99.00	3.00	4.67
T_2 - Wheat straw mulch	115.33	117.00	1.67	120.00	3.00	4.67
T_3 - Saw dust mulch	97.00	98.66	0.34	101.66	3.00	3.34
T_4 - Black polythene mulch	70.00	72.00	2.00	74.00	2.00	4.00
T_5 - White polythene mulch	103.33	104.33	1.00	107.66	3.33	4.33
T_6 - News paper mulch	141.33	143.00	1.67	145.50	2.50	4.17

T_7 - Rice straw mulch	106.33	108.50	2.17	111.33	2.83	5.00
SEm (±)	4.250	4.324	0.069	4.444	0.121	
CD (P = 0.05)	13.017	13.241	0.21	13.611	0.372	

Table 6: Effect of mulching on stem circumference (cm) at centralportion of areole at 30, 60, and 90 DAT

Treatment	Circumference (cm) at central portion of cladode		
	30 DAT	60 DAT	90 DAT
T_0 - Control	11.66	12.16	13.00
T_1- Grass mulch	14.33	14.66	15.33
T_2 - Wheat straw mulch	14.66	15.33	16.33
T_3 - Saw dust mulch	13.66	14.16	15.00
T_4 - Black polythene mulch	14.00	14.66	15.66
T_5 - White polythene mulch	13.00	13.66	14.66
T_6 - News paper mulch	16.00	16.33	17.66
T_7 - Rice straw mulch	14.66	15.16	16.00
SEm (±)	0.654	0.682	0.727
CD (P = 0.05)	2.002	2.090	2.227

Table 7: Effect of mulching on number of branch in per plant

Treatment	Number of branch in per plant					Total increase (cm) From 30 to 90 DAT
	30 DAT	60 DAT	increase at 60 Days	90 DAT	Increase at90 Days	
T0 - (Control)	3.66	5.33	1.67	7.33	2.00	3.67
T1- (Grass mulch)	4.33	5.66	1.33	7.66	2.00	3.33
T2- (Wheat straw mulch)	4.33	5.33	1.00	7.00	1.67	2.67
T3- (Saw dust mulch)	3.33	6.00	2.67	8.66	2.66	5.33
T4 (Black polythene mulch)	4.00	6.66	1.66	8.33	1.67	3.33
T5- (White polythene mulch)	4.00	5.56	1.56	7.33	1.77	3.33
T6 -(Newspapermulch)	4.00	6.00	2.00	9.00	3.00	5.00
T7- (Rice straw mulch)	4.33	6.00	1.67	7.66	1.66	3.33
SEm (±)	0.190	0.118	0.088	0.386	0.098	
CD (P = 0.05)	0.583	0.361	0.27	1.181	0.301	

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RESEARCH ARTICLE

On $(1,2)^*$- RPS - Connected in Bitopological Spaces

A.Arivu Chelvam*

Assistant Professor, PG and Research, Department of Mathematics, Mannar Thirumalai Naicker College, Madurai, Tamil Nadu, India.

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***Address for Correspondence**
A.Arivu Chelvam
Assistant Professor,
PG and Research, Department of Mathematics,
Mannar Thirumalai Naicker College,
Madurai, Tamil Nadu, India.
Email: arivuchelvam2008@gmail.com

ABSTRACT

In this dissertation, we introduce the new type of connected spaces called a (1,2)*-rps-connected spaces.

Keywords: (1,2)*-rps-connected space, (1,2)*-gpr-connected space, (1,2)*-pgpr-connected space, (1,2)*-πgp-connected spaces.

INTRODUCTION

J.C.Kelly [1] was first introduced the concept of a bitopological spaces (X,τ_1,τ_2), where X is a nonempty set and τ_1,τ_2 are topologies on X. Pervin [2] was first to define connectedness and components in bitopological spaces. Connectedness is one of the principal topological properties used to differentiate topological spaces. The concept of rps-closed sets in bitopologicalspace was introduced by K.Indirani and G.Sindhu [10]. In this paper we introduce (1,2)*-rps-connected in bitopological space and investigate the basic properties of (1,2)*-rps-connected space.

Preliminaries

Throughout this paper (X,τ_1,τ_2) represents a bitopological space on which no separation axiom is assumed unless otherwise mentioned. For a subset A of bitopological space X, cl(A) and int(A) denote closure and the interior of A respectively. X \ A denotes the complement of A in X. we recall the following definitions and results.

Definition 2.1:A subset of a space (X,τ_1,τ_2) is called
(i) (1,2)*- regular-open [3] if A= $\tau_{1,2}$-int($\tau_{1,2}$-cl(A)) and (1,2)*-regular closed if A=$\tau_{1,2}$-cl($\tau_{1,2}$-int(A))
(ii) (1,2)*-pre-open [4] if A⊆ $\tau_{1,2}$-int($\tau_{1,2}$-cl(A)) and (1,2)*-pre closed if $\tau_{1,2}$-cl($\tau_{1,2}$-int(A))⊆A

(iii) (1,2)*-semi-pre-open [5] if A⊆ $\tau_{1,2}$-cl($\tau_{1,2}$-int($\tau_{1,2}$-cl(A)) and (1,2)*-semi-pre-closed if $\tau_{1,2}$-int($\tau_{1,2}$-cl($\tau_{1,2}$-int(A))⊆A

(iv) $\tau_{1,2}$-π-open [6] if A is a finite union of (1,2)*- regular open sets. The (1,2)*-semi-pre-closure of a subset A ofX is the intersection of all (1,2)*-semi-pre-closed sets containing A and is denoted by (1,2)*-spcl(A) and the (1,2)*-pre closure of a subset ofX is the intersection of all (1,2)*-pre-closed sets ContainingA and is denoted by (1,2)*-pcl(A).

Lemma 2.2: [8]
For any subset A of a bitopological space X, the following relations hold:
(i) $pcl_{1,2}$A = A ∪ $cl_{1,2}(int_{1,2}$ A)
(ii) $spcl_{1,2}$ A = A ∪ $int_{1,2}cl_{1,2}(int_{1,2}$ A)

Definition 2.3
A subset A of a space X is called
(i) (1,2)*-regular generalized closed or (1,2)*-rg-closed [11] if cl (A) ⊆ U whenever A ⊆ U and U is (1,2)*-regular open.
(ii) (1,2)*-generalized preregular closed or (1,2)*-gpr-closed [9] if (1,2)*-pcl(A) ⊆ U when ever A ⊆ U and U is (1,2)*-regular open. The complement of an (1,2)*-rg-closed set is (1,2)*-rg-open and the complement of a (1,2)*-gpr-closed set is (1,2)*-gpr-open.

Definition 2.4:
A subset A of a space X is called
(i) (1,2)*-regular presemiclosed or (1,2)*-rps-closed [10] if (1,2)*-spcl(A) ⊆ U when ever A ⊆ U and U is (1,2)*-rg-open.
(ii) (1,2)*-π-generalized preclosed or (1,2)*-π gp-closed [7] if (1,2)*-pcl(A)⊆ U when ever A ⊆ Uand U is (1,2)*-π-open.
(iii) (1,2)*-pre-generalized pre-regular closed or (1,2)*-pgpr-closed [12] if (1,2)*-pcl(A)⊆ U whenever A ⊆ U and U is (1,2)*-rg-open. The complement of an (1,2)*-rps-closed set is (1,2)*-rps-open, the complement of a (1,2)*-πgp-closed set is (1,2)*-π gp-open and the complement of a (1,2)*-pgpr-closed set is (1,2)*-pgpr-open.

Definition 2.5: [13]
A function f: (X,τ_1, τ_2)→(Y,σ_1, σ_2) is (1,2)*-rps-continuous if f^{-1}(V) is (1,2)*-rps-closed in (X,τ_1, τ_2) for every closed set V in (Y,σ_1, σ_2).

Definition 2.6: [13]
A function f: (X,τ_1, τ_2)→(Y,σ_1, σ_2) is (1,2)*-rps-irresolute if f^{-1}(V) is (1,2)*-rps-closed in (X,τ_1, τ_2) for every (1,2)*-rps-closed set V in (Y,σ_1, σ_2).

Definition 2.7:
A topological space (X,τ_1, τ_2) is said to be
(i) (1,2)*-pgpr-connected if X cannot be written as the union of two nonempty disjoint (1,2)*-pgpr-open sets in X
(ii) (1,2)*- gpr-connected if X cannot be written as the union of two nonempty disjoint (1,2)*-gpr-open set in X.
(iii) (1,2)*-πgp-connected if X cannot be written as the union of two nonempty disjoint (1,2)*-πgp-open sets in X.

Definition 2.8:
A function f: (X,τ_1, τ_2)→(Y,σ_1, σ_2) is (1,2)*- contra rps-continuous if f^{-1}(V) is (1,2)*-rps-closed in (X,τ_1, τ_2) for each $\tau_{1,2}$-open set V in (Y,σ_1, σ_2).

Lemma 2.9: [13]
Let f:(X,τ_1, τ_2)→(Y,σ_1, σ_2) be a function. Then the following are equivalent.
(i) f is (1,2)*-rps-continuous.
(ii) The inverse image of each $\tau_{1,2}$-closed set in Y is (1,2)*-rps-closed in X.
(iii) The inverse image of each $\tau_{1,2}$-open set in Y is (1,2)*-rps-open in X.

Theorem 2.10: [13]
A function f: $(X,\tau_1,\tau_2)\to(Y,\sigma_1,\sigma_2)$ is (1,2)*-rps-irresolute if and only if the inverse image of every (1,2)*-rps-open set in Y is (1,2)*-rps-open in X.

Lemma 2.11:
For a bitopological space X, the following are equivalent.
(i) X is (1,2)*-pgpr-connected
(ii) The only subset of X which are both (1,2)*-pgpr-open and(1,2)*-pgpr-closed are the empty set and X.

Lemma 2.12:
(i) Every (1,2)*-pgpr-closed set is (1,2)*-rps-closed.
(ii) Every (1,2)*-pgpr-open set is (1,2)*-rps-open.

Remark 2.13:
If A is (1,2)*-rps-closed in (X,τ_1,τ_2), then A is $\tau_{1,2}$-closed in $(X,\tau_{1,2_{rps}})$ provided $\tau_{1,2_{rps}}$ isbitopology.

Definition 2.14
A space (X,τ_1,τ_2) is called (1,2)*regular pre-semi-$T_{3/4}$(briefly (1,2)*-rps-$T_{3/4}$) if every (1,2)*-rps-closed set is (1,2)*-pre closed.

Diagram 2.15
(1,2)*-preclosed ⟶ (1,2)*-pgpr-closed ⟶ (1,2)*-rps-closed.

III $(1,2)^*$-RPS-CONNECTED SPACES

Definition 3.1
A bitopologicalspace X is said to be (1,2)*-rps-connected if (X,τ_1,τ_2) cannot be written As the disjoint union of two nonempty (1,2)*-rps-open sets in (X,τ_1,τ_2).

Definition 3.2:
A subset S of a bitopological space X is said to be (1,2)*-rps-connected relative to X if S cannot be written as the disjoint union of two nonempty (1,2)*-rps-open sets in X.

Theorem 3.3:
For a bitopological space X, the following are equivalent.
(i) X is (1,2)*-rps-connected
(ii) The only subsets of X which are both (1,2)*-rps-open and (1,2)*-rps-closed are the empty set and X.
(iii) Each (1,2)*-rps-continuous function of X into a discrete space Y with atleast two points is a constant map.

Proof
Suppose X is (1,2)*-rps-connected. Let S be a proper subset which is both(1,2)*-rps-open and (1,2)*-rps-closed in X. Then its complement X/S is also (1,2)*-rps-open and (1,2)*-rps-closed. Then X= S∪(X/S). a disjoint union of two nonempty (1,2)*-rps-open sets which contradicts (i). Therefore S = ∅ or X. This proves (i) ⇒ (ii) Suppose (ii) holds. Let X = A ∪ B where A and B are disjoint nonempty (1,2)*-rps-open subsets of X. Since A = X/ B and B = X/ A, A and B are both (1,2)*-rps-open and (1,2)*-rps-closed. By assumption, A = ∅ or X which is a contradiction. Therefore X is (1,2)*-rps-connected. This proves (ii) ⇒ (i). Now to prove (ii) ⇒ (iii). Suppose (ii) holds. Let f:$(X,\tau_1,\tau_2)\to(Y,\sigma_1,\sigma_2)$ be an (1,2)*-rps-continuous function where Y is discrete space with atleast two points. Then $f^{-1}(\{y\})$ is (1,2)*-rps-closed and (1,2)*-rps-open for each y∈ Y. since (ii) holds, $f^{-1}(\{y\})$ = ∅ or X. if $f^{-1}(\{y\})$ = ∅ for all y∈ Y, f will not be a function. That implies $f^{-1}(\{y\})$ = X for some y∈ Y. Therefore for fixed y, f(x) = Y for all x∈X.This proves that f is a constant map. This proves (ii) ⇒ (iii). Now suppose (iii) holds. Let S be both (1,2)*-rps-open and (1,2)*-rps-closed in

X. Suppose S ≠ ∅. Let f: $(X,\tau_1,\tau_2)\to(Y,\sigma_1,\sigma_2)$ be an (1,2)*-rps-continuous function defined by f(s) = {y} and f (X/ S) = {w} for some distinct points y and w in Y. By (iii) f is a constant function. Therefore S = X. Hence (ii) holds. This proves (iii) ⇒ (ii).

Theorem 3.4

Let f: $(X,\tau_1,\tau_2)\to(Y,\sigma_1,\sigma_2)$ be a function.

(i) If X is (1,2)*-rps-connected and if f is (1,2)*-rps-continuous, surjective, then Y is (1,2)*-connected.

(ii) If X is (1,2)*-rps-connected and if f is (1,2)*-rps-irresolute, surjective, then Y is (1,2)*-rps-connected.

Proof

Let X be (1,2)*-rps-connected and f be (1,2)*-rps-continuous surjective. Suppose Y is disconnected. Then Y = A ∪ B, where A and B are disjoint non empty open subset of Y. Since f is (1,2)*-rps-continuous surjective, by using Theorem 2.9, X = $f^{-1}(A) \cup f^{-1}(B)$. where $f^{-1}(A)$, $f^{-1}(B)$ are disjoint nonempty (1,2)*-rps-open subsets of X. This contradicts the fact that X is (1,2)*-rps-connected. Therefore Y is (1,2)*-connected. This proves (i). Let X be (1,2)*-rps-connected and f be (1,2)*-rps-irresolute surjective. Suppose Y is not (1,2)*-rps-connected. Then Y = A ∪ B where A and B are disjoint nonempty (1,2)*-rps-open subsets of Y. Since f is (1,2)*-rps-irresolute surjective, by using Theorem 2.10, X = $f^{-1}(A) \cup f^{-1}(B)$, where $f^{-1}(A)$ and $f^{-1}(B)$ are disjoint nonempty (1,2)*-rps-open subsets of X. This implies X is not (1,2)*-rps-connected, a contradiction. Therefore Y is (1,2)*-rps-connected. This proves (ii).

Theorem 3.5

Every (1,2)*-rps-connected space is (1,2)*-connected.

Proof

Let X be an (1,2)*-rps-connected space. Suppose X is not (1,2)*-connected. Then there exists a proper nonempty subset B of X. which is both $\tau_{1,2}$-open and $\tau_{1,2}$-closed in X. Since every $\tau_{1,2}$-closed is (1,2)*-rps-closed, B is a proper nonempty subset of X which is both (1,2)*-rps-open and (1,2)*-rps-closed in X. Then by using Theorem 3.3, X is not (1,2)*-rps-connected. This proves the theorem. The converse of Theorem 3.5 is true as shown in the following example.

Example 3.6

Let X={a,b,c} with τ_1={X,∅,{a}} and τ_2={X,∅,{a,b}}. Then we see that the bitopological space (X,τ_1,τ_2) is connected. However, since {b},{a,c} are both (1,2)*-rps-open and (1,2)*-rps-closed, X is not (1,2)*-rps-connected.

Theorem 3.7

Every (1,2)*-rps-connected space is (1,2)*-pgpr-connected.

Proof:

Let X be an (1,2)*-rps-connected space. Suppose X is not (1,2)*-pgpr-connected. Then by usingLemma 2.11, there exists a proper nonempty subset B of X. which is both (1,2)*-pgpr-open and (1,2)*-pgpr-closed in X. Using Lemma 2.12, B is a proper nonempty subset of X which is both (1,2)*-rps-open and (1,2)*-rps-closed in X. Then by using Theorem 3.3, X is not(1,2)*-rps-connected. This proves the theorem. The converse of Theorem 3.7 is not true as shown in the following example.

Example 3.8

Let X={a,b,c} with τ_1={X,∅,{a}} and τ_2={X,∅,{c},{a,c}}. Then the bitopological space (X,τ_1,τ_2) is not (1,2)*-rps-connected. The only subsets of X which are both (1,2)*-pgpr-open and (1,2)*-pgpr-closed are the empty set and X. Therefore X is (1,2)*-pgpr-connected. The concepts of (1,2)*-rps-connectedness and π gp-connectedness are independent of each other as shown in the following examples.

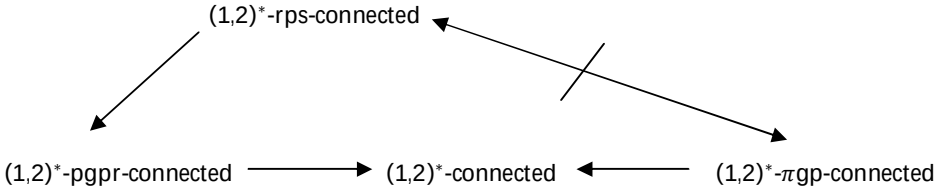
Example 3.9

Let X={a,b,c} with topologies τ_1={X,∅,{c}} and τ_2={X,∅,{b},{b,c}}. Then we see that (X,τ_1,τ_2) is (1,2)*-πgp-connected but not (1,2)*-rps-connected, because {b}, {c}, {a,b} and {a,c} is both (1,2)*-rps-open and (1,2)*-rps-closed and (X,τ_1,τ_2) is (1,2)*-πgp-connected.

Example 3.10

Let X = {a,b,c}τ_1 = {X,∅,{a}} and τ_2 = {X,∅,{a,c}}. Then (X,τ_1,τ_2) is (1,2)*-rps-connected but not (1,2)*-πgp-connected, because {a},{b},{a,b} is both (1,2)*-πgp-closed and (1,2)*-πgp-open in (X,τ_1,τ_2).

Diagram 3.11:



Theorem 3.12:

Suppose X is a bitopological space with $\tau_{1,2_{rps}}$=$\tau_{1,2}$. Then X is connected if and only if X is (1,2)*-rps-connecteed.

Proof:

Suppose X is not (1,2)*-rps-connected. Then there exists a proper non empty subset of B of X which is both (1,2)*-rps-open and (1,2)*-rps-closed in X. Since $\tau_{1,2_{rps}}$= $\tau_{1,2}$, using remark 2.13, every (1,2)*-rps-closed set is $\tau_{1,2}$-closed. Therefore B is both $\tau_{1,2}$-open and $\tau_{1,2}$-closed in X that implies X is not $\tau_{1,2}$-connected. This proves that $\tau_{1,2}$-connectedness implies (1,2)*-rps-connectedness. The converse follows from Theorem 3.5.

Theorem 3.13:

Suppose X is an (1,2)*-rps-$T_{3/4}$ space. Then X is (1,2)*-rps-connected if and only if X is (1,2)*-pgpr-connected.

Proof:

Suppose X is (1,2)*-rps-connected. Then by using Theorem 3.7, X is (1,2)*-pgpr-connected. Conversely, we assume that X is (1,2)*-pgpr-connected. Suppose X is not (1,2)*-rps-connected. Then there exists a proper non empty subset of B of X which is both (1,2)*-rps-open and(1,2)*-rps-closed in X. Since X is (1,2)*-rps-$T_{3/4}$by using Definition 2.14, B is both (1,2)*-pre open and (1,2)*-pre closed in X. Again using Diagram 2.15, B is both (1,2)*-pgpr-open and (1,2)*-pgpr-closed in X which shows that X is not (1,2)*-pgpr-connected, a contradiction. Therefore X is (1,2)*-rps-connected.

Theorem 3.14:

A contra (1,2)*-rps-continuous image of an (1,2)*-rps-connected space is (1,2)*-connected.

Proof:

Let f: $(X,\tau_1,\tau_2)\to(Y,\sigma_1,\sigma_2)$ be a (1,2)*-contra rps-continuous function from an (1,2)*-rps-connected space Xon to a space Y. Assume that Y is disconnected. Then Y= A∪B where A and B are non empty $\tau_{1,2}$-clopen sets in Y with A ∩ B = ∅. Since f is contra (1,2)*-rps-continuous, we have that f^{-1}(A) and f^{-1}(B) are nonempty (1,2)*-rps-open sets in X with f^{-1}(A) ∪ f^{-1}(B) = f^{-1}(A ∪ B) = f^{-1}(Y) = X and f^{-1}(A) ∩ f^{-1}(B) = f^{-1}(A ∩ B) = f^{-1}(∅) = ∅. This means that X is not (1,2)*-rps-connected, which is a contradiction. This proves the theorem.

Theorem 3.15:
Let $\{A_\alpha : \alpha \in \Delta\}$ be a locally finite family of $\tau_{1,2}$-clopen sets in X such that they have a common point. If each A_α is an $(1,2)^*$-rps-connected subspace of X then their union is an $(1,2)^*$-rps-connected subspace of X.

Proof:
Let p be a point A_α for every $\alpha \in \Delta$. Let Y = $\bigcup_{\alpha \in \Delta} A_\alpha$. Then Y is $\tau_{1,2}$-clopen. Suppose Y = C ∪ D where C and D are two disjoint nonempty $(1,2)^*$-rps-open subsets of Y. The point p is in of the sets C or D. If p ∈C then $A_\alpha \subseteq$ C for every α, so that $\bigcup_{\alpha \in \Delta} A_\alpha \subseteq$ C. This shows that D = ∅. Therefore Y = C is $(1,2)^*$-rps-connected.

REFERENCES

1. J.C.Kelly, Bitopological Spaces, Proc.LndonMath.Soc.(3) 13 (1963), pp.71 – 89.
2. W.J.Pervin, Connectedness in bitopological spaces, Indag.Math.,29 (1967), 369-372.
3. M.LellisThivagar, O.Ravi, On Stronger forms of (1,2)*-quotient mapping in Bitopologicalspace, Internet.J.Math.Game theory and algebra, Vol.4, No.6, (2004), pp.481-492.
4. D.Sreeja, Juane P.Sinthya, On $(1,2)^*$-rgα-closed sets in Bitopological Spaces, Malaya J Mat S. 2015; (1): pp.27-41.
5. O.Ravi and M.LellisThivagar, Remarks on λ-irresolute functions via $(1,2)^*$-sets, Advanced in App.Math.Analysis, 5(1) (2010), pp.1-15.
6. P.Bhattacharya and B.K.Lahiri: Semi-generalized closed sets in topology, Indian J.Math.29 (1987), 375-382.
7. J.H.Park, On πgp-closed sets in topological spaces, Indian J.Pure.Appl.Math.
8. R.Chithralekha, M.Anitha and N.Meena, Near and Closer relations in Bitopology, IJRAT,Vol.7, No.1, Jan-2019.
9. S.Leemamary, V.Mahadevan, $(1,2)^*$-gpr-closed set in Bitopological spaces, IJREAM, Vol.4, (2018), pp.501-506.
10. K.Indirani, G.Sindhu, On regular pre-semi closed in Bitopological space, Karpagam IJAM, Vol.3, (2013), pp.574-590.
11. K.Chandrasekhara Rao and N.Palaniappan, Regular generalized closed sets, Kyungpook Mathematical Journal, 33(2) (1993), pp.211-219.
12. M.Anitha and P.Thangavelu, On pre-generalized pre-regular-closed sets, Acta Ciencia Indica, 31M(4) (2005), 1035-1040.
13. T.Shylalsac Mary and P.Thangavelu, On rps-continuous and rps-irresolute functions, International Journal of Mathematical Archive, 2(1) (2011), 159-162.
14. T.Shylalsac Mary and P.Thangavelu, On rps-connected spaces, IJERA, Vol.2, Jan-Feb 2012, pp.768-772.

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RESEARCH ARTICLE

Diversity of Macrophytes and Their Ethno-Beneficial uses in the Deepor Beel, Assam, India

Trishna Kalita[1]*, Sweta Das[1], Richa Sarma[2] and Parag Deka[3]

[1]Research Scholar, Department of Zoology, Cotton University, Guwahati, Assam, India
[2]Post-Graduate Student, Department of Zoology, Pandu College, Guwahati, Assam, India
[3]Assistant Professor, Department of Zoology, Pandu College, Guwahati, Assam, India

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***Address for Correspondence**
Trishna Kalita
Research Scholar,
Department of Zoology, Cotton University,
Guwahati, Assam, India
Email: trishnakalita91@gmail.com

ABSTRACT

The present study highlights the findings of aquatic macrophytes from the famous Ramsar site, DeeporBeel of Assam, India. Deeporbeel, being known to have both biological and environmental importance is taken under consideration for a proper investigation of aquatic macrophytes flourishing here. A total of 20 aquatic macrophytes have been recorded under 15 families and it is found that *Eichhornia crassipes*is most dominant followed by *Pistia stratoites.* Also, of the total Macrophytes found in this area, were mainly classified into terrestrial (7), floating (7), marginal (5) and submerged (1). During the course of this particular study, it was observed that the majority of the macrophytes also have medicinal properties, which not only provides a sense of health security to the locals but also allows for the acquisition of some traditional knowledge regarding the aquatic macrophytes that can be found here.

Keywords: Macrophytes, diversity, ethno-beneficial, deeporbeel, assam

INTRODUCTION

Aquatic environment constitutes a wide variety of parameters, influencing all the organisms thriving in that environment. The composition of the water, which is the primary component of the aquatic environment, plays a vital effect in the growth and development of the organisms that are able to thrive in that particular ecosystem. One such important component present in water bodies and closely related to water is aquatic plants, macrophytes whose proper growth indicates the actual quality of water. Submerged aquatic macrophytes can have significant effects on habitat composition, fish performance, recreational value, and nutrient dynamics. However, problems with water quality, such as high turbidity, herbicides, or salinization, can stunt plant growth and development, so an absence of macrophytes may be cause for concern. Furthermore, an excessive amount of macrophytes could be an indication of

high nutrient levels, which is detrimental to the ecology. Even though India has a diverse array of plant and animal species, there are still parts of the country that have not been explored or studied by scientists. One such place is the north eastern part of the country, where documentation is still absent even to this day. Wetlands once known as wastelands. Before, wetlands were thought of as waste products that should be dumped into drains, without anybody realizing the benefits that come with them in terms of the environment, the economy, education, society, recreation, or aesthetics. The largest majority of floral and animal taxonomic units may be found in wetlands, which are one of the most productive ecosystems and are considered as the home of biodiversity. Because of the regional and local variations in soils, terrain, climate, hydrology, water chemistry, vegetation, and other factors, including human variation, wetland ecosystems are the subject of a significant amount of research. Out of the many benefits, Wetlands also help in controlling the erosion and the roots of wetland plants hold soil in place and help in holding the soil and reduce velocity of stream or river currents. In furthermore, DeeporBeel, which is in Northeast India (Assam) and is located about 10 km southwest of Guwahati, has recently been designated as a globally important wetland. Moreover, DeeporBeel, which has been known as the Ramsar site since 2002, serves as a haven for migratory birds each year, for which it has also been chosen as one of the significant bird area sites by Birdlife International. In spite of being a comfort to rich fauna, it has also been a place for many aquatic macrophytes and many of such being looked over. So, the present study deals with investigation of as many aquatic macrophytes found in this area along with their benefits to the local inhabitants other than them being just indicators of health of water bodies.

MATERIALS AND METHODS

STUDY AREA

The study is carried out in this floodplain wetland which is also regarded as the Ramsar site. DeeporBeel is a known biologically and environmentally significant natural wetland [1]. DeeporBeel is known to be linked with major river systems connected with Brahmaputra River. It is located to the southwest region of Kamrup district of Assam. At maximum floodlight, it is about 4 meter deep; which drops to a depth of about 1 metre during the dry season. The Basistha and Kalmini rivers are the primary water sources. Through the Khonajan canal, the beel empties into the Brahmaputra River, 5 kilometres to the north. It is a prominent wetland type from the province of Burma monsoon forest and one of the biggest and most significant beels in the Brahmaputra valley of Assam. A perennial freshwater lake of riverine origin, DeeporBeel serves as Guwahati City's storm water storage basin and is located 10 kilometres to the southwest of the city.It extends from 26°03’26”-26°09’26”N and90°36’39”-90°41’25”E in the south of mighty river Brahmaputra. DeeporBeel is one of the largest of the many such beels in lower Assam. The northern fringe of the beel is encompassed by Jalukbari Hill and N.H, 37.The Hills, Rani and Garbhanga Reserve Forests from the southern limit.The N.H. 37, Garbhanga, and Moinakhorang villages are the eastern margins of the beel.The B.G. Railway line ,Guwahati to Jogighopa, is stretching along the eastern and western margin of the beel (Figure 1). The study is carried out in the month of March – June, 2022 at DeeporBeel located in the southwest region of the kamrup District of Assam. Four sites of the wetland were selected foe the study (Table 1). The sites are:

The certain pictures of macrophytes were taken up from the above mentioned zones (Figure 2). A survey has been conducted on the collected species. As per sources from the local villagers, macrophytes surprisingly also possess a great medicinal value in addition to its known association to fishes. During the interactive session, data has been collected from the members of the family regardless of the gender, which also led us to another fact about macrophytes having great medicinal value too. In addition to having medicinal benefits, macrophytes offer fish cover and a substrate for aquatic invertebrates. They also generate oxygen and serve as a source of food for some fish and other animals. Macrophytes are employed for generating the simple abundance matrix because they are easily sampled, do not require lab analysis, and respond to a wide range of environmental circumstances.

RESULTS

The present study revealed 20 species of macrophytes including submerged, free floating; emergent alongside and marginal (Table 2). As growth of some macrophyes is higher after the monsoon period and hence more species are not be able to record. Table 2: Showing the information about recorded species of macrophytes. From the above result it was observed that the most abundant family is the Araceae family (Figure 3) and the maximum diversity of macrophytes are of floating type (Figure 4). The dominant species is seen to be *Eichhornia crassipes* followed by *Pistia stratoites* on the basis of survey.

DISCUSSION

In different regions of Assam the diversity of macrophytes has been already studied. The study is carried out in both the Upper Assam and Lower Assam. Diversity of macrophytes studied in Maijan wetland recorded 42 macrophytes included 34 genera belonging to 28 families [2]. Another study carried out in Hojai district of Assam which recorded 62 aquatic macrophytes species [3]. Furthermore, the study carried out in the wetlands of Nalbari district of Assam and the study recorded 137 species of macrophytes [4]. The present investigation on Deeporbeel recorded 20 species of macrophytes . The macrophytes recorded are free floating; some are terrestrial and some are submerged and marginal macrophytes are included as well .Based on investigation it is recorded that the wetland is dominant by *Eichhornia crassipes* followed by *Pistia stratoites*. In aquatic environments, macrophytes are responsible for a wide variety of ecosystem activities and also bring benefits to human society. The intake of dissolved nutrients from water, such as nitrogen (N) and phosphorus (P), is one of the major roles that macrophytes are responsible for doing. In order to rid polluted water of excess nitrogen and phosphorus, macrophytes are frequently utilised in created wetlands across the globe. In addition to the direct intake of nutrients, it is well established that macrophytes also exert an indirect influence on the cycling of nutrients, particularly nitrogen, by exerting an influence on the denitrifying bacterial functional groups that occupy the roots and shoots of macrophytes. By lowering current velocities and preventing erosion, macrophytes encourage the sedimentation of suspended particles. Additionally, macrophytes add spatial variability to an unstructured water column. The diversity of the habitat that macrophytes provide increases the taxonomic richness and density of both fish and invertebrates.

CONCLUSION

The accumulation of a variety of nutrients in an aquatic body, such as Deeporbeel, can lead to eutrophication, which in turn can result in the rapid growth of macrophytes and other types of weeds. It is believed that increased urbanisation and human pressures are the primary factors contributing to the accumulation of nutrients. Various types of macrophytes; emergent, free floating, submerged as well as marginal have been found in Deeporbeel. Free floating macrophytes as *Eichhornia crassipes*, seemed to be dominant in the area which through their roots plays important role in removing the accumulated nutrients. On the other hand, its excessive growth might also lead to certain negatives impacts on the overall status of the wetland. Livelihood around the wetland is dependent on usage of various aquatic resources of the wetland including fishes and others. Most of this vegetation including some macrophytes consumed by the people of surrounding villages directly or indirectly. The high dependence of people on this wetland resources offers for development of rural economy and for this, conservation of biodiversity also be addressed for sustainable development. Seeing the dependence of people on the wetland it is important that knowledge of the utility of macrophytes in optimum level should be promoted among the people.

ACKNOWLEDGEMENT

The authors wish to thank the local people inhabiting nearby areas of Deeporbeel, Assam, India.

REFERENCES

1. Deka SK & Goswami DC. Hydrology, sediment characteristics and depositional environment of wetlands: A case study of DeeporBeel, Assam. Journal of Assam Science Society.1992; 34(2): 62-84.
2. Abujam SS, Shah RK, Deori DJ, & Biswas SP. Diversity of Macrophytes in Maijan Wetland, Upper Assam, India. South Asian Journal of Experimental Biology.2014; 4(4): 164-171.
3. Saikia M. Aquatic macrophytes of the wetlands of Hojai sub division, Nagaon district of Assam, India. Global Journal of Biology, Agriculture and Health Science.2013; 2(3); 141-145.
4. Deka U &Sarma, SK. Present status of aquatic macrophytes of the wetlands of Nalbari district of Assam, India. Asian journal of plant science and research.2014; 4(3): 67-75.

Table 1: Information about the study area divided in 4 zones

Zone	Latitude	Longitude
1	26.1130°N	91.6545°E
2	26.1177°N	91.6494°E
3	26.1129°N	91.6567°E
4	26.1133°N	91.6566°E

Table 2: Showing the information about recorded species of macrophytes

S NO	SCIENTIFIC NAME	FAMILY	HABITAT	TRADITIONAL USE
1	*Centella asiatica*	Apiaceae	Marginal	Effective in diarrhea, weakness and also applied as antiseptic on cuts and wounds
2	*Colocasia esculenta*	Araceae	Marginal	Consumed as food by cooking with lentils or fish
3	*Eichhornia crassipes*	Pontideriaceae	floating	It is used as fertilizer, fodder for cattle, with dried parts being rurally used as fuel, used for controlling water pollution and during winter fishes generally takes shelter under it to escape from cold.
4	*Hydrilla verticillata*	Hydrocharitaceae	submerged	It is used in agriculture as bio-fertilizer
5	*Pteridium aquilinum*	Dennstaedtiaceae	terrestrial	It is used as insecticide , root extract is used as antiseptic
6	*Solanum xanthocarpum*	Solanaceae	terrestrial	It is consumed by tribal communities for treating asthma
7	*Pistia stratoites*	Araceae	Floating	Used as aquarium plant for decoration
8	*Leucas aspera*	Lamiaceae	terrestrial	The flower extract is traditionally used for treating headache
9	*Salvinia cucullata*	Salviniaceae	Floating	Invasive aquatic fern used in aquarium trade
10	*Cyperu spilosus*	Cyperaceae	Terrestrial	It is used to treat itches.

11	*Ipomea carnea*	Convolvulaceae	Terrestrial	The milky juice is externally applied to treat skin infection
12	*Cynodon dactylon*	Poaceae	Marginal	Extraction is used in the treatment of piles, blood dysentery
13	*Amaranthus viridis*	Amaranthaceae	Terrestrial	It is used in the treatment of fever , pain, asthma, diabetes liver disorders etc.
14	*Euphorbia hirta*	Euphorbiaceae	Marginal	Used for treating asthma, bronchitis, and skin infection
15	*Nymphea rubra*	Nymphaeaceae	Floating	It is used as anti-depressants
16	*Mikania micrantha*	Asteraceae	Marginal	It is used as an antiseptic.
17	*Nymphea alba*	Nymphaeaceae	Floating	Stalks are consumed raw as vegetable
18	*Spirodela polyrhiza*	Araceae	Floating	Used for bio remediation.
19	*Ipomoea aquatica*	Convolvulaceae	Floating	Consumed to enhance lactation
20	*Amaranthus dubius*	Amaranthaceae	terrestrial	Consumed as food.

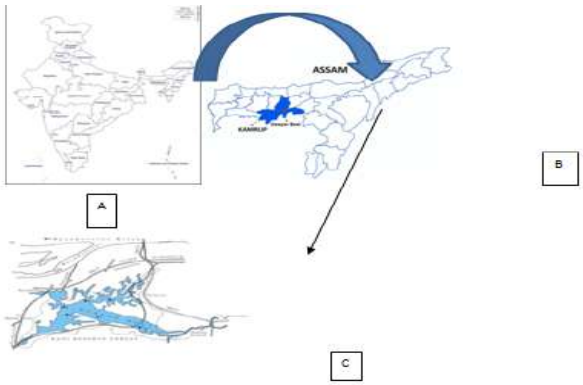

Figure 1: Showing the study area [A] India, [B] Assam, [C] Deeporbeel

A) *Centella asiatica*,

B) *Colocasia esculenta*,

C) *Pteridiuma quilinum*

 **D) *Pistia stratoites***	 **E) *Leucas aspera***	 **F) *Salvinia cucullata***
 **G) *Ipomoea carnea***	 **H) *Cynodon dactylon***	 **I) *Amaranthus virdis***
 **J) *Euphorbia hirta***	 **K) *Nymphea rubra***	 **L) *Amaranthus dubius***
 **M) *Spirodela polyrhiza***	 **N) *Nymphea alba***	 **O) *Ipomoea aquatica***

P) *Mikania micrantha*,

Q) *Cyperus pilosus*

R) *Eichhornia crassipes*

S) *Solanum xanthocarpum*

Figure 2: Some of the pictures of macrophytes located in Deeporbeel *(A) Centella asiatica*, *(B) Colocasia esculenta*, *(C) Pteridiuma quilinum*, *(D) Pistia stratoites*, *(E) Leucas aspera*, *(F) Salvinia cucullata*, *(G) Ipomoea carnea*, *(H) Cynodon dactylon*, *(I) Amaranthus virdis*, *(J) Euphorbia hirta*, *(K) Nymphea rubra*, *(L) Amaranthus dubius*, *(M) Spirodela polyrhiza*, *(N) Nymphea alba*, *(O) Ipomoea aquatica*, *(P) Mikania micrantha*, *(Q) Cyperus pilosus*, (R) *Eichhornia crassipes*, S) *Solanum xanthocarpum*.

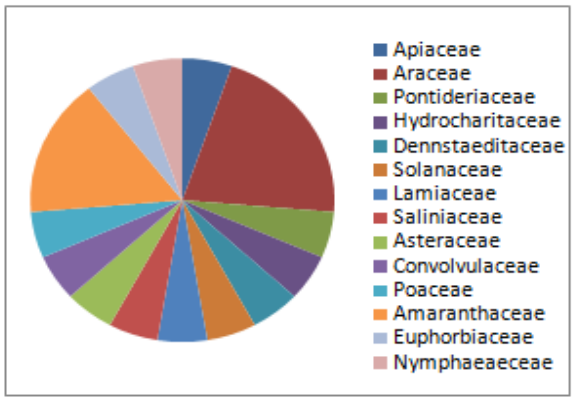

Figure 3: Distribution of macrophyte family.

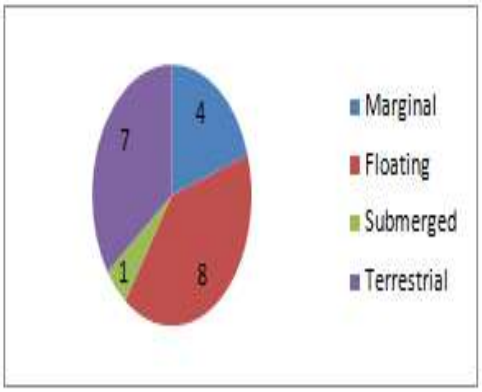

Figure 4: Percentage of habitat distribution

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RESEARCH ARTICLE

Inhibition of Na^+- K^+, Ca^{2+} and Mg^{2+} -ATPase in Different Tissues of Freshwater Fish *Cirrhinus mrigala* (Hamilton)

Sapna S Anigol[1], Srinivas B Neglur[1] and M. Dvaid[2]*

[1]Research Scholar, Department of Zoology, Karnatak University, Dharwad, Karnataka, India.
[2]Professor, Department of Zoology, Karnatak University, Dharwad, Karnataka, India.

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***Address for Correspondence**
M. Dvaid,
Professor,
Department of Zoology,
Karnatak University,
Dharwad, Karnataka, India.
Email: davidkcd@gmail.com

ABSTRACT

Pyrethroids square measure normally used round the home and in agricultural production to control insects. Human contact to one or a lot of pyrethroid pesticides is probably going. various medical specialty studies have evaluated the association between health outcomes in humans and pyrethroid exposure. In the present investigation, an effort has been created to quantify the cyphenothrin accumulated in different tissues (gill, kidney and liver) and observe changes concerned within the levels of Na, K and Ca ions and Na^+–K^+, Mg^{2+} and Ca^{2+} nucleoside triphosphatase (ATPase) activities within the fresh water fish, *Cirrhinus mrigala* on long term exposure to the sub lethal concentration of cyphenothrin. In sublethal concentration (6 mg/l), excretory organ kidney accumulated highest amount followed by gill and liver, which could flow from to the very fact that Cyphenothrin is very lyphophilic. The ion concentration and ATPase activity were found effected in fish exposed to sublethal concentrations of cyphenothrin concentration of Na^+, K^+ and Ca^{2+} ions minimized in gill, muscle and liver on being exposed to sub lethal concentration to a significant level. Whereas the changes weren't extremely seen at sub lethal level indicating low concentration of cyphenothrin and its non-toxic result at chronic exposure. Na^+–K^+, Mg^{2+} and Ca^{2+} ATPases activity were additionally found minimized in correspondence to the ionic change under sub lethal concentrations in target tissues. This might need behavioral activity changes and build wide-spread disturbance within the physiology, ultimately inflicting the death of the fish. The results suggest that in biomonitoring programmes, ions and associated ATPases is a decent diagnostic tool for cyphenothrin toxicity.

Keywords: Cyphenothrin accumulation, ions and associated ATPases, *Cirrhinus mrigala*

INTRODUCTION

A tormenter refers to a placental, insect, nematode, weed, fungus, or the other kind of terrestrial or tracheophyte or virus, bacteria, or alternative microorganisms that damage the foodstuffs, garden plants, home articles, or trees, as a vector of diseases [39,40]. For farmers, pests embrace mites and insects that kill crops and aquatic plants, and cause animal and plant diseases, like fungi, viruses, bacteria, snails, nematodes, and rodents [31]. On the opposite hand, pesticides are mentioned as several chemical compounds that possess numerous biological activities and chemical natures, that are clustered along to extend their capability to eradicate pests [1,41,26,52,12]. Thus, as a broad definition, pesticides are all those substances or their mixture used for bar, destruction, repelling, deterring, resisting, or dominant pests [9]. Water pollution with pesticides could also be due to direct application of those chemicals for dominant aquatic flora and seepage from agricultural lands through agricultural runoffs [13]. These measures wide unfold in each urban and agricultural landscapes [43]; this merely regards the chemical residues or pesticides as major contributors to pollution [20, 25]. Across the world, differing kinds of pesticides measure being employed in numerous ratios, like pesticides, that compose some eighty percent of all pesticides, herbicides (15%), and fungicides (1.46%). Pesticide residues are often sustained for long periods within the fields once application due to their weakened biodegradation properties [33], that may well be absorbed by aquatic organisms, like fish, resulting in negative influences on their health and meat quality, which is able to negatively have an effect on human health. moreover, they need a fast biodegradation rate within the aquatic setting wherever alga and *macrophytes* exist [7].

These pesticides area unit over a hundred times additional toxic for fish to the enhanced sensitivity of fish to harmful agents, to their direct contract to water via gills and absence or the inadequate hydrolytic enzymes for *pyrethroids* [4]. These chemicals area unit reworked within the *hepatocytes*, bile, and blood cells to sulfates and *glucuronides*, inflicting undesirable effects on meat quality and therefore the survival rate of fish [23,58]. The inorganic ions play a vital role in diffusion phenomena and within the regulation of cellular metabolism. These are needed by all animals to supply appropriate medium for protoplasmic activity. Any imbalance within the levels of those ions in animals can cause impairment in varied physiological activities [5]. Freshwater fishes are hyperosmotic to their medium. They gain water osmotically and have a tendency to lose solutes by diffusion. Within the regulation of osmolarity of a system, sodium, potassium and calcium ions play a major role to stay the hyperosmotic properties of those animals [34]. Adinosine triphosphatase (ATPase) enzymes are very important for control biological process, ionic transport, muscle perform alternative and several other membrane transport dependent phenomena. Na^+–K^+ ATPase includes a central role in respiratory organ transepithelial ions transportation in fishes [42]. Mg^{2+}ATPase could be a mitochondrial accelerator concerned not solely within the lysis of ATP however even have a major role within the initiation of ATP synthesis [30]. The flow of ions from exterior to interior or the other way around appearance terribly straightforward however it's a complex electrochemical gradient method.

The freshwater organisms tend to take care of their metabolic stability particularly through in and out flow of ions. There are many reports accessible on the attainable effects of pesticides on the amount of Na, K and Ca ions and therefore the individual ATPase in fresh water species. Pyrethroids are a unit from unremarkably used pesticides worldwide [47,29,44]. Artificial pyrethroid pesticides (such as permethrin, deltamethrin, resmethrin, tetramethrin, -cyhalothrin, and cypermethrin) will cause serious toxicologic impacts on the exposed aquatic organisms [32]. Pyrethroid pesticides belong to a category of chemistry that was derived from pyrethrins, that are found in chrysanthemum flowers as natural pesticides. Pyrethrins are esters of cyclopropane carboxylic acid and a cyclopentenolone alcohol that were synthetically changed to extend insecticidal efficiency and extend longevity within the presence of water, moisture, and daylight (17). The chemical structures of pyrethroids area unit similar across the category and retain the essenstial acid/alcohol composition of pyrethrins. As a result, thought of health effects will be created on the total category of pyrethroids (55). The use of pyrethroids has enlarged over the past twenty years and correspondingly the chance for human exposure within the setting, home and diet. Absorbed pyrethroids area unit quickly metabolized and eliminated from the body.

Though every pyrethroid incorporates a distinctive kinetic profile, the plasma half-life of pyrethroids normally is a smaller amount than eighth (28). Urinary metabolites of pyrethroid pesticides are rumored in population sampling programs (24,14). whereas detection alone doesn't indicate that associate adverse health outcome can occur, there's associate in progress interest within the potential associations of pyrethroid exposure and health effects, significantly at environmentally relevant levels. Cyphenothrin is a racemic mixture of four pairs of diastereoisomers, as (±)-α-cyano-3- phenoxybenzyl (±)-cis-trans- chrysanthemate. The name d,d,trans-cyphenothrin refers to associate enantio-enriched mixture, comprised chiefly of the one stereoisomer(S)-α-cyano-3-phenoxybenzyl(1R,3R)-2,2-dimethyl-3-(2-methylprop-1-enyl) cyclopropanecarboxylate, with alone little proportions of the other stereoisomers. Cyphenothrin is also a form II pyrethroid. It is used as chemical, pesticide, and drugs. reckoning on the type of pyrethroid, repetitive discharges and physical phenomenon block square measure determined in varied regions of the system.

Type II pyrethroids like cyphenothrin contain a cyano group at the alpha-carbon cause nerve membrane modification and block leading to dysfunction. The action is ascribed to modification of nerve membrane sodium channels that finish in really slow gating mechanics. Freshwater fish, *Cirrhinus mrigala* is subjected to investigation as a result of its cosmopolitan Indian major carp that represents majority of the entire Ichthyomass of Indian landmass and its piscary is powerfully captivated with the industrial and recreational demand. India is associate degree agricultural country and also the farmers for dominant agricultural pests and disease-causing vectors use array of pesticides. Cyphenothrin is one in every of the many pesticides employed in great quantity for plant protection purpose. This chemical might reach natural water bodies through totally different modes like run-off from agricultural fields on fresh water, accidental spillage and conjointly by direct application. In the present study, an attempt has been created to quantify the cyphenothrin accumulated in several tissues (gill, kidney and liver) and observe changes concerned within the levels of sodium, potassium and calcium ions and Na^+–K^+, Mg^{2+} and Ca^{2+} ATPase activities within the freshwater fish, *C. mrigala* on exposure to the sublethal concentration of cyphenothrin.

MATERIALS AND METHODS

Procurement of fish and acclimatization

Specimens of *Cirrihinus mrigala* (average length 7–8 cm and weight 100-150 g) were procured from Karnataka Fisheries Development Corporation Limited, Saundatti Fish Farm, Karnataka, India. Before the experiment, fish were held in a large tank for a period of 14 days at 24 ºC temperature and 12-14 hours of photoperiod with continuous aeration. In the present investigation dechlorinated tap water was used with the following physico chemical parameters; salinity (0.191 gms/1), pH (7.4 to 7.6); DO (6 to 7 ml/l); Chlorinity (0.111 gm/l); sodium (1.22 m moles/l); Potassium (30.5 m moles/l); calcium (4.31 m moles/l); carbon di oxide (2. 09 mg/l); oxygen percent saturation (8); Alkalinity 87ppm (as Caco3); Specific gravity (210 m moles/cm); hardness of water 160ppm (Caco3). During acclimatization, fish were fed with balanced nutritious food pellets (Nova, Aquatic P. Feed). Water (three forth of the water) was replaced daily to minimize contamination from metabolic wastes and also to ensure the healthy environment.

Experimental pesticide

Cyphenothrin (Type II pyrethroid) supplied by Sumitomo Chemical India Pvt. Ltd, Gujarat, India, was procured from the local agricultural market of Dharwad, Karnataka, India. The expiry date of the test substance was confirmed prior to the initiation of the exposure. Stock solution was prepared by mixing the calculated volume of the commercial solution with distilled water. Test concentrations for acute toxicity test (i.e.,30 mg/l) and sub-lethal behavioral toxicity test (120 mg/l) were prepared by serial dilution of the stock solution using variable micropipette.

Ionic composition and associated ATPases

The levels of sodium, potassium and calcium ions and the activities of Na^+–K^+ ATPase,Mg^{2+} ATPase and Ca^{2+} ATPases were estimated in gill, liver and muscle of fishes under this study.

Estimation of sodium, potassium and calcium ions

The weighed organs were wet ashed in 50:50 (v/v) concentrated perchloric acid and nitric acid. After keeping the wet ash solutions for 30 min, until the organs were completely dissolved, they were evaporated at 200° C temperature. The residues were dissolved in prefiltered and deionized water and made up to 10 mL. It was filtered through Whatman No. 1 filter paper. Further, appropriate dilutions were made prior to estimations and the sodium, potassium and calcium ions were estimated with the help of flame photometer (Elico make, Model CL–22A). Standard solutions of sodium, potassium and calcium were prepared by using analytical grade chemicals. The values are expressed as mMg1 wet wt of the organ.

Na+–K+, Mg2+ and Ca2+ ATPase activities

Na+–K+, Mg2+ and Ca2+ ATPase activities were estimated separately in the organs by the method described by Watson and Beamish (1981).

Ethical statement

All the experiments performed in the present study abide by the guidelines of the Institutional Animal Ethics Committee (IAEC). The experimental animals used in the study were handled with care according to the guidelines provided by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Statistical analysis

All the results presented in this study were analyzed using SPSS ver. 25 and followed the one-way analysis of variance (ANOVA) with Tukey's post-hoc test. Data were presented as Mean values ± Standard deviation (SD) with statistical significance set at $p < 0.05$.

RESULTS

LC_{50} of cyphenothrin for the fish, *C. mrigala* was found to be 30µg/l and 1/5th of the LC_{50} (6µg/l) was hand-picked as sub lethal concentration for chronic study. varied symptoms of toxicity were determined within the experiment animals exposed to the toxic, cyphenothrin. The shoaling behaviour was disturbed and therefore the fish were determined to be irritable and hyper excited. it absolutely was determined that the fish were makingan attempt to leap out off the chemical medium; associate degree act that may be viewed as an escape development. Restless, irregular, erratic darting movements, loss of equilibrium and excess mucous secretion secretion over the body were different symptoms of intoxication noted throughout the behavioral toxicity study that has been according earlier by the author [13]. Changes within the levels of atomic number 11, metallic element and atomic number 20 ions and activities of associated Na+ - K+, Mg2+ and Ca2+ ATPase in acute and acute exposure regimes in gill, urinary organ and liver of fish, *Cirrihinus mrigala* were determined (Table 1-6).

The uptake and accumulation of cyphenothrin in 3 organ tissues, namely, gill, urinary organ and liver beneath sublethal concentration of cyphenothrin area unit given in Tables one,2 and 3). Levels of atomic number 11, K and atomic number 20 ions (µg/g wet wt) and activities of Na^+–K^+ ATPase, Ca^{2+} ATPase and Mg^{2+} ATPase (µm of supermolecule formed/ mg protein) within the 3 target tissues, specifically gill, urinary organ and liver of seafood, C. mrigala on exposure to sub fatal concentration of cyphenothrin for ten, 20, 30 and 40days area unit given in Figures one and a pair of. A gradual decrease within the Na, K and Ca level was ascertained altogether the 3 tissues, namely, gill, kidney and liver under sub lethal concentration of cyphenothrin. Sublethal concentration recorded a continual decrease within Na, K and Ca| coagulation factor| clotting factor} concentration altogether the tissues up to day 20. Day ten and day thirty registered elevation within the particle concentrations. The activities of Na^+–K^+ ATPase, Ca^{2+} ATPase and Mg^{2+} ATPase are presented in Table no. 4, 5 and 6. Na^+–K^+ ATPase activity showed a concurrence there upon of the ionic strength of Na^+–K^+, that exhibited a reduced price within the sub lethal concentration. Fluctuations within the activity were determined in sublethal concentration up to day ten and eventually day twenty, day thirty

and day forty showed elevation all told the 3 target tissues (gill, kidney and liver). Ca^{2+} ATPase activity showed a gradual decrease at sub fatal concentration and variations at sublethal concentration up to day ten, and sweetening on day twenty, thirty and day forty in gill, excretory organ and liver. Mg^{2+} ATPase failed to dissent in exhibiting the trend of decrease in sublethal concentrations, at the same time showing a rise on day twenty, thirty and day forty of sublethal concentration, that was determined in Na^{+}–K^{+} ATPase and Ca^{2+} ATPase.

DISCUSSION

Fishes breathe in water during which they live and area unit perpetually in direct contact with the close medium. The restless and raised opercula movement within the fish exposed to toxicants gift in close medium area unit characteristics of fish place to hypoxic condition [13] leading to a lot of quantity of toxicants to be brought in reality with the secondary lamellae of the gills inflicting a bigger injury to the metabolism fish tissue, which could have resulted in accumulation of chemical, cyphenothrin within the tissue. Gills area unit concerned within the osmoregulatory method within the fish and bigger uptake of cyphenothrin is also associated with this perform. Since gills area unit perpetually in direct contact with the chemical medium, which could have resulted in fast accumulation of cyphenothrin during this tissue. Moreover, absorption of cyphenothrin molecules by the mucous secretion sheet covering the gills underneath toxic stress may raise the bigger concentration of cyphenothrin within the gill tissue. many authors have according cellular injury to the gill tissue because it is that the initial organ to face chemical medium [25,33,7,45), which provide support to this finding. As the muscle is probably the most important store of fat in fish, Ferrando *et al.* (1991) [19] according that, the bioaccumulation of diazion within the muscle tissue as thanks to the high solubility rate of this pesticide within the muscle fat. Similarly, cyphenothrin is very lyphophilic in nature, and hence, might need accumulated within the macromolecule tissues of the fish.

Apart from the skin, the liver is that the largest visceral organ of the body. several of its functions area unit related to the metabolism of food brought from the gut. All food materials absorbed from the epithelial duct pass on to the liver wherever they're hold on or reborn into another kind pro re nata by the body at that point. within the method it functions because the primary organ for detoxification and thence, it's expected that the toxic, cyphenothrin would reach here in abundance through blood and gut content for storage, detoxification and disposal. Ferrando *et al.* (1992) [18] has according the bioaccumulation of insect powder in high rate in an exceedingly. anguilla that is in accordance with the current study. In fishes, gills form a important site for the particle transport and diffusion water movements, hence, conjointly of pesticides entry. within the gift study the decrease within the levels of Na^{+}, K^{+} and Ca^{2+} ions within the gill, urinary organ and liver exposed to sublethal concentrations of cyphenothrin indicates changes within the leaky properties of the plasma membrane of those organs and of half-crazed Na^{+}, K^{+} and Ca^{2+} ionic pumps because of the probable consequences of tissues harm. From the result, this can be evident that the Na^{+} loss is higher within the case of gill indicating the derangement in Na^{+} transport.

Also, the remittent metallic element content within the tissues of exposed fish indicates changes in leaky properties totally different| of variousbio-membrane systems to different extent by fixing the Na^{+} pump and rupture of the metabolism animal tissue of gill tissue [58]. On exposure to cyphenothrin Ca^{2+} was shrivelled indicating increased chemical process. within the gift study, the restlessness in *C. mrigala* throughout pyrethroid stress may indicate alterations within the laws of Ca^{2+} within the tissue. Moreover, it's been according that shriveled metal content throughout chemical stress corresponds to structural changes in mitochondria integrity. Since mitochondria acts as ‘‘Sinks’’ for living thing Ca^{2+} [47] and principal store homes of Ca^{2+} deposition, it seems that the shrivelled Ca^{2+} within the study may attribute to the disturbances in mitochondrial integrity and later metabolism distress. The decrease in K^{+} content within the tissues of *C. mrigala* exposed to cyphenothrin may be attributed to the derangement in respiration at whole animal as shown by Mushigeri and David (2003) [36]. The main reason for the decrease in sodium, potassium and Calcium levels within the organs of fish, exposed to cyphenothrin can be attributed to the suppressed activities of Na^{+}–K^{+} ATPase, Mg^{2+} ATPase and Ca^{2+} ATPase [42]. The suppression in ATPase activities conjointly suggests a forceful decrease within the gonadotropin unharness, which could be notably liable for the

symptom [25]. Greater level of decrease in Na^+, K^+ and Ca^{2+} levels and also the activities of Na^+–K^+, Mg^{2+} and Ca^{2+} ATPase within the fish exposed to the sub lethal concentration of cyphenothrin indicate severe disruption within the cellular ionic regulation. High concentration of cyphenothrin may need greatly altered the porosity characteristics of the membranes of the organs by interacting with the membrane proteins to serve alterations within the acute transport through destabilizing the membrane enzymes and connected secretion and energy manufacturing method.

Further, the progressive decrease within the ionic levels and progressive suppression of Na^+–K^+, Mg^{2+} and Ca^{2+} ATPase activities within the organs of fish, over time of exposure to the sub lethal concentrations of cyphenothrin indicate the rise within the binding of the cyphenothrin to the active sites of membrane sure catalysts because the level of inhibition relies on the concentration of cyphenothrin on the active sites on enzyme molecules. However, within the sub lethal concentrations vital elevation in ionic levels and additionally within the ATPase activities within the organs of fish, from twenty days of exposure to forty days of exposure, indicate the bigger potency to resist the sublethal concentration of cyphenothrin. the rise in aerobic metabolism additionally might need expedited these animals to elevate the ionic strength by meeting the energy demands the rise within the ionic concentration is also useful to the fish for the upkeep of upper diffusion gradient so as to curb the speedy entry of toxic. Further, the rise in ionic levels could elevate the neuro muscular activity for the improvement in their artificial potentials notably associated with chemical detoxification and domination method. Also, the enhanced ions could facilitate the straightforward uptake of the metabolites and also the structural rigidity in cellular construction. the power to endure the state of imbalance was seen at ten days of exposure, however maintained at twenty days with an initial struggle for survival.

CONCLUSION

The significant elevation within the ionic levels and enzyme activities at ten to twenty days indicate that on prolonged exposure the subacute concentration of cyphenothrin couldn't elicit repressing impact either on the uptake of ions or on the activities of ATPase and instead it stirred the uptake of these factors can be a full of life operation for the elevation in ion levels and ATPase activities within the gills, kidney and liver of fish from thirty to forty days. The redoubled ionic level could also be useful to the animal to avoid the entry of toxic cyphenothrin by maintaining cation concentration gradient.

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CONFLICT OF INTEREST

The authors hereby declare no conflict of interest

REFERENCES

1. Abd El-hameed, S. A., Negm, S. S., Ismael, N. E., Naiel, M. A., Soliman, M. M., Shukry, M., and Abdel-Latif, H. M. 2021. Effects of activated charcoal on growth, immunity, oxidative stress markers, and physiological responses of Nile tilapia exposed to sub-lethal imidacloprid toxicity. Animals, 11(5), 1357.
2. Stara, A., Pagano, M., Capillo, G., Fabrello, J., Sandova, M., Albano, M., ... and Faggio, C. 2020. Acute effects of neonicotinoid insecticides on Mytilus galloprovincialis: A case study with the active compound thiacloprid and the commercial formulation calypso 480 SC. Ecotoxicology and environmental safety, 203, 110980.
3. Alonso, M.B.; Feo, M.L.; Corcellas, C.; Vidal, L.G.; Bertozzi, C.P.; Marigo, J.; Secchi, E.R.; Bassoi, M.; Azevedo, A.F.; Aydın, R.; Köprücü, K.; Dörücü, M.; Köprücü, S. ¸S.; Pala, M 2005. Acute Toxicity of Synthetic Pyrethroid Cypermethrin on the Common Carp (*Cyprinus carpio* L.) Embryos and Larvae. Aquac. Int., 13, 451–458.

4. Bálint, T.; Ferenczy, J.; Kátai, F.; Kiss, I.; Kráczer, L.; Kufcsák, O.; Láng, G.; Polyhos, C.; Szabó, I.; Szegletes, T.;Similarities and Differences between the Massive Eel *(Anguilla anguilla L.*)1997. Devastations That Occurred in Lake Balaton in 1991 and 1995.Ecotoxicol. Environ. Saf., 37, 17–23.
5. Baskin, S. I., Kuhar, K. P., Uricchio, F. J., and Harper, G. R. 1981. The effect of age on five ions of the kidney in the Fischer 344 rat. Reproduction Nutrition Development, 21(5A), 689-694.
6. Bharathi CH, Sandeep BV, Subba Rao BVSSR 2001. The effect of mercuric chloride on the respiration of marine intertidal bivalve, Donax Cuneata. Poll. Res 2001; 20:5–7.
7. Biswas, S.; Mondal, K.; Haque, S 2019. Review on Effect of the Type II Synthetic Pyrethroid Pesticides in Freshwater Fishes. Environ. Ecol., 37, 80–88.
8. Bygrave FL 1978. Mitochondria and the control of intracellular calcium. Biol. Rev; 53:43–46.
9. Coats, J.R.; Symonik, D.M.; Bradbury, S.P.; Dyer, S.D.; Timson, L.K.; Atchison, G.J. 1989.Toxicology of synthetic pyrethroids in aquatic organisms: An overview. Environ. Toxicol. Chem. 8, 671–679.
10. Dall W1967. Hypo-osmoregulation in crustacea. Comp. Blo. Chem. Physiol.;21:653–678.
11. David M. Effect of fenvalerate on behavioural, physiological and biochemicals aspects of freshwater fish, *Labeo rohita*. Ph.D. thesis. S. K. University, Anantapur, India; 1995.
12. Dawood, M.A.O.; Abdel-Tawwab, M.; Abdel-Latif, H.M.R.2020. Lycopene reduces the impacts of aquatic environmental pollutants and physical stressors in fish. Rev. Aquac., 12, 2511–2526. [CrossRef] Animals 2021, 11, 1880 13 of 17
13. Dawood, M.A.O.; El-Salam Metwally, A.; Elkomy, A.H.; Gewaily, M.S.; Abdo, S.E.; Abdel-Razek, M.A.S.; Soliman, A.A.; Amer, A.A.; Abdel-Razik, N.I.; Abdel-Latif, H.M.R. 2020. The impact of menthol essential oil against inflammation, immunosuppression, and histopathological alterations induced by chlorpyrifos in Nile tilapia. Fish Shell fin fish Immunol, 102, 316–325.
14. Dewailly E, Forde M, Robertson L, Kaddar N, Laouan Sidi EA, Cote S Gaudreau E, Drescher O, Ayotte P. 2014. Evaluation of pyrethroid exposures in pregnant women from 10 Caribbean countries. Environ Int. 63:201–206
15. Dorneles, P.R. Pyrethroids: A new threat to marine mammals? Environ. Int. 2012, 47, 99–106.
16. Duncan DM. Mulitple range and multiple tests. Biometrics 1955; 42:1–42.
17. Elliott M. 1995. Chemicals in insect control. In: Casida JE, Quistad GB, editors. Pyrethrum Flowers: production, chemistry, toxicology, and uses. New York: Oxford University Press; p. 3–31.
18. Ferrando MD, Gamon M, Andreu E.1992. Accumulation and distribution of pesticides in Anguilla anguilla from Albufera Lake (Spain). J. Environ. Biol.; 13:75–82.
19. Ferrando MD, Sancho E, Andreu E. 1991. Comparative acute toxicities of selected pesticides to *Anguilla anguilla*. J. Environ. Sci. Health B; 26:491–498.
20. Fetoui, H.; Makni, M.; Mouldi Garoui, E.; Zeghal, N.2010. Toxic effects of lambda-cyhalothrin, a synthetic pyrethroid pesticide, on the rat kidney: Involvement of oxidative stress and protective role of ascorbic acid. Exp. Toxicol. Pathol, 62, 593–599.
21. Finney DJ. Probit Analysis, 3rd edition, London: Cambridge University Press; 1971.
22. Fiske C, Subba Rao Y.1925. The colorimetric determination of phosphorus. J. Biol. Chem ;66:375–400.
23. Gautam, P.; Gupta, A.2008. Toxicity of cypermethrin to the juveniles of freshwater finish*Poecilia reticulata* (Peters) in relation to selected environmental variables. Indian J. Nat. Prod. Resour, 7, 314–319.
24. Hua, J.; Relyea, R. 2014. Chemical cocktails in aquatic systems: Pesticide effects on the response and recovery of 20 animal taxa. Environ. Pollut, 189, 18–26.
25. Kalavathy K, Sivakumar AA, Chandran R.2001. Toxic effects of the pesticide dimethoate on the fish, *Sarotherodon mossambicus*. J. Ecol. Res. Biocon; 2:27–32.
26. Kim K-B, Anand SS, Kim HJ, White CA, Bruckner JV. 2008. Toxicokinetics and tissue distribution of Deltamethrin in Adult Sprague Dawley Rats. Toxicol Sci. 101:197–205.
27. Lehninger L. In: Biochemistry, Ludhiana, New Delhi: Kalyani Publications; 1979.
28. Marigoudar, S.R.; Ahmed, R.N.; David, M. 2013. Ultrastructural responses and oxidative stress induced by cypermethrin in the liver of *Labeo rohita*. Chem. Ecol, 29, 296–308.
29. Metropolitan Areas. Environ. Sci. Technol. 2012, 46, 4297–4303.

30. Molina-Ruiz, J.M.; Cieslik, E.; Cieslik, I.; Walkowska, I. 2015. Determination of pesticide residues in fin fish tissues by modified QuEChERS method and dual-d-SPE clean-up coupled to gas chromatography–mass spectrometry. Environ. Sci. Pollut. Res, 22, 369–378.
31. Morgan TP, Grosell M, Gilmour KM, Playle RC, Wood CM. 2004. Time course analysis of the mechanism by which silver inhibits active Na^+ and Cl uptake in gills of rainbow trout. Am. J. Physiol. Regul. Integr. Comp. Physiol 2004;287: R243–242.
32. Muenstermann, S.; Rinkanya, F.G.R.; Tome, N.R.1988. Tick control in small ruminants with a Cypermethrin 'pour-on' in Kenya. Trop.Pest Manag, 34, 399–401.
33. Mushigeri SB, David M.2003. Assessment of fenvalerate toxicity on oxygen consumption and ammonia excretion in the freshwater fish, *Cirrhinus mrigala.* J. Ecotoxicol. Environ. Monit; 13:191–195.
34. Mushigeri SB, David M.2004. Residue analysis of fenvalerate by gas liquid chromatography in functionally different tissues of freshwater fish, *Cirrhinus mrigala* (Hamilton). Aquacult 5:207–213.
35. Mushigeri SB, David M.2004. Toxicity of synthetic pyrethroid insecticide fenvalerate to the freshwater fish, *Cirrhinus mrigala (*Hamilton). J. Ecobiol. 16:169–174.
36. Naiel, M.A.E.; Ismael, N.E.M.; Abd El-hameed, S.A.A.; Amer, M.S.2020 The antioxidative and immunity roles of chitosan nanoparticle and vitamin C-supplemented diets against imidacloprid toxicity on *Oreochromis niloticus*. *Aquaculture* 523, 735219.
37. Naiel, M.A.E.; Shehata, A.M.; Negm, S.S.; Abd El-Hack, M.E.; Amer, M.S.; Khafaga, A.F.; Bin-Jumah, M.; Allam, A.A.2020. The new aspects of using some safe feed additives on alleviated imidacloprid toxicity in farmed fifish: A review. *Rev. Aquac. 12*, 2250–2267.
38. Oxidative Stress Markers, and Physiological Responses of Nile Tilapia Exposed to Sub-Lethal Imidacloprid Toxicity. Animals 2021, 11, 1357.
39. Ozcan Oruc E, Uner N, Tamer L.2002. Comparison of Na^+K^+-ATPase activities and malondialdehyde contents in liver tissue for three fish species exposed to Azinphosmethyl. Bull. Environ. Contam. Toxicol. 69:271–277.
40. Özkara, A.; Akyıl, D; Konuk, M. Pesticides, environmental pollution, and health. In Environmental Health Risk-Hazardous Factors to Living Species; Intech Open: London, UK, 2016.
41. Potential Sources of Pyrethroid Insecticides in Stream Sediments from Seven U.S.
42. Radhaiah V. Studies on the toxic impact of a phyrethroid insecticide, fenvalerate on some metabolic aspects and histopathology of freshwater teleost, *Tilapia mossambica.* Ph.D. thesis. S. V. University, Tirupathi, Andhra Pradesh, India; 1988.
43. Ranjit Hazarika.2003. A study of neurotoxicity of BHC in relation to residual accumulation on the brain tissue of *Heteropneustes fossilis* (Bloch). J. Environ. Biol. 2003;24:77–80.
44. Richterova, Z.; Machova, J.; Stara, A.; Tumova, J.; Velisek, J.; Sevcikova, M.; Svobodova, Z. 2015. Effects of a cypermethrin-based pesticide on early life stages of common carp (Cyprinus carpio L.). Vet. Med. 2015, 60, 423–431.
45. Sancho E, Fernandez-Vega C, Ferrando MD, Andreu-Moliner E. Eel ATPase activity as biomarkerof thiobencrab exposure. Ecotoxicol. Environ. Saf. 2003; 56:434–441.
46. Sangli AB, Kanabur, VV.2000. Acute toxicity of ammonia to a freshwater fish, *Lepidocephalicths guntea*. Environ. Ecol. 18:874–876.
47. Stehle, S.; Schulz, R. Agricultural insecticides threaten surface waters at the global scale. Proc. Natl. Acad. Sci. USA 2015, 112,5750–5755
48. The effects of dietary clinoptilolite and chitosan nanoparticles on growth, body composition, haemato-biochemical parameters, immune responses, and antioxidative status of Nile tilapia exposed to imidacloprid. Environ. Sci. Pollut. Res. 2021.
49. Thompson JR. In: Analysis of Pesticides Residue in Human and Environmental Samples, Rome: FAO Publications; 1974.
50. Tilak KS, Veeraiah K, Susan TA, Yacobu K.2001. Toxicity and residue studies of fenvalerate to some selected freshwater fishes. J. Environ. Biol.77–180.
51. USEPA. 2013a. Common mechanism grouping for the pyrethrins and synthetic pyrethroids. EPA-HQ-OPP-2011-0746-0003. Washington: USEPA.

52. Watson TA, Beamish WH.1981. Effects of Zinc on bronchial ATPase activity in rainbow trout. Salmo gairdneri. Comp. Biochem. Physiol.66C:77–82.
53. Yamawaki K, Hashimoto W, Fujil K, Koyama JI, Ikeda Y, Ozaki H.1986. Hemochemical changes in carp exposed to low cadmium concentrations. Bull. Jap. Soc. Sci. Fish.;52:459–466
54. Yang, Y.; Ma, H.; Zhou, J.; Liu, J.; Liu, W. Joint toxicity of permethrin and cypermethrin at sublethal concentrations to the embryo-larval zebra fish. Chemosphere 2014, 96, 146–154.

Table No.1 Sodium ion content (µg/g wet wt) in the organs of Fresh water fish *Cirrihinus mrigala* on exposure to the sublethal concentration of Cyphenothrin. Each value is a mean of six estimation. Percent change over control is given parenthesis.

Organs	Control	Exposure in Days			
		10	20	30	40
Gills	54.8562^{A}	43.867^{D}	41.0531^{E}	48.4044^{C}	52.6372^{B}
SD±	0.00023	0.00056	0.00082	0.00062	0.00135
% Change	------	-20.0327	-25.1623	-11.7612	-4.0451
Kidney	66.0374^{A}	56.3432^{E}	50.7762^{D}	58.9792^{C}	64.6662^{B}
SD±	0.00069	0.00047	0.00052	0.00035	0.00059
% Change	------	-14.6798	-23.1099	-10.6881	-2.0763
Liver	59.7331^{A}	53.3324^{C}	49.3631^{E}	51.402^{D}	56.1343^{B}
SD±	0.00049	0.00074	11.09821	0.00056	0.002513
% Change	-------	-10.7154	-17.3605	-13.9472	-6.0248

Mean are ± for a parameter in a row followed by the same letter are not significantly different (P≤ 0.05) from each other according to Duncan's multiple range (DMR) test

Table No.2 Potassium ion content (µg/g wet wt) in the organs of Fresh water fish *Cirrihinus mrigala* on exposure to the sublethal concentration of Cyphenothrin. Each value is a mean of six estimation. Percent change over control is given parenthesis.

Organs	Control	Exposure in Days			
		10	20	30	40
Gills	60.10732^{A}	58.79348^{B}	55.7374^{E}	57.3106^{D}	58.3667^{C}
SD±	0.00034	0.00040	0.000374	0.000374	0.000374
% Change	-----	-2.1859	-7.2701	-4.6528	-2.8958
Kidney	76.7077^{A}	71.5977^{C}	67.4317^{E}	69.3037^{D}	73.3647^{B}
SD±	0.000374	0.000374	0.000374	0.000374	0.000374
% Change	------	-6.6616	-12.0927	-9.6522	-4.3581
Liver	55.2836^{A}	46.6637^{D}	41.3466^{E}	48.1225^{C}	52.9737^{B}
SD±	0.000374	0.000374	0.000349	0.000374	0.000374
% Change	-----	-15.5921	-25.21	-12.9534	-4.1782

Mean are ± for a parameter in a row followed by the same letter are not significantly different (P≤ 0.05) from each other according to Duncan's multiple range (DMR) test

Table No.3 Calcium ion content (µg/g wet wt) in the organs of Fresh water fish *Cirrihinus mrigala* on exposure to the sublethal concentration of Cyphenothrin. Each value is a mean of six estimation. Percent change over control is given parenthesis

Organs	Control	Exposure in Days			
		10	20	30	40
Gills	83.5567^{A}	74.9377^{D}	64.3737^{E}	76.3206^{C}	81.1225^{B}
SD±	0.000374	0.000374	0.000374	0.000374	0.000327

% Change	------	-10.3152	-22.9581	-8.6601	-2.9132
Kidney	66.4446^{A}	55.3115^{D}	51.4686^{E}	58.3036^{C}	64.3677^{B}
SD±	0.000374	0.000374	0.000374	0.000374	0.000374
% Change	------	-16.7555	-22.5391	-12.2523	-3.1257
Liver	75.9577^{A}	70.7326^{C}	69.7648^{E}	67.6647^{D}	73.5437^{B}
SD±	0.000374	0.000374	0.000374	0.000374	0.000374
% Change	-----	-6.8789	-8.1530	-10.9179	-3.1780

Mean are ± for a parameter in a row followed by the same letter are not significantly different (P≤ 0.05) from each other according to Duncan's multiple range (DMR) test

Table No.4 Na^{+}-K^{+} ATPase activity(µm of protein formed / mg protein) in the organs of Fresh water fish *Cirrihinus mrigala* on exposure to the sublethal concentration of Cyphenothrin. Each value is a mean of six estimation. Percent change over control is given parenthesis

Organs	Control	Exposure in Days			
		10	20	30	40
Gills	4.7364^{E}	4.9234^{D}	5.1739^{C}	5.6347^{B}	5.8445^{A}
SD±	0.000374	0.000374	0.000374	0.000374	0.000374
% Change	------	-3.9481	-9.2369	-18.9659	-23.3954
Kidney	7.3017^{E}	7.6317^{D}	7.9448^{C}	8.0017^{B}	8.3437^{A}
SD±	0.000374	0.000374	0.000374	0.000374	0.000374
% Change	--------	-4.5195	-8.8075	-9.5868	-14.2706
Liver	9.1077^{E}	9.4337^{D}	9.8107^{C}	9.9737^{B}	10.1478^{A}
SD±	0.000374	0.000374	0.000374	0.000374	0.000374
% Change	--------	-3.5793	-7.7187	-9.5084	-11.42

Mean are ± for a parameter in a row followed by the same letter are not significantly different (P≤ 0.05) from each other according to Duncan's multiple range (DMR) test

Table No.5 Mg^{2+} ions(µm of protein formed / mg protein) in the organs of Fresh water fish *Cirrihinus mrigala* on exposure to the sublethal concentration of Cyphenothrin. Each value is a mean of six estimation. Percent change over control is given parenthesis

Organs	Control	Exposure in Days			
		10	20	30	40
Gills	6.7337^{E}	6.9747^{D}	7.2417^{C}	7.5436^{B}	7.9218^{A}
SD±	0.000374	0.000374	0.000374	0.00034	0.00033
% Change	------	-3.5790	-7.5367	-12.0276	-17.6441
Kidney	6.9746^{E}	7.1446^{D}	7.4746^{C}	7.9776^{B}	8.2125^{A}
SD±	0.000349	0.000349	0.000349	0.000349	0.000374
% Change	------	-2.4374	-7.1688	-14.3808	-17.7487
Liver	9.1252^{E}	9.5716^{D}	9.8936^{C}	10.0115^{B}	10.2118^{A}
SD±	0.000374	0.000349	0.000349	0.000356	0.00033
% Change	-------	-4.8919	-8.4206	-9.7126	-11.9077

Mean are ± for a parameter in a row followed by the same letter are not significantly different (P≤ 0.05) from each other according to Duncan's multiple range (DMR) test

Table No.6 Ca^{2+} ions(µm of protein formed / mg protein) in the organs of Fresh water fish *Cirrihinus mrigala* on exposure to the sublethal concentration of Cyphenothrin. Each value is a mean of six estimation. Percent change over control is given parenthesis

Organs	Control	Exposure in Days			
		10	20	30	40
Gills	8.1736^{E}	8.6736^{D}	8.9446^{C}	9.1143^{B}	9.6736^{A}
SD±	0.000349	0.000349	0.000374	0.00109	0.00033
% Change	------	-6.11726	-9.4328	-11.509	-18.3518
Kidney	4.6316^{E}	4.8017^{D}	4.9977^{C}	5.215^{B}	5.8728^{A}
SD±	0.000349	0.000374	0.000374	0.00032	0.000374
% Change	-------	-3.6726	-7.9044	-12.5961	-26.7985
Liver	3.7833^{E}	3.9746^{D}	4.1178^{C}	4.4441^{B}	4.6993^{A}
SD±	0.00914	0.000349	0.000374	0.000374	0.000394
% Change	-------	-5.0564	-8.8414	-17.4662	-24.2117

Mean are ± for a parameter in a row followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to Duncan's multiple range (DMR) test

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RESEARCH ARTICLE

A Study on Faculty Performance Development by using Social Media in Selected Private Educational Institutions in Coimbatore

D.Sathish Kumar[1] and S.Najumudeen[2]*

[1]Assistant Professor cum Research Supervisor, Department of MBA, Hindusthan College of Arts and Science, Coimbatore, Tamil Nadu, India.
[2]Assistant Professor cum Research Scholar, Department of Management, Rathinam College of Arts and Science, Coimbatore, Tamil Nadu, India.

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***Address for Correspondence**
S.Najumudeen,
Assistant Professor cum Research Scholar,
Department of Management,
Rathinam College of Arts and Science,
Coimbatore, Tamil Nadu, India.
Email: researcher.najumu@gmail.com

ABSTRACT

Information technology has a vital role to develop the all-over world and making it shrink. In this modern world, most people wanted to get all things within a click of one button for instance Amazon, Flipkart, etc. During the pandemic, teach more about the usage of digital media even non-IT people were working with it. In a few Coimbatore private educational institutions, this research examines how staff's performance has improved as a result of using social media. The institutional faculty leverages their social networks with maturity to promote college admissions, publish research, and engage in discussion about the most recent developments in their particular field in order to learn more. Through the questionnaire survey method, data were collected from 154 respondents from various private institutions in Coimbatore. The statistical software for social science helped to analyze the collected data. The finding of the study explored that faculty performance is affected by social media. The study concluded that LinkedIn is mainly used for sharing knowledge, career development, and collaboration with others. It is linked with faculty performance development. According to this study's final findings, social media's importance in the present age is vital for the better growth of their respective fields, thus institutions need to raise awareness of the best ways to use social media.

Keywords: employee, institutions, LinkedIn, performance development, private, social media.

INTRODUCTION

Globalization opened the country to deliver thoughts, communications, services, and products in and out through information and communication technology. The entrance of the internet made us as an individual do all things. Enact all businesses dealing with fractional movement are encouraged by ICT. Communication was further strengthened by the advent of social media. Through this, we have got the zealous method of world political changes, changes in social thinking, critical strategies, creation of many entrepreneurs, skinning of social ills, and teaching education for modern times. Social media is a network-based communication instrument for connecting the public to interact with each other for sharing knowledge and consuming information. In the 21st century, everyone has social media account even though doesn't have any bank account for savings. login the personal account helps easily feed the news and information that wish to share with others to get more likes.

Usage of Social Media

Industrial and educational persons start their web pages and blogs to post their articles to attach with common people. Generally, people are using Face book, Twitter, YouTube, LinkedIn, and WhatsApp to post their pictures, share their information, discuss with their community, display their professional growth, market their business for development, engage their family connectivity, and their creativity.

Face book

It is a social networking site that makes people easily connect and share with family and friends. These days it has one billion users in the world.

Twitter

It is a social networking site for post user's short posts. It may contain video, text, photos, and links to share their thoughts and views.

WhatsApp

It is a multi-platform messaging app that helps users to make a video and voice calls, text, and show current situational status. It functions with all mobile and computers.

LinkedIn

It is a professional networking site for connecting people with posting professional career development, job seeker sends their CVs to employers, employers' requirements, and Interaction with Industry people.

Youtube

It is a video-sharing site for all categories of people. It enables to start channels for creating videos to upload, watch videos, interact with videos and subscribe to the channels. Some people earn the money through their YouTube channels, which helps to develop their creativity.

Faculty Performances in Private Institutions

Performance of employees refers to how well they do their work, how effectively complete their tasks and how behave with others in the workplace. Organizations provide training programs, workshops, seminars, and monthly tasks for improving the efficiency of employees. College faculty mainly focuses on their classes, study notes preparation, students' career growth development activities, and institution development events to fulfill their fixed tasks. It was tough for teachers to teach a lesson during the period of international spread. Despite the difficult days, they have taught and crossed those paths without fail.

REVIEW OF LITERATURE

Srishti Babu, Hareendrakumar VR, and Suresh Subramoniam (2020) in their study "Impact of Social Media on Work Performance at a Techno park in India" revealed that knowledge share increases the employee performance and innovations. In the IT sector Knowledge procure and delver makes a perfection to lead in their position. Technological interaction increases at the workplace while using social media which makes an effective organization and enhances work performance. N. Boobalakrishnan, R.Jayaseelan, and C. Pichandy (2020) in their study "An Empirical Study on Social Media usage by College Students in Coimbatore, India" disclosed that students are spending more time with social media and it makes them as a creator. Usage of social media as a part of work in their day-to-day life. male and female students are using in Coimbatore drastically increased with awareness of the cyber issues. B. Medina Nilasari (2020) in her study "The Impact of Social Media on Employee Work Performance with Trust as a Mediation Variable" reported that most of the employees are using social media to communicate with co-workers for complete their work and it makes hope in that to move forward without fear for increase work performance. Finally suggested that In this situation organizations should build with experts to deal employees' hurdles to complete their work through social media. P. Panbuselvan and V. Veerakumaran (2018) in their study "The Impact of social media on Students Academic Performance (With Special Reference to Arts and Science College Students in Coimbatore District - Tamil Nadu)" exhibit that most of the students are using social media for checking notes, downloading the study material, sharing the links, and viewing the lecture videos. 102 respondents' data collected by the academic department wise and revealed that most of the students are spending with Facebook and WhatsApp. It shows that strong association between social media and academic performance.

Ali Sukru Cetinkaya and Muhammad Rashid (2018) in their study "The Effect of Use of Social Media on Employee Job Performance" explored that lack of knowledge in the use of social media leads to lagging behind in business techniques. Through this study service sector employees showed their performances increased by the informative knowledge collected by social media. Concluded with the recommendation to the organizations to find the path to the maximum benefit of its use to develop the business process. Irene Polnaya, NaziefNirwanto, and BogeTriatmanto (2018)in their study ""The Evaluation of Lecturer Performance through Soft Skills, Organizational Culture and Compensation on the Private University of Ambon" unclosed those soft skillscompletely rise the lecturer performance and personal development in their working environment. The culture of organization take as a part of increase their performance and development. Finally suggested that proper training need to develop their soft skill and leadership in their organization. Dorcas M. Nyamanya, Stella Omari and Andrew Nyanga'u (2017) in their study "An Assessment of Social Media Use on Employee Performance In Public University Colleges: A Case Of Rongo University, Kenya" explored that They have collected the data from 136 respondents and used the connectivity theories for employee performance with Facebook, WhatsApp, LinkedIn, and Twitter and found that LinkedIn usage very effective for sharing their knowledge and career development. other than that, they do not relate to their work and it consumes efficienttime. This study suggested encouraging the employees to use social media for their specific utility.

Problem of the Study

Social media encourage all people to develop their talent, business, and innovation. College faculty also use social media to develop their knowledge to share with students and enhance their professional growth. In the previous year'sfaculty members did not consider social media because they shared the knowledge of what they have experienced in particular subjects. In the digital media world, teachers must update their knowledge quickly to create fast thinkers and creators. Through social media, most of the faculty members enhanced themselves, their students, and their institution's growth for achieving the next level. Past studies failed to reveal that faculty performance development by using social media.

Scope of the Study

The current study throw slight on the development of faculty performance by using social media in a private institution. It may give the chance to study the faculty performance further using various parameters.

Objective of the Study

1.To study the utilization of social media for developing faculty performance in private educational institutions.
2.To find effective social media for developing faculty performance in private educational institutions.
3.Recommend the suggestions based on the findings.

RESEARCH HYPOTHESIS

H0: There is no relationship between Respondents' experience and using social media to personal development, to students' development and to institution's development.
H1: There is a relationship between Respondents experience and using social media to personal development, to students' development and to institution's development.

Sources of Data

The primary data are collected from various 5 private college employees from various departmentsand the sample size is fixed at 154 from a population of 576. Secondary dataare collected from published articlesin the reputed journals.

Data Analysis and Interpretation

From the table.1 revealed that 34 percent respondents from Rathinan College and 25 percent from Hindusthan College. Each 12 percent respondents from AJK college and KSG college. Least respondents from Sree Saraswathy College (9 percent) and Bishop Amrose college (8 percent). Above table explored that 48 percent respondents are belongs to the age of 31 to 40 and 27 percent are below 30. 16 percent and 9 percent respondents are being in41 to 50 and 51 to 60 respectively. Table.3 clarified that the majority (63 percent) of the respondents are male and 37 percent of respondents are female. The above table.4. Simplified that most (80 percent) of the respondents are working as Assistant professor and 20 percent respondents are as Associate professor. The table.5. explained that 27 percent respondents are have 1 to 10 years' experience and below 1 year experience respondents are 24 percent. 11 to 20 years and 21 to 30 years experienced faculty are 23 percent and 14 percent respectively. least respondents (12 percent) are have above 31 years. From the table. 6. Revealed that 43 percent respondents are mostly using WhatsApp and 24 percent respondents are using YouTube. 16 percent and 9 percent respondents are using Facebook and Twitter respectively. Least respondents (8 percent) are using LinkedIn. The above table.6. disclosed that among 154 respondents,

Social Media using for Personal Development

1. YouTube helpful their academic work for 48 percent of respondents and Linked In for 18 percent. WhatsApp and Facebook for 24 percent and 16 percent respectively. 8 percent of respondents preferred Twitter to help their work.
2. 61 percent of respondents favoured Facebook for help to geta quicker response for their needs and WhatsApp for 24 percent. Twitter helped 15 percent of respondents.
3. From the mentioned social media, for the Majority (47 percent) of respondents LinkedIn motivate to their work hard for improvement and YouTube for 38 percent of respondents. WhatsApp and Facebook help for 10 percent and 4 percent of respondents respectively. 1 percent of the respondents choose twitter to help motivate.
4. 51 percent of respondents' professional growth showed on LinkedIn and Facebook for 42 percent respondents. YouTube and WhatsApp help to show others for 4 percent and 2 percent respectively. At least 1 percent respondent shows professional growth in Twitter.
5. LinkedIn is helpful to getting opportunity for be a resource person in seminars and workshop for 47 percent

respondents and Facebook for 42 percent respondents. YouTube helpful for this opportunity to 5 percent and WhatsApp and Twitter helpful for each 3 percent respondents. Table.8. revealed that among 150 respondents,

Social Media using for Students' Development

1. For 86 percent respondents are getting immediate response from students by WhatsApp and 14% by Facebook.
2. YouTube helps for 69 percent respondents to enhance their student's academic development and WhatsApp for 24 percent respondents. Only 7 % respondents helped by Facebook.
3. For 73 percent respondents are getting genuine information of students' internship and placement from LinkedIn and for 21 percent are getting from Facebook. Just 6 percent respondents are getting from WhatsApp.
4. To collect study materials for 56 percent respondents are using YouTube. 23 percent and 21 percent respondents are using WhatsApp and Facebook respectively.
5. Highest respondents (76 percent) are getting more effective to delivering the knowledge through YouTube. Each 12 percent respondents are getting more effective from WhatsApp and Facebook.

Social Media are using for Institutions Development

1. 51 percent respondents are using Facebook for getting admission to develop their institution and 17 percent are using LinkedIn. 15 percent and 14 percent respondents are using YouTube and WhatsApp respectively. Only 3 percent respondents are using Twitter for their institution's admission.
2. Facebook using respondents are high (54 percent) for get more views regarding the institutional post and 18 percent for LinkedIn. WhatsApp and YouTube are using by 15 percent and 14 percent of respondents for get more views.
3. 44 percent of respondents are using Facebook for getting event detail is from other institutions to participate students with the name of their institution and 22 of percent respondents are using Twitter. 19 percent and 16 percent are using WhatsApp and LinkedIn respectively.
4. LinkedIn is very useful to collect other institutional development strategies for 62 percent of respondents and 24 percent are using Facebook. 12 percent and 1 percent of respondents are using WhatsApp and YouTube.
5. 43 percent of respondents are using LinkedIn for get details of Fundraising projects for institutions and YouTube for 35 percent. 22 percent use Facebook for their institutional fundraising projects.

Hypothesis Statistical Testing

Table.10 described that there is no significant difference between the respondent's experience and Personal development by using social media. One-way ANOVA significance value greater than P value (0.05 < 0.31, 0.3,0.45,0.24,0.15). based on this research hypothesis rejected and null hypothesis selected. Table.11 detailed that there is no significance difference between the respondent's experience and students' development by using social media. One-way ANOVA significance value greater than P value (0.05 < 0.977,0.688,0.169, 0.052, 0.394). based on this research hypothesis rejected and null hypothesis selected. Table.12 explored that there is no significance difference between the respondent's experience and Institutional development aspects are get admission more, more post views, get event details and get fund raising projects by using social media. One-way ANOVA significance value greater than P value (0.05 < 0.828,0.41,0.223, 0.669). based on this research hypothesis rejected and null hypothesis was selected. It showed that there is a relationship between respondent's experience and collect strategies of other institutional development by using social media. One-way ANOVA significance value less than P value (0.05> 0.011). based on this research hypothesis selected and null hypothesis rejected.

Findings

1. From the 154 respondents, Majority of the respondents from Rathinam college of arts and science
2. Most of the respondents age category is 31 to 40.
3. Male respondents higher in this study.
4. Most of the respondents are Assistant professors.
5. Majority of the respondents experience are below 1 to 20 years.
6. Highest frequently using social media is WhatsApp.

7. YouTube helpful to the majority of respondents for academic work.
8. Most of the respondents received quicker response from Facebook.
9. 47 percent respondents are motivating to improve their work, 51 percent respondents showcase their professional growth to others and 47 percent respondents are getting opportunity as a resource person for seminars and workshops in other institutions by LinkedIn.
10. YouTube helps to enhance respondent's students' academic development, collect the notes and to delivering the knowledge more effectively.
11. WhatsApp helpful to getan immediate response from the students.
12. LinkedIn provides genuine information of Internships and placement offers for students at the right time.
13. Majority of the respondents getting help from Facebook for Admission, more views on post and collect event details to participate.
14. LinkedIn helpful to collect other institutions' development strategies and to get details of fundraising projects for institutions.
15. There is no significant difference between the respondent's experience and students' development, Students' development, and Institutional development except for collecting the strategies of institutional development by using social media.

Suggestions

1. Management must encourage the professors to learn the unknown knowledge of the social media even though well know those because they are getting more information from there.
2. Technical expert sessions must conduct to avoid Cybercrime activities, update knowledge, and further current teaching aid to students.
3. Most of the employees are using immediate response social media like WhatsApp and Facebook, so Management can fix those as authenticate for official group or medium of contact.
4. Visualised learnings are working as efficiently for subject understanding, application and innovations. So, YouTube links that subject relevant must be shared with the students before starting the classes to make students productive.
5. LinkedIn professionally gives much attraction for all aspects of development, so Management must focus to enhance the knowledge of their faculty for effective utilization of this kind of social media.

CONCLUSION

After the advent of social media, more and more people were not interested in spending it because they thought it was a waste of more productive time. But nowadays they are used to fill the need for new technology, the expansion of information knowledge, and the rapid cycle of the emergency world. In the pandemic situation, unable to hide that society has gotten more help from the people through social media. Growth trends and mechanisms in the service and manufacturing sectors are irreversible without touching any social media. Educational institutions faced a lot of development difficulties and rectify them through social media. Revived world increasing their usage of social media as a part of their routine work. Management of Institutions has to consider the utilization of social media makes a development tool.

REFERENCES

1. Chandrashekar M. Mathapati (2021), Key Aspects of Digital Technology and Its Impact on Bank Employee Performance, IOSR Journal of Business and Management (IOSR-JBM) e-ISSN: 2278-487X, p-ISSN: 2319-7668. Volume 23, Issue 8. Ser. V (August 2021), PP 01-03.
2. Srishti Babu, Hareendrakumar VR and Suresh Subramoniam (2020). Impact of Social Media on Work Performance at a Technopark in India. Metamorphosis 19(1) 59–71, 2020 DOI: 10.1177/0972622520962949.
3. N. Boobalakrishnan, R.Jayaseelan and C. Pichandy (2020).An Empirical Study on Social Media usage by College

Students in Coimbatore, India. Asian Journal of Multidisciplinary Studies Vol. 3, No. 1, (2020) ISSN 2651-6691 (Print) ISSN 2651-6705 (Online).

4. B. Medina Nilasari (2020).The Impact of Social Media on Employee Work Performance with Trust as a Mediation Variable", JurnalManajemen/Volume XXIV, No. 02, June 2020: 298-312, DOI: http://dx.doi.org/10.24912/jm.v24i2.649.
5. K Seethal,andDr. B Menaka (2019), Digitalisation Of Education In 21ST Century: A Boon Or Bane , International Journal for Research in Engineering Application & Management (IJREAM) ISSN : 2454-9150 Special Issue - ICDOMP'19, DOI : 10.18231/2454-9150.2019.0436.
6. Ali Sukru Cetinkaya and Muhammad Rashid (2018), The Effect of Use of Social Media on Employee Job Performance, journal of internet applications and management 2018/ 9(2).
7. P. Panbuselvan and V. Veerakumaran (2018), The Impact of Social Media on Students Academic Performance (With Special Reference to Arts and Science College Students in Coimbatore District - Tamil Nadu), International Journal of Computer Sciences and Engineering, Volume-6, Issue-3 E-ISSN: 2347-2693
8. Irene Polnaya, NaziefNirwanto, and BogeTriatmanto (2018), The Evaluation of Lecturer Performance through Soft Skills, Organizational Culture and Compensation on Private University of Ambon, Academy of Strategic Management Journal (Print ISSN: 1544-1458; Online ISSN: 1939-6104), 2018, Vol: 17 Issue: 2.
9. Dorcas M. Nyamanya, Stella Omari and Andrew Nyanga'u (2017), An Assessments of Social Media Use On Employee Performance In Public University Colleges: A Case Of Rongo University, Kenya, Africa International Journal of Management Education and Governance (AIJMEG) 2(3): 75-95 (ISSN: 2518 -0827).
10. AgrippahKandiero, Tambudzai Perpetua and Dr. Nelson Jagero (2014) Impact of Access To Social Media On Employee Productivity And Organisational Performance At Econet Wireless Zimbabwe, International Journal of Knowledge and Research in Management & E-Commerce Vol.4, Issue 1, January, 2014 Print-ISSN: 2231-203X e-ISSN: 2231-0339.

Table 1.Colleges of the Respondents

S.NO	COLLEGE	RESPONDENTS	PERCENTAGE
1	Ajk College of Arts And Science	18	12
2	Bishop Ambrose College	13	8
3	Hindusthan Arts And Science College	38	25
4	KSG College of Arts And Science	18	12
5	Sree Saraswathy Thyagaraja College	14	9
6	Rathinam College of Arts And Science	53	34
	TOTAL	154	100

Table 2. Age of the Respondents

S.NO	AGE	RESPONDENTS	PERCENTAGE
1	Below 30	42	27
2	31 to 40	74	48
3	41 to 50	24	16
4	Above 51	14	9
	TOTAL	154	100

Table 3. Gender of the Respondents

S.NO	GENDER	RESPONDENTS	PERCENTAGE
1	MALE	97	63
2	FEMALE	57	37
		154	100

Table 4. Designation of the Respondents

S.NO	DESIGNATION	RESPONDENTS	PERCENTAGE
1	Assistant Professors	123	80
2	Associate Professors	31	20
	TOTAL	**154**	**100**

Table 5. Experience of the Respondents

S.NO	EXPERIENCE	RESPONDENTS	PERCENTAGE
1	Below 1 Year	37	24
2	1 To 10 Years	42	27
3	11 To 20 Years	35	23
4	21 To 30 Years	21	14
5	Above 31 Years	19	12
	TOTAL	154	100

Table 6. Most using Social Media of the Respondents

S.NO	MOST USING SOCIAL MEDIA	RESPONDENTS	PERCENTAGE
1	WhatsApp	67	43
2	Facebook	24	16
3	LinkedIn	12	8
4	You tube	37	24
5	Twitter	14	9
	TOTAL	**154**	**100**

Table 7. Social Media using for Personal Development

S.NO	FOR PERSONAL DEVELOPMENT	What's App	Facebook	LinkedIn	You tube	Twitter	TOTAL
1	Which social media do you think is helpful for the academic work you do?	24 (15%)	16 (10%)	28 (18%)	74 (48%)	12 (8%)	154 (100%)
2	which social media helps in getting a quicker response for your need?	37 (24)	94 (61)	0 (0%)	0 (0%)	23 (15)	154 (100%)
3	which social media motivates you to work hard for improvement?	16 (10)	7 (4)	72 (47)	58 (38)	1 (1)	154 (100%)
4	Which social media showcase your professional growth to others?	3 (2)	64 (42)	79 (51)	6 (4)	2 (1)	154 (100%)
5	which social media helps you in getting the opportunity as a resource person for seminars and workshops in other institutions?	5 (3)	64 (42)	73 (47)	7 (5)	5 (3)	154 (100%)

Table 8.Social Media using for Students' Development

S.NO	FOR STUDENTS DEVELOPMENT	What's App	Facebook	LinkedIn	You tube	Twitter	TOTAL
1	which social media helps in easy interactions with students for getting an immediate response?	132 (86%)	22 (14 %)	0 (0%)	0 (0%)	0 (0%)	154 (100%)
2	Which social media is used to enhance the academic development of students?	37 (24%)	11 (7%)	0 (0%)	106 (69%)	0 (0%)	154 (100%)
3	which social media provides the genuine information of Internships and placement offers for students at the right time	9 (6%)	32 (21%)	113 (73%)	0 (0%)	0 (0%)	154 (100%)
4	Which social media helps to collect the study notes?	36 (23%)	32 (21%)	0 (0%)	86 (56%)	0 (0%)	154 (100%)
5	which social media helps in delivering the knowledge to students more effectively?	18 (12%)	19 (12%)	0 (0%)	117 (76%)	0 (0%)	154 (100%)

Table 9. Social Media are using for Institutions Development

S.NO	FOR THE INSTITUTION DEVELOPMENT	WhatsApp	Facebook	LinkedIn	You tube	Twitter	TOTAL
1	Which social media helps to get admissions more?	22 (14%)	78 (51%)	26 (17%)	23 (15%)	5 (3%)	154 (100%)
2	Which social media helps you get more views on your post for the Institution?	22 (15%)	84 (54%)	27 (18%)	21 (14%)	0 (0%)	154 (100%)
3	which social media helps to get event details of other institutions for participating students with the name of institutions?	29 (19%)	67 (44%)	24 (16%)	0 (0%)	34 (22%)	154 (100%)

4	Which social media helps to collect other institutions' development strategies?	19 (12%)	37 (24%)	96 (62%)	2 (1%)	0 (0%)	154 (100%)
5	which social media helps to get details of Fundraising projects for institutions?	0 (0%)	34 (22%)	66 (43%)	54 (35%)	0 (0%)	154 (100%)

Table 10. Relationship between the Respondent's Experience and Personal Development by using Social Media

ANOVA							
S.NO	**Experience* Personal development**		**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
1	Which social media do you think is helpful for the academic work you do?	Between Groups	7.064	4	1.766	1.2	0.31
		Within Groups	219.43	149	1.473		**Significance 0.31 > 0.05**
		Total	226.49	153			
2	Which social media helps in getting a quicker response for your need?	Between Groups	7.592	4	1.898	1.23	0.3
		Within Groups	229.76	149	1.542		**Significance 0.3 > 0.05**
		Total	237.35	153			
3	Which social media motivates you to work hard for improvement?	Between Groups	3.186	4	0.796	0.94	0.45
		Within Groups	126.95	149	0.852		**Significance 0.45 > 0.05**
		Total	130.14	153			
4	Which social media showcase your professional growth to others?	Between Groups	2.411	4	0.603	1.4	0.24
		Within Groups	64.212	149	0.431		**Significance 0.25 > 0.05**
		Total	66.623	153			
5	Which social media helps you in getting the opportunity as a resource person for seminars and workshops in other institutions?	Between Groups	3.975	4	0.994	1.72	0.15
		Within Groups	85.927	149	0.577		**Significance 0.15 > 0.05**
		Total	89.903	153			

Table 11.Relationship between the Respondent's Experience and Students' Development by using Social Media

ANOVA							
S.NO	**Experience * Students' Development**		**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
1	Which social media helps in easy interactions with students for getting immediate response?	Between Groups	0.058	4	0.014	0.114	0.977
		Within Groups	18.8	149	0.126		**Significance 0.977 > 0.05**
		Total	18.857	153			
2	Which social media is used to enhance the academic development of students?	Between Groups	3.923	4	0.981	0.566	0.688
		Within Groups	258.214	149	1.733		**Significance 0.688 > 0.05**
		Total	262.136	153			
3	Which social media provides the genuine information of Internships and placement offers for students at the right time	Between Groups	2.175	4	0.544	1.633	0.169
		Within Groups	49.592	149	0.333		**Significance 0.169> 0.05**
		Total	51.766	153			
4	Which social media helps to collect the study notes?	Between Groups	15.778	4	3.945	2.408	0.052
		Within Groups	244.118	149	1.638		**Significance 0.052 > 0.05**
		Total	259.896	153			
5	Which social media helps in delivering the knowledge to students more effectively?	Between Groups	4.928	4	1.232	1.031	0.394
		Within Groups	178.111	149	1.195		**Significance 0.394> 0.05**
		Total	183.039	153			

Table 12. Relationship between the Respondent's Experience and Institutional Development by using Social Media

ANOVA							
S.NO	**Experience * Institutional Development**		**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
1	Which social media helps to get admissions more?	Between Groups	1.559	4	0.39	0.372	0.828
		Within Groups	156.006	149	1.047		**Significance 0.828 > 0.05**
		Total	157.565	153			
2	Which social media helps you get more views on your post for the Institution?	Between Groups	3.097	4	0.774	0.998	0.41
		Within Groups	115.559	149	0.776		**Significance 0.41 > 0.05**
		Total	118.656	153			

3	which social media helps to get event details of other institutions for participating students with the name of institutions?	Between Groups	11.104	4	2.776	1.442	0.223
		Within Groups	286.799	149	1.925		**Significance** 0.223 > 0.05
		Total	297.903	153			
4	Which social media helps to collect other institutions' development strategies?	Between Groups	6.731	4	1.683	3.404	0.011
		Within Groups	73.665	149	0.494		**Significance 0.011 < 0.05**
		Total	80.396	153			
5	which social media helps to get details of Fundraising projects for institutions?	Between Groups	1.335	4	0.334	0.591	0.669
		Within Groups	84.068	149	0.564		**Significance 0.669 > 0.05**
		Total	85.403	153			

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RESEARCH ARTICLE

Bio Fabrication of NIO Nanoparticles from *Gliricidia sepium* and *Terminalia arjuna* Leaf Extract

A. Mary George Shemona[1]*, S. Kanagaprabha[2] and H. Johnson Jeyakumar[3]

[1]Research Scholar, Department of Physics, Pope's College, Sawyerpuram, Thoothukudi, (Affiliated to Manonmaniam Sundaranar University, Tirunelveli) Tamil Nadu, India.
[2]Assistant Professor, Department of Physics, Kamaraj College, Thoothukudi (Affiliated to Manonmaniam Sundaranar University, Tirunelveli) Tamil Nadu, India.
[3]Associate Professor, Department of Physics, Pope's College, Sawyerpuram, Thoothukudi (Affiliated to Manonmaniam Sundaranar University, Tirunelveli) Tamil Nadu, India.

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***Address for Correspondence**
A. Mary George Shemona,
Research Scholar,
Department of Physics,
Pope's College, Sawyerpuram, Thoothukudi,
(Affiliated to Manonmaniam Sundaranar University, Tirunelveli)
Tamil Nadu, India.
Email: smilsonshemona@gmail.com

ABSTRACT

In this modern world nanotechnology is playing a vital role in all the fields. The present study which I have done is also based on the nickel nanoparticles which was obtained by Biosynthesis method from the leaf extract of *Gliricidium sepium* and *Terminalia arjuna*. And also, the obtained sample is subjected to various analysis. The X-Ray Powder Diffraction (XRD) showed the high-density peaks. The result obtained from the Fourier Transformation Infrared spectroscopy analysis is about the type of bond and the vibration. Since the obtained sample was Biologically synthesized it was subjected to Anti-bacterial activity against *E. coli* and *Staphylococcus aureus*. The SEM result also gave us additional information about the other chemical compound present including the Nickel particles.

Keywords: Green synthesis, XRD, Anti-bacterial, SEM, FTIR

INTRODUCTION

Nanotechnology refers to an emerging field of science that includes synthesis and development of various nanomaterials. Nanomaterials can be defined as objects ranging in size from 1-100 nm that due to their size may differ from the bulk material. Presently, different metallic nanomaterials are being produced using copper, zinc, titanium, magnesium, gold, alginate and silver. Nanoparticles are being used for diverse purpose, from medical treatments, using in various branches of industry production such as solar and oxide fuel batteries for energy storage, to wide incorporation into diverse materials of everyday use such as cosmetics or clothes [1]. Nanoparticles can be synthesized chemically or biologically. Eco friendly alternatives to chemical and physical methods are biological ways of nanoparticles synthesis using microorganisms [2,3], enzymes [4], fungus [5], and plants or plant extracts [6,7]. Biosynthesis of nanoparticles by microorganisms is a green and eco-friendly technology. The synthesis of nanoparticles may be intracellular or extracellular according to the location of nanoparticles [8,9]. Nickel oxide (NiO) has in recent times attracted the attention of scientists worldwide for various technological applications including antiferromagnetic materials [10], electrode materials for lithium-ion batteries [11], gas sensors absorbents [12], and electro-chemical super capacitors [13]. Various physical and chemical methods such as anodic arc plasma [14], sol-gel[15], precipitation[16], solvothermal [17], sonochemistry [18],pulse laser ablation [19,20,21], microwaves [22], and thermal decomposition[23,34,25] have been used for the synthesis of metal oxide nanoparticles, particularly NiO-NPs. In this study, we reported the green synthesis of NIONPs using the leaves extract of *Gliricidium sepium* and *Terminalia arjuna*. Furthermore, green NiONPs were characterized using XRD, SEM, EDS, MAPPING, PL, FTIR and Antibacterial activities.

MATERIALS AND METHODS

Sample collections

Nickel Nitrate of AR grade was purchased from the sun scientific corporation and the leaves of *Gliricidium sepium* and *Terminalia arjuna* were collected from the neighbour garden and brought up to the laboratory.

Green Synthesis Method

The biological method, which is represented as an alternative to chemical and physical methods, provides an environmentally friendly way of synthesizing nanoparticles. Moreover, this method does not require expensive, harmful and toxic chemicals. Metallic nanoparticles with various shapes, sizes, contents and physicochemical properties can be synthesized thanks to the biological method actively used in recent years. Synthesis can be done in one step using biological organisms such as bacteria, action bacteria, yeasts, molds, algae and plants, or their products. Molecules in plants and microorganisms, such as proteins, enzymes, phenolic compounds, amines, alkaloids and pigments perform nanoparticle synthesis by reduction. Actinobacteria, which performs the production of secondary metabolites such as antibiotics, are aerobic, immobile, and mostly filamentous gram-positive bacteria. They are resistant to the most toxicheavymetals owing to their detoxification property. Soluble toxic metal ions are detoxified by either being degraded by intracellular or extracellular reduction or precipitation. Thus, nanoparticles being antibacterial, antifungal, anticancer, antioxidant, antibio-contamination and having catalytic activity can be produced. Synthesis of nanoparticles can be done as extra-cellular or intra-cellular with enzymes by employing simply-cultured and fast-breeding eukaryotic yeasts and molds with easy biomass design, as. The incubation conditions and the metallic ion solutions used influence the size of the nanoparticles produced. Being pathogenic for humans limits the use of some molds in nanoparticle production [26].

- Green synthesis of nanoparticles using microorganism, plants and fungi were made at room temperature.
- Green synthesis provides an environmentally friendly, simple, economical and reproducible approach for faster metal nanoparticle production.

- Nanoparticles obtained by green synthesis method are used in many application fields such as cancer treatment, drug, bio sensor construction.

Synthesis of Nio Nanoparticles using *Terminalia arjuna* and *Gliricidium sepium* Leaf Extract

Take 2.9g of Nickel Nitrate and dissolved in 100ml de-ionised water. Add the Nickel Nitrate solution to 100ml both leaf extract. The solution is allowed to stir at 500 RPM for 5 hours followed by dispersion of the pellet in the solution until clear solution is formed. The dark green colour was formed for *Terminalia arjuna* leaf extract and it is washed with de-ionize Water for 5 times to remove by products and unreacted compounds. The green colour was formed for *Gliricidium sepium* leaf extract and it is washed with de-ionize Water for 5 times to remove by products and unreacted compounds. The prepared solutions are then placed in hot air oven at 120^{0}c for 3Hours.The dried powder is crushed by using agate motor and it is then Annealed in muffle furnace for annealing at 500^{0}c temperatures. After annealing process, the black coloured NiO nanoparticleis obtained for both extracts. After calcination the sample was again grinded using agate motor and used for characterization purpose. Fig. 1 shows the biosynthesized NiO-NPs of *Gliricidium sepium* and fig. 2 shows the biosynthesized NiO-NPs of *Terminalia arjuna.*

Characterization

The green synthesized NiO-NPs were confirmed through different techniques such as FESEM/EDAX, XRD, PL and FT-IR. The functional groups of nanoparticles were investigated using of FTIR spectrum. The FESEM/ EDAX/PSA images were applied to examine shape, size, and elements existing in the samples. The crystalline structure of synthesized NiO-NPs was examined using the XRD pattern.

RESULTS AND DISCUSSION

XRD Analysis

The crystalline nature of NiO-NPs can be observed throughout the provided XRD pattern in fig. 3,4. In *Gliricidium sepium,* several varying peaks that emerged at 2θof 29.5^o, 37.3^o, 43.3^o, and 62.9^o were corresponding to the crystal planes of (004), (111), (200), and (220), respectively. In *Terminalia arjuna,* several varying peaks that emerged at 2θ of 31.3^o, 37.3^o, 43.3^o, 62.9^o, and 75.4^o were corresponding to the crystal planes of (002), (111), (200), (220) and (311), respectively. This diffraction pattern indicated that the NPs contain very sharp peaks and high crystalline cubic structure that confirms the purity and fine formation of metal-oxide NPs. The crystalsize can be calculated according to Debye-Scherer formula [27].

$$D = \frac{K\lambda}{\beta \cos\theta}$$

Where D corresponds to the crystal size, K is the shape dependent Scherer's constant (0.98), λ is the wavelength of radiation (0.15406) used for the XRD, β is the full peak width at half-maximum (FWHM) of the peak and θ is the Bragg's diffraction angle.

SEM analysis

In order to study the morphology and size of the biosynthesized NiO-NPs, SEM images were recorded at different magnifications. The SEM images of the *Gliricidium sepium* NiO-NPs are shown in fig. 5a shows it is possible to notice that the unitary particles as well as their agglomerates present with irregular and inhomogeneous surface aspects.fig. 5b shows the energy dispersive x-ray spectroscopy (EDS) analysis with SEM, and fig. 5c shows the Scanning electron microscopy (SEM) elemental mapping. The SEM images of the *Terminalia arjuna* NiO-NPs are shown in fig. 6a shows the sponge-like structure of NiO-NPs. fig. 6b shows the energy dispersive x-ray spectroscopy (EDS) analysis with SEM, and fig. 6c shows the Scanning electron microscopy (SEM) elemental mapping. The formation of NiO-NPs, as well as their morphological dimensions in the SEM study, demonstrated that the average size was 30- 35 nm with inter-particle distance.

FTIR Analysis

FTIR analysis was carried out to identify the functional groups in biomolecules that are responsible for the reduction, capping and efficient stabilization of the as-synthesized NiO-NPs. Analysis of the spectra is done and chemical groups identified at different frequency range of *Gliricidium sepiumare* shown in table 1 and *Terminalia arjuna* are shown in table 2.It has been observed from the analysis of the IR spectra that difference functional groups are present in the NiO-NPs such as hydrogen bonded alcohol, phenols, alkanes, alkenes, alcohol, ethers, carboxylic acid and ester. Fig. 7 shows that the *Gliricidium sepium* spectrum shows the major peak at 3313.1 cm^{-1} and the other peaks are at 2981.4, 1639.1, 1045.2 and 879.3 cm^{-1}.Fig. 8shows that the Terminalia Arjuna spectrum shows the major peak at 3303.3 cm^{-1} and the other peaks are at 1635.3, 1043.3 and 431.7 cm^{-1}.In *Gliricidium sepium* it is observed at the bands 3313.1 cm^{-1} corresponds to the hydrogen bonded alcohol and phenols. The band at 2981.4 cm^{-1} corresponds to C-H stretching of alkanes. The band at 1639.1 cm^{-1} corresponds to C=C stretching. The band at 1045.2 cm^{-1} could be related to C-O vibration of alcohol, ethers, carboxylic acid and ester. The band at 879.3 cm^{-1} corresponds to C-H bonds of alkenes. In *Terminalia arjuna* it is observed at the bands 3303.3 cm^{-1} corresponds to the hydrogen bonded alcohol and phenols. The band at 1635.3 cm^{-1} corresponds to C=C stretching. The band at 1043.3 cm^{-1} could be related to C-O vibration of alcohol, ethers, carboxylic acid and ester. The band at 431.7 cm^{-1} corresponds to the C–Br stretch alkyl halides.

Applications

Antibacterial Activity

Evaluation of antibacterial activity was carried out for synthesized NiO-NPs using *Gliricidium sepium* and *Terminalia arjuna* leaf extract.The Antimicrobial effect of NiO-NPs was assessed through an agar diffusion method against gram-negative bacteria *Escherichia coli* and gram-positive bacteria *Staphylococcus aureus*. The *Gliricidium sepium* and *Terminalia arjuna* Fig.9 and Fig. 10 shows the various zones of inhibition (mm), when the panel of bacteria was treated with 150 µg/ml of NiO sample. From the fig.9 it is evident that the NiO-NPs synthesized through green route has excellent antibacterial activity against the evaluated bacterial strains, *Staphylococcus aureus (20 mm), Escherichia coli (*18 mm). In *Terminalia Arjuna,* fig. 10 shows that the evaluated bacterial strains, *Staphylococcus aureus (21 mm), Escherichia coli (*17 mm). However, the activity was more over the gram positive (*Staphylococcus aureus) bacteria, than that of gram-negative bacterial strains, may be due to the fact that it contains only one cytoplasmic membrane and thick wall of multilayers of peptidoglycan, which are more susceptible to damage [28]. Also, a significant activity was noticed against Escherichia coli (*18 mm), a gram-negative bacterium which has a complexed cell walled structure. Moreover, the efficacy of the antibacterial activity also depends on the composition of the nanomaterial, intrinsic properties, surface phenomena, type of the bacterial species, colony size and concentration. The mechanism through which a nanoparticle interacts with the bacterial membrane is not well known, but studies report that when the nanoparticles are treated with the bacterial pathogens, there occurs a significant change in the membrane morphology, which results in the increase of the permeability and involves in the regular transport of the plasma membrane, leaving the bacterial cell incapable of proper regulation which results in cell death [29]. Also, the small size of the NiO nanomaterial, easily penetrates into the cell membrane and binds with the functional groups of proteins, sulfur, oxygen, nitrogen, DNA, and phosphorous with consequent protein denaturation and cell death [30,31].

CONCLUSION

NiO nanoparticles are also prepared by green synthesis using Nickel Nitrate as a precursor along with *Terminalia arjuna* leaves as reducing agent. *Terminalia arjuna* leaves extract have been effectively used for the synthesis of NiO nanoparticles. We have utilized the natural, renewable biomaterial for the synthesis of NiO nanoparticles. The as-prepared samples are subjected to XRD, FTIR, Antibacterial Activity and SEM. The XRD pattern indicates that the sample crystallizes in cubic structure. The elements of the sample have been identified by EDS method. The functional group analysis was carried out by FTIR study. Moreover, the antimicrobial activity of this product was evaluated against gram-negative bacteria and gram-positive bacteria.

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REFERENCES

1. Dubchack S., Ogar A., Mietelski J.W and Tuenau K., Influence of silver and titanium nanoparticles on arbuscular mycorrhiza colonization and accumulation of radio caesium in Helianthus anus, Span. J. Agric. Res., 8(1), 103-108, (2010).
2. Klaus T., Joerger R., Olsson E. and Granqvist C.G., SilverBased Crystalline Nanoparticles, Microbially Fabricated, J. Proc. Natl. Acad. Sci. USA, 96, 13611-13614, (1999).
3. Konishi Y. and Uruga T., Bioreductive Deposition of Platinum Nanoparticles on the Bacterium Shewanella algae, J. Biotechnol., 128, 648-653, (2007).
4. Willner I., Baron R. and Willner B., Growing metal nanoparticles by enzymes, J. Adv. Mater, 18, 1109-1120, (2006).
5. Vigneswaran N., Ashtaputre N.M., Varadarajan P.V, Nachane R.P., Paralikar K.M., Balasubramanian R.H., Materials Letters, 61(6), 1413-1418, (2007).
6. Shankar S.S., Ahmed A., Akkamwar B., Sastry M., Rai A., Singh A. Biological synthesis of triangular gold nanoprism, Nature, 3 482, (2004).
7. Ahmad N., Sharma S., Singh V.N., Shamsi S.F, Fatma A. and Mehta B.R., Biosynthesis of silver nanoparticles from Desmodium triflorum: a novel approach towards weed utilization, Biotechnol. Res. Int. 454090 (1-8), (2011).
8. Hulakoti N.I. and Taranath T.C., Biosynthesis of nanoparticles using microbes: A Review. Colloids surf Bio Interfaces, (121), 474-83, (2014).
9. Mann S., Biomineralization, Principles and Concepts in Bioinorganic Materials Chemistry, Oxford University Press, Oxford, UK, (2001).
10. S. Krishnakumar, M. Liberati, C. Grazioli, M. Veronese, S. Turchini, P. Luches, S. Valeri, C. Carbone, Magnetic linear dichroism studies of in situ grown NiO thin films, J. Magn. Magn. Mater. 310 (2007) 8–12.
11. S. Ni, T. Li, X. Yang, Fabrication of NiO nanoflakes and its application in lithium ion battery, Mater. Chem. Phys. 132 (2012) 1108–1111.
12. Y. Zhang, W. Zeng, New insight into gas sensing performance of nanoneedleassembled and Nano sheet assembled hierarchical NiO nanoflowers, Mater. Lett. 195 (2017) 217–219.
13. J. Zhao, H. Liu, Q. Zhang, Preparation of NiO nanoflakes under different calcination temperatures and their super capacitive and optical properties, Appl. Surf. Sci. 392 (2017) 1097–1106.
14. Z. Wei, T. Xia, L. Bai, J. Wang, Z. Wu, P. Yan, Efficient preparation for Ni nano powders by anodic arc plasma, Mater. Lett 60 (2006) 766–770.
15. Q. Li, L.S. Wang, B.Y. Hu, C. Yang, L. Zhou, L. Zhang, Preparation and characterization of NiO nanoparticles through calcination of malate gel, Mater. Lett 61 (2007) 1615–1618.
16. J. Li, R. Yan, B. Xiao, D.T. Liang, D.H. Lee, Preparation of nano-NiO particles and evaluation of their catalytic activity in pyrolyzing biomass components, Energy Fuels 22 (2008) 16–23.
17. K. Anandan, V. Rajendran, Structural, optical and magnetic properties of welldispersed NiO nanoparticles synthesized by CTAB assisted solvothermal process, Nanosci. Nanotechnology.: Int. J. 2 (2012) 24–29.
18. S.M. Mohseni, S.A. Hosseini, M. Rezaee, S.K. Sadrnezhaad, D. Mohammadyani, Synthesis of wide band gap nanocrystalline NiO powder via a sonochemical method, Ultrason. Sonochem 19 (2012) 841–845.
19. G.G. Guillén, S. Shaji, M.I.M. Palma, D. Avellaneda, G.A. Castillo, T.K.D. Roy, D.I. G. Gutiérrez, B. Krishnan, Effects of ablation energy and post-irradiation on the structure and properties of titanium dioxide nanomaterials, Appl. Surf. Sci. 405 (2017) 183–194.

20. M. Rahmat, A. Rehman, S. Rahmat, H.N. Bhatti, M. Iqbal, et al., Laser ablation assisted preparation of MnO2 nano colloids from waste battery cell powder: Evaluation of physicochemical, electrical and biological properties, J. Molecular Struct. 1191 (2019) 284–290.
21. L. Zbroniec, T. Sasaki, N. Koshizaki, Parameter dependence of nickel oxide nanoparticles prepared by pulsed-laserablation, J. Ceram. Process. Res 6 (2005) 134–137.
22. X. Song, L. Gao, Facile synthesis of polycrystalline NiO nanorods assisted by microwave heating, J. Am. Ceram. Soc 91 (2008) 3465–3468.
23. F. Davar, Z. Fereshteh, M.J. Salavati-Niasari, Nanoparticles Ni and NiO: Synthesis, characterization and magnetic properties, J. Alloys Compd. 476 (2009) 797–801.
24. R. Manikandan, K. Giribabu, R. Suresh, S. Munusamy, S.K. Praveen, S. Muthamizh, A. Stephen, V. Narayanan, Characterization and photocatalytic activity of nickel oxide Nanoparticles, Int. J. Chemtech. Res. 6 (6) (2014) 3395–3398.
25. F. Motahari, M.R. Mozdianfard, F. Soofivand, M. Salavati-Niasari, NiO nanostructures: Synthesis, characterization and photocatalyst application in dye pollution wastewater treatment, RSV Adv. 4 (2014) 27654–27660.
26. Korbekandi H, Iravani S, Abbasi S. Production of nanoparticles using organisms. Critical Reviews in Biotechnology (2009) 29:279– 306. doi:10.3109/07388550903062462.
27. Sharma R, Acharya A D, Moghe S, Shrivastava S B, Gangrade M, Shripathi T, and Ganesan V 2014 Material Science in Semiconductor Processing 23 42.
28. G. Fu, P.S. Vary, C.T. Lin, J. Phys. Chem. B., 109 (2005) 8889.
29. P.K. Stoimenov, P.L. Klinger, G.L. Marchin K. Klabunde, Langmiur 18 (2002) 6679- 6686.
30. S. Suresh, S. Karthikeyan, K. Jayamoorthy, Karbala, Int. J. Mod. Sci., 2 (2016) 69-77.
31. L. Juan, Z. Zhimin, M. Anchun, L. Lei, Z. Jingchao, Int. J. Nanonet., 5 (2010) 261– 267.

Table 1. Analysis of FTIR spectra of *Gliricidium sepium*

Bond	Frequency range	Types of compound	Intensity
O-H	3313.1	Hydrogen bonded alcohol, phenols	Variable, sometimes broad
C-H	2981.4	Alkanes	Strong
C=C	1639.1	Alkenes	Variable
C-O	1045.2	Alcohol, ethers, carboxylic acid, ester	Strong
C-H	879.3	Alkenes	Strong

Table 2. Analysis of FTIR spectra of *Terminalia arjuna*

Bond	Frequency range	Types of compound	Intensity
O-H	3303.3	Hydrogen bonded alcohol, phenols	Variable, sometimes broad
C=C	1635.3	Alkenes	Variable
C-O	1043.3	Alcohol, ethers, carboxylic acid, ester	Strong
C-H	431.7	Alkenes	Strong

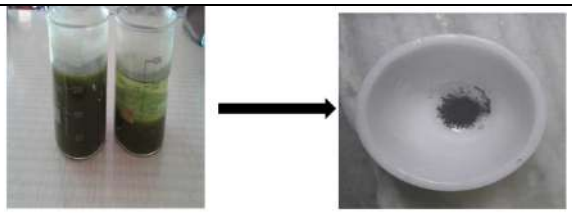


Fig. 1 Prepared NiO nanoparticles of *Gliricidium sepium*

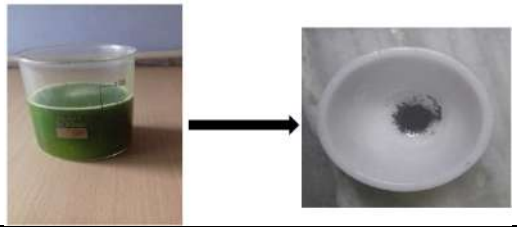


Fig. 2 Prepared NiO nanoparticles of *Terminalia arjuna*

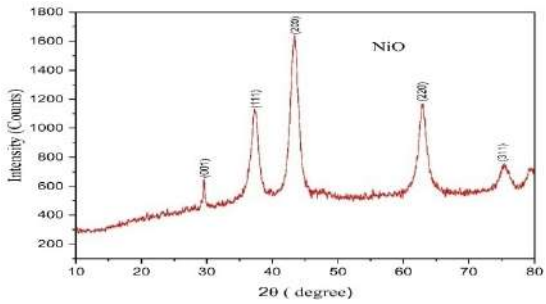


Fig. 3 XRD Pattern of the prepared NiO nanoparticles by Green synthesis using *Gliricidium sepium*

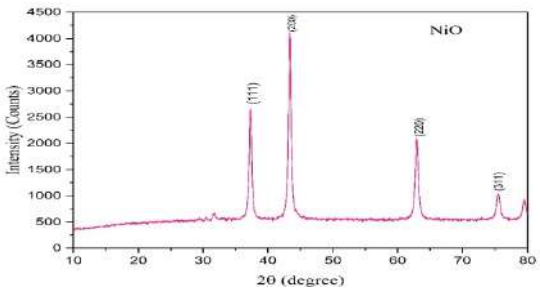
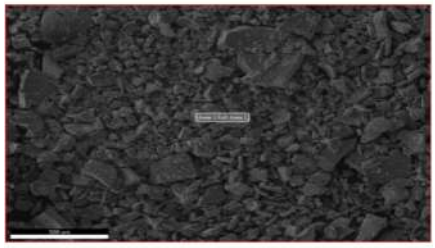
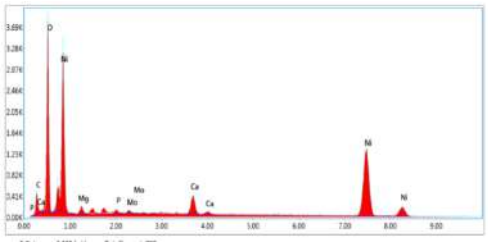


Fig. 4XRD Pattern of the prepared NiO nanoparticles by Green synthesis using *Terminalia arjuna*



(a)Energy dispersive X-ray spectroscopy (EDS)



(b) Scanning electron microscopy (SEM) elemental mapping

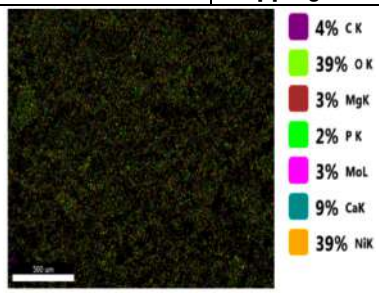
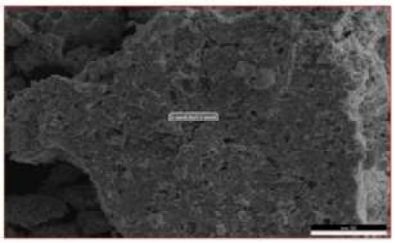
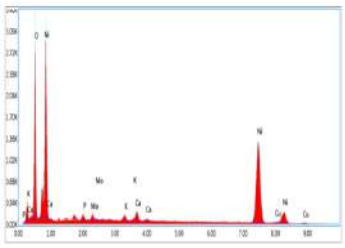


Fig. 5 SEM image(c) of *Gliricidium sepium.*



(a), Energy dispersive X-ray spectroscopy (EDS)



(b), Scanning electron microscopy (SEM) elemental mapping

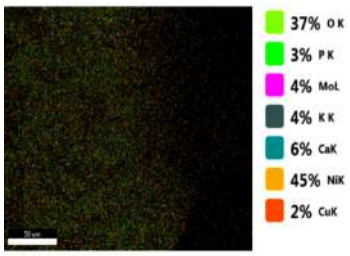


Fig. 6 SEM image(c)of *Terminalia arjuna.*

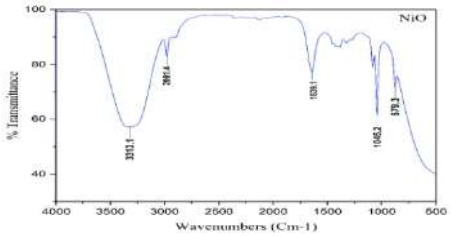


Fig. 7 FTIR spectra for NiO nanoparticles of *Gliricidium sepium.*

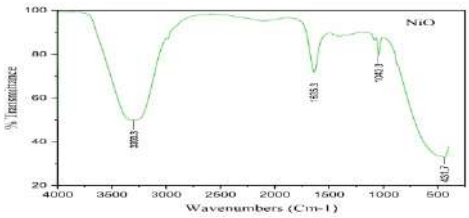


Fig. 8 FTIR spectra for NiO nanoparticles of *Terminalia arjuna.*

Fig. 9 Antibacterial activity of *Gliricidium sepium.*

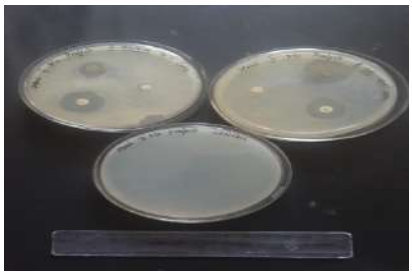


Fig. 10Antibacterial activity of *Terminalia arjuna.*

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REVIEW ARTICLE

A Review on Hypercholesterolemia and Methods for Cholesterol Quantification

Ankita Kalra[1]*, Pallavi Sharma[1] and Sushmita Uniyal[2]

[1]Master of Pharmacy (Pharmacology), Department of Pharmacology, School of Pharmaceutical Sciences, Shri Guru Ram Rai University, Patel Nagar, Dehradun, Uttarakhand, India.
[2]Assistant Professor, Department of Pharmacology, School of Pharmaceutical Sciences, Shri GuruRam Rai University, Patel Nagar, Dehradun, Uttarakhand, India.

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***Address for Correspondence**
Ankita Kalra,
Master of Pharmacy (Pharmacology),
Department of Pharmacology,
School of Pharmaceutical Sciences,
Shri Guru Ram Rai University,
Patel Nagar, Dehradun, Uttarakhand, India.
Email: ankitakalra42@gmail.com

ABSTRACT

Hypercholesterolemia has hierarchically joined the best risk factors causative to the prevalence and severity of coronary heart diseases. It's a medical condition wherever abnormally high levels of cholesterol are measured within the blood. High cholesterol levels will limit blood flow, increasing the danger of an attack or stroke. This malady will increase morbidity and mortality once combined with alternative prevailing diseases like DM, high blood pressure, and other cardiovascular diseases. Therefore, correct analysis and quantification of blood cholesterol are very crucial for people who are at increased risk for these diseases. Multiple analytical ways are developed for the analysis of blood cholesterol, also involving traditional chemical ways, Fluoro metric enzymatic assays, gas and liquid chromatography, and mass spectroscopic analysis (MS). The strategy referred to as ambient ionization mass spectroscopic analysis (AIMS), has been utilized for the quantification of cholesterol. During this review, we tend to summarize the foremost prevailing ways for cholesterol quantification in biological samples.

Keywords: Hyperlipidemia, Cholesterol, quantification, chromatography, mass spectrometry.

INTRODUCTION

Hypercholesterolemia could be defined as a condition where abnormally high levels of lipids mostly cholesterol and triglycerides are found in the blood. This condition can be additionally known as hyperlipidemia or hypolipoproteinaemia. This elevation of plasma lipids is among the key risk factors related to heart diseases (1). The cholesterol circulates within the blood and is concerned with the structure and performance of cells whereas triglycerides (TG) are best viewed as the energy that's either used straight off or kept in fat cells.TG is factory-made within the liver from the foods or by being absorbed from the intestine in the digestive tract. Arteries are commonly swish and patent on the within, but in the case of high lipid levels, a sticky substance known as plaque is made within the walls of arteries. This results in reduced blood flow, consequentially which leads to stiffening and narrowing of the arteries. It's been verified that elevated plasma levels of cholesterol and low-density lipoprotein are to blame for coronary-artery disease in man; also epidemiologic knowledge suggests that elevated plasma levels of high-density lipoprotein have a protecting effect (2).

The main reason for high lipid levels includes changes in lifestyle habits involving a high-fat diet i.e. with a fat intake exceeding 40% of total calories, saturated fat intake exceeding 10% of total calories; and cholesterol intake exceeding 300mg/day. Other lifestyle factors like being overweight, smoking high alcohol use, and lack of exercise can also lead to unusually high cholesterol levels. Alternatively, some ailments can elevate cholesterol levels such as diabetes, kidney disease, pregnancy, polycystic ovary syndrome, and hypoactive thyroid gland. Medications like diuretics, beta-blockers, and anti-depression have also been reported to lift cholesterol levels. Age, gender, and heredity are the modifying factors in the development and progression of hyperlipidemia. Also, factors giving rise to hypercholesterolemia with no prevalence information include Berardinelli-Seip congenital lipodystrophy – hyperlipidemia, Cholestasis, Chromosome 15q, deletion, Neuropathy, hereditary motor and sensory, Okinawa type, chronic renal failure, metabolic syndrome and Nephrotic syndrome – hyperlipidemia(3). Hyperlipidemia is categorised into familial or primary hyperlipidemia that is induced by definite inherited abnormalities and acquired or secondary hyperlipidemia that is induced by changes in plasma lipid and lipoprotein mechanisms and is non-inheritable(4).

Exogenous lipids, which are ingested and broken down in the gut, and endogenous lipids, which are produced in the liver, are the two sources of lipids, which are insoluble in water. Plasma contains LDL cholesterol, which makes up around 7% of the body's total cholesterol. The liver plays a critical role in both cholesterol synthesis and catabolism, which have an impact on plasma cholesterol levels(5). Some forms of fat, including cholesterol, are incapable of dissolving in blood. Additionally, they must be specifically transported to and from cells by specific molecules known as lipoproteins, which are made up of an outer layer of protein and an inner core of cholesterol and triglycerides. Additionally, it has been discovered that lipoproteins are crucial for the movement of cholesterol inside the body. Total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), and very low-density lipoprotein (VLDL) cholesterol are several types of lipids that can be categorized(3,5).

VLDL carries triglycerides in the blood. Extra calories, alcohol, or sugar are converted by the body into triglycerides, which are then stored as fat in the adipose tissue. In order to transport cholesterol and other lipids from tissues back to the liver for breakdown, the liver produces HDL, also known as the good cholesterol. High levels of HDL cholesterol have long been seen as a reliable sign of heart health. LDL, also referred to as bad cholesterol, is a type of lipid that the liver produces and transports to various parts of the body, including the heart, muscles, tissues, and organs. High levels of LDL suggest that there is far too much cholesterol in the bloodstream, which raises the risk of heart disease. Very low density lipoproteins (VLDL), LDL, and HDL are the three main lipoprotein fractions that contain the majority of the circulating cholesterol.

VLDL + HDL + LDL = total cholesterol

According to the following connection, LDL-cholesterol is derived from measured values of total cholesterol, triglycerides, and HDL cholesterol

LDL = TG/5 + HDL - Total Cholesterol

Where all data are reported in mg/dL and [TG]/5 is a rough estimation of VLDL cholesterol.
VLDL cholesterol is comparable to LDL cholesterol in that it primarily consists of fat and little protein. The lipoproteins known as VLDL cholesterol are responsible for transporting cholesterol from the liver to the body's organs and tissues. Triglycerides and cholesterol are combined to create them. Atherosclerosis and heart disease are also linked to VLDLs, which are heavier than LDLs (1, 3).

VLDL = TG/5 can be used to calculate VLDL from the measured triglycerides.

Factors impacting lipid and lipoprotein measurements (7)

Fasting
Recent dietary consumption has little impact on the level of plasma total cholesterol. However, postprandial plasma triglyceride levels rise to a degree that is correlated with fasting triglyceride levels and dietary fat intake. This results from chylomicrons entering the bloodstream following a meal high in fat. After a 12-hour period of fasting, there should be no chylomicrons because they are typically eliminated within 9 to 12 hours. The size of these brief drops in HDL and LDL cholesterol is influenced by the amount of fat in the meal.

Plasma versus serum
Anticoagulants typically have osmotic effects, which cause water to leave cells and move into the plasma, dilating the plasma and reducing the quantities of non-diffusible components. The strength of this impact is influenced by the concentration and type of anticoagulant being taken. Compared to EDTA plasma, serum concentrations of triglycerides and cholesterol are roughly 3–5% higher, whereas HDL concentrations show no appreciable difference between serum and plasma. Therefore, it is likely that the blood concentrations of lipids and lipoproteins more precisely represent the participants' physiological state at the moment of venipuncture.

Sample volume requirements
0.5 ml of total cholesterol and/or triglycerides; 0.2 ml of total cholesterol and 0.5 ml of HDL measured using the direct technique. Any samples that are left over after analyses are finished are sent back to -80^{o}C and then transferred to the serum bank.

Serum shipment and storage container
Plastic, screw-top cryo-vials are utilised as the container for shipping and storing serum. For children aged 3-5 and those beyond 5, different size vials are used.

Sample stability and storage
Serum can be kept at -20°C in a freezer that doesn't self-defrost for up to 4 weeks. They should be kept at -80°C or lower for longer storage (more than 4 weeks). At -80^{o}C or lower, total cholesterol, triglycerides, and HDL cholesterol remain steady for at least a year.

Analytical methods for quantification of cholesterol
The Abell-Kendall modified approach
This is a common reference technique for calculating total blood cholesterol. It is a multi-step, classical chemical process that entails (8)

- Treating the serum with alcoholic potassium hydroxide to dissolve the cholesterol esters and release it from the lipoprotein complexes;

- After diluting the alcoholic solution with water, the cholesterol is extracted into a predetermined volume of petroleum ether.
- Using the Liebermann-Burchard colour reaction to measure the cholesterol in a portion of the petroleum ether layer.

The Liebermann-Burchard reagent can produce colour in the presence of compounds other than cholesterol, hence the counter-current distribution approach was used to test the method's specificity (9).

Fluoro metric-enzymatic assay(7,8,10,11,12)

Using a brand-new fluorogenic H_2O_2 probe called Amplex Red; a Fluoro metric approach for the enzyme-based detection of cholesterol levels has been created. This experiment is carried out on a 96-well micro plate and is a one-step process that can be automated. Our assay, which uses commercially available cholesterol, is 100 times more sensitive than published Fluoro metric and colorimetric approaches, allowing detection of 5pmol (2 ng) cholesterol per well. Since the oxidation product of the Amplex Red method has superior long wavelength spectra and is less sensitive to interference from biological components, it has been shown to be more appealing when used to measure the levels of cholesterol in serum and food samples.

Total cholesterol

Through a chain of linked processes that hydrolyse cholesteryl esters and oxidise the 3-OH group of cholesterol, total cholesterol is detected enzymatically in serum or plasma. H_2O_2 is quantified as one of the processes' by-products in a peroxidase-catalysed reaction that creates colour. At 500 nm, absorbance is measured. The concentration of cholesterol has a direct relationship with colour intensity. The following is the order of the reactions

Cholesteryl ester hydrolase

Cholesteryl ester + H_2O ----------->cholesterol + fatty acid

Cholesterol oxidase

Cholesterol + O_2 ----------> cholest-4-en-3-one + H_2O_2

Peroxidase

$2H_2O_2$ + 4-aminophenazone + phenol ----------> 4-(p-benzoquinonemnoimino) - phenazone + 4 H_2O

Triglyceride

Levels are determined enzymatic ally by measuring the production of glycerol from the hydrolysis of triglycerides in serum or plasma. Then, using glycerol oxidase, glycerol is subjected to oxidation, and one of the reaction products, H_2O_2, is determined as for total cholesterol. At 500 nm, absorbance will be measured. The following is the order of the reactions:

Lipase

Triglycerides + $3H_2O$ ----------> glycerol + fatty acids

Glycerokinase

Glycerol + ATP ----------> glycerol-3-phosphate + ADP

Glycerophosphate oxidase

Glycerol-3-phosphate + O_2 ----------->dihydroxyacetone phosphate + H_2O_2

Peroxidase

H_2O_2 + 4-aminophenazone + 4-chlorophenol ----------> 4-(p-benzoquinone- monoimino)-phenazone + $2H_2O$ + HCl.

HDL

Serum is used to directly measure HDL. The method's fundamental tenet is as follows. The ApoB-containing lipoproteins in the specimen are subjected to a blocking reaction that makes them inert to the assay's enzymatic cholesterol reagent. As a result, the assay effectively excludes the lipoproteins carrying ApoB, and under the assay conditions, only HDL-cholesterol is found. The procedure employs sulphated alpha-cyclodextrin, which binds to ApoB-containing lipoproteins and forms complexes with them, as well as polyethylene glycol-coupled cholesteryl esterase and cholesterol oxidase for the detection of HDL cholesterol. These are the responses:

- ApoB containing lipoproteins + α-cyclodextrin + Mg^{+2} + dextran SO_4 ---> soluble non-reactive complexes with ApoB-containing lipoproteins

PEG-cholesteryl esterase

- HDL-cholesteryl esters ----------> HDL-un-esterified cholesterol + fatty acid

-cholesterol oxidase

- Un-esterified cholesterol + O_2 PEG ----------> cholestenone + H_2O_2
- H_2O_2 + 5-aminophenazone + N-ethyl-N-(3-methylphenyl)-N'_succinyl ethylene diamine + H_2O + H^+ peroxidase >qunoneimine dye + H_2O

Absorbance is measured at 600 nm

Gas and liquid chromatography (13, 14, 15)

These techniques are employed to determine cholesterol on a regular basis. It is employed to separate cholesterol from other species that might interfere. It can be used to measure the amounts of total, esterified, and free cholesterol (based on sample pre-treatment). These techniques offer more precision than conventional chemical and enzymatic techniques. Prior to analysis, thorough sample preparation, including derivatization, is necessary.

GC/GC-MS: gas chromatography-mass spectrometry (16, 17, 18, 19)

Gas chromatography negative ion chemical ionisation mass spectrometry (GC-NCI-MS) is a potentially useful analytical tool for the investigation of cholesterol metabolism because of its high sensitivity. Pentafluorobenzoyl cholesterol was chosen out of a number of derivatives that were prepared for potential use in tracer studies because it formed quickly at room temperature and remained stable for a long time, could be detected at a level of 1 fmol, and produced a mass spectrum in which the molecular ion was the main component. With a coefficient of variation averaging 3.2 percent, hexadeuterated cholesterol tracer ([26,26,26,27,27,27-2H6]cholesterol) could be detected in unlabeled cholesterol at dilutions as high as 2700.

LC/LC-MS: liquid chromatography-mass spectrometry (20, 21, 22)

To measure total cholesterol in serum, we have created a liquid chromatography-isotope dilution mass spectrometry technique. The mass spectrometer and liquid chromatograph were linked using a particle-beam interface. The ions m/z = 386 and m/z = 389 were used for selective ion monitoring of cholesterol and the internal standard [25,26,27-13C]cholesterol after electron impact ionisation. For serum materials, alkaline hydrolysis and an extraction of the cholesterol into the cyclohexane phase are essential sample preparation procedures.

Matrix-assisted laser desorption/ionization mass spectrometry (23, 24, 25)

Matrix-assisted laser desorption/ionization (MALDI) is not a viable ionisation technique for neutral free sterol molecules. However, there is growing proof that MALDI-MS can be used to measure cholesterol. It can be used to analyse the cholesterol content in human serum lipoproteins. The lipids were mixed with the organic matrix (2,5-dihydroxybenzoic acid, or 2,5-DHB), which had been extracted from the serum and dissolved in chloroform-methanol (Folch's solution). Lipoprotein lipid signals were observed in the range of 369e910 m/z, whereas signals from the 2,5-DHB matrix were seen in the region of 154e551 m/z. A signal from dehydrated cholesterol was found at m/z 369 [MH2OH]. Quantitative measurement was achieved by using 4-cholesten-3-one as an internal standard to correct for instrument variation. Infrared matrix-assisted laser desorption electro spray ionisation was improved with the use of silver cat ionization (IR-MALDESI). Silver nitrate (AgNO3) was doped into the ESI solvent in minute

concentrations. Additionally, a MALDI-IM-MS (matrix-assisted laser desorption/ionization/ion mobility-mass spectrometry) strategy for profiling cholesterol and 7-dehydrocholesterol (7-DHC) in human fibroblast cells was reported, suggesting the possibility of investigating disorders of cholesterol biosynthesis using mass spectrometry-based techniques.

Ambient ionization mass spectrometry (26, 27, 28, 29)

For real-time, in-situ, and quick mass spectrometric analysis using ambient ionisation techniques, just a small amount of sample preparation is necessary. A family of ambient ionisation techniques was created as a result of the development of desorption electro spray ionisation (DESI) and direct analysis in real time (DART), which were first described in 2004 and 2005, respectively. These techniques are superior than direct ESI approaches for cholesterol ionisation and can be used to test cholesterol quantitatively. Condensed-phase samples, which are frequently complicated mixtures, are handled in these procedures in a way that causes analytes to be desorbed and ionised. The two phases may take place concurrently while being influenced by a specific substance, or they may take place independently. A phase shift occurs during the desorption stage (e.g., solid to vapour phase). In DESI, a condensed-phase sample is impacted by a spray of charged droplets on a substrate, forming a thin liquid layer. In this liquid film, analytes dissolve, and as new droplets come in contact with it, they splash into the film, forming a shower of microdroplets that contain the dissolved analyte. The secondary droplets are pulled into a mass spectrometer's air intake, where the solvent is destroyed by heat and vacuum and the analyte's ionised form is mass measured. Therefore, in DESI, ionisation occurs via the common electrospray ionisation (ESI) method while desorption is accomplished through the action of charged micro droplets. In DART, an electrical potential is applied to a plasma to produce excited-state atoms and ions from a gas (nitrogen or helium) at atmospheric pressure. The resulting plasma is heated and thrown over the sample, where it reacts with ambient gases like water to produce excited-state atoms and typically ions. Desorbed into the gas phase, analyte molecule ions (usually in the 20–1,000 Da range) are examined by MS. Ionization is primarily caused by ion/molecule reactions with solvated hydronium ions and hydroxyl anions, while desorption is thermally aided.

Direct analysis in real time mass spectrometry of serum cholesterol (30)

Human serum samples were screened for endogenous free cholesterol utilising a DART ion source for direct measurement in real-time mass spectrometry of serum cholesterol. A tiny amount of chromatography paper was loaded with 0.5 mL of serum, followed by the same amount of internal standard. The paper substrates were then secured to the transmission module, which was then driven toward the DART ionisation zone for mass spectrometric analysis. The name of this methodology was pDART-MS. Serum cholesterol quickly interacted with the plasma-induced metastable species and effectively desorbed from the paper substrate. In the mass range of 50–1000 m/z, the signal at m/z 369.5, which corresponds to dehydrated cholesterol, was the predominate signal.

CONCLUSION

Hyperlipidemia, sometimes referred to as hyper-lipoproteinemia or high cholesterol, is a condition marked by unusually high blood lipid (fat) concentrations that are linked to the onset of atherosclerosis, the underlying cause of coronary heart disease (CHD) and stroke. Abnormal lipid and lipoprotein metabolism leads to hyperlipidemia. Numerous human disorders, including heart disease, stroke, type II diabetes, brain ailments, and many more, have been linked to abnormally high amounts of cholesterol or its precursors. Therefore, for people who have a higher risk of developing chronic disorders, proper cholesterol measurement is crucial. Various analytical techniques have been created to date for the analysis of cholesterol, including enzymatic assays, conventional chemical techniques, gas chromatography (GC), liquid chromatography (LC), and mass spectrometry (MS). However, new analytical platforms are being created and used, such as ambient ionisation mass spectrometry. The possibility of using DART-MS, a promising technology for quick and affordable cholesterol quantification, in POCT was raised. To see the spatial distribution of cholesterol and its derivatives in tissue slices, however, methods like MALDI-MSI and DESI-MSI were employed.

REFERENCES

1. Kalita S, Hazarika A., A Mini-review on Plants with Potential Antihyperlipidemic Properties of Northeast India, International Research Journal of Plant Science, 2021; 12(3), 01-11.
2. Madhekar SM, AArsul V, Abrar SM. Evaluation of Anti-hyperlipidemic activity of various fraction & sub-fractions of Momordica charantia (L).
3. Dhaliya S, Surya A, Dawn V, Betty C, Arun K, Sunil C. A review of hyperlipidemia and medicinal plants. Int JA PS BMS. 2013 Oct; 2(4):219-37.
4. Shattat GF. A review article on hyperlipidemia: types, treatments and new drug targets. Biomedical and Pharmacology Journal. 2015 May 3;7(1):399-409.
5. Onwe PE, Folawiyo MA, Okike PI, Balogun ME, Umahi G, Besong EE, Okorocha AE, Afoke AO. Lipid profile and the growing concern on lipid related diseases. IOSR Journal of Pharmacy and Biological Sciences (IOSJPBS). 2015;10:2278-3008.
6. Bradley RD, Oberg EB. Are additional lipid measures useful? Integrative medicine (Encinitas, Calif.). 2008 Dec;7(6):18.
7. Laboratory Procedure Manual for Total Cholesterol, HDL-Cholesterol, Triglycerides, and LDL-Cholesterol performed by Lipid Laboratory Johns Hopkins University School of Medicine Lipoprotein Analytical Laboratory.
8. Li LH, Dutkiewicz EP, Huang YC, Zhou HB, Hsu CC. Analytical methods for cholesterol quantification. Journal of food and drug analysis. 2019 Apr 1;27(2):375-86.
9.] Abell LL, Levy BB, Brodie BB, Kendall FE. A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. J BiolChem 1952;195:357e66
10. Allain CC, Poon LS, Chan CS, Richmond WF, Fu PC. Enzymatic determination of total serum cholesterol. Clinical chemistry. 1974 Apr 1;20(4):470-5.
11. Amundson DM, Zhou M. Fluorometric method for the enzymatic determination of cholesterol. Journal of biochemical and biophysical methods. 1999 Jan 13;38(1):43-52.
12. Carr TP, Andresen CJ, Rudel LL. Enzymatic determination of triglyceride, free cholesterol, and total cholesterol in tissue lipid extracts. Clinical biochemistry. 1993 Feb 1;26(1):39-42.
13. KANAI M. Gas chromatographic separation of sterols and its clinical application. The Journal of Biochemistry. 1964 Sep 1;56(3):266-72.
14. Takatsu A, NIshI S. Total cholesterol in serum determined by isotope dilution/mass spectrometry, with liquid-chromatographic separation. Clinical chemistry. 1987 Jul 1;33(7):1113-7.
15. Ellerbe P, Meiselman S, Sniegoski LT, Welch MJ, White E. Determination of serum cholesterol by a modification of the isotope dilution mass spectrometric definitive method. Analytical chemistry. 1989 Aug 1;61(15):1718-23.
16. Beggio M, Cruz-Hernandez C, Golay PA, Lee LY, Giuffrida F. Quantification of total cholesterol in human milk by gas chromatography. Journal of separation science. 2018 Apr;41(8):1805-11.
17. Ostlund Jr RE, Hsu FF, Bosner MS, Stenson WF, Hachey DL. Quantification of cholesterol tracers by gas chromatography–negative ion chemical ionization mass spectrometry. Journal of mass spectrometry. 1996 Nov;31(11):1291-6.
18. Lian K, Zhang P, Wang W, Dai T, Li L. Determination of Total Cholesterol in Serum by Gas Chromatography-Mass Spectrometry. Asian Journal of Chemistry. 2014 May 1;26(9).
19. Pelletier O, Wright LA, Breckenridge WC. Isotope dilution/mass spectrometry of serum cholesterol with [3, 4-13C] cholesterol: proposed definitive method. Clinical chemistry. 1987 Aug 1;33(8):1403-11.
20. Kock R, Delvoux B, Greiling H. Determination of total cholesterol in serum by liquid chromatography–isotope dilution mass spectrometry. Clinical chemistry. 1997 Oct 1;43(10):1896-903.
21. Duncan IW, Culbreth PH, Burtis CA. Determination of free, total, and esterified cholesterol by high-performance liquid chromatography. Journal of Chromatography B: Biomedical Sciences and Applications. 1979 Mar 1;162(3):281-92.
22. Chen WX, Li PY, Wang S, Dong J, Li JZ. Serum cholesterol determined by liquid chromatography with 6-chlorostigmasterol as internal standard. Clinical chemistry. 1993 Aug 1;39(8):1602-7.

23. Hidaka H, Hanyu N, Sugano M, Kawasaki K, Yamauchi K, Katsuyama T. Analysis of human serum lipoprotein lipid journal of food and drug analysis 27 (2019) 375 e386 385 composition using MALDI-TOF mass spectrometry. Ann Clin Lab Sci 2007;37:213e21
24. Xu L, Kliman M, Forsythe JG, Korade Z, Hmelo AB, Porter NA, McLean JA. Profiling and imaging ion mobility-mass spectrometry analysis of cholesterol and 7-dehydrocholesterol in cells via sputtered silver MALDI. Journal of The American Society for Mass Spectrometry. 2015 Mar 31;26(6):924-33.
25. Meier F, Garrard KP, Muddiman DC. Silver dopants for targeted and untargeted direct analysis of unsaturated lipids via infrared matrix-assisted laser desorption electrospray ionization (IR-MALDESI). Rapid Communications in Mass Spectrometry. 2014 Nov 30;28(22):2461-70.
26. Ifa DR, Jackson AU, Paglia G, Cooks RG. Forensic applications of ambient ionization mass spectrometry. Analytical and bioanalytical chemistry. 2009 Aug;394(8):1995-2008.
27. Hsu CC, Dorrestein PC. Visualizing life with ambient mass spectrometry. Current Opinion in Biotechnology. 2015 Feb 1;31:24-34.
28. Takats Z, Wiseman JM, Gologan B, Cooks RG. Mass spectrometry sampling under ambient conditions with desorption electrospray ionization. Science. 2004 Oct 15;306(5695):471-3.
29. Cody RB, Laramée JA, Durst HD. Versatile new ion source for the analysis of materials in open air under ambient conditions. Analytical chemistry. 2005 Apr 15;77(8):2297-302.
30. Hsieh HY, Li LH, Hsu RY, Kao WF, Huang YC, Hsu CC. Quantification of endogenous cholesterol in human serum on paper using direct analysis in real time mass spectrometry. Analytical chemistry. 2017 Jun 6;89(11):6146-52.

Table 1: Normal Range of Lipid Panel According to National Cholesterol Education Program (Ncep) Guidelines(6)

TOTAL CHOLESTEROL	Desirable (low)	<200 mg/dl
	Borderline high	200-239 mg/dl
	High	240 mg/dl or greater
HDL CHOLESTEROL	Desirable (high)	>60 mg/dl
	Acceptable	40-60 mg/dl
	Low	<40 mg/dl
LDL CHOLESTEROL	Desirable (low)	<100 mg/dl
	Acceptable	100-129 mg/dl
	Borderline high	130-159 mg/dl
	High	160-189 mg/dl
	Very high	190 mg/dl or greater
TRIGLYCERIDES	Desirable (low)	<150 mg/dl
	Borderline high	150-199 mg/dl
	High	200-499 mg/dl
	Very high	500 mg/dl or greater

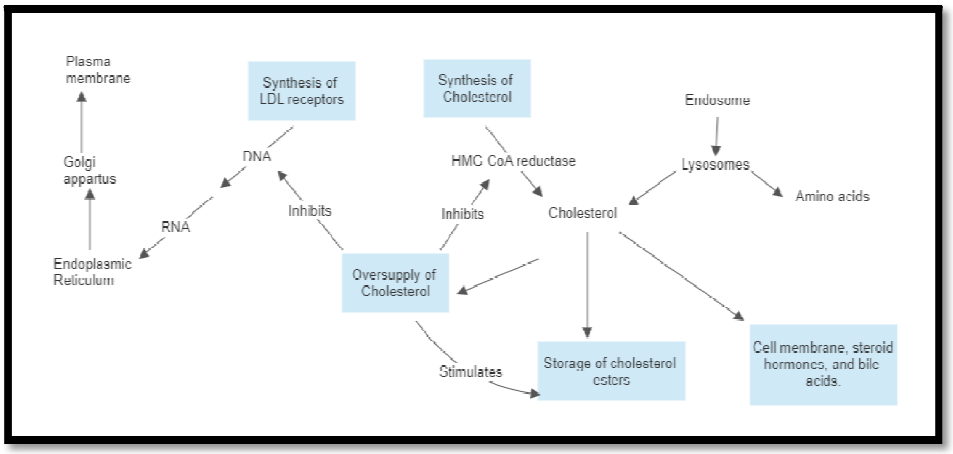

Figure 1: Mechanism of Lipid Transport

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RESEARCH ARTICLE

Recognition of Speech in Noisy Environments to Improve the Speech Recognition by using Various Neural Networks

V.Thiyagu[1] and T.Sheela[2]*

[1]Research Scholar, Department of Electronics and Communication Engineering,Vinayaka Mission's Research Foundation (Deemed to be University), Salem, Tamil Nadu, India.
[2]Associate Professor, Department of Electronics and Communication Engineering, Vinayaka Mission's Kirupananda Variyar Engineering College, Vinayaka Mission's Research Foundation (Deemed to be University), Salem, Tamil Nadu, India.

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***Address for Correspondence**
T.Sheela,
Associate Professor,
Department of Electronics and Communication Engineering,
Vinayaka Mission's Kirupananda Variyar Engineering College,
Vinayaka Mission's Research Foundation (Deemed to be University),
Salem, Tamil Nadu, India.

ABSTRACT

In modern world speech recognition is one of the techniques which is used to capture the voice of human using Microphone to control the appliances, robot, etc, While we using the Human interaction System in highly noisy environmental area recognition of speech capture by the system can't recognizing a actual command we pass, utterance of the words capture by the system get confused to capture actual command. To avoid such a noise problem in the environment during speech recognition, we use the various neural network algorithms to reduce the problem and to recognize a speech in both indoor and outdoor noise environments. In this paper we discussed the various neural network algorithms in speech recognition to get high accuracy rate in results.

Keywords: Neural networks**,** Speech recognition, Human interaction System, robot.

INTRODUCTION

Now a day's communication through voice is easy to access the entire medium and to reduce the effort of human's time. It is more efficient and effective communication through voice rather than using mouse and keyboard for communication purpose. For example while the lecturer present there topic in front of the students the students take the lecturer in the note book for the reference it is not easy to take all lecturing in the SR-mediated lecture acquisition (*SR-mLA*) will helps to copy all the lecturing by using voice recognition this technical will convert the lecturer speech

into transcript so it is easy to covert the speech into text via using speech recognition mlA. If the unwanted noise is produce while taking lecturer to the student the mediator lecturer acquisition get confused to covert speech into text to avoid this kind of problem we have to use the various algorithms to reduces the environmental noise to get high input of recognized the actual speech . In neural network there are a various algorithms to analysis speech recognition such as artificial neural network (ANN), PNCC (Power Normalized Cepstral Coefficients), Convolutiona**l** neural network (CNN). By analysis these algorithm to find which one is suitable to recognize the actual speech in both indoor and outdoor noise environments.

Artificial Neural Network

Artificial neural networks were employed in this study to complete isolated voice recognition. The subject was researched in two stages, first using Digital Signal Processing (DSP) techniques for the pre-processing and then using Artificial Neural Networks for the post-processing (ANN). These two components were briefly explained, and Mat lab speech recognizers were developed using several ANN topologies. Elman, multi-layer back propagation, and probabilistic neural networks are three alternative models of neural networks that were created. Performance comparisons with neural network that are similar to the proposed ANN architectures produce good outcomes. Artificial neural networks are a subset of soft computing technologies that analyze numerical data by connecting computational primitives. These were influenced by the way the human nervous system, which is made up of millions of nerve cells or neurons, functions. Based on the storage, transmission, and processing features of the human nervous system, various artificial neural network topologies have developed over time. These networks typically function in one of two modes, supervised mode or unsupervised mode. The network must be trained using a training set of data in the supervised mode of operation. Topology preservation strategies are used by networks working in the unsupervised mode to learn inputs.

Power Normalized Cepstral Coefficients

The most organic and vocalized form of human communication is speech. The speaker's words are understood by the computer thanks to automatic speech recognition. The feature extraction method known as Power Normalized Cepstral Coefficients (PNCC) makes use of the nonlinearity of the power law. The system loses accuracy as the ambient conditions change. Better acoustical environment enhancement is offered by PNCC. The intelligibility of the improved speech signal is assessed in this work using an objective metric called SNR loss. The evaluation result for the improved speech signal after SNR loss demonstrates that PNCC processing offers strong robustness in acoustical conditions.

Convolutional Neural Network

The employment of a voice recognition model has grown significantly in significance. Speech recognition features were combined with deep convolutional neuro-learning techniques to create a word-tracking model in our study. Speech control has grown in importance. There are six control words (start, stop, forward, backward, right, and left). Words from a range of age groups. Our voice dataset, which is used to train and test suggested deep neural networks, is contributed in an equal split by men and women. Gather information at several locations, like the market, lab, park, and street. For thirty persons, the duration of each word varied from 1 to 1.30 seconds. In order to categorize each word from our pooled data set as a multi-class classification problem, Convolutional Neural Network (CNN) is used as an advanced deep neural network with a completely unidentified speech sample; the suggested deep neural network produced a word classification accuracy score of 97.07 percent. Our data is trained and tested using CNN. Unlike many other articles, which frequently use pre-made and generally reliable data of the isolated word kind, our work stands out While our data is gathered from two forms of speech—isolated words and continuous words—in various noisy situations and under various circumstances.

Analysis of the entire neural network

The recognized speech ratio compares to the Artificial Neural Networks the pre-processing and post processing is highly effective in both indoor and outdoor noise environments. But in post processing the PNCC will good outcome in indoor area. Convolutional Neural Network is used in the deep neural network to achieve the accuracy rate of

97.07% accuracy level in all the environments in this deep neural network no another algorithms will be involved for research process . In machine learning process the performances of artificial neural network will plays the major role to recognize speech in highly better to the entire neural network. It is also a simple neural system which communicates and recognized the speech gradually.

CONCLUSION

As the results of neural network, entire algorithm will have unique quality of recognition the speech. The pronunciation of word will be in actual sound, the speech recognition will do accurately. If not, the system gets confused and results may be failure to recognize the word. Compared to neural network all the three algorithms will be suitable for recognizing speech .Exact result will be word pronunciation and the system actual code will be same, it has been identified in all the environmental areas. In machine and deep neural network the word utterance will be highly noticeable .if we pronounced actual word to the system it activates and system get responded to the speaker in both indoor and outdoor noise area.

REFERENCES

1. Fadlilah A. F., Djamal E. C. (2019) Speaker and speech recognition using hierarchy support vector machine and back propagation. In: 2019 6th International Conference on Electrical Engineering, Computer Science and Informatics (EECSI). IEEE, p. 404-409
2. Shaikh Naziya S., Deshmukh R. R. (2016) Speech recognition system—a review. IOSR J. Comput. Eng, 8.4: 3-8.
3. Aderhold J, Davydov V Yu, Fedler F, Klausing H, Mistele D, Rotter T, Semchinova O, Stemmer J and Graul J 2001 J. Cryst. Growth 222 701 [3] Kesarkar M. P., Rao, P. (2003) Feature extraction for speech recognition. Electronic Systems, EE. Dept., IIT Bombay.
4. Alías F., Socoró J. C., Sevillano X. (2016) A review of physical and perceptual feature extraction techniques for speech, music and environmental sounds. Applied Sciences, 6(5), 143.
5. Student P. G. (2016) Feature Selection and Extraction of Audio Signal. algorithms, 5(3).
6. Abdel-Hamid O., Mohamed A. R., Jiang, H., Deng L., Penn, G., Yu D. (2014) Convolutional neural networks for speech recognition. IEEE/ACM Transactions on audio, speech, and language processing, 22(10), 1533-1545.
7. Li X., Zhou Z. (2017) Speech Command Recognition with Convolutional Neural Network. CS229 Stanford education.
8. Poudel S., Anuradha, R. (2020) Speech Command Recognition using Artificial Neural Networks. JOIV: International Journal on Informatics Visualization, 4(2), 73-75.
9. Song Z. (2020) English speech recognition based on deep learning with multiple features. Computing, 102(3), 663-682.
10. Passricha V., Aggarwal R. K. (2019) Convolutional support vector machines for speech recognition. International Journal of Speech Technology, 22(3), 601-609.
11. Yang X., Yu H., Jia L. (2020) Speech recognition of command words based on convolutional neural network. In: 2020 International Conference on Computer Information and Big Data Applications (CIBDA) (pp. 465-469).
12. Saitoh T., Zhou Z., Zhao, G., Pietikäinen M. (2016) Concatenated frame image based cnn for visual speech recognition. In: Asian Conference on Computer Vision. p. 277-289.15- Phung, Son Lam, and Abdesselam Bouzerdoum. "Visual and Audio Signal Processing Lab University of Wollongong", 2009.
13. Nanni L., Costa Y. M., Aguiar R. L., Mangolin, R. B., Brahnam S., Silla C. N. (2020) Ensemble of convolutional neural networks to improve animal audio classification. EURASIP Journal on Audio, Speech, and Music Processing, 1-14.
14. Patel S. (2020) A Comprehensive Analysis of Convolutional Neural Network Models. International Journal of Advanced Science and Technology, 29(4), 771-777.
15. Kubanek M., Bobulski J., Kulawik, J. (2019) A method of speech coding for speech recognition using a convolutional neural network. Symmetry, 11(9), 1185.

16. Nwankpa C., Ijomah W., Gachagan, A., Marshall, S. (2018) Activation functions: Comparison of trends in practice and research for deep learning. arXiv preprint arXiv:1811.03378.
17. Phung S. L., Bouzerdoum A. (2009) Visual and Audio Signal Processing Lab University of Wollongong.

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RESEARCH ARTICLE

On Kasaj Generalized Semi and Semi Generalized Closed Sets in Kasaj Topological Space

E.Prakash[1]*, P. Sathishmohan[1],K.S.Viplavanjali[2] and K.Rajalakshmi[3]

[1]Assistant Professor, Department of Mathematics, Kongunadu Arts and Science College (Autonomous), Coimbatore,Tamil Nadu,India.
[2]Research Scholar, Department of Mathematics, Kongunadu Arts and Science College (Autonomous), Coimbatore,Tamil Nadu, India.
[3]Assistant Professor, Department of Science and Humanities, Sri Krishna College of Engineering and Technology, Coimbatore, Tamil Nadu, India.

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***Address for Correspondence**
E.Prakash,
Assistant Professor,
Department of Mathematics,
Kongunadu Arts and Science College (Autonomous),
Coimbatore,Tamil Nadu,India.

ABSTRACT

The objective of this paper is to introduce and study the concept of Kasaj Generalized Semi Closed and Kasaj Semi Generalized Closed sets and to investigate some Characterization

AMS Subject Classifications: 54A05, 54B05

Keywords: $KS_R(X)$,KS-Semi-closed, KS-Pre-closed, KS-α-closed, KS-β-closedKS_{gs}-closed set, KS_{sg}-closed set.

INTRODUCTION AND PRELIMINARIES

In 2020, Kashyap.G.Rachchh and Sajeed.I.Ghanchi [1] introduced partial extension of Micro Topological Space namely Kasaj Topological Spaces. A subset P of U is said to be Kasaj generalized closed set [3] if $KS_{cl}(P) \subseteq V$whenever $P \subseteq V$ and V is Kasaj-open set in $KS_R(X)$. The complement of Kasaj generalized closed set is called Kasaj generalized open set. The Kasaj topology is defined by $KS_R(X) = \{(K \cap S) \cup (K' \cap S'): K, K' \in \tau_R(X)$, fixed $S, S' \notin \tau_R(X), S \cup S' = U\}$
The Kasaj topology $KS_R(X)$ satisfies the following postulates:
1. $U, \phi \in KS_R(X)$
2. The union of elements of any subcollection of $KS_R(X)$ is in $KS_R(X)$
3. The intresection of any finite subcollection of elements of $KS_R(X)$ is in $KS_R(X)$

Then $(U, \tau_R(X), KS_R(X))$ is called Kasaj Topological Spaces and the members of $KS_R(X)$ are called Kasaj-open (KS-open)set and the complement of a Kasaj-open set is called a Kasaj-closed (KS-closed)set and the collection of all Kasaj-closed sets is denoted by KSCL(X). KS-Semi-closed [1], if $KS_{int}(KS_{cl}(P)) \subseteq V$; KS-Pre-closed [1], if $KS_{cl}(KS_{int}(P)) \subseteq V$; KS-$\alpha$-closed [1], if $KS_{cl}(KS_{int}(KS_{cl}(P))) \subseteq V$; KS-$\beta$-closed [1], if $KS_{int}(KS_{cl}(KS_{int}(P))) \subseteq V$. The objective of this paper is to introduce and study about KS_{gs}-closed, KS_{sg}-closed sets and to illustrate some characterizations.

KS_{gs}-CLOSED SET AND KS_{sg}-CLOSED SET

In this section, we initiate a new type of sets which we call Kasaj Generalized Semi Closed Sets and Kasaj Semi Generalized Closed Sets in Kasaj Topological Space and investigate some characterization theorems. In this paper we use the following symbolsKasaj closed, Kasaj open, Kasaj Generalized Semi Closed, Kasaj Semi Generalized Closed, Kasaj Generalized Semi open, Kasaj Semi Generalizedopen will be denoted asKS-C , KS-O, KS_{gs}-*C*,KS_{sg}-*C*, KS_{gs}-*O and* KS_{sg}-*O*.

Definition 2.1 A subset P of U in $(U,\tau_R(X),KS_R(X))$ is called a KS_{gs}-C set , if $KS_{scl}(P) \subseteq V$ whenever $P \subseteq V$ and V is KS-O set in $KS_R(X)$. The complement of the KS_{gs}-C in $KS_R(X)$ isKS_{gs}-O set in $KS_R(X)$.

Definition 2.2 A subset P of U in $(U,\tau_R(X),KS_R(X))$ is called a KS_{sg}-C set, if $KS_{scl}(P) \subseteq V$ whenever $P \subseteq V$ and V is KS semi-O set in $KS_R(X)$. The complement of the KS_{sg}-C set in $KS_R(X)$ is KS_{sg}-O set in $KS_R(X)$.

Theorem 2.3 For a KS topological space $(U,\tau_R(X),KS_R(X))$ the subsequent conditions are hold

1. Every KS-C set is KS_{gs}-C set.
2. Every KS-semi C set is KS_{gs}-C set.
3. Every KS-pre-C set is KS_{gs}-C set.
4. Every KS-α-C set is KS_{gs}-C set.
5. Every KS-β-C set is KS_{gs}-C set.
6. Every KS_g-C set is KS_{gs}-C set.
7. Every KS_{sg}-C set is KS_{gs}-C set.

Proof:

1. Let P be a KS-C set of U and $P \subseteq V$ and V is KS-O in U. Since, P is KS-C.
$KS_{cl}(P) = P \subseteq V$. So, $KS_{scl}(P) \subseteq KS_{cl}(P) \subseteq V$. Therefore $KS_{scl}(P) \subseteq V$.
Hence, P is KS_{gs}-C set.
2. (2) to (7) is Obvious.

The reverse of an implications is untrue from the subsequent exemplar.

Example 2.4 *Let U = {அ, ஆ, இ, ஈ, உ} with U/R = {{அ,ஈ},{இ,உ},{ஆ}} and X = {அ,ஆ}*$\subseteq U$.Then$\tau_R(X)$ = {ϕ, U, {ஆ}, {அ, ஆ, ஈ}, {அ, ஈ}} . If we consider S={அ}S'={ஆ,இ,ஈ,உ}
$KS_R(X)$={ϕ,{அ},{ஆ},{ஈ},{அ,ஆ},{அ,ஈ},{ஆ,ஈ},{அ,ஆ,ஈ},{ஆ,இ,ஈ,உ},U}.
Then the subset {ஆ, இ, ஈ} is KS_{gs}-C but not KS-C, KS-semi C, KS-pre-C, KS-alpha-C, KS-beta-C, KS_g-C, KS_{sg}-C.

Theorem 2.5 For a KS topological space $(U,\tau_R(X),KS_R(X))$ the subsequent conditions are hold

1. Every KS-C set is KS_{sg}-C set.
2. Every KS-pre-C set is KS_{sg}-C set.
3. Every KS-α-C set is KS_{sg}-C set.

Proof

1. Let P be a KS-C set of U and $P \subseteq V$ and V is KS semi-O in U. Since, P is KS- C.
$KS_{cl}(P) = P \subseteq V$. So, $KS_{scl}(P) \subseteq KS_{cl}(P) \subseteq V$. Therefore $KS_{scl}(P) \subseteq V$. Hence, P

is KS_{sg}-C set.

2. (2) and (3) Obvious.

The reverse of an implications untrue from the subsequent exemplar.

Example 2.6 *Let U = {அ,ஆ,இ,ஈ,உ} with U/R = {{அ,இ},{ஈ,உ},{ஆ}} and X = {அ,ஆ}*$\subseteq U$.Then $\tau_R(X)$ = {$\phi, U,$ {ஆ}, {அ, ஆ, இ}, {அ, இ}}. If we consider S={இ} S'={அ,ஆ,ஈ,உ}

$KS_R(X)$= {ϕ,{அ},{ஆ},{இ},{அ,ஆ},{அ,இ},{ஆ,இ},{அ,ஆ,இ},{அ,ஆ,ஈ,உ},U}. Then the subset {அ} is KS_{sg}-C but not KS-C, KS-pre-C, KS-α-C.

Remark 2.7 KS_g-C set and KS_{sg}-C are independent to each other it is shown by subsequent exemplar.

Example 2.8 *Let U = {அ,ஆ,இ,ஈ,உ} with U/R = {{இ,ஈ},{ஆ,உ},{அ}} and X = {அ,ஆ}*$\subseteq U$. *Then* $\tau_R(X)$ = {$\phi, U,$ {அ}, {அ, ஆ, *e.* }, {அ, ஆ, இ, ஈ}}. *If we consider S={உ}S´={அ,ஆ,இ,ஈ}*

$KS_R(X)$ ={ϕ,{அ},{ஆ},{உ},{அ,ஆ},{அ,உ},{ஆ,உ},{அ,ஆ,உ},{அ,ஆ,இ,ஈ},U}. Then the subset {அ,ஆ,இ} is KS_g-C but not KS_{sg}-C and the subset {அ} is KS_{sg}-C but not KS_g-C.

Theorem 2.9 The union of any two subset of the KS_{gs}(KS_{sg})-C set is KS_{gs} (KS_{sg})-C.

Proof: Let P and Q be $KS_{gs}(KS_{sg})$-C sets in U. Let V be KS-O(KS-semi O) in U. Such that $P \cup Q \subseteq V$. Then $P \subseteq V$ and $Q \subseteq V$.Since P and Q are $KS_{gs}(KS_{sg})$-C set. $KS_{scl}(P) \subseteq V$ and $KS_{scl}(Q) \subseteq V$. Hence $KS_{scl}(P \cup Q) = KS_{scl}(P) \cup KS_{scl}(Q) \subseteq V$. Therefore $P \cup Q$ is $KS_{gs}(KS_{sg})$-C.

Remark 2.10 The intersection of any two subset of the KS_{gs}-C set is need not be a KS_{gs}-C, as established from subsequent exemplar.

Example 2.11 *Let U = {அ,ஆ,இ,ஈ,உ} with U/R = {{இ,ஈ},{ஆ,உ},{அ}} and*$X = \{அ,ஆ\} \subseteq U$.Then

$\tau_R(X)$ = {$\phi, U,$ {அ}, {அ, ஆ, உ}, {ஆ, உ}}. If we consider S = {உ} S' = {அ,ஆ,இ,ஈ}

$KS_R(X)$={ϕ,{அ},{ஆ},{உ},{அ,ஆ},{அ,உ},{ஆ,உ},{அ,ஆ,உ},{அ,ஆ,இ,ஈ},U}

KS_{gs} = {U, ϕ, {அ}, {ஆ}, {இ}, {ஈ}, {உ}, {அ, இ}, {அ, ஈ}, {அ, உ}, {ஆ, இ}, {ஆ, ஈ}, {ஆ, உ}, {இ, ஈ}, {இ, உ}, {ஈ, உ}, {அ, ஆ, இ}, {அ, ஆ, ஈ}, {அ, இ, ஈ}, {அ, இ, உ}, {அ, ஈ, உ}, {ஆ, இ, ஈ}, {ஆ, இ, உ}, {ஆ, ஈ, உ}, {இ, ஈ, உ}, {அ, ஆ, இ, ஈ}, {அ, ஆ, இ, உ}, {அ, ஆ, ஈ, உ}, {அ, இ, ஈ, உ}, {ஆ, இ, ஈ, உ}}. Consider {அ,ஆ,இ} and {அ,ஆ,ஈ} are KS_{gs}-C sets. But their intersection {அ,ஆ} is not a KS_{gs}-C sets.

Remark 2.12 The intersection of any two subset of the KS_{sg}-C set is KS_{sg}-C, as established from the subsequent exemplar.

Example 2.13 By applying the Example 2.4 we establish the subsequent sets

KS_{sg} = {U, ϕ, {அ}, {ஆ}, {இ}, {ஈ}, {உ}, {அ, ஆ}, {அ, இ}, {அ, ஈ}, {அ, உ}, {ஆ, இ}, {ஆ, உ}, {இ, ஈ}, {இ, உ}, {ஈ, உ}, {அ, ஆ, இ}, {அ, ஆ, உ}, {அ, இ, ஈ}, {அ, இ, உ}, {அ, ஈ, உ}, {ஆ, இ, உ}, {இ, ஈ, உ}, {அ, ஆ, இ, உ}, {அ, இ, ஈ, உ}, {ஆ, இ, ஈ, உ}}. Consider {அ,ஆ} and {அ,இ} are KS_{sg}-C sets. But their intersection {அ} is a KS_{sg}-C sets.

Theorem 2.14 The intersection of the KS_{gs}(KS_{sg})-C set and KS_R(X)-C set is KS_g-C set.

Proof: Let P be a $KS_{gs}(KS_{sg})$-C subset of U and F is a $KS_R(X)$-C set. If V is KS-O set(KS- semi O set) of U with $P \cap F \subseteq V$ then $P \subseteq V \cup (U \backslash F)$. So, $KS_{scl}(P) \subseteq V \cup (U \backslash F)$. Then $KS_{cl}(P \cap F) = KS_{cl}(P) \cap KS_{cl}(F) \subseteq KS_{scl}(P) \cap KS_{cl}(F) = KS_{scl}(P) \cap F \subseteq V$. So, P∩F is a KS_g-C.

Theorem 2.15 If P is KS_{gs}(KS_{sg})-C set in KS_R(X)and P ⊆ Q ⊆ KS_{scl}(P) then Q is KS_{gs}(KS_{sg})-C set.

Proof: Let $Q \subseteq V$, where V is KS-O(KS-semi O) in $KS_R(X)$. Since P is $KS_{gs}(KS_{sg})$-C and $P \subseteq V$. Therefore $KS_{scl}(P) \subseteq V$. $Q \subseteq KS_{scl}(P)$ that implies $KS_{scl}(Q) \subseteq KS_{scl}(P)$. Hence, $KS_{scl}(Q) \subseteq V$ and So, Q is $KS_{gs}(KS_{sg})$-C set.

Theorem 2.16 For any elements அ ∈ U, the set U\{அ} is KS_{gs} (KS_{sg})-C set.
Proof: Suppose $U\backslash\{அ\}$ is not KS-O(KS-semi O), then U is the only KS-O(KS-semi O) set containing $U\backslash\{அ\}$. This implies $KS_{scl}(U\backslash\{அ\}) \subseteq U$. Hence, $U\backslash\{அ\}$ is $KS_{gs}(KS_{sg})$-C set in U.

Theorem 2.17 An KS_{gs} (KS_{sg})-C set P is KS-C if and only if KS_{scl} (P)\P is KS-C.
Proof:(Necessity) Let $KS_{gs}(KS_{sg})$-C set P is KS-C. Then $KS_{scl}(P) = P$ and so, $KS_{scl}(P)\backslash P = \phi$. Which is KS-C. (Sufficiency) Suppose $KS_{scl}(P)\backslash P$ is KS-C. Then $KS_{scl}(P)\backslash P = \phi$ since P is KS-C. That is $KS_{scl}(P) = P$ (or) P is KS-C.

Theorem 2.18 A subset P of $(U,\tau_R(X),KS_R(X))$ is KS_{gs} (KS_{sg})-C if KS_{scl} (P)\P contains no nonempty KS_{gs} (KS_{sg})-C set.
Proof: Suppose if P is $KS_{gs}(KS_{sg})$-C. Then $KS_{scl}(P) \subseteq V$ where $P \subseteq V$ and V is KS-O(KS-semi O). Let F be a KS-C subset of $KS_{scl}(P)\backslash P$. Then $P \subseteq F^c$ and F^c is KS-O(KS-semi O). Since P is $KS_{gs}(KS_{sg})$-C, $KS_{scl}(P) \subseteq F^c$ implies $F \subseteq [KS_{scl}(P)]^c$. That is $F \subseteq KS_{scl}(P)$ and $F \subseteq [KS_{scl}(P)]^c$ implies $F \subseteq \phi$. So F is nonempty.

Theorem 2.19 Let P be a KS_{gs} (KS_{sg})-C set in U. Then KS_{scl} (P)\P ⊉ ϕ KS_g-C set.
Proof: Assume that F is a KS_g-C subset of $KS_{scl}(P)\backslash P$. This implies that $F \subseteq KS_{scl}(P)$ and $F \subseteq U\backslash P$. Since $U\backslash F$ is a KS-O(KS-semi O) set, P is $KS_{gs}(KS_{sg})$-C and $KS_{scl}(P) \subseteq U\backslash F$. Therefore, $F \subseteq KS_{scl}(P) \cap (U\backslash KS_{scl}(P)) = \phi$. Hence, $KS_{scl}(P)\backslash P \not\supseteq \phi KS_g$-C set.

Theorem 2.20 Let P be a KS_{gs} (KS_{sg})-C set in U. Then KS_{scl} (P)\P ⊉ ϕ KS-C set.
Proof: Assume that F is a KS-C subset of $KS_{scl}(P)\backslash P$. This implies that $F \subseteq KS_{scl}(P)$ and $F \subseteq U\backslash P$. Since $U\backslash F$ is a KS-O(KS-semi O) set, P is $KS_{gs}(KS_{sg})$-C and $KS_{scl}(P) \subseteq U\backslash F$. Therefore, $F \subseteq KS_{scl}(P) \cap (U\ KS_{scl}(P)) = \phi$. Hence, $KS_{scl}(P)\backslash P \not\supseteq \phi$ KS-C set.

Theorem 2.21 Let P be a KS_g-C set in U. Then KS_{cl} (P)\P ⊉ ϕ KS_{gs} (KS_{sg})-C set.
Proof: Assume that F is a $KS_{gs}(KS_{sg})$-C subset of $KS_{cl}(P)\backslash P$. This implies that $F \subseteq KS_{cl}(P)$ and $F \subseteq U\backslash P$. Since $U\backslash F$ is a KS-O(KS-semi O) set, P is KS_g-C and $KS_{cl}(P) \subseteq U\backslash F$. Therefore, $F \subseteq KS_{cl}(P) \cap (U\backslash KS_{cl}(P)) = \phi$. Hence, $KS_{cl}(P)\backslash P \not\supseteq \phi KS_{gs}(KS_{sg})$-C set.

Theorem 2.22 If P is KS_g-O and KS_{gs} (KS_{sg})-O set in X, then P is KS-semi C.
Proof: As, P ∈ KS_g-O and $KS_{gs}(KS_{sg})$-C, then $KS_{scl}(P) \subseteq P$,but $P \subseteq KS_{scl}(P)$. Therefore, $KS_{scl}(P) = P$. Hence, P is KS-semi C.

Theorem 2.23 If P is KS-O and KS_{gs} (KS_{sg})-C set in X, then P is KS-semi C.
Proof: As, P ∈ KS-O and $KS_{gs}(KS_{sg})$-C, then $KS_{scl}(P) \subseteq P$, but $P \subseteq KS_{scl}(P)$. Therefore, $KS_{scl}(P) = P$. Hence, P is KS-semi C.

Theorem 2.24 *If* அ ∈ U, *either* $\{அ\}$ *is KS-C (or)* $\{அ\}^c$ *is* $KS_{gs}(KS_{sg})$*-C in* $KS_R(X)$.
Proof: Consider $\{அ\} \notin$ KS-C in U. Then $\{அ\}^c$ is not KS-O(KS-semi O) and unique KS-O(KS-semi O) set $\supseteq \{அ\}^c$ is $V \subseteq U$. That is $\{அ\}^c \subseteq U$. Therefore $KS_{scl}(\{அ\}^c) \subseteq U$ which implies $\{அ\}^c$ is $KS_{gs}(KS_{sg})$-C in $KS_R(X)$.

CONCLUSION

We have attempted an endeavor to concentrate aboutKS_{gs}*-C and*KS_{sg}*-C* sets. Furthermore, we initiate and study KS_{gs}*-C and*KS_{sg}*-C* sets characterizations.

REFERENCES

1. Rachchh.K.G and Ghanchi.S.I, On Kasaj Topological Spaces, Malaya Journal of Matematik.8(4), 2020, 1766-1770.

2. Rachchh.K.G, Ghanchi.S.I and Soneji.A.A, On Kasaj-b-open sets and Kasaj-regular-open sets in Kasaj Topological Spaces, Journal of Emerging Technologies and Innovative Research, 7(12), 2020, 652-657.
3. Rachchh.K.G, Ghanchi.S.I, Soneji.A.A and Ghanchi.S.I, On Kasaj Generalized Closed Sets in Kasaj Topological Spaces, Journal of Emerging Technologies and Innovative Research, 7(12), 2020, 646-651.

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RESEARCH ARTICLE

Adequacy of the Measure of Tibial Torsion in Predicting the Grade of Toe-Walking among Autistic Children of 3 to 8 Years of Age – A Correlational Study

Sumitha Hemavathy.P[1]*, Ramya.K[2] and Senthilkumar.M[2]

[1]Director, CAPAAR, Bangalore, Karnataka, India.
[2]Director, Barath Physio Care, Salem, Tamil Nadu, India.

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***Address for Correspondence**
Sumitha Hemavathy.P,
Director,
CAPAAR,
Bangalore, Karnataka, India.

ABSTRACT

The aim of the study was to establish a correlation between the torsional profile of lower limb and toe walking in autistic children. The study analyzed the correlation between Thigh foot angle, Transmalleolar angle and degree of toe walk in autistic children. A total of 36 children between the age group of 3 and 8 years with diagnosis of autism were included in the study. The rotational values at hip and knee, degree of toe walk, thigh foot angle and foot floor angle data were compared with the controls with age matching. The hip rotations score showed that more than half of the subjects with autism diagnosis exhibited external rotation. There was a weak positive correlation between the toe walk and external tibial torsion. And there was positive correlation between TMA and TFA and toe walking. Hence it was concluded that any measure to correct the tibial torsion and foot floor angle can reduce the degree of toe walk in children and any physiotherapy treatment to address this issue can change the walking pattern of the autistic children.

Keywords: Autism, Toe Walk, Torsion, Transmalleolar axis, Thigh foot angle

INTRODUCTION

Toe walking is a pattern of walking that a growing child develops at an early stage of life. The child walks on the toes and ball of foot without placing the heel on the ground. When most of the toe walking are insignificant and disappears at age of 2, persistent toe walking can be a warning sign of many underlying pathologies. And it has become a major concern among parents as it is one of the parameter to determine the developmental delay. While almost 7% of the paediatric patients represent with toe walking which might be either idiopathic or due to underlying pathology, it is challenging to rule out the cause of such representation. Hence it needs expertise of the

biomechanical features which cause an abnormality in gait pattern. It is documented that around 10%[1] of toe walking are idiopathic and 34.1% are due to any prospective causes. The gait pattern is profoundly linked to sensory process dysfunction [2] and this is where the necessity and need to identify the causative factors comes into play. It is also associated with a speech delay with or without a delay in motor development. Hence a holistic treatment to address all these issues will inhibit the toe walk.

Autism Spectrum disorder is defined as a developmental disorder characterized by difficulty in social interaction and communication. It is manifested as repetitive patterns of thoughts and behavior [3]. The sensory dysfunction and motor delay are the prominent features that differentiate the children socially. These children usually present with a vast motor problem which when identified early can integrate them into socially acceptable norms. One early sign of autistic children is the toe walk. Among the possible reasons identified for the toe walk, equinus deformity at the foot plays a key role followed by the tightness of gastrocnemius. And there is a compensatory mechanical changes in the knee and foot called the Tibial Torsion. Tibial torsion is defined as any twist or rotation on the tibia in longitudinal axis [4]. The Trans-malloeolar axis and the Thigh-foot angle are the torsional factors that produce equinus in foot. The TMA is measured between the line of the longitudinal axis of the thigh and the line perpendicular to the axis that connected the most prominent portions of medial and lateral malleolus. TFA is the angle between the longitudinal axis of the thigh and longitudinal axis of the foot were measured. A negative angle means internal rotation and a positive angle external rotation [5]. Around 20.1% of persistent toe walking and 12% of tight heel cord symptoms were exhibited in children with autistic spectrum disorder [6]. Hence as an early sign of autism the medicos are more keen on reducing toe walking and modulating a normal gait pattern. Tibial torsion is considered as one of the key factor that elicits toe walk. In fact the accurate measurement of torsion of lower limb can pave way for a working model therapy to change the gait pattern and normalize the walk in paediatrics.

MATERIALS AND METHODS

A population of 36 children between the age group of 3 and 8 years, with a history of toe walking and diagnosis of Autistic spectrum disorder and receiving treatment for the same were selected from CAPAAR Bangalore between December 2021 and March 2022. Consent was taken from the parents of the subjects before including into the study. The subjects with a history of surgical intervention of tendoachilles lengthening or osteotomy, cerebral palsy with toe walking were excluded from the study. The anthroprometric data of the subjects like height, weight and head circumference were recorded. The height was recorded using stadiometer and was approximated to 0.5 cm. The weight of the subjects was recorded using digital weighing scale. The independent variable, degree of toe walk was measured through a 5 point gait scale, with measures from 0 to 4.The dependent variable, the tibial torsion profile was measured Trans-malleolar axis, Thigh-foot angle. The trans-malleolar axis was measured in supine position by the angle formed by a line joining the lateral malleolus and medial malleolus to the line joining the lateral and medial femoral condyles. The thigh foot angle was measured in prone lying with knee bent. The angle formed by a line bisecting the foot and line bisecting the thigh is measured and recorded.

Statistical Analysis

The Pearson correlation coefficient was performed to assess the association between the degree of toe walk and trans-malleolar axis and Thigh foot angle. Mean and Standard deviation were calculated for the study. The independent t-test was performed to find the difference between the two independent pearson correlation and Bonferroni was done to correct the significance level. The significance level 0.5 and a confidence level of 95% was fixed for this study.

RESULTS

The anthroprometric characteristics of the 48 subjects were computed and presented in Table 1. To determine the surrogate for measuring the toe walking, the correlation between the degree of toe walk in gait cycle score and other measurable variables like TMA and TFA were done and is presented in Table 2. The analysis showed that the correlations ranged from 0.1744 to 0.1704 for TMA (right and left leg), and from 0.1744 to 0.1704 for TFA (right and left leg). Figure 1- 4 displays the relationship between degree of toe walk and TMA and TFA respectively.

DISCUSSION

Toe walking is a common paediatricorthopeadic problem addressed at the physiotherapy set up initially. When the toe walk does not resolve by physiotherapeutic intervention the chance of surgical correction is brought up. Hence it is the sole responsibility of the therapist to identify the biomechanical causes and rectify it at the earliest to avoid the surgical procedure and the post-operative management. Lee Young [7] identified that the TMD, TFS and Second toe test as the potential tests to be evaluated in assessing the tibial rotational profile. They were not able to establish a correlation between the tibial torsion and autism severity score. But they observed increased chances of occurrence of tibial rotation in the autistic children. Their study threw light on the interrater reliability of the TMA and was followed by TFA and second toe test in assessing the torsional values. Their study stresses on the surgical correction by osteotomy. Despite the causes of toe walking, it is necessary to inhibit the gait to promote normal walking. Though the child surrogates the toe walk gait with tight gastrocnemius; it is necessary to address it for the social acceptance rather than the cosmetic problem. And especially for autism children, the sensory issues of the foot need to be addressed to bring down the foot sensitiveness. Hence a compensatory biomechanical can bring about a change in gait pattern to inhibit toe walk. The current study focus on establishing a relation between the tibial rotational profile and toe walk.

CONCLUSION

On the basis of the current study it is concluded that the tibial rotational profile to a certain extent predict the grade of toe walk. But there is insufficient proof that the correction of tibial torsion might change the toe walk pattern on longer duration. Thus the study recommends that though the relation between the torsional profile and toe walk is obvious there is need for a strategy to inhibit it without any surgical correction.

REFERENCES

1. Fox A, Deakin S, Pettigrew G, Paton R. Serial casting in the treatment of idiopathic toe-walkers and review of the literature. Actaorthopaedicabelgica. 2006 Decmber 1;72(6):722.
2. Stricker SJ, Angulo JC. Idiopathic toe walking: a comparison of treatment methods. Journal of Pediatric Orthopaedics. 1998 May 1;18(3):289-93.
3. Block ME, Block VE, Halliday P. What Is Autism?. Teaching Elementary Physical Education. 2006 Novmber;17(6):7-11.
4. HutterJr CG, Scott W. Tibial torsion. JBJS. 1949 Jul 1;31(3):511-8.
5. Staheli LT, Corbett M, Wyss C, King H. Lower-extremity rotational problems in children. Normal values to guide management. Journal of Bone Joint Surgery Am. 1985 January;67(1):39-47. PMID: 3968103.
6. Barrow WJ, Jaworski M, Accardo PJ. Persistent toe walking in autism. J Child Neurol. 2011 May;26(5):619-21. doi: 10.1177/0883073810385344. Epub 2011 Jan 31. PMID: 21285033.
7. Lee SH, Chung CY, Park MS, Choi IH, Cho TJ. Tibial torsion in cerebral palsy: validity and reliability of measurement. Clinical Orthopaedics and Related Research®. 2009 August;467(8):2098-104.

Table 1. Anthroprometric data of the subjects

N = 36	Mean
Age in years	4.2
Height in cms	132.8
Weight in kgs	14.6

Table 2. Corporal correlation between degree of Toe walk and TMA TFA

N = 36	Degree of Toe walk 1.8333 (1.424)
TMA	0.1744 (R) 0.1704 (L)
TFA	0.1744 (R) 0.1704 (L)

Pearson correlation with Bonferroni correction of significance levels

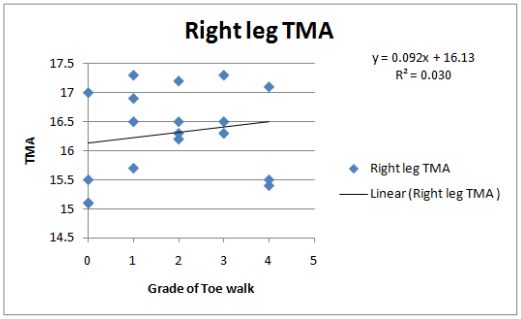

Figure 1. Correlation between Grade of Toe Walk and Right Transamalleolar Axis

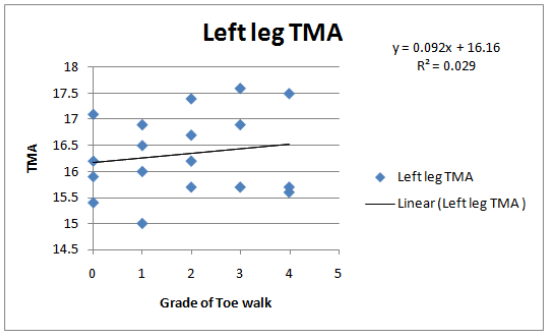

Figure 2 . Correlation between Grade of Toe Walk and Transamalleolar axis Left side

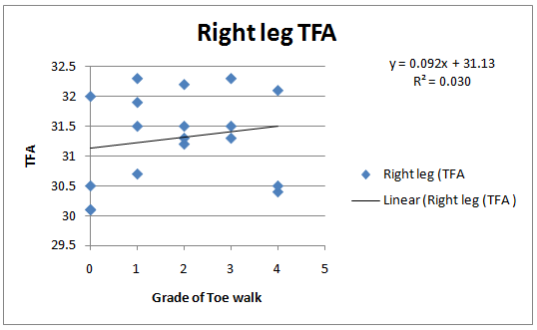

Figure 3. Correlation between Grade of Toe Walk and Right Thigh Foot Angle

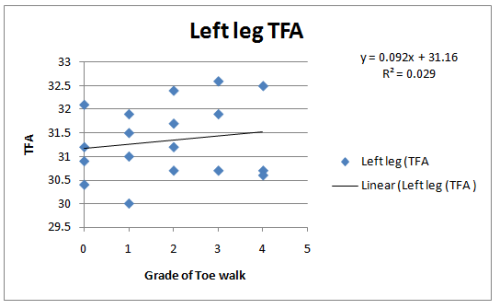

Figure 4. Correlation between Grade of Toe Walk and Left Thigh Foot Angle

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RESEARCH ARTICLE

Value Preferences of Post-Graduate Students in Assam Don Bosco University (ADBU), Guwahati: A Schwartz Approach

Bonnie Amonge Crerar[1]* and Sujit Basumatary[2]

[1]Assistant Professor (Senior), Department of Education, Assam Don Bosco University, Guwahati, Assam, India.

[2]Assistant Professor, Department of Education, Don Bosco Junior College, Bongaigaon, Assam, India.

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***Address for Correspondence**
Bonnie Amonge Crerar,
Assistant Professor (Senior),
Department of Education,
Assam Don Bosco University,
Guwahati, Assam, India.
Email: bonnieamonge1@gmail.com

ABSTRACT

Adopting the Schwartz approach to value preferences, this paper aims to find out the value Preferences of Post graduate Students of Assam Don Bosco University and to compare their value preferences with respect to their gender and departments. Following the cross sectional research design and the normative survey method , data was collected using the TwIVI tool from a sample of 79 post graduate students of the School of Humanities and Social Sciences (ADBU), who were selected by adopting the stratified Random sampling technique. It was found that majority of the PG students of ADBU have a high level of value preference. Further no difference in value preferences was found with respect to gender and departments.

Keywords: value, value preference, ADBU Post-graduate students, Schwartz approach

INTRODUCTION AND RATIONALE

A nation stands strong by the values adhered to by its people. Etymologically 'Value' comes from the Latin word 'Valere' meaning 'be strong', 'be well' and 'be worth'[1]- something that is intrinsically valuable or important**.** Value thus is a matter of one's preference or choice and hence the term 'Value Preference'. Technically preference is used in context of choice/ decision making in the face of alternatives; making explicit one's likes and dislikes. This is aptly stated by Schwartz, (2006) stated that, **'**Value preferences reflect the individual's general motivational goals, which affect the individuals' perception of reality and direct behaviour' [2].

Values are life's driving force. One's value preferences may be desirable or undesirable ideas or behaviour related to one's goals [3]. Further value preferences are learnt as Parson (1951) mentions, 'Value is a culture that influences human selection by the virtues of being internalized by an actor [4]'. Students are no exception. Hüseyin, Sapnaz and Uzunkol (2014) highlighted value preferences as being predictors to students' life goals [5]. Hence, it is important to identify the value preferences of students, so that appropriate strategies and learning environment can be created to channelize their value preferences in the right direction.

Schwartz classification of value

'Value Preference' implies that there is a spectrum of choice. But for the present study Schwartz (2012) classification of values based on associated motivational goals has been considered as follows

Conformity

A conformist restrains actions, inclinations, and impulses that may upset or harm others, violate social expectations or norms, upset social interaction and functioning. They are loyal, self-disciplined, and responsible.

Tradition

A traditionalist upholds customs, culture and religion. They develop practices, symbols, ideas, and beliefs, representing shared expertise and fate of its members. In the long run become valued customs and traditions, that represent the groups' unity, value, that contribute to its survival (Durkheim, 1912/1954; Parsons, 1951). After a period of time they take on the status of religious institutions.

Benevolence

A benevolent individual strives to preserve and enhance the welfare of in-group members. This value arises out of requirement for smooth group functioning (Kluckhohn, 1951) [6] and the Organismic need for affiliation (Maslow, 1965) [7].

Universalism

Opposed to benevolence, universalism is external oriented- comprising of understanding, appreciation, tolerance, and protection for the welfare of all people and nature.

Self-Direction

This value arises out of the need for independence and control. A self-directed person is independent in thought and action, choosing, creating, exploring.

Stimulation

Deci (1975) opined that this value arises out of the need for having a varied, exciting and daring life [8]. Those who go by this value look for excitement, novelty and challenge to maintain a positive active life (Berlyne, 1960) [9].

Hedonism

A hedonistic individual is self-indulgent, looks for pleasure, sensuous gratification to enjoy life.

Achievement

Achievers value personal success by demonstrating competence according to social standards, thereby getting social approval. Competency is crucial to group survival and establishing objectives.

Power

Power loving people are prone to social status and prestige, control or dominance over people and resources.

Security

This value derives from the basic requirement of the individual and group for safety, harmony, and stability of society, relationships and of self (Kluckhohn 1951; Maslow, 1965).

Objectives

i. To find the value preferences of the post graduate students of Assam Don Bosco University.
ii. To compare mean scores of value preferences of postgraduate students with respect to their gender.
iii. To compare mean scores on the 10 dimensions of value preferences of post graduate students with respect to their gender.
iv. To compare mean scores of value preferences of postgraduate students with respect to their departments.
v. To compare mean scores on the 10 dimensions of value preferences of post graduate students with respect to their departments.

Hypotheses

To achieve objective ii, iii, iv and v the following null hypotheses had been formulated:

i. There is no significant difference among mean scores of value preferences of post graduate students with respect to their gender.
ii. There is no significant difference in the mean scores on the 10 dimensions of value preferences of post graduate students with respect to their gender.
iii. There is no significant difference among mean scores of value preferences of post graduate students with respect to their departments.
iv. There is no significant difference in the mean scores on the 10 dimensions of value preferences of post graduate students with respect to their departments.

Delimitations

The study was delimited to:

i. PG Students of ADBU.
ii. The School of Humanities and Social Science.
iii. The 2nd and 4th semester PG students of English, Education, Psychology, and Master of Social Work.
iv. Schwartz concept of value.

Method

Normative survey method and cross-sectional research design was used to collect data from the sample during the same period with regard to the phenomenon at hand.

Variables

Independent: Gender & Departments
Dependent: Value Preference

Population and Sample

The population for the study comprised of the total number of postgraduate students in the second and fourth semesters of the School of Social Sciences and Humanities studying in Assam Don Bosco University, Tapesia Campus. Adopting the stratified Random sampling technique only 79 students formed the sample for the study. Gender being a variable care had been taken to draw a representative sample from the given population. Randomization was assured by adopting the lottery method. Distribution of the population and sample for each Department with respect to male and female students is given in Table 1, and in Fig. 1 and Fig. 2 respectively

Tool used

Tool used for data collection was the TwIVI – Twenty Item Value Inventory developed by Sandy, Gosling, Schwartz, and Koelke in 2016. The inventory consists of ten dimensions and 20 items i.e. two items for each dimension. The identified dimensions and items are given in Table 2. The scoring for each item is given in Table 3.

RESULTS AND DISCUSSION

Table 4 and Fig. 3 depicts that no PG student of ADBU showed low level of value preference. Out of 100 %, 69.6 % of PG students have a high level of value preference and only 30.4% of PG students have an average level of value preference. Further, the mean scores and ranking of the values as given in Table 5 and Fig. 4 indicate that PG students of ADBU gave first preference to the value of universalism followed by benevolence, conformity, self-direction and so on, with power being the less preferred value. Fig. 3 gives the value preferences of the PG students as per their mean values and ranking. Table 6 and Fig. 5, shows that the computed t-value is 1.6 which is less than the table t-value (2) at 0.05 level of significance for 77 df and has not been considered as significant. Hence, the hypothesis got retained. It means that "There will be no significant difference between the mean scores of value preference of PG male and PG female students of ADBU.'' Though there is no overall difference in the mean score of value preference of the male and female students as depicted in Table 6, but difference is seen in the hierarchical rank order (given within brackets) of the mean scores in the 10 dimensions of value preference between the male and female students as given in Table 7 and Fig. 6. The male PG students gave 1st preference to benevolence but female PG students gave 1st preference to universalism. Second in order of value preference for male PG students was conformity but for female PG students it was benevolence. However, it is interesting to note that the 6th, 8th, 9th and 10th order of value preference for both male and female students are the same i.e. stimulation, achievement, tradition and power respectively. Table 7 and Fig. 6 makes it clear that on the dimensions of conformity, achievement, hedonism and stimulation the difference in the mean scores between the male and female students is not significant since calculated t value with df 77 is much less than the tabulated t value at 0.05 level of significance. Hence, for these dimensions the null hypothesis is retained and it can be stated that 'There is no significant difference in the mean scores on the dimensions of conformity, achievement, hedonism and stimulation of value preferences of post graduate students with respect to their gender'.

However, significant difference in the mean scores of male and female students is seen on the dimensions of tradition, security, universalism, benevolence, power and self-direction whose calculated 't' value 66.66, 2.85, 4.57, 3.6, 12.3 and 6.2 respectively is much greater than the tabulated 't' value 2 and 1.99 with df 70 and 80 at 0.05 level of significance. Hence, the null hypothesis 'There is no significant difference in the mean scores on the dimensions of tradition, security, universalism, benevolence, power and self-direction of value preferences of post graduate students with respect to their gender' is rejected and an alternative hypothesis formulated, ''There is a significant difference in the mean scores on the dimensions of tradition, security, universalism, benevolence, power and self-direction of value preferences of post graduate students with respect to their gender'. The comparison of the mean scores on the 10 dimensions of value preferences between male and female PG students has been represented in the following Fig.7. From Table 8, it is clear that calculated 'F' 1.67 with df 3/75 is not greater than the table value 4.04 at 0.01 level of significance, and hence not significant. Thus, at 0.01 level of significance the H_0, There is no significant difference among mean scores of value preferences of post graduate students with respect to their departments, is accepted. Table 9 and Fig. 8 reveals the departments of English, Education, Psychology and Master of Social Work did not differ significantly on any of the 9 domains of value preference. However, on the domain of tradition the calculated value 6.23 is much greater than the table value 4.04 at 0.01 degree of significance. And hence the difference between the four departments is significant.

CONCLUSION

Value being the driving force to one's existence, it is notable to report that majority PG students of the School of Humanities and social Sciences of ADBU have a high level of value preference. Hierarchically, the first preferred dimension was universalism, with power being the least preferred value. No significant difference was reported in the overall level of value preference with respect to gender and department. But significant difference was recorded only on the dimension of tradition respectively. Interestingly the boys scored higher mean than girls on the tradition value, implying that the PG boys were more traditional compared to the girls. Power drives one to achieve social

recognition, direct one's own life, and control resources **[10]**. Hence, low preference for power among the students needs to be further investigated to identify the factors responsible.

REFERENCES

1. Harper, D. (n.d.). *Etymology of value*. Online Etymology Dictionary. Retrieved February 24, 2022, from https://www.etymonline.com/word/value.
2. Schwartz, S. H. (2007). Basic human values: Theory, measurement, and applications. *Revue française de sociologie*, *47*(4), 929.
3. Schwartz, S. H. (1992). Universals in the content and structure of values: Theoretical advances and empirical tests in 20 countries. In *Advances in experimental social psychology* (Vol. 25, pp. 1-65). Academic Press.
4. Parsons, T. (1951). The Social System. Glencoe: Free Press. Retrieved 26/08/2022 from https://voidnetwork.gr/wp-content/uploads/2016/10/The-Social-System-by-Talcott-Parsons.pdf
5. Hüseyin, C., Sapmaz, F. & Uzunk, E. (2015, October). Value Preferences of University Students of Life Goal. *Social Indicators Research: An International and Interdisciplinary Journal for Quality of life Measurement.* 124 (1), 111-125.Retrieved from https://econpapers.repec.org/article/sprsoinre/v_3a124_3ay_3a2015_3ai_3a1_3ap_3a111-125.htm
6. Kluckhohn, C. (1951). Values and value-orientations in the theory of action: An exploration in definition and classification. In Schwartz, S. H. (2012). An Overview of the Schwartz Theory of Basic Values. Online Readings in Psychology and Culture, 2(1), p.5. https://doi.org/10.9707/2307-0919.1116
7. Maslow, A. H. (1965). Eupsychian management. Homewood, IL: Dorsey. In Schwartz, S. H. (2012). An Overview of the Schwartz Theory of Basic Values. Online Readings in Psychology and Culture, 2(1), p.5. https://doi.org/10.9707/2307-0919.1116
8. Deci, E. L. (1975). Intrinsic motivation. New York: Plenum. In Schwartz, S. H. (2012). An Overview of the Schwartz Theory of Basic Values. Online Readings in Psychology and Culture, 2(1), p.5. https://doi.org/10.9707/2307-0919.1116
9. Berlyne, D. E. (1960). Conflict, arousal and curiosity. New York: McGraw-Hill. In Schwartz, S. H. (2012). An Overview of the Schwartz Theory of Basic Values. Online Readings in Psychology and Culture, 2(1), p.5. https://doi.org/10.9707/2307-0919.1116
10. Hashiem & Driscoll. (October, 2013). Values (Shalom H. Schwartz) – Power. *Cross Cultural Psychology. 3.* https://doi.org/10.1002/9781118339893.wbeccp597

Table 1: Distribution of the sample

Department	Total Population		Total	Sample		Total
	M	F		M	F	
Education	7	12	19	3	4	7
MSW	48	64	112	19	16	35
Psychology	13	36	49	4	12	16
English	16	63	79	3	18	21
	Total		259			79

Table 2: Distribution of items Dimension wise

Sl. No.	Dimensions	No. of Items	Total
1.	Conformity	1,11	2
2.	Tradition	2 ,12	2
3.	Benevolence	3,13	2
4.	Universalism	4 , 14	2
5.	Self-direction	5 , 15	2

6.	Stimulation	6 , 16	2
7.	Hedonism	7 ,17	2
8.	Achievement	8 , 18	2
9.	Power	9 , 19	2
10.	Security	10,20	2
Total			20

Table 3: Scoring Procedure

6	5	4	3	2	1
Very much like me	**Like me**	**Somewhat like me**	**A little like me**	**Not like me**	**Not like me at all**

Table 4: Level of value preferences of PG students of ADBU

Levels	Classification	No. of Students	Percentage
LOW	20 – 53	-	-
AVERAGE	54 – 86	24	30.4%
HIGH	87- 120	55	69.6%
		79	100%

Table 5: Value preference mean ranking

Value preferences	Mean	Ranks
Universalism	10.16456	1
Benevolence	9.848101	2
Conformity	9.607595	3
Self-direction	9.392405	4
Security	9.379747	5
Hedonism	9.202532	6
Stimulation	9.177215	7
Achievement	8.683544	8
Tradition	7.987342	9
Power	7.126582	10

Table 6: Summary of Means, SD, SED, df, and t-value of the value preference scores of ADBU PG students.

Groups	N	Mean	SD	SED	df	t-value	Significance
Male	29	92.44	11.2	2.7	77	1.6	Not significant at 0.05 level
Female	50	88.1	12.1				

Table 7: Mean score comparison of male and female students on different dimensions of Value preferences

Dimensions	Gender	Mean	N	SD	SED	df	t value	Significance
Tradition	Male	8.448276 (9)	29	0.46	0.0108	77	66.66	Significant at 0.05 level
	Female	7.72 (9)	50	0.44				
Conformity	Male	9.896552 (2)	29	0.39	5.79	77	0.07	Not significant at 0.05 level
	Female	9.44 (4)	50	-40.95				
Security	Male	9.586207 (4)	29	0.48	0.112	77	2.85	Significant at 0.05 level
	Female	9.26 (5)	50	0.49				
Universalism	Male	9.862069 (3)	29	0.47	0.105	77	4.57	Significant at 0.05 level
	Female	10.34 (1)	50	0.43				
Benevolence	Male	10.10345 (1)	29	0.45	0.11	77	3.6	Significant at 0.05 level
	Female	9.7 (2)	50	0.48				

Achievement	Male	8.62069 (8)	29	0.47	0.11	77	0.9	Not significant at 0.05 level
	Female	8.72 (8)	50	0.48				
Power	Male	7.931034(10)	29	0.46	0.103	77	12.3	Significant at 0.05 level
	Female	6.66 (10)	50	0.41				
Hedonism	Male	9.344828 (5)	29	0.49	0.11	77	2	Not significant at 0.05 level
	Female	9.12 (7)	50	0.49				
Stimulation	Male	9.310345 (6)	29	0.49	0.11	77	1.9	Not significant at 0.05 level
	Female	9.1(6)	50	0.49				
Self-direction	Male	8.965517 (7)	29	0.49	0.11	77	6.2	Significant at 0.05 level
	Female	9.64 (3)	50	0.49				

Table 8: Department wise comparison value preferences of post graduate students

Stream	Sum of squares	df	Mean squares	F	Significance
Between groups	629.73	3	209.91	1.67	Not significant at 0.01 level
Within groups	9455.89	75	126.078		
Total	10,085.62	78			

Table 9: One way ANOVA depicting significance of difference in the mean scores on the 10 dimensions of value preferences of post graduate students with respect to their departments

Dimensions	Stream	Sum of squares	df	Mean squares	F	F ratio for 0.01 level of significance	Remark
Tradition	**Between groups**	86.79	3	28.93	6.23	4.04	Significant
	Within groups	347.74	75	4.64			
	Total	434.53	78	33.57			
Conformity	**Between groups**	17.0816	3	5.69	1.56	4.04	Not significant
	Within groups	273.5736	75	3.65			
	Total	290.6552	78	9.34			
Security	**Between groups**	6.7859	3	2.26	0.72	4.04	Not significant
	Within groups	233.9621	75	3.12			
	Total	240.748	78	5.38			
Universalism	**Between groups**	2.5624	3	0.85	0.24	4.04	Not significant
	Within groups	261.98	75	3.49			
	Total	264.5424	78	4.34			
Benevolence	**Between groups**	10.22	3	3.4	0.89	4.04	Not significant
	Within groups	286.92	75	3.83			
	Total	297.14	78	7.23			
Achievement	**Between groups**	12.99	3	4.33	0.61	4.04	Not significant
	Within groups	529.27	75	7.1			
	Total	542.26	78	11.43			
Power	**Between groups**	48.2736	3	16.0912	2.0024	4.04	Not significant
	Within groups	602.7416	75	8.036			
	Total	651.0152	78	24.1272			
Hedonism	**Between groups**	2.81	3	0.94	0.21	4.04	Not significant
	Within groups	338.05	75	4.5			
	Total	340.86	78	5.44			
Stimulation	**Between groups**	3.72	3	1.24	0.34	4.04	Not significant
	Within groups	267.84	75	3.6			

	Total	271.56	78	4.84			
Self-direction	**Between groups**	18.03	3	6.01	1.6	4.04	Not significant
	Within groups	282.55	75	3.8			
	Total	300.58	78	9.81			

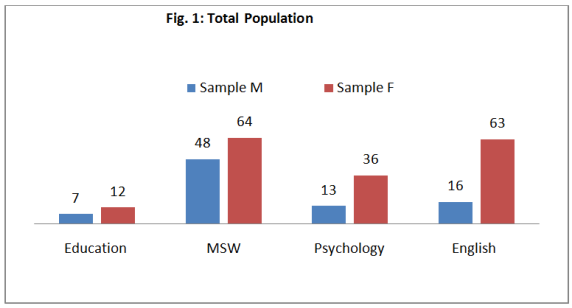

Fig. 1: Total Population

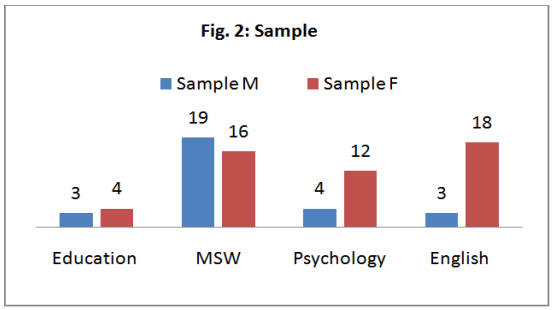

Fig. 2: Sample

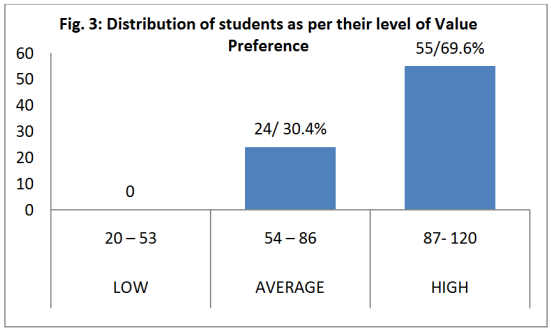

Fig. 3: Distribution of students as per their level of Value Preference

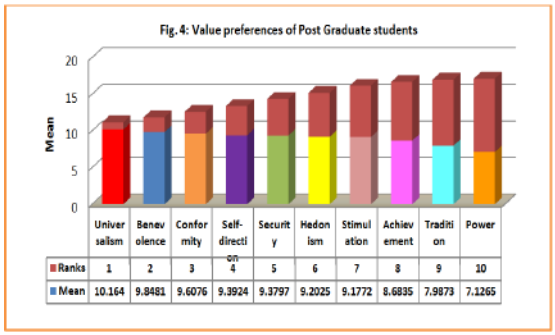

Fig. 4: Value preferences of Post Graduate students

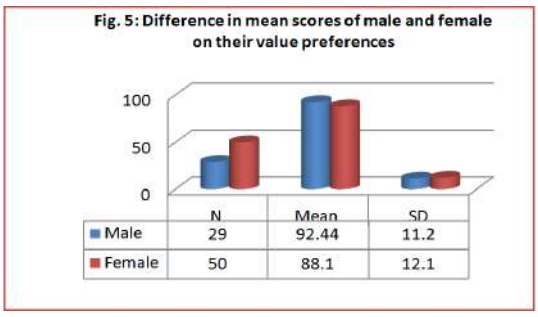

Fig. 5: Difference in mean scores of male and female on their value preferences

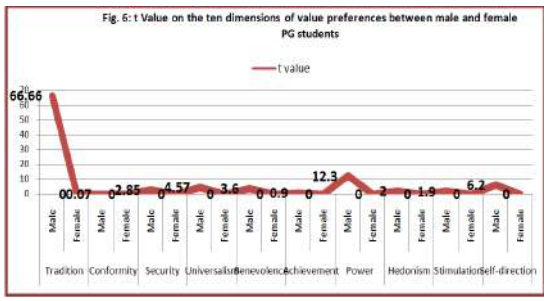

Fig. 6: t Value on the ten dimensions of value preferences between male and female PG students

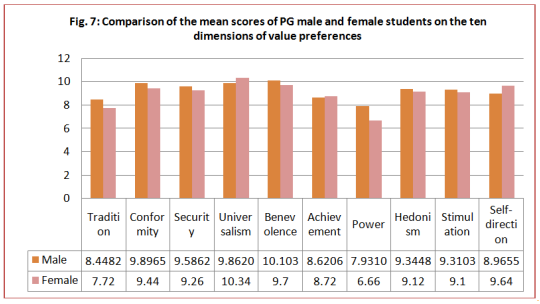

Fig. 7: Comparison of the mean scores of PG male and female students on the ten dimensions of value preferences

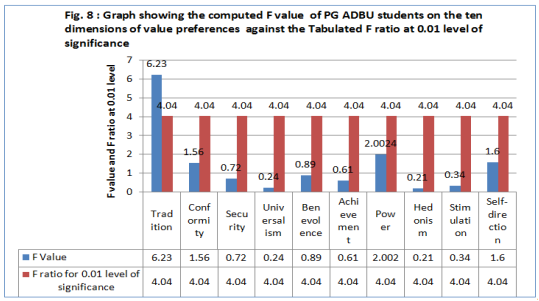

Fig. 8 : Graph showing the computed F value of PG ADBU students on the ten dimensions of value preferences against the Tabulated F ratio at 0.01 level of significance

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REVIEW ARTICLE

Preparation of Agricultural Waste Biomass and its Characterization for Treating Dye Effluents: A Review

Beautlin Nisha.R[1], Abisha.B.R[1], Daniel Sam.N[1], Anish.C.I[1] and M. Jaya Rajan[2]*

[1]Research Scholar, Department of Chemistry and Research Centre, Annai Velankanni College, (Affiliated to Manonmaniam Sundaranar University, Tirunelveli) Tholayavattam, Kanniyakumari, Tamil Nadu, India.

[2]Associate Professor, Department of Chemistry and Research Centre, Annai Velankanni College, (Affiliated to Manonmaniam Sundaranar University, Tirunelveli) Tholayavattam, Kanniyakumari, Tamil Nadu, India.

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***Address for Correspondence**
M. Jaya Rajan
Associate Professor,
Department of Chemistry and Research Centre,
Annai Velankanni College,
(Affiliated to Manonmaniam Sundaranar University, Tirunelveli)
Tholayavattam, Kanniyakumari,
Tamil Nadu, India.
Email: jayarajanm1983@gmail.com

ABSTRACT

Adsorption activities are done many harmful materials, in general harmful materials are collected from bio waste sources, such as hazardous metals/elements and organics. It has many advantages over the conventional materials due to its low cost abundance, effective adsorption capacity, and recyclability. Dyes are mainly organics with different structures and molecular weight; they are harmful to the environment and organisms if they were not efficiently treated before releasing. This review highlights and provides an overview of these activated carbons prepared by agricultural wastes and their application for dye removal. The adsorption kinetics and isotherms were illustrated and tabled for selected studies to show the adsorbents behaviors and adsorption mechanisms.

Keywords: Adsorption, Activated Carbon, Bio waste, Isotherm, Recyclability.

INTRODUCTION

In general, water pollution is described as harmful substance or unexpected materials that found in the water is sufficient quantities. Dyes are important class of water pollutants eliminated from dye manufacturing and textile industries. The waste chemicals and dye effluents must be treated properly. Many physical and chemical treatment methods including adsorption, coagulation, precipitation, filtration, electro dialysis, membrane separation and oxidation have been used for the treatment of dye containing effluents. The adsorption process is one of the most efficient methods of removing pollutants from waste water. In this review selected studies that deal with the removal of the most reported dyes using agricultural waste .This work give a general up to date view on exploiting the bio waste as adsorbents to remove dyes, where a wide range of adsorption results was reported.

Activated carbon Vs Activating Agent

Sugar beet pulp [1], Oil palm empty fruit bunch [9], activated carbon prepared by using H_3PO_4. The carbonization step was carried out at a temperature of 110^{0}C for 12 hrs. The product obtained was activated under same condition as carbonization but at different temperature (350, 400, 450, 500 and 550^{0}C) activation held for time (0.5, 1, 1.5, 2 and 2.5 hrs). Respectively oil palm empty fruit bunch carbonized step carried out at temperature of 105^{0}C for 24 hrs. The product was activated at 450^{0}C for 2 hrs. Betel nut husk [2], Rice husks [13] and Pumpkin peel [17] activated carbon prepared by using NaOH. Betel nut husk was mixed with powdered NaOH at different ratios (1:1, 1:2 and1:3) for 24 hrs at room temperature .The product were activated by 500^{0}C for 1h. Rice husks carbonization step carried out temperature of 120^{0}C for 12 hrs. Activated by different temperature (650, 700, 750 and 800^{0}C) for 60 min. Dried pumpkin peel is heated (250, 350, 450 and 550^{0}C) for 1 hr. Mango seed [4], coir pith [6], Sugarcane bagasse [7], Date stones [8], Mangosteen peel [16] and *Dipterocarpus alatus fruits* (wing, pericarp and endocarp) [20] activated carbon prepared by using $ZnCl_2$. Mango seed carbonized step carried out at a temperature of 100^{0}C for 30 min and activated at 500^{0}C for 1hr. Coir pith was dried under sun light for 5 hrs. It was stirred in a boiling solution containing $ZnCl_2$ in the weight ratio 2:1 drying temperature 700^{0}C under controlled condition. Date stones impregnated in $ZnCl_2$ for activation were carried out at room temperature for 24 hrs and activated by 500^{0}C for 1 hr. Sugarcane bagasse soaked with $ZnCl_2$ for 0.5 hr then pyrolyzed at 500^{0}C. Mangosteen peel powder impregnated in $ZnCl_2$ and calcinated at 500-800^{0}C time ranging from 0.5hr to 3hrs. *Dipterocarpus alatus* fruits (wing, pericarp, and endocarp) were chemically activated by using $ZnCl_2$, $FeCl_3$, H_3PO_4 or KOH for 24 hrs and carbonized at 500^{0}C in N_2 atmosphere for 1 hr. Physical activation was carried out at 400^{0}C in N_2 atmosphere for 1 hr followed by 850^{0}C in CO_2 for 1 hr.

The activated carbons prepared from Indian almond shell, ground nut shell, areca nut shell, cashew nut shell [3] were calcinated at 400^{0}C. Activated by treatment with acid and heated at 120^{0}C for 1 hrs. Palm seed coat [10] was thermally activated in carbon dioxide atmosphere at 850-900^{0}C for 3hrs. Activating agent KOH used in a several adsorbents such as Rubber seed shell [11], Sunflower pith [12], Coconut shell [14] and fallen coconut leaves [18]. Rubber seed shell mixed with KOH at 1:1 ratio then activated at 500^{0}C for 3 hrs. Sunflower pith was activated by using KOH and NaOH at 700^{0}C for 1 hr. Coconut shell carbonization step carried out at a temperature of 400^{0}C for 0.5hr. The product obtained was activated by 600^{0}C for 2 hrs. Fallen coconut leaves carbonized out at 110^{0}C for 24 hrs, and then the product was activated at 700^{0}C for 1 hr. Raw papaya seed shells [5] were washed with distilled water to remove its dust particles. It was left at room temperature for 1 day to ooze out excess water and was dried in air dry oven at 105^{0}C for 24 hrs. The orange peel [15] was activated by using $ZnCl_2$ and K_2CO_3. Carbonization step carried out at temperature by 400-500^{0}C for 1hr. *Sargassum hemiphyllum* [19] untreated algae were washed with deionised water three times to remove its impurities and salt. It was dried at 333K for 24 hrs.

Adsorption and kinetic studies:

Activated carbon prepared from Betel nut husk [2], Coir pith [6], Oil palm empty fruit bunch [9] and Pumpkin peel [17]. Adsorption studies show pseudo- second order rate kinetics. The equilibrium data tested by Langmuir and Freundlich isotherms. Date stones [8], *Dipterocarpus alatus* fruits (wing, pericarp and endocarp) [20] equilibrium adsorption data were analysed by the Langmuir and Freundlich isotherm models kinetic studies derived from

pseudo- first order, pseudo- second order. Papaya seed [5], mangosteen peel [16], fallen coconut leaves [18] and *Sargassum hemiphyllum* [19], kinetic uptake profiles as well described by the pseudo – second order model, while adsorption equilibrium data describes the Langmuir isotherm. Sugar beet pulp [1] results show that the adsorption kinetics followed the pseudo- second order. Indian almond shell, ground nut shell, areca nut shell, tamarind shell, cashew nut shell [3]. The adsorption equilibrium data agreed well Langmuir, freundlich, Redlich-peterson isotherm and followed pseudo- second order kinetics. Palm seed [10] equilibrium data could be described well by the Freudlich isotherm model. The kinetic studies derived from the pseudo- first order. Sunflower pith [12], equilibrium data were analysed by the Langmuir Freundlich isotherm models. Kinetic studies derived from pseudo- first order.

Characterization Studies

Scanning Electron Microscopy (SEM)

Indian almond shell, ground nut shell[3], sunflower pith[12], Rice husks[13], *mangosteen* peel[16], Pumpkin peel[17], Fallen coconut leaves[18], *Sargasteen hemiphyllum*[19], *Dipterocarpus alatus* Fruits [20] activated carbons were characterized by SEM. Indian almond shell is used as adsorbent for Azure A (AA) dye. Before adsorption of AA dye both the carbons appeared as fine structure. In contrast after adsorption of AA dye the porous structures of both the carbons disappeared; this indicates that AA dye molecules are strongly absorbed by the adsorbents. Indian Almond Shell Carbon (IASC) and Ground nut Shell Carbon (GSC). SEM Analysis of sunflower pith activated carbon (SPAC) shows good pore formation and good structure of the resulting activated carbons. SEM analysis of Rice husk activated carbon sample shows it has porous structure with cracks and crevices. SEM images of *Magosteen peel* activated carbon shows that the surface of the activated carbon is rough and porous containing cracks, crevices, and holes of various sizes. SEM images of the modified Beetroot Activated Carbon (BAC) adsorbent shows high pore volume well distributed pore structures, different sizes of pores uneven and rough surface morphology. Activated Carbon prepared from Fallen Coconut Leaves is activated using potassium hydroxide (KOH). Before adsorption it is highly heterogeneous pores with different size and shape are also clearly visible. The morphology and surface structure of *Sargassum hemiphyllum* before and after bio sorption of Methylene blue was determined by SEM micrographs. The surface of *Sargassum hemiphyllum* became smoother after loading Methylene blue. This phenomenon may arise from the MB molecules attaching around the algal surface and filling the pores.

The SEM image of *Dipterocarpus alatus* fruit activated carbon shows that the surface formation of various sized cavities which make up distinct micropores and mesoporous. The surface morphology of ZnCl2 activated carbon shows irregular and porous surface activated carbon was observed. Oil palm empty fruit bunch [9], Rubber seed shell [11] activated carbon were characterized by SEM. The SEM image of Oil palm empty fruit bunch shows many large pores were clearly found on the surface of the activated carbon . The SEM micrographs of Rubber seed shell activated carbon shows that morphological characteristics are must different between raw materials and the three activated carbons. The SEM image reveals the nature of its surface. Orange peel [15] SEM images showed that pores of different size and different shapes were obtained from different chemical activation agents. K_2CO_3 impregnated sample forms honeycomb-like morphology; $ZnCl_2$ impregnated samples forms irregular and heterogeneous surface morphology with a well-developed porous structure after activation. The SEM micrographs of betel nut husk [2] activated carbon and the state of BNH-AC after adsorption AT 3000 × magnification. Due to NaOH activator the pore areas are developed containing dense surface area. Coconut shell [14] SEM image showed the presence of well-developed pores on the surface of the activated carbon sample.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis of sugar beet pulp[1] activated carbon (SBPAC) shows different functional group such as hydroxyl groups and carbonyl groups. The peak at 3447cm-1 is characteristic of the stretching vibration of hydrogen bonded to the due to –OH groups. The signal at 2349cm^{-1} was assigned to the alkynes. FTIR analysis of Betel nut Husk [2] activated carbon show changes occurring in the adsorption process after modifying the raw Betel nut Husk (BNH) to Betel nut Husk Activated Carbon (BNH- AC), which is visualized by band points in spectra. The peak points of 3681 and 3750cm^{-1} indicate O-H stretching of alcohols and phenols. A large number of peak points at 1756 and 1720cm^{-1} correspond to C=O stretch. FTIR spectra of activated carbon prepared from sugarcane bagasse [7]. shows the

presence of C=O at 1574cm-1. The absorbance peak at 1170cm-1 is associated with C-O- bands due to phenolic structure. The FTIR spectra of activated carbon prepared from Indian almond shell carbon (IASC) [3] The peak at 752.2 may be due to N-H bending. The functional groups present on the surface of Indian almond shell carbon (IASC) are responsible for adsorption of AA dyes. The FTIR analysis was utilized to evaluate the chemical structure of sun flower pith [12]. Activated carbon (SPAC) and functional group changes after carbonization and activation process. The broad band near 3300cm^{-1} in FTIR spectrum is due to O-H and N-H groups. Band at 2900cm^{-1} represents C-H stretching vibration in the biomass. Peaks at 1730 and 1600cm^{-1} is related to the C=O stretching vibration of carbonyl group. FTIR spectra obtained from both Sodium hydroxide– Sunflower pith activated carbon (N-SPAC) and potassium hydroxide -Sunflower pith activated carbon (K-SPAC) showed very similar trends. The FTIR result of Rice Husk [13] .Activated carbon indicates the spectra have similar shapes with most of the bands located on the same wave number range. The band at 3425cm^{-1} is due to the O-H stretching of hydroxyl groups. The bands at 2924 and 1393cm^{-1} are attributed to C-H stretching of aliphatic carbon. The band appearing at 1627cm^{-1} corresponds to the C=O vibration of lactonic, carboxyl or anhydric groups. FTIR analysis of Coconut shell [14] activated carbon shows the different functional groups like allene at 2100cm^{-1}.A very small peak near 1700cm-1 is assigned to the C=O stretching vibrations of ketones, aldehydes, lactones or carboxyl groups. The FTIR spectrum Orange peel [15], activated carbon showed the presence of different oxygen groups and olefin and aromatic carbon structures in the raw shell. Subsequent heat treatment resulted in the aromatization of the carbon structure and a decrease in the oxygen groups. The FTIR analysis of Mangosteen peel [16] activated carbon shows that the broad band located in the region of 3500-3100cm^{-1} related to O-H stretching vibration still exists but shifted to the lower wave number. The broadening of the peak at 1171cm^{-1} of the carbonization reveals the presence of many O-H groups on the surface of activated carbon. The appearance of many small peaks around 1100cm^{-1} after adsorption of Methylene Blue confirms that the MB dye cation adsorbed to the surface of activated carbon. [16]. FTIR analysis of Pumpkin peel [17] activated carbon shows the different group, the broad band at 3460 cm^{-1} is related to OH groups. The peak located at 1560 cm^{-1} could be related to the stretching vibrations of C-C and the peaks of C-O-H were observed at 1445.8 cm^{-1}.

The interpretation of functional groups FT-IR spectral analysis of Fallen coconut leaves [18] was performed. FT-IR analysis of before methylene blue adsorption shows the bands at 1550, 1064, 1007 and 623 cm^{-1} which are characteristic of stretching vibrations for carboxylate, alkane, secondary cyclic alcohol, and alkene groups respectively. After the methylene blue adsorption as new peak appeared and many functional groups are either frequency shifted.The FTIR analysis of Sargassam hemiphyllum [19] activated carbon showed that hydroxyl amine and carboxyl groups present on the surface of the algae are responsible for the bio sorption of MB. The strong vibration and broad band at approximately 3500-3200 cm^{-1} represents the O-H group. The peak at 1033.78 cm^{-1} could be related to the C-O stretching vibration of carboxylic acids and alcohols. Activated carbon prepared from *Dipterocarpus alatus* [20] its FTIR analysis shows the different functional groups. The bands at 1160 and 1093cm-1 are attributed to C-O stretching. The peaks observed at 1093cm-1 is due to aromatic C-H in plane deformation.

Brunauer-Emmett-Teller (BET)

Coconut shell [14], orange peel [15] activated carbon was characterized by BET. The BET surface analysis of Activated carbon from Coconut shell reveals that the synthesized sample is a mesoporous solid. Pore volume at 1.032 cm^3/g was found by the BET surface analysis. BET surface area of Activated Carbon prepared from orange peel, shows the sample with higher burn-offs presented higher apparent, for the K_2CO_3 series the values were between 9 and 1352 m^2 g^{-1} and micro pore volume, by the as method, between 0.01 and 0.79 cm^2 g^{-1}. $ZnCl_2$ series results were a little lower with BET surface area between 804 and 1215 m2 g^{-1}, and micro pore volume within the range 0.10–0.16 cm^3 g^{-1}.

X-ray Diffraction (XRD)

Coconut shell [14] activated carbon was characterized by XRD analysis. It proved that the sample had a perfect crystallite structure. Peaks corresponding to potassium metal and potassium oxides usually appear at 42^o and 49^o (2θ) with 42^0 denoting the "K" metal and 49^0 (2θ) the K2CO3 compound. The XRD patterns of orange peel [15], activated carbon did not exhibit well defined peaks in any region, which indicated that no discrete mineral phase

was detected. Thus the orange peel has predominantly amorphous structure of the activated carbon prepared, transforming it into a structure of greater crystalline at higher temperatures.

CONCLUSION

In this review, the inexpensive and effective method of removing dyes by using agriculture waste biomass as replacements for existing commercial materials were investigated. Adsorption process is a powerful technique that can be used for efficient removal or uptake of toxic materials from gas and liquid phases. Activated carbon is one of the most important adsorbent that can be employed for these purposes. The use of these low-cost biosorbents is recommended since they are relatively cheap or of no cost, easily available, renewable and show highly affinity for dyes. Reviewing a group of papers that deal with dye removal by agriculture waste, it can conclude that agricultural waste biomass is very promising not only for removal of dyes but for other pollutants such as hazardous, heavy metal substances and oxidation.

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REFERENCES

1. D.Li, J.Yan, Z.Liu Adsorption kinetic studies for removal of methylene blue using activated carbon prepared from sugar beet pulp, International Journal Environmental science Technol. (2016) 13:1815-1822. http://dx.doi.org/10.1007/s13762-016-1012-5
2. Mondira Bardhan, Tamanna Mamun Novera, Mumtahina Tabassum, Md. Azharul Islam, Ali H. Jawad and Md. Atikul Islam Adsorption of methylene blue onto betel nut husk-based activated carbon prepared by sodium hydroxide activation process. The Authors water science and Technology82.9|2020. (http:// creativecommons.org/licenses/by/4.0). doi:10.2166/wst.2020.451
3. S.Sivakumar, P. Muthirulan, M. Meenakshi Sundaram Adsorption kinetic and isotherm studies of Azure A on various activated carbons derived from agricultural wastes Arabian Journal of chemistry (2019) 12, 1507-1514. http://dx.doi.org/10.1016/j.arabjc.2014.10.028
4. G.D. Akpen, I.L.Nwaogaziie and T.G. Leton adsorption characteristics of mango (Magniferaindica) seed shell activated carbon for removing phenol from waste water Journal of Applied Science and Technology(JAST),Vol.19,Nos.1&2,2014,pp.43-48. http://www.researchgate.net/publication/277652644
5. Deji K.C. and Khagendra Prasad Bohara adsorptive removal of chromium (VI) by papaya seed from aqueous media Journal Nepal chem. Soc., Vol. 39, 2018. http://dx.doi.org/10.3126/jncs.v39i0.27015
6. C. Namasivayam, D. Sangeetha kinetic studies of adsorption of thiocyanate onto $ZnCl_2$ activated carbon from coir pith, an agricultural solid waste chemosphere 60 (2005) 1616-1623. https://doi.org/10.1016/j.chemosphere.2005.02.051
7. W. T. Tsai, C. Y. Chang, M. C. Lin, S. F. Chien, H. F. Sun, M. F. Hsieh adsorption of acid dye onto activated carbons prepared from agricultural waste bagasse by $ZnCl_2$ activation Chemosphere 45 (2001) 51-58. https://doi.org/10.1016/S0045-6535(01)00016-9
8. Muthanna J. Ahmed, Samar K. Dhedan equilibrium isotherms and kinrtics modelling of methylene blue adsorption on agricultural wastes based activated carbons. Fluid phase Equibria 317 (2012) 9-14. https://doi.org/10.1016/j.fluid.2011.12.026

9. F.W. Shaarani, B.H. Hameed batch adsorption of 2,4-dichlorophenol onto activated carbon derived from agricultural waste. Desalination 255 (2010) 159-164. https://doi.org/10.1016/j.desal.2009.12.029
10. S. Rengaraj, Seung-Hyeon Moon, R. Sivabalan, B. Arabindoo, V. Murugesan agriculture solid waste for the removal of organic adsorption of phenol from water and wastewater by palm seed coat activated carbon. Waste management 22 (2002) 543-548. https://doi.org/10.1016/S0956-053X(01)00016-2
11. AzryBorhan and Ahmad FikreeKamil preparation and characterization of activated carbon from Rubber-seed shell by chemical activation. Journal of Applied Sciences 12 (11): 1124-1129, 2012. http://dx.doi.org/10.3923/jas.2012.1124.1129
12. Mustafa Baysal, Kaan Bilge, Bengisu Yilmaz, MelihPapila, YudaYurum, preparation of high surface area activated carbon from waste-biomass of sunflower piths kinetics and equilibrium studies on the dye removal, Journal of Environmental Chemical Engineering https://doi.org/10.1016/j.jece.2018.02.020.
13. Khu Le Van, Thu Thuy Luong Thi, activated carbon derived from rice husk by NaOH activation and its application in supercapacitor, Progress in Natural Science: Materials International 24(2014) 191-198. http://dx.doi.org/10.1016/j.pnsc.2014.05.012
14. MdShariful Islam, Bee Chin Ang, Samira Gharehkhani and AmalinaBinti Muhammad Afifi, adsorption capability of activated carbon synthesized from coconut shell,Original Articles Carbon Letters Vol. 20, 1-9 (2016). http://dx.doi.org/10.5714/CL.2016.20.001
15. EdaKoseoglu, CananAkmil-Basar, preparation, structural evalution and adsorptive properties of activated carbon from agricultural waste biomass, Advanced Power Technology APT 965 (2015) No. of pages 8. Model 5G. http;//dx.doi.org/10.1016/j.apt.2015.02.006
16. AsmaNasrullah, Bahruddinsaad, A.H. Bhat, Amir Sada Khan, Mohammed Danish, Mohamed Hasnain Isa, Abdul Naeen, Mangosteen peel waste as a sustainable precursor for high surface area mesoporous activated carbon: Characterization and application for methylene blue removel, Journal of Cleaner Production (2018), doi: http://doi.org/10.1016/j.jclepro.2018.11.094.
17. Ramlah Abd Rashid, Ali H. Jawad, Mohd Azlan Mohd Ishak and Nur Nasulhah Kasim, KOH-activated carbon developed from biomass waste: adsorption equilibrium, kinetic and thermodynamic studies for methylene blue uptake ,Desalination and water Treatment, https://doi.org/10.1080/19443994.2016.1167630
18. Jamshaid Rashid, FakhraTehreem, AdeelaRehman, Rajeev Kumar, Synthesis using natural functionalization of activated carbon from pumpkin peels for decolourization of aqueous methylene blue, Science of the Total Environment 671 (2019) 369-376. http://doi.org/10.1016/j.scitotenv.2019.03.363
19. Jianfeng Liang, Jianrong Xia and Jianyou Long, Biosorption of methylene blue by non-living biomass of the brown macroalga Sargassum hemiphyllum, IWApublishing 2017 water science and Technology 76.6 2017. https://doi.org/10.2166/wst.2017.343
20. Chantakorn Patawat, Ketsara Silakate, Somchaichuan-Udom, Nontipa Supanchaiyamat, Andrew J.Hunt and YuvaratNgernyen, Preparation of activated carbon from dipterocarpusalatus fruit and its application for methylene blue adsorption, The Royal society of Cemistry Adv., 2020,10,21082-21091. https://doi.org/10.1039%2Fd0ra03427d

Table-1: Comparison table for Biomass adsorbent and their characterization

S. No	Adsorbent	Activating agent	Temperature	Adsorption studies	Kinetic studies	Characterization
1	Sugar beet pulp	H_3PO_4	Impregnated by 110^{0}C for 12hrs activated by (350,400,450,500 and 550^{0}C) time (0.5,1,1.5,2 and 2.5hrs)	-	Pseudo-second order	BET, FTIR
2	Betel nut husk (BNH)	NaOH	BNH was mixed with powdered NaOH at three (1:1,1:2 and 1:3) ratios for	Langmuir, Freundlich	Pseudo-second order	SEM, FTIR

			24hrs at room temperature. Activated by 500 ^{0}C for 1hrs			
3	Indian Almond shell carbon,ground nut shell carbon, areca nut shell carbon, tamarind shell, Cashew nut shell	Acid	Impregnated by 400 ^{0}C, Activated by 120^{0}C for 1 hr	Langmuir, Freundlich, Redlich-Peterson	Pseudo-second order	SEM, FTIR
4	Mango seed	$ZnCl_2$	Impregnated by 100^{0}C for 30 minutes, activated by 500^{0}C for1hr	Phenol adsorption	-	-
5	Papaya seed	-	RPS washed with distilled water to remove the dust particles. It was left at room temperature for1 day. Dried in oven at 105^{0}C for 24hrs	Langmuir	Pseudo-second order	-
6	Coir pith	$ZnCl_2$	Drying temperature 700^{0}C	Langmuir, Freundlich	Pseudo-second order	-
7	Sugarcane bagasse	$ZnCl_2$	Soaking $ZnCl_2$ for 0.5 hrs then pyrolysis at 500^{0}C.	Langmuir, Freundlich	-	FTIR
8	Date stones	$ZnCl_2$	Impregnated by30^{0}C for 24 hrs. Activated by 500^{0}C for 1hr	Langmuir, Freundlich	Pseudo-first order, Pseudo-second order	-
9	Oil palm empty fruit bunch	H_3PO_4	Impregnated by 105^{0}C for 24hrs activated by 450^{0}C for 2hrs	Langmuir, Freundlich	Pseudo-second order	SEM
10	Palm seed	-	Thermal activation in carbon dioxide atmosphere at 850-900^{0}C for 3hrs.	Freundlich	Pseudo-Firstorder	
11	Rubber-seed shell	KOH	Powered RSS mixed with KOH at 1:1 ratio then activated by 500^{0}C time 3hrs.	-	-	SEM
12	Sunflower pith	KOH and NaOH	Activated by 700^{0}C for 1hr	Langmuir, Freundlich	Pseudo-First order	SEM, FTIR
13	Rice husks	NaOH	Impregnated by 120 ^{0}C for 12hrs activated by 650,700,750 and 800^{0}C for 1hr	-	-	FTIR, SEM, TGA, DTA

14	Coconut shells	KOH	Impregnated by 400^{0}C for 30min.Activated by 600^{0}C for 2hrs	-	Pseudo-second order	BET, XRD, FTIR, SEM
15	Orange peel	$ZnCl_2$ and K_2CO_3	Impregnated by110^{0}C for 1hr.$ZnCl_2$ activated by 400-500^{0}C. K_2CO_3 activated by 900-950^{0}C.	-	-	BET, SEM, XRD
16	Mangosteen peel	$ZnCl_2$	MP powered mixed with $ZnCl_2$ in different ratios and impregnated. 500-800^{0}C various time ranging from0.5hr to 3hrs	Langmuir	Pseudo-second order	FTIR, SEM
17	Pumpkin peel	NaOH	Dried PP was heated250,350,450and 550^{0}C for 1hr,	Langmuir, Freundlich	Pseudo-second order	SEM, FTIR
18	Fallen coconut leaves	KOH	Impregnated by110^{0}C for 24hrs activated by700^{0}C for 1h.	Langmuir	Pseudo-second order	FT-IR
19	Sargassum hemiphyllum	-	Dried at 333K for 2 hrs	Langmuir	Pseudo-second order	SEM, FT-IR
20	Dipterocarpus alatus fruits (wing, pericarp, endocarp)	$ZnCl_2$	Chemically activated by immersed in $ZnCl_2$, FeCl3, H3PO4 or KOH for 24 hrs and carbonized at 500^{0}C in N_2 atmosphere for 1 hrs. Physically activated by 400^{0}C in N_2atmosphere for 1 hrs followed by 850^{0}C in CO_2 for 1 hr.	Langmuir, Freundlich	Pseudo-first order, & second order	SEM, FTIR

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REVIEW ARTICLE

Exploring the Medicinal Aspects of Various Plant Varieties of Uttarakhand: A Review

Namrata Singh[1], Rashmi Verma[2]*, Aayushi Bhakta[1], Anushka Kala[1] and Rabia Basri Aziz[1]

[1]Ph.D Scholar, Department of Biotechnology, School of Basic and Applied Sciences Shri Guru Ram Rai University, Dehradun, Uttarakhand, India.
[2]Assistant Professor, Department of Biotechnology, School of Basic and Applied Sciences, Shri Guru Ram Rai University, Dehradun, Uttarakhand, India.

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***Address for Correspondence**
Rashmi Verma
Assistant Professor,
Department of Biotechnology,
School of Basic and Applied Sciences,
Shri Guru Ram Rai University,
Dehradun, Uttarakhand, India.
Email: rashmi210417@gmail.com

ABSTRACT

From the beginning of the human civilization, the main natural resource for the primary health care system is medicinal plants. Uttarakhand an 'Herbal state' is a rich source of traditional medicine knowledge of medicinal plants. A lot of information about medicinal plants of Uttarakhand is carried out in different forms and different spaces. From ancient times, humans have been handed over their knowledge on the use of several plant species to their next generation. The natives of Uttarakhand have good knowledge of the medicinal plants and traditional therapy. The natives of this state use plants for their primary health care system and depend on traditional knowledge of medical practices of medicinal herbs. The Medicinal and Ayurvedic plant assortment of Uttarakhand hills has been provided and conducted by natives to view for different species of medicinal plants. Many researchers have done studies to investigate the role of various medicinal plants of Uttarakhand in treatment of various diseases. Till date various studies reported the medicinal and clinical use of numerous plant varieties of uttarakhand and still various plant species are yet to be explored in terms of its medicinal properties. The present study is a review on some previously reported medicinally important plant varieties of Uttarakhand and the aim is to record the information about the use of these plants for medicine. These species of plants are found in different forms e.g. trees, shrub and herbaceous. Studies reported that different parts of plant like root, leaf, shoots, bark, bulb, flowers, fruits, tuber as well as rhizome are important for preparing herbal medicine for curing various ailments. The information about these

reported plants will be helpful in further research and explore various other plant varieties of Uttarakhand and their potential use as therapeutic medicines.

Keywords: Medicinal plants, Traditional medicine, biodiversity, clinical, bacterial strains.

INTRODUCTION

Medicinal plant bestowed the human study their importance since civilization. India is regarded as having a wealth of genetic diversity, species diversity, and habitat diversity. Uttarakhand the part of Himalayan region is also considered for its diversity wealth, including medicinal plant, aromatic plant and species. More than 7,500 species have been considered and reported to have medicinal uses. Out of so many species such as 17,000 angiosperm species, 64 gymnosperms, 1,200 pteridophytes, 2,850 bryophytes, and 2,021 lichen [1]. Out of the total, 7,500 species have been reported to have medicinal uses [2]. Indian part of the Himalaya have been provided diverse topography and climatic conditions made it unique area which is especially in rich medicinal plants where as alpine areas is considered major source of important medicinal plant. Because of its position and the mild to chilly climate it experiences, the Uttarakhand Himalayas are thought to have a rich diversity. According to World Health Organization (WHO) 25% of modern medicines have been developed from plant sources which were used traditionally, and 75% of herbal drugs discovery these on the basis of research on traditionally medicinal herbal plant [3-4]. People of rural and remote area considered medicinal plant not only major component of their health take care system but also their economic benefits.

Various factors for improvisation of medicinal plants conservation and their production

It is important to encourage the cultivation of those therapeutic plants having a sizable market. A location should be chosen that has favorable agro-ecological conditions and comparatively little economic growth. All the cultivation of important medicinal plants research and development is needful for caring out to understand the favorable conditions should be done. This can aid in enhancing productivity and production of medicinal and herbal plants by fostering more collaboration between researchers and farmers. Such shopper should be identified in the place of market that can assure to purchase of whole manufacturing at a best price that higher return can other crops and increase their trade in the state. The locality of medicinal plants and aromatic from cultivation should be increased on hilly barren land. Although villagers are becoming more aware of herbal medicines and dietary supplements, many are still unaware of the importance of the pharmaceutical and food industries. Understanding how cultural influences affect the usage and production of medicinal plants will be crucial for maintaining biodiversity. It is crucial to implement low-cost processing and the cluster technique, which increases production and productivity. It is required to fix support prices by the government for long term plantation. Road network at village level due to the difficult physical geography of the hill villages in the state has a very important role. In order to grow and enhance the production of medicinal principles and to perform R&D to develop green products, the government of Uttarakhand must strengthen its technological and scientific capabilities. State forest policies in Uttarakhand that support preservation and sustainable use of medicinal plants should be revised by the government.

Importance of medicinal plants

The use of medicinal plants is seen to be quite safe, as there are no or very few adverse effects. The fact that these medicines are in tune with nature is the largest advantage. The golden truth is that herbal remedies can be used by people of all ages and genders. Herbs, according to ancient experts, are only answers to a variety of health-related problems and disorders. They undertook extensive research and experimentation in order to get reliable conclusions about the usefulness of various therapeutic herbs. The majority of the medications created in this manner have no negative side effects or responses. This is why herbal medicine is becoming increasingly popular around the world. These medicinal herbs provide a sensible approach of treating a variety of interior ailments that would otherwise be difficult to treat.

Literature review on medicinal plants of Uttarakhand

Plants that are growing in Uttarakhand are already consumed by local people of Uttarakhand villages as medicines of certain common sickness. Many researchers have studied and explored various plant varieties of Uttarakhand till date and the previous studies reported the importance of uttarakhand plant variety as an important and effective source of medicine for the treatment of many physiological disorders and diseases. Studies revealed different medicinal plants and have explored there importance and role in treatment and cure of various diseases like cardiovascular, arthiritis, diabetes, high blood pressure, hypertension, constipation, cancer, and many more. Some reviewed studies on the medicinally important plants, and their specific use in a particular disease have been listed in the given table 1.

DISCUSSION

A table has been created that lists some of the therapeutic properties of plants from Uttarakhand. All of these plants' botanical data were verified and analysed on the Plants of the World Online database website. This study is pointed out the plants, shrubs and herbaceous which are used in preserve different diseases. Some of this genus is applied in health drink and herbal tea. Different parts of medicinal plants are used as medicine by the natives of Uttarakhand. These parts are root, bark, leaf, stem, bulb, tuber, rhizome, flowers, fruits and seeds. Applied of different genus persist whole in the research from beginning due to remote hilly area. Some important species were exploited a lot due to lack of awareness and commercial interests. *Zanthoxylum armatum* belongs to the *Rutaceae* family, is a medicinal plant and is commonly known as Timur in Uttarakhand. Singh(2010) studied that *Z. armatum* is popularly used in the Indian system of medicines. The natives of Uttarakhand use this plant as a source of food and medicines from centuries. Natives have tradition of worship it in some region. Young shoot are usually used as toothpaste and also used as a cure for gum diseases. The fruits are used for removing round worm present in the stomach. Plant extract is also used for the treatment of Pneumonia and tick infection caused by eactoparasite. *Rhododendron arboretum* belongs to the Eraceae family and is a medicinal plant. It is commonly known as Burans in Uttarakhand. Ayurvedic buransh juice prevents heart, skin, liver and body pain. According to some research Buransh is also rich in nutrients and is suitable for overall health. Buransh juice is beneficial in diabetes, anemia and body weakness. It has painkilling ability. It makes liver and skin healthy. This plant is rich in anti-inflammatory properties.

Mentha piperita, also known as wild *Mentha balsamea*. It belongs to the Lamiaceae family. Common name of this plant is peppermint. It has a cooling effect that's why used topically for nerve pain and muscle pain. It provides relief from itiching and used as a fragrance. *Pyracantha crenulata* belongs to the Rosaceae family. In Uttarakhand, common name of this medicinal plant is Ghigharu. This plant is used in the treatment of hypertension, myocardial weakness, paroxysmal tachycardia cardiac failure. This plant is a good source of beta carotene, iron and potassium. It reduces joint pains and act as appetizer. *Tinospora cordifolia* belongs to the Menispermaceae family. The common name of this medicinal plant is Giloy in Uttarakhand. This plant is a universal herb that helps boost immunity. It is a powerhouse of antioxidant properties. It helps remove toxin, purifies blood, treat diabetes, and reduces stress and anxiety. It helps in arthritis and improve respiratory problem. *Ficus auriculata* belongs to the Moraceae family. The fruits, leaves and root of this plant are used to prepare the medicine of diarrhea and constipation. It leaves are also beneficial in diabetes and high cholesterol. Many medicinal plants like *Ficus auriculata*, Bedu and Ghigharu etc have been used in various pharmacological activities. The development activities, tourism, population growth, construction work and deforestation ultimately affect the traditional system of plant use for medicine. It also affects the diversity of convenient plant species. We need to conserve the biodiversity of medicinal plants. It requires close observation of the natural world as well as knowledge of customary medical procedures, elements of natural and regional medicinal herbs, etc. These traditionally used, widely accessible, and cost-effective therapeutic plants for health were heavily employed by hill natives.

Future of Medicinal plants industry in Uttarakhand

The production and cultivation of medicinal plants in Uttarakhand is mostly unorganized. An equipped supply chain management and formation of farmer associations can improve the scenario. Improvement is required in sell and production of medicinal plants in Uttarakhand.

CONCLUSION

Medicinal plants are the principle health care resources among the villagers in India. These plants of Uttarakhand play an important role for treatment of various ailments like cuts, wounds, diarrhea, dysentery and dental problems etc. These plants are also used in various pharmacological activities as well as the food items due to high nutritional value. Medicinal plant also possesses antimicrobial, antifungal, antibacterial, antiviral property. Most of the regions of Uttarakhand are covered by the forests with a very scattered population mainly the tribal community. This population depends on folk medicine. These people prefer to consult the local traditional healers or elderly person in the family for the treatment. These people only moved hospitals in case of any serious health trouble. The present study covered numerous antibacterial, antifungal, antiviral properties found in medicinal plant. These plants are very helpful for health and have no side effects. These plants are easily available, less expensive and much important that no side effects compare to modern medicine. Medicinal plants are used in new drug discovery due to antioxidant properties.

REFERENCES

1. Joshi B, Pant SC. Ethnobotanical study of some common plants used among the tribal communities of Kashipur, Uttarakhand. Indian Journal of Natural Products and Resources. 2012; 3(2):262-266
2. M. P. Shiva, Inventory of Forestry Resources for Sustainable Management and Biodiversity Conservation, Indus Publishing Company, New Delhi, India, 1996.
3. Mallik, A. R., Chaudhary, S., & Shrestha, S. Useful valuable plants of Maithili community in Eastern Nepal: An ethnobotanical study. *Bangladesh Journal of Plant Taxonomy*, 2020, *27*(2), 439-446.
4. World Health Organization (WHO), WHO Traditional Medicine Strategy, WHO, Geneva, Switzerland, 2002.
5. Garima Tamta, Nisha Mehra and S. Tandon, (2021) "Traditional uses, Phytochemical and Pharmacological properties of Ficus auriculata: A Review *Journal of Drug Delivery & Therapeutics.* 11(3):163-169.
6. Ramachandraiah, C., Nandish, S. K. M., JayannaKengaiah, C., Srinivas, A. S., Martin, S. S., &ManoharShinde, D . Macrotylomauniflorum seed aqueous extract exhibits anticoagulant, antiplatelet and clot dissolving properties. *Asian Journal of Pharmacy and Pharmacology*, (2019),5(3), 589-603.
7. Nadiroğlu, M., Behçet, L., & Çakılcıoğlu, U. An ethnobotanical survey of medicinal plants in Karlıova (Bingöl-Turkey). *Indian Journal of Traditional Knowledge*, (2019),18(1), 76-87.
8. Shan, S., Huang, X., Shah, M. H., & Abbasi, A. M. Evaluation of Polyphenolics Content and Antioxidant Activity in Edible Wild Fruits. *BioMedical research international,* 2019, 1381989-1381989.
9. Kumar, S., Kumar, A., & Kumar, R. Himalayan (Himachal region) cedar wood (Cedrusdeodara: Pinaceae) essential oil, its processing, ingredients and uses: *A review. Journal of Pharmacognosy and Phytochemistry,* 2019,8(1), 2228-2238.
10. Negi, V. S., Maikhuri, R. K., Maletha, A., &Phondani, P. C. Ethnobotanical Knowledge and Population Density of Threatened Medicinal Plants of Nanda Devi Biosphere Reserve, Western Himalaya, India. *Iranian Journal of Science and Technology, Transactions A: Science,* 2019,43(1), 63-73.
11. Apu, A. S., Al-Baizyd, A. H., Ara, F., Bhuyan, S. H., Matin, M., & Hossain, M. F. Phytochemical analysis and bioactivities of Argemonemexicana Linn. leaves. *Pharmacology online*, 2012,3, 16-23.
12. Topwa, M. Review on Important Ethno- Medicinal Plants in Uttarakhand. *International Journal of Pure & Applied Bioscience,* 2018, 6(2), 455–464.
13. Bandana, K., Raina, R., Kumari, M., & Rani, J. Tagetesminuta: An overview. *International Journal of Chemical Studies,* 2018, 6(2), 3711-3717.

14. Singh, A., Nautiyal, M. C., Kunwar, R. M., &Bussmann, R. W. Ethnomedicinal plants used by local inhabitants of Jakholi block, Rudraprayag district, western Himalaya, India. *Journal of Ethnobiology and Ethnomedicine,* 2017, 13(1), 1–29.
15. Kaur, K., Sidhu, M. C., & Ahluwalia, A. S. Angiosperm diversity in Doaba region of Punjab, India. *Journal of Threatened Taxa,* 2017, 9(8), 10551-10564.
16. Bozorgia M, Amin G, Shekarchi M, Rahimia R. Traditional medical uses of Drimia species in terms of phytochemistry, pharmacology and toxicology. *Journal of Traditional Chinese Medicine*. 2017, 37(1):124-139.
17. Sharma IP, Kanta C, Semwal SC, Goswami N. Wild Fruits of Uttarakhand (India): Ethnobotanical and Medicinal Uses. *International Journal of Complementary & Alternative Medicine.* 2017, 8(3):1-8. 2
18. Kumar, V., Malhotra, N., Pal, T., & Chauhan, R. S. Molecular dissection of pathway components unravel atisine biosynthesis in a non-toxic Aconitum species, A. heterophyllumWall. 2016,3. *Biotech*, 6, 106, 1-10.
19. Joshi, R. K., Satyal, P., &Setzer, W. N. Himalayan aromatic medicinal plants: a review of their ethnopharmacology, volatile phytochemistry, and biological activities. Medicines, 2016, 3(1), 1-55.
20. Nisar, M., Ali, S., Muhammad, N., Gillani, S. N., Shah, M. R., Khan, H., &Maione, F. Antinociceptive and anti-inflammatory potential of Rhododendron arboreum bark. *Toxicology and industrial health*, 2016, 32(7), 1254-1259.
21. Purnima, B. M., &Kothiyal, P. A review article on phytochemistry and pharmacological profiles of Nardostachysjatamansi DC-medicinal herb. *Journal of pharmacognosy and phytochemistry,* 2015, 3(5), 102-106.
22. Ghosh, S., Parihar, V. S., More, P., Dhavale, D. D., &Chopade, B. A. Phytochemistry and therapeutic potential of medicinal plant: *Dioscorea bulbifera*. Med chem, 2015, 5(4), 154-159.
23. Singh, K. J., & Thakur, A. K. Medicinal plants of the Shimla hills, Himachal Pradesh: a survey. *International Journal of Herbal Medicine,* 2014, 2(2), 118-127
24. Sharma S, Satpathy G, Gupta RK. Nutritional, phytochemical, antioxidant and antimicrobial activity of Prunus armenicus. *Journal of Pharmacognosy and Phytochemistry*. 2014, 3(3):23-28.
25. Rahman AHMM, Hossain MM, Islam AKMR. Taxonomy and Medicinal Uses of Angiosperm Weeds in the Wheat Field of Rajshahi, Bangladesh. *Frontiers of Biological and Life Sciences.* 2014, 2(1):8-11. 8
26. Adkar,P.P. and Bhaskar,V.H. Pandanus odoratissimus (Kewda): A Review on Ethnopharmacology, Phytochemistry, and Nutritional Aspects. *Advances in Pharmacological Sciences,* Article ID 120895, 2014, 19 pages.
27. Siddiqui, M. H., Al Whaibi, M. H., Faisal, M., & Al Sahli, A. A. Nanosilicon dioxide mitigates the adverse effects of salt stress on Cucurbitapepo L. *Environmental toxicology and chemistry,* 2014, 33(11), 2429-2437.
28. Prasad, R. Synthesis of silver nanoparticles in photosynthetic plants. *Journal of Nanoparticle,* Article ID 963961, 2014, 8 pages.
29. Bhattacharjee, P., & Bhattacharyya, D. Medicinal plants as snake venom antidotes. *Journal of Experimental and Applied Animal Sciences,* 2013, 1(1), 156-181.
30. Ozturk M, Uysal I, Gucel S, Altundag E, Dogan Y, Baslar S Medicinal Uses of Natural Dye-Yielding Plants in Turkey. *Research Journal of Textile and Apparel.* 2013, 17(2):69-80.
31. Gopinath K, Venkatesh KS, Ilangovan R, Sankaranarayanan K, Arumugam A . Green synthesis of gold nanoparticles from leaf extract of Terminalia arjuna, for the enhanced mitotic cell division and pollen germination activity. Industrial Crops and Products. 2013, 50:737–742.
32. Mawa, S., Husain, K., &Jantan, I. Ficuscarica L.(Moraceae): Phytochemistry, Traditional Uses and Biological Activities. *Evidence-based complementary and alternative medicine*: eCAM, 2013, 974256-974256.
33. Velayutham, K., Rahuman, A. A., Rajakumar, G., Santhosh Kumar, T., Marimuthu, S., Jayaseelan, C.&Elango, G. Evaluation of Catharanthusroseus leaf extract-mediated biosynthesis of titanium dioxide nanoparticles against Hippoboscamaculata and Bovicolaovis. *Parasitology research,* 2012, 111(6), 2329-2337.
34. Handral, H. K., Pandith, A., &Shruthi, S. D. A review on Murrayakoenigii: multipotential medicinal plant. *Asian Journal of Pharmaceutical and Clinical Research*, 2012, 5(4), 5-14.
35. Batish DR, Kaur M, Harminder P, Singh R, Kohli K. Phytotoxicity of a medicinal plant, Anisomeles indica, against Phalaris minor and its potential use as natural herbicide in wheat fields. Crop Protection. 2007, 26(7):948-952.
36. Singh, D., Singh, B., &Goel, R. K. Traditional uses, phytochemistry and pharmacology of Ficusreligiosa: A review. *Journal of Ethnopharmacology,* 2011, 134(3), 565–583.

37. Khosravi AR, Shokri H, Kermani S, Dakhili M, Madani M, Parsa S. Antifungal properties of Artemisia sieberi and Origanum vulgare essential oils against Candida glabrata isolates obtained from patients with vulvovaginal candidiasis. *Journal de Mycologie Médicale.* 2011, 21(2):93-99.
38. Rahmatullah M, Mollik MAH, Azam ATMA, Islam MR, Chowdhury MAM, Jahan R, Chowdhury MH, Rahman T. Ethnobotanical survey of the Santal tribe residing in Thakurgaon District, Bangladesh. *American-Eurasian Journal of Sustainable Agriculture.* 2009, 3:889–898.
39. Vadivelan, R., Bhadra, S., Ravi, A. V. S., Singh, K., Shanish, A., Elango, K., & Suresh, B. Evaluation of anti-inflammatory and membrane stabilizing property of ethanol root extract of Rubusellipticus Smith in Albino rats. *Journal of Natural Remedies,* 2009, 9(1), 74-78.
40. Qureshi RA, Ghufran MA, Gilani SA, Yousaf Z, Abbas G, Batool A. Indigenous Medicinal Plants used by Local Women in Southern Himalayan Regions of Pakistan. *Pakistan Journal of Botany.* 2009, 41(1):19-25.
41. Uniyal SK, Singh KN, Jamwal P, Lal B. Traditional use of medicinal plants among the tribal communities of Chhota Bhangal, Western Himalaya. *Journal of Ethnobiology and Ethnomedicine.* 2006, 2(14):1-8.
42. Naghibi F, Mosaddegh M, Mohammadi MM, Ghorbani A. Labiatae Family in folk Medicine in Iran. *Journal of Ethnobiology and Ethnomedicine.* 2005, 4(2):63-79.
43. Singh, S. S., Pandey, S. C., Srivastava, S., Gupta, V. S., Patro, B., & Ghosh, A. C. Chemistry and medicinal properties of Tinosporacordifolia (Guduchi). *Indian journal of pharmacology*, 2003, 35(2), 83-91.
44. Manandhar NP. Plants and People of Nepal. Timber Press Oregon. 2002; 7(12):599.
45. CCRS. An appraisal of Tribal- folk medicines. Vijay nagar, New Delhi, 1999.
46. Fayaz, M., Jain, A. K., Bhat, M. H., & Kumar, A. (2019).Ethnobotanical Survey of Daksum Forest Range of Anantnag District, Jammu and Kashmir, India. *Journal of herbs, spices & medicinal plants,* 25(1), 55-67.
47. Nisar, M., Ali, S., Muhammad, N., Gillani, S. N., Shah, M. R., Khan, H., &Maione, F. (2016).Antinociceptive and anti-inflammatory potential of Rhododendron arboretum bark. *Toxicology and industrial health*, 2016, 32(7), 1254-1259.

Table 1: Some Medicinal Plants of Uttarakhand state of India.

S. No.	Local Name	Botanical Name	Family	Part Used	Uses	Reference
1	Timla	Ficus *auriculata*	Moraceae	Fruit, bark and leaves	Maintain blood pressure, hypertension, constipation	Garima Tamta *et al*(2021)[5]
2	Kulthi bean/Gahat	Macrotyloma *uniflorum*	Fabaceae	Seed	It utilized as food, common cold, fever, throat infection, urinary diseases.	Ramachandraia *et al* (2019)[6]
3	Kandali/ Bichchughas	Urtica *dioical*.	Urticaceae	Leaf, fruit	Inflammation, cancer, rheumatism	Nadirogl *et al*(2019)[7]
4	Wild apricot	Prunus *Armeniaca*	Rosaceae	Fruit	It used as cooking oil, Constipation, and carminative.	Shan *et al* (2019)[8]
5	Deodar	Cedrus *deodara*	Pinaceae	Leaves, bark, wood root	It's used in Ayurvedic medicine. It utilized in diabetes, Rheumatism, stomach disease, cancer, inflammation.	Kumar *et al* (2019)[9]
6	Banwangun/Bankakri	Podophyllum *hexandrum Royle*	Berberidace	Rhizome, roots	It is used in wounds, leucorrhoea, Vermifuge, diabetes and constipation	Negi *et al* (2019)[10] Fayaz *et al* (2019)[46]
7	Premathandu/ kandra	Argemone *Mexicana L*	Papaveraceae	Leaf, stem, seed	It's used in skin diseases, toothache, dropsy, malaria.	Apu *et al*(2018)[11]

8	Arandi	Ricinus *communisL*	Euphorbiaceae	Leaf	Internal injury, arthritis, constipation	Topwa *et al.*(2018)[12]
9	Jungli Genda	Tagetes *erecta*	Asteraceae	Flower	It's used in sedative, stomach problem.	Bandana *et al.*(2018)[13]
10	Amedu	Rumex *hastatusD.Don*	Polygonaceae	Whole plant	Wounds, cuts, burns, stomachache	Singh *et al*, (2017)[14]
11	Bhilmoru	Oxalis *corniculata L*	Oxildaceae	Whole plant	Astringent, burning sensation, hepatitis, diarrhea, dysentery, dysmenorrhoea.	Kaur *et al.* (2017)[15]
12	Banpyaja	Drimia *indica (Roxb.) Jessop*	(Asparagaceae)	Buib	Among the illnesses for which bulb juice is utilised are cough, bronchitis, nematode infection, pyrexia dropsy, respiratory sickness, bone and joint difficulties, skin disorders, epilepsy, and cancer.	Bozorgia M, *et al* (2017)[16]
13	Ghigharu	Pyracantha *crenulata*	Rosaceae	Fruit	It utilized in cardiac failure, burgor's disease, arteriosclerosis, paroxysmal tachycardia, hypertension.	Sharma *et al.,* (2017)[17]
14	Kutki	Picrorhiza *scrophulariflora Pennel*	Scrophulariaceae	Root	It utilized in Jaundice, blood troubles, leucoderma, burning sensations, biliousness.	Kumar *et al.,* (2016)[18]
15	Basya	Eupatorium *adenophorum*	Asteraceae	Aerial parts	It is also used in treat wounds and cuts.	Joshi *et al.,* (2016)[19]
16	Burans	Rhododendron *arboretum Sm.*	Ericaceae	Flower, leaves, bark	It used in high blood pressure maintain, Chronic eczema, diarrhea, menstrual disorders, heart attack.	Uniyal & Shiva, (2005)[20] Nisar *et al.,* (2016)[47]
17	Jatamansi	Nardostachys *jatamansi (D.Don) DC.*	Valerianaceae	Rhizome	It is used in hypertension, heart diseases, Epilepsy, cerebral ischemia, liver damage, and insomnia.	Purnima & Kothiyal., (2015)[21]
18	Genthi	Dioscorea *bulbiferaL.*	Dioscoreaceae	Tuber, stem, leaf	Food, Syphilis, eye disease, goitre, and lung bleeding are all treated with it.	Ghosh *et al.,* (2015)[22]
19	Ogal	Fagopyrum *acutatum*	Polgonaceace	Whole plant	Anti-diabetic, and anti-tumour.	Singh & Thakur(2014)[23]
20	Khubani	Prunus *armeniaca L.*	Rosaceae	Fruit	This fruit's high levels of carotene and vitamin C make it a valuable source	Sharma S, *et al* (2014)[24]

					of food, a cancer treatment, and it also has antioxidant and antimicrobial properties. The entire plant is used orally for the treatment of cough, asthma, and fever.	
21	Guma	Leucas *cephalotes (Roth) Spreng.*	Lamiaceae	Whole plant	It's orally use of whole plant used in fever and decoction, Asthma, cough.	Rahman AHMM, *et al*;(2014)[25]
22	Kewda/ Ketki	Pandanus *fascicularis Lam.*	Pandanaceae	Leaf, Flower, Root, Fruit	It utilized in antispasmodic, aphrodisiac, cold/flu, leprosy Perfumery, dysuria, hemorrhoids, and hepatitis.	Adkar & Bhaskar (2014)[26]
23	Kafal	Myrica *esculenta*	Myricaceae	Flower, Fruit, Bark, Root	Asthma, arthritis, rheumatoid, menorrhagia menstrual disorders, cough and cholera.	Siddiqui *et al.,* (2014)[27]
24	Kingod	Berberis *aristataDC.*	Berberidaceae	Root	Jaundice, hepatitis, haemorrhage, infections of the skin and eyes, and malaria.	Prasad (2014)[28]
25	Kamach	Mucuna *pruriens*	Fabaceae	Seed	It's used in Snake venom.	Bhattacharjee, (2013)[29]
26	Bhotia badam	Corylus *colurna L.*	Betulaceae	Nut	Use of nuts directly or their tonic use as a diuretic or aphrodisiac.	Ozturk M, *et al* (2013)[30]
27	Arjun	Terminalia *arjuna*	Combretaceae	Bark	The usage of bark for pneumonia, ulcers, hepatic hypocholesterolemic fractures, antibacterial, and anti-HIV properties is highly beneficial.	Gopinath K, *et al* (2013)[31]
28	Fig	Ficus *caricaL.*	Moraceae	Fruit	Maintain bp, Colic, indigestion, loss of appetite, coughs, bronchial problems and cardiovascular disorders.	Mawa *et al.,* (2013)[32]
29	Amla	Emblica *officinalis Gaertn.*	Euphorbiaceae	Leaves, Fruit, root,	Heart conditions, excess weight, Alzheimer's, Parkinson's, and Huntington's chorea. It is heightened immune capacity.	Velayutham *et al.,* (2012)[33]

30	Karipatta	Murraya *koenigiiL.Spreng*.	Rutaceae	Leaf	It is used to treat nausea, vomiting, dangerous animal bites, and to make food taste better.	Handral *et al.,* (2012)[34]
31	Catmint	Anisomeles *indica (L.) Kuntze*	Lamiaceae	Leaf	Volatile oil found in both fresh leaves and greenish portions of the plant is used to cure snakebites, chronic rheumatism, psoriasis, and coughs and colds.	Joshi B, (2012)[1]
32	Ban Nimbu	Glycosmis *pentaphylla (Retz.) DC.*	Rutaceae	Seed	Plant seeds that are used to treat vomiting.	Joshi B, (2012)[1]
33	Timur	Zanthoxylum *armatum DC.*	Rutaceae	Bark, fruit,	Fever, dyspepsia, dental troubles, snake bite	Singh & Singh (2011)[36]
34	Vantulsi	Origanum *vulgare L.*	Lamiaceae	Whole plant	By drinking a decoction of the entire plant, various activities, urinary disorders can be treated.	Khosravi AR (2011)[37]
35	Brahati	Solanum *violaceum Ortega*	Solanaceae	Fruit	To reduce cough and other symptoms including hypertension, four ripe fruits are given orally at once.	Rahmatullah M, *et al* (2009)[38]
36	Himalayan raspberry	Rubus *ellipticus Sm.*	Rosaceae	Root, fruit	Diabetes mellitus, inflammatory disorders and ulcers	Vadivelan *et al.*, (2009)[39]
37	Laljari	Geranium *wallichianum D.Don*	Geraniaceae	Root	Root extract beneficial for backaches, gout, bone building, hepatitis, liver issues, and preterm labour.	Qureshi RA *et al.,* (2009)[40]
38	Jangali palak	Rumex *nepalensis Spreng.*	(Polygonaceae)	Leaf	Leaf juice or powder is beneficial for skin conditions with wound healing and anti-allergic characteristics, as well as for stomach colic.	Uniyal SK Singh (2006)[41]
39	Pipermint	Mentha *longifolia (L.)*	Lamiaceae	Leaf	Common cold, coryza, rheumatism, diarrhoea, and dyspepsia can all be treated with pipermint leaf juice or leaf powder.	Naghibi F *et al* (2005)[42]
40	Giloy	Tinospora *cordifolia*	Menispermaceae	Stem, root	Uses include treating viral fever, dengue, dyspepsia, jaundice, skin conditions, snakebite, and type 2 diabetes.	Singh *et al.,* (2003)[43]

41	Lalanchu	Rubus *ellipticus Sm*	Rosaceae	Fruit	It offers free energy drink and high antioxidant characteristics.	Manandhar NP. (2002) [44]
42	Sanjwanboata	Kalanchoe *pinnata (Lam.)*	Crassulaceae	Leaf	Leaf paste is applied to wounds to promote healing.	CCRS. (1999) [45]
43	Van Ajwain	Thymus *serpyllum L*	Lamiaceae	Leaf	Spice made from leaves to treat dyspepsia.	CCRS. (1999) [45]

A B

Fig. 1: A: showing leaves of *Zanthylum armatum*, B: showing tree of *Rhododendron arboretum*

A B

Fig. 2: A: Showing leaves of *Mentha piperita*, B: Showing tree of *Pyracantha creulata*

A B

Fig. 3: A: Showing leaves of *Tinospora cordifolia*, B: Showing small tree of *Ficus auriculata*

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RESEARCH ARTICLE

A Study on *In vitro* Propagation and Effect of Kn+ NAA, Kn + IAA and KN+ 2,4-D on Nodal Explants of *Buchanania lanzan* on MS Medium

Sakeena Gani[1]* and V.K. Pandey[2]

[1]Research Scholar, Department of Science, RKDF University, Bhopal, Madhya Pradesh, India
[2]Associate Professor, Department of Science, RKDF University, Bhopal, Madhya Pradesh, India

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***Address for Correspondence**
Sakeena Gani
Research Scholar,
Department of Science,
RKDF University,
Bhopal, Madhya Pradesh, India
Email: shokha965@gmail.com

ABSTRACT

Buchanania lanzan known as an important tree species is found in deciduous forest through larger piece of India. It is found in plenitude on clayey soils. In its common living space, the most extreme temperature changes from 40°C to 46° and least from - 1 °c to 13°C and normal precipitation shifting from 750 mm to 2120 mm. The natural product is a drupe void, black when ready. The kernels which have flavor fairly between that of pistachio and almond are eaten crude or broiled and are ordinarily utilized in the planning of milk based sweetmeats however the wood is of poor quality. Its sylviculture importance lies in extraordinary plenitude in certain normal kind of forests and its utility for attire dry slopes.

Keywords: *Buchanania Lanzan*, Kn, NAA, 2,4-D, IAA, Micropropagation.

INTRODUCTION

Many traditional plant based remedies are back in use and find increasing application as, (i) source of direct therapeutic agent, (ii) as a raw material base for the elaboration of more complete semi-synthetic chemical, (iii) as model for new synthetic compounds for the production (Dixon, 2001). The consumption and international trade in medicinal plant and phyto-medicines is increasing day by day, therefore, are growing and expected to grow in future quite significantly. With this growth in global demand for medicinal plants and a large base of local demand for plant based traditional medicine, demand for medicinal plants has increased tremendously during the last few decades (Ramachandra Rao and Ravishankar, 2002). Tissue culture is a means of preserving species that are rare and threatened and provides an alternative source of plant for commercial, horticulture and traditional medicinal trade. Tissue culture is the aseptic culture of plant protoplast, cell tissue or organ on a culture medium. The main difficulty in growing indigenous plants in large quantities for obtaining sufficient plant material is that in many plants the

seeds may germinate erratically, which makes production too slow to warrant their introduction as new commercial crops. *In vitro* methods are used to speed up propagation. The success of this system lies in the development of protocols for each species. This involves improving decontamination procedures and determining the effect of the cultural factors on plant growth, both in vitro and *ex vitro* and to establish optimum growing condition (Mei-Chun Lu, 2005).

Micropropagation

Tissue culture techniques are becoming increasingly popular as an alternative means of vegetative propagation. In recent years, in vitro techniques are considered as promising tools for selection of cell lines, mutants and somaclones (Groghan et al, 1978). Efficient methods for in vitro clonal propagation have been developed for many tree species but success has largely been restricted to poplars, willows. Eucalyptus, and pines (Konar and Nagamani, 1974; Bonga, 1987; Winton, 1978; Sommer and Brown, 1979; Tisserat, 1981). In this context, reports on successful. The gum subsequent to mixing with goat milk is utilized as a pain relieving. Products of chironji are diuretic and used to ease thirst, burning of the body and fever. Kernels of organic products are utilized as ointment in skin diseases (Das and Agrawal, 1991). The tree is leafless or about in this way, for a short time during the late spring season. Blossoms show up from January to March and their color is greenish-white. Natural products mature in the long periods of May–June (Troup, 1986). The organic products become red in the wake of ripening. The organic product assortment begins from mid-April and finishes by mid-June; however, its harvesting is commonly finished in 15-20 days as it were. In forests, its regular regeneration is extremely insufficient because of informal and pre-full grown harvesting of its seeds and site debasement by virtue of growing biotic weight. Chironji is an income generating produce of forest ward networks. On a normal, 40–50 kg new organic products are delivered per tree, which yields 8–10 kg on drying, resulting in 1–1.5 kg of finished produce per tree (Tewari, 1995). Normal yearly seed assortment is 300 to 1200 quintals in Madhya Pradesh (Prasad, 1989).

MATERIALS AND METHODS

The present investigation entitled "A Study on Effect of Kn + NAA and Kn + 2,4-D and Kn + IAA on Nodal Explants of *Buchanania lanzan* on MS Medium" was carried out in the Department of Botany, RKDF University, Bhopal MP India. *Buchanania lanzan* was the plant entitled for the project. Mature seeds of the plant were brought from the local market of Bhopal. Seeds were sown in poly bag and these grown plantlets were transferred into earthen pots, which were well maintained in the department premises. These preconditioned plantlets were then used as a source of explants for the project. The plants were maintained and used for the present investigation of the research work.

Preparation of culture medium:

a) Medium for plant tissue culture consists of a

1) Group of Macro elements
2) Group of Micro elements
3) Amino acids (Glycine)
4) Sucrose (which serves as a source of carbon and is a carbohydrate for plants)

The amount of salts to be taken are to be weighed as per the requirement and are dissolved in deionized water, care has to be taken to avoid precipitation of salts as it prevents availability of salts to plants. Salts with higher solubility are to be dissolved first.

b) Adjustment of the PH of the medium

1. Generally, plants can take up nutrients at a pH ranging from 5.6-5.8.
2. After the medium attains the desired pH, it is ready to be poured in Erlenmeyer's flask or 250 ml test tubes.
3. Semi-solid medium has to be prepared, the method followed is th weigh 0.75-0.8g of agar agar. This is to provide moisture to cultures.

c) Pouring of Medium

1) 100 ml medium is carefully poured into each 250ml Erlenmeyer flask and are plugged using cotton plug
2) Sterilization of culture medium in autoclaving

The media prepared and the equipment's are autoclaved at high temperature of 120^0C and pressure 15 Pascal.

Preparation of Explant

After the Explant is collected they are immediately immersed in a clean water to avoid entrance of air bubbles, microbes and containments from the cuts or exposed parts. After bringing the Explants to the laboratory surface sterilization is done for this the Explant is cut into smaller size with the help of scissors and the explants are put into Petri dish. In the next step the Explant is cleaned with a mild solution of detergent or Tween 20 as a wetting agent then the Explants are put in a clean beaker containing a mild solution of fungicide or an antibiotic. The Explants are put in it for some time then washed several times with deionized water. Finally, the Explants are taken into inoculation chamber.

Preparation of De-Ionized Double Distilled Water

The deionization treatment was done to remove most of the ionic impurities from water by passing through a deionizer (INDION). Double distillation process removed large organic molecules, pyrogens and micro-organisms (Brown and Thorpe 1984). A double borosilicate glass distillation unit was used for simultaneous supply of single and double distilled water.

Explant E-1 and E-2

Collection of E-1 and E-2

Shoot tip of 1-2 cm size were excised from the 1-2-year old plants grown at RKDF University Bhopal and 1-2-year-old plants of Chironji grown under greenhouse conditions by sterilized scissor in the morning. These excised shoot tips were collected in a culture bottle containing tap water.

Inoculation of Explants

1) While entering the inoculation chamber it is important to wear a clean lab court and take an air shower.
2) Inoculation means transferring of explants into media under aseptic conditions.

Transplantation of the Plantlets:

Acclimatization

Transfer of plantlets from in- vitro to natural condition involves delicate stages as these plants find it extremely hard to withstand the sudden change in environmental condition. Vij et al. (1995) suggested gradual change in humidity (70 - 60 per cent) and increase in temperature (28° - 30° C) during acclimatization. Acclimatization was done in the laboratory before transferring them to natural environment.

RESULT

Effect of Kinetin and NAA on nodal explants of *Buchanania lanzan* on MS medium

Kinetin and 2,4-D:

i) On MS medium supplemented with 0.2 mg/l of Kn and 0.3 mg/l of 2,4-D nodal explants did not develop callus. Single shoot with few roots was obtained in 120 days.

ii) On the medium with 0.3 mg/l of Kn and 0.6 mg/l of 2,4-D callus developed' and only one shoot with some roots was obtained in 120 days.

iii) When 0.3 mg/l of Kn was added to 0.9 mg/l and 2,4-D callus developed from the whole explant. Single plantlet transferable to soil developed in 120 days.

iv) From the callus multiple shoots developed in 65 days on MS medium supplemented with 0.5 mg/l of Kn and 1.5 mg/l of 2,4-D and total 14 number of plantlets were obtained.

v) In the medium with 0.9 mg/l of Kn and l.5 mg/1 of 2,4-D callus developed and multiple shoots developed from this callus in 69 days. Total 17 numbers of plantlets were obtained.
vi) When 2,4-D concentration was increased to 2.5 mg/l and Kn was kept at 0.2 mg/1 single plantlet transferable to soil developed in 120 days. No callus.

Kinetin and IAA

i) Nodal explants cultured on MS medium supplemented with lower concentration of IAA (0.1mg/l) along with lower concentration of kinetin (0.1mg/l) developed single shoot with roots but no callus. In 120 days' single plantlet was capable of being transferred to soil.
ii) In the medium with 0.1 mg/l of Kn plus 0.3 mg/l of IAA explants showed well developed roots with shoots. No callus.
iii) Callus developed from the explant on MS medium supplemented with 0.7 mg/l of Kn plus 0.6 mg/l of IAA. In 120 days 7 numbers of plantlets with well-developed roots transferable to soil were obtained.
iv) The best result was obtained with 0.9 mg/l of Kn plus 0.6 mg/l of IAA which resulted into multiple shoot formation in 55 days and more than 16 plantlets were obtained.
v) Response was slightly lower in 1.4+0.9 mg/l (8 shoots with roots) and 1.3+1.5 mg/l (5 shoots with roots) of kinetin plus IAA.

DISCUSSION

Response of Kn and NAA

Nodal explants cultured on MS medium added with different concentrations of kinetin and NAA showed positive response. This combination leads to shoot regulation only but no growth of root and callus**.** When nodal explants were supplied with 1.1 mg/l of NAA plus 0.6 mg/l of Kn, no callus but two shoots were seen coming out after 30 days, later 9 shoots developed within 120 days without roots When MS medium was supplied with 0.2 mg/l of Kn and 0.3 mg/l of 2,4-D nodal explants did not develop callus but a single shoot with few roots developed within 120 days.

Response of Kinetin and IAA

When MS medium was supplied with 0.1 + 0.1 mg/l of IAA and Kn, no callus, no root but opened axillary bud was seen after 30 days. 4 roots of one shoot was noticed after 60 days, later after120 days only 1 plantlet was transferred to soil. MS medium supplied with 0.6 + 0.9 mg/l of IAA and Kn, 16 well developed plantlets with well-established roots were noted

REFERENCES

1. Amasino, R. (2005). "1955: Kinetin Arrives. The 50th Anniversary of a new Plant Hormone". Plant Physiology. 138 (3): 1177-1184.
2. Bonga, J.M., Durzan, D.J. (1987). Introduction. In: Bonga, J.M., Durzan, D.J. (eds) Cell and Tissue Culture in Forestry. Forestry Sciences, vol 24-26. Springer, Dordrecht. https://doi.org/10.1007/978-94-017-0992-7_1.
3. Das, D. and Agrawal V.S. 1991. Fruits drug plants of India", Kalyani Publishers, New Delhi, pp. 250.
4. Dixon, 2001; Dixon and Paiva, 1995; Grayer and Harborne, 1994; Hammer Schmidt,1999; Kuc", 1995; Mansfield, 2000.
5. KONAR RN 1974 *In vitro* studies on Pinus. I. Establishment and growth of callus. Physiol Plant 32: 193–197
6. Nagamani, R. (1987). Tissue Culture of Himalayan Conifers and Allied Gymnosperms. In: Bonga, J.M., Durzan, D.J. (eds) Cell and Tissue Culture in Forestry. Forestry Sciences, vol 24-26. Springer, Dordrecht. https://doi.org/10.1007/978-94-017-0992-7_12.

7. Prasad, R. 1989. Research Needs in Minor Forest Produce in Madhya Pradesh. In: Minor Forest Produce and Tribal Development. Proceeding National Seminar October, 1989, Institute of Deciduous Forests, Jabalpur, India, pp. 32-34.
8. Ramachandra Rao sathuluri and Ravishankar Gokare, June 2002. Biotechnology advances 20(2): 101-53; DOI: 10. 1016/S0734-9750 (02) 00007-1.
9. Schwartz, Dale. "Carlos O. Miller" (PDF). Retrieved 15 November 2011.
10. Singh J, Patra AK, Nandeshwar DL, Meshram PB, Negi KS. Effect of growth regulators on the rooting of root cuttings of Chironji (Buchanania lanzan Spreng). Proceedings of National Workshop on Conservation of Medicinal Plants; 2002. p. 128.
11. Singh J, Patra AK, Nandeshwar DL, Meshram PB, Negi KS. Effect of growth regulators on the rooting of root cuttings of Chironji (Buchanania lanzan Spreng). Proce. of Nat. Workshop. Conserv. of Medic. Plants. 2002;128(6):11-12.
12. Singh, J., Banerjee, S.K. and Francis, A. 2002. Vegetative propagation of Buchanania lanzan Spreng. Root cuttings. Indian Forester, 128 (6): 700-704.
13. Tewari 1995. Bulletin on Buchanania lanzan (Chironji), Indian Council of Forest Research and Education, pp. 1–6.
14. Tewari, R.K. and Bajpai, C.K. 2001. Propagation of Chironji (Buchanania lanzan Spreng.) by chip budding. Agroforestry Newsletter. 13(1 and 2), pp.1-2.
15. Troup, R.S. 1986. The Sylviculture of Indian Trees. IBP Publishers, Dehra Dun (Reprinted). 1: 240–243.
16. World Health Organization. Traditional Medicine Strategy 2002-2005 (WHO/EDM/TRM/2002.1). Geneva, Switzerland: World Health Organization; 2002.

Table 1: - Different explants used for *in-vitro* propagation of Chironji

S.No.	Explant code	Explant	Source
1	E_1	Shoot tips	1-2 year old plants
2	E_2	Nodes	1-2 year old plants
3	E_3	Roots	1-2 year old plants
4	E_4	Young Leaves	1-2 year old plants maintained at Green house

Table 2: Effect of Kinetin and NAA on nodal explants of *Buchanania lanzan* on MS medium

MS medium+ PGRs (mg/l)		Intensity of development			Gradual development in day			
NAA	**Kn**	**R**	**S**	**C**	**30 days**	**60 days**	**90days**	**120days**
0.6	0.2	-	+	-	No root, no callus only two shoot	Single shoot of size 1.0 cm from axillary bud	Single shoot> 1.6	No plantlet capable of being transferred to soil
1.1	0.6	-	++	-	No callus only two shoots of size 1.1 cm & 1 cm from axillary bud	No callus but shoots branched into four	No roots but eight shoots of size 1.8 from axillary bud	9 shoots > 2.5 without roots
2.0	0.6	-	+	-	No development	No root only one shoot	No callus only one shoot of size 0.7 cm	No plantlet capable of being transferred to soil.

R = Roots: S = Shoot: C = Callus

Response types: +++: Excellent, ++: Good, +: Positive

Table 3 : Effect of Kinetin and NAA on nodal explants of *Buchanania Lanzan* on MS medium

MS medium + PGRs (mg/l)		Intensity of development			Gradual development in days			
2,4-D	Kn	R	S	C	30 days	60 days	90 days	120 days
0.3	0.2	+	+	_	1 shoot of size 1.3 cm from axillary bud but no root was developed	Single shoot of size 2.5 cm & 4 roots were developed	Single shoot > 2.8 cm & 5 roots >1.5 cm	1 Plantlet
0.6	0.3	+	+	+	No callus only single shoot of size 1.5 cm & 2 roots were developed	Small callus, single shoot of size 2 cm & 5 roots of size 1.7 were developed	Single shoot > 2.5 cm & 8 roots > 2 cm	1 plantlet
0.9	0.3	+	+	++	Single shoot of size 1.7 cm from axillary bud & callus at base	1 shoot of size 2.5 cm & 3 roots >1.5 cm	1 shoot >2.8 cm with 6 roots > 1.5 cm	1 plantlet
1.5	0.5	++	+++	+++	Base with callus, 5 roots & single shoot of size 1.7 cm from axillary bud	Base with callus, 7 roots > 1.5 cm & single shoot of size 2.5 cm from axillary bud	After 65 days multiple shoots started coming out	14 plantlets with few roots were capable of being transferred to soil
1.5	0.9	++	+++	+++	Base with callus, single shoot of size 2.5 cm from axillary bud & 3 roots were developed	White callus at base, single shoot of size 2.7 cm & 6 roots were developed	After 69 days multiple shoots were seen growing out	Root rich 17 plantlets were transferrable to soil
2.5	0.2	+	+	_	No root , no callus only a single shoot was developed	Single shoot of size 1.5 cm from axillary bud	No callus, no root only 1 shoot > 2.5 cm.	1 plantlet with roots

R: Roots, S: Shoot, C: Callus Response types = +++: Excellent, ++: Good, +: Positive.

Table 4: Effect of Kinetin and IAA on nodal explants of *B. Lanzan* on MS medium

MS medium+ PGRs (mg/i)		Intensity of development			Gradual development in days			
IAA	Kn	R	S	C	30 days	60 Days	90 days	120 days
0.1	0.1	+	+	_	No callus, no root but opened axillary bud	4 roots of one shoot with shoot length of 1cm	Shoot of length 1.7 & 6 roots > 1.5 cm	1 plantlet capable of being transferred to soil
0.3	0.1	++	+	_	3 roots, no callus but 1 shoot seen coming out from axillary bud	Single shoot of size 1.7 cm & 5 roots > 1.5 cm	10 roots > 1.5 cm & single shoot > 2.5 cm	1 plantlet capable of being transferred to soil
0.6	0.7	++	++	+	No callus but shoot of size 1 cm was obtained	Callus with 3 roots at base & shoot of size 2 cm	Callus with 4 shoots of size 2.5 cm & 5 roots	Well-developed 7 plantlets with well-developed roots were transferred to soil

0.6	0.9	+++	+ + +	++	Callus with 4 roots & single shoot of size 1.5 cm from axillary bud	Single shoot of size 2.5 cm & 8 roots > 1.7 cm	12 shoots of size 3 cm & 14 roots from callus	16 plantlets with well-developed roots were transferred to soil
0.9	1.4	+++	+ +	++	Callus with 4 roots followed by a single shoot of size 1.5 cm	4 shoots of size 2.5 cm & 8 roots of size 1.5 cm from callus	6 shoots of size 3.2 cm & 11 roots > 2 cm from callus	8 plantlets were transferred to soil
1.3	1.5	++	+ +	++	No callus but a single shoot of size 2 cm was obtained	Callus with 5 roots & single shoot of size 2.8 cm	3 cm 3-4 shoots & 8 roots of size > 1.5 cm	5 plantlets were transferred to soil

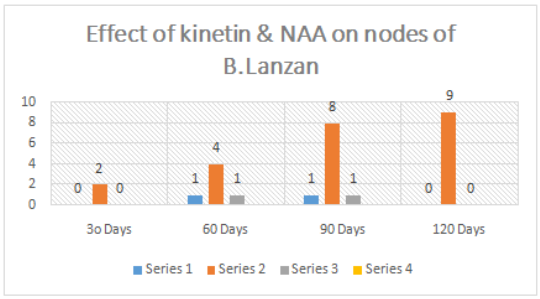


Fig 1: Inhibited growth with 0.6 + 0.2 mg/l of Kn + NAA a) Kn 0.2, NAA 0.6 b) 0.6 Kn, 1.1 NAA c) 2.0 NAA, 0.6 Kn

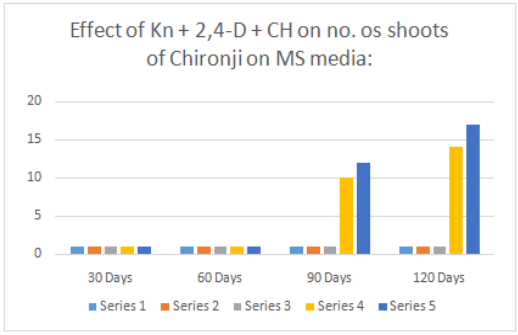


Fig 2: Callus with multiple shoots in 0.9 + 1.0 mg/l of Kn + 2,4-D

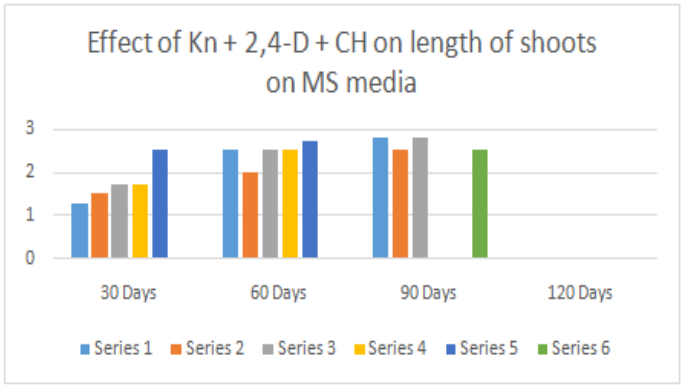
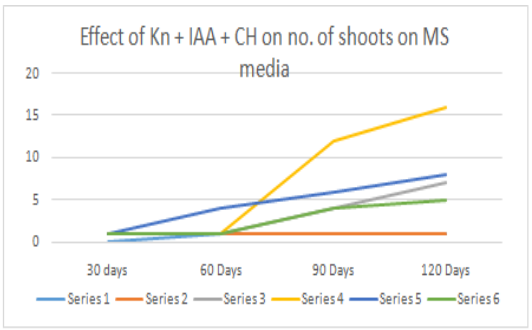


Fig 3: Effect of Kn +2,4-D+ CH On length of schoots on MS media
A = 0.3 2,4-D + 0.2 Kn + 50 mg/l B = 0.6 2,4-D + 0.3 Kn + 50 mg/l
C = 0.9 2,4-D + 0.3 Kn + 50 mg/l D = 1.5 2,4-D + 0.5 Kn + 50 mg/l
E = 1.5 2,4-D + 0.9 Kn + 50 mg/l F = 2.5 2,4-D + 0.2 Kn + 50 mg/



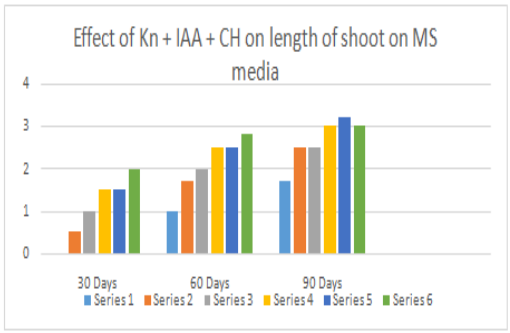


Fig.4: Callus with multiple shoots in 0.6 + 0.9 mg/l of IAA + Kn

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RESEARCH ARTICLE

An Efficient Detection of Manipulated Digital Photographs using Neural Network

S.Velmurugan[1]* and T.S.Subashini[2]

[1]Research Scholar, Department of Computer and Information Science, Annamalai University, Annamalai Nagar, Chidambaram, Cuddalore, Tamil Nadu, India.
[2]Professor, Department of Computer Science and Engineering, Annamalai University, Annamalai Nagar, Chidambaram, Cuddalore, Tamil Nadu, India.

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***Address for Correspondence**
S.Velmurugan,
Research Scholar,
Department of Computer and Information Science,
Annamalai University, Annamalai Nagar,
Chidambaram, Cuddalore, Tamil Nadu, India.
Email: ssvelmurugan@yahoo.com

ABSTRACT

In today's digital world tampering pictures has become a common issue. There are a lot of tampered images floating around digital media. Especially with the advancement of image manipulating software tools which are available online, tampering a digital image has become very easy task. Even though tampered images provide us a significant aesthetic view of the picture they can be used for malicious intent too. These images are much difficult to be detected with human eye which challenges the reliability of digital images as real-world events. The main objective is to detect a digital photograph whether it is genuine or manipulated. The manipulated digital photographs are detected by trained neural network using Error Level Analysis along with a supporting feature Metadata Extractor. The metadata analyzer is basically a tag searching algorithm. The rate of compression of the external content in a manipulated image will be divergent from that of a genuine photograph which can be identified by Error-Level analysis. The error level analyzed image is given as input to the Multi-Layered Perceptron Network. A Multilayer Perceptron (MLP) is a feed forward artificial neural network (ANN) which consists of input, hidden and output layers. The output layer has two neurons representing fakeness and realness. The digital image is classified as fake or real by a neural network which is trained with the highest success rate and using this desktop application will greatly reduce the spread of the manipulated photographs.

Keywords: Neural Network, Manipulated detection, Multi-layer Perceptron, Metadata extractor, Error Level Analysis.

INTRODUCTION

Manipulated or forging an image means altering a digital image by adding or removing few parts from it thereby creating new photographs. Sometimes, it is difficult to identify the processed area of the authentic image [1]. In the 21st century, digital images are being used everywhere, not just on social networks, but also in business, government, military, legal, industrial, forensic, medical and fashion institutions. Some of the digital images editing tools are Photoshop, Pixlr, Gimp. Today, these effective images modifying software's allow people to alter an image and regulate those images without difficulty in a quick length of time. Despite the fact that an image can be altered to hide some traces and provide an aesthetic view of it, it can also sometimes be used with malicious intent, making it difficult for us to find and judge the authenticity of those images. Thus, developing techniques to test the genuineness or realness of the digital image is essential, mainly thinking about that those images are provided as evidence in all fields. There are two different digital image tampering approaches. They are Active and Passive approach.

Active image forgery approaches typically involve coming up with varied sorts of fingerprints or watermarks from the image's content and grouping them into a single digital image [2]. Digital watermarking is commonly used active approach. Media broadcastings usually use digital watermarking to protect their video or image content from copyright issue. In the authentication phase, previously embedded fingerprints or watermarks are extricated and explored to know whether or not the initial image is manipulated and if so, where its locale is. These active approaches will accurately find digital image manipulation. However, they are not widely used because it is not possible to want all the digital images on the network to be watermarked before they are shared. Therefore, passive forensic approaches became a lot of common option. Passive image approaches uncover digital images by analysing specific intrinsic traces or patterns that occur in the digital image creation / modification section [3]. On comparing active approaches with passive approaches, passive approaches are not based on past or current information. Therefore, they may have a wider application in the analysis of forensic images. Types of editing operations applied on images are Content modifications, Geometric modifications and Enhancement.

Content modifications are mostly used for malicious intent which includes copy-move, cut/copy-paste includes Copy-move and Image Splicing. Copy-Move technique or (Cloning) includes copying some of the parts from the picture and pasting them into the original picture. Image Splicing Cut-paste or Image Splicing means copying some of the parts from the original picture and then pasting them into another picture which will create a new picture. If the splicing is done accurately, the edges between the spliced areas may not be visually noticeable [4]. Splicing is more common than copy-move because of its flexibility and allows creating an image with very different content with respect to the original. Geometric modifications consist of rotation, translation, zoom, cropping and shearing. Image rotation turns the image clockwise or counter-clockwise. Translation moves the image to a new location without changing its original look. Reflection flips the image across a line. Zooming and cropping enlarges the required areas of the image. Image Enhancement includes color modification, color adjustment, filtering. Enhancement is essentially changing the look and feel of the picture. It is done by making sure that there is a nice balance of color theme throughout the picture. Image Retouching is the process of removing specific defects from an image. These can be smaller objects such as dust or dirt on the camera lens or sensor.

In this model two methods are implemented, first is the Metadata analysis, Metadata analysis is basically a tag searching algorithm. Photoshop, Gimp, Corel, Paint are some of the commonly used editing tools. If these keywords are the present in the metadata, the image is said to be tampered. Second level is analysing an image using Error Level Analysis technique which detects the digital image as fake or real.

LITERATURESURVEY

Kim and Lee [6], worked on an algorithm for detecting forged digital images using deep learning technology, which has achieved remarkable results in modern research. First, a converted neural network is applied to image processing. A high pass filter is used to get the hidden features in the image instead of the semantic information in the image. To implement the model edited images are created using the intermediate filter and Gaussian blur with the addition of Gaussian white noise. Marra F *et al* in 2018 [7] proposed a model to detect images generated by GAN. The authors examined the performance of various image forgery detectors against image-to-image translation, both under ideal conditions and with the compression in place, which is routinely performed when uploading to social media. The study, which was carried out on a data set of 36, 302 images, shows that the recognition accuracy in compressed data is up to 89%.

Bunk *et al* in 2017 [8], proposed two methods for detection of manipulated images by the combination of re-sampling features and deep learning. In the first method the forged regions are located using a method called Random Walker Segmentation. In the second method, re-sampled features of picture patches will be passed through Long Short-Term (LSTM) for classification and then these two results are compared by confirming that both methods are active in detecting fraud digital pictures. E. Jeevitha *et al*, proposed Fraud Document Detection of educational certificates [9] using Convolutional Neural Network. In the initial phase the system will scan the QR-code of the document and then in the second phase features like the logo of the institute, stamp, sign of the document will be analysed by using Deep Learning algorithm CNN. In the last step classification is done whether the scanned document is forged or not using Minimum Distance Classifier. Bo Liu *et al* in 2014 [10] proposed a forensic technique to detect copy-move and splicing techniques in a picture. To do this, a special descriptor has been created for each block which combines the artificial grid characteristic of the JPEG block with that of the noise estimate. And the direct image quality assessment procedure adapts these different characteristics by defining appropriate weights and is effective in detecting forgeries regardless of the JPEG compression rate of the picture.

PROPOSED METHODOLOGY

A supervised deep learning model is developed by providing a two-level analysis for the image. Foreign content in a tampered picture has a distinct compression rate than the authentic picture, which is recognized by an error-level analysis. This error-level analyzed image is sent as an input to the trained neural network and detects authenticity of the digital image. Another feature used in conjunction with error-level analysis is image Metadata which is a supporting parameter in detecting whether the image is fake or real.

Metadata Analysis

At the first level, metadata of the image will be analyzed. Every digital image contains the information (metadata) about it. The metadata provide information about the origin of a digital image, for example the type of camera used to take the picture, size of it. Most commonly used image formats are JPEG and PNG. A digital image which is in JPEG format usually includes a lot of details about the image, such as camera settings, date of creation, image resolution, Exif information, focal length and aperture information, and time stamps. Usually, images in PNG format contain very little information about the image, unless it is converted from a JPEG or edited in Photoshop. Source file format metadata can be included in converted PNG files. Some common things to look for when analysing the metadata information are: The date and time information, software information like Photoshop, size of the image. For extracting the metadata from a digital image, the metadata extraction library is used. Metadata-Extractor [12] is capable of extracting metadata information from a large number of different images. The metadata analyser is basically a tag searching algorithm. If the keywords like Photoshop, Gimp, Adobe, etc. from the table 1 are found in the data, then the image is said to be tampered. Two different variables namely realness and fakeness will be maintained. Each parameter will have different pre-defined weights representing fakeness and realness of an image. If a keyword is found in the metadata, the corresponding variable is incremented by pre-defined weights

(from table 1). After analysing all the tags, the final values of fakeness and realness from metadata analysis will be displayed.

Error-Level analysis

Before tipping input to the neural network, we are applying a fake image identification method called Error Level Analysis on that image. So, the input of the neural network is the error-level analysed image. Error level analysis works by resaving a particular image with a certain error rate. Each resave introduces a different amount of error rate and after that calculates the difference between two photos. An image is first saved with 100%quality, and then it is again saved with 90%quality.The dissimilarity between these two images is found by the difference method. The resulting image is the required Error Level Analysis (ELA) image. A beak was photo shopped to the person's nose (Fig 3), ELA identifies changes of the areas that are no longer at their minimum error level. Since Photoshop merges information from different layers, it actually changed many pixels, additional areas of the image show a bit more instability. The modifications will result in changing the image features in such a way that the stable areas (no additional errors) become unstable. In the original image, almost all pixels are not at their local minima. Large areas where pixels have reached their minimum are visible during the first save (75%). Other areas which have reached their local minima are entered in the second resave. Since small scale changes in images are difficult to detect with the naked eye, machine learning can be used to detect irregularities in the error level analysed images.

Now this ELA image is saved as a buffered image and sent to the neural network for further processing. Deep learning is carried out using Neuroph [13] library in java to implement neural network because of its simplicity and easiness to implement them. In the second level, when a digital image is chosen for evaluation, the image is converted into an error-level analysed format and will be resized to a 100x100 pixel image. These pixels include red, green, and blue components; therefore, 10,000 pixels will have 30,000 values. Then this error-level analysed formatted image will be given as input to the trained neural network (multi-layer perceptron). The Multilayer Perceptron (MLP), is a type of artificial neural network that has an input, output and one or more hidden layers between them. Each layer consists probably different, but a fixed number of neurons. Each neuron in the network layer is connected to every neuron (expect input neurons since they have no previous layer and output neurons since they have no next layer) and has weights associated with the output connection. The output layer consists of two neurons representing a fake image and a real image respectively. The given image will be classified as real or fake depending on the activation value of these two neurons. If the activation for the fake neuron is more than the real image then the fake neuron is set to 1, real neuron is set to 0 and the image is considered as fake image. If the activation for the real neuron is more than fake neuron then the real neuron is set to 1 and the image is considered as a real image. During testing, the image matrix will be inserted into the input neurons and the values of the output neurons will be taken to display the analysis result.

Dataset

To train the neural network, number of layers and other requirements are ought to be calculated first. The multilayer perceptron has three layers. Weighted sum is the input function of the network. Suppose, N is a neuron which is connected to n neurons in the previous layer in the network. The weighted sum A of the inputs to neuron N is calculated by adding up the product of each connection's input value at times the connection's weight wacrossalln inputs is given by the formula[14].

$$A_N = \sum_{i=1}^{n} a_i w_j \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

The output function determines whether a neuron passes its output signal to all of the neurons in the next layer of the network. The Sigmoid function is the most commonly used activation function in MLP network because of its non-linearity and it is best-suited when working with neural networks. For any weighted sum A for a given neuron, the Sigmoid value V of A is given by the formula [14].

$$V_A = \frac{1}{1 + e^{-A}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

The neural network is trained with the dataset called CASIA [15]. The dataset consists of 7492authentic images and 5124 tampered images of various sizes and formats. All these images are first pre-processed to 100x100 pixels so that total pixel values to be fed into the neural network will be 30,000. From this dataset, 5000 realand4000 fake images for training and the remaining pictures were used for testing of the neural network. During training the total neural network error is calculated and the connection weights in the network are modified. Table 2 shows various configurations of neural network and corresponding neural network efficiency. The highest efficiency is achieved when the parameters learning rate is set to 0.4 and momentum to 0.9. Fig. 4. shows iterations during training of the neural network were expected to minimize the error from 0.225 to 0.001. In MLP networks, total network error function is commonly used. Basically, the average "distance" between the actual value calculated by the training program and the expected value from the training data will be calculated by mean squared error function. The connection weights are changed starting from the output layer and moving backwards (in the direction of the input layer), by gradually adjusting the weight of each neuron. This is known as back propagation of error which is significantly used technique for training neural network.

RESULTS

Theoutputgeneratedbythefirststagethatismeta-dataanalysishasbeengivena weight age of around 40 percent only. The reason behind this is the meta-data of an image can be altered using meta editing tools but it is able to detect anomalies in any retouched pictures with very little processing, making it reliable on its own. Fig 5 shows the result from the first level which is metadata analysis. The weight age of fakeness and realness will be represented in the form of apiechart. A long with the weight age, the model will display on which soft ware the digital image is tampered. However, the compression rate of external content in a tampered digital picture differs from the genuine digital picture and is identified using error level analysis. After metadata data analysis the ELA image is given as input to the neural network. Fig 6 shows the result interface which provides the results from neural network. The results will display whether the given digital image is real or fake with confidence. The output generated by the neural network stage is more reliable and thus it has been given a weight age of around 60%. At a certain high success rate, the trained neural network can recognize the images as fake or real.

CONCLUSION

As the internet advances rapidly in modern society, may software editing tools have been used not only for good reasons but also some take the misuse them for negative purposes. Under these circumstances, crimes against digital images are appearing for illegal purposes. Digital forensics need to detect such illegal purposes. The trained neural network was able to identify the image as tampered or genuine with a maximum success rate of 94%. It has been shown that this desktop application will greatly reduce the spread of tampered digital images on the social media and can also be used as a fake evidence technique to know the legitimacy of the digital image when needed. By integrating the results of the metadata analysis (40%) and the neural network output (60%), a reliable model for fake digital image detection is developed and tested. In future, this model will focus on designing a more efficient network architecture and finding high level information for better decision making. This model can further be implemented on larger datasets with other machine learning techniques to attain a better accuracy by focusing on the design, the most useful network configuration and some best high-quality specifications.

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REFERENCES

1. M. Sridevi, C. Mala, SiddhantSanyam, "Comparitive Study of Image Forgery and Copy-Move Techniques" Advances in Computer Science, Engineering & Applications, pp 715-723.
2. G. Zhou, D. Lv, "An overview of digital watermarking in image forensics", Proceedings of 2011 Fourth International Joint Conference on Computational Sciences and Optimization; 2011 Apr 15–19; Yunnan, China, IEEE Computer Society, Washington, DC (2011), pp. 332-335.
3. H. Farid, "How to detect faked photos", Am Sci, 105 (2) (2017), pp. 77-81.
4. AbhishekKashyap, Rajesh Singh Parmar, Megha Agarwal, Hariom Gupta, "An Evaluation of Digital Image Forgery Detection Approaches", arXiv:1703.09968v2 [cs.MM] 30 Mar 2017.
5. D.-H. Kim, & H.-Y. Lee, (2017). Image Manipulation Detection using Convolutional Neural Network. International Journal of Applied Engineering Research, 12(21), 11640-11646.
6. Marra, F. Gragnaniello, D. Cozzolino and Verdoliva.L "Detection of Gan-Generated Fake Images over Social Networks", IEEE Conference on Multimedia Information Processing and Retrieval (MIPR), Miami, 10-12 April 2018, 384-389.
7. J. Bunk, J. Bappy, H. Mohammed, T. M. Nataraj, L., Flenner, A., Manjunath, B., *et al*. (2017) "Detection and Localization of Image Forgeries using Resampling Features and Deep Learning" University of California, Department of Electrical and Computer Engineering, USA.
8. E. Jeevitha, A. Abitha, A. Shalini, B. Swetha "Fake Education Document Detection using Image Processing and Deep Learning", International Journal of Engineering Research & Technology (IJERT) ISSN: 2278-0181, ICRADL-2021 Conference Proceedings.
9. Bo Liu, Chi-Man Pun and Xiao-Chen Yuan, Department of Computer and Information Science, University of Macau, Macau, China "Digital Image Forgery Detection Using JPEG Features and Local Noise Discrepancies" in The Scientific World Journal, 2014, Article ID 230425.
10. Abburi M.N.S.P. Pavani, ArunSekarRajasekaran, B.T.Geeta, Radhamanvi, MuthuKumar Subramanian, AshwinPerti, "Machine Learning Systems in Image Manipulation and Fake Detection" Turkish Journal of Physiotherapy and Rehabilitation; 32(2) ISSN 2651-4451 | e-ISSN 2651-446X, pp. 2631.
11. https://github.com/drewnoakes/metadata-extractor
12. Metadata-extractor is a straightforward Java library for reading metadata from image files.
13. https://neuroph.sourceforge.net
14. Neuroph Framework Neuroph is lightweight Java neural network framework to develop common neural network architectures.
15. https://www.ibm.com/in-en/cloud/learn/neural-networks Neural Networks - By IBM Cloud Education
16. http://forensics.idealtest.org/casiav2.0

Table1: Keyword Listings For Analysis Meta Data

Keyword	Realness/ Fakeness	Increment Value
Photoshop	Fakeness	5
Gimp	Fakeness	5
Adobe	Fakeness	5
PicsArt	Fakeness	3
Corel	Fakeness	5
Paint	Fakeness	2
Camera Tags	Realness	2
ExifInfo	Realness	2

Table 2: Neural Network Training Results

Learning Rate	Momentum	Epoch	Efficiency
0.1	0.6	500	82%
0.2	0.8	500	86%
0.2	0.9	1000	90%
0.4	0.9	1000	94%

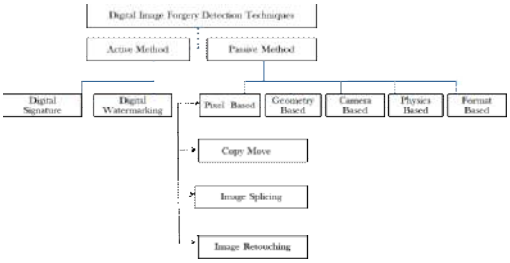

Fig. 1: Different Digital Image Forgery Detection Techniques

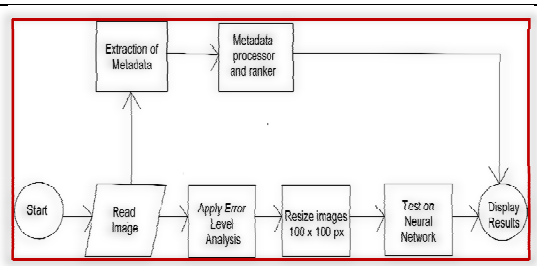

Fig. 2: Architecture of the proposed model

Fig. 3: Tampered Image on the left and ELA image on the right

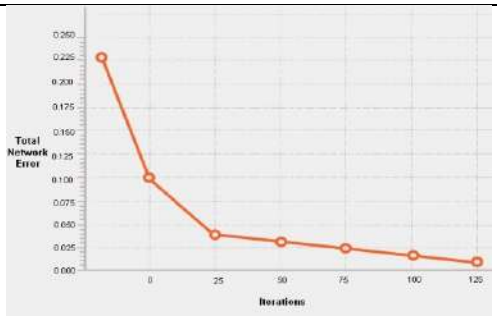

Fig. 4: Total network error graph training

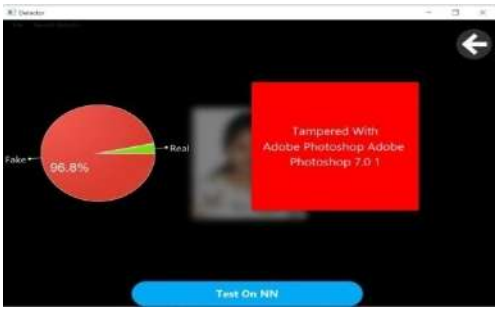

Fig. 5. Metadata analysis result of an image

Fig. 6: The final result of the image

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REVIEW ARTICLE

Presence of Toxicity Caused by Heavy Inorganic Metals in the Environment: A Systematic Review

Shaik Annar[1]* and Bukke Siva Sankar Naik[2]

[1]Lecturer, Department of Chemistry, MRR. Government Degree College, Udayagiri, (Affiliated by Vikrama Simhapuri University, Nellore), Andhra Pradesh, India.
[2]Lecturer, SKBR Government Degree College, Macherla, (Affiliated by Acharya Nagarjuna University, Guntur), Palnadu, Andhra Pradesh, India.

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***Address for Correspondence**
Shaik Annar,
Lecturer,
Department of Chemistry,
MRR. Government Degree College, Udayagiri,
(Affiliated by Vikrama Simhapuri University, Nellore),
Andhra Pradesh, India.
Email: skannar1973@gmail.com

ABSTRACT

In the developing countries like India, a number of developmental activities are undertaken, which would contribute enormously to the release of toxic metals in to the atmosphere. They have exposed people to heavy metals, which get accumulated in their bodies. And metal toxicity is determined by the amount of the toxic metals being absorbed by the human body, how it has entered the human body and the duration of its presence in the human body. One such toxic metal is arsenic, which is the most prevalent heavy metal that causes poisoning in the human body and affects thousands of people across the world. It is naturally present in the ground water at high levels in many countries, contaminating drinking water, food and irrigation of crops. Mercury is a liquid metal found in the environment from natural and anthropogenic sources. It is highly harmful to ecosystems and human beings. In India, human activities and industrial activities lead to the release of high amounts of this toxic mercury into the atmosphere every year. Like arsenic, it also affects human health adversely if they are exposed to it for longer durations. Besides, it exists in several forms such as mercury vapor, mercurous (Hg+), mercuric (Hg++) and Inorganic mercury. Thirdly, cadmium, a toxic non-essential heavy metal, is vastly used in batteries, plating, coating, alloys, welding etc. in various industries. Even tobacco smoke transmits cadmium into lungs and then to the rest of the body through blood. The bioaccumulation of cadmium in human body and in food chain leads to acute and chronic intoxications due to biomagnifications. Long-term exposure to cadmium through air, water, soil and food causes cancer and toxicity of organs, which affects reproductive, urinary, skeletal, cardiovascular, central and peripheral

nervous and respiratory systems. Exposure to cadmium primarily occurs through the ingestion of contaminated food and water. The liver and kidneys are extremely sensitive to cadmium exposure. This may be due to the ability of these tissues to synthesize metallothioneins (MT), which are Cd-induced proteins that protect the cell by tightly binding the toxic cadmium ions. Recent investigations have revealed the capability of sunflower (*Helianthus annulus* L.), Indian mustard (*Brassica juncea*) and river red gum (*Eucalyptus camaldulensis*) to remove cadmium from polluted soil and water. Nano particles of Titanium Oxide and Aluminum Oxide have been used to efficiently remove cadmium from waste water and soil. Microbial fermentation has been studied as a promising method for removing cadmium from food. In this manuscript, I reviewed the sources of Cd in the environment and its ecological importance in interdisciplinary studies. This review provides an update on the effects of Arsenic, Mercury and Cadmium exposure on human health.

Keywords: Heavy metals, Exposure, toxicity, chronic poisoning, Decontamination, Chelating agents, global production, health effects.

INTRODUCTION

Arsenic is also called "king of poisons" because of its potency and the discreetness. The metal is classified as a "heavy metal" if in its standard state has a specific gravity of more than 5g/cm^3. It is a harmful heavy metal for the public. It is one of the 10 chemicals of major public health concern as per the WHO. WHO set the permissible limit of arsenic in drinking water at 10µg/L. It is a neutral metalloid chemical that can be found in ground water. Fish samples from various outlets in Hyderabad and Secunderabad were found to have large concentrations of heavy metals like Arsenic, according to a study published by scientists from National Institution of Nutrition. The properties of Arsenic are shown in Table 1.

Arsenic has certain minerals which include:
i) Arsenopyrite, which is made up of Iron arsenic sulphide;
ii) arsenic sulphide, which is also described as "ruby of arsenic"; and
iii) Orpiment, which contains arsenic sulphide mineral. Generally, Arsenic is used in alloys of Lead. It is a common n-type dopant in semiconductor electronic devices. The Arsenic compounds are used in the production of pesticides, herbicides and insecticides. These applications have now been discouraged due to the toxicity of Arsenic and its compounds.

The heavy metals have a specific gravity of more than 5g/cm^3. Mercury is a heavy toxic non-radioactive metal, which is at times also called "Quick Silver" because of its silvery white appearance. It has a wide range of commercial applications in industries, mercurial catalysts, and hospitals, wherein it's extensively used in Thermometers, Sphygmomanometers (Each Sphygmomanometer has an approximately 60 g of Mercury), dental amalgams (mercury vapors from dental amalgam are the most dangerous form of mercury), etc. Due to extensive commercial use, mercury consumption is increasing in leaps and bounds so is the human exposure. Mercury is a heavy, odorless, lustrous liquid metal that can mix with water so thoroughly that is very difficult differentiate. Besides, it is very flexible and changes to malleable mass which can be solidified at -39oC. Once solidified, it can be cut with a knife. The properties of Mercury are shown in Table 2.

Mercury is extracted by heating Cinnabar (HgS) in a current of air and condensing the vapor.

$$HgS + O_2 \longrightarrow Hg + SO_2$$

Cadmium (Cd) is a soft, malleable, ductile silvery bluish-white metal with low melting point. It is chemically akin to the other metals Zn and Hg. It was first identified by German chemist Prof Friedrich Strohmeyer. Cadmium is a common impurity of zinc compounds. The average concentration of Cadmium in Earth's crust is 0.1 – 0.5 ppm. The most commonly-found mineral of cadmium is greenockite (CdS), which is nearly associated with sphalerite (ZnS). Unlike most other metals, cadmium is resistant to corrosion and is used as a protective plate on other metals. Cadmium is produced mainly as a byproduct of mining, smelting and refining sulfidic ores of zinc. Cadmium forms several important compounds in nature, including $Cd(OH)_2$ (this compound is seen in the Nickel – Cadmium battery), CdO (This compound is used in a variety of reactions including acting as a catalyst in redox reactions, hydrogenation reactions ,polymerization and cleavage) and $CdSO_4$ (It is used in electroplating, fluorescents, pigments and batteries). At present, it is considered to be a pollutant of the atmosphere with global ramifications.

Sources of Exposure

There are two major sources for heavy metals to enter the environment – natural and anthropogenic. Natural sources comprise volcanic eruptions, forest fires and the weathering of rocks and soil while the anthropogenic sources include emissions from industry, traffic, thermal power plants and coal combustion in rural areas. These sources are shown in table 4. Arsenic (As) is a natural component of the earth's crust and is widely distributed throughout the environment in the air, water and land. Assam, Chhattisgarh, Bihar and West Bengal are the major States affected by Arsenic contamination of water. 26 million people were affected by the Arsenic contamination in West Bengal. All the metals can be divided into three groups based on the extensive Carcinogenic studies by the International Agency for Research on Cancer (IARC) are shown in Table 5.

Water: Arsenic found in water is almost entirely in the inorganic form and can be stable as both arsenite and arsenate
Food: The key organic arsenic compounds that can be routinely found in food (depending on food type) include monomethyl arsenic acid (MMAs), DMAs, arsenocholine, arsenosugars. DMAs or MMAs can be found in various types of fin fish, crabs and mollusks but often at very Low levels. Arsenisugars are detected mainly in seaweed but are also found to a lesser extent in marine.

Soil: Heavy metals can be found generally at trace levels in soil and vegetation. Heavy metal toxicity has an inhibitory effect on plant growth, enzymatic activity, stoma function, photo synthesis activity and accumulation of other nutrient elements and also damages the system. Arsenic in soil is almost entirely in the inorganic form. In soil, pentavalent Arsenic predominates due to oxidation of trivalent arsenicals. Arsenic concentration is ranging from 0.055mg/kg to 0.220mg/kg in Nellore district, Andhra Pradesh.
Air: Generally Arsenic comes from high temperature processes such as coal-fired power plants, burning vegetation and volcanic activity. Natural and anthropogenic activity has caused the release of particulate matter (PMs), especially fine particles and dust.

According to the report on human exposure to hazardous chemicals (National Health and Nutrition Examination Survey 1V – NHANES), women are the most affected under the category of non occupational setting. Children are majorly exposed to these chemical through food, especially fish, notwithstanding the fact that rice could be a major source for methyl mercury in Asia. Chlor – alkali industries have been found to be the major source of mercury release to the environment. Thermal power plants, steel industries, cement plants, paper and pulp industries, plastic industries, certain agriculture and pharmaceutical industries contribute substantially to mercury production. More than 90% of mercury is released by these seven countries – USA, Spain, Yugoslavia, former Soviet Union, China and Mexico. Amalgam fillings are the largest source of methyl mercury exposure to people who are not working in any industry. Approximately, 1g of Hg in a typical clinical thermometer is enough to contaminate water body with a surface area of about 20 acres, to the degree that the fishes living there would become unsafe to be consumed. Methyl mercury (MeHg) and Di-Methyl mercury (Me_2Hg) usually originate from biological sources, mainly fresh or salt water fish. Nearly 3000 lakes in the U.S have been closed to fishing due to Hg contamination.

Global Production of Hg

The estimates for Global primary production of Hg, as reported by the US Geological Survey, are shown in table7 . Estimated World production of primary (mined) mercury (Metric tons)

Hg in the Environment

Path Ways of Hg to the Environment

Emissions from power plants

↓

Atmospheric Transport and deposition

↓

Mercury transform in to methyl mercury in soils and water, then can bioaccumulate in fish

↓

Humans and wild life affected primarily by eating fish containg mercury

↓

Best documented impacts on the developing fetus: impaired motor and cognitive skills.

Production of Cd

Cadmium occurs mainly in association with the sulfide ores of Zinc, Lead and Copper. Cd is a byproduct of the zinc industry. The average annual production of Cd throughout the world increased from only 20 tonnes in the 1920s to about 12000 tonnes in the period 1960-1969, and it reached 17000 tonnes in 1970-1984. Since 1987, however, it has started fluctuating around 20,000 tonnes. The world production of Cd was estimated at 25,600 tonnes in 2018. Cd is mainly produced in China, Republic of Korea, Japan, Canada, Kazakhstan, Russia, Mexico and Peru. As per Mineral Commodity Summaries, 2020 of USGS Report, "the world refinery production of Cd was estimated at 25,100 & 25,000 tonnes in 2018 and 2019 respectively". The data is also shown in the Table 9. Cadmium is a pollutant that is pumped into the environment as a consequence of the rapid developments taking place in industries and modern technologies. Humans are exposed to cadmium through different sources such as air, water, soil, food and smoking. Cadmium can be released into the environment in the following ways: a) Natural activities such as volcanic eruptions, weathering and erosion. b) Human activities such as tobacco smoking, mining, smelting and refining of non-ferrous metals, fossil fuel combustion, incineration of municipal waste (especially cadmium containing batteries and plastics).

Industrial Processes

Cadmium is predominantly applied in Nickel-Cadmium batteries, coatings and plating, as a stabilizer in plastics and other areas which include non-ferrous alloys, semiconductors and photovoltaic devices. Cadmium production and refining, and nickel cadmium battery manufacturing are the occupations that cause major exposures to humans. The disposal and recycling of electronic and electrical e-waste has also been identified as a important source of exposure to cadmium, particularly for children.

Food and Drinking Water

Drinking water contains deep traces of Cd, usually in the range of 0.01 - 1.00µg/litre. In a survey done in the Netherlands, "about 99% of drinking water samples in 1982 contained less than 0.1µg/litre". In polluted areas, well

water may contain very high concentrations of cadmium (exceeding 25 µg/litre). Highest cadmium levels are found in the kidney and liver of mammals fed with cadmium-rich diets and in certain species of oysters, scallops, mussels and crustaceans. Some crops such as rice can have high concentrations of cadmium if grown on cadmium-polluted soil and water.

Smoking

The tobacco plant naturally takes relatively high amounts of Cd in its leaves. Cigrette smoking can cause significant increases in the concentration of cadmium in the kidney. Cadmium exposure from smoking cigarettes may vary depending on the type of cigarettes. For instance, one cigarette contains 1-2 µg Cd; a person smoking 20 cigarettes per day will absorb about 1 µg Cd. About 10% of cadmium with a 40% - 50% absorption rate gets accumulated in the lung. Inhaled cadmium is linked to smoking-related respiratory diseases such as pulmonary disease and lung cancer. Depending on the brand (i.e. mainly on the origin of the tobacco), cigarettes produced in Europe or the United States of America contain Cd at a concentration of 0.5 - 2.0 µg/g (dry weight) of tobacco, of which 10% can be absorbed.

Health Hazards of Arsenic

Arsenic exposure affects virtually all organ systems including the cardiovascular, nervous, renal, gastro intestinal and respiratory systems. The immediate symptoms of acute arsenic poisoning are shown in table 11 , which include vomiting, abdominal pain and diarrhea, etc. The first symptoms of long term exposure to high levels of inorganic arsenic can normally be seen in the skin. They include pigmentation changes, skin lesions and hard patches on the palms and soles of the feet (hyperkeratosis). In China (province of Taiwan), arsenic exposure has been linked to "Black foot disease", which is a severe disease of blood vessels leading to gangrene.

Diagnosis of Arsenic Toxicity

The urine test is the most reliable test for arsenic exposure. Urine testing needs to be done within 24-48 hours for an accurate analysis of an acute exposure. Hair is a potential bio-indicator for arsenic exposure due to its ability to store trace elements from blood.

Treatment of arsenic toxicity

Dimercaprol and dimercaptosuccinic acids are chelating agents that separate the arsenic from blood proteins and are used in treating acute arsenic poisoning. Various techniques have been developed for the removal of arsenic and the most frequently used absorbents are activated carbon, aluminium oxide and electro coagulation by nanoparticles.

Toxicity of Hg

The severity of health effects from mercury exposure is determined by following factors:
a) Chemical form of Mercury (Inorganic/Organic) b) Hg dose c) Duration of exposure d) Age and health status of the person exposed e) Route of exposure (inhalation/ingestion/dermal contacts)

Inorganic Hg

Hg in all forms damages cellular function by changing the tertiary and quaternary structure of proteins. The most-affected organ of Hg vapor is the brain. With massive acute exposure to mercury vapor, erosive bronchitis and bronchiolitis are caused, which potentially lead to respiratory failure accompanied by CNS symptoms such as erethism. At low level exposures, non specific symptoms like weakness, anorexia, fatigue, weight loss and gastrointestinal disturbance have been found.

Mercuric Mercury (Hg^{2+})

Acute poisoning with mercuric salts (typically $HgCl_2$) targets the gastrointestinal tract and the kidneys. Extensive precipitation of enterocyte proteins occurs with abdominal pain, vomiting, and bloody diarrhea with potential necrosis of the gut mucosa.

Organic Hg (MeHg)

Methyl mercury reacts with sulfhydryl groups throughout the body. Thus, it potentially affects the functioning of any cellular or sub-cellular structure. Methyl mercury interferes with DNA transcription and protein synthesis [5]. Recent studies suggest that methyl mercury, when combined with cystein, has a structure, which resembles methionine and enters brain and other cells via the neutral amino acid carriers. The Central Nervous System (CNS) is the target organ for CH_3Hg toxicity. The earliest symptoms include paraesthesia, malaise and blurring of the vision. More severe exposure leads to the constriction of the visual fields, deafness, dysarthria, ataxia and finally mental derangement, coma and death.

Minamata Disease

Minamata disease was discovered for the first time in the world at Minamata City , Japan in 1956. This case was attributed to the methyl mercury that was generated in the process for producing acetaldehyde using mercury as catalyst. Methyl mercury had accumulated in fishes and shellfishes and those who consumed them had been poisoned with it. Such type of exposure to methyl mercury was highly uncommon and unusual, although the number of victims eventually certified with Minimata disease was over 2,200.

Methyl Mercury Poisoning in Iraq

In Iraq, three epidemic poisonings have been reported in 1955 – 60 and the largest outbreak in 1971 – 1972. These outbreaks were caused by the distribution of seed grains treated with methyl mercury. Rural people consumed the grains by using it to make homemade bread, instead of planting the seeds. The total number of victims was 6530, including 459 deaths.

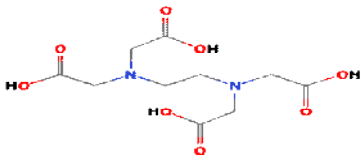
Alternative available for Mercury Commercial Use

(Source :-Global Mercury Assessment , UNEP Chemicals).The substitution of Mercury with mercury free alternatives is one of the preventive actions against mercury release to environment and its toxicity. Increased awareness is required for substitution of mercury with mercury free alternatives for major uses of mercury. The mercury alternatives available are detailed belowin table 13.

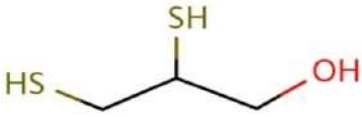
Toxicity of Cadmium

Cadmium (Cd) is a well- known global environmental pollutant. Cadmium is considered as a toxic metal and is hazardous to both human and wild life. According to International Agency for Research on Cancer (IARC) [3]. Human exposure to Cd compounds may create a serious health problem. Itai - Itai disease was one of these conditions caused by chronic cadmium contaminated rice fields. The number of patients affected by the disease was estimated around 400 patients from 1910 to 2007. The chief organ toxic impact in the human is the kidney. About 30% of body cadmium is deposited in the kidney tubule region. Diabetics are more susceptible to renal tubular damage from Cd exposure than controls. Cadmium may also impair vitamin D metabolism in the Kidney. Cadmium decreases serum osteocalcin levels in rats. Cadmium also directly induces oxidative stress, increases lipid peroxidation and depletes glutathione. Hematopoeisis is adversely affected, most notably in itai - itai disease where severe anemia is observed. Cadmium has considerable endocrine disruption capacity, apparently disregulating all pituitary hormones. Male infertility in rats from Cd exposure is due to damage to the blood - testis barrier, decreasing germ cell adhesion leading to germ cell loss, reduced sperm count and subfertility or infertility. Cadmium exposure is a known risk factor for developing insulin resistance. The United States Environmental Protection Agency considers Cd to be a Class B_1 carsinogen. There is contradictory evidence linking Cd exposure to breast cancer and denying that link. Prostate cancer is also correlated with Cd consumption as is pancreatic cancer. Cadmium may interact with different hormonal signaling transduction along estrogen. Low doses of cadmium affect the cardiovascular system.

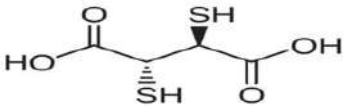
Detoxification (By Chelating Agents)



EDTA



BAL (Dimercaprol)



DMSA

CONCLUSION

Awareness should be created among the communities regarding its sources of exposure, features regarding its sources of exposure, features of toxicity. In the revie5, I reviewed the toxicity of arsenic on the environment and living organisms. Toxic metals (As) cause genomic instability. Defects in DNA repair following the induction of oxidative stress and DNA damage by these metals is considered as the cause of their carcinogenicity. This could be another aspect of heavy metals to be reviewed in the future. Mercury pollution has now become a global phenomena and thus with growing concern number of international organizations have mandate to address the impacts of mercury on health and environment. Awareness should be created among the communities regarding its sources of exposure, features of toxicity. Awareness programmes should be launched to educate the population about the risk and impact of mercury. In this review, I reviewed the toxicity of mercury and its compounds on the environment and living organisms. Toxic metals (Hg) cause genomic instability. Defects in DNA repair following the induction of oxidative stress and DNA damage by these metals is considered as the cause of their carcinogenicity. This could be another aspect of heavy metals to be reviewed in the future.

REFERENCES

1. Arsenic: Envoronmental Chemistry, Health threats and waste Treatment. Editor, Kevin Henke march 2009.ISBN; 978-0-470-02758-5.
2. Abate E, Hussien S,Laing M, Mengistu F.(2013). Aluminium toxicity tolerance in cereals: Mechanisms,genetic control and breeding methods. Afr J Agric Res 8(9):711-722.
3. Hughes JP, Polissar L, Van Belle G.(1988). Evaluation and synthesis of health effects studies of communities surrounding Arsenic producing industries. Int J Eidemiol 17: 407 -413.
4. Sheramet I.and Varma A.,eds.,Soil Heavy Metals, Soil Biology, Springer- Verlag, Berlin, Heidelberg, 19 (2010)
5. Duffus J.H., "Heavy metals" – a meaninless term ? Pure App.Chem.,74,793-807 (2002)
6. Colin Baird, Environmental Chemistry, Freeman W.H. and Compa6((1995)
7. Ziemack G., Viviano G.and Merli F., Heavy metals: Sources and environmental presence, Ann. Dell Isttit. Super. Di San., (Roma) 25(3), 531 – 536 (1989).
8. Abirami,N.,Raju, V.S., and Rajathi, K.(2007). Effect of semecarpus anacardium against lead induced toxicity in rats.ANC.Sci.Lif 27 (2), 24-27.
9. Department of Health and Human Services, Public Health Service. Agency for Toxic substances and Disease registry. Toxicology Profile for Lead. Available at: http://www.atsdr.CDC.gov/toxfaq.html. Accessed August 30, 2008.
10. National Institute of Nutr, Hyderabad, Role of Nutrients in Environmental Toxicity. Annual report 2005 – 2006, pp 62-64.
11. Gupta PK. Elements of Biotechnology, 3Rd Edition. Rastogi Publicatioms,Meerut.2010.
12. V.Masindi,K.L.,Muedi, Envoronmental contamination by heavy metals,in: Heavy Metals, In Tech,2018.

13. L.K.Wang, Heavy Metals in the Environment, CRC Press, 2009.
14. C.H.Walker, R.M.Sibly, S.P. Hopkins, D.B.P., in: Principles of Eco toxicology; Group,T. And F., Ed: 4th Edition, CRC Press, 2012.

Table 1. Properties of Arsenic

Property	Value
Atomic number of As	33
Density	5.75g/Cm^3
Abundance	55th
Allotropic forms	3
Group	15 (Pnictogens)
Period	4
Electronic configuration	[Ar] $3d^{10}$ $4s^2$ $4p^3$
Block	P
Phase at STP	solid
Heat of fusion	24.44 kJ/mol
Heat of vaporization	34.76 kJ/mol
Molar heat capacity	24.64 J/ (mol.K)
Electro negativity	2.18 (pauling scale)
Main Isotopes	^{73}As, ^{74}As, ^{75}As

Table 2 Properties of Mercury

Chemical symbol	Hg (Hydrorgyrum)
Density	13.456 g/Cm^3
Atomic number	80
Atomic weight	200.61
Boiling Point (B.P)	356.9°C
Melting Point (M.P)	-38.8°C
Group	12 th
Period	6 th

Table 3. Physical Properties of Cd

S.No	Physical Property	Value
1	Atomic number	48
2	Group	12 (II B)
3	Period	5
4	Electronic Configuration	[Kr] $4d^{10}5s^2$
5	Boiling point	767 °C
6	Melting point	321 °C
7	Density	8.65 g/cm^3
8	Oxidation states	+2 but few compounds show +1 (Cd_2^{+2})
9	Electro negativity (EN)	1.69 (Pauling scale)
10	Stable Isotopes (8)	^{106}Cd, ^{108}Cd,^{110}Cd,^{111}Cd,^{112}Cd,^{113}Cd,^{114}Cd and ^{116}Cd
11	Natural Radioactive Isotopes	^{113}Cd (beta decay, $t_{1/2}$ = 7.7 X 10^5 Years) And ^{111}Cd (two neutrino double decay, $t_{1/2}$ = 2.9 X 10^{19} years)

Table 4 .Sources of exposure

Heavy metal	Industrial Source	Major heavy metals Contaminated Site in India
As	Geogenic / Natural processes, smelting operations, Thermal power plants, Fuel burning.	Tuticornin, Tamilnadu, West Bengal, Balia and other Districts, UP

Table 5. Classification of heavy metals by IARC

Metal/Metalloid	IARC classification	Type of cancer causes
As and its compounds, Ni, Cd	Group 1	Lung, liver, urinary bladder cancer
Pb(Inorganic compounds)	Group 2 A	Lung
Pb (metallic)	Group 2B	Lung
Hg, Cr,	Group 3	Lung, nose and nasal sinuses

Table. 6.Industrial Sources of Mercury

Heavy metal	Industrial Source
Hg	Mining and refining of Hg, Organic mercurial's used in pesticides, laboratories using mercury.

Table.7. US Geological Survey

COUNTRY	2019
China	4000
Mexico	240
Tajikistan	100
Peru	40
Argentina	30
Kyrgystan	20
Norway	20
Others	20

Table 8. Natural and Anthropogenic Sources

Natural Sources	Anthropogenic Sources
1.Natural degassing of earth's crust	**A. From mobilization of Hg impurities**
2. Degradation of minerals and forest fires	1) Coal fired power generation and heat production
	2) Petroleum production
	3) Cement production (Mercury in lime)
	4) Energy production from other fossil carbon fuels
	5) Mining and other metallurgical activities
3. Elemental and oxidized forms of Hg	**B. From waste treatment and cremation**
	1) Land fills
	2) Recycling and storage
	3) Municipal, medical and hazardous water incineration
	C. From intentional Extraction and use of Hg
	1) Use of batteries, fireworks and laboratory chemicals
	2) Chlor – alkali production
	3) Products such as thermometers, manometers and other instruments

Table.9.World production of Cd (in tonnes)

Country	2016	2017	2018	2020
World: Total(rounded)	**25700**	**25800**	**24400**	**24000**
Chain	8222	8200	8200	8200
Korea	4000	4000	4900	3000
Japan	1989	2310	2108	1800
Russia	1500	1800	1900	900
Canada	2305	1802	1676	1800
Kazakhstan	2682	1500	1500	1500
Mexico	1244	1142	1307	1300
Peru	820	797	765	700
Netherlands	620	620	620	1100
USA	400	550	550	500
Germany	400	500	500	450
Norway	355	416	399	320
Other Countries	1146	1119	1148	2300

Source: World Mineral Production, BGS

Table 10. Industrial Processes

Pollutant	Major sources
Cadmium (Cd)	Cadmium producing Industries, Electroplating, welding, By products from refining of Pb, Zn and Cu, Fertilizer Industries, Pesticide manufactures, Cadmium - Nickel batteries, nuclear fission plants.

Table.11.Symptoms of acute Arsenic toxicity

Bodily system affected	Symptoms/signs
Systemic	Thirst, Hypovolemia, Hypotension
Gastrointestinal	Garlic or metallic taste, Burning mucosa ,Nausea and Vomiting, Diarrhea, Abdominal pain
Hematopoietic system	Hemolysis, Hematuria, Lymphopenia
Pulmonary	Cough, Dyspnea, Chest pain, pulmonary edema
Liver	Jaundice, Fatty degeneration, Central necrosis
Kidney	Proteinuria, Hematuria, Acute renal failure

Table 12. Organic Hg (MeHg)

Mercury compounds	Symptoms / Signs/ Diseases/ Disasters
CH_3Hg / Hg / $HgCl_2$	Depression, Emotional instability , memory reduction andirritability.
	Defects in hearing , vision and speech
	Difficulty in writing, delays in motor and language development, 49852in ability to walk properly
	Death in extream case
	Minamata disease*
	Pink disease
	Methyl mercury poisoning episode in Iraq **

Table.13. Alternative available for Mercury Commercial Use

S.NO	Chemical / Product of Application	Available alternatives
01	Mercury (+2) Oxide	Copper Catalyst
02	Mercuric Chloride	Magnesium Chloride / H_2SO_4 / Zinc
03	Mrcuric nitrate for anti-fungal uses	Ammonia / Copper Sulphate, mycin
04	Zenkar's solution	Zinc formalin
05	Mrcuric Sulphate	$AgNO_3$ / k_2SO_4
06	Mercury cell process in Chlor – alkali industry	Membrane technology
07	Mercury used in dental amalgam	Gold, Silver , Ceramic, porcelain , polymers, composites
08	Thermometers	Other liquids, gas, electric and electronic sensors

(Source :-Global Mercury Assessment , UNEP Chemicals)

Table.14 Impact of Cd Toxicity on Different Plant Species

Plant Species	Cd level	Medium	Duration	Effects
Pea	250 µM $CdCl_2$	Nutrient	1month	Reduction in rate of photosynthesis .
Rice	5 mM $CdCl_2$	Petri dishes hydroponically	12 days	Leaf chlorosis,
Georgia wild flower	10 mM $Cdcl_2$	Petri dishes hydroponically	**5** days	Inhibited seed germination
Wheat	1 mg/L $CdCl_2$	Plastic container	2 months	Reduced the root elongation

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RESEARCH ARTICLE

The Sustainable Management of Crop Residues in the Environmental and Agricultural Perspectives

Atul Sharma[1], Heena Choudhary[2], Mukesh Kumar[1] and Raj Singh[3]*

[1]Research Scholar, Department of Biotechnology, Maharishi Markandeshwar (Deemed to be University) Mullana-Ambala, Haryana 133207, India.
[2]ICMR-National Institute of Malaria Research, New-Delhi,110077,India.
[3]Professor, Department of Biotechnology, Maharishi Markandeshwar (Deemed to be University) Mullana-Ambala, Haryana 133207, India.

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***Address for Correspondence**
Raj Singh,
Professor,
Department of Biotechnology,
Maharishi Markandeshwar (Deemed to be University),
Mullana-Ambala,
Haryana 133207, India.
Email: dr.rajsingh@gmail.com

ABSTRACT

In the past few years, India is fronting many issues in the agriculture sector for buttress soil fertility, making food grains and environmental degradation along with food reliability of the country as the cultivable land is decreasing and food grains demand is increasing day by day. Our natural resources are under considerable strain so maintenance of the cultivable land and soil fertility are the major tasks. Moreover, the use of mechanical gadgets and different technological advances for crop harvesting left huge quantities of crop residues behind, which are burnt by farmers as they found burning is the cheapest and easy method with the delusion that burning of crop residues helps in controlling the insects and pest and also enhances soil fertility. From different studies, it is deduced that there is a heavy loss of organic carbon as well as soil nutrients by the burning of crops. It emits an enormous quantity of trace gases like CO_2, Sulphur SO_2, CO, smoke, and submicron aerosols this is an evolving problem to the environment and a hazard to the human health as well. According to the Ministry of India, about 500 Mt of renewable energy from crop residues are produced yearly that is used in many ways like animal feeding, Manure, Soil mulching, composting, mushroom production, etc. Taking the above point into consideration, data was collected and technical and policy options for crop residue management were

suggested that can forbid crop residue burning, and used to enhance the fertility of soil along with the prevention of environmental degradation.

Keywords: Crop Residue, Natural resources, Burning, Human health Hazards, Soil Fertility.

INTRODUCTION

The rising demand for food in the world led to a surge in food production in India as well. Hence an Agro-based activity gives a hugely profitable business to developing countries. Talking about the staple crop in the world rice has the first place, approximately 486 million tons of rice were produced around 2017 and 493 million tons around 2018. Infect, in India 111.52 million tons of rice were cultivated in 2017- 2018. Rice straw consists of about 50% of the dry weight of the rice plant and a huge quantity of the straw is assembled as an outgrowth of rice cultivation annually. Farmers avoid the integration of rice straw in the crop field because of its slow degradation rate, disease infestation, unstable nutrients, and it also reduced yield caused by the short-term negative effect of nitrogen immobilization [1]. They used a very common method to decompose it by burning it in the open field which led to the emergence of carbon dioxide, carbon monoxide, methane, nitrous oxide, and Sulphur dioxide into the atmosphere. This process also emits harmful air pollutants such as polychlorinated dibenzo-p-dioxins (PCDD˝s) and polychlorinated dibenzofurans (PCDF˝s) they are very toxic and are notable, potential carcinogens that cause impacts on human health [2]. Rice straw is a serious concern for the whole world that's why it needs a protocol for proper decomposition. For the safe disposal of rice straw in a short period, non-hazardous, environment-friendly, and sustainable techniques have to be introduced. To recycle the rice straw into compost, microbial composting is an effective environmentally friendly method. It nurtures sustainable agriculture along with environmental protection and also improves the soil's biological activity which gives better plant growth and yield. Composting of rice straw requires a pre-treatment due to the presence of lignocellulose to make it convenient for biodegradation. Inherently a few microbes can depolymerize lignin. Fungi are having the ability to compost lignocellulosic waste because they are filamentous and produce prolific spores, which can seize substrates quickly (Table 1). Furthermore, mixed cultures can better influence the colonization of the substrate by increasing the production of enzymes as well as resistance to contamination by other microbes.

Application of crop residues

Use of crop residue for soil improvement

Soil aeration is enhanced by crop residue mulch by promoting the interchange of gases among the atmosphere and soil [3]. To improve the overall drainage and improves the structural stability, and porosity, and decrease surface crusting. In unmulched conditions, the oxygen diffusion rate is low while in mulch conditions it is higher. Remitting crop residues in the soil will enhance many soil's physical properties by increasing the moisture content of the soil, decreasing bulkiness, and increasing the porosity and stability [4]. Crop residue addition in the soil can elevate its moisture content by increasing the holding capacity of the soil, direct evaporation that enhances saturated water conductivity of soil, and infiltration of water. In the beginning adsorption of crop residue and microorganism in soil water content will be reduced and gradually rises [5].

Environmental protection

In India and other Asian countries due to mechanized farming Crop residue burning is a common practice. Before 1986 in India, the method of harvesting crops like rice and wheat was manual by permitting the roots of crops in the field. The manual harvesting of crops was adopted in India in since1986 when the farmers found easy and economical ways to burn the residues of crops like rice and wheat in the fields. The farmer prepares their field for the wheat crop after harvesting the rice during the pre-winter season [6].To prepare the field for new crops in the season the burning of the residues of the rice and wheat was found more suitable. When the temperature is around 15–22°C and the boundary layer is low the burning of the rice crop is carried out. However, sometimes in-between mid-May

to mid-June wheat crop residues is burnt. During pre-Crop residue management in the rice-wheat cropping system is done during the monsoon season when the temperature is around 25–35°C in northern India [7, 8]. Punjab state gives birth to this method of crop burning initially and then other neighbouring states start following the same. Smoke spreads all over due to the burning of rice at low temperatures and crosses the boundaries that leading to pollution and a higher AQI value. From different studies, it is deduced that there is a heavy loss of organic carbon as well as soil nutrients by the burning of crops (Fig. 1). It also depends on the speed of the wind, usually; the smoke even reaches up to other countries like Bangladesh. People facing health issues due to this smoke. With the burning of crop residues, people from Delhi faced many problems. Delhi weather conditions were affected by this smoke plume due to the burning of the crop residues in Haryana which create a very dense fog called haze. In Haryana, paddy straw burning is considered the main cause of the pollution and the government passes different bills on that but there were many clashes between the government and farmers so government retracts all of them (Table-2). In Punjab state and in IGP, the western part of India is the agriculturally productive region [9]. For rice and wheat crops the use of fertilizers is in abundance by the farmers in states like Punjab and Haryana. These crops are the main consuming food in India. Ammonia emission in the environment occurs due to the use of an excessive amount of fertilizers. The growing global population is the main concern of the production of an enormous amount of food and the use of fertilizers. Decomposing the crop residues and making fertilizers from them will make correct use of that by decreasing the use of the other artificial fertilizers and these are also environment friendly [10].

Crop residues as fodder, Cattle shed and Bed Preparation

Now a day, the use of the nutritional values of the crop residue is the main concern of the research and development as the crop harvests and its residue is directly linked to the by-product of the quantity of its harvest and linked to the factor that how it will make available to fulfill the nutritional values of the animals [11]. Although of the crop residues are generally considered poor in nutrition and high in fiber content. Feeding these residues to the animals may lead to the low performance of the animals and also can cause disease. Like other plant residues, crop residue also has cellulose that could be a good source of carbon and energy in animal feed. It also contains microbial proteins, enzymes, and bio factors that can be directly used as animal feed or can be treated with other nutritional products. However, crop residues are not easily degraded as they are having high lignocellulose content. Due to this their utility in animal feed production is limited. To make it suitable for livestock, pre-treatment technology is used to enhance digestibility and increase its nutritional value. Different methods are available for the valorization of crop residue into stock feed that increases its protein content. To produce protein-rich stock feed solid-state fermentation is the most suitable option. This technology removes toxins and anti-nutritional factors present in crop residues. These days, research and development are widely working in the fermentation of cellulosic materials into high protein feed [12]. Farmers used it for bedding purposes for the animals but not for feeding. Therefore, it is necessary to have all the information on the quality and quantity of these crop residues to fulfill the animal's needs and this can be achieved through different processes like milling, mixing different enrichment things to it like the molasses, increasing nitrogen content, etc. The best replacement for the burning of the paddy straw is making the cattle shed, bed for cattle, and fodder. The paddy straw is normally not used as fodder due to the presence of high silica and lingo-cellulosic content which is not easily digestible for cattle except straw of Basmati rice and wheat straw. The frequently used cattle fodder is wheat straw. In India, widely used as cattle fodder is basmati rice straw. However, crop residue burning affects the basmati paddy area by approximately 7-10%. The paddy straw is much used as bedding to prevent the chances of injury to the animals and use as a shed for cattle as it also protects cattle from extreme cold in winters [8].

Crop residue as packaging materials

In India, plastic waste decomposition is the main concern about 26,000 tons of plastic generated per day. Plastic wastes that are generally not collected are creating pollution in the environment. It affects all the natural bodies of the land like seas, rivers, oceans, etc. The use of plastic as a packaging material is the oldest method and its implementation is increasing day by day but the plastic packaging is not properly collected and is a serious concern these days, approximately 80% of plastic is generated as waste and 40% is considered uncollected [5].To make the best use of the crop residue and overcome this problem the rice straw is a convenient alternative, which will resolve

the problem of the plastic packaging use along with the problem of management of paddy straw. In the context of renewable material, paddy straw packaging is considered environmentally friendly as conventional plastic is made from petroleum-based raw materials. For two major environmental concerns i.e., crop waste disposal and plastic pollution, this can be a solution [13]. Rice straw can be converted into paper and cardboard for packaging by using chemical pulping technology as it is having starch, cellulose, and lignin contents. Approximately 65% of the rice straw is used in making paper and cardboard where rice straw is converted into pulp and this method is based on the extraction of the cellulose from the rice straw. Rice straw is a turnout a very good packaging material because of its compression and elasticity [14].

Production of Food materials

A Vast Amount of agro-industrial by-products and lignocellulosic agricultural crop residues are annually generated that are rich in organic compounds and worth being transformed. In solid-state fermentation (SSF) processes these residues have been obtain as feedstock by using *basidio mycetes* fungi for the generation of different products like medicinal compounds, food for mushroom, feed for animals, and few enzymes [15]. In addition, these above-mentioned microorganisms are used in the process of bioremediation of hazardous compounds and waste detoxification. These days mushroom cultivation is doing by Biotechnological industry worldwide by using solid-state-fermentation process for the recovery of food protein from lignocellulosic materials [16]. Furthermore, the cultivation technologies of *Agaricusbisporus, Pleurotus* sp., and *Lentinula edodes* consist of inoculum preparation, substrate preparation, and widely in mushroom growing technique like inoculation, substrate colonization. Lastly, the two medicinal mushroom genera, *Pleurotus* and *Lentinula*, mainly cultivated for their nutritional value and mainly researched for their biodegradation capabilities helps in the conversion of the residues into fruiting bodies [17].

In Production of renewable energy

In India, the most common topics of discussion are renewable energy and crop residues, as crop residues are composed of lignocellulosic materials that are potential sources of renewable energy. The synthesis of biofuels from the crop residues and mixing them with the conventional fuels can help in the exploitation of fossil fuels [18]. For the generation of liquid fuels like ethanol or biodiesel crop residues can be used directly or in processed form. With efficient commercial technologies, there is a huge potential in crop residues to generate biofuels. Potentially, Dry crop residues can produce 250-350L of ethanol from each metric ton [19]. In review, annual ethanol production from 20% of the world's rice straw is 40 billion liters, which can replace about 25 billion liters of gasoline-type fossil fuel [20]. To make good use of these commercial technologies has to develop as 70 million tons of CO_2 reduction is noticed in net greenhouse gas emissions. Presently, for bioenergy production, only 10% of total rice residues are used in India [5]. To reduce greenhouse gas emissions, protect fossil fuels and enhance the rural economy, the generation of renewable energy is very important [15,21]. Many techniques are present to generate biofuels such as biomethane, bio hydrogen, etc. by using crop residue as raw material. The generation of renewable energy from biomass depends on several factors like availability, cost, and environmental implications and how it will be beneficial for human use. The effect of energy products on the environment is an important factor in decision-making processes for making new policies [22].

Generation of the Bioethanol

Biofuels are considered as the non-greenhouse gas emitting, clean and eco-friendly, and play important role in environmental protection because it is produced from different crop residues. However, the process of conversion of the crop residue is much more complex and expensive in comparison to fossil fuels. To lower the production cost, the production of high-value biochemicals and biomaterials like benzene, toluene, xylene, styrene, micro-fibrillated cellulose, or cumene along with the production of renewable energy could be a promising strategy. Many biorefineries and biomass energy industries are already on the market, and many demonstration plants are emerging worldwide to check the generation efficiency of ethanol from lignocellulosic biomass. The main bio-based products produced from the conversion of biomass are cellulose, starch, oil, and lipids. Presently ethanol and biodiesel are produced from biomass [22]. Crop residues are now considered the source of bioethanol production. A total of 491

GL per year of bioethanol is produced from crop residue and wasted crops. The countries like Brazil and the USA are the major ethanol producers in the world which give about 62% of global ethanol production that can be used in power generation as well [22,12,23]. It consists of the process of fermentation and downstream processing, fermentation of cellulosic waste provide high yields of ethanol. To minimize the production cost and increase energy input bioprocesses such as simultaneous scarification and fermentation can be adopted [9,24]. Researchers are now working on the genetic manipulation of *yeast, Clostridium thermocellum*, and *C. phytofermentans* to overcome this issue. *Saccharomyces cerevisiae* is the most predominant species for bioethanol production, pure ethanol is collected by the process of distillation [25].

CONCLUSION

In the above paper, the potential application of crop residues is discussed. Understanding the different approaches that lead to the decomposition of the crop residues by the use of the different fungal species is explained. Giving the most used applications of the crop residues is important these days as the burning of the crop residues is the major concern for the government as it is creating a clash between the views of government and farmers. Burning of the crop residues leads to environmental pollution getting rid of the burning and making the best use of the crop residues is important. Making paper for packaging from the crop residues will create employment followed by decreasing the pollution. Crop residues as fodder for the animals will create a new dynamic by understanding their nutritional value of it. Crop residues as a Bio-Fuel are at their urge as paddy straw is the most commonly used for the production of ethanol.

Conflict of interest

There are no conflicts of interest declared by the author.

REFERENCES

1. Pandey, A.K., Gaind, S., Ali, A. & Nain, L. (2009). Effect of bioaugmentation and nitrogensupplementationoncompostingofpaddystraw.*Biodegradation*, 20 (3), 293-306.
2. Gadde, B., Bonnet, S., Menke, C. &Garivait, S.(2009). Air pollutant emissions from rice strawopenfieldburninginIndia,ThailandandthePhilippines.*EnvironmentalPollution*, 157 (5),1554-1558.
3. Haris, M., Hamid, Y., Usman, M., Wang, L., Saleem, A., Su, F., Guo, J. & Li, Y. (2021). Crop-residues derived biochar: Synthesis, properties, characterization and application for the removal of trace elements in soils. *Journal of Hazardous Materials*, 416, 126212.
4. Fu, B., Chen, L., Huang, H., Qu, P. &Wei, Z. (2021). Impacts of crop residues on soil health: a review. *Environmental Pollutants and Bioavailability*, 33 (1), 164–173.
5. Prasad, S., Singh, A., Korres, N. E., Rathore, D., Sevda, S. & Pant, D. (2020). Sustainable utilization of crop residues for energy generation: A life cycle assessment (LCA) perspective. *Bioresource technology*, *303*, 122964.
6. Grzyb, A., Wolna-Maruwka, A. &Niewiadomska, A. (2020). Environmental Factors Affecting the Mineralization of Crop Residues. *Agronomy,* 10(12), 1951.
7. Shurpali, N., Agarwal, A.Kumar. & Srivastava, V.K. (2019). Energy, Environment, and Sustainability Greenhouse Gas Emissions (Challenges, Technologies and Solutions) *Crop Residue Burning: Effects on Environment,* 127–140.
8. Bolo, P., Kihara, J., Mucheru-Muna, M., Njeru, E. M., Kinyua, M. & Sommer, R. (2021). Application of residue, inorganic fertilizer and lime affect phosphorus solubilizing microorganisms and microbial biomass under different tillage and cropping systems in a Ferralsol. *Geoderma*, *390*, 114962.
9. Kumar, P. & Singh, R. K. (2020). Selection of sustainable solutions for crop residue burning: an environmental issue in northwestern states of India*. Environment, Development and Sustainability,* 23, 3696-3730.
10. Mira, A.B., Cantarella, H., Souza-Netto, G.J.M., Moreira, L.A., Kamogawa, M.Y. &Otto, R. (2017). Optimizing urease inhibitor usage to reduce ammonia emission following urea application over crop residues. *Agriculture, Ecosystems & Environment*, 248, 105-112.

11. Oschwald, W.R. (1978). Crop Residue Management Systems Factors Affecting the Decomposition of Crop Residues by Microorganisms. *ASA Special Publication*, 1-15.
12. Deshwal, G. K., Alam, T., Panjagari, N. R. & Bhardwaj, A. (2021). Utilization of Cereal Crop Residues, Cereal Milling, Sugarcane and Dairy Processing By-Products for Sustainable Packaging Solutions. *Journal of Polymers and the Environment, 29(7), 2046–2061.*
13. Devi, S., Gupta, C., Jat, S. L. &Parmar, M.S. (2017). Crop residue recycling for economic and environmental sustainability: The case of India. *Open Agriculture*, 2(1), 486-494.
14. Downing, A. S., Kumar, M., Andersson, A., Causevic, A., Gustafsson, O, Joshi, N. U. &Crona, B. (2022). Unlocking the unsustainable rice-wheat system of Indian Punjab: Assessing alternatives to crop-residue burning from a systems perspective. *Ecological Economics*, *195*, 107364.
15. Singh, R. & Kumar, S. (2019). A review on biomethane potential of paddy straw and diverse prospects to enhance its bio digestibility. *Journal of Cleaner Production,* 217, 295-307.
16. Muhammad, W., Vaughan, S.M., Dalal, R.C. & Menzies, N.W. (2011). Crop residues and fertilizer nitrogen influence residue decomposition and nitrous oxide emission from a Vertisol. *Biology and fertility of soils*, 47 (1), 15–23.
17. Hellequin, E., Monard, C., Quaiser, A., Henriot, M., Klarzynski, O. &Binet, F. (2018). Specific recruitment of soil bacteria and fungi decomposers following a biostimulant application increased crop residues mineralization. *PloS one*, *13*(12), e0209089.
18. Wang, H., Liu, X., Yang, Q., Zheng, L., Li, W., Cai, M. & Yu, Z. (2017). Insect biorefinery: a green approach for conversion of crop residues into biodiesel and protein. *Biotechnology for biofuels*, *10*(1), 1-13.
19. Hiloidhari, M., Das, D. &Baruah, D.C. (2014). Bioenergy potential from crop residue biomass in India. *Renewable and Sustainable Energy Reviews*, 32, 504–512.
20. Reijnders, L. (2008). Ethanol production from crop residues and soil organic carbon. *Resources, Conservation and Recycling*, *52*(4), 653-658.
21. Kamusoko, R., Jingura, R. M., Parawira, W. &Chikwambi, Z. (2021). Strategies for valorization of crop residues into biofuels and other value-added products. *Biofuels, Bioproducts and Biorefining,* 15(6), 1950-1964.
22. Khaiwal, Singh, T. &Mor, S. (2018). Emissions of air pollutants from primary crop residue burning in India and their mitigation strategies for cleaner emissions. *Journal of Cleaner Production,* 208, 261-273.
23. Maris, S. C., Capra, F., Ardenti, F., Boselli, R., Pochintesta, D., Beone, G. M. &Fiorini, A. (2021). The interaction between types of cover crop residue and digestate application methods affects ammonia volatilization during maize cropping season. *Journal of Environmental Quality*, *50*(2), 504-512.
24. Kaushal, L. A. &Prashar, A. (2020). Agricultural crop residue burning and its environmental impacts and potential causes – case of northwest India. *Journal of Environmental Planning and Management,* 264-284.
25. Das, P. K., Das, B. P. & Dash, P. (2020). Potentials of postharvest rice crop residues as a source of biofuel. *Refining Biomass Residues for Sustainable Energy and Bioproducts*, 275–301.
26. Singh, R., Upadhyay, S. K., Upadhyay, T.K., Singh, B.J., Rani, A. & Singh, C. (2021). Association analyses among fungi colonizing wheat crop residues during decomposition for sustainable and environment-friendly management of renewable natural resources.*Biointerface Research in Applied Chemistry,*11(5), 13754 – 13764.
27. Singh, R., Yadav, D. B., Ravisankar, N., Yadav, A. & Singh, H. (2020). Crop residue management in rice–wheat cropping system for resource conservation and environmental protection in north-western India. *Environment, Development and Sustainability*, *22*(5), 3871-3896.

Table1: Types of fungi involved in different stages of composting [26].

Composting stage	Involved organisms in the composting stage	Types of micro-organisms
Mesophilic (composting before mixing the pile)	*Aspergillus fumigatus*	Fungi
At the end of composting procedure	*Aspergillus fumigatus, Emericella sp. Aspergillus ochraceus, Aspergillus terraus and Penicilliumoxalicum*	Fungi
Mesophilic and thermophilic	*Chaetomium sp. Thermophile sp. Malbrancheasulfurea,Thermomyceslanuginosus and Torulathermophila*	Fungi
Thermophilic	*Aspergillus sp., Fusarium sp., Penicillium sp., Humicola sp., Mycothypa sp., Scopurialopsis sp. Cephalosporium sp.*	Fungi

Table 2:Rice growing districts of Haryana and paddy straw burning area of Haryana [27].

Districts	Paddy straw burning area with different years (In thousand hectare)				
	2013	2014	2015	2016	Average
Karnal	54.3	34.4	44.0	45.8	44.6
Fatehabad	32.7	38.8	40.6	34.5	38.4
Kurukshetra	39.8	26.6	28.9	39.8	34.2
Kaithal	41.4	29.1	23.8	40.8	31.6
Sirsa	19.6	18.1	6.3	18.1	15.5
Ambala	12.3	10.8	9.0	9.9	10.5
Jind	4.2	3.7	4.0	8.4	5.1
Yamunanagar	2.0	4.5	5.7	3.0	3.8
Sonipat	1.2	1.8	0.1	0.5	0.9
Panipat	0.8	1.2	0.5	0.9	0.8
Total	208.3	168.9	163.0	201.5	185.5

Table3: Technologies for the generation of energy from crop residue [5]

S.No	Crop residue	Technology	Energy Type	Energy Yield	Country
1.	Rice Crop Residue	Bioconversion, Enzymatic saccharification, Fermentation	Bioethanol	4 g/L ethanol with fermentation efficiency 55– 66%	India
2.	Agricultural crops, Agricultural waste and residue	Biochemical conversion	Second generation biofuels	About 0.35, 3.84, 1.07 biodiesel, bioethanol, bio butanol respectively can be produced	Iran
3.	Microalgae and rice residue	Co-fermentation	Biohydrogen & volatile fatty acids generation	Highest H2 yield of 201.8 mL/g VS	China
4.	Residual biomass	Bioenergy (utilizing forest and agricultural residues)	To produce bioheat in a rural area in Portugal, Estremoz	53 t/km2 /year of residual biomass is produced in the given area	Montado area, Southwestern Europe
5.	Agricultural residues	Bioenergy	Direct combustion	Potential nearly 141 TWh/year generation(bio)	Brazil

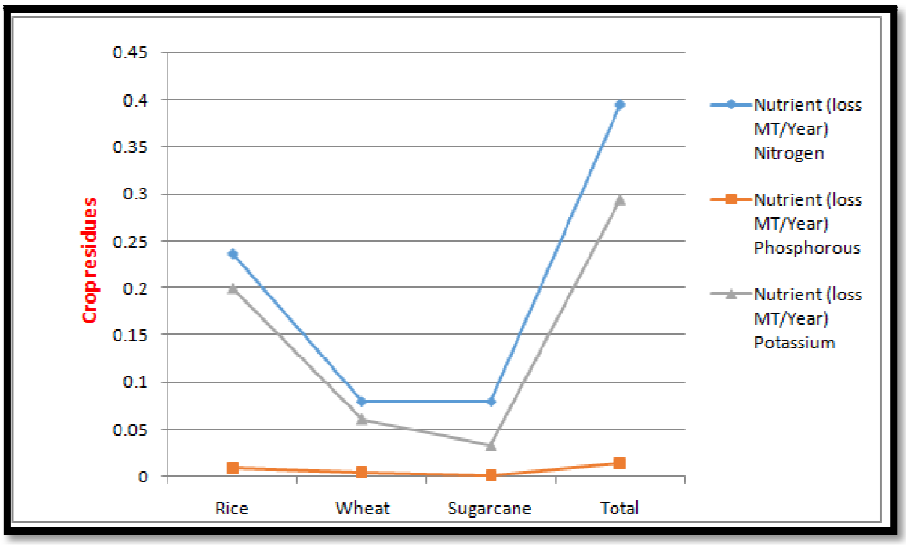

Fig. 1: Loss of nutrition due to burning of the crop residue in India (adapted from NITI, 2019).

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RESEARCH ARTICLE

Coping Styles and Occupational Stress among Healthcare Professionals: A Review Paper

Kanika Khanna[1], Gargi Sharma[2]* and Charu Dhankar[2]

[1]Research Scholar, Department of Psychology, Manipal University Jaipur, Rajasthan , India.
[2]Assistant Professor, Department of Psychology, Manipal University Jaipur, Rajasthan , India.

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***Address for Correspondence**
Gargi Sharma,
Assistant Professor,
Department of Psychology,
Manipal University Jaipur, Rajasthan , India.
Email: gargi.sharma@jaipur.manipal.edu

ABSTRACT

A number of research had focused on the rising mental issues among patients and their care givers, but for a good measure, equal attention should be lent to healthcare professionals in addition to patients. This study takes a leverage to employ theoretical approach towards understanding the relation of coping styles and occupational stress among health care professionals. The rise in occupational stress among healthcare professionals has become a potential concern. Almost a third of them face some or the other kind of a mental health problem. Although doctors and hospital staff have excessive workload, go through sleep deprivation, works day and night, and have repetitive exposure of emotionally charged situations, no effective measures or management have been carried out to lower their workload. Individual differences arise while choosing the coping styles, and this makes a similar situation different for everyone. The present study is an attempt to do qualitative research on the coping styles used by healthcare professionals to see their level of occupational stress. From the literature, it is summarized that healthcare professionals with high occupational stress tend to use avoidant coping styles. Individuals with high burnout have emotion-oriented coping approach, particularly avoidance strategies, reports high level of occupational stress. This study suggests the need for awareness for normalizing stigma associated with the mental health of health care professionals.

Keywords: Health Care Professionals, Occupational Stress, Coping Styles.

INTRODUCTION

Background

Unhealthy lifestyles are causing the need for healthcare services in India and around the world to rise. One of the most stressful industries for individuals to work in is healthcare. Healthcare workers are required to be present at all times in order to uphold a professional demean our and care for terminally ill patients. Patients who require a lot of emotional support make the situation worse. Because they are so exhausted on a physical, emotional, and psychological level, healthcare workers must find appropriate coping mechanisms in order to avoid burnout and occupational stress. Each career has its own pros and cons, a doctor's career can be incredibly rewarding but comes with many underlying challenges. An elevated risk of depression, suicide, anxiety, and substance misuse are found in doctors as compared to the wider population (Mason S, O'Keeffe C, Carter A, *at al.* ; shanafelt TD, Boone S, Tan L, *et al.*). The factors like long working shifts, heavy workload, and high-pressured environment results in high level of stress and can have adverse effects on the overall well-being of doctors (Garbarino S, Lanteri P, Durando P, *et al.* ; Costa G, Accattoli MP, Garbarino S, *et al.*).

Occupational Stress And It's Effects

Stress is a widespread and frequent problem that affects worker efficiency and is a harsh truth of life in the present workplace. Stress is a factor in high error rates, poor organisational performance, high staff turnovers, poor employee performance, reduced work quality, and absenteeism brought on by healthiness issues like depression, anxiety, and emotional disorders as well as other conditions like persistent headaches, obesity, and cardiac arrests (Samuel Ajayi, 2018) Occupational stress has become a universal challenge and is ubiquitous in every sector of workplace. The increasing level of workload, and lower job satisfaction has been resulting in developing stress that affect both the individual and association. At the singular level, low degree of occupation fulfillment and elevated degree of occupation stress are danger to mental and actual wellbeing, personal satisfaction, objective accomplishment, and self-awareness. The individual distress than correlates with the working environment and these circumstances lead to expanded non-appearance, struggle and turnover, and decreased quality and amount of work. Work pressure is a perceived issue in medical care workers and specialists and are viewed as at specific gamble of endlessly pressure related psychosocial issues. Specialists have more significant level of mental breakdown, self-destructive tendencies, and liquor reliance than controls of practically identical social class. Caplan revealed that about portion of senior clinical staff experiences elevated degree of stress and a comparable extent experiences uneasiness. Additionally, Firth-Cozens observed that portion of the lesser specialists in their pre-enrolment year were experiencing enthusiastic unsettling influence.

The conveyance of great clinical consideration adds to further developed wellbeing results. Specialist's work fulfillment influences nature of clinical considerations that he/she gives, patient's fulfillment with the specialist, patient's adherence to treatment and diminishes specialist's turnover. Studies from West find that long working hours and over-work are significant elements for work disappointment and stress among specialists. Over the past two decades, there has been an increase in awareness of the major impact that stress has on the workplace. Employee well-being has repeatedly been linked to workplace stress (Weinberg & Creed, 2000; Gummer, 1996; Zeidner and Endler, 1996). The study has amply demonstrated the detrimental impacts of workplace pressure on employee health and quality of life, productivity, absenteeism, and worker turnover (Sauter & Murphy, 1995; Hotopf & Wessely, 1997; Stahl & Hauger, 1994). Hospital workers have also received a lot of attention on work-related stress (Janssen, Jonge, and Baker, 1999; Fischer, Calame, Dettling, Zeier, and Fanconi, 2000; Omdahl& O'Donnell, 1999), but social workers have not received nearly as much attention (Soderfeldt, Soderfeldt, & Warg, 1995 ;Egan & Kadushin, 1993).

Workplace stress was correlated with elements linked to the job, the workplace, interpersonal relationships, and organisational issues. One-fourth of workers gave their job stress a high rating. Inadequate pay, workplace inequality, an excessive amount of work, a staffing shortage, time pressure, poor recognition and promotion, job instability, and a lack of management assistance were the main causes of occupational stress. High levels of

occupational stress have been linked to an increased risk of cardiovascular disease, physical injuries, high blood pressure, depression, and an increase in negative personality traits like anxiety, aggressiveness, and irritability. The likelihood of people leaving their jobs was strongly correlated with occupational stress (Mosadeghrad, 2014). Mergers, downsizing activity, increased rivalry, and changing technology are some of the factors that may contribute to a poor worker-environment fit and accompanying occupational stress in the healthcare industry. individual social workers and human service organisations must develop effective coping mechanisms for handling the pressures of their professions if they hope to maintain their wellness and productivity. We understand the need of considering both occupational stress levels and coping mechanisms among healthcare workers in light of these critical challenges.

Stress and Coping In People

Coping involves reducing the negative consequences of professional stress on physical and mental health in order to avoid distress, burnout, and psychological maladjustment. Depending on how much and what kind of coping technique is employed, coping may have a moderating effect. Lazarus contends that the stressor, the individual, and the working circumstances all have an impact on coping mechanisms. For instance, nurses who experienced occupational stress due to excessive workloads used more problem-solving techniques, whereas those who reported patient demands and domestic problems as their primary stressors employed social support techniques (Tyson *et al*. 2002). To deal with a stressor, you need to accept or tolerate unpleasant realities or circumstances in order to cope, all the while trying to keep your emotional equilibrium and feeling of self-worth strong. Coping happens when there are believed to be stressful life adjustments. Losing a loved one or a job are two examples of unpleasant life changes that are frequently linked to psychological stress. But every shift necessitates some form of adaptation. Adjusting to exceptional tasks or pressures is part of coping. This necessitates exerting more effort and energy than what is required in ordinary activities. Long-term effort mobilisation can result in increased levels of stress-related hormones, bodily deterioration, and sickness. Among the most influential psychologists in the stress and coping field, Lazarus and Folkman (1984) described coping as attempts to manage pressures that may exceed our resources. It is crucial to emphasise from this definition that someone will experience stress if they feel as though their current situation is taxing and beyond what they can handle. The hunt for coping mechanisms that can lessen the effects of exposure to stressors has taken a lot of consideration in latest years. Problem-focused, emotion-focused, and avoidant coping behaviours have all been extensively studied in the literature (Carver, Scheier, and Weintraub, 1989; Ben-Zur, Yagil, and Oz, 2005; Lazarus, and Folkman, 1984), respectively.

It is anticipated that problem-focused coping strategies will alter the environment to lessen the likelihood that the incident will recur in the future. The emotions connected to or triggered by a stressor are reduced event eliminated through emotion-focused coping behaviours (Carver *et al*., 1989) Five different coping strategies are often identified by studies on coping (Folkman & Moskowitz, 2005 ;Clarke, 2006; Skinner, *et al*., 2003). They consist of the subsequent: There are four types of coping strategies: problem-focused, emotion-focused, seeking-understanding, and helping others. Emotion-focused coping entails seeking an explanation of the issue and seeking a meaning for the experience, as opposed to emotion-focused coping, which involves expressing emotions or engaging in emotional release activities like practising meditation, yoga, or exercising. Problem-focused coping entails immediately addressing the issue by acting, making plans, or formulating solutions. Last but not least, people may respond to pressures by attempting to avoid both the problem and any potential solutions. The problem-focused and emotion-focused modes of coping with stress have been distinguished by Lazarus and Folkman (1984). Problem-focused coping involves actions done to control or modify the stressful situations. In contrary, emotion-focused coping involves trying to control painful feelings by employing strategies that avoid confronting the stressor directly. Problem-focused coping has been shown to be successful for lowering stress at work in numerous research (Latack, 1986; Latack, Kinicki, & Prussia, 1995;Havlovic& Keenan, 1995; Parasuraman & Cleek, 1984), but itsnot obvious whether emotion-focused coping is also useful for doing so (Long, 1990; Zevon, Donnelly, & Starkey, 1990).

Since there isn't a single standard instrument for measuring coping, measuring the idea of coping may be difficult, which may contribute to uncertainty regarding the success of emotion-focused techniques (Latack, 1992; Danoff-Burg, Revenson, & Ayala, 2000). But under Lazarus' idea, coping is defined as a person-environment exchange, and this seems to be the general consensus in the study (Lazarus, 2000; Gottlieb, 1997; Folkman & Moskowitz, 2000). According to some researchers, there are two main categories for emotion-focused coping mechanisms: (a) problem reappraisal, which involves efforts to control how stressful an event is assessed, and (b) avoidance, that includes attempts to de-escalate tension by avoiding dealing with the problem (Latack *et al*., 1995; Folkman, 1997; Menaghan & Merves, 1984).

Additionally, studies have shown that problem-reappraisal styles of emotion-focused coping are linked to symptom levels that are lower and positive adjustment, whereas avoidance styles are linked to negative psychological outcomes (Folkman & Moskowitz, 2000; Folkman, 1997; Parkes, 1990). Accordingly, it is crucial to define emotion-focused coping has two different categories, avoidance techniques, andproblem-reappraisal, based on the empirical data. According to the literature, a worker's degree of work satisfaction is inversely correlated with their experience with a stressful work environment (Spector, 1997; Jackson and Schuler, 1985). In the social work literature, job satisfaction has been investigated as an outcome variable that is closely associated to occupational stress. (McLean & Andrew, 2000). Job satisfaction may be described as the outcome of an employee's assessment of how well their work environment meets their needs (Dawis, Rounds, & Lofquist, 1987). According to this formula, an individual's level of job satisfaction depends on how much stress they perceive and how they cope with it.

Coping among Health Care Professionals

Workplace stress among healthcare workers has been extensively studied in many different nations. At an effort to better understand the type and severity of role stress faced by doctors in government hospitals across gender, experience levels, areas of speciality, and geographical locations in India, a study was conducted. Additionally, an effort has been made to investigate the various coping mechanisms used by doctors to manage job stress. The results of the study demonstrated that female doctors typically experience higher levels of stress than male doctors. Geographically, doctors in disturbed environments score much higher on the stress scale than those in serene environments. Additionally, there are differences in the type and degree of role stress experienced by doctors from diverse specialties and levels of expertise. According to the results based on "Role Pics," The number of experts adopted an avoidance coping strategy, which was accompanied by an inconsistent coping strategy (approach coping). The rising stress levels must be addressed with stress management interventions. More emphasis needs to be placed on the necessity of stress management for medical professionals (Irfana Rashid, Parvaiz Talib).

It is frequently discovered that doctors have developed coping mechanisms that focus on emotions and problems. They give up their personal preferences in favour of work obligations. However, the situation becomes traumatising when there is an imbalance between the demands of the profession and one's ability to handle them (Ratanawongsa, Wright, Carrese, 2007). Their low self-esteem causes them to react by being irate with their patients and resentful of their bosses, which causes further harm to both themselves and their patients (Isikhan, Comez, Daniz, 2004). These people frequently exhibit higher levels of emotional weariness and depersonalization (Le Blanc, Schaufeli, 2003; Doolittle, Windish, 2015). However, developing personalised coping strategies like bingeing or overeating during night shifts can help you stay awake and alert (Wallace, Lemaire, 2013). Similar to taking breaks from work, engaging in leisure activities, and mentally and emotionally unplugging from work during downtime are all beneficial to employees (Stoller, Papp, Aikens, Erokwu, & Strohl, 2005). This tactic is more common in conscientious people, especially female doctors. Medical residents then employ a variety of coping mechanisms to lessen the emotional and physical strain brought on by their high-acuity careers. Unfortunately, no formal curriculum has been developed to address these obstacles associated with lengthy service, and neither has any formal instruction or training (Wild, Scholz, Ropohl, Bräuer, Paulsen, & Burger, 2014; Satterfeld, Becerra, 2010).

According to a study, the inhabitants utilised both problem- and emotion-focused coping mechanisms. In order to balance their personal desires with their professional obligations, doctors use certain coping mechanisms (Ratanawongsa, Wright, & Carrese, 2007). Reflexion on behaviour, self-control, and mood regulation were discovered as the medical residents in this study's coping techniques. A similar study (Patel, Sekhri, Bhimanadham, Imran, & Hossain, 2019) has demonstrated the benefits of resilience training, reflections, shared experiences, and stress management techniques. A study revealed that physician stress has been managed by a variety of methods, including meditation, gratitude, exercise, and healthy relationships with friends and family (Satterfeld& Becerra, 2010). This self-control would focus their critical thinking and energy on their aim. The individuals in this study also listed exercise, family time, and short breaks from work as coping mechanisms.

Strategic planning for the future, taking a break, working out, and spending time with family can all help reduce stress associated to work (Lemaire & Wallace, 2010). Additionally, a recent research of Australian health workers revealed that sustaining exercise and social connections were their most frequently reported adaptive coping mechanisms (Smallwood, Karimi, Pascoe, Bismark, Putland, Johnson, *et al*., 2021). Adaptive or Maladaptive coping mechanisms are both possible. Consider how social support and issue solutions seem to be more adaptive than avoidance. Avoidant coping was substantially linked to a negative impact at work, but problem-solving techniques and problem-appraising were linked to a good impact (Bowman and Stern 1995).

CONCLUSION

This study has shed light on the issue of occupational stress among healthcare workers and identified the underlying causes of it. The goal of the article is to provide them with clear guidance and support in creating an effective stress management programme. Physician burnout is two times higher than that of other professions (Shanafelt, Boone, Tan, & *et al*, 2012). Physician burnout has negative repercussions on their well-being as well as how they view the care they deliver and the healthcare system as a whole. Short interventions do not work to stop burnout and workplace stress. As a result, lengthy intervention strategies are required to implement the plan. The number of patients seen each day, the length of those visits, the doctor's control over working hours, and the number of shifts is all actions that can lessen occupational stress. Violence and the conditions that are hypothesised to lead to burnout, such as occupational stress, may also contribute to a decline in sleep quality. Our study serves as a first step in identifying some of the problems related to stress management for healthcare professionals. The ramifications of these findings for upcoming study are numerous. First, more research should be done in the health care industry under varied work contexts on personal orientations in terms of coping mechanisms. Second, by offering cognitive restructuring and stress reduction groups that try to change people's perceptions of work-related stressors, coping abilities may be enhanced. Through the use of encouraging self-talk, focusing on stressful situations, and support group discussions on potential solutions, techniques could be used to promote the wellbeing of healthcare workers. Making changes to the employment environment to lessen potential stressors at work could be another option.

REFERENCES

1. Ajayi, S. (2018). Effect of stress on employee performance and job satisfaction: A case study of Nigerian banking industry. *Available at SSRN 3160620*.
2. Ben-Zur, H., Yagil, D., & Oz, D. (2005). Coping strategies and leadership in the adaptation to social change: The Israeli kibbutz. *Anxiety, Stress & Coping*, *18*(2), 87-103.
3. Carver, C. S., Scheier, M. F., & Weintraub, J. K. (1989). Assessing coping strategies: a theoretically based approach. *Journal of personality and social psychology*, *56*(2), 267.
4. Costa, G., Accattoli, M. P., Garbarino, S., Magnavita, N., &Roscelli, F. (2013). Sleep disorders and work: guidelines for health surveillance, risk management and prevention. *La Medicina del lavoro*, *104*(4), 251-266.

5. Doolittle, B. R., & Windish, D. M. (2015). Correlation of burnout syndrome with specific coping strategies, behaviors, and spiritual attitudes among interns at Yale University, New Haven, USA. *Journal of Educational Evaluation for Health Professions*, *12*.
6. Fischer, J. E., Calame, A., Dettling, A. C., Zeier, H., & Fanconi, S. (2000). Objectifying psychomental stress in the workplace–an example. *International archives of occupational and environmental health*, *73*(1), S46-S52.
7. Folkman S, Lazarus RS, Gruen RJ, DeLongis A. (1986). Appraisal, coping, health status, and psychological symptoms. *J Pers Soc Psychol,* 50(3), 571.
8. Folkman, S., & Moskowitz, J. T. (2000). Positive affect and the other side of coping. *American psychologist*, *55*(6), 647.
9. Garbarino, S., Lanteri, P., Feeling, N. R., Jarczok, M. N., Quintana, D. S., Koenig, J., &Sannita, W. G. (2020). Circadian rhythms, sleep, and the autonomic nervous system: A position paper.
10. Gummer, B. (1996). Stress in the workplace: Looking bad, telling lies, and burning out. *Administration in social work*, *20*(1), 73-88.
11. Hotopf, M., &Wessely, S. (1997). Stress in the workplace: unfinished business. *Journal of Psychosomatic Research*, *43*(1), 1-6.
12. Isikhan, V., Comez, T., &Danis, M. Z. (2004). Job stress and coping strategies in health care professionals working with cancer patients. *European Journal of Oncology Nursing*, *8*(3), 234-244.
13. Lazarus, R. S. (2000). Toward better research on stress and coping.
14. Lazarus, R. S., & Folkman, S. (1984). *Stress, appraisal, and coping*. Springer publishing company.
15. Le Blanc, P. M., & Schaufeli, W. B. (2003). Burnout among oncology care providers: Radiation assistants, physicians and nurses. In *Occupational stress in the service professions* (pp. 157-182). CRC Press.
16. Lemaire, J. B., & Wallace, J. E. (2010). Not all coping strategies are created equal: a mixed methods study exploring physicians' self reported coping strategies. *BMC health services research*, *10*(1), 1-10.
17. Manzoor, S., Sajjad, M., Anwar, I., & Rafi, A. (2022). Coping strategies adopted by medical residents in dealing with work-related stress: a mixed-methods study. *BMC Medical Education*, *22*(1), 1-9.
18. Mosadeghrad, A. M. (2014). Occupational stress and its consequences: Implications for health policy and management. *Leadership in Health Services*, *27*(3), 224-239.
19. Omdahl, B. L., & O'Donnell, C. (1999). Emotional contagion, empathic concern and communicative responsiveness as variables affecting nurses' stress and occupational commitment. *Journal of Advanced Nursing*, *29*(6), 1351-1359.
20. Patel, R. S., Sekhri, S., Bhimanadham, N. N., Imran, S., & Hossain, S. (2019). A review on strategies to manage physician burnout. *Cureus*, *11*(6).
21. Rashid, I., & Talib, P. (2015). Occupational stress and coping styles among doctors: role of demographic and environment variables. *Vision*, *19*(3), 263-275.
22. Ratanawongsa, N., Wright, S. M., &Carrese, J. A. (2007). Well-being in residency: a time for temporary imbalance?. *Medical Education*, *41*(3), 273-280.
23. Satterfield, J. M., & Becerra, C. (2010). Developmental challenges, stressors and coping strategies in medical residents: a qualitative analysis of support groups. *Medical education*, *44*(9), 908-916.
24. Sauter, S. L., & Murphy, L. R. (1995). The changing face of work and stress.
25. Shanafelt, T. D., Boone, S., Tan, L., Dyrbye, L. N., Sotile, W., Satele, D., ... &Oreskovich, M. R. (2012). Burnout and satisfaction with work-life balance among US physicians relative to the general US population. *Archives of internal medicine*, *172*(18), 1377-1385.
26. Smallwood, N., Karimi, L., Pascoe, A., Bismark, M., Putland, M., Johnson, D., ... & Willis, K. (2021). Coping strategies adopted by Australian frontline health workers to address psychological distress during the COVID-19 pandemic. *General hospital psychiatry*, *72*, 124-130.
27. Söderfeldt, M., Söderfeldt, B., &Warg, L. E. (1995). Burnout in social work. *Social work*, *40*(5), 638-646.
28. Stoller, E. P., Papp, K. K., Aikens, J. E., Erokwu, B., &Strohl, K. P. (2005). Strategies resident-physicians use to manage sleep loss and fatigue. *Medical Education Online*, *10*(1), 4376.
29. Wallace, J., & Lemaire, J. (2013). Physician coping styles and emotional exhaustion. *Relations Industrielles/Industrial Relations*, *68*(2), 187-209.

30. Weinberg, A., & Creed, F. (2000). Stress and psychiatric disorder in healthcare professionals and hospital staff. *the Lancet*, *355*(9203), 533-537.
31. Wild, K., Scholz, M., Ropohl, A., Bräuer, L., Paulsen, F., & Burger, P. H. (2014). Strategies against burnout and anxiety in medical education–implementation and evaluation of a new course on relaxation techniques (Relacs) for medical students. *PloS one*, *9*(12), e114967.
32. Zeidner, M., &Endler, N. S. (Eds.). (1995). *Handbook of coping: Theory, research, applications* (Vol. 195). John Wiley & Sons.
33. Zevon, M. A., Donnelly, Jr, J. P., & Starkey, E. A. (1990). Stress and coping relationships in the medical environment: A natural experiment. *Journal of Psychosocial Oncology*, *8*(1), 65-77.

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RESEARCH ARTICLE

Activated Carbon Loaded Cellulose Composite Bead for Remediation of Methylene Blue from Aqueous Solution

Pankaj Sarkar*

Assistant Professor, Nabadwip Vidyasagar College, Nabadwip, West Bengal, India

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***Address for Correspondence**
Pankaj Sarkar
Assistant Professor,
Nabadwip Vidyasagar College,
Nabadwip, West Bengal, India
Email: pankaj@nvc.ac.in

ABSTRACT

The adsorption process is frequently used for the decontamination of toxic chemicals such as synthetic dyes. The use of the agricultural product as adsorbents makes the process cost-effective. In the present study, sugarcane bagasse and cellulose powder are taken as the primary materials to synthesize a novel adsorbent, activated carbon loaded cellulose composite (AC-CC) bead. Xanthation and sol-gel conversion of cellulose were the key steps in the synthesis of adsorbent. The synthesized adsorbent was characterized by Fourier transformed infrared spectroscopy (FT-IR), Scanning electron microscope (SEM), and energy dispersive X-ray analysis (EDS). The synthesized novel adsorbent was employed for the decontamination of methylene blue. The batch adsorption process was carried out to optimize the process parameters. The effect of different process parameters such as contact time, adsorbent dose, shaking speed, initial dye concentration, and temperature were studied and explained. Both Langmuir and Temkin model was found to fit well with the experimental data. The maximum adsorption capacity of AC-CC bead was found to be 28.32 mg g^{-1}.The pH of the dye solution was not adjusted during the experiment, i.e., the natural pH of MB solution was used to study the adsorption experiment. The pseudo-second-order model was found to fit well compared to the pseudo-first-order model. A thermodynamic study divulged that the process was spontaneous and endothermic.

Keywords: methylene blue, Xanthation and sol-gel, Temkin model, initial dye concentration

INTRODUCTION

A significant portion of the world's total population is directly or indirectly dependent on the textile sector for livelihood. With the advancement of civilization, the trend of using textile products has increased tremendously among people worldwide. As a result, the production of textile products continues to grow rapidly. Synthetic dyes create more attraction to buyers for use in different colored dyes in textile products. Consequently, the production of

synthetic dyes has been found to increase rapidly. It is reported that $8x10^5$ tones of synthetic dyes are produced globally per year[1,2]. A large amount of these dyes remain unfixed and enter the environment as textile effluent which causes serious environmental pollution. These effluents released from the textile industry primarily pollute the lake and river water. The addition of colored dyestuff to the water may cause harmful effects on the aquatic plant and animals and adversely affect the ecosystem. Methylene blue, a cationic dye, is widely used in the textile industry. It is generally used to dye cotton, silk, leather, paper, and wool in the textile sector [3]. Remarkable use of methylene blue has been observed in medication. Methylene blue was first developed to stain and inactivate certain microbes [4]. It was recommended to treat malaria, in 1891 [4].Once upon a time, methylene blue was used to treat cyanide poisoning and urinary tract infections. Nowadays, it is recommended to treat methemoglobinemia[5]. Eye burn inhumans or aquatic animals is one of the most common harmful effects of methylene blue. Besides that, it may cause nausea, vomiting, irritation of the gastrointestinal tract, and increased heartbeat [3].

The use of agricultural waste has great importance in the removal of synthetic dyes as they are easily available and cost-effective materials. Modification of agricultural waste is sometimes needed to enhance the effectiveness of the adsorbent. Agricultural waste is often converted to activated carbon and it is frequently employed for decontamination of dye from an aqueous solution. In the present study, sugarcane bagasse is selected for the preparation of activated carbon. Another cost-effective material, cellulose, is also chosen for the development of the novel adsorbent, activated carbon loaded cellulose composite bead (AC-CC). The removal of MB from aqueous solution using several techniques has been reported by the researchers. The most frequently used methods are advanced oxidation[6,7], membrane filtration[8,9], bioremediation[10], ion exchange [11,12], reverse osmosis [13], electrodialsis[14]and adsorption[15,16,17,18]. The other methods are more complicated and expensive compared to adsorption. Hence, adsorption is selected for the present study because of its simplicity, feasibility, and cost-effectiveness.

REAGENTS AND MATERIALS

Methylene blue and carbon disulfide were purchased from Merck, India. Cellulose powder and sodium hydroxide were purchased from Loba Chemie, India. Double distilled water was used to prepare all the experimental solutions. Sugarcane bagasse and lemon were collected from local street hawkers of Nabadwip (23.40^0, 88.36^0), West Bengal, India.

ADSORPTION EXPERIMENT

The batch adsorption study was selected for the present study. Initially, a definite amount of adsorbent was taken in a 100 cm^3 conical flask and 10 ml of dye solution was added to the flask. The flask was shaken in a rotary shaker (Remi 12RS) until the equilibrium was achieved. The absorbance of the dye solution was measured by a UV-vis spectrophotometer (Make-Lab India). The concentration of MB was measured from a standard curve. The percent removal and adsorption capacity of dye solution was measured using Eq.1 and Eq.2 respectively.

$$\frac{C_0-C_e}{C_0}x100 \quad (1)$$

$$\frac{(C_0-C_e).V}{m} \quad (2)$$

whereC_0 and C_e are the initial and equilibrium dye concentration ($mgdm^{-3}$) respectively. V is the volume of dye solution (dm^3) and m is the mass of adsorbent (g). The contact time and shaking speed varied from 0 to 120 min and 75 to 175 rpm respectively. The adsorbent dose varied from 0.030 to 0.150 g while the initial dye concentration varied from 25 to 100 mg dm^{-3}. Three different temperatures viz. 288, 298, and 308 K were chosen for the present study. The pH of the dye solution was not adjusted during the experiment.

SYNTHESIS OF ADSORBENT

Activated carbon was prepared from waste sugarcane bagasse collected from a local street hawker. At the initial stage, sugarcane bagasse was washed several times with deionized water to remove any dirt or unwanted substances. Thereafter, the sugarcane bagasse was dried in sunlight and cut into small pieces. It was dried again in a hot air oven at 125^0C for 6 h to remove any moisture before carbonization at 550^0C with a heating rate of 10^0C per min. Thereafter, it was sieved to standardized particle size. In order to make activated carbon, the ash obtained was kept with a definite volume of lemon juice (to form a paste) in an air-tight container for 48 h. The obtained product was dried in a hot air oven and stored in an air-tight container. In order to make a suspension of cellulose, 1.5 g of cellulose powder was soaked in 25 cm^3 of 20% NaOH solution for 36 h. To the suspension of cellulose, 2.0 cm^3 of carbon disulfide was added (for xanthation) and the mixture was shaken for 8 hours at 180 rpm. At the next stage, 15.0 cm^3 of 20% NaOH solution was added to the mixture and the mixture was shaken again for 2 h to obtain a reddish-orange sol. The sol was settled for at least 72 h to make cellulose gel by the sol-gel approach [19]. In order to make an activated carbon loaded cellulose composite bead, 120 mg of activated carbon was added to the cellulose gel and the mixture was stirred well to make a homogeneous mixture. Thereafter, the mixture was added drop-wise to methanol from a 5 cm^3 syringe to obtain a spherical black bead. The bead was washed several times with deionized water. At the final stage, the bead was dried in a hot air oven at 150^0C for 6 h and stored for further use as an adsorbent. A schematic diagram of the synthesis of adsorbent is represented in Scheme 1.

RESULT AND DISCUSSION

Characterization of adsorbent

AC-CC bead was characterized by Fourier transformed infrared spectroscopy (FT-IR), scanning electron microscope (SEM), and X-ray energy dispersive analysis (EDS). FT-IR analysis of the adsorbent was carried out using a Fourier transformed infrared spectrometer (make-Perkin Elmer). The FT-IR spectrum of the adsorbent is represented in Fig.1. Peaks at 3785 and 3409 cm^{-1} were attributed due to O-H stretching of the free O-H group and H-bonded O-H group respectively. A characteristic peak at 2922 cm^{-1}appeared due to the asymmetric vibration of CH_2groups [20].A peak corresponding to O-H bending due to absorbed water molecules appeared at 1640 cm^{-1} [21]. Peaks at 1380 and 1058 cm^{-1} were attributed due to C-H bending (in plane) and C-O stretching respectively. Peaks were assigned at 772 and 608 cm^{-1} due to the presence of O-H bending (out of plane) and C-H deformation respectively. The surface morphology of AC-CC bead and the dye-loaded AC-CC bead was analyzed using a scanning electron microscope (Zeiss EVO LS 10). A fixed magnification of 2500x was chosen for both samples at 20.00 kV. Fig.2.(a) and Fig.2.(b) represent the SEM images of AC-CC and MB-loaded AC-CC beads respectively. The presence of a large number of spherical beads on the surface of the adsorbent was confirmed from the SEM image of AC-CC bead. The surface of the bead was also found to be an irregular structure with a lot of roughness. On the other hand, the surface of the adsorbent was found to be smoother after the attachment of MB molecules on the surface of AC-CC bead (Fig.2.(b)).

Energy dispersive X-ray analysis (EDS) of AC-CC and MB-loaded AC-CC bead was carried out using an EDS attachment to the SEM instrument (Zeiss EVO LS 10). EDS spectrum of AC-C and MB-loaded AC-C composite bead is represented in Fig.3.(a) and Fig.3.(b) respectively. The adsorbent was composed of carbon and oxygen (hydrogen does not appear in EDS spectrum) only and the peaks of carbon and oxygen were found below 1.0 keV. On the other hand, peaks of carbon, oxygen, nitrogen, and sulphur appeared in the EDS spectrum of dye-loaded adsorbents indicating the attachment of MB molecules on the surface of the adsorbent. Table 1 and Table 2 represent the elemental composition of AC-CC and MB-loaded AC-CC composite beads respectively. The percent weight of carbon and oxygen was found to be 52.56 and 47.44 respectively while their atomic percent was found to be 59.61 and 40.39 respectively (Table 1). On the other hand, the percent weight of carbon, nitrogen, oxygen, and sulphur was found to be 36.31, 16.55, 47.07, and 0.06 respectively. The atomic percent of carbon, nitrogen, oxygen, and sulphur was found to be 42.29, 16.53, 41.15, and 0.03 respectively.

Effect of contact time

Contact time is one of the most important parameters in the study of the adsorption of solute molecules on the surface of the adsorbent. In order to determine the equilibrium contact time of a particular adsorption process, the concentration of solute in the solution is to be determined at certain time intervals. In the present study, the effect of contact time of MB adsorption on AC-CC bead surface was investigated by varying contact time from 0 to 120 min and the result is represented in Fig.4.(a).Initially, the rapid adsorption of MB was found due to the availability of a large number of active sites on the surface of the adsorbent. Thereafter, the extent of adsorption was found to increase slowly up to 90 min. No further increase of adsorption was found beyond 90 min because of electrostatic repulsion between cationic dye molecules on the adsorbent surface and for the presence of a limited number of active sites.

Effect of shaking speed

The effect of shaking speed was investigated by varying speeds from 75 to 175 rpm. The effect of shaking speed of MB adsorption on the surface of AC-CC bead is represented in Fig.4.(b). The result demonstrated that the amount of MB adsorbed at equilibrium (q_e) was found to increase from 5.343 to 7.162, 9.053 to 13.30, and 13.85 to 20.50 mgg^{-1} corresponding to dye concentrations of 25, 50, and 100 $mgdm^{-3}$ when shaking speed was increased from 75 to 125 rpm. This is due to a decrease in the film boundary layer around the adsorbent particles and thus an increase in the diffusion rate of the outer film as well as the rate of absorption [22]. The adsorption capacity of MB was found to decrease slowly when the shaking speed was increased from 125 to 175 rpm. This is probably due to the desorption of some dye molecules at a higher shaking speed.

Effect of adsorbent dose

In the present study, the effect of the adsorbent dose was investigated by varying the dose from 0.030 to 0.150 g. The effect of the dose of AC-CC bead on the removal of MB is represented in Fig.4.(c) The percent removal of MB was found to increase with increasing dose from 0.030 to 0.090 g. The number of active sites of the adsorbent increases with increasing dose and thus the percent removal of MB is found to increase with increasing dose of AC-CC bead. The percent removal of MB was found to remain constant beyond the adsorbent dose of 0.090 g. On the other hand, the adsorption capacity of AC-CC bead was found to decrease gradually with increasing doses. The q_e was found to decrease from 7.16 to 1.61, 13.50 to 3.15, and 21.41 to 5.99 mgg^{-1}corresponding to MB concentration of 25, 50, 100 $mgdm^{-3}$with increasing doses from 0.030 to 0.150 g.

Effect of temperature

The temperature has a pronounced effect on the adsorption process. In this study, the effect of temperature was investigated for the removal of MB on AC-CC bead varying the temperature from 288 to 308 K and the result is represented in Fig.4.(d). The extent of MB adsorbed on AC-CC was found to increase with temperature. The amount of MB adsorbed on the surface of AC-CC was found to increase from 6.83 to 7.77, 12.56 to 14.57, and 20.55 to 23.19 mg g^{-1} corresponding to MB concentrations of 25, 50, and 100 mg dm^{-3} respectively as the temperature was increased from 288 to 308 K. The kinetic energy of MB molecules in the solution was increased with increasing temperature. As a result, the movement of MB molecules to the surface of AC-CC bead accelerated to increase the extent of adsorption of MB [23].

Effect of initial dye concentration

The effect of initial dye concentration on the removal of MB was performed by varying the dye concentration from 25 to 100 $mgdm^{-3}$. Three different temperatures viz. 288, 298, and 308 K were chosen for the study. The amount of MB adsorbed at equilibrium on to AC-CC was found to increase from 6.83 to 20.55, 7.16 to 21.41, and 7.77 to 23.19 mgg^{-1}corresponding to temperatures 288, 298, and 308 K respectively as the concentration of MB were increased from 25 to 100 $mgdm^{-3}$ (Fig.4.(e)). With increasing concentration, more MB molecules get attached to the active sites of AC-CC resulting increase in the adsorption capacity of AC-CC.

Isotherm analysis

Langmuir, Freundlich, and Temkin isotherm models were applied to analyze the adsorption process. The linear form of the model equations is represented in Table 1. The distribution of active sites and the type of the adsorption layer of the solute molecules on the adsorbent surface can be obtained from the parameters involved in the isotherm equations [24]. Fig.5.(a) represents the Langmuir plot of MB adsorption on AC-CC bead. The linear regression values were found to be 0.9986, 1.0000, and 0.9986 corresponding to temperatures288, 298, and 308 K respectively implying good fitting of data.The values of the Langmuir constant (K_L)were determined to be 6.797x10^{-2}, 10.21x10^{-2}, and 21.83x10^{-2} dm^{3}mg^{-1}corresponding to temperatures 288, 298, and 308 K respectively. The maximum adsorption capacity was determined to be 28.32 mgg^{-1} from the Langmuir model. The plot of the Freundlich isotherm model ($\ln q_e$ against $\ln C_e$) of MB adsorption is represented in Fig.5.(b). The values of $\frac{1}{n}$were found to be 0.512, 0.465, and 0.375 corresponding to temperatures 288, 298, and 308 K respectively. The adsorption process is considered to be favorable as all the values of $\frac{1}{n}$ lie between 0 to 1 [25]. The values of K_F were found to be 3.270 dm^{3}mg^{-1}at 288 K, 4.249 dm^{3}mg^{-1} at 298 K, and 6.711 dm^{3}mg^{-1} at 308 K. A plot of the Temkin isotherm model (plot of q_e against $\ln C_e$) is represented in Fig.5.(c). The linear regression values were found to be 0.9964, 0.9996, and 0.9997 at 288, 298, and 308 K respectively. The values of A_T and B_T were determined from the intercept and slope of the plot respectively. The values of A_T were determined to be 0.992, 0.998, and 1.010 dm^{3}mg^{-1} corresponding to temperatures 288, 298, and 308 K respectively. The values of B_T were found to be 373.34 Jmol^{-1} at 288 K, 403.93 Jmol^{-1} at 298 K, and 480.50 Jmol^{-1} at 308 K. The linear regression values of the Langmuir and Temkin model suggest that both models are fitted well with the experimental data compared to the Freundlich model.

Kinetics of adsorption

The study of the kinetics of an adsorption process has great importance to determine the rate and the mechanistic pathway of the process. In this study, pseudo-first-order, pseudo-second-order, and intra-particle diffusion models have been studied for the adsorption of MB on AC-CC bead. The linear form of the model equations is mentioned in Table 2. The pseudo-first-order plot of ln (q_e-q_t) against t is represented in Fig.6.(a). The linear regression values were found to be 0.8725, 0.8787, and 0.9468 corresponding to MB concentrations of 25, 50, and 100 mgdm^{-3} suggesting that the experimental data were not fitted suitably with this model. The rate constants (k_1) were determined to be 7.44x10^{-2}, 4.03x10^{-2}, and 5.42x10^{-2} min^{-1} corresponding to MB concentration of 25, 50, and 100 mgdm^{-3} respectively. The plot of the pseudo-second-order kinetic model of MB adsorption is represented in Fig.6.(b). The linear regression values of the plot t/q_t against t were found to be 0.9989, 0.9890, and 0.9974 corresponding to MB concentrations of 25, 50, and 100 mgdm^{-3} respectively. The linear regression values imply that the model is suitably fitted with the experimental data. The model q_e values were calculated to be 8.04, 15.57, and 24.39 mgg^{-1} corresponding to MB concentrations of 25, 50, and 100 mgdm^{-3}respectively. The rate constants of the process were determined to be11.95x10^{-3}, 3.93x10^{-3}, 3.26x10^{-3}g mg^{-1}min^{-1}for MB concentrations of 25, 50, and 100 mgdm^{-3} respectively. The model q_e values were found very close to the experimental values. So, it can be concluded that the adsorption process follows the pseudo-second-order kinetic model rather than the pseudo-first-order model.

Thermodynamics of adsorption

The equilibrium constant of an adsorption process is represented by Eq.(3).

$$K_c = \frac{C_a}{C_e} \quad (3)$$

Where C_a(mgdm^{-3}) is the concentration of dye on the adsorbent surface and C_e(mgdm^{-3}) is the amount of MB remaining in the solution at equilibrium. The change in Gibbs free energy (ΔG^0) was determined using Eq.(4).

$$\Delta G^0 = -RT\ln K_c \quad (4)$$

The change in Gibbs free energy (ΔG^0) is related to the change in enthalpy (ΔH^0) and change in entropy (ΔS^0) by Eq.(5).

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \quad (5)$$

A plot of RlnKc against 1/T is used to determine ΔH^0 and ΔS^0. In the present case, the plot of RlnK$_c$ against 1/T of MB adsorption on AC-CC is represented in Fig.7. The values of thermodynamic parameters are represented in Table 4.

The negative values of ΔG^0 confirmed the spontaneity of the process while the positive ΔH^0values suggested the endothermic nature of adsorption.

CONCLUSIONS

The novel adsorbent, AC-CC bead was synthesized by a sol-gel approach for the decontamination of MB from an aqueous solution. The experimental results suggested that the synthesized adsorbent was quite effective to remove MB. The amount of MB adsorbed on AC-CC was found to increase up to optimum condition with an increase in contact time, shaking speed, temperature, and contact time. Isotherm analysis of experimental data suggested that the process followed both Langmuir and Temkin isotherm models. The maximum adsorption capacity was determined to be 28.32 mg g^{-1}. The pseudo-second-order kinetic model fitted best with the experimental data. The process was spontaneous as all the values of ΔG^0 were determined to be negative. The positive values of ΔH^0 suggested the endothermic nature of the adsorption process.

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REFERENCES

1. Bhatia D, Sharma NR, Singh J, Kanwar RS. Biological Methods for Textile Dye Removal from Wastewater: A Review. Crit Rev Environ SciTechnol 2017;47:1836–1876.
2. Jamee R, Siddique R. Biodegradation of Synthetic Dyes of Textile Effluent by Microorganisms: An Environmentally and Economically Sustainable Approach. Eur J MicrobiolImmunol 2019; 9:114–118.
3. Mulushewa Z, Dinbore WT, Ayele Y. Removal of methylene blue from textile waste water using kaolin and zeolite-x synthesized from Ethiopian kaolin. *EnvironAnalHealth Toxicol* 2021;*36*(1).
4. Ryding S. (2019, July 01). Potential Health Benefits of Methylene Blue.News-Medical.Retrieved on August 18, 2022 from https://www.news-medical.net/health/Potential-Health-Benefits-of-Methylene-Blue.aspx.
5. Silva M, Caro V, Guzmán C, Perry G, Areche C, Cornejo A. α-Synuclein and tau, two targets for dementia. *Studies in natural products chemistry* 2020; *67:* 1-25.
6. Jaramillo-Sierra B, Mercado-Cabrera A, Hernández-Arias A N, Peña-Eguiluz R, López-Callejas R, Rodríguez-Méndez BG, Valencia-Alvarado R. Methylene blue degradation assessment by advanced oxidation methods. *JApplResTechnol*2019;*17*(3):172-179.
7. Sindhu TK, Pavithran R, Vidhya A. Design of 3D-supramolecular metal organic framework of zinc as photocatalyst for the degradation of methylene blue through advanced oxidation process. JMolStruct 2021;*1245*:131039.
8. Zhao R, Li Y, Sun B, Chao S, Li X, Wang C, Zhu G. Highly flexible magnesium silicate nanofibrous membranes for effective removal of methylene blue from aqueous solution. ChemEng J 2019; 359:1603-1616.
9. Lyubimenko R, Busko D, Richards BS, Schäfer AI, Turshatov A. Efficient photocatalytic removal of methylene blue using a metalloporphyrin–poly (vinylidene fluoride) hybrid membrane in a flow-through reactor. *ACS Appl Mater interfaces* 2019;*11*(35): 31763-31776.
10. Upendar G, Dutta S, Bhattacharya P, Dutta A. Bioremediation of methylene blue dye using Bacillus subtilis MTCC 441. Water SciTechnol 2017;*75*(7):1572-1583.
11. Keong CC, Vivek YS, Salamatinia B, Horri B A. Green synthesis of ZnO nanoparticles by an alginate mediated ion-exchange process and a case study for photocatalysis of methylene blue dye. In *Journal of Physics: Conference Series* (Vol. 829, No. 1, p. 012014). IOP Publishing, 2017.
12. Shao X, Wang J, Liu Z, Hu N, Liu M, Duan C, et al. Cellulose based cation-exchange fiber as filtration material for the rapid removal of methylene blue from wastewater. *Cellulose* 2021;*28*(14):9355-9367.

13. Li H, Lin Y, Luo Y, Yu P, Hou L. Relating organic fouling of reverse osmosis membranes to adsorption during the reclamation of secondary effluents containing methylene blue and rhodamine B. *J Hazard Mater* 2011;*192*(2):490-499.
14. Lafi R, Mabrouk W, Hafiane A. Removal of Methylene blue from saline solutions by adsorption and electrodialysis. *Membr. Water Treat* 2019;*10*(2):139-148.
15. Zhang, Y., Zheng, Y., Yang, Y., Huang, J., Zimmerman, A. R., Chen, H., ...&Gao, B. (2021). Mechanisms and adsorption capacities of hydrogen peroxide modified ball milled biochar for the removal of methylene blue from aqueous solutions. *BioresourTechnol* 2021;*337*:125432.
16. Gemici BT, Ozel HU, Ozel HB. Removal of methylene blue onto forest wastes: Adsorption isotherms, kinetics and thermodynamic analysis. *Environ TechnolInnov*2021;*22*:101501.
17. You X, Wang R, Zhu Y, Sui W, Cheng D. Comparison of adsorption properties of a cellulose-rich modified rice husk for the removal of methylene blue and aluminum (III) from their aqueous solution. Ind Crops Prod2021;*170*:113687.
18. Tsade Kara H, Anshebo ST, Sabir FK, Adam Workineh G. Removal of methylene blue dye from wastewater using periodiated modified nanocellulose. *Int J ChemEng2021*;9965452, https://doi.org/10.1155/2021/9965452
19. Santra D, Joarder R, SarkarM.Taguchi design and equilibrium modeling for fluoride adsorption on cerium loaded cellulose nanocomposite bead. *CarbohydrPolym*2014;111:813-821.
20. Mateus T, Almeida I, Costa A, Viegas D, Magalhães S, Martins F, et al. Fourier-Transform Infrared Spectroscopy as a Discriminatory Tool for Myotonic Dystrophy Type 1 Metabolism: A Pilot Study. Int J Environ Res Public Health 2021;18(7):3800.
21. Cichosz S, Masek A. IR study on cellulose with the varied moisture contents: Insight into the supramolecular structure. *Materials* 2020;*13*(20):4573.
22. Abbas A, Murtaza S, Shahid K, Munir M, Ayub R, Akber S. Comparative study of adsorptive removal of Congo red and brilliant green dyes from water using peanut shell. Middle East J Sci Res 2012;11 (6):828-832.
23. Jiang LL, Yu HT, Pei LF, Hou XG. The effect of temperatures on the synergistic effect between a magnetic field and functionalized graphene oxide-carbon nanotube composite for Pb^{2+} and phenol adsorption. J Nanomater2018.https://doi.org/10.1155/2018/9167938
24. Embaby MS, Elwany SD, Setyaningsih W, Saber MR. The adsorptive properties of UiO-66 towards organic dyes: A record adsorption capacity for the anionic dye Alizarin Red S. Chin J ChemEng 2018;*26*(4):731-739.
25. Erdogan FO.Freundlich, Langmuir, Temkin, DR and Harkins-Jura isotherm studies on the adsorption of CO_2 on various porous adsorbents.*Int J Chem React Eng*2018; 20180134.I.

Table 1: Elemental composition of AC-CC and MB-loaded AC-CC

Element	AC-CC		MB loaded AC-CC	
	Weight %	Atomic %	Weight %	Atomic %
C K	52.56	59.61	36.31	42.29
N K	-	-	16.55	16.53
O K	47.44	40.39	47.07	41.15
S K	-	-	0.06	0.03
Total	100	100	100	100

Table 2: Isotherm equations

Isotherm	Equation	Terms involved
Langmuir	$\frac{C_e}{q_e} = \frac{1}{q_m K_L} + \frac{C_e}{q_m}$	C_e(mg dm^{-3}) = equilibrium concentration of adsorbate, q_e(mg g^{-1}) = amount of adsorbate, K_L (dm^3mg^{-1}) and q_m(mg g^{-1}) = Langmuir adsorption constants related to energy and adsorption capacity respectively.

Freundlich	$lnq_e = lnK_F + \frac{1}{n}lnC_e$	$\frac{1}{n}$ and K_F (g mg^{-1}) (mg $dm^{-3})^{-1/n}$ = Freundlich constants expressing the intensity and the capacity of adsorption respectively
Temkin	$q_e = \left(\frac{RT}{B_T}\right) lnA_T + \left(\frac{RT}{B_T}\right) lnC_e$	A_T(dm^3 mg^{-1}) and B_T(J mol^{-1}) = adsorption capacity and the heat of adsorption respectively

Table 3: Kinetic models of adsorption

Kinetic model	Equation	Terms involved
Pseudo-first-order	$\ln(q_e - q_t) = \ln q_e - k_1 t$	q_t (mg g^{-1}) = amount of solute adsorbed, q_e (mg g^{-1}) = amount of dye adsorbed at equilibrium, k_1 (min^{-1}) = Pseudo-first-order rate constant, t (min) = time
Pseudo-second-order	$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e}$	k_2(g mg^{-1} min^{-1}) = Pseudo-second-order rate constant

Table 4: Thermodynamic parameters

Concentration ($mgdm^{-3}$)	Temp. (K)	$-\Delta G^0$ (KJ mol^{-1})	ΔH^0 (KJ mol^{-1})	ΔS^0 (KJ $mol^{-1}K^{-1}$)
25	288	3.482	40.87	0.154
	298	5.022		
	308	6.562		
50	288	2.682	30.15	0.114
	298	3.822		
	308	4.962		
100	288	1.142	12.97	0.049
	298	1.632		
	308	2.122		

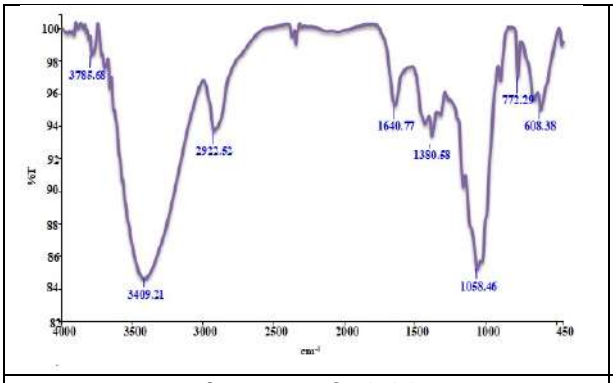


Fig.1. FTIR of AC-CC

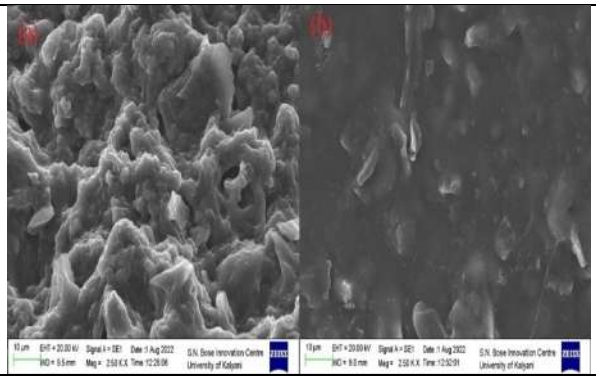


Fig.2. SEM image of (a) AC-CC and (b) MB loaded AC-CC

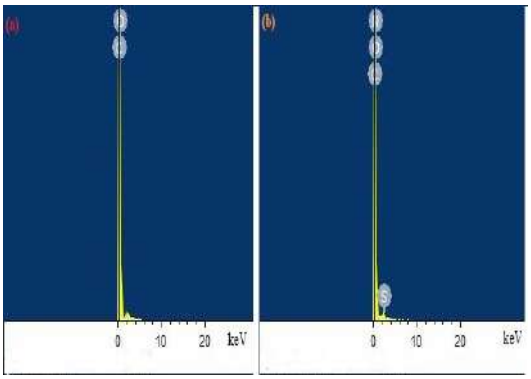


Fig. 3. EDS of (a) AC-CC and (b) MB-loaded AC-CC

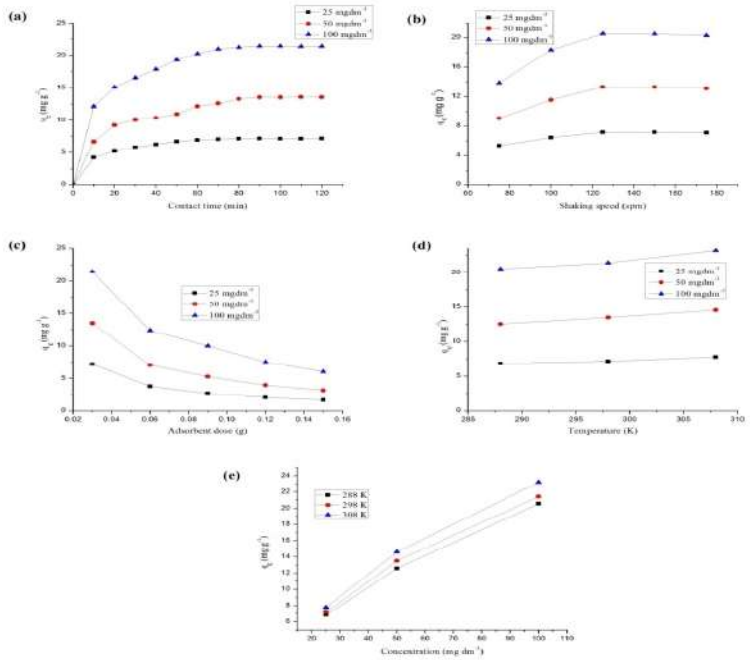


Fig. 4. Effect of process parameters (a) contact time, (b) shaking speed, (c) adsorbent dose, (d) temperature and (e) concentration

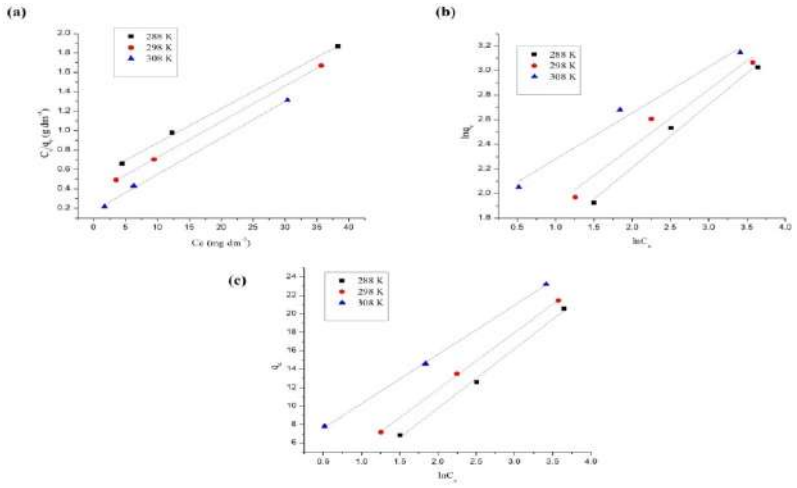


Fig.5. Plot of (a) Langmuir model, (b) Freundlich model and (c) Temkin model

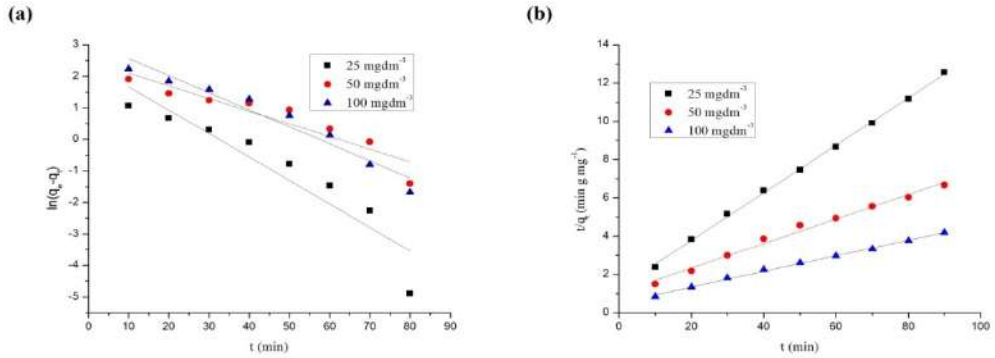


Fig.6. Plot of (a) Pseudo-first-order model and (b) pseudo-second-order model

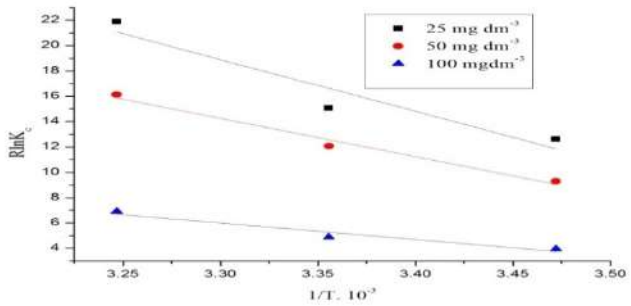
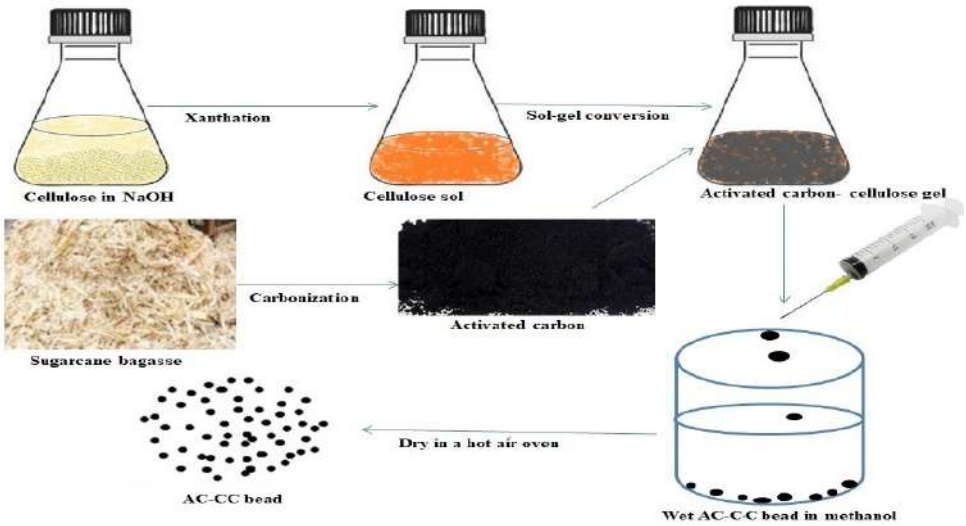


Fig.7. Plot of RlnK$_c$ against 1/T



Scheme 1: Preparation of AC-CCB

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RESEARCH ARTICLE

Effectiveness of Awareness Programme on Knowledge Regarding Alzheimer's Disease and its Prevention among Middle age Adults in Selected Community Areas at Kanpur

S. Andal[1]*, Firdous Siddiqui[2], Devsmita Shukla[2], Mayank Dixit[2], Shiksha Singh[2], Sonam Yadav[2] and Soni Rajput[2]

[1]Professor cum HoD, Mental Health Nursing, Faculty of Nursing, Rama University, Kanpur, Uttar Pradesh, India.
[2]Student Nurses, Faculty of Nursing, Rama University, Kanpur, Uttar Pradesh, India.

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***Address for Correspondence**
S. Andal
Professor cum HoD,
Mental Health Nursing,
Faculty of Nursing, Rama University,
Kanpur, Uttar Pradesh, India.

ABSTRACT

One of the most enervating prolonged disease among the elderly is Alzheimer disease. It is one of an unchangeable condition that led to gradual decline in reasonable intellectual somatic and psychosocial functions. The study was focused to assess the effectiveness of Awareness Programme on knowledge of Alzheimer disease among middle-aged adults in selected areas of community in Kanpur. A pre-experimental one group pretest posttest design was used for this study. A purposive sampling technique was used to select the sample size as 60. A multiple choice questionnaire was used to collect the data on knowledge and the data was analyzed by using statistical methods. For the present study when assess the knowledge of middle-age adults were in the age group of 36 - 40 years (40%) and 41 - 45 year (23%) respectively 46 - 50 years (20%) and 51 - 55 years (17%). Awareness Programme were in the group of gender 29, (48%) and whereas 31, (52%) female majority of the middle-aged adults were Hindu 34, (57%) and Muslim 19, (32%). Most of the middle-aged adults were graduate 19, (22%). Predominant of the middle- aged adults were married 50, (83%). Majority having knowledge by books 6 (32%). The assessment of mean SD of Pre- test knowledge of middle-aged adults regarding Alzheimer disease pre mean score (7.41) further the post mean score was (17.95) the score reveals that middle adults have poor knowledge prior to the administration of awareness Programme, but after implementation of awareness Programme many of them have average and good knowledge. The difference between pre and post mean score is (10.04) hence, it is interpreted that the Awareness Programme was effective in improving the knowledge level regarding disease among the middle- aged adults. There is an association between pretest knowledge score with the selected demographic variables of subjects like education, gender,

occupation of middle-aged adults at (0.05) level. As one piece of an overall plan to improve health pattern to people at a risk of having Alzheimer's disease this research supports the role of introducing systematic specific education or training regarding complete knowledge on Alzheimer disease.

Keywords: Effectiveness, Awareness Programme, Alzheimer's disease, Middle age adult

INTRODUCTION

Alzheimer's disease could be a gradual neurological Condition that leads the brain to condense (atrophy) and allows brain cells to die. Alzheimer's is that the most typical explanation for dementia - an eternal decrease in thinking, activity and social skills that alters somebody's ability to operate severally [1]. Alzheimer's isn't a standardly a segment of maturing. the best renowned risk issue is increasing age, and therefore the predominant of individuals with Alzheimer's are sixtyfive years and older [2]. Alzheimer's sickness is taken into account to be younger-onset Alzheimer's if it affects someone beneath sixty five [3]. Alzheimer's is that the most typical explanation for insanity, a general term for cognitive state and alternative psychological feature skills serious enough to interfere with existence. Alzheimer's} accounts for 60-80% of dementia [4]. Alzheimer's worsens over time. it could be a moving forward gradual developing disease, wherever insanity signs worsen over step by day by day for many years. In its beginning stages, cognitive state is delicate, however with late-stage Alzheimer's, people lose the flexibility to hold on a speech communication and answer their atmosphere. Alzheimer's is that the sixth- leading explanation for death within the us. On average, someone with Alzheimer's lives four to eight years once identification however will live as long as twenty years, betting on alternative factors [5].

Need for the study

Alzheimer's, symptoms 1st seems during the mid-60s. approximately, however specialists counsel that over six million Americans, most of them age sixty-five or older, might have dementedness caused by Alzheimer's illness is presently graded as sixth grade reason behind death within the United States., however recent values indicate that the disease might rank third, simply behind cardiac condition and cancer, as a reason behind death for older folks. Caring for someone with Alzheimer's illness will have high physical, emotional, and money prices [6]. The stress of daily care, changes in family roles, and selections regarding placement in an exceedingly care facility is troublesome. Their area unit many evidence-based approaches and programs which will facilitate, and researchers' area unit continued to seem for brand spanking new and higher ways that to support caregivers. changing into well- aware regarding the illness is one necessary long-run strategy. Programs that teach families regarding the assorted stages of Alzheimer's and regarding ways that to handle troublesome behaviors and different care giving challenges will facilitate [7]. sensible cope skills, a robust support network, and respite care area unit different ways in which facilitate caregivers handle the strain of caring for a honey with Alzheimer's illness. for instance, staying physically active provides physical and emotional edges[8].There is growing realization that the care of older folks with incapacity makes huge demands on their care givers. terms like dementedness and Alzheimer's illness area unit currently higher understood. However, this wasn't the case once the Alzheimer and connected for the most part hidden drawback in Asian nation, particularly in those a part of Asian nation wherever poorness and illiteracy levels area unit high [9]. In Asian nation expectancy at birth has magnified from forty-two years since independence to sixty eight years at the moment and it's higher for girls than men. Increasing era of illness like dementedness and therefore the variety of individuals with dementedness is foreseen to exceed eighty million by 2040 [10].

Statement of Problem

Effectiveness of awareness Programme on knowledge regarding Alzheimer disease and its prevention among the middle age adult in selected community area at Kanpur.

Objective of the Study

To assess the level of knowledge regarding Alzheimer's disease among the middle adults.

To assess the effectiveness of awareness Programme on knowledge regarding Alzheimer's disease among middle adults.

To associate the pre-test level of knowledge regarding Alzheimer's disease among middle adults with their selected demographic variables.

Hypothesis-

H_1: There is No significant difference between pre and post-test knowledge score of middle age adults on knowledge regarding Alzheimer's disease.

H_2: There is No significant association between selected demographic variable with knowledge score middle age adult regarding Alzheimer's disease.

METHODOLOGY

Research Approach

Quantitative Evaluatory Research Approach

Research Design

Pre-Experimental (one group pre-test and post –test) Research design.

Variables

Independent variables

The Independent variable of the present study is Awareness Programme regarding Alzheimer's disease and its prevention.

Dependent variable

The dependent variable is Knowledge level of middle age adults.

Setting of the Study

The present research study setting was at selected area of community Kukradev, Kanpur.

Population

In this study the population comprises of middle age adult.

Sample

In the present study the sample consisted of 60 middle age adults.

Sampling technique

For the present study Non probability convenient sampling technique was used

Description of tool

The tool consists of two sections.

Section A

It deals with the demographic variables like respondent Age of parents, religion, type of family, education of the respondents, parent's employment status, number of children with sex, caretaker of children, previous knowledge of child abuse and if yes source of knowledge on child abuse.

Section B

Alzheimer's disease Knowledge Scale consists of 30 questions to assess the level of knowledge on Alzheimer's disease. It consists of two options like true or false. For every true the score is 1 and for every false the score is 0. Total score is 30. The knowledge level was categorized as follows

The result is organized and presented under the following broad headings.

Section I: Distribution of knowledge among middle age adult according to their pre-test and post-test knowledge.

Section II: Effectiveness of awareness programme on knowledge regarding Alzheimer disease and its prevention among middle age adults.

Section III: Association of the pre-test level of knowledge regarding Alzheimer disease and its prevention among middleage adult with their demographic variables.

Section-I

(Table 2 Fig.1) explains that in pre-test 47middle age adults (78%)were having poor level of knowledge, 13 (22%) had average level of knowledge and no one was having good level of knowledge. With regard to post-test 5 middle age adults (8%) were having poor level of knowledge, 40(67%) had average level of knowledge and 15(25%) were having good level of knowledge. Table 3 show that the middle age adults had pre mean score (7.41) with SD (3.21) and after the implementation of awareness program post mean score was (17.45) with SD (4.70)and there was mean difference of 10.04

Section-II

Table 3 shows that the "t" value was 24.53 and p value was at 0.05 level which clearly show that "Awareness Programme" was effective in increasing the knowledge of Middle Age adults regarding Alzheimer's Disease. H_1 is accepted.

Section-III

Association between Pre-Test Knowledge score with selected demographic variable.

There is no significance association of pre-test level of knowledge among middle age adults with selected demographic variables. Hence H_2 is rejected.

Implications

Nursing Practice

Conduct awareness programs among middle age adults knowledge regarding Alzheimer's disease. These findings of the study helps to increase the knowledge level among middle age adults regarding Alzheimer's disease

Nursing Education

The results of this study can be abase for conducting a similar study on large number of population. The studies can be measure for the future studies to expand and prompt to conduct additional studies. The suggestion of the study can help to prompt the nurses to conduct research in future areas regarding enhancement of knowledge of Alzheimer's disease among Middle age adults.

Nursing Research

The result of this study can be employed for conducting research on the effectiveness of awareness programme on various aspect of knowledge regarding Alzheimer's disease. The finding scan be used to plan further research in this area.

Nursing Administration

As Nurse administrator they may distribute materials and imparting motivation for further study over in rural and in urban areas. In service education programme may be planned and organized to distribute the findings of the research among the nursing staffs.

REFERENCES

1. Sukkran. Old age problem. 2011. Available from: URL: http://www. squidoo.com/old-age-problem.
2. Agrawal N ,Jhingan HP. Life events and depression in elderly. Indian JournalofPsychiatry2002; 44 (1);34-40.
3. Rubinsztein DC. The role of itracellular protein degradation pathway in neurodegneartion 2006 oct; 443(7113);780-6.,
4. Yaari R, JodyCorey-Bloom,Alzhiemer'sDiseaseSeminarsinNeurology[Cited2011Dec6];27(1):321-41.
5. Alzheimer's Association. Alzheimer's disease Facts and Figures 2009:5(3):7-9.
6. Nathan E, Lavid MD Neuroscience Applied to clinicalpsychiatry 2002-2001: Availavle from: URL<http://www.nathanlavidmd.com/psychiatric-description/dementia.html>.
7. Sharma SK. Nursing Research and statistics; Research problem 1st ed. NewDelhi: Elsevier A division f Reed Elsevier (India) Private limited;2011 pageno.30,34.
8. SelfInstructiondefinition.http://www.staff.ncl.ac.uk/f.r.jones/definition.htm
9. Knowledge-Wikipedia.https://en.wiki/knowledge
10. BasvanthappaBT. Nursing Research,2nded: Jaypeepublishers:2007.pageno148-164
11. Botha,M.E.(1989),"Theory Development in Perspective. The Role of conceptual Frameworks and Models in Theory Development", journal ofadvance nursing14(1),49-55
12. Carpenter BD, ZollerSM, Balsis S, Otilingam PG,Gatz M Demographic andc on textual factors related to knowledge aboutAlzheimer's disease. American Journal of Alzheimer's Disease. 2011 march ;26(2)15
13. Blay SL,FurtadoA,Peluso ET. Knowledge and beliefs about help–seek in behaviour and helpfulness of interventions for Alzheimer'sdisease.2008 sep; 12(5):577-86
14. BlaySL,Toledo pisaPeluso E. Public stigma : the community tolerance of Alzheimer disease AM JGeriatr Psychiatric. 2010 feb;18(2):577-86
15. LowLF, AnsteyKJ. Dementialiteracy: recognition and beliefs on dementia of the Australian public 2009 Jan ;5(1): 43-9
16. Connell CM, ScottRobertsJ , Mc Laughlin SJ. Publicopinion about Alzheimer disease among blacks, Hispanics, and whites: results from a national survey. 2007july–Sep :21:(3):232-40

Table 1 : Grading of Level of Knowledge

S.No	Score	Level of Knowledge
1	0 – 10	Inadequate Level of Knowledge
2	11 – 20	Moderately Adequate Knowledge
3	21 – 30	Adequate Knowledge

Table 2: Distribution of knowledge among middle age adults according to their Knowledge Level

N=60

Knowledge Level	Pre Test		Post Test	
	Frequency	Percentage	Frequency	Percentage
Inadequate Knowledge	47	78	5	8
Moderate Adequate Knowledge	13	22	40	67
Adequate Knowledge	00	00	15	25

Table 3: Mean and Standard deviation of knowledge level on Alzheimer's disease

Knowledge Level	Mean	Standard Deviation
Pre Test	7.41	3.21
Post Test	17.45	4.70

Table 4: Effectiveness of Awareness Programme on Knowledge regarding Alzheimer's Disease

Area	Calculated t Value	Degree of Freedom	Table Value	Inference
Knowledge	24.53	59	2.01	Significance

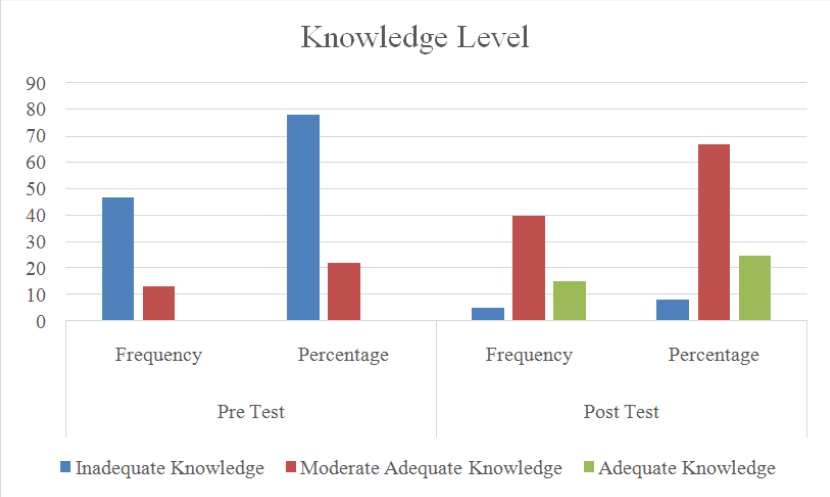

Fig 1: Bar Diagram shows the level of knowledge regarding Alzheimer's disease

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RESEARCH ARTICLE

Hepatoprotective Efficacy Study of Polyherbal Churna and Polyherbal Syrup Containing *Butea monosperma, Flemingia strobilifera* and *Moringa oleifera* against CCl4 Induced Hepatotoxicity

Shital Devlekar[1]*, Jignyasha Raval[2], Anubha Khale[3], Jayesh Dwivedi[2], and Dheeraj Randive[4]

[1]Research Scholar, Pacific Academy of Higher Education and Research University, Udaipur, Rajasthan, India
[2]Professor, Pacific Academy of Higher Education and Research University, Udaipur, Rajasthan, India
[3]Dean, Faculty of Sciences and Technology, SNDT Women's University, Mumbai, India
[4]Assistant Professor, Department of Pharmaceutics, Rajarambapu College of Pharmacy, Kasegaon, Sangli, MS, India.

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***Address for Correspondence**
Shital Devlekar
Research Scholar,
Pacific Academy of Higher Education and Research University,
Udaipur, Rajasthan, India
Email: spednekaring@gmail.com

ABSTRACT

Investigation of the pharmacological activity of medicinal plants and their extract preparations with the aim of rationalizing their therapeutic potential is one of the major aspects towards their standardization. There is a plethora of investigations reporting the hepatoprotective activity of plant extracts *in vitro* and *in vivo*. In the present work, efficacy of Polyherbal Churna and Polyherbal Syrup containing standardized powders and hydroalcoholic extracts of Polyherbal combination of plants (1:1:1), was conducted *in vivo* in female rats to determine its hepatoprotective potential against CCl4 induced hepatotoxicity. Hepatoprotective activity was assessed against CCl4 induced liver intoxication. CCl4 induced sprague dawley rats were treated with different doses of polyherbal churna and Polyherbal syrup containing standardized powders and hydroalcoholic extracts of combination (1:1:1) plants, *Butea monosperma* (BM), *Flemingia strobilifera* (FS) and *Moringa oleifera* (MO). The results were compared with established positive control, Silymarin. The treatment of two different formulations provided concentration dependent percent protection which was evident by changes in level of biochemical parameters (like SGOT, SGPT, ALP, GGT, TB, CHO and TG) in sprague dawley rats and were at par with the effect shown by Silymarin.

The observations of biochemical parameters and histopathology endorse an overall promising effect of novel formulations against liver disorders.

Keywords: *Butea monosperma, Flemingia strobilifera, Moringa oleifera,* Hepatoprotective, Carbon tetrachloride

INTRODUCTION

Medicinal plants have been in use in the form of plant extracts from ancient times to cure a wide range of ailments and are gaining popularity among the people of both urban and rural areas [1]. Investigation of the pharmacological activity of medicinal plants and their extract preparations with the aim of rationalizing their therapeutic potential is one of the major aspects towards their standardization [2]. There is a plethora of investigations reporting the hepatoprotective activity of plant extracts *in vitro* and *in vivo* [3,4]. For evaluating the hepatic efficacy of herbal extracts, several chemicals have been used for the development of liver diseases in animal models. CCl_4 is a potent hepatotoxic agent, causing hepatic injury including centrilobular hepatic necrosis, steatosis, and inflammation [5]. It is widely used in animal models for induction of acute and chronic injury. Liver injuries induced by CCl_4 are the best characterized system of xenobiotic-induced hepatotoxicity and commonly used models for the screening of anti-hepatotoxic and/or hepatoprotective activities of drugs. Since the changes associated with CCl_4-induced liver damage are similar to that of human infective hepatitis, CCl_4-mediated hepatotoxicity was chosen as the experimental model. Many scientists have evaluated the hepatoprotective effects of the plant extracts in CCl_4 induced rats model [3,6]. A number of herbal plants have been documented in Ayurveda and other ancient Indian texts and their preparations have been found to be important in the treatment of various diseases Knowledge about active phytoconstituents are not fully explored as per the present need [7]. Advancement in ayurvedic medicine has been revolution-raised from the screening of phytochemicals, pharmacological activities to elucidating their mechanisms and sites of action [8]. Nowadays, there is an increased interest in herbal drugs and remedies for treatment of chronic diseases[9]. Liver damage is a common complication. Liver has to detoxify many toxic substances. Most of the hepatotoxic chemicals damage liver cells by producing reactive species which form covalent bond with the lipids of the tissue. Due to excessive exposure to hazardous chemicals like CCl4, alcohol, etc., the number of free radicals generated is so high that they overpower the natural defence system of the body causing hepatic damage and lead to jaundice, cirrhosis and fatty liver etc. In the present work, efficacy of Polyherbal Churna and Polyherbal Syrup containing standardized powders and hydroalcoholic extracts of combination *Butea monosperma, Flemingia strobilifera* and *Moringa oleifera* (1:1:1) respectively, was conducted *in vivo* in female Sprague dawley rats to determine its hepatoprotective potential against CCl_4 induced hepatotoxicity.

MATERIALS AND METHOD

Plant Materials

Butea monosperma was collected from Thane, Maharashtra) and the herbarium of the sample, *Flemingia strobilifera* was collected from Amby Valley and *Moringa oleifera* was collected from Yeoor, Thane, and Maharashtra). The herbarium of the sample was authenticated (HRL/AUTH/2021/03, HRL/AUTH/2021/02, HRL/AUTH/2021/04respectively). After collection, the plant materials Sample was carefully segregated, cleaned and oven dried at 37° C to constant weight, powdered, sieved (BSS 85) and stored in airtight containers.

Chemicals and Reagents

CCl4 (GR grade, Merck Specialties Pvt. Ltd) and Silybon tablets (Silymarin as silybin 70 mg, (Wyeth Ltd, India) were procured from market. All other chemicals used were of analytical grade.

For induction
Carbon tetrachloride (Merck Specialities Pvt. Ltd., Mumbai, India); liquid paraffin (Ashwin Fine Chemicals and Pharmaceuticals, India).

For dosing
Silymarin tablets - 70 mg, Silybon-70 (as silybin) (Wyeth Ltd, India); distilled water.

For blood withdrawal
Heparin injection IP - Hep 25, 25000 IU (Gland Pharma Ltd, Hyderabad, India; heparinized capillaries (Top Tech Lab Equipment Pvt. Ltd, Mumbai, India); sterilized cotton swab.

For excision of liver
Surgical blade (Deepak Healthcare, India), Sodium Chloride (Parental Surgicals Ltd, India).

For histopathological evaluation
Bouin's fixative (picric acid, formalin, glacial acetic acid); ethanol (Changshu Hongsheng Fine Chemicals Co, Ltd, China); distilled water.

Procurement of kits for biochemistry
ALT, AST, alkaline phosphatase, total proteins, cholesterol, HDL, total and direct bilirubin and triglycerides kits were obtained from Span Diagnostics Ltd, Surat, India whereas GGT kit was procured from Erba Mannheim, Transasia Bio-medicals Ltd, India.

Preparation of Polyherbal formulations
Syrup: Syrup was prepared by dissolving hydroalcoholic extract of three plants (1:1:1) in sucrose syrup containing Sodium benzoate as preservative.

Churna: Plant powders were passed through 80 # sieve and then mixed together in 1:1:1 proportion to get uniformly blended churna.

Quality Control
The quality of Syrup was evaluated for Color, Odor, Viscosity, Specific gravity, PH, Residual solvent test and Churna was evaluated for color, odor, LOD, water content bulk density, extractive values.

Animals:
Adult Sprague dawley rats (female 200-250 g) procured from Bharat Serum (Thane, Mumbai, India) were used in this study. The animals were maintained under standard laboratory conditions at a temperature of 22 ± 5ºC, relative humidity of 60 ± 5% and 12/12 h dark/light cycle in a well ventilated rooman ambient temperature of 25 ± 2º C with 12-h light and dark cycle in an animal house with standard facilities under CPCSEA/315 approvals. They were fed with feed pellets supplied by Amrut Laboratory Animal Feed (Manufactured by Pranav Agro Industries Limited; Sangli, Maharashtra, India) enriched with stabilized vitamins such as vitamin A, B_1, B_2, B_3, B_9, B_{12}, $D_3$and supplemented with minerals and microelements. (AMRUT feed) and water ad libitum. The experimental procedures and protocol (Protocol No. RRC/IAEC/01/2019) for this study were reviewed and approved by Institutional Animal Ethics Committee (IAEC) of Ramnarain Ruia Autonomous College, Matunga, Mumbai, India. The guidelines for animal care were followed as recommended by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Preparation of test sample
Dose of Polyherbal Churna and Polyherbal Syrup was calculated with reference to the body weight of each animal. Churna was weighed separately (300 and 600mg/kg body weight) and suspended in distilled water just prior to

administration. Syrup was administered (100 and 200 mg/kg (extracts) body weight) in the form of syrup. Similarly, the positive control for the study: Silybon-70 {Silymarin tablets (as silybin)} at a dose of 70 mg/kg body weight was prepared in distilled water [10].

Safety evaluation

Safety study of hydroalcoholic extracts of combination containing *Butea monosperma, Flemingia strobilifera and Moringa oleifera* was conducted in rat as per OECD guidelines (No. 420, fixed dose procedure).The rat were fasted overnight for 10-14 hours and administered with hydroalcoholic extracts of combination containing *Butea monosperma, Flemingia strobilifera and Moringa oleifera* (2.0 g/kg) orally. The animals were observed individually during the first 30 min for all reflexes, periodically during the first 48 hours with special attention given during the first 4 hours (short-term toxicity) and daily thereafter for a total of 14 days (long-term toxicity) for alteration from general behavior and clinical symptoms like alteration of skin and fur texture, ptosis, excessive salivation, breathing problems, diarrhea etc. Daily body weight, food and water intake record was also maintained. The results were compared with control group (orally administered with DW [11].

Assessment of Hepatoprotective activity against CCl4 induced liver intoxication

Carbon tetrachloride intoxication in rats is an experimental model widely used to study necrosis and pathophysiological status of liver [12]. In this study Sprague dawley rats were randomly divided into 8 groups with six animals in each. The group details are as follows:

- Group I: Normal control
- Group II: CCl4 control (0.7 ml/kg)
- Group III: CCl4 control treated with daily dose of Silymarin (0.070 g/kg b.w.)
- Group IV: CCl4 control as natural recovery group
- Group V: Hepatotoxicity induced rats treated with Polyherbal churna (300 mg/kg body weight)
- Group VI: Hepatotoxicity induced rats treated with Polyherbal churna (600 mg/kg body weight)
- Group VII: Hepatotoxicity induced rats treated with Polyherbal syrup (100 mg/kg body weight (extract))
- Group VIII: Hepatotoxicity induced rats treated with Polyherbal syrup (200 mg/kg body weight (extract))

The animals were fasted overnight before the initiation of the study. Animals from Group I received an intraperitoneal injection of 0.5 ml liquid paraffin/animal on the first day of the study and were treated as normal control. Those from Groups II, III, IV, V, VI. VII and VIII received an intraperitoneal injection of 0.7 ml/kg CCl4 [13] [14] in 0.5 ml liquid paraffin/animal on the first day of the study. The animals from Groups I received an oral dose of 0.5 ml of liquid paraffin. On the same day (1 h after the induction), the animals from Group I, II and IV were administered 2 mL of distilled water each as a sham treatment, induction control and natural recovery whereas animals from group III (positive control) were administered with silymarin tablets (Silybon-70) at a dose of 70 mg/kg body weight, daily for 3 days, group V to VI were administered with the Polyherbal Churna in 2 mL of distilled water at a dose of 300 mg/kg body weight and 600 mg/kg body weight, respectively for 3 days. The animals from group VII to VIII received Polyherbal Syrup equivalent to100 mg/kg body weight and 200 mg/kg body weight, combination extracts respectively for 3 days. The route of administration for dosing the Polyherbal Churna and Polyherbal Syrup, modern drug and distilled water was oral, via gavage using needle No. 16 (force tube feeding) daily once for 3 days (1st to 3rd day).

The animals from Groups I, II, IV, V, VI, VII and VIII were sacrificed on the fourth day (72 hr after dosing) and those from Group III were sacrificed on seventh day of the study for comparative evaluation of the natural recovery in the study. Daily record of body weight and food and water intake was also maintained. Healthy adult female (8-12 weeks old) Sprague dawley rats, weighing 209.30 ± 1.23 g (mean ± SE) at the beginning of the experiment, were procured from Bharat Serum (Thane, Mumbai, India). The animals were randomly selected and kept in Animal House Facility (CPCSEA/315) at Ramnarain Ruia Autonomous College, Matunga, Mumbai, India. The animals were housed in clean and dry polypropylene cages provided with clean and dry rice husk bedding and acclimatized at a temperature of 22 ± 5ºC, relative humidity of 60 ± 5% and 12/12 h dark/light cycle in a well ventilated room. The

cages were placed randomly on the racks and their positions were changed daily to avoid any bias or influence due to the specific location of the cage. The cages were cleaned daily and other standard hygiene procedures were implemented. Each cage was tagged having the description of study number, test substance code, dose, animal number, cage number, date of initiation and date of completion of the experiment. The animals were marked to permit individual identification with the help of picric acid and were kept in their cages for at least seven days prior to the start of dosing to allow for acclimatization to the laboratory conditions. Animals were fed with feed pellets supplied by Amrut Laboratory Animal Feed (Manufactured by Pranav Agro Industries Limited; Sangli, Maharashtra, India) enriched with stabilized vitamins such as vitamin A, B_1, B_2, B_3, B_9, B_{12}, $D_3$and supplemented with minerals and microelements. Filtered drinking water (Water filter: Aqua Sure Xtra Tuft, manufactured by Forbes Aquatech Ltd., Dehradun, Uttarakhand) was supplied to every cage *ad libitum*. The experimental procedures and protocol (Protocol No. RRC/IAEC/01/2019) for this study were reviewed and approved by Institutional Animal Ethics Committee (IAEC) of Ramnarain Ruia Autonomous College, Matunga, Mumbai, India. The guidelines for animal care were followed as recommended by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Preparation of hepatotoxicant-Carbon tetrachloride (CCl_4)

Hepatotoxicity was induced by oral administration of CCl_4 at a doseof 0.7 mL/kg body weight which was calculated with reference to the body weight of each animal, measured individually and suspended in liquid paraffin (NMT 1 mL/100 g body weight) just prior to induction[15].

Preparation of test sample

Dose of Polyherbal Churna and Polyherbal Syrup was calculated with reference to the body weight of each animal. Churna was weighed separately (300 and 600mg/kg body weight) and suspended in distilled water (NMT 1 mL/100 g body weight) just prior to administration. Syrup was administered (100 and 200 mg/kg (extracts) body weight) in the form of syrup. Similarly, the positive control for the study: Silybon-70 {Silymarin tablets (as silybin)} at a dose of 70 mg/kg body weight was prepared in distilled water[10].

Experimental design

Animals were randomized into eight groups of six animals each and housed in separate appropriately labeled cages. Animals from each cage were marked on tail, head and back to permit individual identification. Animals were fasted for 12 h prior dosing, where food was withheld and drinking water was provided *ad libitum*. Reversible liver damage was induced by an intraperitoneal (ip) injection of carbon tetrachloride (CCl_4) at a concentration of 0.7 mL/kg body weight in 0.5 mL of liquid paraffin using sterile, latex free BD 1 mL syringe with 26G needle [15][10] to each animal from Group II to Group VIII on 1st day. The animals from Group I served as normal control and were administered with an intraperitoneal injection of 0.5 mL of liquid paraffin as sham treatment. On the same day (1 h after the induction), the animals from Group I, II and IV were administered 2 mL of distilled water each as a sham treatment, induction control and natural recovery whereas animals from group III (positive control) were administered with silymarin tablets (Silybon-70) at a dose of 70 mg/kg body weight, daily for 3 days, group V to VI were administered with the Polyherbal Churna in 2 mL of distilled water at a dose of 300 mg/kg body weight and 600 mg/kg body weight, respectively for 3 days. The animals from group VII to VIII received Polyherbal Syrup equivalent to100 mg/kg body weight and 200 mg/kg body weight, combination extracts respectively for 3 days. The route of administration for dosing the Polyherbal churna and Polyherbal Syrup, modern drug and distilled water was oral, via gavage using needle No. 16 (force tube feeding) daily once for 3 days (1st to 3rd day).The animals were provided with food and water and observed for any abnormal behavior or side effects. Daily body weight, food and water intake of animals was recorded. The group details and dosage regime is given in Table 1. The schematic representation of schedule of the efficacy study is shown in Table 2.

Use of CCl_4

For evaluating the hepatic efficacy of herbal extracts, several chemicals have been used for the development of liver diseases in animal models. CCl_4 is a potent hepatotoxic agent, causing hepatic injury including centrilobular hepatic

necrosis, steatosis, and inflammation. It is widely used in animal models for induction of acute and chronic injury. Liver injuries induced by CCl_4 are the best characterized system of xenobiotic-induced hepatotoxicity and commonly used models for the screening of anti-hepatotoxic and/or hepatoprotective activities of drugs. Since the changes associated with CCl_4-induced liver damage are similar to that of human infective hepatitis, CCl_4-mediated hepatotoxicity was chosen as the experimental model. Many scientists have evaluated the hepatoprotective effects of the plant extracts in CCl_4 induced rats model[3][6]. In the present work, efficacy of standardized Polyherbal churna and polyherbal Syrup of hydroalcoholic extract of combination containing *Butea monosperma, Flemingia strobilifera* and *Moringa oleifera* was conducted *in vivo* in female Wistar rat to determine its hepatoprotective potential against CCl_4 induced hepatotoxicity.

Blood collection and sacrifice

Blood sample will be withdrawn on the day of sacrifice by decapitation under diethyl ether anesthesia by retro orbital plexus technique using heparinized capillaries and collected into sterile, heparinized vials ensuring that there is no haemolysis. The blood samples will then centrifuged at 5000 rpm for 10 min to separate plasma. The vials with plasma samples were immediately stored at 4^0C. The plasma samples will be analyzed immediately (within 8 h of collection) for clinical biochemical parameters like levels of Gamma-Glutamyl Transferase(GGT), Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline Phosphatase (ALP), Triglycerides (TG), Total Cholesterol (CHO), Total bilirubin (TB) using commercially available reagents kits. The animals from group I, II, III, V-VIII will be sacrificed on 4^{th} day immediately after the blood collection and the ones from Group IV will be sacrificed on 7^{th} day of the study. Animals will be sacrificed by CO_2euthnesesia. Liver will be excised during autopsy, rinsed in 0.9% saline and blotted dry of saline and excess of blood and weighed. The small piece of the central portion of the liver will be cut and fixed in Bouin's fixative for the histopathological examination. Liver will be cut into small pieces, weighed as required for analysis of tissue biochemical parameter like liver glycogen and also further will be used for histopathological studies. Liver samples will be stored at -70°C till further studies.

Parameters considered for evaluation and observation

The extent of liver damage and subsequent status of the liver after treatment will be assessed and evaluated by studying body and organ weight, hematological, tissue and histopathological parameters:

- **Body weight**
- **Food consumption and water intake**
- **Evaluation of biochemical and tissue parameters:** SGOT, SGPT, ALP, GGT, TG, CHO and TB
- **Histopathological evaluation:** Light Microscopy of Liver tissue

The extent of liver recovery was compared with known hepatoprotectant, Silymarin. Percent protection in individual biochemical parameters from their elevated values caused by the hepatoprotection was calculated as 100 × (values of CCl4 Control - values of sample) / (values of CCl4 control - values of vehicle) [12].

Statistical analysis

All values were expressed as mean ± S.E and statistically analyzed for % protection, comparing group I with the other groups.

RESULTS AND DISCUSSION

Medicinal plants and their derivatives have been used since ancient time in various forms for the treatment of liver disorders. Medicinal uses of these three plants can be traced back to previous research work, such as anti-diabetic, anti-cancer, anti-inflammatory, anti-asthmatic, anti-oxidant, anticonvulsant, anti-microbial, antiviral and hepatoprotective. These medicinal uses have been demonstrated in recent in vivo and in vitro research. Though work has been done on hepatoprotective efficacy of *Butea monosperma*, *Moringa oleifera* and *Flemingia* species, there exist no work done on *in vitro* hepatoprotective efficacy for combination of these three plants in different dosage forms (Churna and Syrup).Thus, in the present work, Churna and Syrup of BM, MO and FS were evaluated as an

alternative cure on CCl4 induced hepatotoxicity in rats. The quality of Churna was found to be as per the prescribed limits in API for parameters like Color, Odor, LOD, Water content, Bulk density and Extractive value. The quality of syrup was evaluated with respect to Color, Viscosity, Specific gravity, pH and residual solvent test and found to be within limit as per Ayurvedic pharmacopoeia. The Assay evaluation of both Churna and Syrup have been performed using validated HPTLC method using biomarker from phytosterol group. The safety of the standardized hydroalcoholic extract combination containing BM, FS and MO was established by acute oral toxicity study which was carried out on rats at 2.0 g / kg body weight. The extract was found to be safe as it showed no abnormal fluctuation in body weights and food and water intake of the animals. Clinical symptoms of toxicity were also found to be absent during the period of the study and no mortality was recorded. The safety study of the extract revealed that hydroalcoholic extract can be considered safe with a wide margin for oral use. CCl4 induced hepatotoxicity has been chosen as the experimental model since the changes associated with the CCl4 induced liver damage are similar to those of viral hepatitis [16]. Carbon tetrachloride is commonly used for inducing liver damage because it causes peroxidative degeneration in adipose tissue and is metabolized to trichloromethyl radical and trichloromethyl peroxy radicals which are involved in pathogenesis of liver [15] [17]. CCl4 metabolites react with polyunsaturated fatty acids and form covalent adducts with lipids and proteins. These events lead to lipid peroxidation and destruction of cell membranes with the consequent liver injury[18]. Hepatic damage induced by CCl4 results in an increase in the level of biochemical parameters like SGOT, SGPT, ALP, GGT, TG, CHO and TB [15] [6]. CCl4 induction also causes classical fatty liver as indicated by significant increase in cholesterol. Group II showed marked increase in all biochemical parameters analyzed and showed severe loss of hepatic architecture with intense peripheral and central vein necrosis, fatty changes and crowing of the central vein histopathologically [Figure 1(b)].

The characters such as centrilobular hepatic necrosis, fatty changes sinusoidal degeneration, infiltration by lymphocytes and inflamed hepatocytes with vacuoles were observed. Fatty degeneration with severe hepatic necrosis of parenchyma cells was observed in the central lobular region. Along with these characters sinusoidal congestion and broad infiltration of kupffer cells was also evident. The established standard drug silymarin was used as modern control. Group IV animals were treated with Silymarin, at a dose of 0.07 g/kg. Silymarin treated rats showed marked recovery in biochemical parameters (SGOT, SGPT, ALP, GGT, TB, CHO and TG) and offered percentage protection of 101.54%, 103.10 %, 98.06%, 97.90%, 90.72%, 96.99% and 93.49% respectively. Histopathological findings supported the biochemical data as the treated animals showed near normal hepatic architecture with mild degree of necrosis that signifies recovery and protective effect of the drug formulations[Figure 2 (d)]. Animals from the groups V and VI were treated with two different doses of polyherbal churna (PC) formulations orally at doses of 300mg/kg and 600mg/kg respectively. The percentage protection offered by these two doses of PC formulation in terms of the reduction in the level of SGOT, SGPT, ALP, GGT, TB, CHO and TG levels is mention in Table 2. Animals from the groups VII and VIII were treated with two different doses of polyherbal syrup (PS) formulations orally at doses of 100mg/kg and 200 mg/kg respectively. The percentage protection offered by these two doses of PS formulation in terms of the reduction in the level of SGOT, SGPT, ALP, GGT, TB, CHO and TG levels is mention in Table 2. The histopathological results also showed marked recovery in the hepatic architecture and reduction in liver damage and cellular necrosis. PC and PS at higher doses showed better results in terms of percent protection and histological findings when compared with the lower doses [Figure 3 (F) and Figure 4 (H)].

Reduction in food and water consumption coupled with the decrease in body weight after CCl4 treatment indicates toxic response, whereas any significant increase observed in these parameters after treatment indicates its protective action. Elevated levels were observed in the CCl4 induction group as per the results obtained from biochemical parameters (Table 1), which indicate CCl4 induced damage to the liver. The significant reductions in the levels of SGOT, SGPT, ALP, GGT, TB, CHO and TG in case of Silymarin control and in the groups treated with the two different doses of PC and PS, suggest possible stabilization of the plasma membrane and the repair and recovery of hepatic tissue damage caused by CCl4 intoxication [19]. This may also imply that the serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes [20]. Liver sections of the animals of the group III [(Figure 2:(C)], which were not given any treatment, showed a histoarchitecture similar to the animals from group II. Severely distorted sinusoids and invasion of kupffer cells was observed in these

animals suggesting liver damage. Treatment by silymarin (Group IV) restored histoarchitectural damage caused by the intoxication of CCl_4. Treatment by low dose of both the Polyherbal Churna and Polyherbal Syrup [(Figure 3: (E) and Figure 4: (G)] showed suggestive improvements in the liver architecture, but dilation of sinusoids, congestion of central vein and presence of kupffer cells was evidences to suggest that tissue injuries were not yet completely recovered. The treatment by high dose of Polyherbal Churna and Polyherbal Syrup [Figure3: (F) and Figure 4 (H)] were more successful than the low dose, in prevention of cellular changes due to hepatotoxicity by CCl_4. Polyherbal combination treatment reduced the presence of vacuoles in cytoplasm and congestions in the veins. The dilation of sinusoids was also lesser and regeneration was evident. The treatment by high dose of Polyherbal Syrup (Figure4:H) remarkably reduced the severeness of hepatic lesions and damage induced by CCl_4. The histoarchitecture was observed to have returned to normalcy and at par with the positive treatment, Silymarin. These results of histopathology hence further support the results of biochemical analysis. A comparative histopathological study of liver from different groups confirmed the hepatoprotective efficacy of *Polyherbal churna and Polyherbal syrup* against CCl_4 induced liver damage, with significantly better recovery with high dose of *Polyherbal syrup.*

CONCLUSION

Findings of the present investigation adequately prove the hepatoprotective potential of Polyherbal churna and Polyherbal syrup of combination of *Butea monosperma, Flemingia strobilifera* and *Moringa oleifera* with significant recovery with high dose of Polyherbal syrup. The therapeutic potential shown by both the formulations in the management of hepatic dysfunction may be due to presence of phytochemical constituents in BM, FS and MO, acting synergistically. Extraction, isolation and characterization of the constituents responsible for the therapeutic efficacy of Polyherbal Churna and Polyherbal Syrup of combination of *Butea monosperma, Flemingia strobilifera* and *Moringa oleifera* followed by evaluation of their pharmacological action against liver damage can be carried out to identify an even efficient hepatoprotective dosage form.

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Author's contribution

SD wrote the proposal, participated in sample and data collections, analyzed the data and drafted the manuscript. JR and AK designed the study concept, and drafted an initial proposal and approved the subsequent proposal, participated in sample collection and analysis, revised and finalised the subsequent drafts of the paper. JD and DR provided access to Hepatoprotective studies. All of the authors read and approved the final manuscript.

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Data availability -The data supporting results of this study and the information about the materials used is included within the article.

Compliance with ethical standards:

Declarations:

Competing Interests: The authors declare no competing interests.

Ethical approval: (Protocol no. RRC/IAEC/01/2019)

REFERENCES

1. Pani M, Nahak G, Sahu RK. Review on larvicidal activity of medicinal plants for malaria vector control. Int J Curr Pharm Rev Res. 2015;6:94–114.
2. Kunle. Standardization of herbal medicines - A review. Int J Biodivers Conserv. 2012;4:101–112.
3. Hanafy A, Aldawsari HM, Badr JM, et al. Evaluation of Hepatoprotective Activity of Adansonia digitata Extract on Acetaminophen-Induced Hepatotoxicity in Rats. Evidence-based Complement Altern Med. 2016;2016.
4. Agrawal SK, Jat RK, Chhipa RC. Pharmacological evaluation of hepatoprotective activity of Clerodendrum serratum. Int J Pharmacol Toxicol. 2013;3:67–70.
5. Ramasamy P, Subhapradha N, Shanmugam V, et al. Protective effect of chitosan from Sepia kobiensis (Hoyle 1885) cuttlebone against CCl4 induced hepatic injury. Int J Biol Macromol. 2014;65:559–563.
6. Shailajan S, Chandra N, Sane RT, et al. Effect of Asteracantha longifolia Nees. against galactosamine induced liver dysfunction in rat. Indian J Exp Biol. 2007;14:7–13.
7. Randive DS, Shejawal KP, Bhinge SD, et al. Green synthesis of gold nanoparticles of isolated citrus bioflavonoid from orange: Characterization and in vitro cytotoxicity against colon cancer cell lines colo 320DM and Ht29. Indian Drugs. 2020;57:61–69.
8. Randive DS, Bhinge SD, Wadkar GH, et al. Comparative standardization of marketed formulations of fermented biomedicine - Arjunaristha. Indones J Pharm. 2016;27:220–225.
9. Shejawal KP, Randive DS, Bhinge SD, et al. Green synthesis of silver, iron and gold nanoparticles of lycopene extracted from tomato: their characterization and cytotoxicity against COLO320DM, HT29 and Hella cell. J Mater Sci Mater Med [Internet]. 2021;32:19. Available from: http://dx.doi.org/10.1007/s10856-021-06489-8.
10. Pingale Shirish S. Hepatoprotection study of leaves powder of Azadirachta indica a. Juss. Int J Pharm Sci Rev Res. 2010;3:37–42.
11. Devlekar S, Raval J, Khale A. Acute Toxicity Study in Rat for Hydroalcoholic Extract of Combination Containing Butea monosperma , Flemingia strobilifera and Moringa oleifera. 2021;11:13–19.
12. Rao BG, Rao YV, Rao TM. Hepatoprotective activity of Spillanthes acmella Extracts against CCl4-induced liver toxicity in rats. Asian Pacific J Trop Dis. 2012;2:208–211.
13. Koh PH, Mokhtar RAM, Iqbal M. Andrographis paniculata ameliorates carbon tetrachloride (CCl(4))-dependent hepatic damage and toxicity: diminution of oxidative stress. Redox Rep. 2011;16:134–143.
14. Nayak, Y. , Abhilash, D. , Vijaynarayana, K. and Fernandes J. Antioxidant and Hepatoprotective Activity of Pimenta Dioica Leaves Extract. J Cell Tissue Res. 2008;8:1571–1576.
15. Frank PR, Suresh V, Arunachalam G, et al. Evaluation of hepatoprotective effect of adiantum incisum forsk leaf extract against CCL4 induced hepatotoxicity in rats. Int Res J Pharm. 2012;3:230–234.
16. Shailajan S, Joshi M, Singh D, et al. Efficacy of a traditional unani formulation Jawarish-e-Amla Sada against CCl4 induced liver toxicity in Albino Wistar rats: Comparison with silymarin treatment. Int J Green Pharm. 2015;9:100–103.
17. Shailajan S, Joshi M, Tiwari B. Hepatoprotective activity of Parmelia perlata (Huds.) Ach. against CCl4 induced liver toxicity in Albino Wistar rats. J Appl Pharm Sci. 2014;4:70–74.
18. Recknagel RO, Glende EAJ, Dolak JA, et al. Mechanisms of carbon tetrachloride toxicity. Pharmacol Ther. 1989;43:139–154.
19. Thabrew MI, Joice PDTM, Rajatissa W. A comparative study of the efficacy of Pavetta indica and Osbeckia octandra in the treatment of liver dysfunction. Planta Med. 1987;53:239–241.
20. Anusha M, Venkateswarlu M, Prabhakaran V, et al. Hepatoprotective activity of aqueous extract of Portulaca oleracea in combination with lycopene in rats. Indian J Pharmacol. 2011;43:563–567.

Table 1:Group details and dose regimen

Group	No. of animals	Group details	Dose	Volume
I	6	Normal control (sham treated rats, administered with distilled water) (No induction)	--	NMT 1 mL/100 g body weight
II	6	CCl_4 induced hepatotoxicity (Induction control)	0.7 ml/kg body weight	
III	6	Hepatotoxicity induced rats treated with Silybon tablets (Positive control)	70 mg/kg body weight	
IV	6	Hepatotoxicity induced, rats left untreated for 7 days (Natural recovery)	--	
V	6	Hepatotoxicity induced rats treated with Polyherbalchurna*	300 mg/kg body weight	
VI	6	Hepatotoxicity induced rats treated with Polyherbalchurna*	600 mg/kg body weight	
VII	6	Hepatotoxicity induced rats treated with Polyherbal syrup*	100 mg/kg body weight(extract)	
VIII	6	Hepatotoxicity induced rats treated with Polyherbal syrup *	200 mg/kg body weight(extract)	

*Note: 1) **Polyherbal churna contains powders and Polyherbal Syrup** contains hydroalcoholic extracts of combination containing *Butea monosperma, Flemingia strobilifera* and *Moringa oleifera*in 1:1:1 proportion. 2) Dose was administered once daily for three days (day 1 – day 3).

Table 2: Percentage Protection

Biochemical parameter	Group Number	%Protection value
SGOT	V	57.46
	VII	69.64
	VII	67.26
	VIII	95.87
SGPT	V	70.71
	VII	93.21
	VII	85.97
	VIII	106.11
ALP	V	54.71
	VII	59.77
	VII	73.80
	VIII	84.87
GGT	V	56.57
	VII	73.61
	VII	80.34
	VIII	95.37
TB	V	59.54
	VII	68.81
	VII	71.13
	VIII	79.64
CHO	V	53.80
	VII	83.02

Biochemical parameter	Group Number	%Protection value
	VII	56.41
	VIII	96.24
TG	V	63.77
	VII	78.15
	VII	95.83
	VIII	96.16

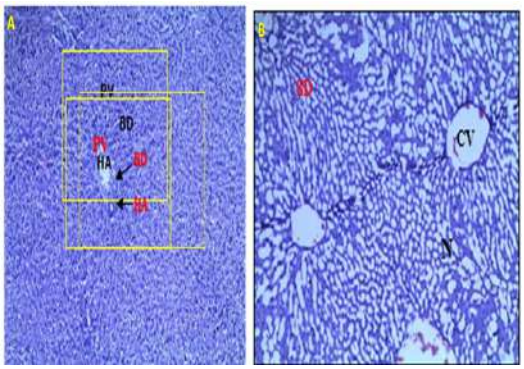

Figure 1: Histopathology of liver sections (A): Normal Control – Liver section of control animal demonstrating normal structure, (B): Induction Control – Liver in CCl4 intoxicated animal,

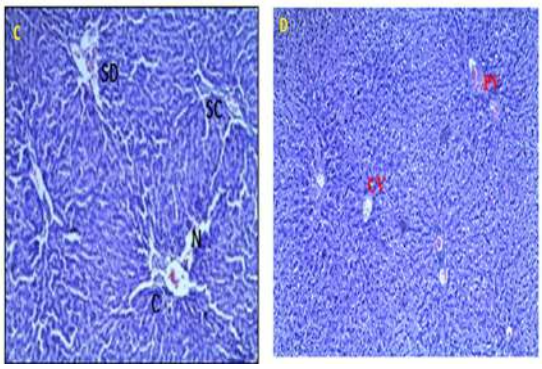

Figure 2: Histopathology of liver sections (C): Natural Recovery – Liver in CCl4 intoxicated animal after a natural recovery period of 7 days, (D): Modern Control – Liver in CCl4 intoxicated animals treated daily with 0.07 g/ kg silymarin,

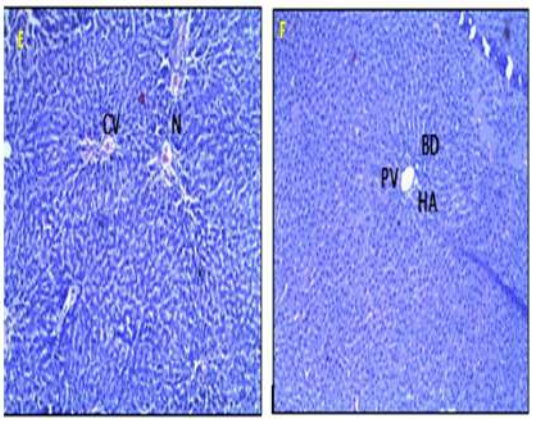

Figure 3: Histopathology of liver sections (E) PC 0.3 g/ kg – Liver in CCl4 intoxicated animals treated daily with 0.3 g/ kg PC, (F):PC 0.6 g/kg – Liver in CCl4 intoxicated animals treated daily with 0.6 g/ kg PC

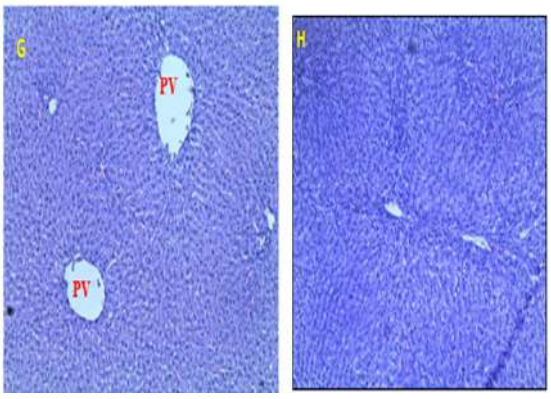

Figure 4: Histopathology of liver sections (G): PS 0.6 g/ kg – Liver in CCl4 intoxicated animals treated daily with 0.1 g/ kg PS (H): PS 0.2 g/kg – Liver in CCl4 intoxicated animals treated daily with 0.2 g/ kg PS

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RESEARCH ARTICLE

Role of Job Embeddedness among Teachers in Educational Sector

Surya S[1]*, Jacob P.M[2] and Afsal E.M[3]

[1]Research Scholar, Kerala University of Fisheries and Ocean Studies, Ernakulam district, Kerala, India
[2]Research Guide and Director, Naipunnya Business School, Ernakulam District, Kerala, India
[3]Associate Professor and Head, School of Management and Entrepreneurship, Kerala University of fisheries and ocean studies, Kerala, India

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***Address for Correspondence**
Surya.S,
Research Scholar,
Kerala University of Fisheries and Ocean Studies,
Ernakulam district, Kerala, India

ABSTRACT

Job Embeddedness is a new concept which aims to reduce turnover intention and helps to measure the factors that makes employees intention to stay at work. The Job Embeddedness model developed by Mitchell and Lee (2001) has 6 main dimensions that is Fit to community, fit to organization, Link to Community, Link to Organization, Sacrifice to Community and Sacrifice to Organization. The objective of this paper is to study on Job Embeddedness among College teachers. A sample of 50 teachers were selected using Snowball Sampling method. The data was collected using 30 items Job Embeddedness scale questionnaire. The findings indicate that Job Embeddedness stands on a medium level among college teachers. The dimension Fit considered to be in the highest Rank and Link considered to be in the lowest rank position. There is a greater reliability for the items in the scale. The demographic factors such as age, gender, experience, designation, education qualification etc. and different types of college teachers across Govt, Aided and Self financing also shows a significant difference on Job Embeddedness items of Link and Sacrifice.

Keywords: Job Embeddedness, Link, Fit and Sacrifice.

INTRODUCTION

Retention of employees is very necessary for the survival of the organization. It is very important for the achievement of organizational goals. The organization which cannot retain employees can leads to failure of productivity. This new concept Job embeddedness focus on factors that can make employees to stay on their job. This job embeddedness factor helps to improve work life that can definitely reduce attrition and increases performance and productivity of the organization. Mitchell (2001) says that embeddedness suggests that there are several strands that tie an employee and his or her family in a social, psychological, and financial web that include work and non-

work friends, groups, the community, and the physical environment in which he or she lives". According to Mitchell, Holtom, Lee, Sablynski, and Erez, (2001). "Job embeddedness is defined as the on-the-job and off-the-job factors associated with individual links, fit, and sacrifice". This concept came in education sector from management and organization psychology. The job embeddedness model by Mitchell and Lee (2001) has 6 original dimensions. Organization links and community links (individual connections with people in the organization and community), organization fit and community fit (individual perception of fit within an organization and community), organization sacrifice and community sacrifice (what the individual gives up when leaving the organization or community. This is a rich concept and it is very necessary to understand to its deepness. The turnover studies are in management and organizational psychology. Later it was studied by different researchers like, Hom and Griffeth (1991), on employee turnover. The number of literatures identified the factors for the turnover. But it is difficult to say that there is a single reason for employee turnover (Afsar & Badir, 2016; Akgunduz and Sanli, 2017; Charlier, *et al.,* 2016; Clinton, NgKnight, and Guest, 2012). Researchers explain that many demographic factors are the reasons for turnover such as age, gender, work experience and marital status etc. Today, researchers are interested in exploring the potential forces that retain an employee on his job. This occurrence of this condition in the life of an employee is called job embeddedness.

Literature Review A Conceptual Definitions

Job embeddedness (JE) is a construct developed to explain why people stay in their jobs (Mitchell, Holtom, Lee, Sablynski, & Erez, 2001). JE theory suggests that we are held in our jobs and the communities in which we live tries to connect with other people, groups, organizations, places, and things.

Literature Studies

The applications of JE in relation to the manufacturing and service sectors using different factors. (J. Cheng and O-Yang, 2018). In the services sector, which includes academics, hospital industry, business centres, IT firms, home-based jobs, and banking sectors, JE has been found to be the key issues of job performance, commitment, and turnover intention (Chan *et al*., 2019). The JE has been discussed in manufacturing sector, including the chamber of commerce and industry, oil and gas companies, and the automotive industry (Wheeler *et al.,* 2007) Hasnizawati, H (2017). A study on Job Embeddedness and Organizational Climate of 114 disabled employees in the private sector by the Social Welfare Department. The results have shown that organizational climate has a significant positive relationship with the dimensions in job embeddedness namely fit, link and satisfaction which provide support to the hypothesis. The results are consistent with past studies whereby disabled employees are able to embed with their companies due to the support given by the employer by providing a better organizational climate.

Young-bohk Cho *et al* (2009). In his study on Organizational Citizenship Behaviours in Relation to Job Embeddedness, Organizational Identification, Job Performance, Voluntary Turnover Intention in Korea, for determining whether there is positive or the negative association between Organizational Citizenship Behaviours and Job Embeddedness, Organizational Identification, Job Performance, Voluntary Turnover Intention in Korea. Organization-related sacrifice significantly had negative effects on voluntary turnover intention and positive effects on job performance, organizational identification and that Organizational citizenship behaviour mediated the relationship between on-the-job embeddedness and job performance, voluntary turnover intention, organizational identification. In a study conducted by Lev and Koslowsky (2014) on the relationship between the job embeddedness of teachers and their organizational citizenship and organizational commitment, the findings indicate a positive relationship between the job embeddedness of teachers and their sense of responsibility, job performance and contextual performance.

Statement of the Problem

Job Embeddedness consists of factors that attract employees to stay within an organization. Their intention to leave the organization will be less as they are surrounded by a network of people and activities around them. The relationship between the people and activities in a network that makes the existing employees to their current job is known as Job Embeddedness. Those who are highly embedded will have more connections to the organization and

community. Job embeddedness is necessary as this factor makes employees to predict their work life. If Quality of Work Life decreases among teachers it can lead to Attrition. Turnover of employees can result in low productivity and job performance. To know more about it is necessary to investigate into the factors contributing job embeddedness towards the organization and community among college teachers and to find whether any differences exists.

Objectives of the Study

To measure the levels of Job Embeddedness among college teachers.
To explore the influence of different dimensions of job embeddedness among college teachers.
To identify Job Embeddedness between different demographic factors such as age, gender, experience, education qualification and designation among college teachers.
To compare Job embeddedness across different types of college teachers.

Hypothesis for the Study

Hypothesis 1: There is a significant difference in Job Embeddedness between demographic factors among college teachers.
Hypothesis 2: There is a significant difference on the Job embeddedness across government, aided and self-financing college teachers.

RESEARCH METHODOLOGY

Research Design

The study uses exploratory research method for identifying the factors contributing to job embeddedness among college teachers. The research type is descriptive in nature.

Population and Sample of The Study

The total population for the study is 200 teachers which is taken from Perumbavoor, Ernakulam district, Kerala. The sample selected for the study is 50 teachers.

Data Collection Tool

Primary data collected through Pretested Questionnaire were used and was distributed through google forms to college teachers. Research data were collected through 30 Items Job Embeddedness scale measuring Link, Fit and Sacrifice developed by Mitchell *et al* (2001). First 6 variables were the demographic profile of the teachers such as age, gender, experience, designation, educational qualification and type of college. Next were the dimensions used to measure the job embeddedness factors on college teachers which consist of 11 items measuring Fit,7 items measuring Link and 12 items measuring sacrifice.

Scale of Measurement

Total of 30 items of Job embeddedness used. 5-point Likert scale was used to record the responses of the respondents. The scale represented the rating from "strongly disagree" to "strongly agree". JE questionnaire has good internal consistency. The fit factor consisted of 11 items (α=.775), Link consisted of 7 items (α=.66), and sacrifice contained 12 items (α=.793), the scale has good reliability, with a Cronbach alpha coefficient reported of .939

Sampling Technique

Sampling technique used here is Snowball sampling method.

Data Analysis and Interpretation

Software packages like SPSS and several statistical tools were used for analysing the data. The Descriptive analysis was done to know the levels of Job embeddedness among college teachers. Exploratory factor analysis was done to

analyse the dimensions and ANOVA and t test used to know the differences among different demographic characters such as age, gender, experience, educational qualification, designation and different types of college teachers. Cronbach's alpha reliability coefficient normally ranges between 0 and 1. However, there is actually no lower limit to the coefficient. The closer Cronbach's alpha coefficient is closer to 1.0, the greater the internal consistency of the items in the scale. Here alpha is greater than .9 so it is considered to be highly reliable.

Objective

To Measure the levels of Job embeddedness among college teachers.

Descriptive statistics

Interpretation

College faculties stands on medium level on Job Embeddedness scale with an overall mean of 3.83. It is found that that there is a high level for the dimension Link towards community.

Objective

To explore the influence of different dimensions of Job Embeddedness among college teachers. Exploratory Factor analysis was done to check the influence of dimensional factors of Job Embeddedness among College teachers.KMO for the factors Fit, Link and Sacrifice is .775,.666,.793 which is greater than 0.5. thus, it indicates that factor analysis is appropriate for the data. Bartlett's Test of Sphericity tests the null hypothesis; here the null hypothesis is rejected. Significant value of Bartletts Test is .001 which is less than 005.Bartelltts test seems to be appropriate. The mean score of the factor Fit is 0.713 is considered to in the highest rank as compared with other dimensions link and sacrifice. Mean value of the factor link 0.53 is considered to be in the lower rank.

Objective 3 and 4

To identify Job Embeddedness between different demographic factors such as age, gender, experience, education qualification and designation among college teachers. The demographic factors show differences in certain items of Link and Sacrifice. Overall, there is no significant differences found as all P values are greater than .05.

DISCUSSIONS

Job Embeddedness stands on medium level among college teachers with an overall mean of 3.83. Job Embeddedness scale constitutes a high reliability with Cronbach's Alpha. 939.The exploratory factory analysis done for the dimensions such as Link, Fit and Sacrifice considered to be appropriate. Fit contributes highest rank among college teachers and Link to the lowest rank position. Demographic factors shows significant difference in certain items of Link and Sacrifice but no such differences regarding the dimension Fit. Age shows differences in the item of sacrifice for the years between 37-47 and 48-58. T test was done to check the differences regarding gender. Male and female shows significant differences in the items of Link. Expereince and Designation also vary significantly with the dimensions Link and Sacrifice. Types of college teachers across Govt, Aided and Self-financing also vary significantly with the dimension Sacrifice.

SUGGESTIONS AND CONCLUSION

It is suggested to improve the levels of Job Embeddedness among college teachers. Inorder to increase connections towards organization and communities, provide them with a fair working Environment, have more interaction with their co-workers, involves them in a decision-making process. Consider female employees into the priority as most of the colleges are functioning with the female employees. Due to poor promotional facilities, retirement benefits and other incentives, employees sacrifice a lot when they leave an institution. This all are the reasons that shows a difference in Job Embeddedness towards the dimensions Link and Sacrifice. If these factors are improved, they will definitely show an intention to stay in their institution. Only then the work life and productivity can be improved.

Otherwise it can lead to attrition among employees. From the previous studies shown a negative impact on demographic factors with Job Embeddedness on various areas. The present study proved that there are differences with regard to the age, gender, experience, designation, qualification, type of college teachers. High age groups, experiences, Professors with higher qualification are less embedded in their jobs. Thus, study makes contribution to the organization literature to identify the factors that makes them to stay within an organization.

REFERENCES

1. Afsar, B., & Badir, Y. F. (2016). Person-organization fit perceived organizational support, and organizational citizenship behaviour: The role of job embeddedness. Journal of Human Resources in Hospitality & Tourism, 15(3), 252-278.
2. Akgunduz, Y., &Sanli, S. C. (2017). The effect of employee advocacy and perceived organizational support on job embeddedness and turnover intention in hotels. Journal of Hospitality and Tourism Management, 31, 118-125
3. Barber, L. K., &Grawitch, M. J. (2009) service oriented and emotion-oriented emotion regulation in police officers. Applied Psychology in Criminal Justice, 5(2)
4. Bergiel, E. B., Nguyen, V. Q., Clenney, B. F., & Taylor, G. S. (2009). Human resource practices, job embeddedness and intention to quit. Management Research News, 32(3), 205– 219. https://doi.org/10.1108/01409170910943084
5. Chafra, J., &Erkutlu, H. (2016). Authentic leadership and organizational job embeddedness in higher education. Hacettepe University Journal of Education, 32, 413–426. https://doi. org/10.16986/HUJE.2016018528
6. Chan, W. L., Ho, J. A., Sambasivan, M., & Ng, S. I. (2019). Antecedents and outcome of job embeddedness: Evidence from four and five-star hotels. International Journal of Hospitality Management, 83, 37–45. https://doi.org/10.1016/j. ijhm.2019.04.011
7. Charlier, S. D., Guay, R. P., & Zimmerman, R. D. (2016). Plugged in or disconnected? A model of the effects of technological factors on employee job embeddedness. Human Resource Management, 55(1), 109-126
8. Cheng, J., & O-Yang, Y. (2018). Hotel employee job crafting, burnout, and satisfaction: The moderating role of perceived organizational support. International Journal of Hospitality Management, 72, 78–85. https://doi.org/10.1016/j. ijhm.2018.01.005
9. Clinton, M., Ng-Knight, T., & Guest, D. (2012). Job Embeddedness: A New Attitudinal Measure (Vol. 20).
10. Cotton, J. L., & Tuttle, J. M. (1986). Employee turnover: A meta-analysis and review with implications for research. Academy of management Review, 11(1), pp. 55-7
11. Duan, X., Wang, Y. (2019). The impact of workplace violence on job satisfaction, job burnout, and turnover intention: The mediating role of social support. Health and Quality of Life Outcomes, 17(1), Article 93. https://doi.org/10.1186/s12955-019- 1164-3
12. Gelfand, M. J., Raver, J. L., & Ehrhart, K. H. (2002). Methodological issues in crosscultural organizational research. Handbook of Research Methods in Industrial and Organizational Psychology, pp. 216-246
13. Greene, J., Mero, N., & Werner, S. (2018). The negative effects of job embeddedness on performance. Journal of Managerial Psychology, 33(1), 58–73. https://doi.org/10.1108/JMP-02- 2017-0074
14. Hasnizawati,h (2017). Job Embeddedness and Organizational Climate. Asian Journal of Quality of Life, 2(6), p.31-42
15. Lee, Koslowsky (2014). The story of why we stay: A review of job embeddedness. Annual Review of Organizational Psychology and Organizational Behaviour, 1, 199– 216.
16. Lewin, K. (1951). Field theory in social science: Selected theoretical papers (edited by Dorwin Cartwright), 1951.
17. Mitchell, T. R., Holtom, B. C., Lee, T. W., Sablynski, C. J., & Erez, M. (2001). Why people stay: Using job embeddedness to predict voluntary turnover. Academy of Management, 44(6), pp. 1102-1121. Retrieved from http://www.amj.aom.org
18. Pavot, W., Diener, E., Colvin, C. R., & Sandvik, E. (1991). Further validation of the Satisfaction with Life Scale: Evidence for the cross-method convergence of wellbeing measures. Journal of personality assessment, 57(1), 149-161

19. Plooy & Roodt, (2013). Biographical and demographical variables as moderators in the prediction of turnover intentions.JournalofIndustrialPsychology39(1),01-12.
20. Royalty, A.B. (1998), 'Job-to-Job and Job-to-Non employment: Turnover by Gender and Education Level, Journal of Labor Economics, 16(2), pp. 392–443.
21. Tanova, C., & Holtom, B. C. (2008). Using job embeddedness factors to explain voluntary turnover in four European countries. International Journal of Human Resource Management, 19(9), 1553-1568.
22. Wheeler, A. R., Coleman Gallagher, V., Brouer, R. L., &Sablynski, C. J. (2007). When person-organization (mis)fit and (dis)satisfaction lead to turnover. Journal of Managerial Psychology, 22(2), 203–219. https://doi.org/10.1108/02683940710726447.
23. Young-bohk Cho etal (2009). Organizational Citizenship Behaviours in Relation to Job Embeddedness, Organizational Identification, Job Performance, Voluntary Turnover Intention in Korea. International business and economic research journal 8, no 7.

Table.1: Levels of Job Embeddedness

Factor	N	Mean	Level
Fit to community	50	3.92	Medium
Fit to Organization	50	3.94	Medium
Link to community	50	4.19	High
Link to Organization	50	3.82	Medium
Sacrifice to community	50	3.86	Medium
Sacrifice to Organization	50	3.28	Medium
Overall mean of the study	50	3.83	Medium

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RESEARCH ARTICLE

Design of Repetitive Group Sampling (RGS) Plan using Fuzzy Parameter

P.Kavi Priya[1]* and A.R.Sudamani Ramaswamy[2]

[1]Research Scholar, Department of Mathematics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, Tamil Nadu, India.
[2]Professor, Department of Mathematics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, Tamil Nadu, India.

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***Address for Correspondence**
P.Kavi Priya,
Research Scholar,
Department of Mathematics,
Avinashilingam Institute for Home Science and Higher Education for Women,
Coimbatore, Tamil Nadu, India.
Email: kaviarun88@gmail.com

ABSTRACT

This study deals with the concept of RGS Plan using fuzzy parameter. FOC values for RGS Plan are calculated. The design parameter of the sampling plan is determined by satisfying two risks at the specified quality levels. Then sum of the risks is also minimized for the given plan and optimum value of the sample size is obtained.

Keywords: Repetitive Group sampling plan, FOC curve, Fuzzy number, Trapezoidal fuzzy number

INTRODUCTION

A new acceptance sampling plan was introduced by Sherman in 1965 is called Repetitive Group Sampling Plan. It comes below special purpose sampling plans. RGS Plan is intermediate in sample size efficiency among single sampling plan and sequential probability ratio test plan. Soundarajan and Ramaswamy [12, 13] tabulated values for the selection of RGS plan indexed through (AQL, AOQL); (p_0,h_0);(p^*, h^*). Govindaraju [3] has shown that the OC function for RGS plan. Romboski [9] single sampling quick switching system and dependent stage sampling plan of Wortham and Mogg [17] are strikingly the same. Subramani [14] has studied the RGS Plan involving minimum sum of risks. Further Suresh [15] has constructed tables for designing RGS Plan based on the relative slopes at the points (p1, 1-α) and (p2, β) considering the filter and incentive effects for the selection of plans. Fuzzy acceptance sampling plans are developed by Kahraman and Kaya [5]. Turanoglu , Kaya and Kahraman [16] have studied OC curve using fuzzy parameters in acceptance sampling.

Basic definitions of fuzzy number, trapezoidal fuzzy number, operating procedure for RGS Plan and its flow chart are included in this work. Then FOC Curve or band values are intended using fuzzy number. The specified plan for the given $(\widetilde{AQL})$ and $(\widetilde{LQL})$ value is determined to satisfy the inequality conditions and also to minimize the risks. The results are presented in tables.

Definitions

Fuzzy Number (Zadeh [18] and Dubis & Prade [1]) : " Fuzzy set that are characterized on the arrangement of real numbers having the structure $\breve{E}$: R tends to [0,1] are known as fuzzy number. A fuzzy number $\breve{E}$ will be a fuzzy set in the real line that fulfills the state of both normal and convexity".

Trapezoidal fuzzy number (Zadeh [18] and Dubis & Prade [1]):"If trapezoidal fuzzy numbers (TrFNs) are $\breve{E}$ = (e_1, e_2 , e_3 , e_4) then its membership function is as follows"

$$\mu_{E(y)} = \begin{cases} 0 & , \quad otherwise \\ \frac{y-e_1}{e_2-e_1} & , \ e_1 \le y \le e_2 \\ 1 & , \quad e_2 \le y \le e_3 \\ \frac{e_4-y}{e_4-e_3} & , \ e_3 \le y \le e_4 \\ 0 & , \quad otherwise \end{cases} \quad \ldots\ldots\ldots (1)$$

"The interval of confidence of trapezoidal fuzzy number defined by γ cuts can be written as follows"

$$\breve{E}[\gamma] = [e_1 + (e_2 - e_1)\gamma,\ e_4 - (e_4 - e_3)\gamma] \quad \ldots\ldots\ldots (2)$$

Operating procedure of RGS plan

"According to Sherman [11] as

Step 1: Draw a random sample of size n from the lot.

Step 2: Count the number of defectives in the sample.

Step 3: If d <c_1 , accept the lot

Step 4: If d>c_2 , reject the lot

Step 5: If c_1< d≤ c_2 , repeat Steps 1and step 2."

Fuzzy Probability of Acceptance (FPA) and Fuzzy Proportion of defective (FPrD)

Probability of acceptance is calculated using binomial distribution. γ cut of trapezoidal fuzzy number is used to solve RGS plan such that $\breve{p}_s$ = ($s, e_2 + s, e_3 + s, e_4 + s$) Where $e_i = b_i - b_1$, i = 2,3,4 and $s \in [0,1 - e_4]$ $\breve{p}_s[\gamma]$ = $[s + e_2\gamma,\ e_4 + s - (e_4 - e_3)\gamma]$ and taking γ=0,1 then we get fuzzy interval of proportion defective $\breve{p}_s[\gamma] = [\breve{p}_s^{lb}, \breve{p}_s^{ub}]$ and interval value of fuzzy probability of acceptance $\breve{P}_{As}[\gamma] = [\breve{P}_{As}^{lb}, \breve{P}_{As}^{ub}]$

The Operating Characteristic function is given by

$$\breve{P}_{As} = L(\breve{P}) = \left[\frac{\breve{P}_{as}}{(\breve{P}_{rs}+\breve{P}_{as})}\right] \quad \ldots\ldots\ldots(3)$$

$$where\ \breve{P}_{as} = \sum_{i=0}^{c_1} \binom{n}{i} \breve{p}_s^{\,i}(1-\breve{p}_s)^{n-i}$$

$$and\ \breve{P}_{rs} = 1 - \sum_{i=0}^{c_2} \binom{n}{i} \breve{p}_s^{\,i}(1-\breve{p}_s)^{n-i}$$

$$L(\breve{P}) = \left[\frac{\sum_{i=0}^{c_1}\binom{n}{i}\breve{p}_s^{\,i}(1-\breve{p}_s)^{n-i}}{(1-\sum_{i=0}^{c_2}\binom{n}{i}\breve{p}_s^{\,i}(1-\breve{p}_s)^{n-i})+\sum_{i=0}^{c_1}\binom{n}{i}\breve{p}_s^{\,i}(1-\breve{p}_s)^{n-i}}\right] \quad \ldots\ldots\ldots(4)$$

$$\breve{P}_{As}^{lb} = min\left[\frac{\sum_{i=0}^{c_1}\binom{n}{i}\breve{p}_s^{\,i}(1-\breve{p}_s)^{n-i}}{(1-\sum_{i=0}^{c_2}\binom{n}{i}\breve{p}_s^{\,i}(1-\breve{p}_s)^{n-i})+\sum_{i=0}^{c_1}\binom{n}{i}\breve{p}_s^{\,i}(1-\breve{p}_s)^{n-i}}\right] \quad \ldots\ldots(5)$$

$$\breve{P}_{As}^{ub} = max\left[\frac{\sum_{i=0}^{c_1}\binom{n}{i}\breve{p}_s^{\,i}(1-\breve{p}_s)^{n-i}}{(1-\sum_{i=0}^{c_2}\binom{n}{i}\breve{p}_s^{\,i}(1-\breve{p}_s)^{n-i})+\sum_{i=0}^{c_1}\binom{n}{i}\breve{p}_s^{\,i}(1-\breve{p}_s)^{n-i}}\right] \quad \ldots\ldots(6)$$

Here in RGS plan where c_2 is greater than c_1 ; whereas c_1 is equal to c_2 means it represents ordinary SSP. One can observe that when the parameter 's' value is very small or nearer to zero then the acceptance value of fuzzy probability is approximately equal to unity.

Real life Application

A diary factory produces milk powder after production they packed neatly in packets. Then some samples of milk powder packets are inspected by supermarket manager using Repetitive Group Sampling Plan. Let us consider $\breve{p}_s$ = (0.005,0.006,0.007,0.008) as fuzzy number, sample size n= 20, acceptance numbers $c_1 = 0$ and $c_2 = 1$. Count the number of defective (d) is less than zero, and then accept the lot. If defective (d) is greater than one, then reject the lot. If defective is greater than zero and less than or equal to one then repeat the process otherwise inform the dairy factory to improve the packing quality of the milk powder.

Example 1

For the above real life application when γ cut of trapezoidal fuzzy number is used to get fuzzy proportion defective value as $\breve{p}_s[\gamma = 0]$ = [0.005 0.013] and $\breve{p}_s[\gamma = 1]$ = [0.011 0.012] and fuzzy probability of acceptance is calculated as $\breve{P}_{As}[\gamma = 0]$ = [0.9951 0.9655] and $\breve{P}_{As}[\gamma = 1]$ = [0.9755 0.9707] .

Fuzzy Operating characteristic (FOC) curve

OC Curve we have upper bound and lower bound therefore it is called as FOC curve. From the Figure1and Figure 2, when γ value increases from zero to one then FOC curve value becomes closer.

Fuzzy probability of acceptance when sample size varies

Let us assume that $\breve{p}_s$ = (0.002,0.003,0.004,0.005) and the sample size n varies from 5 to 50 then γ cut of trapezoidal fuzzy number is used to calculate FPrD. The interval is obtained as $\breve{p}_s[\gamma = 0]$ = [0.002 0.007] , $\breve{p}_s[\gamma = 1]$ = [0.005 0.006]. Table 2, reveals that when the sample size values decreases the width of FOC curve decreases.

Determination of sample size

"The risk of producer is denoted by $\widetilde{\alpha_f}$ and the risk of consumer is denoted by $\widetilde{\beta_f}$. Accepting quality level (AQL) is denoted as $\breve{p}_{1f}$ and Limiting quality level (LQL) is denoted as $\breve{p}_{2f}$. Here RGS plan is designed using the fuzzy parameter. The sample size n is calculated in order to satisfy both the inequalities for $\breve{P}_{1fa}$ and $\breve{P}_{2fa}$ simultaneously for the given values of $\widetilde{\alpha_f}$ and $\widetilde{\beta_f}$. $\breve{P}_{1fa} \geq 1 - \widetilde{\alpha_f}$ and $\breve{P}_{2fa} \leq \widetilde{\beta_f}$, $\widetilde{\alpha_f}$ = 0.05 and $\widetilde{\beta_f}$ = 0.10 is fixed so that the interval of fuzzy probability of acceptance satisfies the conditions $\breve{P}_{1fa} \geq 0.95$ and $\breve{P}_{2fa} \leq 0.10$ for different sample sizes".

$$\widetilde{P_{1fa}} = \left[\frac{\sum_{i=0}^{c_1}\binom{n}{i}\breve{p}_{1f}^{\,i}(1-\breve{p}_{1f})^{n-i}}{\left(1-\sum_{i=0}^{c_2}\binom{n}{i}\breve{p}_{1f}^{\,i}(1-\breve{p}_{1f})^{n-i}\right)+\sum_{i=0}^{c_1}\binom{n}{i}\breve{p}_{1f}^{\,i}(1-\breve{p}_{1f})^{n-i}}\right] \geq 0.95 \qquad \text{.....(7)}$$

$$\widetilde{P_{2fa}} = \left[\frac{\sum_{i=0}^{c_1}\binom{n}{i}\breve{p}_{2f}^{\,i}(1-\breve{p}_{2f})^{n-i}}{\left(1-\sum_{i=0}^{c_2}\binom{n}{i}\breve{p}_{2f}^{\,i}(1-\breve{p}_{2f})^{n-i}\right)+\sum_{i=0}^{c_1}\binom{n}{i}\breve{p}_{2f}^{\,i}(1-\breve{p}_{2f})^{n-i}}\right] \leq 0.10 \qquad \text{....(8)}$$

Minimizing the sum of risks

The mathematical expression to minimize the sum of risk is $\widetilde{\alpha_f} + \widetilde{\beta_f} = 1 - \breve{P}_{1fa} + \breve{P}_{2fa}$. The sum of risks is obtained as interval of fuzzy. The sample size is calculated so as to minimize the sum of the risks for Repetitive Group sampling plan and its values are displayed in Table 4.

$$\widetilde{\alpha_f} + \widetilde{\beta_f} = 1 - \breve{P}_{1fa} + \breve{P}_{2fa}$$

$$= 1 - \left[\frac{\sum_{i=0}^{c_1}\binom{n}{i}\breve{p}_{1f}^{\,i}(1-\breve{p}_{1f})^{n-i}}{\left(1-\sum_{i=0}^{c_2}\binom{n}{i}\breve{p}_{1f}^{\,i}(1-\breve{p}_{1f})^{n-i}\right)+\sum_{i=0}^{c_1}\binom{n}{i}\breve{p}_{1f}^{\,i}(1-\breve{p}_{1f})^{n-i}}\right] + \left[\frac{\sum_{i=0}^{c_1}\binom{n}{i}\breve{p}_{2f}^{\,i}(1-\breve{p}_{2f})^{n-i}}{\left(1-\sum_{i=0}^{c_2}\binom{n}{i}\breve{p}_{2f}^{\,i}(1-\breve{p}_{2f})^{n-i}\right)+\sum_{i=0}^{c_1}\binom{n}{i}\breve{p}_{2f}^{\,i}(1-\breve{p}_{2f})^{n-i}}\right]$$

CONCLUSION

RGS Plan is designed using fuzzy parameters. Binomial distribution is used for RGS Plan . The interval value of fuzzy proportion defective and the fuzzy probability of acceptance is calculated for trapezoidal fuzzy number. By fixing the quality levels, the optimum value of n is calculated such that inequality conditions are satisfied and simultaneously minimizing the sums of the risks.

REFERENCES

1. D. Dubis and H.Prade, "Operations of fuzzy Numbers", International Journal System Vol 9, pp 613-626, 1978.
2. A.J. Duncan, "Quality control and industrial statistics", Richard D.Irwin Inc, Homewood, IL, 1986.
3. K. Govindaraju , "An interesting observations in Acceptance Sampling", Economic Quality Control Journal, Vol.2, No.4, pp.89-92, 1987.
4. J. Jothikumar and C. Raju, "A table of Repetitive Group Sampling Plans", Economic Quality Control, Vol.13, No.4, pp.221-226, 1998 .
5. C. Kahraman and I. Kaya , "Fuzzy acceptance sampling plans, In C. Kahraman & M Yavuz, (Eds.), Production engineering and management under fuzziness pp 457-481 springer 2010.
6. V. Kuralmani, "Studies on Designing Minimum Inspection Attribute Acceptance Sampling Plans", Ph.D Thesis, Department of Statistics, Bharathiar University, Tamilnadu, India, 1992 .
7. D.C. Montgomery, "Introduction to statistical quality control", John wiley and sons, NewYork, N.Y. USA, 2004.
8. M.M. Ramasamy ,"Repetitive Group Sampling (RGS) plan"—A Review M.phil., Thesis, Bharathiar University, 1983.
9. L.D.Romboski,"An Investigation of Quick Switching Acceptance Sampling Systems",Ph.D Thesis, Rutgers- The State University, New Brunswick, New Jersey, 1969.
10. E.G. Schiling , " Acceptance sampling plan in Quality control", Marcel Dekker, Inc, New York, 1982.
11. R.E. Sherman,"Design and Evaluation of Repetitive group sampling plan , Technometrics, Vol 7, No 1, Feb 1965, pp 11 to 21. "
12. V. Soundararajan and M.M. Ramaswamy , "Designing of Repetitive group sampling (RGS) plan indexed by AQL and LQL", IAPQR Transactions, vol 9 issue No 1, pp 9-14, 1984.
13. V. Soundararajan and M.M. Ramaswamy M.M, "Procedures and tables for construction of Repetitive group sampling plan", the QR Journal vol 13 issue No 1, pp 19-21, 1986.
14. Subramani, "Studies on designing attribute acceptance Sampling plans with emphasis on chain sampling plans", Ph. D. Thesis, Department of Statistics, Bharathiar University, Coimbatore, TN, 1991.
15. Suresh, "Constructed tables for designing RGS Plan based on the relative slopes at the points (p1, 1-α) and (p2, β) has considering the filter and incentive effects for the selection of plans", 1993.
16. E. Turanoglu, C. Kahraman and I. Kaya "Fuzzy acceptance sampling and characteristic curves," International journal of computational intelligence systems, Vol 5 No 1, pp 13-29, 2012.
17. A.W. Wortham and J.M. Mogg, "Dependent stage sampling Inspection", The International Journal of Production Research , Vol8 issue 4, pp385-395, 1970.
18. L.A. Zadeh , Fuzzy sets, Information and control, 8, 338-353, 1965.

Flow chart for RGS plan

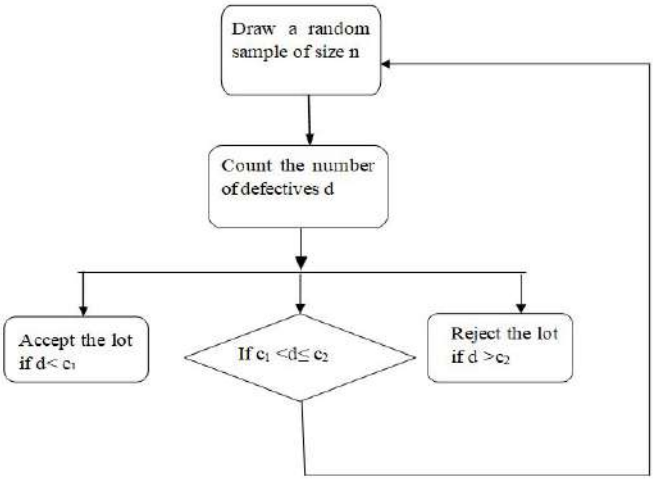

Table 1: Fuzzy probability of acceptance when n=20, c_1=0 and c_2 =1

$\breve{p}_s = (s, e_2 + s, e_3 + s, e_4 + s)$	$\breve{p}_s[\gamma = 0]$	$\breve{\breve{P}}_{As}[\gamma = 0]$	$\breve{p}_s[\gamma = 1]$	$\breve{\breve{P}}_{As}[\gamma = 1]$
(0.000, 0.001, 0.002, 0.003)	[0 .000 0.003]	[1.0000 0.9983]	[0.001 0.002]	[0.9998 0.9992]
(0.001, 0.002, 0.003, 0.004)	[0.001 0.005]	[0.9998 0.9951]	[0.003 0.004]	[0.9983 0.9969]
(0.002, 0.003, 0.004, 0.005)	[0.002 0.007]	[0.9992 0.9902]	[0.005 0.006]	[0.9951 0.9929]
(0.003, 0.004, 0.005, 0.006)	[0.003 0.009]	[0.9983 0.9837]	[0.007 0.008]	[0.9902 0.9872]
(0.004, 0.005, 0.006, 0.007)	[0.004 0.011]	[0.9969 0.9755]	[0.009 0.010]	[0.9837 0.9798]
(0.005, 0.006, 0.007, 0.008)	[0.005 0.013]	[0.9951 0.9655]	[0.011 0.012]	[0.9755 0.9707]
(0.006, 0.007, 0.008, 0.009)	[0.006 0.015]	[0.9929 0.9539]	[0.013 0.014]	[0.9655 0.9599]
(0.007, 0.008, 0.009, 0.01)	[0.007 0.017]	[0.9902 0.9406]	[0.015 0.016]	[0.9539 0.9474]
(0.008, 0.009, 0.01, 0.011)	[0.008 0.019]	[0.9872 0.9257]	[0.017 0.018]	[0.9406 0.9333]
(0.009, 0.010, 0.011, 0.012)	[0.009 0.021]	[0.9837 0.9093]	[0.019 0.020]	[0.9257 0.9177]
(0.010, 0.011, 0.012, 0.013)	[0.010 0.023]	[0.9798 0.8914]	[0.021 0.022]	[0.9093 0.9005]
(0.011, 0.012, 0.013, 0.014)	[0.011 0.025]	[0.9755 0.8723]	[0.023 0.024]	[0.8914 0.8820]
(0.012, 0.013, 0.014, 0.015)	[0.012 0.027]	[0.9707 0.8519]	[0.025 0.027]	[0.8723 0.8622]
(0.013, 0.014, 0.015, 0.016)	[0.013 0.029]	[0.9655 0.8305]	[0.027 0.028]	[0.8519 0.8413]
(0.014, 0.015, 0.016, 0.017)	[0.014 0.031]	[0.9599 0.8081]	[0.029 0.030]	[0.8305 0.8194]
(0.015, 0.016, 0.017, 0.018)	[0.015 0.033]	[0.9539 0.7850]	[0.031 0.032]	[0.8081 0.7966]
(0.016, 0.017, 0.018, 0.019)	[0.016 0.035]	[0.9474 0.7612]	[0.033 0.034]	[0.7850 0.7731]
(0.017, 0.018, 0.019, 0.02)	[0.017 0.037]	[0.9406 0.7368]	[0.035 0.036]	[0.7612 0.7491]
(0.018, 0.019, 0.02, 0.021)	[0.018 0.039]	[0.9333 0.7122]	[0.037 0.038]	[0.7368 0.7245]
(0.019, 0.02, 0.021, 0.022)	[0.019 0.041]	[0.9257 0.6873]	[0.039 0.040]	[0.7122 0.6997]

Table 2: Fuzzy probability of acceptance when sample size varies

n	$\check{P}_{As}[\gamma = 0]$	$\check{P}_{As}[\gamma = 1]$
50	[0.9988 0.9675]	[0.9885 0.9793]
45	[0.9990 0.9738]	[0.9907 0.9834]
40	[0.9992 0.9794]	[0.9927 0.9869]
35	[0.9994 0.9844]	[0.9945 0.9901]
30	[0.9996 0.9886]	[0.9960 0.9928]
25	[0.9997 0.9922]	[0.9972 0.9950]
20	[0.9998 0.9951]	[0.9983 0.9969]
15	[0.9999 0.9973]	[0.9990 0.9983]
10	[1.0000 0.9988]	[0.9996 0.9993]
5	[1.0000 0.9997]	[0.9999 0.9998]

Table 3: Optimum value of the parameter n, when $\check{P}_{1fa} \geq 0.95$ and $\check{P}_{2fa} \leq 0.10$

$(\widetilde{AQL})$	$(\widetilde{LQL})$	n
(0.001,0.0011,0.0012,0.0013)	(0.05,0.051,0.052,0.053)	103
(0.001,0.0011,0.0012,0.0013)	(0.06,0.061,0.062,0.063)	98
(0.001,0.0011,0.0012,0.0013)	(0.07.0.071,0.072,0.073)	93
(0.001,0.0011,0.0012,0.0013)	(0.08,0.081,0.082,0.083)	89
(0.001,0.0011,0.0012,0.0013)	(0.09,0.091,0.092,0.093)	85
(0.002,0.0021,0.0022,0.0023)	(0.05,0.051,0.052,0.053)	103
(0.002,0.0021,0.0022,0.0023)	(0.06,0.061,0.062,0.063)	100
(0.002,0.0021,0.0022,0.0023)	(0.07.0.071,0.072,0.073)	96
(0.002,0.0021,0.0022,0.0023)	(0.08,0.081,0.082,0.083)	90
(0.002,0.0021,0.0022,0.0023)	(0.09,0.091,0.092,0.093)	84
(0.003,0.0031,0.0032,0.0033)	(0.05,0.051,0.052,0.053)	93
(0.003,0.0031,0.0032,0.0033)	(0.06,0.061,0.062,0.063)	90
(0.003,0.0031,0.0032,0.0033)	(0.07.0.071,0.072,0.073)	88
(0.003,0.0031,0.0032,0.0033)	(0.08,0.081,0.082,0.083)	86
(0.003,0.0031,0.0032,0.0033)	(0.09,0.091,0.092,0.093)	84
(0.004,0.0041,0.0042,0.0043)	(0.06,0.061,0.062,0.063)	71
(0.004,0.0041,0.0042,0.0043)	(0.07.0.071,0.072,0.073)	69
(0.004,0.0041,0.0042,0.0043)	(0.08,0.081,0.082,0.083)	66
(0.004,0.0041,0.0042,0.0043)	(0.09,0.091,0.092,0.093)	63
(0.005,0.0051,0.0052,0.0053)	(0.08,0.081,0.082,0.083)	58
(0.005,0.0051,0.0052,0.0053)	(0.09,0.091,0.092,0.093)	53

Table 4: Optimum value of the parameter n which minimizes sum of risks $When\ \widetilde{\alpha_f} \cong 0.05\ and\ \widetilde{\beta_f} \cong 0.10$

n	$\check{p}_{1f}$ [γ = 0]	$\check{P}_{1fa}$[γ = 0]	$\check{p}_{2f}$ [γ = 0]	$\check{P}_{2fa}$[γ = 0]	$\widetilde{\alpha_f}$ + $\widetilde{\beta_f}$
103	[0.001 0.0013]	[0.9946 0.9908]	[0.05 0.053]	[0.0052 0.0037]	[0.0106 0.0129]
98	[0.001 0.0013]	[0.9951 0.9917]	[0.06 0.063]	[0.0024 0.0017]	[0.0073 0.0100]
93	[0.001 0.0013]	[0.9956 0.9925]	[0.07 0.073]	[0.0012 0.0009]	[0.0056 0.0084]
89	[0.001 0.0013]	[0.9960 0.9932]	[0.08 0.083]	[0.0006 0.0004]	[0.0046 0.0072]
85	[0.001 0.0013]	[0.9963 0.9938]	[0.09 0.093]	[0.0003 0.0002]	[0.0067 0.0064]
103	[0.002 0.0023]	[0.9779 0.9707]	[0.05 0.053]	[0.0052 0.0037]	[0.0273 0.0330]
100	[0.002 0.0023]	[0.9792 0.9724]	[0.06 0.063]	[0.0021 0.0015]	[0.0229 0.0291]
96	[0.002 0.0023]	[0.9809 0.9746]	[0.07 0.073]	[0.0009 0.0007]	[0.0200 0.0261]
90	[0.002 0.0023]	[0.9832 0.9777]	[0.08 0.083]	[0.0006 0.0004]	[0.0174 0.0227]
84	[0.002 0.0013]	[0.9854 0.9806]	[0.09 0.093]	[0.0004 0.0003]	[0.0150 0.0197]
93	[0.003 0.0033]	[0.9592 0.9506]	[0.05 0.053]	[0.0088 0.0065]	[0.0496 0.0559]
90	[0.003 0.0033]	[0.9618 0.9537]	[0.06 0.063]	[0.0039 0.0029]	[0.0421 0.0492]
88	[0.003 0.0033]	[0.9635 0.9558]	[0.07 0.073]	[0.0017 0.0013]	[0.0382 0.0455]
86	[0.003 0.0033]	[0.9652 0.9578]	[0.08 0.083]	[0.0008 0.0006]	[0.0356 0.0428]
84	[0.003 0.0033]	[0.9668 0.9598]	[0.09 0.093]	[0.0004 0.0003]	[0.0336 0.0405]
71	[0.004 0.0043]	[0.9578 0.9512]	[0.06 0.063]	[0.0131 0.0103]	[0.0553 0.0591]
69	[0.004 0.0043]	[0.9602 0.9539]	[0.07 0.073]	[0.0069 0.0055]	[0.0467 0.0516]
66	[0.004 0.0043]	[0.9636 0.9579]	[0.08 0.083]	[0.0042 0.0033]	[0.0406 0.0454]
63	[0.004 0.0043]	[0.9669 0.9617]	[0.09 0.093]	[0.0027 0.0022]	[0.0358 0.0405]
58	[0.005 0.0053]	[0.9561 0.9506]	[0.08 0.083]	[0.0083 0.0068]	[0.0522 0.0562]
53	[0.005 0.0053]	[0.9634 0.9589]	[0.09 0.093]	[0.0070 0.0058]	[0.0436 0.0469]

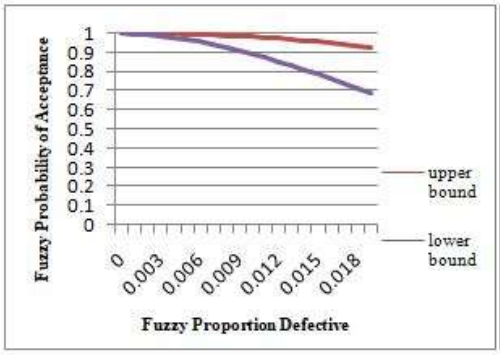

Fig. 1. FOC curve for RGS Plan when $\gamma = 0$

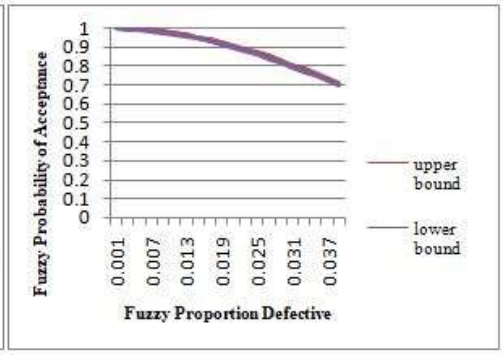

Fig. 2. FOC curve for RGS Plan when $\gamma = 1$

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RESEARCH ARTICLE

A Study on Efficacy of Conventional Mode Tens with Neural Tissue Mobilisation in Cheiralgia Paresthetica

S.Senthilkumar[1]*, S.Jeyakumar[1], Anjali Suresh[2] and Prasanna Mohan[3]

[1]Professor and Research Supervisor, School of Health Sciences, Department of Physiotherapy, Garden City University, Bangalore, Karnataka, India.
[2]HoD and Professor, School of Health Sciences, Department of Physiotherapy, Garden city University, Bangalore, Karnataka, India.
[3]Associate Professor, School of Health Sciences, Department of Physiotherapy, Garden city University, Bangalore, Karnataka, India.

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***Address for Correspondence**
S.Senthilkumar
Professor and Research Supervisor,
School of Health Sciences,
Department of Physiotherapy,
Garden City University,
Bangalore, Karnataka, India.
E.Mail: senthil.k@gardencity.university

ABSTRACT

Cheiralgia paraesthetica is a compression of superficial branch of radial nerve neuropathy of the hand. It will be affect from the posterior aspect of the hand at the base of the thumb and near the anatomical snuffbox. The Symptoms include Pain and numbness, tingling with the sensory radial nerve branch and there is no motor impairment. To prove the effect of transcutaneous electrical nerve stimulation with neural tissue mobilisation to relieve pain in cheiralgia parasthetica. The twenty convenient samples of subjects was taken from saveetha medical college and hospital, Chennai. The participants were n=20 and age range = 20 - 50 years. Subjects were then allocated in two groups. Group A (experimental group) Conventional mode TENS with Neural tissue mobilisation were given for 20 minutes continuously 1 week. Group B (control group) Conventional mode TENS with wrist extension exercises were given. Randomized clinical trial, Experimental design. NUMERICAL PAIN RATING SCALE .The analyzed data was tabulated and statistically updated. The Pain were calculated by compare the pre and post intervention, unpaired t test were used. The results were compared with two groups, two tailed P value is less than 0.0001 by considered to be extremely statistically significant from the experimental Group.

This study concluded that to prove the effectiveness of Conventional Mode Tens with neural tissue mobilisation was very effective treatment of chieralgia parasthetica.

Keywords: Conventional Mode TENS, Cheiralgia Parasthetica

INTRODUCTION

Cheiralgia paraesthetica is otherwise known as the Wartenberg's syndrome. The compression neuropathy of the hand and generally caused by trauma or any pressure over the superficial branch of the radial nerve. The posterior aspect of the hand at the base of the thumb, near the anatomical snuffbox most affected, but may extend up the back of the thumb and index finger and across the posterior of the hand. The symptoms of chieralgia includes numbness, tingling, burning sensation or pain occur in the nerve root and there is no motor impairment. The chieralgia was described in 1932 by Wartenberg, who suggested the name chieralgia paraesthetica and as known the entrapment of the superficial sensory branch of the radial nerve. There are different causes for chronic nerve entrapments have been described this syndrome, the most common cause is thought to be constriction of the wrist, as with a bracelet or watch band hence reference to "wristwatch neuropathy" and sometimes this condition is the use of handcuffs refers to as handcuff neuropathy. Patients complaints of dysesthesia on the dorsal radial forearm, radiating to the thumb and index finger. When sensory disturbances are associated with the posterior interosseous nerve innervated muscles, and consider alternative diagnosis, such as a more proximal lesion of the cervical spine and posterior cord of the brachial plexus. The radial nerve proper or perhaps a mass in the radial tunnel and to irritation of the sensory nerve root often occurs in the region of the first dorsal compartment, compression may be confused with the symptoms of de Quervain's disease owing to pain with ulnar deviation of the wrist. This study focus on conservative treated from this condition, especially Transcutaneous Electrical Nerve stimulation with neural mobilization was very effective treatment for relieve symptoms in cheiralgia Parasthetica.

AIM AND OBJECTIVE OF THE STUDY

To prove the Effectiveness of Conventional mode TENS with Neural tissue mobilization on pain in chieralgia parasthetica

SUBJECTS AND METHODS

A randomized clinical trial was designed which is divide into a control group and an experimental group. The convenient samples of 20 subjects was taken from Saveetha medical college hospital, Chennai in the year of 2018. Participants were n=20 and age range = 20 - 50 years. Each group had a physiotherapist who carried out all interventions. Group A (experimental group) Conventional mode TENS with Neural tissue Mobilisation were given. Group B (control group) Conventional Mode TENS with Wrist exercises were given.

Inclusion criteria

Patients with Cheiralgia parasthetica last three months, forearm fracture after removal of POP, tight wrist band, pronation twisting injury at the wrist, Posterior interosseous nerve neuropathy.

Exclusion criteria

Infections, tumor, crush injuries, mal united fracture, material insufficiency, deformities, surgery with metal implantation. All the patients were screened and randomized after finding their suitability as per inclusion and exclusion criteria. Numerical Pain Rating Scale (NPRS) was used to measure pain.

PROCEDURE

The control group received wrist exercises and hot packs for fifteen minutes. The Experimental group received Transcutaneous electrical nerve stimulation applied patient in comfortable sitting position hand placed over the

pillow supported. Skin preparation was done and coupling gel was used between the electrodes and skin contact. The electrodes were placed in the posterior aspect of the wrist conventional mode Tens include pulse width of 50 to 125microsecods, 50 to 100pps, frequency of 1 to 200 Hz. In the both groups treatment was given once per day for 10 days with outcome variables were measures prior to the treatment on day one and end of the treatment sessions at day ten.

Neural Mobilization: The patients were in a comfortable supine lying position. The physiotherapist maintained walk standing B position. The shoulder depression, elbow extended with arm internally rotated, wrist, thumb and fingers were flexed. These movements were stressed the radial nerve root, and then shoulder droop maintained with elbow flexion and wrist extension. The wrist and fingers were fixed prior to the elbow extension test that was performed gently, extending the elbow for approximately 2 seconds just into the range where the patient felt only the tension and then flexing the elbow. This is for 3 sets of 6 to 8 oscillations were performed.

RESULTS

The data was analysed and tabulated by using SPSS Package. The Pain were calculated and compare pre and post intervention, parametric statistical tests, dependent t sample test and t test were used.

DISCUSSION

The study was compared the results were used for the treatment strategies of patients with cheiralgia parasthetica. The Numerical pain rating scale was commonly measured in the treatment of cheiralgia parasthetica for the both groups. The findings obtained in this randomized controlled trial imply that transcutaneous electrical nerve stimulation with neural mobilization was effective intervention of Cheiralgia parasthetica. The present study showed that there was a significant improvement in the experimental group. For the outcome measures of NPRS showed significant statistical difference (p<0.05) between pre and post-test measurements.The results shows that the post intervention phase NPRS scale 95% confidential interval and t value is 20.44, standard error of difference 0.313.The two tailed p value was less than 0.0001 and this difference is considered to be extremely statistically significant in the experimental group. Chieralgia paraesthetica reported 2005 (Lanzettaetal) 74% success rate in 55 patients who underwent physiotherapy **.**

SUGGESTIONS AND LIMITATIONS

1. A small sample study
2. In future research EMG biofeedback used to analysed.
3. Other physiotherapy modalities are use in future research

CONCLUSION

The Watrenberg's described the compression neuropathy superficial branch of the radial nerve was implicated. It is the condition from the damage to the radial nerve can lead to disability, especially if the patient's dominant hand is involved. Radial nerve sensory branch characterized by movements of the arm and wrist will be decreased, and loss of sensations on the back of the arm, forearm, or hand. It is an excellent treatment like transcutaneous electrical nerve stimulation along with Neural tissue mobilization for decreasing pain and inflammation in muscles and tendons, especially the radial nerve at the wrist. The treatment of chieralgia parestheticastates that up to 71% of patients treated non- operatively have good to excellent outcomes.

Ethical Considerations

This study was approved by ethical committee of Saveetha Institute of Medical and technological sciences, Chennai at 2018.The study conform that there is no conflict of interest.

ACKNOWLEDGMENT

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REFERENCES

1. Abrams RA, Ziest RJ, Liebert RL, Botte MJ: Anatomy of the radial nerve motor branches in the forearm. J Hand Surg 22A:232-237, 1997
2. Berry PR, Wallis WE: Venipuncture nerve injuries. Lancet 1:1236-1237, 1977
3. Braidwood AS: Superficial radial neuropathy. J Bone Joint Surg 57:380-383, 1975
4. Dang AC, Rodner CM: Unusual compression neuropathies of the forearm, Part I: radial nerve. J Hand Surg Am 34A:1906-1914, 2009
5. Dee R: Principles of Orthopaedic Practice ed 2. New York: McGraw-Hill; 1989, pp125-130
6. Dellon AL, Mackinnon SE: Radial sensory nerve entrapment. Arch Neurol 43:833-835, 1986
7. Dellon AL, Mackinnon SE: Radial sensory nerve entrapment in the forearm. J Hand Surg Am 11A:199-205, 1986
8. Glanvill R, Boon JM, Birkholtz F, Meiring JH, van Schoor AN, Greyling L: Superficial radial nerve injury during standard K-wire fixation of uncomplicated distal radial fractures. Orthopedics 29:639-641, 2006
9. Hochwald NL, Levine R, Tornetta P 3rd: The risks of Kirschner wire placement in the distal radius: a comparison of techniques. J Hand Surg Am 22A:580-584, 1997
10. Horowitz SH: Peripheral nerve injury and causalgia secondary to routine venipuncture. Neurology 44:962-964, 1994.
11. Chodoroff G, Honet JC. Cheiralgiaparesthetica and linear atrophy as a complication of local steroid injection. Arch Phys Med Rehabil. 1985 Sep;66(9):637-9. [PubMed]
12. Patel A, Pierce P, Chiu DTW. A fascial band implicated in Wartenberg syndrome. PlastReconstr Surg. 2014 Mar;133(3):440e-442e. [PubMed]
13. Khalifeh A, Khashab T, Huffner M, Rezvani ZN, Kwan J, Toursavadkohi S. Radial neuropathy following arterial line removal: A rare complication from a routine ICU procedure. SAGE Open Med Case Rep. 2018;6:2050313X18760740. [PMC free article] [PubMed]
14. Dellon AL, Mackinnon SE. Radial sensory nerve entrapment. Arch Neurol. 1986 Aug;43(8):833-5. [PubMed]
15. Gaspar MP, Kane PM, Vosbikian MM, Ketonis C, Rekant MS. Neurolysis with Amniotic Membrane Nerve Wrapping for Treatment of Secondary Wartenberg Syndrome: A Preliminary Report. J Hand Surg Asian Pac Vol. 2017 Jun;22(2):222-228. [PubMed]
16. Zöch G, Aigner N. [Wartenberg syndrome: a rare or rarely diagnosed compression syndrome of the radial nerve?]. HandchirMikrochirPlastChir. 1997 May;29(3):139-43. [PubMed]
17. Tagliafico A, Cadoni A, Fisci E, Gennaro S, Molfetta L, Perez MM, Klauser AS, Martinoli C. Nerves of the hand beyond the carpal tunnel. SeminMusculoskeletRadiol. 2012 Apr;16(2):129-36. [PubMed]
18. Kleinert JM, Mehta S. Radial nerve entrapment. OrthopClin North Am. 1996 Apr;27(2):305-15. [PubMed]
19. Spies CK, Müller LP, Oppermann J, Neiss WF, Hahn P, Unglaub F. [Surgical decompression of the superficial radial nerve: Wartenberg syndrome]. Oper OrthopTraumatol. 2016 Apr;28(2):145-52. [PubMed]
20. Tosun N, Tuncay I, Akpinar F. Entrapment of the sensory branch of the radial nerve (Wartenberg's syndrome): an unusual cause. Tohoku J Exp Med. 2001 Mar;193(3):251-4. [PubMed]
21. Braidwood AS. Superficial radial neuropathy. J Bone Joint Surg Br. 1975 Aug;57(3):380-3. [PubMed]
22. Doughty CT, Bowley MP. Entrapment Neuropathies of the Upper Extremity. Med Clin North Am. 2019 Mar;103(2):357-370. [PubMed]

23. Knutsen EJ, Calfee RP. Uncommon upper extremity compression neuropathies. Hand Clin. 2013 Aug;29(3):443-53. [PubMed]
24. Popinchalk SP, Schaffer AA. Physical examination of upper extremity compressive neuropathies. OrthopClin North Am. 2012 Oct;43(4):417-30. [PubMed]
25. Jacobson JA, Fessell DP, Lobo Lda G, Yang LJ. Entrapment neuropathies I: upper limb (carpal tunnel excluded). SeminMusculoskeletRadiol. 2010 Nov;14(5):473-86. [PubMed]
26. Latef TJ, Bilal M, Vetter M, Iwanaga J, Oskouian RJ, Tubbs RS. Injury of the Radial Nerve in the Arm: A Review. Cureus. 2018 Feb 16;10(2):e2199. [PMC free article] [PubMed]

Table.1 Pre Intervention

No	STATISTICAL MEASUREMENT	EXPERIMENTAL GROUP	CONTROL GROUP
1.	Mean	8.3	8.5
2.	Standard Deviation	0.48	0.52

Table.2 Post Intervention

No	STATISTICAL MEASUREMENT	EXPERIMENTAL GROUP	CONTROL GROUP
1.	Mean	5.4	27.7
2.	Standard Deviation	1.42	3.46

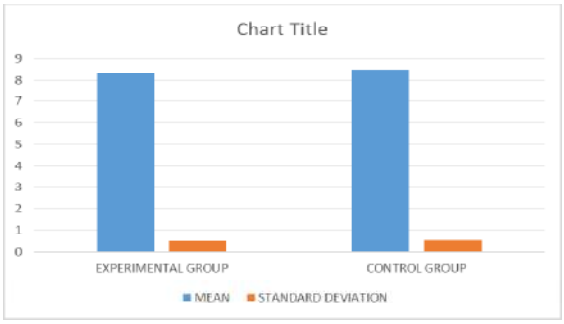

Fig.1. Pre Intervention

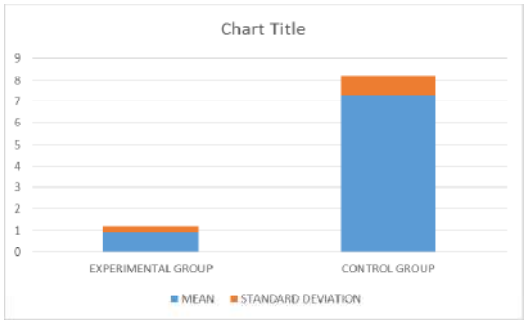

Fig.2. Post Intervention

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RESEARCH ARTICLE

To Study Additive Effect of task Oriented Exercise in Improving Hand Function in Subacute and Chronic Hemiparetic Patients

Krupa H Parekh[1]* and Karishma B Jagad[2]

[1]Assistant Professor, Shri K.K Sheth Physiotherapy College, Rajkot, Gujarat, India
[2]Senior Lecturer, Government Physiotherapy College, Jamnagar, Gujarat, India

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***Address for Correspondence**
Krupa H Parekh
Assistant Professor,
Shri K.K Sheth Physiotherapy College,
Rajkot, Gujarat, India
Email: krupaparekh25@gmail.com

ABSTRACT

The purpose of the study was to investigate the effects of task oriented exercise in improving hand function in patients with subacute and chronic hemiparetic patients.Method:24 hemiparetic patients were included in the study with age group above 40 years. The patients were randomly allocated to intervention group and control group. Pre and Post treatment measurement of hand function was done using Jebson Taylor Hand Function Test. There was significant improvement in the hand function in the task oriented exercise group compared to control group. Post exercise duration of Jebsen Taylor Hand Function Test showed significant reduction with P value <0.05 in the intervention group. Task oriented exercise reduced the amount of duration of task performance compared to the control group which implies improvement in hand function in subacute and chronic hemiparetic patients.

Keywords: Hemiplegia, Jebsen Taylor Hand Function Test, Task oriented exercise.

INTRODUCTION

Hemiplegia or Hemiparesis is characterized by paralysis or weakness, typically on the side of the body opposite the side of the lesion[1]. The term hemiplegia is often used generically to refer to the wide variety of motor problems that result from stroke[1]. Stroke is the sudden loss of neurological function caused by the interruption of the blood flow to the brain [1]. Cerebrovascular accident (CVA) is used interchangeably with stroke to refer to the vascular conditions of the brain [1]. Hemiplegia is the most obvious sign of CVA and major concern of therapist [2]. Other symptoms that are equally disabling include sensory dysfunction, aphasia or dysarthria, visual field defects and mental and intellectual impairment [2]. Stroke affects 15 million people in the world each year and approximately and one third will live with the sequel of this disease [3]. Owing to high incidence of MCA (middle cerebral artery) strokes, the upper extremity is frequently more affected than lower extremity. The rehabilitation of the affected arm

and hand remains a challenge [4]. Stroke rehabilitation is an organized endeavor to help patients to maximize all opportunities for returning to an active lifestyle [5,6]. Neuro-rehabilitation is a method for relearning a previously learned task in a different way either by compensatory strategy or by adaptively recruiting alternative pathway [7]. Based on newer theories of motor control one of the newer approach to retraining is the task-oriented approach. This approach argues that it is critical to recognize that movement emerges from an interaction between the individual, the task and the environment in which the task is being performed [8].The nature of the task being performed in part determines the type of movement needed [8]. In task related training, the practice of goal directed functional movement is carried out in natural environment [9]. Task related practice is advocated during stroke rehabilitation to improve functional performance of daily activities such as walking and reaching to grasp objects (Carr and Shepherd 2002, Sonoda 1999) [10]. A task oriented approach establishes an intervention strategies designed to achieve following goals – a) resolve, reduce, or prevent impairments b) develop effective and efficient task specific strategies. The goals are not approached sequentially, but rather concurrently. Intervention strategies are designed to focus on one or more of the goals within same therapy session [8]. The Jebsen Taylor Test (JTT) described in 1969 was taken as an outcome measure which evaluates grasping, holding and manipulating objects that are ADLs. The main advantage of Jebsen Taylor Test is it provide objective measure of hand functions, employing functionally relevant task with good intra and inter-rater reliability [11]. So, the present study is to find additive effect of task oriented exercise along with conventional treatment on hand function of subacute and chronic hemiparetic patients.

METHOD

A comparative interventional study was conducted in various physiotherapy centres at Rajkot after approval from the ethical committee at K.K.Sheth Physiotherapy College, Rajkot. A total of 26 hemiparetic patients were taken which were divided into interventional group and control group through convenient sampling technique. Inclusion criteria for the study were age> 40 years, both males and females, post stroke duration – from 3 months to 12 months, must be diagnosed as having suffered first stroke, having some upper extremity mobility, no severe spasticity (Modified Ashworth Scale < 2) or tremor on the affected upper extremity and patient should understand instruction [4,12,13]. Exclusion criteria were patient having any orthopedic problem like fracture of upper limb, patient having any hemispatial neglect, attention and memory deficit, patient having any neurological deficit related to metastatic disease and patient having history of recurrent stroke were excluded from the study [12].

Procedure

Basic demographic data regarding age, gender, occupation, tone, etc was taken prior to treatment planning. Before initiation of the treatment, all the patients were explained about the treatment procedure. Out of 26 patients, 2 patients discontinued the treatment due to personal reasons. So total 24 patients continued the treatment for 40 minutes per day, for 5 days in a week for 4 weeks. Pre treatment measurement of hand function was done using Jebsen Taylor Test (JTT).

Testing Procedure

Patient was made to seat on a chair with its height in accordance with table height such that elbow is kept at 90^{o} flexion with affected hand at the centre of the table. Then the patient was asked to do each activity with the command of 'go' and duration required to complete each activity was recorded by stop watch and noted and then summed up. If the object was dropped, the subject should pick it up and then continue. There were 6 subtest taken in the study, excluding writing subtest. For card turning – each of 5 cards of 3x5 inch size are placed on table 5 cm apart. For manipulating small objects – 5 small objects like paper clip, bottle cap, and coin were taken and placed on table 5 cm apart. One empty tin can was kept at the centre in which all the objects were kept one by one and duration of it was noted. For feeding using teaspoon and 5 kidney beans, for stacking checkers, for picking up large light objects (11cm height x 7.5cm diameter), for picking up large heavy objects (425 gm) activity was performed similar to those performed in manipulating small objects [14].

Intervention Group

Patients in experimental group were given task oriented exercise. The task oriented exercise includes –drinking, moving glass out, combing, moving tray, reaching straight out and presses buzzer, reaching-grasping-transfer and release of bottles of different size and weight, turning 3x5 inch cards, picking up small objects like paper clip, coin, bottle cap. Stacking checkers, picking up kidney beans with spoon, drawing an arc. Patients in control group were given active and passive range of motion exercises, strengthening and stretching exercises were given. Strengthening exercise includes repetitions against resistance using free weights, theraband strips, and gripping exercise for fingers. Post measurement of hand function was taken by 'Jebsen Taylor Hand Function Test' (JTT). Jebsen test was done only on the affected side and not for both dominant and non dominant hand. After recording pre and post treatment JTT duration they were compared using statistical analysis.

Statistical Analysis

All Statistical analysis was done by software SPSS 20.0 version & Microsoft Office Excel 2007 version. Mean was found as a measure of central tendency. Paired t-test was used for within group analysis and Unpaired t-test was used for between group analysis.

RESULTS

26 hemiparetic patients were taken for the study. From this study, on comparison of JTT mean of pre intervention in control and experimental group was found to be 202.91 and 200.66 respectively whereas mean of post intervention was found to be196.25 and 192.25 respectively. As shown in Graph 1.

DISCUSSION

The study compared the additive effect of 4 week of task oriented training in intervention group versus conventional treatment in control group in improving hand function in sub acute and chronic hemiparetic patients. The study revealed that in control group pre-exercise and post-exercise duration of Jebsen Taylor Hand function Test was 202.91 $\pm$ 8.67 (SD) and 196.25 $\pm$ 8.38 (SD) respectively. However, in the intervention group the pre and post exercise duration was 200.66 $\pm$ 6.70 (SD) and 192.25 $\pm$ 6.06 (SD) respectively. Thus, the mean duration difference between the control and experimental group through unpaired t-test showed $t = 3.46$ which has P value < 0.05. Consequently, the experimental group showed more recovery than control group. The result of the present study is supported by the study of Jannette Blennerhassett and Wayne Dite [15]. Larger gains observed in upper limb group which received task oriented exercise were due to the type of additional practice received during therapy. The enhanced upper limb performance achieved by extra practice has potential benefit for arm use in daily life. Upper limb group could perform hand dexterity test 6.5 seconds faster. Thus, the above study provided the support for use of task related training [15].Task related training strategies provide opportunity to drive neural reorganization and optimize functional effectiveness. Reorganization includes dendritic and axonal sprouting, change in sensitivity of certain sites to neurotransmitters. Concurrent with brain changes, muscles and other soft tissues also reorganize and adapt according to the patterns of use [11].

Lee and Van Donkelaar and Sung Ho Jang pointed out that recovery after task oriented exercise must be due to other mechanism called 'plasticity' which include unmasking of pathways previously functionally inactive, sprouting of fibers from surviving nerve cells with formation of new synapse and increased cortical activation in primary sensori-motor cortex and secondary motor areas such as supplementary motor area and pre-motor cortex [11,16]. In contrast, G Kwakkel et al., (2002) in his study to determine effect of intensity of training in stroke patients who participated in upper limb and lower limb task oriented therapeutic approach found no significant difference for treatment given 6 months onwards[17]. Statistical analysis of the study showed that both the group showed better improvement in hand function after having physiotherapy treatment for 4 week. Analysis done using unpaired t-test for comparison between control and experimental group showed there is difference in hand function between the two groups.

Experimental group which underwent task oriented exercise for 4 week showed greater improvement in hand function compared to control group which underwent only conventional therapy. The greater improvement in experimental group might be because the task oriented exercise uses the exercises that are similar to the activities of daily living that individual perform so they can easily get over to that activity after short duration of treatment, whereas conventional therapy does not use exercise done in natural environment so it requires greater amount of time for recovery. The other possible reason may be that brain is capable of undergoing reorganization and that reorganization in turn depends on the pattern of use. In task oriented exercise, task similar to that of activities of daily living are performed which leads to rapid similar reorganization processes as practiced during exercise. Also there may be increase in cortical activity in that area of brain.

Limitations

The limitations of the present study were it has limited sample size. Moreover, the age group was very wide from 40 years and above which may bring about minor changes in the results. Apart from that, the duration of the study was short of only 4 weeks and drop out of patients was of 7.69% and this may cause bias in the outcome of the study.

Further Recommendations

The present study can be carried out in patient with severe arm impairment or those who have difficulty in active movement. Secondly, more than two approaches can be compared as well as study can be done using large sample size and can compare difference in amount of recovery between acute, sub acute and chronic patients. Other outcome measures that measures arm impairment can also be taken.

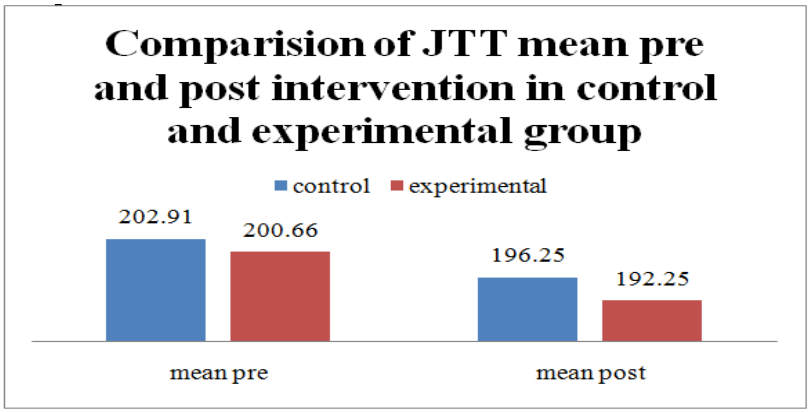
CONCLUSION

From the above study it can be concluded that, both control and experimental group showed improvement in hand function after undergoing treatment. However, when compared with each other there was significant difference between two treatment approaches and that experimental group showed better results post intervention. Thus, task oriented exercise proved to be better in improving hand function in sub acute and chronic hemiparetic patients within 4 week duration.

REFERENCES

1. O'Sullivan SB, Schmitz TJ. Physical Rehabilitation. 5th edition. New Delhi: Jaypee Brothers.
2. Darcy Umphred A. Neurological Rehabilitation. 5th edition. Philadelphia: Elsevier Publication; 2007.
3. World Health Organization.Global Burden OfStroke.In the atlas of heart disease and stroke. Accessed February 2005 from http://www.who.int/cardiovascular _diseases/en/cvd_atlas15_burden_stroke.pdf2.
4. Johanne Higgins, Nancy M. Salbach, Sharon Wood-Dauphinee, Carol L. Richards, Robert Cote, Nancy E Mayo. The effect of a task-oriented intervention on arm function in people with stroke: a randomized controlled trial. Clinical Rehabilitation. 2006; 20:296-310.
5. Aichner F, Adelwohrer C, and Haring H.P. Rehabilitation approaches to stroke. Journal of Neural Transmission. Supplementum 63, 59-73.
6. Gresham G.E., Alexander D., Bishop D.S., Giuliani C., Goldberg G., Holland A., Kelly Hayes., Linn R.T., Roth E.J.., Statson W.B. and Trombly C.A. Prevention and rehabilitation of stroke. American Heart Association Prevention Conference. IV. 1997; 28(7):1522-1526.
7. Matthews P.M., Johansen-Berg, H. and Reddy H. Non-invasive mapping of brain functions and brain recovery: applying lessons from cognitive neuroscience to neurorehabilitation. Restorative Neurology and Neuroscience. 2004; 22(3-5):245-260.
8. Anne Shumway Cook, Marjorie Woollacott. Motor Control – Translating Research Into Clinical Practice. 3rd edition.

9. Ada L, Canning CG, Carr JH, Kilbreath SL, Shepherd RB. Task specific training of reaching and manipulation. In: Bennet KM, Casteillo U, editors. Insights into the reach to grasp movement. Amsterdam: Elsevier; 1994; 239-264.
10. Jannette Blennerhassett and Wayne Dite. Additional task-related practice improves mobility and upper limb function early after stroke: A randomized controlled trial. Australian Journal of Physiotherapy. 2004; 50:219-224.
11. Karina N. Ferreiro, Renata L. dos Santos, Adriana B. Confronto. Psychometric properties of the Portuguese version of the Jebsen-Taylor Test for adults with mild hemiparesis. Rev Bras Fisioter. 2009.
12. Sung Ho Jang, Yun-Hee Kim, Sang-Hyun Cho, Jin-Hee Lee, Ji-Won Park and Yong-Hyun Kwon. Cortical reorganization induced by task-oriented training in chronic hemiplegic stroke patients. Lippincott Williams & Wilkins. 2003; 14:137-141.
13. Gergory T. Thielman, Catherine M. Dean, A.M. Gentile. Rehabilitation of Reaching After Stroke: Task-Related Training Versus Progressive Resistive Exercise. Arch Phys Med Rehabil. 2004; Vol 85: (1613-1618).
14. Patricia Agnew, Frederick Maas. An interim Australian version of the jebsen test of hand function. The Australian Journal Of Physiotherapy. 1982; 28 (2): 23 – 29.
15. Dean CM, Carol L. Richards, Francine Malouin. Task related circuit training improves performance of locomotor tasks in chronic stroke: A randomized, controlled pilot trial. Arch Phys Med Rehabil. 2000; 81:409-407.
16. Gowland C, deBruin H, Basmajian J, et al. Agonist and antagonist activity during voluntary upper limb movement in patients with stroke. Phys Ther. 1992; 72:624-633.
17. Shanta Pandian, Kamal Narayan Arya, E. W. Rajkumar Davidson. Comparison of Brunnstrom movement therapy and motor relearning program in rehabilitation of post-stroke hemiparetic hand: A randomized trial. Journal of Bodywork and Movement Therapies. 2012; 16(3):330-337.



Graph 1: The above graph shows Mean of JTT pre and post intervention between control and experimental groups.

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RESEARCH ARTICLE

In silico Design, Synthesis, Characterisation and Biological Evaluation of 1,3- Benzothiazole Derivatives

Midhula C.C[1], Anilkumar.V[2], Amrutha P.G[2], Cyril Mathews Jacob[2], Shadiya. C.K[3] and Akash Marathakam[4]*

[1]M.Pharm Student, Department of Pharmaceutical Chemistry, National College of Pharmacy, Kozhikode, Kerala, India
[2]Assistant Professor, Department of Pharmaceutical Chemistry, National College of Pharmacy, Kozhikode, Kerala, India
[3]Associate Professor, Department of Pharmaceutical Chemistry, National College of Pharmacy, Kozhikode, Kerala, India
[4]Professor and Head, Department of Pharmaceutical Chemistry, National College of Pharmacy, Kozhikode, Kerala, India

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***Address for Correspondence**
Akash Marathakam
Professor and Head,
Department of Pharmaceutical Chemistry,
National College of Pharmacy,
Kozhikode, Kerala, India.
Email: akashmarathakam@gmail.com

ABSTRACT

Benzothiazole core fills in as extraordinary and flexible frameworks in heterocyclic science because of its rising significance in drug revelation. Many strategies are accessible for orchestrating the benzothiazole core and its subordinates. Among these 2-amino benzothiazole have been orchestrated by involving aniline and ammonium thiocyanate in presence of a corrosive. Further they go through Schotten-Baumann response. Among the orchestrated mixtures, ABT2 show critical anticancer action situated in-vitro cytotoxicity concentrate on DLA cell lines. ABT2 show conspicuous calming action on protease restraint measure and the compound ABT5 display most prominent cell reinforcement action by nitric oxide searching examine. The current review shows that the orchestrated mixtures have expansive range in-vitro natural exercises, for example, anticancer, calming and cancer prevention agent movement. Further examinations on these mixtures might change these subordinates in to a clever class of anticancer, mitigating and cell reinforcement specialists.

Keywords: Benzothiazole; Anticancer; Anti-Inflammatory; Antioxidant activity; Molecular docking.

INTRODUCTION

The beginning and headways of restorative science and medication revelation are entwined in nature [1]. Restorative science is a specific science which manages an expansive scope of disciplines worried about the revelation, plan, and improvement of medication like mixtures for helpful use, in light of sub-atomic cooperations as far as atomic designs or its physicochemical properties included. Additionally, it incorporates disengagement, portrayal, and substance combination of novel mixtures that can be utilized in medication for the counteraction, treatment, and fix of sickness [2]. It worries with an exhaustive comprehension of medication component of activity, SAR, physicochemical properties, ADMET profiles. These days the methodology has been defended in numerous ways. Drug revelation and improvement is a cycle by which new medications are found or planned with healing advantage. Developing and arising another medication is exceptionally costly and tedious cycle that go on through a few phases from target ID to lead revelation and streamlining, preclinical approval and clinical preliminaries. It is a coordinated creating discipline which means a time of the custom-made drug [3]. Drug revelation process have been zeroing in on profoundly powerful leads that cooperate with single targets, delivering quantifiable results. When a lead substance with a helpful organic impact has been uncovered, the restorative scientist should embrace a progression of primary changes to decide an example of underlying movement connections prompting an upgrading of the valuable natural impact. New innovations for orchestrating a clever medication against a chose focus for a particular illness typically includes combinatorial science (combichem), microwave helped natural blend (MAOS) and high-throughput (HTS) organic screening strategies. Finding a particular medication targets has become step by step a huge strategy with the improvement in the field of genomics and proteomics.

The significant missions to accomplish in the medication disclosure process are:-
I) Identification of lead particles utilizing new advances, for example, HTS and combinatorial science which have the ideal natural action.
ii) The lead adjustment or improvement should be possible by utilizing structure-movement examination (SAR).

MATERIALS AND METHODS

All the synthetics and reagents utilized in this examination work were of scientific or manufactured grade. Compounds acquired were appropriately purged and dried utilizing standard circumstances before use, any place important. Synthetics and reagents are Aniline, Ammonium thiocyanate, Ethanol, Conc. Hydrochloric corrosive, Bromine water, Methanol, Petroleum ether, Benzoyl chloride, 4-Nitrobenzoyl chloride, 4-Flurobenzoyl choride, Propionyl chloride, 4-Chloro - benzoyl chloride. Sub-atomic docking done by utilizing programming projects like ACD Chemsketch11.0, Schrodinger programming and Molinspiration. Liquefying focuses were estimated with a lab dissolving point device. Additionally we have utilized Magnetic stirrer, Hot air stove, and Electric water shower. The NMR spectra were recorded utilizing a JEOL 400YH spectrometer working at 400 MHz for 1H, 100 MHz for 13C in DMSO. HRMS of mixtures were acquired on Agilent 6545 Q-TOF LC/MS instrument. Likewise we have utilized ATR spectrophotometer.

***In Silico* Drug Design**

Atomic docking studies were acted in Maestro 11.1 utilizing float (Schrodinger, LLC, New York, NY, 2018). This is an intelligent sub-atomic illustrations program from docking estimations, for recognizable proof of the plausible restricting site of the biomolecules, and for imagining ligand-receptor connections. All mixtures were construct utilizing Maestro assemble sheet and enhanced to bring down energy conformity utilizing Ligprep which utilizes an OPLS_2005. The direction for chemical (PDB: ID) were taken from RCSB Protein Data Bank and ready for docking involving 'protein arrangement wizard' in Maestro 11.1. Water particles in the construction were eliminated and ends were covered by adding ACE and NMA buildup. The bond requests and formal charges were added for hetero gatherings and hydrogens were added to all iotas in the strucuture side chain that are not near restricting depression and don't takes part in salt extensions were killed. After arrangement, the design was refined to enhance the

hydrogen bond network utilizing opls_2005 force field. The minimization was ended when the energy united or the RMSD arrived at a most extreme end of 0.30A0. Frameworks were then characterized around the refined construction by focusing on ligand utilizing default box size. The additional accuracy (XP) docking method of the relative multitude of mixtures was performed on a created lattice of protein structure. The last assessment of ligand protein restricting was finished with skim score (docking score) [51].

General procedures

Synthetic Procedure

Step 1: General method for the synthesis of 2-amino benzothiazole

0.05 mol of aniline and 0.05 mol of ammonium thiocyanate were dissolved in absolute ethanol containing 4 ml of conc. Hydrochloric acid. To this mixture bromine water (0.125mol) was added and the reaction mixture was refluxed for 1:30 hr. After the completion of the reaction, the reaction mixture was poured into crushed ice. The precipitate obtained was filtered, washed with cold water and dried. The crude product was recrystallized from ethanol [52].

Step 2: General method for the synthesis of 2-amino benzothiazole derivatives

Schotten- Baumann Reaction

In a 250 ml iodine flask, 0.25 g of 2-amino benzothiazole, 2.5 ml of 10% sodium hydroxide solution and added 0.4 ml of acid chloride derivatives, all at the same time. Corked the flask and shaked it vigorously for about 15 minutes until the components were completely dissolved. 5 ml of cold water was added to the flask. The crude product formed was filtered in a Buchner funnel with suction and washed with cold water. Drained and dried in hot air oven at 100 ^{0}C. The product was recrystallized from ethanol.

Synthesis of ABT1

In a 250 ml iodine flask, 0.25 g of 2-amino benzothiazole, 2.5 ml of 10% sodium hydroxide solution and added 0.4 ml of nitro benzoyl chloride, all at the same time. Corked the flask and shaked it vigorously for about 15 minutes until the components were completely dissolved. 5 ml of cold water was added to the flask. The crude product formed was filtered in a Buchner funnel with suction and washed with cold water. Drained and dried in hot air oven at 1000C. The product was recrystallized from ethanol.

Synthesis of ABT2

In a 250 ml iodine flask, 0.25 g of 2-amino benzothiazole, 2.5 ml of 10% sodium hydroxide solution and added 0.4 ml of 4-flurobenzoyl chloride, all at the same time. Corked the flask and shaked it vigorously for about 15 minutes until the components were completely dissolved. 5 ml of cold water was added to the flask. The crude product formed was filtered in a Buchner funnel with suction and washed with cold water. Drained and dried in hot air oven at 100^{0}C. The product was recrystallized from ethanol.

Synthesis of ABT3

In a 250 ml iodine flask, 0.25 g of 2-amino benzothiazole, 2.5 ml of 10% sodium hydroxide solution and added 0.4 ml of 4-chlorbenzoyl chloride, all at the same time. Corked the flask and shaked it vigorously for about 15 minutes until the components were completely dissolved. 5 ml of cold water was added to the flask. The crude product formed was filtered in a Buchner funnel with suction and washed with cold water. Drained and dried in hot air oven at 100 ^{0}C. The product was recrystallized from ethanol.

Synthesis of ABT4

In a 250 ml iodine flask, 0.25 g of 2-amino benzothiazole, 2.5 ml of 10% sodium hydroxide solution and added 0.4 ml benzoyl chloride, all at the same time. Corked the flask and shaked it vigorously for about 15 minutes until the components were completely dissolved. 5 ml of cold water was added to the flask. The crude product formed was filtered in a Buchner funnel with suction and washed with cold water. Drained and dried in hot air oven at 100 ^{0}C. The product was recrystallized from ethanol.

Synthesis of ABT5

In a 250 ml iodine flask, 0.25 g of 2-amino benzothiazole, 2.5 ml of 10% sodium hydroxide solution and added 0.4 ml of propionyl chloride, all at the same time. Corked the flask and shaked it vigorously for about 15 minutes until the components were completely dissolved. 5 ml of cold water was added to the flask. The crude product formed was filtered in a Buchner funnel with suction and washed with cold water. Drained and dried in hot air oven at 100 ^{0}C. The product was recrystallized from ethanol.

Method of characterization

IR spectra

The peak in IR spectrum gives an idea about the probable structure of the compound IR region ranges between 4000-500 cm^{-1}. Quanta of radiation from this region of the spectrum correspond to energy difference between different vibrational levels of molecules. The compounds were recorded using ATR ALPHA-E spectrometer.

1H NMR spectra

1H NMR of the synthesized compounds was recorded in DMSO-d_6 on Bruker's 400 MHz.
Chemical shifts were reported (ppm) relative to Tetra Methyl Silane (TMS) as internal standard.

MASS spectra

Mass spectra of the synthesized compounds were recorded using MASS spectrometry.

ABT1 Pale yellow powder; (Yield: 58%); Melting point: 2390C; IR Vmax/cm, (KBr): 2926 cm-1(Ar-CH str), 1518 cm-1 (Ar-C=C str), 3174 cm-1 (NH str), 1693 cm-1 (C=O str), 686 cm-1 (C-S str), 1286 cm-1 (C-N str), 1605 cm-1 (C=N str), 1346 cm-1 (Ar-NO2), 748 cm-1 (Ar-H); [Annexure 1] . 1H NMR ppm (400 MHz in DMSO-d_6) data; this synthesized compound complies with standard protocol of ABT1. [Table No. 7], [Annexure 6]; Mass: molecular ion peak at m/z: 300.043Da, Molecular formula: C14H9N3O3S; Molecular weight (g/mol): 299.31

ABT3 White color powder; (Yield: 55%); Melting point: 2510C; IR Vmax/cm, (KBr): 2988 cm^{-1}(Ar-CH str), 1528 cm-1 (Ar-C=C str), 3344 cm^{-1} (NH str), 1722 cm^{-1} (C=O), 642 cm^{-1} (C-S str), 1322 cm^{-1} (C-N str), 1653 cm^{-1} (C=N str), 792 cm^{-1} (C-Cl), 690 cm^{-1} (Ar-H); [Annexure 3] .1H NMR ppm (400 MHz in DMSO-d_6) data; this synthesized compound complies with standard protocol of ABT3. [Table No. 9], [Annexure 8]; Mass: molecular ion peak at m/z: 289.019Da $[M+H]^{+}$, Molecular formula: $C_{14}H_9ClN_2OS$; Molecular weight (g/mol): 288.76

ABT4 Whitish yellow powder (Yield: 72%); Melting point: 2080C; IR Vmax/cm, (KBr): 2978 cm^{-1}(Ar-CH str), 1526 cm^{-1} (Ar-C=C str), 3233 cm^{-1} (NH str), 1600 cm^{-1} (C=O), 604 cm^{-1} (C-S str), 1249 cm^{-1} (C-N str), 1560 cm^{-1} (C=N str), 746 cm^{-1} (Ar-H); [Annexure 4] . 1H NMR ppm (400 MHz in DMSO-d_6) data; this synthesized compound complies with standard protocol of ABT4. [Table No. 10], [Annexure 9] Mass: molecular ion peak at m/z: 255.058Da, Molecular formula: $C_{14}H_9ClN_2OS$; Molecular weight (g/mol): 254.31

ABT5 Light greyish powder; (Yield: 65%); Melting point: 1370C; IR Vmax/cm, (KBr):3165 cm^{-1}(Ar-CH str), 1510 cm^{-1} (Ar-C=C str), 3272 cm^{-1} (NH str), 1607 cm-1 (C=O), 632 cm^{-1} (C-S str), 1254cm^{-1} (C-N str), 2995 cm^{-1} (CH3 str), 1441 cm^{-1} (CH2 str), 743 cm^{-1} (Ar-H), [Annexure 5]; 1H NMR ppm (400 MHz in DMSO-d_6) data; this synthesized compound complies with standard protocol of ABT5. [Table No. 11], [Annexure 10] Mass: molecular ion peak at m/z: 207.058Da, Molecular formula: $C_{10}H_{10}N_2OS$; Molecular weight (g/mol): 206.27

Biological Activity

In-vitro cytotoxicity study

The in-vitro cytotoxicity of the analogues was screened using tryphan blue expansion method on Dalton's lymphoma as cites (DLA) cell lines. The tumor cells aspirated from the peritoneal cavity of tumor bearing mice were washed thrice with PBS or normal saline. Cell viability was determined by tryphan blue exclusion method. Viable cell suspension (1*106 cells in 0.1ml) was added to tubes containing various concentrations of the test compounds

and the volume was made up to 1ml using phosphate buffered saline (PBS). Control tube contained only cell suspension. These assay mixture were incubated for 3 hour at 370C. Further cell suspension was mixed with 0.1 ml of 1 % tryphan blue and kept for 2-3 minutes and loaded on a haemocytometer. Dead cells take up the blue color of tryphan blue while live cells do not take up the dye. The numbers of stained and unstained cells were counted separately.

$$\% \text{ Cytotoxicity} = \frac{\text{No. of dead cells X 100}}{\text{No. of live cells + No. of dead cells}}$$

Anti-inflammatory activity

Protease inhibition assay

100 ml of bovine albumin was added to 0.1 ml of sample. This was incubated at room temperature for 5 minutes. Reaction was inhibited by the addition of 0.25ml of tryps in followed by centrifugation. The supernatant was collected, and absorbance was observed at 210 nm. Naproxen was used as a control. The experiment was carried out and percent inhibition of protease inhibition was estimated [53, 54, 55].

% Inhibition=100– ((A1-A2)/A0)*100)
Where A1 = absorbance of the sample,
A2 = absorbance of the product control
A0 = absorbance of the positive control.

Anti-oxidant activity

Nitric oxide (NO) scavenging activity

NO scavenging activity of sample was determined by adding 400 µL of 100 mM sodium nitroprusside, 100 µL of PBS (pH - 7.4) and 100 µL of different concentration of sample. This reaction mixture was kept for incubation at 25°C for 150 minutes. To 0.5 mL of above solution, 0.5 mL of Griess reagent was added (0.1 mL of sulfanilic acid and 200 µL naphthyl ethylene diamine dichloride (0.1%) w/v)). This was kept on incubation at room temperature for 30 minutes, and finally absorbance is observed at 540 nm [56, 57]. The percentage inhibition was calculated by using the formula:

% Inhibition= ((A0-A1)/A0)*100
Where A0 = absorbance of the control
A1= absorbance of the sample.

RESULTS

***In silico* molecular modeling**

In silico molecular analysis of five different analogues of 2-amino benzothiazole has been done. None of the compound violate Lipinski rule of five. Compounds which were predicted to have optimal activity by the drug design software were selected for wet lab synthesis.

DISCUSSION

The present study involves synthesis of 2-amino benzothiazole derivatives and its evaluation of anti-cancer, anti-inflammatory and antioxidant activity. The premilinary *insilico* design of different analogues of 2-amino benzothiazole was performed. Docking studies of five different analogues were carried out against the cancer target 1XKK and also anti-inflammatory against 3NT1 target. Docking scores were tabulated.

The synthetic method involves two steps

In the first step, aniline reacts with ammonium thiocyanate in presence of anacidic environment. Bromine is used as a catalyst in this reaction to yield 2 aminobenzothiazole. In second step, 2-aminobenzothiazole reacts with acyl chloride inpresence of a base such as sodium hydroxide to yield N-(1, 3-benzothiazol-2-yl)-4-nitro benzamide, *N*-(1, 3-benzothiazol-2-yl)-4-fluorobenzamide, *N*-(1, 3-benzothiazol-2-yl) -4- chlorobenzamide,*N*-(1,3-benzothiazol-2-yl) benzamide,*N*-(1,3-benzothiazol-2-yl) propanmide. The structures of ABT1, ABT2, ABT3, ABT4, and ABT5, series of compounds were established by IR, ^{1}HNMR, and mass spectra. In- vitro cytotoxicity studies of synthesized compounds were carried out by tryphan blue dye exclusion method on DLA cell lines and the result revealed that all the test compounds had anticancer activity against DLA cell lines. It seems that ABT2containing fluro compound on 2-amino benzothiazole shows significant anti-cancer activity. The typhan blue exclusion method is used to establish the number of viable cells present in a cell suspension. The principle is based on the live cells possess whole cell membranes that exclude certain dyes, such as tryphan blue, whereas dead cells do not. A viable cell will have a clear cytoplasm whereas a nonviable cell will have a blue cytoplasm. In-vitro anti-inflammatory activity of synthesized compounds was carried out using protease inhibition assay. ABT2 containing a fluro group increases anti-inflammatory activity. Trypsin inhibiting assay is a method used to investigate the anti-inflammatory activity of synthesized compounds. Trypsin cleaves peptides on the C-terminal side of lysine and arginine amino acid residues. Bovine albumin was used as the substrate for trypsin. In-vitro antioxidant activity of synthesized compounds was carried out using Nitric oxide scavenging assay. Nitric oxide is produced from sodium nitroprusside at physiological pH. It results in the formation of nitrite ions which reacts with Griess' reagent to form a complex which can be measured by UV. All the compounds show antioxidant activity. Among them ABT5 containing electron withdrawing group (such as alkyl group) shows prominent antioxidant activity.

CONCLUSION

The present study was carried out for the synthesis of some effective therapeutic derivatives of 2-amino benzothiazole using novel synthetic schemes with expected biological activities such as cytotoxicity, anti-inflammatory activity, and anti-oxidant activity. The new synthetic schemes were designed according to the standard protocol. From these schemes a series of new benzothiazole derivatives were synthesized in moderate to good yield. The target compounds were carried out for molecular docking studies to know its molecular interactions towards the protein. The spectral studies were performed for all the synthesized compounds for their confirmation. The compounds were further subjected to IR,NMR and MASS study for confirmation of functional group, presence of total number of proton of compounds and molecular weight. Five synthesized compounds namely ABT1, ABT2, ABT3, ABT4, ABT5 shows prominent anti-cancer, anti-inflammatory, antioxidant activity. The present study showed that ABT2 containing fluro group shows enhanced cytotoxic activity. ABT1, ABT3, ABT5 shows moderate cytotoxic activity. The compound ABT2 shows moderate inflammatory activity and ABT5 shows significant antioxidant activity. 2-amino benzothiazole can be an important building block for the development of new drug applicant. The present study concludes that the synthesized compounds possess broad spectrum *in-vitro* biological activities such as anti-cancer, anti-inflammatory and antioxidant activity. Further studies on these compounds may transmute these derivatives into a novel class of anti-cancer, anti-inflammatory and antioxidant agents.

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REFERENCES

1. Faruk Khan MO, Deimling MJ, Philip A. Medicinal chemistry and the pharmacy curriculum. American Journal of Pharmaceutical Education. 2011Oct10;75(8):161
2. Hart T. Medicinal Chemistry-Progress through Innovation. DDW Drug DiscoveryWorld.2006;7(3):52.
3. Kalyani G, Sharma D, Vaishnav Y, Deshmukh VS. A review on drug designing, methods, its applications and prospects. International Journal of PharmaceuticalResearchandDevelopment.2013Jul;5(5):15-30.
4. PatilSA.RoleofMedicinalChemistintheModernDrugDiscoveryandDevelopment.OrganicChemistry:CurrentResearch.2012 Jan;1(3):1-2.
5. Ekins S, Mestres J, Testa B. *In silico* pharmacology for drug discovery: applications to targets and beyond. British journal of pharmacology. 2007 Sep1;152(1):21-37.
6. PugazhendhiD,UmamaheswariTS.InsilicoMethodsinDrugDiscovery-AReview.InternationalJournal.2013May;3(5):680-683
7. Gupta Praveen Kumar, Agrawal Pushpa, Shiva Kumar neeta, Hiremath S uhasini. *In silico* modelling and drug design- A Review. International Research Journal ofPharmacy.2011Sep12;2(9):15-17.
8. Rao VS, Srinivas K. Modern drug discovery process: an *In silico* approach. Journal of Bioinformatics and Sequence Analysis. 2011Jun30;3(5):89-94.
9. Maria Antony Dhivyan JE, Anoop MN. Virtual screening and lead optimisation to identify novel inhibitors forHDAC-8.2012Sep13:1-58.
10. Phoebe Chen YP, Chen F. Identifying targets for drug discovery using bioinformatics. Expert opinion on therapeutic targets.2008Apr1;12(4):383-9.
11. Chaudhary KK, Mishra N. A Review on Molecular Docking: Novel Tool for DrugDiscoverydatabases.2016Aug18;4(3):1-4.
12. Mukesh B, Rakesh K. Molecular docking: a review. International Journal of Research in Ayurved a and Pharmacy. 2011Dec 18;2(6):1746-1751.
13. Yadav PS, Devprakash D, Senthilkumar GP. Benzothiazole: different methods of synthesis and diverse biological activities.ChemInform.2011Oct4;42(40):1-7.
14. Hunasnalkar S G, Gazi S, Patil S M, Surwase U S. Synthesis and biological activities of some benzothiazole derivatives. Asian Journal of Research in Chemistry. 2010;3(2):421-7.
15. Jena JI. Significance of benzothiazole moiety in the field of cancer. International Journal of Pharmacy and Pharmaceutical Sciences. 2014;6(2):16-22.
16. Gill RK, Rawal RK, Bariwal J. Recent advances in the chemistry and biology of benzothiazoles. Archivder Pharmazie.2015Mar1;348(3):155-78.
17. RajuDN, DeviDS, AghastinaDG, RaoKG, KumarKS. Synthesis, Characterization and Analgesic Activity of somenovel Substituted 2-Amino Benzothiazole Derivatives. World Journal of Pharmacy and Pharmaceutical Science. 2015Mar15;4(5):1815-27.
18. Facchinetti V, da R Reis R, RB Gomes C, RA Vasconcelos T. Chemistry andbiological activities of 1, 3-benzothiazoles. Mini-Reviews in Organic Chemistry.2012Feb1;9(1):44-53.
19. March J, Smith MB. March's advanced organic chemistry: reactions, mechanisms, and structure. Molecules. 2001Jan1;6(12):1064-5.
20. Sharma HL, Sharma KK. Principles of Pharmacology. First edition, New Delhi,2007;862-863
21. Leelaprakash G, Dass SM. In vitro anti-inflammatory activity of methanol extract of *Enicostemma axillare*. International Journal of Drug Development and Research.2011Sep;3(3):186-196.
22. Hotamisligil GS. Inflammation, meta flammation and immunometabolic disorders. Nature. 2017 Feb;542(7640):177.
23. Pisoschi AM, Negulescu GP. Methods for total antioxidant activity determination :a review. Biochemistry and Analytical Biochemistry. 2011;1(1):106.
24. Moharram HA, Youssef MM. Methods for Determining the Antioxidant Activity: A Review. Alexandria Journalof Food Science and Technology. 2014June8;11(1):31-42.

25. Singh Y, Kaur B, Kaur A, Gupta VK, Gupta M. Synthesis, spectral studies and biological activity of 2, 3-disubstituted imidazo [2, 1-b] benzothiazole derivatives.IndianJournalofPharmaceuticalSciences.2018;6(1):1-8.
26. Yang ML, Zhang H, Wang WW, Wang XJ. Design, Synthesis, and Evaluation ofBisBenzothiazole Derivatives as DNA Minor Groove Binding Agents. Journal ofHeterocyclic Chemistry.2018Jan1;55(1):360-5.
27. Labib MB, Philoppes JN, Lamie PF, Ahmed ER. Azole-hydrazone derivatives: Design, synthesis, invitro biological evaluation, dual EGFR/HER2inhibitoryactivity, cell cycle analysis and molecular docking study as anticancer agents.Bioorganic chemistry.2018Feb28;76:67-80.
28. Uremis MM, Yaglioglu A S, Budak Y, Ceylan M. Synthesis, characterisation, invitro antiproliferative and cytotoxicity effects of a new class of 2-((1R, 2S)-2-((E)-4-substitutedstyryl) cyclooctyl)benzo [d]thiazole derivatives. Organic Communications. 2017Jul;10(3);190.
29. Belal A, Abdelgawad MA. New benzothiazole/benzoxazole-pyrazole hybrids withpotential as COX inhibitors: design, synthesis and anticancer activity evaluation. Research on Chemical Intermediates. 2017 Jul1;43(7):3859-72.
30. Osmaniye D, Levent S, Ardic CM, Atli O, Ozkay Y, Kaplancıkli ZA. Synthesis and anticancer activity of somenovel benzothiazole- thiazolidine derivatives. Phosphorus, Sulfur, and Silicon and the Related Elements.2017Nov14:1-8.
31. ShaikTB,HussainiSA,NayakVL,SucharithaML,MalikMS,KamalA.Rationaldesignandsynthesisof2-anilinopyridinyl-benzothiazole Schiff bases as antimitoticagents. Bioorganic & Medicinal Chemistry Letters. 2017 Jun 1;27(11):2549-58.
32. Eshkil F, Eshghi H, Saljooghi A S, Bakavoli M, Rahimizadeh M. Benzothiazolethiourea derivativesasanti cancer agents: Design, synthesis, and biological screening. RussianJournalofBioorganic Chemistry.2017Sep;43(5):576-82.
33. Dipesh K, Noolvi M N. Synthesis, characterization and evaluation of some benzothiazole derivatives bearing oxindole moiety as potential anticancer agents. International Journal of Pharmaceutical Chemistry and Analysis. 2016; 3(3):143-9.
34. Prabhu PP, Panneerselvam T, Shastry CS, Sivakumar A, Pande SS. Synthesis andanticancerevaluationof2-phenylthiaolidinonesubstituted2-phenylbenzothiazole-6-carboxylic acid derivatives. Journal of Saudi Chemical Society.2015Mar31;19(2):181-5.
35. Sathiya A, Dr. Sundara Pandian M, Dr. Palanivelu M, Sathya Devi S, Jothirani P,Nagarajan K. Synthesis and In-vitro Antioxidant, Anticancer Activity of Schiff's Bases andAzetidinone of 6-ethoxy 2-aminobenzothiazole derivatives. World Journal of Pharmacy and Pharmaceutical Sciences. 2018 March 18 7(4): 804-8013.
36. AbdelgawadMA, Lamie PF,Ahmed OM. Synthesis of New Quinolone Derivatives Linked to Benzothiazole or Benzoxazole Moieties as Anticancer andAnti-OxidantAgents.JournalofMedicinalChemistry.2016;6:652-7.
37. Batra C, Chawla HK. Synthesis, Characterization and Biological Evaluation ofQuinazolinone substituted Benzothiazole via Schiff Base for Antioxidant Activity.Global journal of pharmaceutical education and research. 2018 Jan 29;3(1-2):14-16.
38. Parle A, Amin S. Synthesis, Characterization and Antioxidant Activity of Novel2-ArylBenzothiazoleDerivatives.DerPharmaChemica.2018;10(1).
39. Kumari B, Reji F, Abbs TF. Antioxidant potential of novel 2-[2, 4-bis (arylamino)thiazol-5-oyl]benzothiazoles. International Journal of Pharmaceutical, Chemical&BiologicalSciences.2017Oct1;7(4):426-429
40. Cabrera-Perez LC, Padilla-Martinez II, Cruz A, Mendieta-Wejebe JE, Tamay-Cach F, Rosales-Hernández MC. Evaluation of a new benzothiazole derivativewith antioxidant activity in the initial phase of acetaminophen toxicity. ArabianJournalofChemistry.2016Feb17:1-12
41. Choudhary S, Kini SG, Mubeen M. Antioxidant activity of novel coumarin substituted benzothiazole derivatives. Der Pharma Chemica.2013;5(4):213-2.
42. KhoobiM,EmamiS,DehghanG,ForoumadiA,RamazaniA,ShafieeA.SynthesisandFreeRadicalScavengingActivityofCoumarinDerivativesContaining a 2-Methylbenzothiazoline Motif. Archiv der Pharmazie. 2011 Sep1;344(9):588-94.

43. Karalı N, Guzel O, Ozsoy N, Ozbey S, Salman A. Synthesis of new spiroindolinones incorporating abenzothiazolemoiety as antioxidant agents. European journal of medicinal chemistry.2010Mar31;45(3):1068-77.
44. TariqS,Alam O, Amir M. Synthesis, p38α MAP kinase inhibition,anti-inflammatory activity, and molecular docking studies of 1, 2,4-triazole-basedbenzothiazole-2-amines. Archivder Pharmazie. 2018Apr;351(3-4):1-15.
45. Patil V, Asrondkar A, Bhadane V, Bobade AS, Chowdhary A. Synthesis and Anti-inflammatory activity of 2-amino-6-methoxy benzothiazole derivative. Journal ofAppliedChemistry.2015Jan;8(1):1-2.
46. Mahtab R, Srivastava A, Gupta N, Kumar S, Kushwaha At. Synthesis of novel 2-benzylbenzo [d] thiazole-6-sulfonamide derivatives as potential anti-inflammatoryagent.JournalofChemicalandPharmaceuticalScience.2014Jan;7(1):34-8.
47. KharbandaC, AlamMS, HamidH, JavedK, BanoS,DhulapA, AliY,NazreenS, Haider S. Synthesis and evaluation of pyrazolines bearing benzothiazole asanti-inflammatoryagents.Bioorganic&medicinalchemistry.2014Nov1;22(21):5804-12.
48. Shafi S, Alam MM , Mulakayala N, Mulakayala C, Vanaja G, Kalle AM, Pallu R,Alam MS. Synthesis of novel 2-mercapto benzothiazole and 1, 2, 3-triazole basedbis-heterocycles: their anti-inflammatory and anti-nociceptive activities. European journal of medicinal chemistry.2012Mar1;49:324-33.
49. SatheBS,JagtapVA,DeshmukhSD,JainBV.Screeningofin-vitroanti-inflammatoryactivityofsomenewlysynthesizedfluorinatedbenzothiazoloimidazolecompounds.InternationalJournalofPharmacyPharmaceuticalSciences.2011;3(3):220-2.
50. Deshmukh VK, Raviprasad P, Kulkarni PA, Kuberkar SV. Design, synthesis andbiological activities of novel 4H-pyrimido [2, 1-b][1, 3] benzothiazole derivatives.InternationalJournalofChemTechResearch.2011;3:136-42.
51. SameerI.Shaikh, Zahid Zaheer, SantoshN. Mokale, DeepakK.Lokwani. Development of new pyrazolehy brid sasantitubercular agents: Synthesis, biologicale valuation and molecular docking study. International Journal of Pharmacy and Pharmaceutical Sciences.2017;9(11):50-56.
52. Lafta SJ. Synthesis and Characterization of Some New 2-AminobenzothiazoleDerivatives.Al-MustansiriyahJournalofScience.2012;23(5):105-18.
53. KeroJemal,Sandeep BV,Sudhakar Pola. *In vitro* anti-inflammatory and anti-microbial properties of AllophylusSerratus Leaf Extract. International Journal ofAdvancedResearch.March2017;5(3):71-80.
54. Reshma A K, Brindha P Invitro anti-inflammatory, anti oxidant and nephroprotective studies on leaves Aeglemarmeloes and Ocimum sanctum, Asian journal of Pharmaceutical and clinical research, June 2014;7(4):121-129
55. Patel SS, Zaveri MN. Trypsin and Protein Denaturation Inhibitory Activity of Different Fractionation and Isolated Compound of Leaf and Root of Justicia Gendarussa. International Journal of Pharmaceutical Sciences and Reasearch. Nov2014;5:5564-71.
56. Parul R, Kundu SK, Saha P. In vitro nitric oxide scavenging activity of methanolextracts of three Bangladeshi medicinal plants. The pharma innovation. 2013 Feb1;1(12,PartA):83-88.
57. Awah FM.Antioxidant activity, nitric oxide scavenging activity and phenolic contents of Ocimumgratis simum leaf extract. Journal of Medicinal Plants Research. 2010Dec4;4(23):2479-87.

Table. 1: List of Schotten – Baumann derivatives:

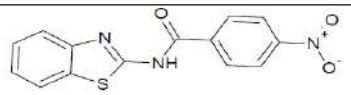
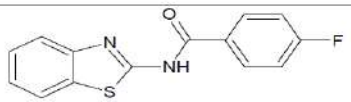
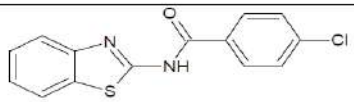
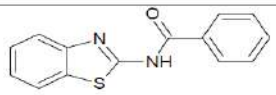
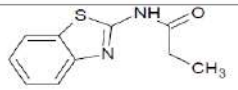
Compound code	Structure of the compound	IUPAC name of the compound
ABT1		*N*-(1,3-benzothiazol-2-yl)-4-nitrobenzamide
ABT2		*N*-(1,3-benzothiazol-2-yl)-4-fluorobenzamide
ABT3		*N*-(1,3-benzothiazol-2-yl)-4-chlorobenzamide
ABT4		*N*-(1,3-benzothiazol-2-yl)benzamide
ABT5		*N*-(1,3-benzothiazol-2-yl)propanamide

Table. 2: Molecular properties of proposed Schotten- Baumann derivatives

Compound code	Molar volume (cm $^{-1}$)	Parachor (cm^{-1})	Polarizability (10-24 cm^3)
ABT1	196.9 ± 3.0 cm3	583.5 ± 4.0 cm3	32.38 ± 0.5 10-24cm3
ABT2	189.3 ± 3.0 cm3	535.1 ± 4.0 cm3	29.78 ± 0.5 10-24cm3
ABT3	197.0 ± 3.0 cm3	563.9 ± 4.0 cm3	31.73 ± 0.5 10-24cm3
ABT4	185.1 ± 3.0 cm3	528.0 ± 4.0 cm3	29.79 ± 0.5 10-24cm3
ABT5	155.8 ± 3.0 cm3	431.9 ± 4.0 cm3	23.57 ± 0.5 10-24cm3

Table. 3: Lipinski rule analysis of proposed derivatives by Molinspiration

Compound code	milog P	MW	nON	nOHNH	N rotb	N violation
ABT1	3.34	299.31	6	1	3	0
ABT2	3.55	272.30	3	1	2	0
ABT3	4.06	288.76	3	1	2	0
ABT4	3.38	254.31	3	1	2	0
ABT5	2.55	206.27	3	1	2	0

Table. 4: Docking scores of proposed derivatives against EGFR Kinase.

Compound code	EGFR Kinase (PDB ID: 1XKK)
	Docking score
ABT1	-8.144
ABT2	-8.449
ABT3	-8.27
ABT4	-8.286
ABT5	-7.298

Table. 5 Docking scores of proposed derivatives against COX-2 receptor

Docking Code	COX 2 (PDB ID: 3NT1)
	Docking Score
ABT1	-6.116
ABT2	-6.802
ABT3	-6.458
ABT4	-6.928
ABT5	-7.36

Table. 6: In-vitro cytotoxicity of synthesized compounds against DLA cell lines

Compound code	Percentage cell death (concentration in μg/ ml)					IC50
	10 μg/ ml	20 μg/ ml	50 μg/ ml	100 μg/ ml	200 μg/ ml	
ABT1	5	12	22	28	38	259.5253
ABT2	4	10	25	58	78	113.7253
ABT3	6	14	24	30	42	232.7134
ABT4	3	7	12	20	32	315.4632
ABT5	1	3	6	17	27	356.7765

Table. 7: In-vitro anti-inflammatory activity of synthesized compounds

Compound code	IC50
ABT1	73.107
ABT2	33.807
ABT3	124.18
ABT4	302.74
ABT5	330.00
Indomethacin	11.57

Table. 8: Nitric oxide scavenging activity

Compound	Concentration (mcg/ml)	Absorbance	% inhibition	IC50
Control	-	1.649 ±0.001	-	-
Ascorbic acid	50	1.28±0.0890	22.377	111.973
	100	0.91±0.0314	46.634	
	150	.56±0.005	66.04	
	200	0.21±0.020	87.26	
ABT1	50	1.59±0.03	3.578	225.66
	100	1.42±0.005	13.89	
	150	1.18±0.001	28.44	
	200	0.92±0.002	44.21	
ABT2	50	1.46±0.001	4.46	163.970
	100	1.18±0.02	28.44	
	150	0.82±0.005	50.23	
	200	0.68±0.030	58.76	
ABT3	50	1.57±0.005	6.61	178.29
	100	1.28±0.003	22.38	
	150	0.98±0.011	40.57	
	200	0.7±0.004	57.55	
ABT4	50	1.38±0.001	16.31	137.262
	100	1.12±0.02	32.08	

	150	0.63±0.04	61.79	
	200	0.48±0.03	70.89	
ABT5	50	1.29±0.005	21.77	121.61
	100	1.010±0.005	38.75	
	150	0.570±0.002	65.43	
	200	0.339±0.001	79.44	

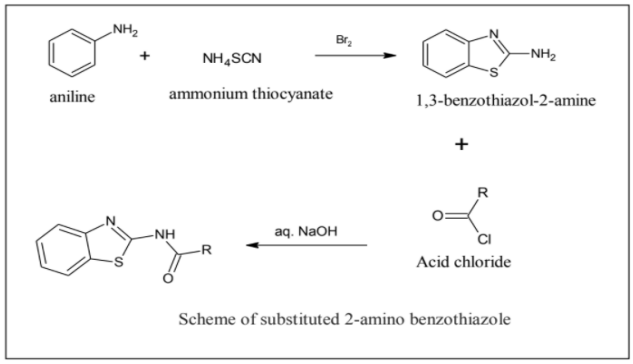


Fig. 1: Methods employed

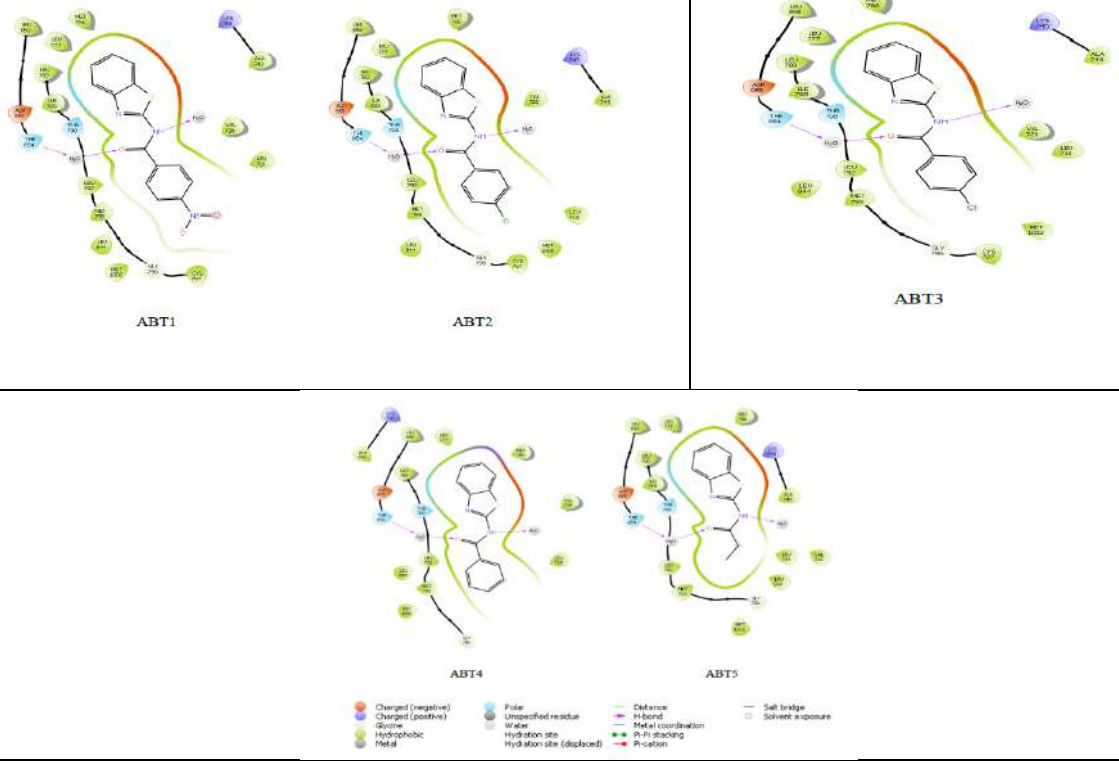


Fig . 2: Docking images of proposed derivatives binding to EGFR

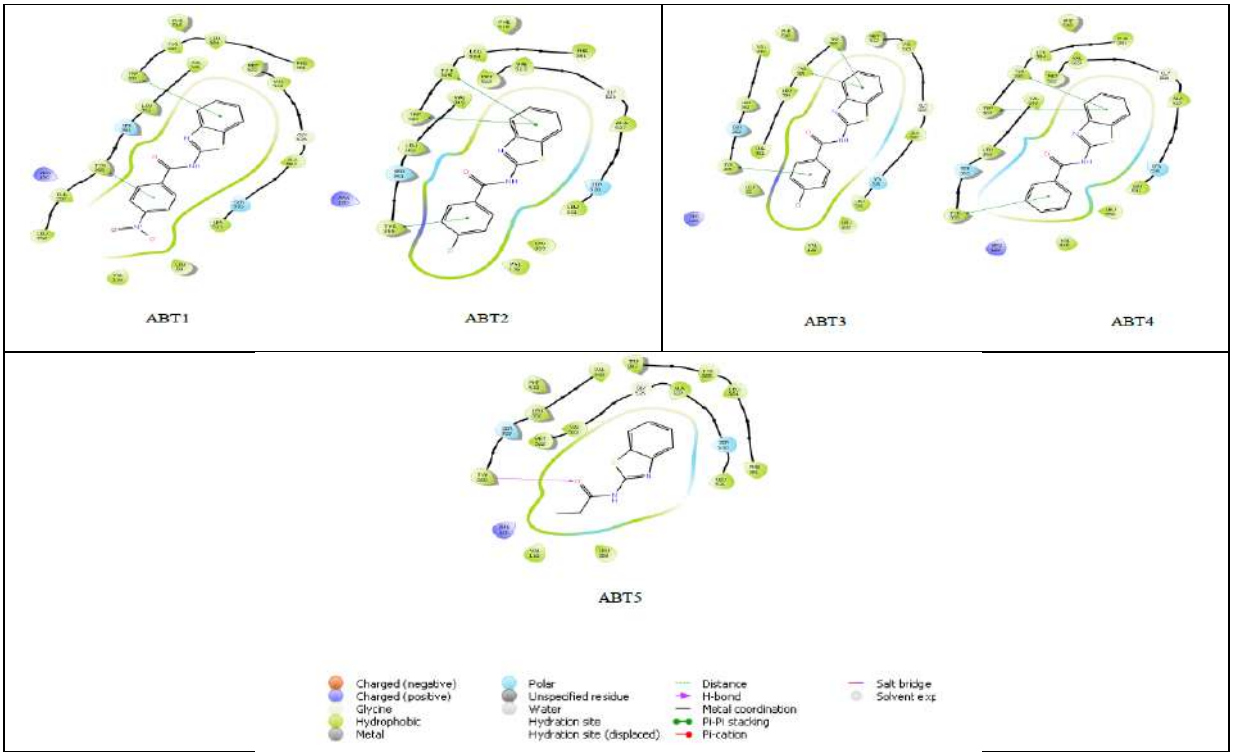


Fig. 3. Docking images of proposed derivatives binding to COX 2

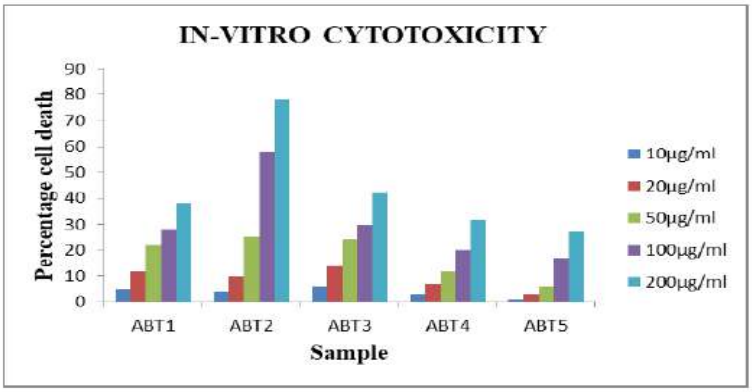


Fig. 4. *In-vitro* cytotoxicity of synthesized derivatives against DLA cell lines

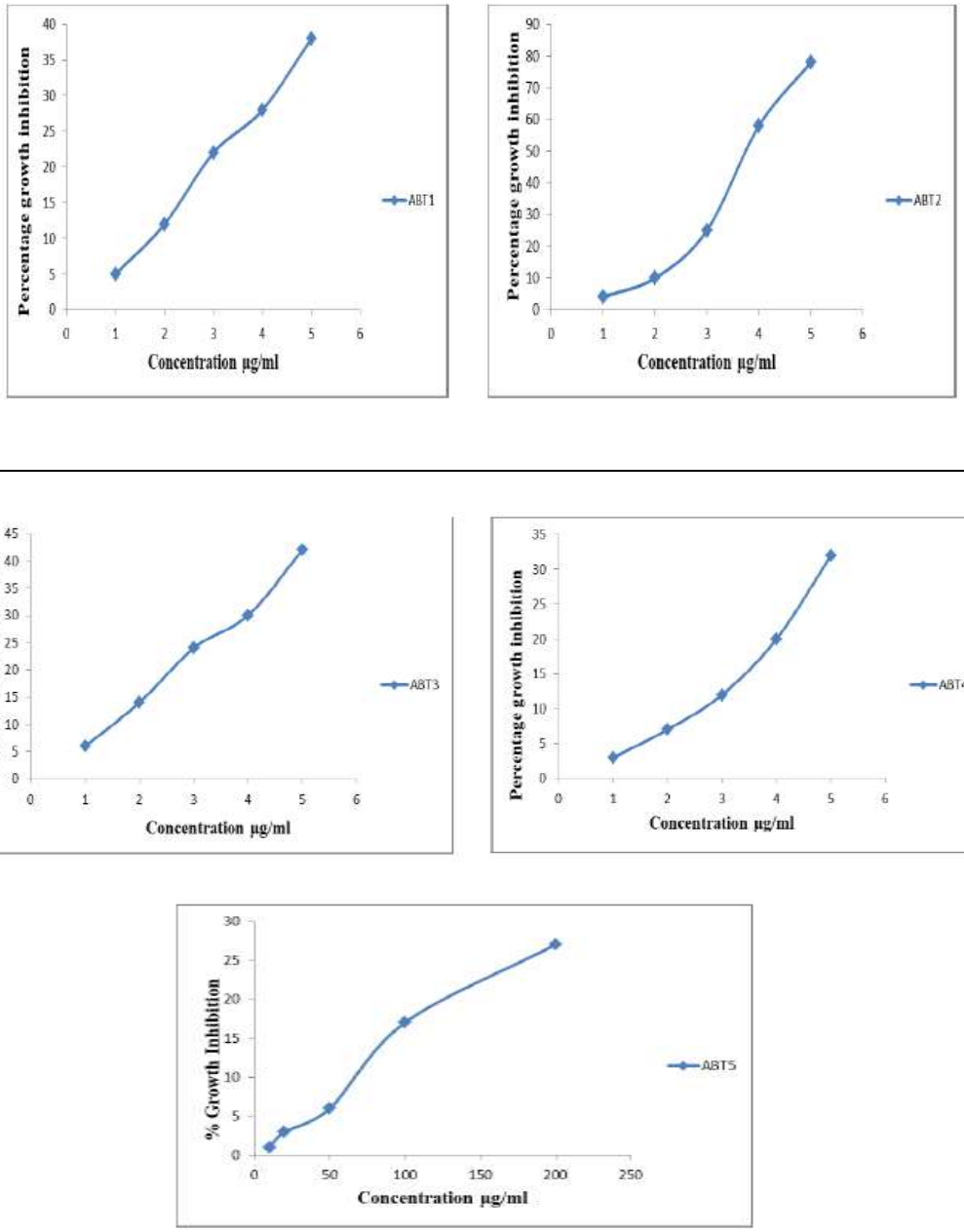


Fig. 5*: In-vitro* anti-inflammatory activity of synthesized compounds

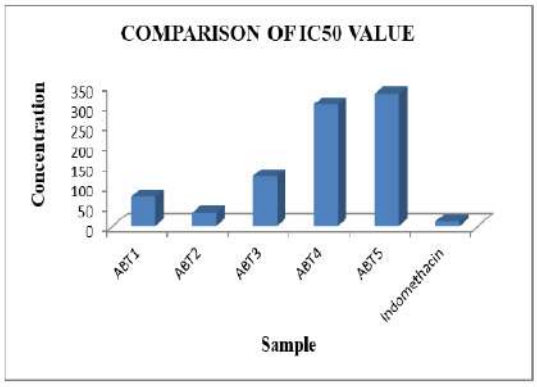


Fig. 6. Comparison of IC50 value by Protease inhibition assay

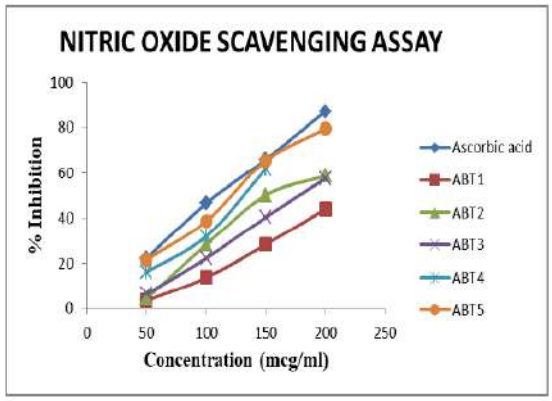


Fig. 7. Nitric oxide scavenging assay

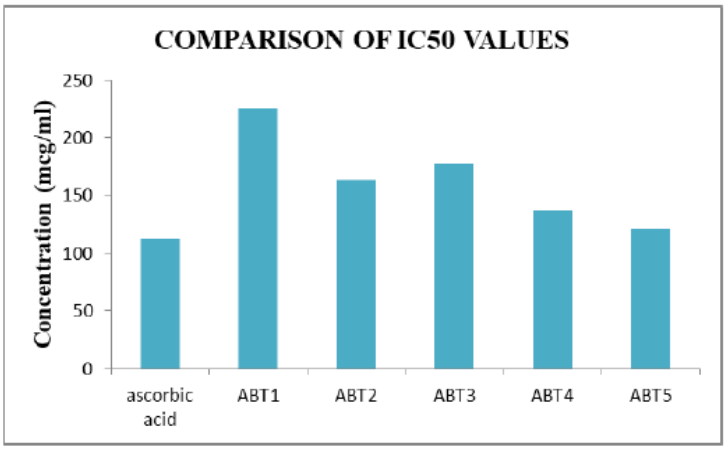


Fig. 8. Comparison of IC50 value by Nitric oxide scavenging assay

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RESEARCH ARTICLE

Case Study of Man Vin Farmer Producer Organisation in Thiruchirappalli District of Tamil Nadu

R. Azhagesan[1]*, R. Selvakumar [2] and S. Radhakrishnan[1]

[1]Research Scholar, Department of Agricultural Economics, Annamalai University, Annamalai Nagar, Tamil Nadu, India.
[2]Assistant Professor, Department of Agricultural Economics, Annamalai University, Annamalai Nagar, Tamil Nadu, India.

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***Address for Correspondence**
R. Azhagesan,
Research Scholar,
Department of Agricultural Economics,
Annamalai University,
Annamalai Nagar,
Tamil Nadu, India.
Email: azhagesanravi@gmail.com

ABSTRACT

Agriculture is a mainstay of the Indian economy and plays a crucial role in its growth and development. More than two thirds of Indians rely on agriculture and related industries either directly or indirectly. In Indian agriculture, Cultivators are mostly small and marginal farmers (85%). Their access to markets, information, funding, and agricultural extension services is constrained. However due to the high transaction expenses, the food sector finds it challenging to buy agricultural products directly from farmers. Ineffective supply chains as a consequence lead to concerns about food safety, transparency, and traceability. For the purpose of integrating Farmers into the value chain, many organisational designs are developing. Farmer Producer Organizations (FPOs) are one such project that aims to assist farmers in gaining the advantages of economies of scale via collectivization and aggregation. MAN VIN Farmer Producer Organization in Thiruchirappalli District, registered on November 7, 2014, is the subject of this case study, which aims to trace its progress while also identifying the major difficulties it encountered and the lessons it learned along the way. This information will be used to develop other FPOs in the future.

Keywords : Agriculture, Small and Marginal Farmers, Processing, Marketing, Food Safety and FPO.

INTRODUCTION

Agriculture is a mainstay of the Indian economy and plays a crucial role in its growth and development. More than two thirds of Indians rely on agriculture and related industries either directly or indirectly. In Indian agriculture, the bulk of cultivators (85%) are small and marginal farmers. They only have a limited number of options for markets, information, funding, and agricultural extension services. But purchasing agricultural goods directly from farmers is a challenge for the food industry due to the expensive transaction fees. Ineffective supply chains as a consequence lead to concerns about food safety, transparency, and traceability. Numerous organisational designs are being developed with the goal of incorporating Farmers into the value chain. One such initiative is Farmer Producer Organizations (FPOs), which intends to help farmers benefit from economies of scale via collectivization and aggregation. In this case study, the Thiruchirappalli District's MAN VIN Farmer Producer Organization's journey is being followed. MAN VIN Farmers Producer Company limited is a private company incorporated on 7th November 2014. It is classified as Non-Government Company and is registered as registrar of companies, Chennai. Its authorized share capital is Rs.25, 00,000 and its paid up capital is Rs.8, 84,000. It is involved in the Agricultural and Services related to animal husbandry, with the exception of veterinary services, are often provided on farms on a fee or contract basis. The most recent Annual General Meeting (AGM) of MAN VIN Farmers Producer Company Limited took place on September 21, 2020, and it's most recent balance statement was filed on March 31, 2020, according to MCA data. The figure I shows that Organization Structure of MAN VIN Farmer Producer Company Limited.

Business of MAN VIN Farmer Producer Company Limited

There are three subcommittee to oversee the whole range of company for efficient operation and sharing of responsibility. Unless additional examination is necessary, the board accepts the subcommittees' decisions.

- Pesticide, Bio-pesticide
- Seed license to carry on business of a dealer
- Fertilizer, micro nutrients, bio-fertilizers.

The primary activity of FPO is the provision of agricultural inputs to its members. FDC provided inputs like as fertiliser, seed, bio-input, and insecticide. For roughly 530 members, the FPO levies a small fee of RS. 10-15 on each bag of seeds and a margin of 8–10% on pesticides as part of its licensing for the sale of different inputs. All of these inputs are available to its members for 85% less than the going rate. This figure may rise if FPO started selling items using credits instead of only cash and carry, which it does not yet. Maize, Paddy, Cotton, and Bhendi are the principal crops farmed in the region. The FPO participates in the purchase of corn. In addition to these operations, FPO also offers farmers minor agricultural equipment. Sprayers are in high demand, thus FPO offers them to its member farms for 30% less than the market rate. 83% of their business revenue comes from non-member companies. The degree of member satisfaction and patronage is comparatively high. Table.1 demonstrates that farm equipment. Provided by MAN VIN Farmer Producer Company Limited and the Table.2 Shows that Profile of Membership of MAN VIN Farmer Producer Company Limited.

Financial Status

Authorised Capital- Rs.25, 00,000
Paid up Capital- Rs.8, 84,600
The Table.3 State that Strength of MAN VIN Farmer Producer Company Limited.

Facilities Provided by FPO (MAN VIN Farmer Producer Company Limited)

Processing

Paddy Processing

One of the major activities done in the FPO. Some paddy processing activities are,

Cleaning

1.Following the arrival of rice from rice fields for milling in the rice mill, paddy rice is cleaned in the second stage of the rice milling system.
2.Paddy always emerges with an abundance of extraneous things, such as weed, dirt, seeds, etc.
3.Cleaning has traditionally been a crucial step in the rice milling process.
4.These extraneous items must be taken out before hulling the grain, to ensure that milling and the huller operate as efficiently as possible.
5.The huller's efficiency and milling recovery would be lower if the paddy is not cleaned before hulling.

Dehusking

Removal of husk from paddy is known as Dehusking or Dehulling.

Milling

By doing so, the bran layer of the rice is removed, converting it from brown to white.

Polishing

By putting rice through a succession of rollers, the surface was smoothed and given a gloss.

Grading

It is a procedure in which broken rice is isolated and divided into various lengths.

Sorting

This stage increases the value of rice by removing immature, yellow, and discolored rice.

Packaging

The finished product is then packaged and kept in storage until it is delivered to cherished clients.

Technology used

Modern Technology

Warehouse and Other Facilities

1.Rural and Urban Godowns
2.Cold Storage Unit

Other Facilities and Programmes Organized

1.FIRST MOBILE GRAIN DRYER was Introduced (2019).
2.Marketing Facilities are provided in the upcoming year 2022.
3.Value Added Product was also started from upcoming years.
4.Seed production process was started in December 2021.
5.Solar dryer was present in Renganathapuram, Trichy.
6.Seminars organized to the organic farmers.
7.Training programme organized to the farmers under free of cost.
8.KVK (Sirugamani) gives free seeds to FPO and it was given to the farmers.

Challenges Faced in the Process of Setting Up- MAN VIN Farmer Producer Company Limited

Constrains

1. It is difficult to formalise individual farmers into a systematic organisation. It could be difficult to persuade lone farmers and producers to establish an organisation. The target audience is hesitant to participate in an activity that is unique, such as organisational development, in return for payment. After FPO formation, developing a company strategy and raising share capital are significant hurdles.

2.It is essential to have legal and technical understanding of laws and regulations. Often, professional counsel is required.

Solutions

1.It is crucial to provide farmers a clear understanding of the advantages of a well-organized farmer's organization. They are prepared to go above and beyond to guarantee the success of the FPO once they are convinced of the benefits of a collective.
2.Begin financially viable short-term ventures while only making slight modifications to established manufacturing procedures, such as employing a cluster-based approach to find potential business strategies.
3. MAN VIN is being used as a model for sustainable development in all areas thanks to an integrated framework that considers agriculture from the perspectives of business, environmental sustainability, and farmer livelihood.
4.A solid institutional framework for the FPO is made possible by farmers' high knowledge, engagement, and ownership, as seen by their frequent attendance, transparency, and trust in communities surrounding the FPO.

Policy Suggestions

1.If concrete answers to their concerns are offered, farming communities will support the collective.
2.Promoting minor revenue-generating initiatives is necessary for the long-term growth of any community group.
3.Community organisation helps the FPO succeed and be sustainable over time by promoting services and infrastructure and supporting local agriculture.
4.FPO capacity development is a gradual process that adds new functions without making their operation more difficult. Before tackling more difficult problems, they should first have some modest success. For instance, the FPO may serve as a procurement hub and dispense MSP.
5.The development of value chains is a continuous process that calls for long-term planning in the fields of sourcing, grading, sorting, and value addition. An FPO requires at least 5-7 years of guidance and assistance before becoming organically self-sustaining.
6.By developing stronger business models, it is crucial to change the focus from scaling up to making the current FPOs self-sustainable.
7.There is considerable regulatory compliance requirements that are challenging for newly formed FPOs to satisfy, in addition to the statutory compliances that FPOs are expected to comply with under their various registration acts. Growth would be boosted by exempting FPOs from a punitive provision for the first five years in the event of certain non-compliances.
8.FPO will develop significantly if they are treated as Agri-startups. As a result, they ought to have access to the same advantages as startups.

REFERENCES

1. Bikkina. Nalini., Turaga. Rama Mohana, and Bhamoriya. Vaibhav, “Farmer Producer Organizations as Farmer Collectives: A Case Study from India,” *IIMA Working Papers,* Indian Institute of Management Ahmedabad, Research and Publication Department, vol. 1, pp. 1-38, 2015.
2. D. K. Krishna., Sanjay Kumar Gupta, and Sujay Kademani, “Farmer Producer Organizations: Implications for Agricultural Extension,” *Agricultural Extension Journal,* vol. 2, issue. 3, pp. 196-200, September 2018.
3. Ereneus. K. Marbaniang, J.K. Chauhan, and Pynbianglang Kharumnuid, “Farmer Producer Organization (FPO): the need of the hour,” *Agriculture & Food: E-Newsletter,* vol. 1, issue. 12, pp. 292-297, December 2019.
4. Mohd. Ameer, and R.A. Siddique, “Farmer Producer Organization (FPO): Empowering Indian Farming Community,” *International Journal of Current Microbiology and Applied Sciences,* special issue. 11, pp. 2089-2099, June 2021.
5. Preeti Sawairam, “Case Study of Farmer Producer Organization in Maharashtra in the Era of Globalization,” *IBMRD Journal of Management & Research,* vol. 4, issue. 2, pp. 1-7, 2015.

Table.1. Farm Equipment Provided by MAN VIN Farmer Producer Company Limited

S. No	Items Name	Charge Per Hour	Quantity
1	Rotovator	500	1
2	Tractor	350	2
3	Straw Baler	Free of Cost	1

Table.2. Profile of Membership of MAN VIN Farmer Producer Company Limited

Parameter	Units
Members	530
Non Members	55
% of Total Business from the Members	90%

Table.3. Strength of MAN VIN Farmer Producer Company Limited

S. No	Particulars	Factors	Observation
1.	Ownership	Farmer members are the owner of the FPO	Farmer claims the ownership and any member can put forth their views on its business via their representatives.
		Farmers members on their own manage the FPO	MAN VIN farmer producer company limited is managed by professional paid staff with help of the resource institution
		Frequency of attendance in meetings	More than 80% attendance in annual general meeting is witnessed as per records
		Accountability	The business decision and profit statements are shared with members.
2.	Awareness on Role and Function of FPO	Member are aware of FPO's structure	Farmers are aware of the structure, role and responsibility of BODs but not completely in tune with the management of the FPO
		Farmer members understand their role in running the FPO	Farmers consider their responsibility to produce organic and sell it to the FRP
3.	Satisfaction	Satisfaction amongst farmer members	High satisfaction, based on high returns on investments and supports in newer technologies of doing farming.
4.	Processes and Protocols	Maintenance of records, bye laws and its effective implementation	All records of attendance, input transaction, crops procurement and business are duly maintained.
5.	Capital	% of produce sold to the FPO by farmers	All the produce by member farmers are sold to the FPO Expect for milk
		Financial capital	MAN VIN Farmer Producer Company limited has taken loan from the NABARD

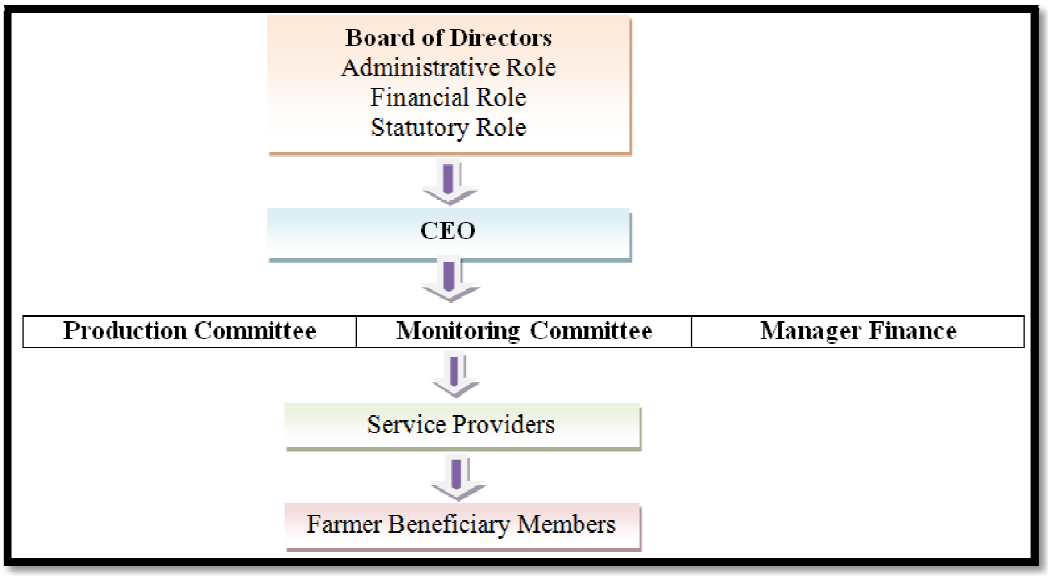

Figure 1: Organization Structure of MAN VIN Farmer Producer Company Limited

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RESEARCH ARTICLE

An Economic Analysis of Area and Production of Amla in Tiruppur District

R. Selvakumar[1]*, R. Azhagesan[2] , D. Velmurugan [3] and M. Mangaiyarkarasi[4]

[1]Assistant Professor, Department of Agricultural Economics Annamalai University, Annamalai Nagar, Tamil Nadu, India.
[2]Research Scholar, Department of Agricultural Economics Annamalai University, Annamalai Nagar, Tamil Nadu, India.
[3]Associate Professor**,** Department of Agricultural Economics, Annamalai University, Annamalai Nagar, Tamil Nadu, India.
[4]P.G Scholar, Department of Agricultural Economics, Annamalai University, Annamalai Nagar, Tamil Nadu, India.

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***Address for Correspondence**
R. Selvakumar,
Assistant Professor,
Department of Agricultural Economics,
Annamalai University, Annamalai Nagar,
Tamil Nadu, India.
Email: selvakumaragri83@gmail.com

ABSTRACT

Amla (*Embilica Officinalis*) is a deciduous plant with high Vitamin C belongs to the family of Euphorbiaceae and is native to India. In India, the cultivation of Amla is mainly done in Uttar Pradesh, Madhya Pradesh, Tamil Nadu, Gujarat, Chhattisgarh, Assam, etc. Uttar Pradesh is the lead state with a production of 397.76 tonnes fallowed by Madhya Pradesh occupies the second position with a production of 361.24 tonnes. Tamil Nadu occupies the Third position with a production of 178.01 tonnes (National Horticultural Board 2020). Tamil Nadu had a major production with an area of about 8,40,000 hectares during the year 2020. The problem in production of Amla is Non-availability of labour, water problem and high initial investment was the major problem expressed by farmers. The foremost problem in marketing of Amla is that the cultivators are forced to sell their produce as soon as the harvest is over because of poverty and prior indebtedness. Based on the problems the specific objective were to analyze the production of Amla in selected area, to analyse the trend and growth rate in area and production of Amla in selected area.

Keywords: Amla, Area, Production, Trend Analysis, Growth Rate.

INTRODUCTION

Amla (*Embilica officinalis*) is a deciduous plant with high Vitamin C belongs to the family of Euphorbiaceae and is native to India. Tamil Nadu had a major production with an area of about 8,40,000 hectares during the year 2020. Amla grows to a height of 8 to18m planted through deciduous of tropical India and on the hill slopes up to 200mt. It starts bearing fruit after about 4 to 5 years of planting depending on the cultivar grown. Amla is cultivated in many parts of the world. Indonesia was the top producer of Amla with a production of 18.3 MT in 2017. Next are Philippines which produce 15.35 MT in 2017. India is the world's third largest Amla producer with a production of 11.93 MT in 2017. Amla or Indian Gooseberry or Nelli is an important crop in India with high medicinal value. The fruit is valued as an anti scorbutic, diuretic, laxative, antibiotic and anti- dysenteric. It has good demand from the industries for the preparation of various health care products also like hair oil, dye, shampoo, face cream, and tooth powder. In India, the cultivation of Amla is mainly done in Uttar Pradesh, Madhya Pradesh, Tamil Nadu, Gujarat, Chhattisgarh, Andhra Pradesh, etc. Uttar Pradesh is the lead state with a production of 397.76 tonnes fallowed by Madhya Pradesh occupies the second position with a production of 361.24 tonnes. Tamil Nadu occupies the Third position with a production of 178.01 tonnes (National Horticultural Board 2020). Tamil Nadu occupies the third position in terms of area and production with 8, 40,000 hectare under cultivation producing of 171.47 million tonnes (Horticulture Statistics Division, Department of Agriculture Cooperation and Farmers Welfare 2019 to 2020). The fruits are transferred to major markets such as Koyembedu market, Chennai, Ottanchathiram etc.

Objective of the study

1) To analyse the trend and growth rate in area and production of Amla in selected area.

Review of literature

Growth Rate

Karthikeyan (2021) in his study estimated the compound growth rate analysis showed that both the area under carrot and production of carrot had positive and more or less same growth i.e., 0.034 and 0.029 percent respectively. The productivity had negative growth which indicated that every year the productivity of carrot was decreasing by meager amount i.e., 0.005 tonnes per hectare. Kathiroli (2021) in his study worked out the compound growth rates were 2.98, -1.68 and -1.19 percent of area, production and productivity of papaya respectively. It is inferred that there is increasing growth in area under papaya, whereas production shows negative growth. The test of significance showed that there is scope for increasing the area, production and productivity of papaya in erode district. Sethuraman (2021) in his study estimated the compound growth rate under Chilli production and productivity was -1.094, -1.488 and -1.5873 respectively. It is inferred that the compound growth rate is negative for area, production and productivity is decreasing significantly at 5% level of significance which shows a decreasing trend in area, production and productivity of Chilli. The exponential trend line witnessed that there is a decreasing trend in the area, production and productivity of Chilli in Ramanathapuram district during 2009 to 19. The trend line for area, production and productivity showed a negative trend which may be due to the affected in the rainfall. The last seven years rainfalls are deficit.

DATA AND METHODOLOGY

Study Area

In the selection of study area, multistage purposive sampling method was followed. In the first stage Tiruppur district was purposively selected because of high area of Amla fallowed by Tirunelveli district of Tamil Nadu. Tiruppur district formed the universe of the study. In the second stage among the 13 blocks in Tiruppur district, Vellakoil and Udumalaipet blocks were purposively selected because of high area of Amla cultivation. In the third stage further in the selected block the villages namely Mettupalayam, Udaiyam, were purposively selected, since area wise these villages occupy the first two places in Amla cultivation in Vellakoil block. Elayamuthur, Sellappampalayam, are another two villages which occupies first and second place in Udumalaipet block.

Selection of Sample Respondents

The block selected as per the procedure described above formed the first stage unit of sampling and it is furnished in the Table 1. The selected villages formed the second stage unit of sampling. From each of the selected villages 30 farmers were selected at random. Totally 120 sample respondents were stratified random sampling technique. The study is based on both primary and secondary data. The primary data referred to those which are collected first hand by the researcher. The respondents in the selected villages are interviewed personally and the required data were collected with the help of a well-structured interview schedule. The schedule covered all the aspects, viz., general characteristics, details on production and marketing of Amla and the problem faced by the producer. The secondary data required for the study were collected from the published source's are, Ministry of Agriculture- Government of India, Department of Statistics in Tiruppur, Horticulture Statistics Division, Department of Agriculture Cooperation and Farmers Welfare, Horticulture Office and Assistant Director of Horticulture at Vellakoil and Udumalaipet block and other organizations like FAO.

Tools of analysis

The data collected were processed and tabulated for subsequent analysis. Keeping in view the objective of the study, appropriate tools were employed to analyze the data. The analytical techniques used in the study are presented below.

Growth Rate

In order to analyse growth in area, production and productivity of Amla, compound growth rate is estimated using the function as under

Where,

Y=Dependent variable (area/ productivity/ production)

a = Intercept term

b = (1+r) and r is the compound growth rate

T = Time

E^u = error term

In the logarithmic form the function could be expressed as,

Log Y = log a + log b + u

Log a and Log b were obtained using the Ordinary Least squares (OLS) procedure and (Antilog of log (b +1)* 100 gave the per cent growth rate.

RESULTS AND DISCUSSION

Growth Rate of Area and Production of Amla in Tiruppur District

The table 2, figure I and II shows that area, under Amla cultivation has decreased from 719.19 hectares in 2005-06 to 467.55 hectares in 2019-20. The production of Amla also decreased from 13.9 tonnes in 2005-06 to 10 tonnes in 2019-20. The estimated compound growth rate was -1.98 per cent of area,-0.99 per cent of production.

CONCLUSION

The estimated compound growth rate was -1.98 per cent of area,-0.99 per cent of production. The fluctuation in area and production of Amla were found negative over the years of Thriruppur District. In the context of our new economic policy, plantations of Amla orchards may be encouraged as a focus are for diversification of agriculture. It has great potential of generating higher income per unit area and time besides, earning foreign exchange through export of Amla products and FPO may further help in safeguarding the interest of the Amla producers.

REFERENCES

1. A. King. Stephen., Nathan Howell, Martin Stone and Todd Willian, "Economic feasibility of Table Grape Production in Kentucky," *Southern Agricultural Economics Association (SAEA) Annual Meeting,* Orlando, Florida, pp.3-5, 2013.
2. Anpu Kumar Chattopadhyay and Purnendu Sekar Das, "Estimation of Growth Rtae: A critical Analysis with Reference to West Bengal Agriculture**,"** *Indian Journal of Agrcultural Economics*, 55(2): 116-135, 2000.
3. G. Ramanan, "Cost of Production and Capital Productivity of Grape Cultivation in Tamil Nadu, India," *Indian Streams Research Journal,* 2(1): 1-6, 2012.
4. M. Karthikeyan, "An Economic Analysis of Production and Marketing of Papaya in Modakuruchi Block of Erode District," *(Unpublished M.Sc. (Ag) Thesis, Department of Agricultural Economics, Faculty of Agriculture, Annamalai University),* 2021.
5. M. Sethuraman, "An Economic Analysis of Production and Marketing of Chilli in Ramanathapuram District," *(Unpublished M.Sc. (Ag) Thesis, Department of Agricultural Economics, Faculty of Agriculture, Annamalai University),* 2021.
6. Mirdu Pavan Chitney, "Production and Marketing of Turmeric in Assam," *International Education and Research Journal,* 5(3): pp.217-219, 2017.
7. S. A. Naphade and A. S. Tingre, "Economics of Production and Marketing of Guava in Buldhana District of Maharashtra," *Indian Journal of Agricultural Marketing,* 22(2): 32-41, 2008.
8. S.S. Singla., Chalal and P. Kataria, "Economics of Production of Green Peas in Punjab," *Agricultural Economics Research Review,* 19:237-250, 2006.
9. T. Kathiroli, "An Economic Analysis of Production and Marketing of Papaya in Modakuruchi Block of Erode District," *(Unpublished M.Sc. (Ag) Thesis, Department of Agricultural Economics, Faculty of Agriculture, Annamalai University),* 2021.
10. T.G. Mamtha., K.C. Gummagolmath and P.V. Shripad, "Trend Analysis in Production and Export of Cashew in India," *The Cashew Journal***,** 16(1):13-17, 2002.
11. Y. Radha., D.S. Prasad and S. Jayarama Reddy, "Economic Analysis of Production and Marketing of Grape in Andhra Pradesh," *Indian Journal of Agriculture Research,* 40(1): 18-24, 2006.

Table.1. Sampling Distribution of Farmers in the Selected Villages

S. No	Village Name	Sample Respondents
1	Mettupalayam	30
2	Udaiyam	30
3	Elayamuthur	30
4	Sellappamapalayam	30
	Total	**120**

Table.2. Area and Production of Amla in Tiruppur (2005-2020)

S. No	Year	Area (in ha)	Production ('000 MT)
1	2005-06	719.19	13.90
2	2006-07	690.64	12.70
3	2007-08	681.382	13.25
4	2008-09	684.24	12.70
5	2009-10	671.17	12.38
6	2010-11	680.00	13.00
7	2011-12	714.17	13.17
8	2012-13	738.33	14.20
9	2013-14	730.35	13.05

10	2014-15	647.24	11.66
11	2015-16	594.52	11.66
12	2016-17	597.66	12.93
13	2017-18	568.42	11.46
14	2018-19	498.615	10.38
15	2019-20	467.55	10.09

Source: Statistical Department, Tiruppur

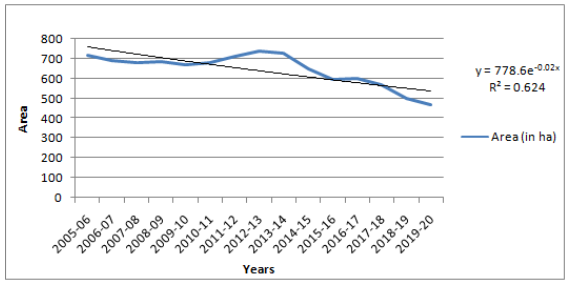

Figure 1: Growth Trend of Amla Cultivation Area in Tiruppur

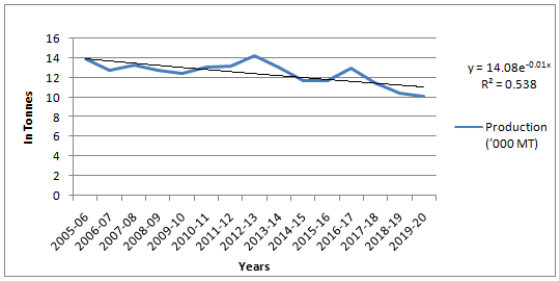

Figure 2: Growth Trend of Amla Production in Tiruppur

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RESEARCH ARTICLE

Optimization of 2.5d Milling Parameters using RSM and ANN-GA for Inconel 718

Gourav Kalra[1]* and Arun Kumar Gupta[2]

[1]Research Scholar, Department of Mechanical Engineering, Maharishi Markandeshwar (Deemed to be University), Mullana, Haryana, India.
[2]Professor, Department of Mechanical Engineering, Maharishi Markandeshwar (Deemed to be University), Mullana, Haryana, India.

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***Address for Correspondence**
Gourav Kalra
Research Scholar,
Department of Mechanical Engineering,
Maharishi Markandeshwar (Deemed to be University),
Mullana, Haryana, India.
Email: gkalra89@gmail.com

ABSTRACT

Inconel 718 is indeed a difficult to cut material to manufacture, and its products actually require a high level of surface quality in aerospace, marine engineering. Thus, it is critical for the assessment and prediction of surface roughness, as it affects the deflection of the product and the performance of machining of the machined Inconel 718 workpiece to be created. Speed (v_c), depth of cut (d_a), feed per tooth (f_t), and nose radius (n_t) are few of the most influencing machining parameters to the surface quality during milling. Therefore, the Box–Behnken design (BBD) with three levels for each parameter has been explored in this investigation. Artificial Neural Network has been applied to predict the surface quality of the machined component. The surface quality of the components has been observed with the help of Mitutoyo surftest S-310. ANOVA was performed for the significance and applicability of the proposed model, as well as the impacts of process parameters on surface quality. It has been observed that the ANN provides more accurate results as compared to Regression RSM. The relationship between the input parameters and surface roughness has been evaluated using an artificial neural network modelling approach, and then further GA optimization. Five structural trials have also been conducted to validate this novel strategy, which has been proven to be more successful.

Keywords: Inconel 718, RSM, BBD, Operational parameters, Surface Roughness, 2.5d Milling, Artificial Neural network.

INTRODUCTION

Inconel 718 is indeed a super-alloy that finds extensive use in the aerospace, marine and automotive sectors. In these sectors, Inconel 718 parts or components must have high strength and an excellent surface quality. As it is a difficult-to-cut assisted with high cutting forces and temperatures, which can degrade the surface texture under certain machining operations. As a result, in production processes, it is critical for evaluating and predicting surface roughness of machined Inconel 718 workpieces [1]. Modelling and optimization of the machining operation are two key issues in milling that researchers in the machining studies extensively investigated and discussed. Modelling is the process to predictthe machining performance with respect to input parameters, whereas optimization is the process of determining the best possible level of machining responses under the optimal circumstances. Surface quality and Dimensional accuracy are the most frequent machining performance metrics that most machinists are concerned with [2]. Surface roughness is a statistic that can be used to determine if something has improved in terms of quality (Ra). In general, machining settings, cutting phenomena, work piece quality and cutting tool characteristics affect the Ra value [3]. When machining a component with different operational parameters, it was found that geometry of insert also played important role on surface texture of the product. Studies have shown that an improvement in surface roughness can be achieved by using round inserts combined with small depth of cut as well as small Cutting speed [4]. R_a in Inconel 718 end milling was tested under various cutting parameter combinations, and it was discovered that the lowest value of R_a was achieved when the tiniest chip burrs were generated [5].

Sarkar et al. analysed surface quality of the machined product under dry as well as wet conditioned with the experimentational process, it was found that depth of cut is a crucial factor which significantly affect Ra [6]. Dimensional accuracy as well as surface quality which is generally required in most of the product manufactured with Inconel 718 alloy has been analysed and it was found with the statistical analysis that feed per tooth is the most significant parameter than Cutting speed and Doc [7]. Jonas et al. succeeded in demonstrating that using ceramic tools is able to improve the surface quality of the machined Inconel 718 [8]. It has been demonstrated with the experimental study that Speed, Feed and tool angles are the major operational parameters which affect surface texture [9]. It has been experimentally concluded that by optimizing machine parameters with CBN tool can minimize the R_a value [10]. Kasim et al revealed that product topology of end milled machined Inconel 718 was observed to be lower in the feed direction. It has also determined that the Step over are to blame for the variations in Ra. The carbide particle phenomenon was found during low-feed rate machining, resulting in the additional third body abrasion on the machined part of the product. This will be caught in between the fragments zone and the tear part of the work piece, increasing the R_a of the work piece's surface. The feed rate has an effect on the quality of the machined surface, whereas WoC governs the variation in Ra, according to the combined effect between WoC and fz [11]. It has been experimentally proved that the coating on a cutting tool is critical for boosting the instrument's wear resistance and longevity. And it was found that nose radius of the tool and the feed rate was the most active parameters. It was established that carbide inserts coated with TiAlN have a higher surface quality than carbide inserts that are not coated [12].

Modelling strategies for determining the theoretical minimum value of surface quallity, such as Ra, may be classified into two categories. Traditional methodologies, such as the Regression methodology, provide explicit models that need a deep physical knowledge of the modelling process. Using non-conventional or artificial intelligence (AI), provides implicit models which are easier to execute. As they have inbuild function with ion the system. As a consequence, optimising machining parameters such nose radius (r_n) Cutting speed (v_c) and Feed per tooth (F_z), and DoC(d) on the Ra on end milled Inconel-718 have been considered in the present study. The objective of this study is to develop a prediction model based on ANN of surface quality during 2. 5D milling of Inconel 718 alloy using various selected parameters. The surface quality of the components has been measured with the help of Mitutoyo surftest S-310. ANOVA has been used to determine the significance and suitability of the suggested model, as well as the impacts of process parameters on surface quality. It has been observed that the ANN provides more accurate results as compared to Regression RSM. The relationship between the input parameters and surface roughness has

been evaluated using an artificial neural network modelling approach, and then further GA optimization. In addition, five conformational tests have been conducted on the optimal parameter combination indicated by GA to validate this novel strategy, which has been proven to be more successful. Further, a comparison of the proposed model with the RSA optimization strategy referred to as the desirability approach.

Experimental setup and Work-piece Material

To investigate the relationships between input parameters and surface roughness, three levels of each selected parameters were evaluated based on the material characteristics and the machine tool specification. Experiments are performed on the milling machine (Bharat Fritz Werner, Agni++, BMV45++ TC24 VMC) (in figure 1) . Surface finish has been observed by Mitutoyo surface tester S-310 tester as shown in figure 2. The range of temperature measure is from -270°C to 1250°C, with precision of ±0.75 to ±2%, and Special Limits of Error: +/- l°C or 0.4%. The responses were chosen for the experimental work at various input variables using Inconel 718 super alloy as a work-piece and As a cutting tool, a 20 mm diameter, two-fluted, a flat-ended tungsten carbide with was employed. The chemical composition and various characteristics of Inconel 718 are shown in Table 1. The chromium component of the work material enhances its hardness, making it harder to treat conventionally [13]. The rectangular work piece material was provided with dimensions of 100mm × 50mm ×20 mm for experimenting. The work piece is secured to the worktable by means of an integrated component. The tool with two flutes and a 20 mm diameter is fastened on the machine tool's tool holder by default. The tool holder had a mechanism for adjusting the electrode's alignment with regard to the work piece.

Design of experiments

The experiment has been designed on design expert software. The RSM-BBD design of experiment has suggested 26 experiments for four input parameters with three level each. Instead of analyzing one component at a time, the impact of all independent parameters on responses is examined collectively during this procedure. For the purposes of this study, four control criteria were employed to arrange the trials. The input control factors with their range and levels are illustrated in Table 2. The suggested combination of input parameters as per BBD are shown in Table 2. The trials are carried out in a certain order to ensure the machine's stability.

RESULTS AND DISCUSSION

The responses are tabulated in table 3 shows the findings of the experiments, which were used to create several response surfaces each having two input parameters along the X and Y axes and one machine response such as R_a along the Z axis. For each output parameter, SR, the response surface is presented. The data is analyzed using the computer programmed Design Expert-12. Table 4 provide the results of the ANOVA tables for each answer. The findings are assessed by employing the normal distribution curve, the P-value, and a lack of fitness test to determine whether or not the model is a good match for the data utilizing the SR that was obtained from the experimental investigation.

Surface Finish Analysis

The fit summary shows that the two interaction (2FI) model is significant for investigation.. Table 4 summarizes the ANOVA results for the Ra. As ANOVA contains no quadratic terms, but the interactions term converts it to a 2FI model. As shown, the model f value is 49.87, P-value is 0.05 and the lack of fit is more than 0.05. Thus, the model is significantly useful, although the LOF is not significant. SF analysis is used to conduct all the tests for the presence of a fine model. R^2, adjusted R^2, anticipated R^2, and an appropriate precision are all indicators of a solid model. In coded and actual factors, a mathematical model of SF is given by equations 1 and 2.

SF **=** + SF=+0.052544-0.000019* x(1) +0.116947*x(2) +1.43814* x(3) +0.121732* x(4) +0.000018 *x(1)*x(4) -0.480292* x(2)*x(3) -0.044002* x(2)*x(4) -0.726615* x(3)*x(4)

(1)

SF =0.052544 - 0.000019* Cutting Speed +0.116947*DoC+1.43814*Feed +0.121732*Nose Radius +0.000018* Cutting Speed * Nose Radius -0.480292* DoC * Feed -0.044002*DoC * Nose Radius -0.726615* Feed * Nose Radius (2)

In normal probability (Figure 2), the majority of residual points lie along a straight line., indicating a good fit. The anticipated versus residuals figure (Fig. 2) also suggests a good model, as the residuals are dispersed randomly. Figure 3 illustrates the 3d surface plots and the contour plots of various interactions of independent parameters for influencing Ra. Contributions of the input parameters in the calculations for the degree of Surface finish have been observed from table 4. Feed and Nose radius were found to be the major contributed factor analyzed by the ANOVA.

Artificial Neural network

In the last two decades, the application of neural networks (NN) has shown considerable potential in addressing challenges in a various sector. The Adaptation, generalization, global function approximation, and other benefits of Neural Network in various application areas, such as pattern recognition, classification, prediction, optimization, and control systems, NN can solve a variety of issues. NN has been widely employed in the manufacturing industry for decades and has been proven to be successful at modelling nonlinear and highly correlated data sets. ANNs are also known as information processing systems since they can solve a variety of issues such as modelling and prediction. Back propagation (BP) neural network design has been discovered to be the most extensively employed among the numerous types of neural network architectures in process modeling [14][15][16], [17]. It contains three layers: input units, one or more hidden layers, and output layers. The structure of a ANN is depicted in the figure 4. Training is the initial stage in the ANN. The ANN is given an input along with the intended outputs, and randomly distributed weights. When the network reaches the required level of performance, the training will be terminated. The weights calculated at this step are used to make judgments about output assessment. The NN codes of Matlab were utilised for ANN training and testing in this study. Feed forward Back Propagation was used in the ANN study (BP). The neurons in the hidden layer part and the Levenberg-Marquardt (LM) training technique is used to optimise the ANN model. Three neurons represent the speed (vc), depth of cut (da), feed per tooth (ft), and nose radius in the input layer (nt). Surface roughness is the result. During training and testing, the tansig activation function is employed in the hidden as well as output layer. The ANN projected values were derived from the outcomes of 26 data experiments. Training, validation and testing was done with the default setting of ANN such that70% of experiment values were taken for training the functional network, 15% used for validation and 15% for the testing purpose. A size of 14 neurons were taken for the hidden layer for defining the fitting of the neural network as shown in figure 4. In the next step training of network were done by using Levenberg-Marquardt back propagation methodology. Mean square error has used for checking the performance of the algorithm i.e. for training, validation, and testing as shown in figure 5. Surface roughness of the machined Inconel 718 component was calculated by successfully implementing different models and their values are illustrated in below table. Root mean square has used for calculating the standard error in between experimental findings with RSM and ANN which is also shown in table. It has been clearly seen from the table the prediction made by artificial intelligence model i.e ANN are very close to the experimental findings, Consequence to this ANN model is better predictor tool for calculating the surface roughness value. A chart better illustrates in the figure 6 it could be clearly seen that predicted value from ANN is overlapped with experimental values.

Optimization using integrated ANN and GA approach

In this study, the optimal milling process parameters for Inconel 718 alloy were determined using an integrated model incorporating ANN and GA. The search range for the four input parameters is set at 3000-5000 rpm for the speed parameter, 0.05-0.15mm/tooth for the feed parameter, 0.5-1.5 mm for the depth of cut parameter, and 0.4-1.2 mm for the nose radius parameter. Each search range is dispersed in 100 equal intervals after normalisation. Figure 8 depicts the projected result of using the Matlab GA tool to optimize/minimize the surface roughness value. It has been shown that, for up to 30 generations, the fitness value decreases constantly, with only minor fluctuations thereafter. The black dots reflect the best fitness, while the blue dots represent the average fitness. The best and average fitness are closed to converge at the 18th generation, however the local minimums are attained during the

33rd generation and coincide for the remainder of the generations. For the end milling process parameter problem, it can be determined that local minima may be reached in 25-35 generations, while global minima can be reached in 51 generations using the trial-and-error approach. MATLAB (R2011a) is utilised to determine the optima parameters in the Inconel 718 milling operation by embedding ANN and GA, within which variable X1, X2, X3, and X4 symbolise cutting speed, depth of cut, feed per tooth, and Nose radius, and parameter Y1 signifies the Surface roughness in the work piece during machining. Before and throughout optimization, the ideal response values are compared in Table 7. As fitness metrics for GA, the output of an ANN trained using the surface roughness of a product as inputs may be used. In the GA approach, this fitness function is then utilised to determine the fitness of each chromosome. The operating settings for GA used in this research are as follows: population size = 60, crossover rate = 0.95, mutation rate = 0.01, and maximum generations = 1500. The evolutionary algorithm has selected the ideal solution to be (X1, X2, X3, X4) = (4566, 0.52, 0.055, 0.4) with a response value (Y1) of 0.148. The minimum value in Table 2 is adjusted from 0.183 to 0.148. From the lowest temperature rise numbers in Table 7, we find an improvement of more than 4 percent. Conformational experiments show that the measured results of surface roughness for the best values of parameters by the GA-ANN approach match up well with very small differences, as shown in Table 7.

CONCLUSION

It has been studied from literature that the 2.5 D milling technique is one of the most essential Inconel 718 machining methods. The process must be enhanced in terms of surface quality, i.e. the surface roughness of the machined sample. In this work, an integrated system is developed to detect and regulate factors in the 2.5 D milling process so that a minimal value of the work piece's surface roughness may be obtained, therefore facilitating the attainment of a high level of performance and quality. The combination of GA and ANN was able to minimise surface roughness, which is regarded an improvement in machining conditions, and work piece experiments were able to validate the results. In addition, it can be stated that the established approach is effective and sufficient for estimating the optimal performance parameter values in the 2.5 D end milling process for Inconel 718 alloy. As an extension, the suggested integrated technique may be utilised to solve additional optimization issues for other processes.

REFERENCES

1. Y. Feng, Tsung-Pin Hung, Yu-Ting Lu, Yu-Fu Lin, Fu-Chuan Hsu, Chiu-Feng Lin, Ying-Cheng Lu, Xiaohong Lu, Steven Y. Liang., “Surface roughness modeling in Laser-assisted End Milling of Inconel 718,” Mach. Sci. Technol., vol. 23, no. 4, pp. 650–668, 2019, doi: 10.1080/10910344.2019.1575407.
2. I. S. Jawahir, A. K. Balaji, K. E. Rouch, and J. R. Baker, “Towards integration of hybrid models for optimized machining performance in intelligent manufacturing systems,” J. Mater. Process. Technol., vol. 139, no. 1-3 SPEC, pp. 488–498, 2003, doi: 10.1016/S0924-0136(03)00525-9.
3. P. G. Benardos and G. C. Vosniakos, “Predicting surface roughness in machining: A review,” Int. J. Mach. Tools Manuf., vol. 43, no. 8, pp. 833–844, 2003, doi: 10.1016/S0890-6955(03)00059-2.
4. A. C. Arunachalam, R.M.; Mannan, M.A.; Spowage, “Residual stress and surface roughness when facing age hardened Inconel 718 with CBN and ceramic cutting tools.,” Int. J. Mach. Tools Manuf., vol. 44, no. 9, pp. 879–887., 2004.
5. J. Ning, F.; Wang, F.; Jia, Z.; Ma, “Chip morphology and surface roughness in highspeed milling of nickel-based superalloy Inconel 718.,” Int. J. Mach. Mach. Mater., vol. 15, no. (3/4), pp. 285–294.
6. B. Sarkar, M. M. Reddy, and S. Debnath, “Effect of machining parameters on surface finish of Inconel 718 in end milling,” vol. 02009, 2017.
7. M. Suhaily, A. K. M. N. Amin, and A. U. Patwari, “PREDICTION OF SURFACE ROUGHNESS IN HIGH SPEED MACHINING OF INCONEL 718,” vol. 265, pp. 1193–1198, 2011, doi: 10.4028/www.scientific.net/AMR.264-265.1193.

8. J. Holmberg, A. Wretland, J. Berglund, and T. Beno, "A detailed investigation of residual stresses after milling Inconel 718 using typical production parameters for assessment of a ff ected depth," Mater. Today Commun., vol. 24, no. January, p. 100958, 2020, doi: 10.1016/j.mtcomm.2020.100958.
9. H. A. Sonawane and S. S. Joshi, "Modeling of machined surface quality in high-speed ball-end milling of Inconel-718 thin cantilevers," 2015, doi: 10.1007/s00170-014-6759-6.
10. I. O. P. C. Series and M. Science, "The Comprehensive Review on machining of Inconel 718 superalloy," 2021, doi: 10.1088/1757-899X/1033/1/012069.
11. M. S. Kasim et al., "Investigation of surface topology in ball nose end milling process of Inconel 718," Wear, vol. 426–427, no. December 2018, pp. 1318–1326, 2019, doi: 10.1016/j.wear.2018.12.076.
12. S. Kumar, D. Singh, and N. S. Kalsi, "Investigating the Effect of Approach Angle and Nose Radius on Surface Quality of Inconel 718," J. Inst. Eng. Ser. C, 2017, doi: 10.1007/s40032-017-0411-9.
13. V. R. Khullar, N. Sharma, S. Kishore, and R. Sharma, "RSM- and NSGA-II-Based Multiple Performance Characteristics Optimization of EDM Parameters for AISI 5160," Arab. J. Sci. Eng., vol. 42, no. 5, pp. 1917–1928, 2017, doi: 10.1007/s13369-016-2399-5.
14. S. Kumar, A. K. Gupta, and P. Chandna, "Minimization of Non-Productive Time during 2 . 5D Milling," vol. 8, no. 6, pp. 1155–1160, 2014.
15. C. Series, "Application of RSM and ANN in Predicting Surface Roughness for Side Milling Process under Environmentally Friendly Cutting Fluid Application of RSM and ANN in Predicting Surface Roughness for Side Milling Process under Environmentally Friendly Cutting Flu", doi: 10.1088/1742-6596/1198/4/042016.
16. L. Imani, A. R. Henzaki, and R. Hamzeloo, "Modeling and optimizing of cutting force and surface roughness in milling process of Inconel 738 using hybrid ANN and GA", doi: 10.1177/0954405419889204.
17. B. Choudhury and M. C. D. Devarasiddappa, "Development of ANN modelling for estimation of weld strength and integrated optimization for GTAW of Inconel 825 sheets used in aero engine components," J. Brazilian Soc. Mech. Sci. Eng., vol. 7, 2020, doi: 10.1007/s40430-020-02390-7.

Table 1: Inconel 718 chemical composition and characteristics.

Chemical Composition	**Element**	**Cr**	**Ni**	**Mo**	**Nb**	**Ti**	**Cobalt**	**Al**	**Iron**
	%	0.165	0.515	0.028	0.042	0.0112	0.0088	0.008	Balance
Physical and Mechanical Properties	**Property**	Density (g/cc)	Melt. Temperature (°C)	Modulus of elasticity (GPa)	Thermal cond. (W/mK)	Specific heat (J/KgK)	Poisson's ratio	Tensile strength (MPa)	Hardness Rockwell C scale (HRC)
	Inconel 718	8.19	1260-1336	205	11.4	435	0.284	1100	36
	TiAlNCarbaide	15.25	2870-3000	560	84.02	512	0.2	370	85

Table 2: Independent parameters with their levels.

Control Variable Name	Units	Range	Levels		
			-1	0	+1
Cutting Speed	Rpm	3000-5000	3000	4000	5000
DoC	Mm	0.50-1.50	0.50	1.00	1.50
Feed	mm/tooth	0.05-0.15	0.05	0.10	0.15
Nose Radius	Mm	0.40-1.20	0.40	0.80	1.20
Tool Diameter	Mm	20			

Table 3: Experimental results

Run	Cutting Speed	DoC	Feed	Nose Radius	SR
1	5000	1	0.15	0.8	0.263
2	3000	1	0.1	0.4	0.230
3	4000	0.5	0.1	1.2	0.279
4	4000	1	0.15	1.2	0.281
5	5000	1	0.05	0.8	0.223
6	5000	1	0.1	0.4	0.219
7	5000	0.5	0.1	0.8	0.228
8	3000	1	0.15	0.8	0.285
9	4000	1	0.15	0.4	0.242
10	3000	1	0.05	0.8	0.233
11	3000	0.5	0.1	0.8	0.235
12	4000	1.5	0.1	0.4	0.242
13	3000	1.5	0.1	0.8	0.285
14	4000	1.5	0.1	1.2	0.288
15	4000	1	0.05	0.4	0.183
16	4000	1.5	0.15	0.8	0.271
17	4000	0.5	0.1	0.4	0.198
18	4000	1.5	0.05	0.8	0.258
19	4000	1	0.05	1.2	0.280
20	4000	1	0.1	0.8	0.255
21	4000	0.5	0.05	0.8	0.201
22	4000	0.5	0.15	0.8	0.262
23	4000	1	0.1	0.8	0.260
24	5000	1	0.1	1.2	0.288
25	3000	1	0.1	1.2	0.270
26	5000	1.5	0.1	0.8	0.262

Table: 4 ANOVA table for Surface Finish

	Summations of Squares	DF	Mean of Squares	F-value	p-value	Significant (Yes/No)	Contribution
Model	0.0214	08	0.0027	49.87	< 0.0001	Yes	
Vc	0.0003	01	0.0003	4.82	0.0423	Yes	01.40%
Da	0.0034	01	0.0034	63.52	< 0.0001	Yes	15.89%
Fz	0.0043	01	0.0043	79.24	< 0.0001	Yes	20.09%
Rn	0.0116	01	0.0116	215.19	< 0.0001	Yes	54.20%
Vc*Fz	0.0002	01	0.0002	3.92	0.0643	Yes	00.93%

Da*Fz	0.0006	01	0.0006	10.74	0.0044	Yes	02.80%
Da*Rn	0.0003	01	0.0003	5.77	0.0280	Yes	01.40%
Fz*Rn	0.0008	01	0.0008	15.73	0.0010	Yes	03.73%
Residuals	0.0009	17	0.0001				
LOF	0.0009	16	0.0001	4.50	0.3563	No	
Pure Error	0.0000	1	0.0000				
Cor Total	0.0223	25					
R^2	0.96			**Predicted R^2**	0.90		
Adjusted R^2	0.94			**Adequate Precision**	24.00		

In coded and actual factors, a mathematical model of SF is given by equations 1 and 2.

Table: 5 The used DoE and the model results with % error

Run	Cutting Speed	DoC	Feed	Nose Radius	EXP SR	RSM SR	% error (Exp vs RSM)	ANN SR	% error (Exp vs ANN)
1	5000	1	0.15	0.8	0.263	0.330	25.6	0.265	1.0
2	3000	1	0.1	0.4	0.230	0.275	19.6	0.234	1.5
3	4000	0.5	0.1	1.2	0.279	0.295	5.7	0.277	0.9
4	4000	1	0.15	1.2	0.281	0.351	24.9	0.270	3.7
5	5000	1	0.05	0.8	0.223	0.249	11.8	0.212	4.9
6	5000	1	0.1	0.4	0.219	0.252	14.9	0.211	3.7
7	5000	0.5	0.1	0.8	0.228	0.251	10.2	0.232	1.7
8	3000	1	0.15	0.8	0.285	0.339	19.0	0.283	0.8
9	4000	1	0.15	0.4	0.242	0.318	31.5	0.242	0.2
10	3000	1	0.05	0.8	0.233	0.258	10.9	0.235	0.8
11	3000	0.5	0.1	0.8	0.235	0.260	10.8	0.236	0.4
12	4000	1.5	0.1	0.4	0.242	0.311	28.3	0.245	1.2
13	3000	1.5	0.1	0.8	0.285	0.337	18.3	0.284	0.3
14	4000	1.5	0.1	1.2	0.288	0.355	23.1	0.281	2.3
15	4000	1	0.05	0.4	0.183	0.208	13.9	0.192	5.2
16	4000	1.5	0.15	0.8	0.271	0.372	37.2	0.264	2.7
17	4000	0.5	0.1	0.4	0.198	0.216	9.1	0.205	3.7
18	4000	1.5	0.05	0.8	0.258	0.293	13.7	0.258	0.1
19	4000	1	0.05	1.2	0.280	0.299	6.8	0.280	0.1
20	4000	1	0.1	0.8	0.255	0.294	15.5	0.263	3.2
21	4000	0.5	0.05	0.8	0.201	0.214	6.5	0.208	3.7
22	4000	0.5	0.15	0.8	0.262	0.297	13.5	0.268	2.1
23	4000	1	0.1	0.8	0.260	0.294	13.3	0.263	1.2
24	5000	1	0.1	1.2	0.288	0.328	13.7	0.286	0.5
25	3000	1	0.1	1.2	0.270	0.322	19.4	0.296	9.5
26	5000	1.5	0.1	0.8	0.262	0.328	25.4	0.261	0.3

Table 6. The optimal combination of cutting settings for response

ANN-GA	Machining Parameters				Surface Roughness	Reduction
	Vc	Da	Ft	Nr		
Before optimization	5000	1	0.1	0.4	0.183	~4%
After optimization	4566	0.52	0.055	0.42~0.4**	0.148	

** As 0.42 mm Nose radius tool was not available so we use 0.4mm tool.

Table 7. Conformational Experiments

Exp No	Cutting Speed	Depth of Cut	Feed Rate	Nose radius	Surface Roughness (experimental)	Optimum Value of Surface Roughness	Variation
1.	4566	0.52	0.055	0.4	0.155	0.148	0.007
2.	4566	0.52	0.055	0.4	0.143	0.148	0.005
3.	4566	0.52	0.055	0.4	0.151	0.148	0.003
4.	4566	0.52	0.055	0.4	0.144	0.148	0.004
5.	4566	0.52	0.055	0.4	0.156	0.148	0.008

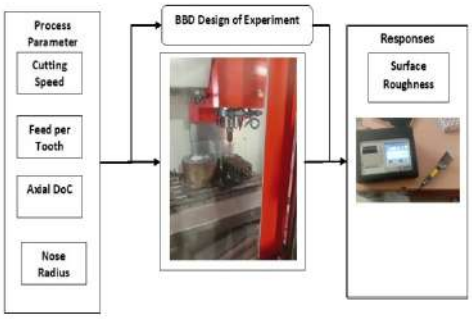


Figure 1: Experimental setup

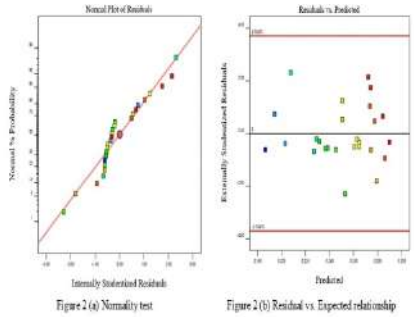
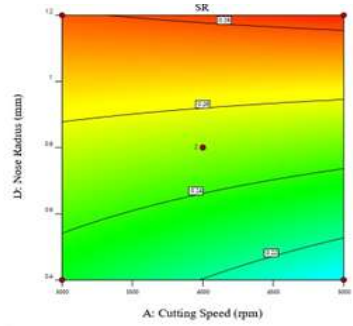
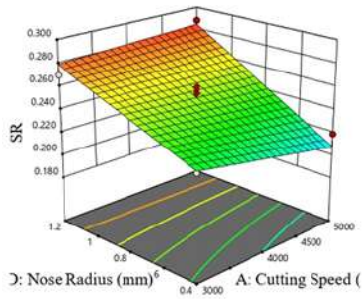
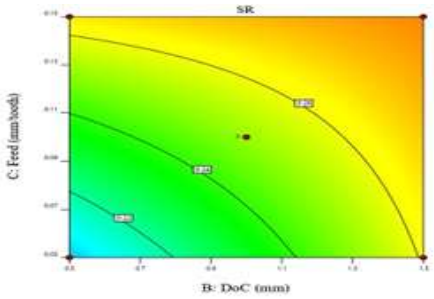
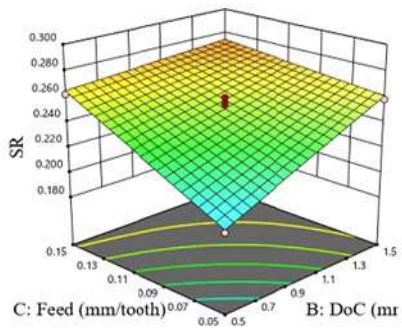


Figure 2: Normality test and Residual vs. Expected relationship









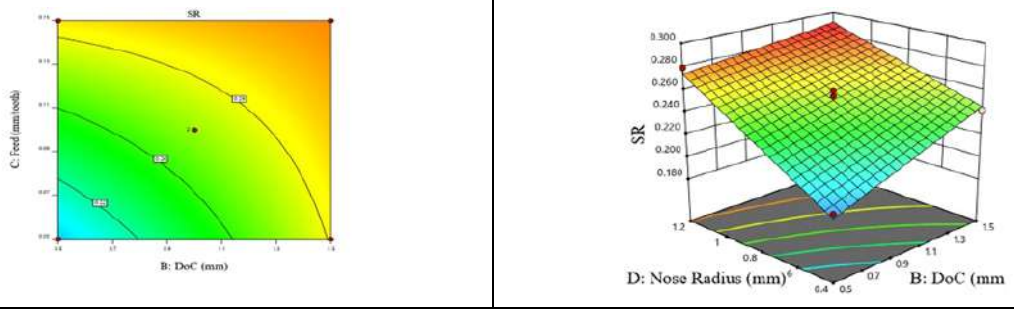


Figure 3: Surface and contour plots

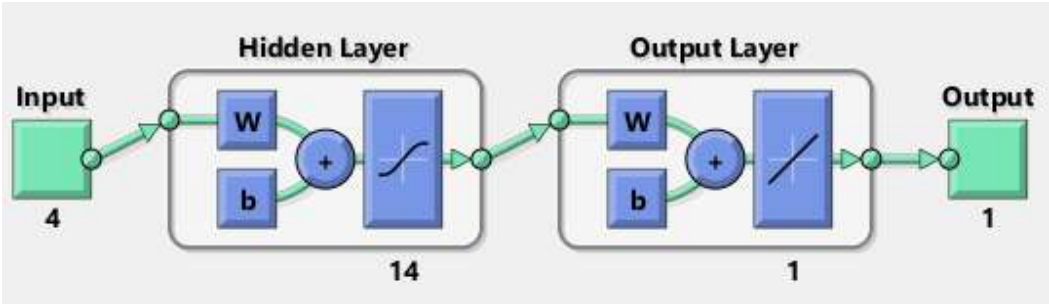


Fig: 4 Structure of ANN

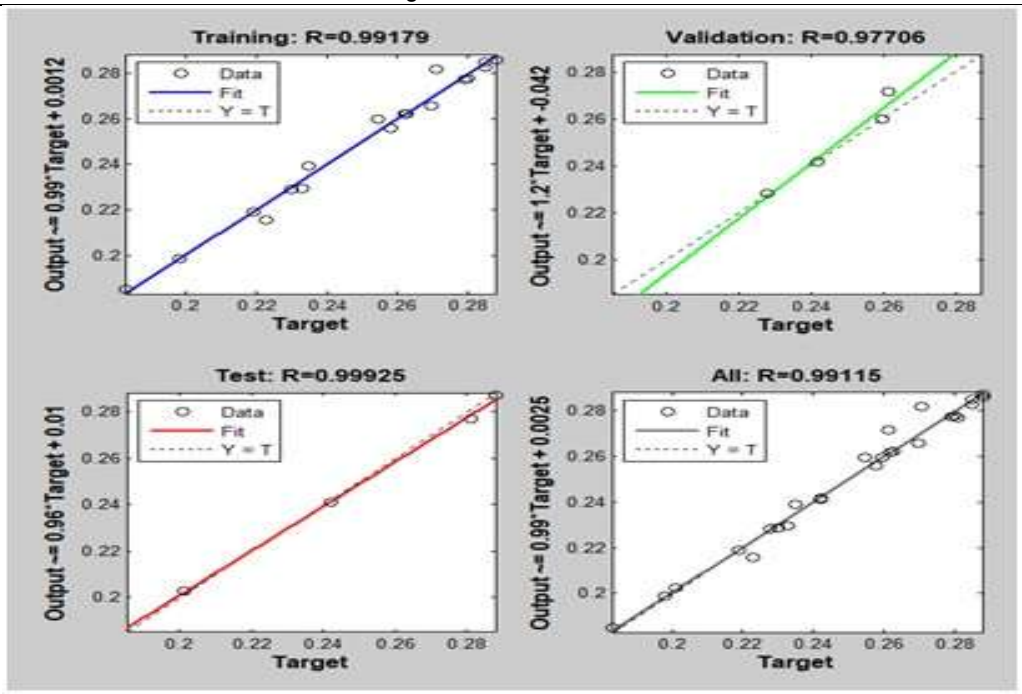


Figure 5: Performance of ANN

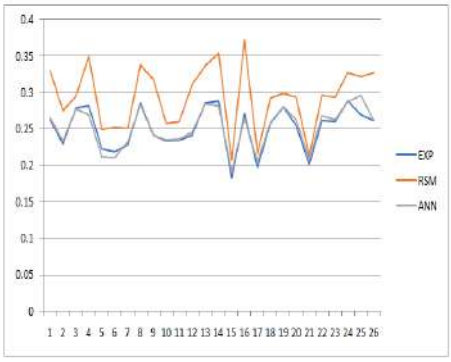


Fig: 6 Comparison charts between experimental values vs predicted values

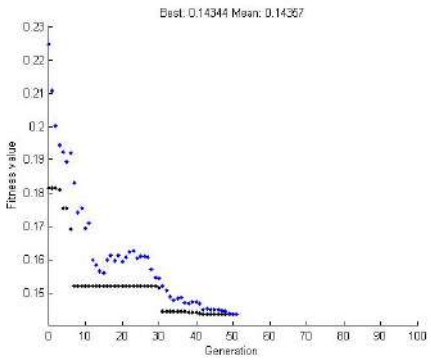


Figure: 7 Optimized process parameters obtained from genetic algorithm

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REVIEW ARTICLE

A Phytochemical and Pharmacological Activity of *Adhatoda vasica.* An Review

Rupak Kumar Swain[1]* and Satya Narayan Tripathy[2]

[1]Assistant Professor, Department of Pharmacology, Roland Institute of Pharmaceutical Sciences, Berhampur, Odisha, India
[2]Assistant Professor, Department of Pharmaceutical Analysis, SoPLS, Centurion University of Technology and Management, Bhubaneshwar, Odisha, India

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***Address for Correspondence**
Rupak Kumar Swain
Assistant Professor,
Department of Pharmacology,
Roland Institute of Pharmaceutical Sciences,
Berhampur, Odisha, India
Email: rupak.55@gmail.com

ABSTRACT

Traditional use of herbal medicines is in commendable use since ages because of its better acceptability and lesser side effects. Bioactive compounds have been the source of attraction which are obtained from natural sources covering a wide spread of diseases both chronic and acute. Further the secondary metabolites play a crucial role in treatment of many diseases. Mainly acting as cardiotonic, hepatoprotective, antioxidant, antidiabetic, antitussive, anti-inflammatory and many more. Adhatoda vasica or vasica is such a herbal plant of much importance for its therapeutic activity. The plant has been widely used in ancient indigenous system of Indian medicine like Ayurveda and unani. Currently this article conveys information relating to the medicinal prospects of the plant Adhatoda vasica. Mainly deals in treatment of respiratory ailments like cough, cold, influenza, bronchitis and also acting against number of diseases. The plant is found to posses quinazoline alkaloids like vasicine, vasicinone and vasicol, adhatodine, and terpenoids, flavonoids, tannins, phenols and glycosides.

Keywords: *Adhatoda vasica*, bronchodilator, hepatoprotective, vasicine, quinazoline alkaloids

INTRODUCTION

Adhatoda vasica most popularly named as Vasaka, or Malabar Nut belonging to family Acanthaceae [1]. It is also familiar by the name *Justice adhatoda nees* and *Adhatoda zeylanica*, it is a evergreen shrub, with bitter taste and unpleasant odour[2]. it is a native plant of asia that is widely distributed in Indian subcontinents in parts like west

Bengal, assam and covers other parts of the plain of india and also found at a height of 1300 m above sea level[3]. Plants play a vital role in imparting good health and improving human life since decades so it finds its special place in siddha, unani, ayurvedic and homeopathic system of medicine [4]. The regular *Adhatoda vasica* is included in the WHO manual stating the importance and usage of traditional medicine in the primary health care treatment [5]. It is quite and well informed that *J.adhatoda* contains quinazoline alkaloids like vasicine. Presence of other alkaloids alike vasicinone, vasicinol, adhatodinine, and vasicinol and many more. vasicine is mainly considered as a bronchodilatory and respiratory stimulant drug [6] [7] .It was also found that Vasicine is having oxytocic and uterine stimulanting activity [8][9]. The whole plant i.e leaves, roots and flowers are of great medicinal importance [10]. other chemical constituents are vitamin C, flavonoids, saponins, fatty acids and steroids [11]. Few plants have reported to be antimicrobial activity [12]. it is widely used for the treatment of common cold, cough, asthma, bronchitis [13], wide spectrum of phytoconstituents isolated from *Adhatoda vasica* have been found to posses property like antitussive, anti-inflammatory, antimicrobial, abortifacient activity [14] [15].

Plant Description

Adhatoda vasica is a quite small, compact and densely branched shrub with a height of 1-2 to 6 metres. Belongs to family Acanthaceae. Leaves are simple, dark green with tapering ends with 7-19 cm long and 4-7 cm wide. Stem is woody and herbaceous. Flowers are big, thick with spikes terminally, white, pink or purple in colour, with average length of 2 cm and 2-0.8 cm breadth [16], [17] .The plant has a bitter taste and unpleasant odour [18]. The fruit is small, longitudinally capsulated with four globular seeds with 5-6mm in length [19].

Leaves

Vasicine and vasicinone are the important alkaloids isolated from the leaves and roots of the plant [20]. other alkaloidal contents like Vascinone, Vascinol, Adhatodine, Adhatonine, Adhavasinone are present. Apart from this it also contains some essential oils, some steroids and alkanes [21],[22],[23].

Flower

It contains flavoinoids like Kaempferol, Quercetin, apigenin and some triterpenes like alpha-amyrin [24], [25], [26].

Root

It contains Vitamin C, fats, carbohydrates and steroids like daucosterol and Alkaloids like vascine, vasicinal, vasicinolone, vasicinone , fiber and adhatonine. sitosterol and deoxyvascine are other components located in the root[27].

Seeds

It contains a yellow coloured oil consist of glycerides of arachidic acid, lignoceric acid, oleic acid, and linoleic acids [28].

Taxonomical status of the plant

Kingdom	: Plantae
Division	: Angiosperms
Class	: Eudicots
Order	: Lamiales
Family	: Acanthaceae
Genus	: *Justicia*
Species	: *J. adhatoda*
Common name	: Adulsa (Vasaka) [29],[30]

PHYTOCHEMISTRY

Adhatoda vasica finds a place in Ayurveda for its expectorant and mucolytic properties. Presence of special bio components like alkaloids, glycosides, phenols, sterols. Quinazoline Alkaloids like vasicine, vasicinone, vasicinolone vasicol, vasicoline [31]. The presence of alkaloid vasicine is having major potent pharmacological actions of the plant [32]. The leaves are found to contains alkaloids like vasicinone, Vasicinol, adhatodine[33]. Apart the plant also contains essential oils, sugars , resins, gums and amino acids and vitamin C.[34] the root of the plants also have found to contain sitosterol, β-glucoside-galactose and deoxyvasicine[35]. the flowers are also found to contain a-amyrin, astragalin, kaempferol, quercetin[36]. vasicine is also found to contain uterine stimulating effect with similar effects like oxytocin [37]. The absorption spectroscopy has noticed the presence of major elements like Na, K, Ca, and Mg along with some trace elements like Cu, Zn, Cr, Ni, Pb and Fe [38].

Pharmacological Activity of *Adhatoda vasica*

Antimicrobial activity

The alcoholic extract prepared from the leaves and roots exhibited antibacterial activity against *S.aureus, E.coli* and the aqueous extract was associated with antibacterial activity in counter to *S.aureus* [55]. Moreover the ethanolic extract of the leaves demonstrated antimicrobial activity against Staphylococcus epidermidis, *Bacillus subtilis*, *Proteus vulgaris* and *Candida albicans* [56]. The methanolic extract of the plant was able to exhibit the activity against *S. aureus* and *B. subtilis* [57]. The presence of bioactive components in the plant showed the antibacterial activity[58]. Above all the bioactive compounds found in the plant extract, the phenolic compounds were mainly responsible for the activity against bacteria [59].

Anti-diabetic activity

The anti-diabetic study of *Adhatoda vasica* has been reported in many studies[60]. The experimental study plan observed that the ethanolic extract of *Adhatoda vasica* whole plant possess significant hypoglycaemic property when evaluated in wistar rats [61]. Ethanolic extract of leaves and roots have a significantly lowers blood glucose levels at a dose of (50 and 100mg/kg) [62]. Another study reveals the non nitrogenous component of the leaf given in the form of suspension at 25 mg/kg decreased blood glucose level in rabbits at a instance [63] [64].

Anti-asthmatic activity

It is a popular traditional drug to treat upper respiratory tract infections. It works as an expectorant by loosening the phlegm. Histamine and acetylcholine produces broncho constriction in guinea pigs [65]. The antiasthmatic activity of vasaka is due to stabilization of mast cells, inhibiting the enzyme cycloxygenase [66]. vasicinone (an oxidized product of vascinine) is a more potent bronchodilator [67]. In an another study guinea pigs were induced broncho-constriction using histamine and was studied for the effect of anti-asthma using vasaka leaves. Animals were kept fasting overnight and exposed to allergens in a air tight shell. [68] it showed that vasicine has strong bronchodilatory activity [69]. The quinazoline alkaloids like vasicine, deoxyvasicine, vasicinone possesses expectorant, and bronchodilatory effect [70] vasicine has stronger antitussive action than codeine [71].

Oxytocic activity:

An important alkaloid like vasicine in *Adhatoda vasica* has oxytocic activity by mediating the release of prostaglandin and there by mediating uterine contraction during parturition [72]. Guinea pigs were used as the experimental study with vasicine resulting as abortifacient due to release of prostaglandin. The dose chosen for this study was between 2.5 to 10 mg/kg [73]. vasicine has found to stimulate uterus and myometrium layer **[**74]. Researchers suggest similar action of vasicine like oxytocin and ergometrine [75]. Orally administered hydroalcoholic preparation of leaf extract (175mg/kg) for 10 days shows abortifacient activity due to vasicine [76].

Hepatoprotective activity

The ethyl acetate extract of the plant at dose of 100mg/kg, and 200mg/kg shows remarked protective action of carbon tetrachloride induce liver toxicity in albino rats. It elevates the liver enzymes lost due to the liver damage [77].

Another research study suggest the l protection of liver by using the whole plant as a drug [78]. It also exhibits hepatoprotective activity at a dose of 50-100 mg/kg induced by D-galactosamine in rats [79**] .**

Analgesic activity

The anti-inflammatory activity was observed in case of methanolic extract of *Adhatoda vasica* (200-400mg/kg/oral) by using carrageenan induced rat paw model. And the analgesic activity was determined hot plate method using wistar albino rats. It was found that potent and similar to diclofenac sodium drug [80]. The aqueous plant extract demonstrates its inhibitory action by inhibiting the cyclooxygenase pathway and the butanol part of the plant extract shows inhibitory action against platelet activating factor [81] **.**The anti-inflammatory action is due to many bioactive compounds like vasicine, vasicinone [82] **.**

Anti-Allergic Activity

Research studies suggest that vascinol and vasicine are the plant chemical compounds responsible for showing anti-allergic activity in *Candida albicans* allergic reactions in animal models like rats and guinea pigs [83]. The methyl alcoholic extract of the plant administered through oral route or nasal route at a dose of 6mg/kg or 2.5gm/kg respectively shows anti-allergic activity [84].

Anti-ulcer activity:

The antiulcer activity was studied on the ethanol induced and aspirin induced experimental animal models rats and compared with a control group and found to be of great potent as a anti-ulcer drug[85] .Highest rate of activity was noticed in case of ethanol–induced ulceration models [86]. So apart from its tremendous medicinal and pharmacological activities it also has anti-ulcer activity [87] .

Anti-viral activity

The methanolic and aqueous extract of *Adhatoda vasica* plant posseses marked antiviral activity by inhibiting the attachement of virus to the host cell surface[88] **.**

PHARMACOKINETIC PROFILE:

It has been found that vasicine and its various metabolites are chiefly excreted in urine. With a oral administration of the drug in mice only 18% drug excreted during 24hrs was vasicine. with intravenous and intramuscular administration around 55% of the drug was excreted in 18 and 22 hours respectively [101]. Clinical trial was conducted in healthy volunteers using vasicine to explore its pharmacokinectic parameters and suggested that 1.5mg/kg body weight of bolus intravenous has reached plasma peak of 65 ±5µmol /L in just 25 minutes [102]. The study on absorption and distribution was carried out in both rats and mice and both showed similar results**.** Vasicine is well absorbed at a dose of 20mg/kg administered intravenous which attained maximum concentration of 56 µg/ml in blood in both normal and pregnant rats and about 10 µg/ml in amniotic fluid. Good concentration of vasicine was obtained in the uterus within 5 minutes of administration and peak level was obtained after 10 minutes[103].

CONCLUSION

From the above study it was observed that the plant *Adhatoda vasica* has extensive use in bronchodilation, anti-asthmatic, mucolyte. Mainly the chemical compounds like vasicine and its derivatives are widely used against many diseases. This herb has a good combination of alkaloids and other phytoconstituents having a tremendous preventive and therapeutic usage. Apart from its action on respiratory system it also covers a broad aspect of pharmacological action like anti-diabetic, anti-inflamatory, hepatoprotective, analgesic, oxytocic and anti-viral. It is also widely known for its application in ayurvedic, siddha and unani system of medicine. Due to the increase risk of adverse effects in synthetic drugs the there is a demand for the herbal drugs which would foster and prevent the disease concerned in the patients economically.

REFERENCES

1. Gedam A, Kshirsagar R, Sawate A, Patil B. Studies on physico chemical characteristics and comparative study on extraction yield of Adulasa (Adhatoda vasica) leaf. J Pharmacogn Phytochem. 2017;6(5):244-247.
2. Patel VK, Venkata-Krishna- Bhatt H. In vitro study of antimicrobial activity of Adhatoda vasica (L) (Leaf extract) on gingival inflammation- A preliminary report. Ind J Med Sci, 1984; 38: 70-72.
3. Gedam A, Kshirsagar R, Sawate A, Patil B. Studies on physico chemical characteristics and comparative study on extraction yield of Adulasa (Adhatoda vasica) leaf. J Pharmacogn Phytochem. 2017;6(5):244-247.
4. Kumar M, Dandapat S, Kumar A, Sinha M. Determination of Nutritive value and Mineral elements of Five-leaf Chaste Tree (Vitex negundo L.) and Malabar nut (Adhatoda vasica Nees). Academic Journal of Plant Sciences. 2013;6(3):103-108.
5. World Health Organization, 1990. The use of traditional medicine in primary health care. A Manual for Health Workers in South-East Asia, SEARO Regional Health Papers, No 19, New Delhi, pp. 1–2.
6. Gupta.O.P.,Sharma ,M.L., Ghatak,B.J.R.,Atal.C.K., 1977. Pharmacological investigation of vasicine and Vasicinone, the Alakloids of *Adhatoda Vasica*. Indian Journal of Medical research 66,680-691.
7. Chakraborty, A., Brantner,AH., 2001. Study of Alkaloids from *Adhatoda Vasica* Nees on their Antiinflammatory activity .Phytotherapy Research 15, 532-534.
8. Gupta, O.P., Sharma, M.L., Ray Ghatak, B.J., Atal, C.K., 1977a. Pharmacological investigations of vasicine and vasicinone-the alkaloids of Adhatoda 6asica. Indian Journal of Medical Research 66, 680–691
9. Gupta, O.P., Sharma, M.L., Ray Ghatak, B.J., Atal, C.K., 1977b. Potent uterine activity of alkaloid vasicine. Indian Journal of Medical Research 66, 865–871.
10. Desai S, Patel D. Phytochemical Screening and antibacterial activity of Adhatoda vesica. Inventi J (P) Ltd.2015:37-49.
11. Wealth of India A Dictionary of Indian Raw Materials: Volume II: B (revised), Publications and Information Directorate, New Delhi, India, 1998.
12. Sharma A, Kumar A. Antimicrobial activity of Justicia Adhatoda. World J Pharm Res.2016.
13. Karthikeyan A, Shanthi V, Nagasathya A. Preliminary Phytochemical and antibacterial screening of crude extract of the leaf of Adhatoda vasica (L). Int J Green Pharm, 2009; 3: 78-80.
14. Karthikeyan A, Shanthi V, Nagasathya A, 2009. Preliminary Phytochemical and antibacterial screening of crude extract of the leaf of Adhatoda vasica (L). Int. J. Green Pharm., 3: 78-80.
15. Maurya S, Singh D. 2010. Quantitative analysis of total phenolic content in Adhatoda vasica Nees extracts. Int. J. Pharm. Tech. Res., 2: 2403-2406.
16. Shinwari ZK, Shah M, Awan R. The Ethnobotany of Kharan Distt. In Baluchistan. Proc. Symp. Med. Pl 1995; 44: 29-35.
17. Panara K, Singh S, Joshi K, Kumar AP, Karra N. Review on research studies of Vasapatra (Leaf of Adhatoda vasica Nees.). Int J Pharmacogn 2014; 1: 168-73.
18. Pa R, Mathew L. Antimicrobial activity of leaf extracts of Justicia adhatoda L. in comparison with Vasicine. Asian Pacific Journal of Tropical Biomedicine 2012 Jan 1; 2(3): S1556-60.
19. Dhale DA, Kalme RK. Pharmacognostic characterization of stem and root of Adhatoda zeylanica Medicus. International Journal of pharmaceutical sciences and research 2012 Nov 1; 3(11): 4264.
20. Haq ME, Ikram M, Warsi SA. Chemical composition of Adhatoda vasica (L.) II. Pak. J. Sci. Ind. Res 1967; 10: 224-5.
21. Lahiri PK, Pradhan SN. Pharmacological investigation of Vasicinol-alkaloid from Adhatoda vasica Nees. Indian Journal of Experimental Biology 1964 Jan 1; 2(4): 219.
22. Bhattacharyya D, Pandit S, Jana U, Sen S, Sur TK. Hepatoprotective activity of Adhatoda vasica aqueous leaf extract on D-galactosamine-induced liver damage in rats. Fitoterapia 2005 Mar 1; 76(2): 223-5.
23. Joshi BS, Newton MG, Lee DW, Barber AD, Pelletier SW. Reversal of absolute stereochemistry of the pyrrolo [2, 1-b] quinazoline alkaloids Vasicine, vasicinone, vasicinol and vasicinolone. Tetrahedron: Asymmetry 1996 Jan 1; 7(1): 25-8.

24. Haq ME, Ikram M, Warsi SA. Chemical composition of Adhatoda vasica (L.) II. Pak. J. Sci. Ind. Res 1967; 10: 224-5.
25. Rawat MS, Pant S, Badoni YS. Negi. Biochemical investigation of some wild fruits of Garhwal Himalayas, Prog Hortic 1994; 26: 1-2.
26. Bhartiya HP, Gupta PC. A chalcone glycoside from the flowers of Adhatoda vasica. Phytochemistry 1982 Jan 1; 21(1): 247.
27. Jain MP, Koul SK, Dhar KL, Atal CK. Novel nor-harmal alkaloid from Adhatoda vasica. Phytochemistry 1980 Jan 1; 19(8): 1880-2.
28. Dweck AC. The William garden collection of Chinese medicinal plants; 1995. p. 31.
29. Hossain MT, Hoq MO. Therapeutic use of Adhatoda vasica. Asian Journal of Medical and Biological Research 2016 Aug 9; 2(2): 156-63.
30. Nadkami KM, Nadkarni AK. Dr KM Nadkarmi's Indian Materia Medica: With Ayurvedic, Unani-Tibbi, Siddha, Allopathic, Homeopathic, Naturopathic and Home Remedies, Appendices and Indexes. Popular Prakashan; 1976. p. 736-737.
31. Kumar A, Ram J, Samarth RM, Kumar M. Modulatory influence of Adhatoda vasica Nees leaf extract against gamma irradiation in Swiss albino mice. Phytomedicine. 2005;12(4):285-293.
32. Gangwar AK, Ghosh AK. Medicinal uses and pharmacological activity of Adhatoda vasica. Int J Herb Med. 2014;2(1):88-91.
33. Chowdhury b, bhattacharyya p. Vasicine and related-compounds. 4. Adhavasinone-a new quinazolone alkaloid from adhatoda-vasica nees. In.: soc chemical industry 14 belgrave square, london, england sw1x 8ps; 1987. P. 35-36.
34. Bhat, V.S., Nasavatl, D.D., Mardikar, B.R. 1978. Adhatoda vasica an Ayurvedic plant. Indian Drugs, 15: 62-66.
35. Jain, M.P., Koul, S.K., Dhar, K.L. and Atal, C.K. 1980. Novel nor-harmal alkaloid from Adhatoda vasica. Phytochem., 19: 1880-1882.
36. Huq, M.E., Ikram, M., Warsi, S.A., 1967. Chemical composition of Adhatoda vasica Linn. II. Pakistan Journal of Scientific and Industrial Research 10, 224–225.
37. Gupta, O.P., Sharma, M.L., Ray Ghatak, B.J., Atal, C.K., 1977a. Pharmacological investigations of vasicine and vasicinone-the alkaloids of Adhatoda 6asica. Indian Journal of Medical Research 66, 680–691.
38. Jabeen, S., Shah, M.T., Khan, S. and Hayat, M.Q. 2010. Determination of major and trace elements in ten important folk therapeutic plants of Haripur basin, Pakistan. J. Med. Plants Res., 4: 559-566.
39. Claeson UP, Malmfors T, Wikman G, Bruhn JG. Adhatoda vasica: a critical review of ethnopharmacological and toxicological data. J Ethnopharmacol. 2000;72(1-2):1-20.
40. Gupta OP, Sharma ML, Ghatak BJ, Atal CK. Pharmacological investigations of vasicine and vasicinone--the alkaloids of Adhatoda vasica. Indian J Med Res. 1977;66(4):680-691.
41. Roy DC, Shark M, Faruquee HM. A Brief Review On Phytochemistry and Pharmacological Properties of Adhatoda vasica. Journal of Tropical Medicinal Plants. 2013;14.
42. Gangwar AK, Ghosh AK. Medicinal uses and pharmacological activity of Adhatoda vasica. Int J Herb Med. 2014;2(1):88-91.
43. Gupta OP, Sharma ML, Ghatak BJ, Atal CK. Pharmacological investigations of vasicine and vasicinone--the alkaloids of Adhatoda vasica. Indian J Med Res. 1977;66(4):680-691.
44. Jain M, Koul S, Dhar K, Atal C. Novel norharmal alkaloid from Adhatoda vasica Phytochemistry. 1980;19(8):1880-1882.
45. Singh SK, Patel JR, Dangi A, Bachle D, Kataria RK. A complete over review on Adhatoda vasica a traditional medicinal plants. Journal of Medicinal plants studies. 2017;5(1):175-180.
46. Jha DK, Panda L, Lavanya P, Ramaiah S, Anbarasu A. Detection and confirmation of alkaloids in leaves of Justicia adhatoda and bioinformatics approach to elicit its anti-tuberculosis activity. Appl Biochem Biotechnol. 2012;168(5):980-990.
47. Duraipandiyan V, Al-Dhabi NA, Balachandran C, Ignacimuthu S, Sankar C, Balakrishna K. Antimicrobial, antioxidant, and cytotoxic properties of vasicine acetate synthesized from vasicine isolated from Adhatoda vasica L. Biomed Res Int. 2015;2015:727304.

48. Banerji J, Chatterjee A, Saha M, Dhara K, Kanrar S, Mukherjee P, Neuman A, Prange T. Reactions and rearrangements of triterpenoids-3- epitaraxerol and its transformation products. 1999.
49. Bhambhani S, Karwasara VS, Dixit VK, Banerjee S. Enhanced production of vasicine in Adhatoda vasica (L.) Nees. cell culture by elicitation. Acta physiologiae plantarum. 2012;34(4):1571-1578.
50. P Singh T, M Singh O, B Singh H. Adhatoda vasica Nees: phytochemical and pharmacological profile. The Natural Products Journal. 2011;1(1):29- 39.
51. Bhambhani S, Karwasara VS, Dixit VK, Banerjee S. Enhanced production of vasicine in Adhatoda vasica (L.) Nees. cell culture by elicitation. Acta physiologiae plantarum. 2012;34(4):1571-1578.
52. Dhankhar S, Kaur R, Ruhil1 S, Balhara1 M, Dhankhar S and Chhillar AK. A review on Justicia adhatoda: A potential source of natural medicine. Afr J Plant Sci, 2011; 5(11): 620-627.
53. Karthikeyan A, Shanthi V, Nagasathya A. Preliminary Phytochemical and antibacterial screening of crude extract of the leaf of Adhatoda vasica (L). Int J Green Pharm, 2009; 3: 78-80.
54. Shinwari ZK, Khan I, Naz S, Hussain A. Assessment of antibacterial activity of three plants used in Pakistan to cure respiratory diseases. Afr J Biotechnol, 2009; 8: 7082-7086.
55. Hassan MH, Ismail MA, Moharram AM, Shoreit AA. Phytochemical and antimicrobial of latex serum of Calotropis procera and its silver nanoparticles against some reference pathogenic strains. J Ecol Health Environ 2017;5:65-75.
56. Baydar H, Sagdic O, Ozkan G, Karadogan T. Antibacterial activity and composition of essential oils from Origanum, Thymbra and Satureja species with commercial importance in Turkey. Food control 2004;15:169-72.
57. Aiman R. Recent research in indigenous antidiabetic medicinal plants--an over-all assessment. Indian J Physiol Pharmacol. 1970;14(2):65-76.
58. Patil MY, Vadivelan R, Dhanabal SP, Satishkumar MN, Elango K, Antony S. Anti-oxidant, anti-inflammatory and anti-cholinergic action of Adhatoda vasica Nees contributes to amelioration of diabetic encephalopathy in rats: Behavioral and biochemical evidences. International Journal of Diabetes in Developing Countries 2014 Mar 1; 34(1): 24-31.
59. Gulfarz M, Ahmad A, Asad MJ, Sadiq A, Afzal U, Imran M, Anwar P, Zeenat A, Abbasi KS, Maqsood S, Qureshi RU. Antidiabetic activities of leaves and root extract of Justica Adhatoda Linn against alloxan induced diabetes in rats. African Journal of Biotechnology. 2011;10(32):6011-6013.
60. Dhar ML, Dhar MM, Dhawan BN, Mehrotra BN, Ray C. Screening of Indian Plants for Biological Activity : I. Indian Journal Of Experimental Biology. 1968;6(4):232-247.
61. Madak AT, Rao R. Hypoglycemic activity of anon nitrogenous principle from the leaves of Adhatoda vasica. Indian Journal Of Pharmacy. 1996; 105-106.
62. Dangi A. Phytochemical screening and assessment of Adhatoda vasica (Leaf) for antiasthmatic activity. Panacea Journal of Pharmacy and Pharmaceutical Sciences ISSN: 2349-7025. 2015;4(3):680-704.
63. Singh P, Anand A, Kumar V. Recent developments in biological activities of chalcones: A mini review. European journal of medicinal chemistry. 2014;85:758-777.
64. Nilani P, Kasthuribai N, Duraisamy B, Dhamodaran P, Ravichandran S, Ilango K, Suresh B. Invitro antioxidant activity of selected antiasthmatic herbal constituents. Anc Sci Life. 2009;28(4):3-6.
65. Ahmad R, Raja V, Sharma M. 2013. Hepatoprotective Activity of Ethyl Acetate Extract of Adhatoda Vasicain Swiss Albino Rats. Int J Cur Res Rev, 5:16-21.
66. Mohan Y, Patil R, Vadivelan SP, Dhanabal MN, Kumar S. 2014. Anti-oxidant, anti-inflammatory and anticholinergic action of Adhatoda vasica Nees contributes to amelioration of diabetic encephalopathy in rats: Behavioral and biochemical evidences. International Journal of Diabetes in Developing Countries, 34(1):24-31.
67. Ahmad S, Garg M, Ali M, Singh M, Athar MT, Ansari SH. A phyto-pharmacological overview on Adhatoda zeylanica Medic. syn. A. vasica (Linn.) Nees. 2009.
68. Gupta R, Thakur B, Singh P, Singh HB, Sharma VD, Katoch VM, Chauhan SV. Anti-tuberculosis activity of selected medicinal plants against multidrug resistant Mycobacterium tuberculosis isolates. Indian J Med Res. 2010;131:809-813.
69. Roy CD, Shaik MM, Munnan M, Faruqee, & Md, H. A brief review on phytochemistry and pharmacological properties of Adhatoda vasica. Journal of Tropical Medicinal Plants. 2013;14:115-124.

70. Chandhoke N. Vasicine, the alkaloid of Adhatoda vasica. Indian Drugs. 1982;24:425-426.
71. Soni S, Anandjiwala S, Patel G, Rajani M. Validation of Different Methods of Preparation of Adhatoda vasica Leaf Juice by Quantification of Total Alkaloids and Vasicine. Indian J Pharm Sci. 2008;70(1):36-42.
72. Singh SK, Patel JR, Dangi A, Bachle D, Kataria RK. A complete over review on Adhatoda vasica a traditional medicinal plants. Journal of Medicinal plants studies. 2017;5(1):175-180.
73. Gangwar AK, Ghosh AK. Medicinal uses and pharmacological activity of Adhatoda vasica. Int J Herb Med. 2014;2(1):88-91.
74. Ahmad R, Raja V, Sharma M. Hepatoprotective activity of ethyl acetate extract of Adhatoda vasicain Swiss albino rats. Int J Cur Res Rev. 2013;5(06).
75. Afzal U, Gulfraz M, Hussain S, Malik F, Maqsood S, Shah I, Mahmood S. Hepatoprotective effects of Justicia adhatoda L. against carbon tetrachloride (CCl4) induced liver injury in Swissalbino mice. African Journal of Pharmacy and Pharmacology. 2013;7(1):8-14.
76. Bhattacharyya D, Pandit S, Jana U, Sen S, Sur TK. Hepatoprotective activity of Adhatoda vasica aqueous leaf extract on D-galactosamine-induced liver damage in rats. Fitoterapia. 2005;76(5):10-14.
77. C. Balachandran K, Chennakesava Rao, Arun Y, Emi N. Yamamoto N, Inaguma Y, Okamoto A, Perumal PT. 2016. Synthetic investigation on chirally pure Mannich derivatives of pseudophenylpropanolamine and their anticancer properties against HepG-2 cells with inhibition of JAK2/STAT3, RSC Advances, 6:96946–96962.
78. Ahmad R, Raja V, Sharma M. Hepatoprotective activity of ethyl acetate extract of Adhatoda vasicain Swiss albino rats. Int J Cur Res Rev. 2013;5(06).
79. Singh B, Sharma RA. Anti-inflammatory and antimicrobial properties of pyrroloquinazoline alkaloids from Adhatoda vasica Nees. Phytomedicine. 2013;20(5):441-445.
80. Megraj KVK, Raju K, Balaraman R, Meenakshisundaram K. Biological activities of some Indian medicinal plants. Journal of Advanced Pharmacy Education & Research. 2011;1(1):12-44.
81. P Singh T, M Singh O, B Singh H. Adhatoda vasica Nees: phytochemical and pharmacological profile. The Natural Products Journal 2011 Jul 1; 1(1): 29-39.
82. Chaturvedi GN, Rai NP, Dhani R, Tiwari SK. Clinical trial of Adhatoda vasica syrup (vasa) in patients of non-ulcer dyspepsia(Amlapitta). Ancient Science Of Life. 1983;3(1):19.
83. Srivastava, Banerjee, Nivsarkar, Manish, 2006. Anti-ulcer activity of Adhatoda vasicaNees. Journal of Herbal Pharmacotherapy; vol. 6; nb. 2; 43 – 49.
84. Shrivastava N, Shrivastava A, Banarjee A, Nivsarkar M. AntiUlcer Activity of Adhatoda vasica Nees. Journal of Herbal Pharmacotherapy. 2009;6(2):43-49.
85. Chavan R, Chowdhary A. In vitro inhibitory activity of Justicia adhatoda extracts against influenza virus infection and hemagglutination. Int J Pharm Sci Rev Res. 2014;25(02):231-236.
86. Dorsch W, Wagner H. New anti-asthmatic drugs from traditional medicine?. International Archives of Allergy and Immunology 1991; 94(1-4): 262-5.
87. Dhuley JN. Antitussive effect of Adhatoda vasica extract on mechanical or chemical stimulation-induced coughing in animals. Journal of Ethnopharmacology 1999 Nov 30; 67(3): 361-5.
88. Lahiri PK, Pradhan SN. Pharmacological investigation of Vasicinol-alkaloid from Adhatoda vasica Nees. Indian Journal of Experimental Biology 1964 Jan 1; 2(4): 219.
89. Wagner H. Search for new plant constituents with potential antiphlogistic and anti-allergic activity. Planta medica 1989 Jun; 55(03): 235-41.
90. P Singh T, M Singh O, B Singh H. Adhatoda vasica Nees: phytochemical and pharmacological profile. The Natural Products Journal 2011 Jul 1; 1(1): 29-39.
91. Gupta OP, Anand KK, Ghatak BJ, Atal CK. Vasicine, alkaloid of Adhatoda vasica, a promising uterotonic abortifacient 1978; 16: 1075-1077.
92. Chandokhe N, Gupta OP, Atal CK. 353. Abortifacient activity of the alkaloid Vasicine through the release of prostaglandins. Journal of Steroid Biochemistry 1978 Sep 1; 9(9): 885.
93. Chandokhe N. Vasicine-the alkaloid of Adhatoda vasica a novel abortifacient. Indian drugs 1987; 24: 425-9
94. Rao MN, Krishnan S, Jain MP, Anand KK. Synthesis of Vasicine and vasicinone derivatives for oxytocic and bronchodilatory activity. Chemischer Informationsdienst 1983 Sep 27; 14(39).

95. Bhatt BP, Panwar MS. Plants with fertility regulating potential of Garhlwal Himalayas. J Econ Phytochem 1990; 1: 33-4.
96. Bhattacharyya D, Pandit S, Jana U, Sen S, Sur TK. Hepatoprotective activity of Adhatoda vasica aqueous leaf extract on D-galactosamine-induced liver damage in rats. Filoterapia 2005 Mar 1; 76(2): 223-5.
97. Singh SK, Patel JR, Dangi A, Bachle D, Kataria RK. A complete over review on Adhatoda vasica a traditional medicinal plants. Journal of Medicinal Plants 2017; 5(1): 175-80.
98. Shrivastava N, Srivastava A, Banerjee A, Nivsarkar M. Anti-ulcer activity of Adhatoda vasica Nees. Journal of herbal pharmacotherapy 2006 Jan 1; 6(2): 43-9.
99. Patil MY, Vadivelan R, Dhanabal SP, Satishkumar MN, Elango K, Antony S. Anti-oxidant, anti-inflammatory and anti-cholinergic action of Adhatoda vasica Nees contributes to amelioration of diabetic encephalopathy in rats: Behavioral and biochemical evidences. International Journal of Diabetes in Developing Countries 2014 Mar 1; 34(1): 24-31.
100. Lahiri PK, Pradhan SN. Pharmacological investigation of Vasicinol-alkaloid from Adhatoda vasica Nees. Indian Journal of Experimental Biology 1964 Jan 1; 2(4): 219.
101. Atal CK. 1980. Chemistry and Pharmacology of vasicine: A new oxytocin and abortifacient. Indian Drugs, 15: 15-18.
102. V. Amla, G. Bano, R.K. Johri, et al. Pharmacokinetics of vasicine in healthy Indian volunteers. Acta Pharmacol. Sin., 1987, 8: 190-192.
103. Zutschi U, Rao PG, Soni A, Gupta OP, Atal CK. 1980. Absorption and distribution of vasicine, a novel uterotonic. Planta Medica, 40: 373-377.
104. Atal CK. 1980. Chemistry and Pharmacology of vasicine: A new oxytocin and abortifacient. Indian Drugs, 15: 15-18. Avula, Bahrathi, Begum, Ahmed, Choudhary, Khan 2008.

Table. 1 Phytochemical constituents of *Adhatoda vasica*.

Sl.No.	Parts of Plant	Chemical constituents	Pharmacological activity	References
1	Leaves, Roots & Flowers	Vasicine	Bronchodilator, Respiratory stimulant, Uterine stimulant, Anti-inflammatory	39, 40, 41
2.	Leaves, Roots	Vasicinone	Anti-Tussive,Bronchodilator, Uterine activity, Anti-cancer	42,43
3.	Roots	B-sitosterol	Anti-inflammatory, Anti-diabetic, Anti-cancer	44, 45,46
4.	Roots	B-glucosidegalactose	Anti-diabetic, Hepatoprotective	47
5.	Flowers	Kaempferol	Anti-inflammatory, hepatoprotective	48
6.	Flowers	Quercetin	Anti-ulcer, Anti-bacterial, Anti-inflammatory	49
7.	Leaves	Adhatodine	Anti-allergic, Anti-tubercular	48, 49
8.	Roots	Deoxyvasicine	Acetylcholinesterase Inhibitor	50
9.	Roots	Epitaraxerol	Anti-thyroid Activity	48,51
10.	Whole plant	Carotene	Anti-oxidant	52
11.	Seeds	Arachidic acid	Hepatoprotective, Insecticidal	53
12.	Seeds	Linoleic acid	Hepatoprotective, Anti-cancer, Anti-inflammatory	53
13.	Seeds	Oleic acid	Analgesic, Anti-inflammatory	53
14.	Seeds	Be- henic acid	Hepatoprotective, Insecticidal	54

Table. 2 Experimental studies on *Adhatoda vasica* plant

Type of Extract	Experimental animal model	Pharmacological activity	Reference
Leaf and Root extract of *A.vasica*	Guinea pig, Rabbit	Anti-asthmatic, bronchodilator activity	89,90,91
Methanolic extract	Mice, Rat, Guinea pig	Anti-allergic and Anti-asthmatic activity	89,90
Aqueous Extract	Albino rats, Guniea pig, rabbit	Abortifacient and uterotonic activity	91,92,93,94
Petroleum ether	Mice	Expectorant	95
Ethyl acetate extract	Swiss albino rat	Hepatoprotective	96
Alcoholic and aqueous extract	*Staphylococcus aureus* and *E. coli*	Anti-microbial activity	97
Ethanolic extract	Wistar rat	Anti-ulcer and Anti-diabetic activity	98, 99, 100

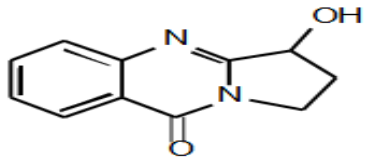

Fig.1. Vasicine

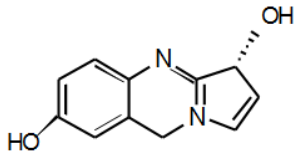

Fig. 2. Vasicinol

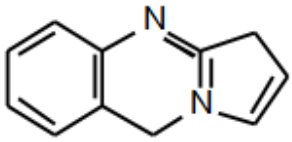

Fig. 3. Deoxyvasicine

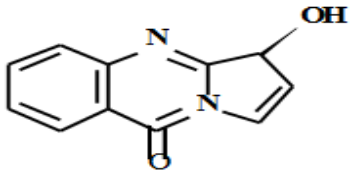

Fig. 4. Vasicinone

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RESEARCH ARTICLE

Urinary Tract Infection in Pregnancy

Dhwani Pandya[1] and Anupama Shrivastav[2]*

[1]Student, M.Sc Microbiology, Department of Life Sciences, Parul Institute of Applied Sciences, Parul University, Vadodara, Gujarat, India.
[2]Institute for Technology and Management- ITM Vocational University, Faculty of Life Health and Allied Sciences, Vadodara, Gujarat, India.

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***Address for Correspondence**
Anupama Shrivastav
Institute for Technology and Management- ITM Vocational University,
Faculty of Life Health and Allied Sciences,
Vadodara, Gujarat, India.
Email: Anupama.shrivastav82045@paruluniversity.ac.in

ABSTRACT

Urinary tract infections are a major problem for many women, and they are associated with multiple visits to various health care providers and quite often repeated tests and treatments, with a higher risk of potential risks. It is well established that urinary tract infections are more common in female patients of all ages. Up to 80% of females will have at least one UTI in their lifetime, with up to 45 percent having recurrent UTIs. Given their prevalence, urinary tract infections (UTIs) pose a significant burden—without prompt and effective treatment, symptoms can be debilitating for several days and have an impact on work and daily routines.

Keywords: UTI in their lifetime, common in female patients, Urinary tract infections

INTRODUCTION

The condition in which bacteria are established and multiplied within the urinary tract is called urinary tract infection. Urinary tract infection is common in pregnancy needs to be studies because it is potentially lethal and preventable. An understanding of the predisposing factors and their prevention will help reduce the morbidity and mortality from the disease. Here, bacteria of the maternity has drawn attention of accoucheur everywhere the planet owing to its effects on the mother and vertebrate. Pregnant girls square measure at accrued risk for UTIs. starting from half dozen and peaking throughout weeks 22-24[47].Several factors increase the chance of UTI in gestation. These factors embrace relative obstruction of the ureters, sleek muscle relaxation of the canal and bladder, and symptom, which give a positive setting for the expansion of the microorganism. *E. coli* is that the most typical organism isolated from the cultures, Proteus Mirabilis and Klebsiella. Respiratory diseases are determined. Less common agents embrace type B *Streptococci* and *staphylococcus saprophyticus*[53].Up tp70% of pregnant ladies develop glycosuria, which Inspire bacterial growth in urine. In urinary the lower urinary tract to resist occupy

microbe. Acute pyelonephritis throughout maternity may be a serious general ill health that may accomplish maternal infection, labor and premature delivery. The maternal and infant complication of a UTIs throughout maternity are often devasting.

HISTORICAL ASPECTS

Urinary tract infection is a frequent problem confronting the Physician. Infection could involve the higher tract or lower tract. though microbic etiology of tract or lower tract. Though microbic etiology of urinary infection is that the same throughout, the urinary clinical options, response to medical care and supreme prognosis are verify by the positioning of infection. an outsized variety of antibiotics are obtainable for the treatment of UTI. Over the years there has been a gradual amendment within the organisms inflicting tract infection and a rise within the incidence of multiple drug resistant bacterium, leading to therapeutic difficulties. it's additionally been ascertained that pattern of sensitivity of bacterium disagree from hospital to hospital and from population to population reflective the degree of exposure to a selected medicinal drug agent. Urinary tract infection was one of the familiar infections to occur in pregnancy. Specific physiological changes during pregnancy, changes in ureter, decrease in the bladder tone, sluggish rate of urine flow, mechanical effects of pregnant uterus and hormonal changes may predispose to infection[18]. A study of physiological condition, urinary tract infection and prematureness states that bacteria is common with a generality of 100 % throughout physiological condition [30]. The recommendation is to screen pregnant ladies at their 1st antenatal visit and through the trimester and not once more, unless their initial take a look at result's positive, or they develop symptoms. urinary tract infection happens in 0.3-13% of pregnancies however doesn't seem to be associated with ASB. pyelonephritis happens in 1-2% of pregnancies.

Complications of tract infection embrace internal organ lump and acute metastasis distress syndromes, transient viscus pathology, anemia, preterm delivery [40].The study on UTIs & physiological state shows that infection of the tract is most typical form of infection in pregnant ladies, with a prevalence starting from 5-10%. the bulk of symptomatic UTIs happens in ladies with pre-existent well bacteriuria, because of the changes the tract undergoes throughout physiological state. so as to avoid probably serious complications touching maternal and fetal health, AN early diagnosing followed by immediate and adequate care is necessary[4]. Hoja, Hefner and Smith in 1964 by analysis of one thousand patients disclosed 143 United Nations agency had bacteriuria (14.3%). They explicit a moderate increase in clinical tract sickness in patient with bacteriuria[24]. A study was conducted on UTIs in physiological state shows that tract infection is one of the general medical complications of physiological state, occurring in roughly 100 percent about all pregnancies. The clinical entitles most ordinarily seen area unit bacteriuria, acute urinary tract infection, and urinary tract infection. Relative statis of excretion because of pregnancy-induced changes within the tract could be a vital motive issue. *E. coli* is that the most typical accountable organisms.

HOST DEFFENCE MECHANISM

At one time microbe reach the urinary tract, 2 components discover whether contamination ensues

(I) The Pathogenicity and inoculum size about microorganism.

(II) Adequacy of the host defense mechanism.

Defense mechanism in the urine:

The urine supports the growth of non-fastidious bacteria. This is understandable because.

- The urine has an allowable chemical construction for bacterial accumulation.
- Human urine lacks effective immunological and biological defense mechanism in case of bacteria. (14)
- Presence of ammonia ion in urine interferes with the action of complement.
- Urine from traditional people is also restrictive or perhaps antiseptic for microorganisms to blame for UTIs once the inoculant is little. restrictive factors are;
- Prone Osmolarity: a really dilute water inhibits microorganism growth and high osmolarity with low pH scale is extremely restrictive. Therefore, high fluid intake throughout medical care provides many benefits.
- PH: restrictive activity tends to extend as pH scale is down

- Urea- Experimental evidence suggests that antibacterial activity is more of a function of urea contents of urine.
- Organic acid- In undissociated form organic acids can penetrate bacterial cell membrane and expert bacteriostatic effect.

Defense in urethra

The exact nature of urethral defense mechanism is unknown. The urine flow may washout these bacteria. Bacterial accumulation in the usual urethra is prohibited by the normal resident flora[8].

Defence in the bladder

The normal vesicle appears to be constitutionally immune to contamination. The inherent resistance to infection is explained by evacuation bladder which is able to take away the majority of infected bacterium ability of the mucous membrane to exert a antiseptic impact presence of glucosamine glycans on the surface of the bladder and therefore the supermolecule connected from the nephritic tubules. This supermolecule is wealthy in mannose residue and *E. coli* expressing kind one fimbriae, get hooked up to the current and kind clumps and are expelled so they're prevented from penetrating bladder mucous membrane[17].

Defence in the Kidney

The cortex of the kidney is relatively resistant to infection. About 100,000 microorganisms must be injected into the cortex to produce infection. In will produce infection. Several mechanisms explain the difference in defense mechanism between cortex and medulla[16].

I. The concertation of the ammonia in renal medulla interferes with phagocytosis and complement c4.
II. Relative anoxia of medulla due to decreased blood supply.
III. Hyperosmolality of renal medulla interfering with leucocyte migration.
IV. PH of renal medulla interference with complement activation.
V. ‘ L ’ forms persisting in renal medulla.

Immunological Aspects

Recent studies indicate that genitourinary area forms composing a secretary immune mechanism. It has been shown that when infections involve the renal parenchyma antibodies appear in the serum. IgM antibodies control the initial reaction to the upper urinary tract infection. The presence of antibodies synthesized within the kidney of invading microorganisms by local phagocytic cell. The IgG and IgM antibodies activate the complement. It has been suggested that immunoglobulin inside the urine may operate in other ways to stop contamination of specific importance is IgA. Low levels of IgA may be associated with increased urinary tract infection.

Route of Infection

Bacteria will reach the tract by three routes.
I. Hematogenous
II. Lymphatic
III. Ascending

Hematogenous

This account for fewer than third-dimensional cases of tract infections and urinary tract infection. the key organisms inflicting hemopoietic infections *are staphylococci aureus*, *Salmonella*, genus *Pseudomonas* and fungus, different bacterium that are uncommon causes of hematogenous infection are *coccobacillus*, *Nocardia*, *zoonotic* disease, TB[10].

Lymphatic

Through the infection along the lymphogenous route is not clearly determined many workers think that it may be of importance.

Ascending route

95% of urinary tract infection is form ascending route to the kidney. Conquest the microorganisms inside the urethra from external sources constitute the general tract for urinary tract infection, mostly organisms of enteric origin, example Escherichia coli and *Enterobacteriaceae*. In females, the urethra is small and is more responsible to contaminate with colonic flora that occupy on the perineal skin [51].

CLINICAL ASPECTS

Symptoms of the urinary tract infection:

The indicator of urinary tract infection are variable. Some patients with UTIs are without any significant symptoms, while in other symptoms are incapacitating. Symptoms help in localizing urinary tract infection, selection of lab test and follow up of patients.

Diagnosis

The corner stone for diagnosis of urinary tract infection is urine culture. Collection of urine specimen should be achieved in a way that bacteria from the outer epithelial mucosa does not poison the sample. In female the epithelial area and labia are cleaned with antiseptic. To minimize contaminations the labia should be help open during micturition. The midstream urine is obtained for culture. Catheterization is an alternative way for acquire the urine from women. However, this method should be avoided as insertion of catheter that damage the uroepithelium introduce bacteria and cause infection. If the culture of voided urine contains more than 10^5 bacteria per ml in a patient not on antibiotics the probability of UTI is approximately 85%. If the same results are obtained in the second culture the probability is 95%. 5% false rate are due to inadequate cleaning of periurethral area of urine that was not cultured within 2 hours of collection. If the urine contained less than 10^5 bacteria per ml it represented contamination. Although most gram-negative bacilli separate soon after division and divide again, gram positive cocci tend to stick together and divide slowly therefore lower colony count of 10^4/ml or perhaps even 10^3/ml in gram positive isolates. In more than 95% of true urinary tract infection a single organism is responsible for infection. Polymicrobial infection is seen when there is catheter induced, foreign body induced infection or when patients have neurogenic bladder. Whenever, the differentiation between contamination and true infection is in doubt a suprapubic bladder aspiration can be performed.

Gram Stain

If there's a bacterium on a dried drop of global organization centrifuges pee than in 80-90% there'll be vital bacteriuria on culture. Gram method helps to differentiate gram positive from negative bacterium.

Urine Microscopy

Here range of cells in global organization centrifuged pee per high power field is measured. every WBC/HPF represents regarding 0.5 1,000,000 excreted twenty-four hours betting on variables like pee volume. once a couple of corpuscle square measure seen in association with varied squamous animal tissue cells extraneous urinary contamination is probably going. In AN otherwise clean catch specimen even a little range of corpuscle properly indicate inflammation. The presence of 10 or a lot of being leucocytes per high field, on research examination is important. If pee proves sterile on culture, it's known as sterile symptom. the chance of infectious disease should be thought of and dominated out. The absence of symptom though uncommon doesn't exclude tract contamination.

Culture

Uropathogens differ in their growth requirement and often selective media are used to encourage the growth of certain organisms while repressing the growth of other bacteria. Most of the uropathogens grow at 37 ºC. A know amount of urine usually (o,1) is placed into agar medium and incubated. After 18-24 hours of incubation the agar medium is investigate for colonies of microbes. A single bacterium put on the agar medium will increase to make clear colonies containing millions of changeable organisms. Thus, colony constitute single pathogen from the initial specimen and is known colony forming unit.

Identification of few common bacterial based on colony morphology

E. coli- is a dry opaque pink colour colony, the pink colour of the colony diffuses deeply into deoxy chocolate medium. Klebsiella- Shiny, heaped up pink colonies the surrounding deoxy cholate does not turn pink as in cases of *E. coli*. *Pseudomonas*- On blood agar appears greenish grey colour. It has a typical aromatic odour. *Proteus mirabilis*- It forms colour less colonies on deoxy cholate , but on blood agar it spreads as a grey film. *Streptococcus faecalis*- On blood agar they form small grey, more or less translucent dew drop colonies without hemolysis.

Chemical Method

Nitrite-Nitrite teat: Bacteria reduce nitrite in urine to nitrate. The presence of nitrite can be measured calorimetrically. Tetrazolium reduction: Triphenyl tetrazolium is decrease to bright red colored triphenyl formazan in the presence of significant bacterium.

Glucose Oxidase

Little amount of sugar present in urine is digest by bacteria. Absence can be detected with by dip stick.

Catalase

When urine containing catalase and hydrogen peroxide, mixed bubbles of oxygen are released. However, these tests are not of much clinical use.

Antibiotic Sensitivity of the Testing the Uropathogen

Kirby-Bauer method: Here antibiotic coated disc is placed on the surface of agar. Incubate the plates at 37º C for 18-24 hours. After the overnight incubation, measure the thickness of zone of inhibition all over the disc using a scale. Each zone was interpreted as resistant, Intermediate, or sensitive with reference to the Kirby-Bauer chart.

CONCLUSION

E.coli is the most ordinary causative agents of UTI in pregnancy.UTI is the most ordinary bacterial infection in pregnancy. Screening of bacteriuria in pregnancy should be a must, as drug resistant is common among bacteria causing UTI. Proper safe antibiotic therapy be considered since; urinary tract infections are associated with risk to bothmotherandthefetus.For many cases, antimicrobial agents continue to stay the core component of treatment and p revention, and more research into the benefits and risks associated with these approaches is required.

REFERENCES

1. Andriole VT, Patterson TF. Epidemiology, natural history, and management of urinary tract infections in pregnancy. Med Clin North Am 1991;75:359-73
2. Chart R. Adherence of cervical, vaginal and distal urethral normal micro flora to human uro epithelial cells. Urol.1984;131:596
3. Christensen. Genito urinary TB review of 102 case. Med 1974;53:577
4. Delcroix M, zone V, Cheront C. Urinary infection in the pregnant women. Obstet Gynaecol.1994;89:277-84.
5. Fleming A. On a remarkable bacteriolytic element found in tissue and secretions. Proc Voy society. 1922;93:906.
6. Freedman: Experimental pyelonephritis Yale J Bio Med 1958;30:406-16.
7. Gilstrap LC. An aggressive approach to UTI during pregnancy. Obstet Gynecol 1987;30:23
8. Hoja WA, Hefner JD, Smith MR. Bacteriuria in pregnancy. Obstet Gynecol 1964;24: 458.
9. Kaitz AL, Hodder EW. Bacteriuria and pyelonephritis. Med. 1961; 265:667.
10. Kass EH. Pregnancy, pyelonephritis and prematurity. Clin obstet Gynecol 1970; 13:239-54.
11. Lucas MJ, Cunningham FG. Urinary infection in pregnancy. Clin Obstet Gynecol. 1993;36:855-68.

12. Mc Gregor JA, French JI, Parker R, Draper D, Patterson E, Jones W, et al. Prevention of premature birth by screening and treatment for common genital tract infection : Results of a prospective controlled evaluation. Am J Obstet Gynecol 1995;173:157-67.
13. McNeeley SG. Treatment of urinary tract infections during pregnancy. Clin Obstet Gynecol. 1988;31:480-7.
14. Patterson TF, Andriole VT. Bacteriuria in pregnancy. Infect Dis Clin North Am. 1987;1:807-22.
15. Pfau A, sacks T. The bacterial flora of the vaginal vestibule, urethra and vagina in premenopausal women with recurrent urinary tract infections. J Urol 1981;12:630.
16. Porpiglia M, Cellura A. Urinary tract infection during pregnancy. Minerva Ginecol. 1990;42:263-5.
17. Qureshi RN, Khan KS. Bacteriuria and pregnancy outcome: a prospective hospital-based study in Pakistani women. Pak Med Assoc. 1994;44:12-30.
18. Roschia H : Experimental pyelonephritis ill influence of localised injury in different parts of kidney. Yale J. Bio Med 1958;30: 341.
19. Schnarr J, Smaill F. Asymptomatic and symptomatic urinary tract infections in pregnancy. Eur Clin Invest. 2008;2:5-7
20. Sobel JD, Kaye D; Produced uromucoid secretion in elderly J Int. disease 1985;152:653.
21. Stamey TA : The immunological based of recurrent bacteriuria. Med. 1978;57: 47.
22. Wait RB. Urinary tract infection during pregnancy. Asymptomatic bacteriuria, acute cystitis and acute pyelonephritis. Protgrad Med. 1984;75: 153-7
23. Zinner SH, Kass EH. Long-tern(10-14) follow-up of bacteriuria of pregnancy. N Engl J Med 1971; 285:820-4.

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RESEARCH ARTICLE

In silico Screening and Identification of Active Compounds Characterized From Fruit Peel of *Psidium guajava* against Estrogen Receptor of Breast Cancer

Vino Udappusamy[1] and Rajan Thinagaran[2*]

[1]Research Scholar (Ph.D), Department of Biochemistry, PSG College of Arts and Science, Coimbatore, Tamil Nadu, India

[2]Assistant Professor, Department of Biochemistry, PSG College of Arts and Science, Coimbatore, Tamil Nadu, India

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***Address for Correspondence**
Rajan Thinagaran
Assistant Professor,
Department of Biochemistry,
PSG College of Arts and Science,
Coimbatore, Tamil Nadu, India
Email: drrajan2012@gmail.com

ABSTRACT

Breast cancer is one of the most significantly identified cancers in women. The existing anti-cancer antibiotics are known to produce side effects. The active phytochemicals could be used as potential therapeutic agents for breast cancer that could resolve the defects of existing antibiotics. The fruit extract of Guava (*Psidium guajava*) is a prominent source of bioactive phytochemicals used to treat and manage various diseases. Based on this, our research focused on identifying a bioactive compound in fruit peel extract of *Psidium guajava*, which acts as an antagonist to the receptor and inhibit the synthesis of estrogen. The fruit peel extract of *Psidium guajava* was prepared and characterized with GC-MS. The pharmacokinetic properties of the obtained bioactive compounds are evaluated using SWISS-Adme tool. Additionally, molecular docking was done to determine the potential usage of the phytochemical compound of fruit peel extract of *Psidium guajava* targeting, estrogen receptor alpha in breast cancer treatment. The network pharmacology was performed to determine the mode of action of estrogen receptor alpha towards breast cancer. The *in vitro* anticancer activity of the fruit peel was assessed in MTT assay. Totally seven bioactive compounds were identified in fruit peel of *Psidium guajava*. It was observed that all bioactive compounds pass the rule of five and have good pharmacokinetic properties. The docking analysis revealed that lauren diterpenol exhibited prominent and stable interaction with the target. About 53 interactive genes were identified in network pharmacological studies. The anticancer activity revealed that *Psidium guajava* exhibit 46.52% of cell death at 75 µg/ml concentration against MCF-7 cells. Based on the results obtained, lauren diterpenol has a significant binding relationship and it

could work as an estrogen inhibitor. Hence, the fruit peel extract of *Psidium guajava* was found as apotential candidate for breast cancer treatment.

Keywords: Breast cancer, Estrogen receptor, *Psidium guajava,* Lauren diterpenol, Doxorubicin

INTRODUCTION

Breast cancer is a commonly occurring cancer and is ranked the second leading cause of cancer mortality in females. It was found that 1 in 8 females are diagnosed with breast cancer globally. About 1.7 million individuals have breast cancer and it is estimated that the incidence of breast cancer will increase by more than 20%, with a mortality rate of 14% and projected to increase by 2030. Breast cancer has become an alarming cause of death in the least developed countries. There are four stages of breast cancer, ranging from Stage I to Stage IV. Among these, stage IV is considered the most aggressive type and typically spreads to the bones, lungs, liver or brain. Based on their molecular profiles, it is categorized into hormone receptor-positive [ER (Estrogen Receptor) +], Human EGFR (Epidermal Growth Factor Receptor) 2 / HER2-positive and Triple-negative breast cancer. About 80% of breast cancer are found to be ER(+). The currently available therapies like hormone therapies exhibit a 5-year survival rate of 10%, which was found to be higher than ER (-). Beyond five years, the survival rate reduced or disappeared and the individuals of ER (+) developed resistance to hormone therapies [1,2]. Thus, researchers have focused on implementing phytochemicals as an anti-cancer drug in treating breast cancer patients. Medicinal plants find an important place in medical systems worldwide. Nearly 60% of currently available anti-cancer drugs are derived from natural sources, including phytochemicals of plants. About 94 plant species have been utilized in contemporary medicine. About 80% of plants have to be exploited for therapeutic applications. Hence, investigating the bioactive compounds of plants used in traditional medicine is crucial to identifying and developing newer therapeutics [3]. *Psidium guajava*, known as guava, is a tropical tree that belongs to the phylum Magnoliophyta, class Magnoliopsida and family Myrtaceae. *Psidium guajava* is an evergreen shrub-like tree that reaches the height of 6 to 25 feet and is abundantly grown for harvesting fruits. Guava fruit ranges from small to medium-sized, with a length of 3 to 6 cm. It appears yellow, pear-shaped and has a musky, pleasant odour when ripened.

Its pulp is slightly darker in colourand contains slightly yellowish seeds. The guava fruit contains vitamin A, vitamin C, iron, phosphorus, calcium and phytochemicals like saponin, oleanolic acid, lyxopyranoside, arabopyranoside, guaijavarin, quercetin and flavonoids. *Psidium guajava* is consumed as food and folk medicine in subtropical areas worldwide due to its pharmacologic activities, including anticancer activity. Guava is frequently employed to cure gastroenteritis disorder, hypertension, diabetes, caries, pains and wounds. Phytochemical analysis revealed that the guava fruit is a rich source of triterpenoids and polyphenols, which help to cure cancerous cells [4,5]. Currently, computational-based studies have been widely performed to evaluate the therapeutic application of the desired product in a shorter time. Based on this, Network pharmacology becomes a promising tool to systematically assess the relationship between targets, diseases, and drug molecules. This analysis is utilized to construct a compound–target–gene-disease network that makes it easy to understand the multi-target mechanism of natural product constituents. Molecular docking is a theoretical simulation method that mainly studies intermolecular interactions and predicts their binding mode and affinity. It can determine the binding sites and affinity of the target and drug molecule[6].Based on the literature studies, we found limited research on the exploring medicinal properties of phytochemical constituents of fruit peel extract of guava. In particular, beneficial characteristics involving the chemoprevention of breast cancer have not yet been exploited. Therefore, this work aimed to find the bioactive phytochemical of fruit peel extract of *Psidium guajava* that can suppress the estrogen synthesis that mediates breast cancer treatment.

MATERIALS AND METHODS

Preparation and Characterization of fruit peel extract of *Psidium guajava*

The fresh fruits of *Psidium guajava* were collected from Palani, Dindigul, Tamil Nadu, India. The collected fruits were washed thoroughly with water to remove the surface contaminants. Then the skin of the fruit was peeled and surface-sterilized with distilled water. It was shade-dried, pulverized and soaked in ethanol for 24-48 hours. The crude extract obtained was filtered using Whatman No 1 filter paper and centrifuged at 12,000 rpm for 15 minutes. The ethanolic fruit peel extract was evaporated, concentrated and stored at 4^{o}C[7]. The concentrated ethanolic fruit peel extract of *Psidium guajava* was subjected to GC-MS (Gas chromatography-Mass spectrometry), Scion 436-GC Bruker, US, for the identification of active phytochemical constituents. The GC-MS was done the on Scion 436-GC Bruker model coupled with a fused silica capillary column BR-5MS (5 percent Diphenyl/95 percent Dimethylpolysiloxane). Triple Quadruple Mass Spectrophotometer with a length of 30 m, internal diameter of 0.25 mm and thickness of 0.25 μm. The helium gas (99.999 %) was used as the carrier gas at a steady flow rate of 1 ml/min, and an injection volume of 2 μl was used (split ratio of 10:1). The obtained spectra were analyzed and compared with NIST (National Institute of Standards and Technology) database [8].

ADME studies

The pharmacokinetic properties (ADME) of the fruit peel extract of *Psidium guajava* were determined by SwissADME prediction (http://www.swissadme.ch/). The molecular descriptors, including molecular weight, hydrogen bond acceptor, hydrogen bond donor, LogP (Lipophilicity), molar refractivity, number of rotatable bonds, topological polar surface area, and violations of Lipinski's rule of five were calculated to establish their pharmaceutical credibility [9].

PASS prediction

The obtained major phytoconstituents of fruit peel extract of *Psidium guajava*, namely, 5-hydroxy methyl furfural, dopamine, butyl lactate, β-caryophyllene, levomenol, cyclorphan and laurenditerpenol were investigated for evaluating their biological activities by using PASS online program (www.way2drug.com/passonline)[10].

Molecular docking

The molecular docking was performed between the target protein and potential ligand inhibitors using AutoDock-Version 4.2. The PDB file of the target protein was obtained from RCSB-PDB (http://www.rcsb.org/) and the water molecules were removed. The 3D structure of ligand molecules, including 5-hydroxy methyl furfural, dopamine, butyl lactate, β-caryophyllene, levomenol, cyclorphan, laurenditerpenol and the standard drug doxorubicin were obtained from PubChem database (http://pubchem.ncbi,nlm.nih.gov/). The calculation for docked target protein and ligand molecules were analyzed with Lamarckian Genetic Algorithm (LGA). Pymol software were used for visualization ofprotein-ligand complex [11].

Network Pharmacology based approach

The STITCH (http://stitch.embl.de/) database were used to identify the active compound, lauren diterpenol of *Psidium guajava*. The SMILE string of the lauren diterpenol(Compound CID: 11174259) obtained from the PubChem database. The Gene Card database (http://www.genecards. org/) were used to identify the targets of breast cancer. The Cluster Profiler package (version 3.16.1) in R software (version 4.0.2) was hired for GO and KEGG pathway enrichment analysis. The GO terms and the KEGG pathways were considered statistically significant when q-value ≤0.05. Then, each top 10 GO terms of molecular function (MF), cellular components (CC) and biological process (BP) and the top 20 KEGG pathways were selected for further analysis. To further probe the relationships among lauren diterpenol, targets and pathways in the treatment of breast cancer, the data of interaction between the targets and the pathways, retained in the results of KEGG enrichment analysis. It was then visualized to construct the drug-targets-pathway network by using the merge function of Cytoscape (version 3.6.0). The PPI for the predicted targets were

calculated on the web of the Retrieval of Interacting Genes (STRING) database (https://string-db.org/). The PPI networks were visualized by Cytoscape [12].

***In vitro* anticancer activity of *Psidium guajava* using MTT assay**

The anticancer activity of *Psidium guajava* against cancer cell lines (MCF-7) was evaluated by using MTT assay. Briefly, $2.5x10^4$ cell/well was seeded onto 96 well micro-titer plates and incubated at 37°C for 12 hours with 5% CO_2. Then, various concentrations (5, 10, 25, 50, 75, 100, 125, 150, 175, 200 µg/ml)of *Psidium guajava* were added and further incubated for 24, 48 and 72 hours, respectively. The one without extract was served as negative control. Followed by incubation, 20 µl of MTT solution was added and further incubated for 3 hours. About 150 µl of media was replaced with DMSO and mixed well to dissolve insoluble formazan crystals. It was measured at 540 nm spectrophotometrically and percentage of cell survival was calculated. The one which has survival percentage below 50 was considered as significant for anticancer potential of fruit peel extract of *Psidium guajava* [13].

RESULTS

Plant sample preparation and Gas Chromatography-Mass Spectroscopy

The ethanolic fruit peel extract of *Psidium guajava* was analyzed using GC-MS. The extract was known to contain a mixture of phytochemical components (Figure 1; Table 1). The active phytochemical constituents, including 5-hydroxy methyl furfural (5.83 RT), dopamine (7.94 RT), butyl lactate (8.18 RT), β-caryophyllene (8.33 RT), levomenol (10.22 RT), cyclorphan (23.24 RT) and laurenditerpenol (22.93 RT).

ADME studies

The pharmacological potential of active ligands of fruit peel extract of *Psidium guajava* was evaluated and tabulated (Table 2).According to Lipinski's rule (Pfizer's rule, Lipinski's rule of five, RO5), the active drug has no more than one violation of the following properties including molecular weight (MW) ≤ 500, LogP ≤ 5, hydrogen bond acceptors ≤ 10, and hydrogen bond donors ≤ 5. Based on the drug likeness analysis, all the phytochemical compounds do not violate the Lipinski's rule of five.

PASS prediction

A total of 7 phytoconstituents namely,5-hydroxy methyl furfural, dopamine, butyl lactate, β-caryophyllene, levomenol, cyclorphan and lauren diterpenol were screened for the antineoplastic (breast cancer) activity with the help of the PASS program. All the compounds exhibited higher Pa thanPi (Figure 2). The compounds dopamine and laurenditerpenol showed highest Pa value for anticancer activity of breast cancer has exhibited a significant pharmacological activity for breast cancer.

Molecular Docking

The structure of target protein, estrogen receptor alpha (3ERT) was retrieved from PDB database (Figure3).To better understand the interaction of target protein estrogen receptor alpha with the ligand molecules, 5-hydroxy methyl furfural, dopamine, butyl lactate, β-caryophyllene, levomenol, cyclorphan and laurenditerpenol, molecular docking was performed. The doxorubicin was chosen as standard drug. The results of molecular docking analyses of active phytochemical compounds of fruit peel extract of *Psidium guajava*was summarized in Table 3; Figure 4. Based on docking score, it was found that laurenditerpenol has a highest binding affinity than the other phytochemical compounds. Laurenditerpenol interacts with estrogen receptor alpha with PRO: 325 residue and pi interactions at PRO A: 324, MET A: 357, LEU A: 387 amino acid residues.

Network pharmacology approach

Based on string analysis, about 53 common genes were observedamong100 potential genes of lauren diterpenol and 6000 potential genes associated with breast cancer. To evaluate characteristics and functions of these 53 predicted targets, analyses of GO enrichment and KEGG pathway enrichment were performed (Figure 5, 6, 7). The top 5

pathways significantly correlated with breast cancer were G-protein coupled receptor signaling pathway, sensory perception of pain, regulation of blood pressure, adenylate cyclase-modulating G-protein coupled receptor signaling pathway, as well as muscle cell proliferation, which were all reported to involve in the occurrence, development, metastasis and treatment of breast cancer. To further characterize the relationships between lauren diterpenol, targets and signaling pathways associated with breast cancer, a Drug-Target-Pathway network was constructed by using Cytoscape. It was observed that GO and KEGG enrichments validated that all 53 predicted genes have involved in the characteristics and functions as the therapeutic targets of lauren diterpenol for treating breast cancer.

***In vitro* anticancer activity of *Psidium guajava* using MTT assay**

The effect of various concentrations of fruit peel extract of *Psidium guajava* was assessed against MCF-7 breast cancer cell line (Figure 8). There was a significant reduction in MCF-7 cells was observed when concentration increased from 5 µg/ml to 200 µg/ml. Followed by 72 hours of incubation, 46.52% of cell death was occurred at 75 µg/ml.

DISCUSSION

Breast cancer is one of the predominantly occurred cancer with an incidence of more than 1,000,000 new cases and 370,000 deaths annually worldwide and it remains a major challenge today. When localized or regionally advanced, the disease is potentially curable with local and systemic therapy [14,15]. The widely adopted therapeutic approaches for cancer treatment, including surgery, chemotherapy radiotherapy and combinatorial therapy. Despite of successful treatment of primary cancer, metastasis of breast cancer is a hurdle and becomes a major reason for death of cancer individuals. Hence, researchers focused on treating breast cancer to overcome existing limitations and challenges in drug discovery[16]. Recently, there has been a lot of focused on using phytochemical compounds for effective treatment of breast cancer. *Psidium guajava* was traditionally used as medicine in many countries. It is predominantly used for gastrointestinal disorders. Especially, the fruit of *Psidium guajava* was found to contain higher number of phytochemical compounds, such as phenols, flavonoids, carotenoids, triterpenes, tannins and quinones. The assortment of fruit of *Psidium guajava* might have immense potential in treating diarrhoea, allergies, gastroenteritis, cough, wounds, diabetes, acne, cardiovascular disorder, dental plaque, degenerative muscular disease, malaria, inflammatory ailments, liver disorder, etc. It also exhibits anti-oxidant, anti-inflammatory and anti-cancer activity [17]. But thorough characterization of anti-cancer potential of fruit peel extract of *Psidium guajava* was least explored. Hence, we exploit *in silico* and *invitro* anticancer potential of fruit peel extract of *Psidium guajava* for drug discovery towards breast cancer with regards to estrogen receptor. The GC-MS characterization of fruit peel extract of *Psidium guajava* revealed that laurenditerpenol was highly present and found to be responsible for anticancer activity. Previous studies reported that laurenditerpenol is a marine-derived dicyclic terpene obtained from the marine red algae, *Laurenica intricata*.

It was the first isolated component and found to inhibit mitochondrial oxygen consumption and it also acts as the inhibitor for Hypoxia-inducible factor 1 (HIF-1). HIF-1 is said to be an important transcription factor in solid tumours to activate the oncogenes and inhibits the tumour suppressor genes[18].The molecular docking approach was adopted to examine the potential molecular targets for the reported anticancer drug derived from phytochemicals of guava fruit peel. It was suggested that the fruit peel extract of *Psidium guajava* could act as potential estrogen receptor alpha inhibitor in the treatment of breast cancer. The network was constructed for breast cancer through the plant bioactive compound, laurenditerpenol followed by recognition of targets associated with breast cancer pathway. The network reveals the potential of 100 bioactive laurenditerpenol to modulate the breast cancer by the interactions of 6000genes through multiple pathways. About 53 gene components are mapped which are responsible for inhibition of cells proliferation, induces cell cycle arrest and apoptosis in cancer cell. GO and KEGG analysis predicts the characteristic feature related disease and disorders for the mapped genes [19]. The GO enrichment analysis revealed the direct involvement of bioactive in the regulation of breast cancer. KEGG pathway analysis showed that estrogen signaling pathway could be key signaling pathway in the selected network which helps to support that laurenditerpenol of fruit peel extract of *Psidium guajava* may be used for breast cancer treatment. The cytotoxicity

assays were done to determine the inhibition of MCF-7 cell growth by various concentrations of fruit peel extract of *Psidium guajava*. Upon increased concentration of fruit peel extract, there is a decrease in cancer cell viability. Further, *Psidium guajava* was consistent in its effect on MCF-7 cell lines with an average IC50 value of75 µg/ml. Hence, the fruit peel extract of *Psidium guajava* has proved to eradicate breast cancer, and it increased the sensitivity of breast cancer cells to standard drugs. Owing to the fact that breast cancer cells relative to estrogen receptor are challenging to eliminate and the observed cytotoxicity of fruit extract is promising [20]. To be concluded, our results suggest that the fruit peel extract of *Psidium guajava* could be significantly promising candidate in the development of anticancer drug. The underlying mechanism and interaction of cytotoxicity of these compounds are well correlated with the results obtained from docking and network pharmacology studies.

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REFERENCES

1. Thakur KK, Bordoloi D, Kunnumakkara AB. Alarming burden of triple-negative breast cancer in India. Clinical breast cancer 2018;18(3):e393-399.
2. Israel BE, Tilghman SL, Parker-Lemieux K, Payton-Stewart F. Phytochemicals: Current strategies for treating breast cancer. Oncology letters 2018;15(5):7471-7478.
3. Jamieson S, Wallace CE, Das N, Bhattacharyya P, Bishayee A. Guava (*Psidium guajava* L.): a glorious plant with cancer preventive and therapeutic potential. Critical Reviews in Food Science and Nutrition 2021;25:1-32.
4. Naseer S, Hussain S, Naeem N, Pervaiz M, Rahman M. The phytochemistry and medicinal value of *Psidium guajava* (guava). Clinical Phytoscience 2018;4(1):1-8.
5. Li Y, Xu J, Li D, Ma H, Mu Y, Huang X, *et al*. Guavinoside B from Psidium guajava alleviates acetaminophen-induced liver injury via regulating the Nrf2 and JNK signaling pathways. Food & function 2020;11(9):8297-8308.
6. Zhang L, Yang K, Wang M, Zeng L, Sun E, Zhang F, *et al*. Exploring the mechanism of Cremastra Appendiculata (SUANPANQI) against breast cancer by network pharmacology and molecular docking. Computational Biology and Chemistry 2021;94:107396.
7. Sneeha V, Safreen SD, Kumaresan R. Anti-cancer efficacy of ethanolic extracts from various parts of Annona Squamosa on MCF-7 cell line. Journal of Pharmacognosy and Phytotherapy 2016;8(7):147-154.
8. Jordán MJ, Margaría CA, Shaw PE, Goodner KL. Volatile components and aroma active compounds in aqueous essence and fresh pink guava fruit puree (*Psidium guajava* L.) by GC-MS and multidimensional GC/GC-O. Journal of Agricultural and Food Chemistry 2003;51(5):1421-1426.
9. Yende SR, Shah SK, Arora SK, Moharir KS, Lohiya GK. In silico prediction of phytoconstituents from *Ehretialaevis* targeting TNF-α in arthritis. Digital Chinese Medicine 2021;4(3):180-90.
10. Auniq RB, Chy MN, Adnan M, Roy A, Islam MA, Khan TN, *et al*. Assessment of anti-nociceptive and anthelmintic activities of Vitex Peduncularis Wall. leaves and in silico molecular docking, ADME/T, and PASS prediction studies of its isolated compounds. Journal of Complementary Medicine Research 2019;10(4):170-.
11. Yalcin S. Molecular Docking, Drug Likeness, and ADMET Analyses of Passiflora compounds as P-glycoprotein (P-gp) inhibitor for the treatment of cancer. Current Pharmacology Reports 2020;6(6):429-40.
12. Li XM, Li MT, Jiang N, Si YC, Zhu MM, Wu QY, *et al*. Network Pharmacology-Based Approach to Investigate the Molecular Targets of Sinomenine for Treating Breast Cancer. Cancer Management and Research 2021;13:1189.
13. Khaghani S, Yajloo MM, Paknejad M, Shariftabrizi A, Pasalar P, Razi F. Selective cytotoxicity and apoptogenic activity of Hibiscus sabdariffa aqueous extract against MCF-7 human breast cancer cell line. Journal of Cancer Therapy 2011;2(03):394.
14. Hassan MS, Ansari J, Spooner D, Hussain SA. Chemotherapy for breast cancer. Oncology reports 2010;24(5):1121-31.

15. Anampa J, Makower D, Sparano JA. Progress in adjuvant chemotherapy for breast cancer: an overview. BMC medicine 2015;13(1):1-3.
16. Neal JT, Li X, Zhu J, Giangarra V, Grzeskowiak CL, Ju J, *et al*. Organoid modeling of the tumor immune microenvironment. Cell 2018;175(7):1972-88.
17. Bontempo P, Doto A, Miceli M, Mita L, Benedetti R, Nebbioso A, *et al*. *Psidium guajava* L. anti-neoplastic effects: induction of apoptosis and cell differentiation. Cell proliferation 2012;45(1):22-31.
18. Hossain CF, Kim YP, Baerson SR, Zhang L, Bruick RK, Mohammed KA, *et al*. Saururus cernuus lignans—Potent small molecule inhibitors of hypoxia-inducible factor-1. Biochemical and biophysical research communications 2005;333(3):1026-33.
19. Sakle NS, More SA, Mokale SN. A network pharmacology-based approach to explore potential targets of Caesalpinia pulcherima: An updated prototype in drug discovery. Scientific reports 2020;10(1):1-6.
20. Koroth J, Nirgude S, Tiwari S, Gopalakrishnan V, Mahadeva R, Kumar S, *et al*. Investigation of anti-cancer and migrastatic properties of novel curcumin derivatives on breast and ovarian cancer cell lines. BMC complementary and alternative medicine 2019;19(1):1-6.

Table 1. Active phytochemical constituents of fruit peel extract of *Psidium guajava*

S.No	Retention Time (RT)	Name of the compound	Molecular formula	Molecular weight	Peak area %
1	5.83	5-Hydroxymethylfurfuryl	$C_6H_6O_3$	126	6.49
2	7.94	Dopamine	$C_8H_{11}NO_2$	153	7.66
3	8.18	Butyl lactate	$C_7H_{14}O_3$	146	2.04
4	8.33	β-caryophyllene	$C_{15}H_{24}$	204	0.47
5	10.22	Levomenol	$C_{15}H_{26}O$	222	0.28
6	23.24	Cyclorphan	$C_{20}H_{27}NO$	297	0.42
7	22.93	Laurenditerpenol	$C_{20}H_{34}O_2$	306	1.09

Table 2. ADME properties of fruit peel extract of *Psidium guajava*

S.No	Compound ID	Molecular weight (<500 Da)	Rotatable bond	H-bond acceptor (<10)	H-bond donar (<5)	LogP (<5)	Lipinski violation
1	47947	297.13 g/mol	3	2	1	-0.87	0
2	681	153.18 g/mol	2	3	3	1.27	0
3	8738	146.18 g/mol	5	3	1	2.06	0
4	5281515	204.35 g/mol	0	0	0	3.27	1
5	442343	222.37 g/mol	4	1	1	3.46	0
6	10017546	297.43 g/mol	2	2	1	3.12	0
7	11174259	306.48 g/mol	4	2	1	3.87	0

Table 3. Molecular docking of target protein and active ligand molecules

S.No	Name of the compound	Binding affinity Kcal/mol
1	5-Hydroxymethylfurfuryl	-5.2
2	Dopamine	-5.6
3	Butyl lactate	-5.2
4	β-caryophyllene	-5.2
5	Levomenol	-6.6
6	Cyclorphan	-5.7
7	Laurenditerpenol	-7.8
8	Doxorubicin	-3.45

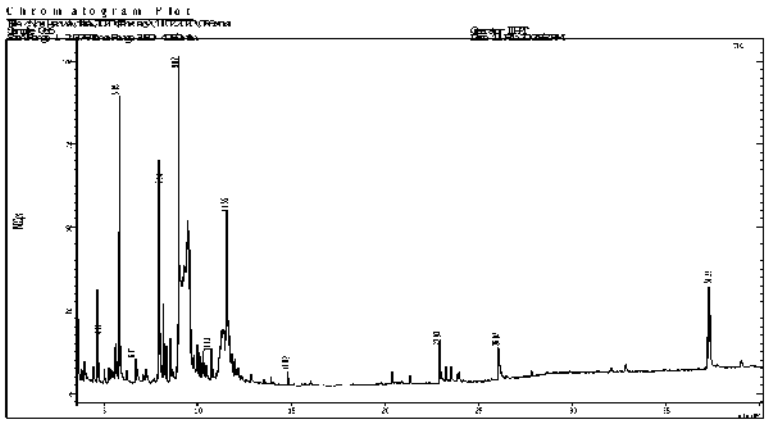
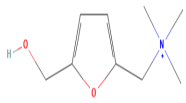
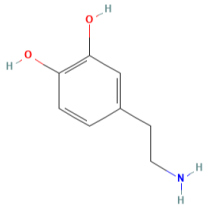
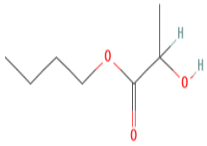
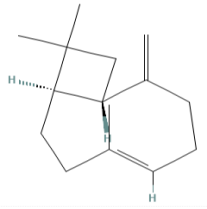
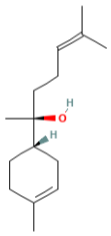


Figure 1. GC-MS characterization of fruit peel extract of *Psidium guajava*

S.No	Compound Name and ID	Compound Structure	Compound activity
1	5-hydroxy methyl furfural 47947		0,446 0,125 Calcium channel (voltage-sensitive) activator 0,366 0,045 DNA-(apurinic or apyrimidinic site) lyase inhibitor 0,383 0,063 Amine dehydrogenase inhibitor 0,331 0,011 Acetylenecarboxylate hydratase inhibitor 0,339 0,020 Lombricine kinase inhibitor 0,348 0,029 [acyl-carrier-protein] S-acetyltransferase inhibitor 0,357 0,037 CYP2B11 substrate 0,344 0,025 Pseudouridylate synthase inhibitor 0,398 0,079 Vasoprotector 0,334 0,015 2-Dehydropantoate aldolase inhibitor 0,334 0,016 Apoptosis antagonist ★ 0,339 0,021 Farnesyltranstransferase inhibitor 0,489 0,004 Antineoplastic (renal cancer) ★
2	Dopamine 681		0,725 0,005 Antimutagenic 0,729 0,009 Fatty-acyl-CoA synthase inhibitor 0,736 0,016 Fusarinine-C ornithinesterase inhibitor 0,723 0,003 Carnosine synthase inhibitor 0,738 0,019 Glutamyl endopeptidase II inhibitor 0,721 0,003 GABA C receptor agonist 0,726 0,012 2-Hydroxyquinoline 8-monooxygenase inhibitor 0,713 0,003 Phenylalanine 4-hydroxylase inhibitor 0,730 0,021 Sphinganine kinase inhibitor 0,717 0,009 Radioprotector 0,726 0,019 Dehydro-L-gulonate decarboxylase inhibitor 0,721 0,015 JAK2 expression inhibitor

3	Butyl lactate 8738		0,322 0,012 Laxative 0,323 0,013 ATP adenylyltransferase inhibitor 0,322 0,012 Malate-CoA ligase inhibitor 0,320 0,010 UGT1A5 substrate 0,323 0,013 Omega-amidase inhibitor 0,349 0,040 Cancer associated disorders treatment 0,315 0,006 Thiol S-methyltransferase inhibitor 0,322 0,013 NAD+ kinase inhibitor 0,323 0,014 Furin inhibitor 0,324 0,017 Oxytocic 0,328 0,020 Aspartoacylase inhibitor 0,340 0,033 Myosin ATPase inhibitor
4	β-caryophyllene 5281515		0,245 0,086 DELTA14-sterol reductase inhibitor 0,300 0,141 CYP2A1 substrate 0,318 0,160 Thioredoxin inhibitor 0,305 0,149 Carboxypeptidase Taq inhibitor 0,268 0,111 FMO1 substrate 0,228 0,072 CYP2A11 substrate 0,176 0,021 Estrogen agonist 0,164 0,010 Anabolic 0,315 0,161 Aminobutyraldehyde dehydrogenase inhibitor 0,160 0,007 Hypercalcemia treatment 0,617 0,044 TP53 expression enhancer 0,572 0,008 Antimetastatic
5	Levomenol 442343		0,557 0,015 Anticarcinogenic 0,660 0,008 Chemopreventive 0,654 0,011 Dermatologic 0,638 0,004 Antimetastatic 0,652 0,022 Antiinflammatory 0,631 0,003 Transcription factor NF kappa B inhibitor 0,657 0,034 Antineoplastic 0,635 0,016 CYP3A4 inducer 0,622 0,005 Transcription factor inhibitor
6	Cyclorphan 10017546		0,196 0,094 Stroke treatment 0,138 0,037 Estrogen antagonist 0,197 0,097 Antidiarrheal 0,246 0,147 MMP9 expression inhibitor 0,167 0,069 CC chemokine receptor 2B antagonist 0,213 0,115 DELTA14-sterol reductase inhibitor 0,236 0,139 Dolichyl-diphosphooligosaccharide-protein glycotransferase inhibitor 0,205 0,109 5 Hydroxytryptamine 7S antagonist 0,134 0,040 Acetylcholine antagonist 0,197 0,163 Antimetastatic 0,192 0,159 Chloride channel blocker 0,053 0,020 Estrogen beta receptor agonist

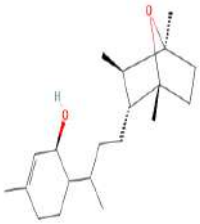
7	Laurenditerpenol 11174259		0,625 0,004 Antimetastatic 0,667 0,062 CYP2J substrate 0,648 0,046 Fibrinolytic 0,664 0,067 Testosterone 17beta-dehydrogenase (NADP+) inhibitor 0,601 0,013 UGT1A substrate 0,604 0,020 UDP-glucuronosyltransferase substrate 0,591 0,009 Antileukemic 0,641 0,067 CYP2C12 substrate 0,603 0,033 HIF1A expression inhibitor 0,577 0,016 Antipruritic, allergic 0,562 0,010 UGT2B substrate 0,519 0,014 Chemopreventive

Figure 2. Biological activity of phytochemical constituent of *Psidium guajava*

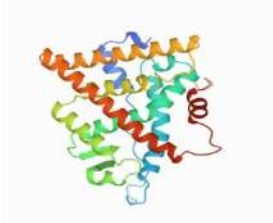
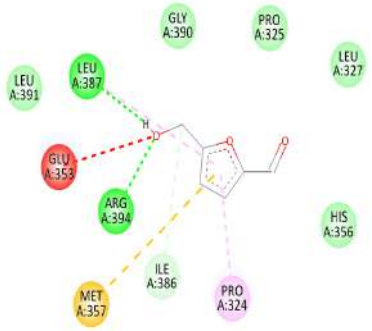
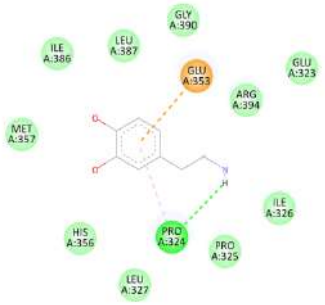
Organism Name	Name of target protein & ID	Structure
Homo sapiens	Estrogen receptor alpha 3ERT	

Figure 3. Structure of target protein – estrogen receptor alpha

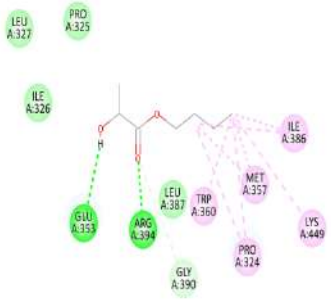
Interaction of 5-hydroxy methyl furfuryl & 3ERT



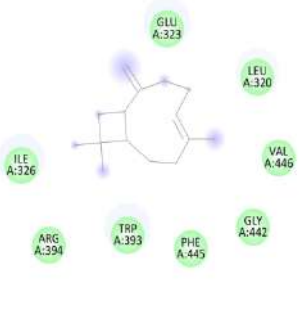
Interaction of dopamine & 3ERT

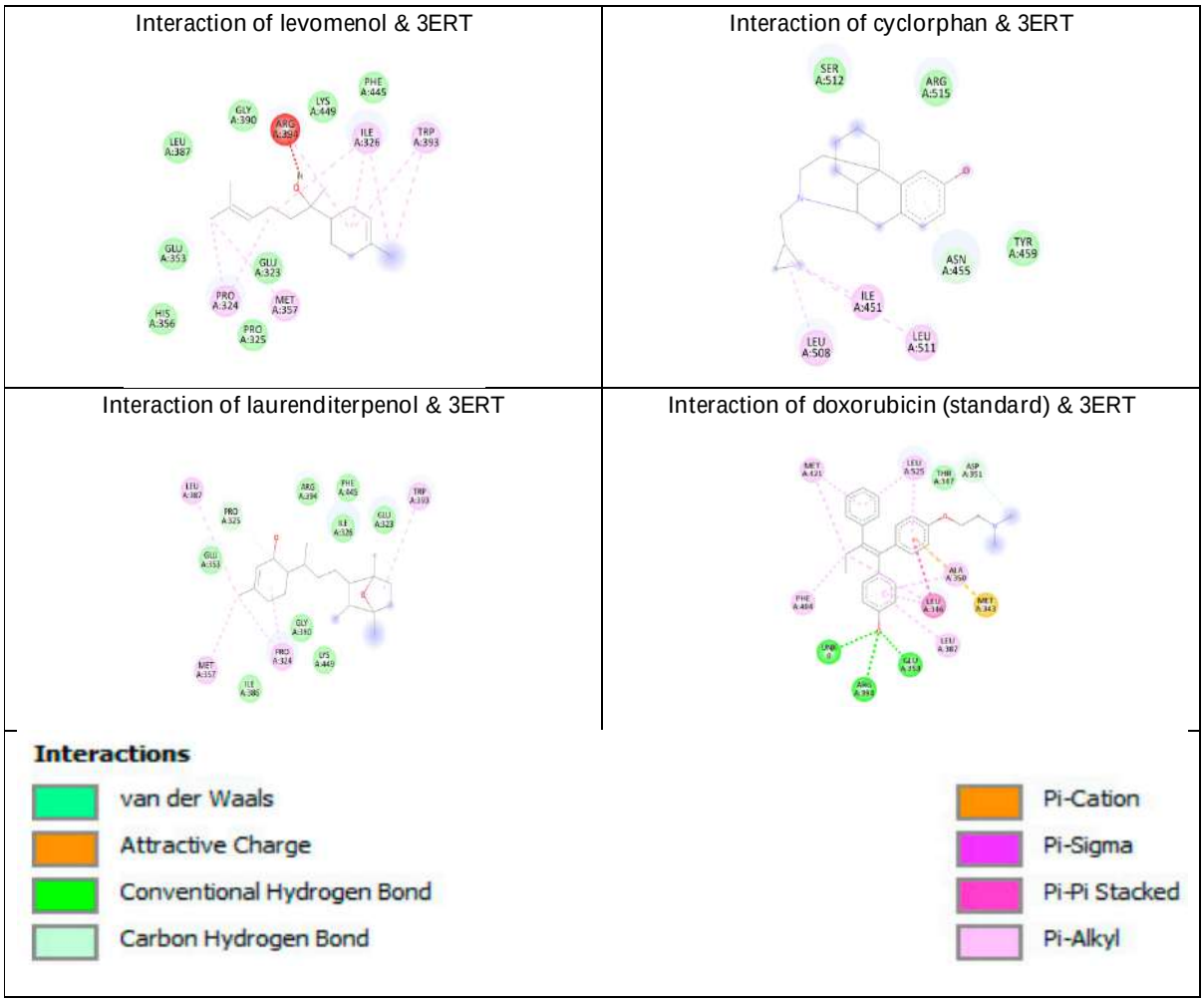


Interaction of butyl lactate & 3ERT



Interaction of β-caryophyllene & 3ERT





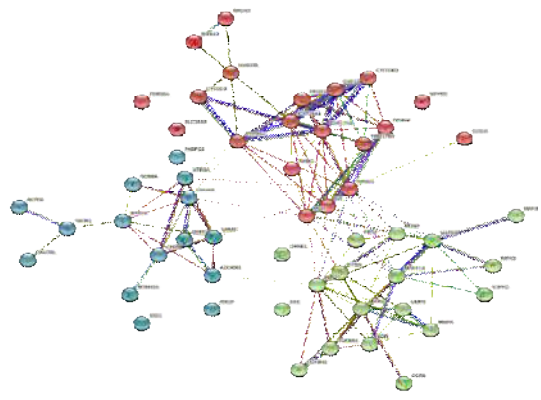


Figure 5. Protein-Protein interaction between laurenditerpenol and breast cancer

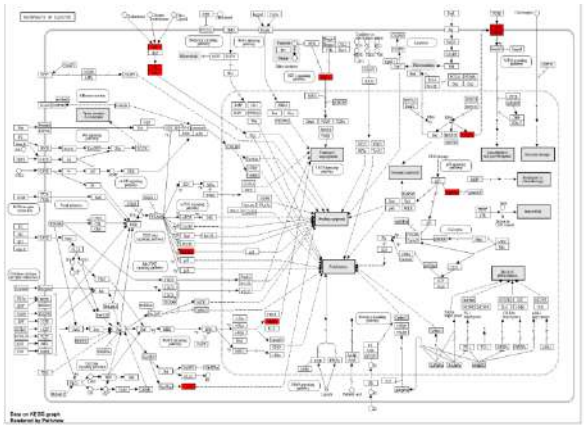


Figure 6. KEGG analysis of breast cancer pathway

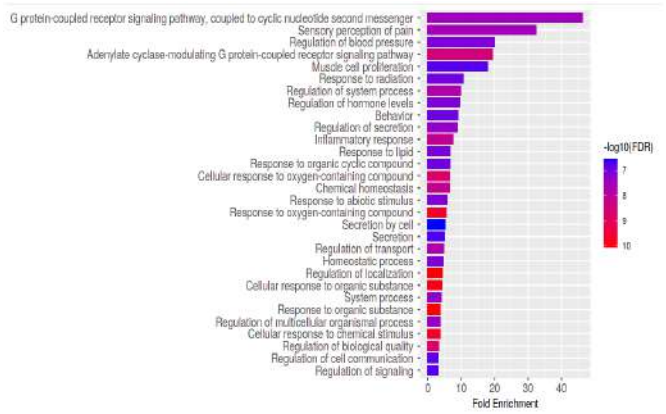
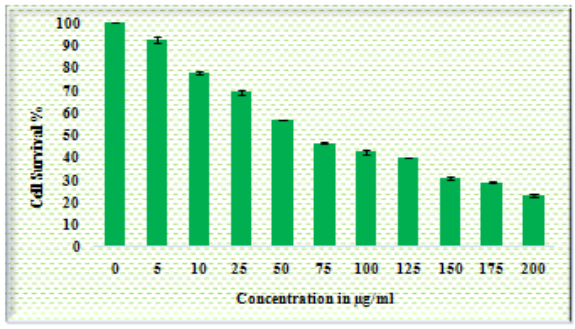


Figure 7. GO enrichment analysis of predicted genes of laurenditerpenol



Data expressed as mean ± SD (n=3)

Figure 8. Anticancer activity of *Psidium guajava* against MCF-7 cells

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RESEARCH ARTICLE

Income and Quantity of Different Form of Energy Consumption: an Analysis of Rural Household in Cuddalore District, Tamil Nadu

S. Velmurugan[1]* and A. Antony Joseph[2]

[1]Ph.D.,Research Scholar, Department of Economics, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India.
[2]Associate Professor, Department of Economics, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India.

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***Address for Correspondence**
S.Velmurugan,
Ph.D.,Research Scholar,
Department of Economics,
Annamalai University,
Annamalai Nagar,
Chidambaram, Tamil Nadu, India.
Email:

ABSTRACT

A study has been conducted to assess the pattern of rural household energy consumption in the Cuddalore district of Tamil Nadu state. The sample was comprised of 427 households who were randomly selected from five rural households dominating blocks in the Cuddalore district. A well-structured interview schedule was constructed to accomplish the objectives of the study, in which one of the objectives was to know the relationship between level of income and quantity of consumption of different forms of energy such as firewood, LPG, kerosene, petrol, diesel, bran, dung cake, and agricultural residues of the rural households in order to testify the Keynes assertion. It was found that three types of tendencies exist between the income level of rural households and the quantity of different forms of commercial energy consumption. On the contrary, the consumption of traditional sources of energy of a rural household has a negative relationship with their income level, which applies to all traditional sources taken into consideration for the analysis.

Keywords: Household fuel choice Determinant of household fuel choice, income and household fuel consumption**.**

INTRODUCTION

Energy is a basic requirement for economic development and has been universally recognized as one of the most important inputs for economic and human development. There is also a strong two-way relationship between economic development and energy consumption (kumara, R., Sethi, N.,&Yadav, Y.K. 2011) Even though it brings environmental pollution and degradation, which affects sustainable development. Utilization of both commercial and non-commercial energy sources causes environmental pollution and degradation, Environmental pollution and degradation could be decreased by the household's choice of selecting a particular energy carrier for service which has less pollution emission or effective burning efficiency than categorized as "clean" or "non-cleaned" energy. (Danlami, A.H., Islam, R., and Applanaidu, S. D. 2015). An individual's or family's propensity to choose a particular energy carrier is generally determined by income level, price of the energy, availability of energy, size of the family, education, and occupation, etc. Even though the choice of selecting a carrier The level of energy consumption is expected to vary among individuals because they have different influences on energy choices. The level of income and price are considered the main factors in the different forms of household energy consumption. M.N. Rao and B.S. Reddy (2007). Energy consumption and income of households are one of the major indicators of the economic condition of both individuals and the economy, which highly influences economic and social behavior, particularly consumption behavior (Singh, S., Jadhav,P., &Khanna, A. 2020). In economic theory, there is a direct and positive relationship between income and the consumption behavior of individuals (Keynes, 1936). Accordingly, it is equally applicable to the pattern of the energy consumption of different energies and different income groups of rural households in Cuddalore district under the study. An attempt has been made to test whether the income levels of the rural households of Cuddalore district have a direct and positive relationship with their energy consumption pattern and, through knowing the relationship between income and quantity of each form of energy consumption of rural households, give the suggestion to policy makers to decrease indoor air pollution

MATERIALS AND METHODS

A multistage random sampling technique was used for the study. In the first stage, Cuddalore district was selected since it is one of the rural-oriented and agriculture-dominant districts in Tamil Nadu. In the second stage, to cover all the regions of the district, five blocks were selected as the representative regions of Cuddalore district under the criterion of the highest percentage of rural population, as the study is based on rural households' energy consumption patterns. Applying required sample size: Where SS = formula on the total rural household population of selected blocks, 427 sample households arrived at stage three. These sample rural households were surveyed in the representative blocks of Cuddalore district to identify the quantity of different types of energy consumption according to income levels of households, and these rural households of Cuddalore district were classified into four income groups, namely: households which earn less than 7000 rupees (Low Income Group LIG), 7000–10,000 (Lower Middle Income Group, LMIG), 10000–13000 (Upper Middle Income Group, UMIG) and more than 13000 (High Income Group**).** The quantification of fuel wood/crop residue was done, considering one head load to have 25 kg. One dung cake was considered as 1 kg, and kerosene, petrol, and diesel consumption were measured in liters. The data were obtained from the block households' electricity bills, and the unit of measurement was kilo-watt-hour (KWh). The quantification of LPG was done, considering one cylinder as having 14.2 kg of gas. To get the energy consumption for various activities in terms of megajoules (MJ), the quantity of consumed energy sources for a particular activity was multiplied by their respective energy values.

DESCRIPTIVE DISCUSSION OF THE STUDY

The total electricity consumption inferred from study rural households is 30184 MJ/month, which accounts for 32 percent of the total different forms of energy consumption of the rural households in the study area, in which electricity is majorly consumed by LMIG of rural households(176160.36 MJ/month), which constitutes 58 percent of the total derived electricity consumption of different income groups (193842 MJ/month), followed by 24 percent, 14

percent, and 3 percent by UMIG, LIG, and HIG of rural households respectively. Therefore, we find from the study that the majority of the quantity of electricity energy consumed by LMIG of the studied rural households is mainly used for lighting, cooling, accessory activity, and occasionally heating purposes. In addition to electricity, LPG occupies a significant portion, i.e., 264355 MJ/month, accounting for 28 percent of total different forms of energy consumption of the study rural households. In the case of LPG, LMIG of rural households consumed a significant proportion (161227 MJ/month), i.e., 66 percent of total LPG consumption of rural households in the study area (264355 MJ/month), followed by UMIG (47940.35 MJHence, it could be found from the study that LPG energy is the first most households' cooking energy and it is chiefly consumed by LMIG of rural households. According to LPG energy, petrol is consumed by the majority of rural households, who consume 161568 MJ per month, accounting for 17.31% of the total different types of energy consumption of the study rural households. In the case of petrol energy consumption, LMIG of rural households also dominated in the consumption of petrol, i.e., 115098 MJ/month, which formed 71.23 percent of total petrol consumed by the different income groups of sample rural households (161568 MJ/month) of the Cuddalore district, followed by 17.02 percent, 9.40 percent, and 2.35 percent by UMIG (27507 MJ/month), LIG (15194 MJ/month), and HIG (3769 MJ/month) respectively.

Thus, it is ascertained from the study that petrol energy is used by households for mainly transport purposes, and it was predominantly consumed by rural households in the study. Following petrol consumption, firewood is primarily used by rural households, i.e., 96755.4 MJ/month, representing a 10.36 percent share of the total different forms of energy consumption of the study households, in which firewood is primarily used by LMIG of rural households, i.e., 61618.08 MJ/month, representing 63.68 percent of the total quantity of firewood consumption of the study households. As a result, it could find from the study that majority of the quantity of firewood energy utilized by LMIG of households in study area. The use of firewood in rural households has declined since the government intervened in the provision of LPG to households, but it is quickly becoming the next choice of cooking energy for rural households due to its free availability. The total diesel energy consumption in the study is 56392 MJ/month, which contributes 6.04 percent of the total different types of energy consumption, with a significant amount of diesel consumed by two income groups, namely UMIG (29747 MJ/month) and LMIG (20894 MJ/month), which accounted for 59.50 percent and 37.05 percent of the total diesel consumption of the households, respectively, and the remaining 5751 MJ/month (10 percent) consumed by HIG, It could be found from the study that diesel energy is utilized for transport purposes like the utilization of petrol energy. A major quantity of diesel energy is consumed by UMIG of rural households in the study. Following diesel consumption, kerosene occupies the majority of the total different forms of energy consumption, i.e. 28046 MJ/month, which contributes 3% of the total different forms of energy consumption in the study households, in which LMIG households consume 15748 MJ/month, which has a share of 55.67% in the total kerosene consumption of the study's different income groups, followed by LIG (8133 MJ/month, 14%), UMIG Therefore, we could find from the study the majority of the quantity of kerosene energy consumed by the LMIG group of rural households in the study.

After that to the diesel consumption, the dung cake consumption of the study households derived from the different income groups is 8233.02 MJ/month, which is less than 1 percent (0.88 percent) of the total different forms of energy consumption of study households, in which LMIG consumes more than half (59.50 percent, i.e., 4893.21 MJ/month) of the total dung cake consumption of the different income groups of the study, followed by LIG (2740.1 MJ/month, 33.20 percent) and UMIG (599.71 MJ/month, 12.26 percent). We could find from the study that the high-income group does not consume dunk cake while LMIG consumes more than half the quantity of dung cake energy in the study. Subsequently, total bran consumption which is gathered from different income groups of the study households is 11145 MJ/month, which constitutes just 1.19 percent of total energy consumption, in which LIG consumes 5758 MJ/month, accounting for half of the total bran consumption of different income groups, followed by LMIG (4123.65 MJ/month, 38.21 percent) and UMIG (1128.35 MJ/month, 10.12 percent). We could find from the study that the high income group did not consume bran while the low income group consumed half the quantity of bran energy in the total bran consumption of the different income groups. And lastly, agricultural residues consumed by the study households are very meager, which calculated 4878.07 MJ/month, i.e., half of one (0.52 percent) of the total different forms of energy consumption, in which LIG consumes more than two thirds, i.e., 70 percent of total

agricultural residues consumption of different income groups, followed by LMIG (1463.7 MJ/month, 30 percent). We could find from the study that agricultural residues are only consumed by two income groups, namely LIG and LMIG. UMIG and HIG do not consume agricultural residues, while LIG consumes more than two thirds of the total quantity of agricultural residues consumed by different income groups in the study.

ANALYTICAL DISCUSSION OF THE STUDY

A household's LIG consumes an average of 571 MJ of electricity per month, while LMIG, UMIG, and HIG consume an average of 696.28 MJ, 862.40 MJ, and 915 MJ of electricity per month. This trend reveals that when the income level of a household rises, their quantity of electricity consumption also increases. LIG of a household consumes an average of 156.40 MJ of kerosene energy per month, while LMIG, UMIG, and HIG of a household consume an average of 132.33 MJ, 95.32 MJ, and 70.4 MJ of kerosene energy per month. This tendency shows that when the income level of a household increases, their quantity of kerosene energy consumption decreases. LIG of a household consumes an average of 519 MJ of LPG energy per month, while LMIG, UMIG, and HIG of a household consume an average of 702.52 MJ, 856.07 MJ, and 961.2 MJ of LPG energy per month. This trend reveals that when the income level of a household rises, their quantity of LPG consumption also increases. A household consuming LIG consumes an average of 403.82 MJ of firewood energy per month, while LMIG, UMIG, and HIG consume an average of 296 MJ, 169.47 MJ, and zero MJ of firewood energy per month. This tendency reveals that when the income level of a household raises, who degreases the quantity of firewood energy consumption. In the case of the petrol consumption of a household, LMIG of a household consumes an average of 605 MJ of petrol energy, which is higher than the HIG, UMIG, and LIG of a household's average petrol energy consumption, and UMIG of a household consumes an average of 436 MJ of petrol energy, which is higher than the average petrol consumption of a household HIG and LIG of a household.

A household using LIG consumes an average of 323 MJ of petrol energy per month, which is higher than the average petrol consumption of a household using HIG (314.08 MJ). This trend shows that there is an asymmetric trend between the income level of a household and their quantity of petrol consumption. The tendency of diesel consumption in a household's income group is also consistent with the tendency of household behavior on the quantity of petrol consumed in rural areas. LIG of a household consumes an average of 106 MJ of bran energy per month, while LMIG, UMIG, and HIG of a household consume an average of 85.17 MJ, 62.68 MJ, and 0 MJ of bran energy per month. This tendency reveals that when the income level of a household raises, who degreases the quantity of bran energy consumption?. The LIG of a household consumes an average of 94.84 MJ of agricultural residues energy per month, while LMIG, UMIG, and HIG of a household consume just an average of 34.85 MJ, 14.63 MJ, and 0 MJ of agricultural residues energy per month. This trend reveals that when the income level of a household increases, their quantity of agricultural residue energy consumption decreases. A household consumes an average of 51.51 MJ of dung cake energy per month, while LMIG, UMIG, and HIG of a household consume an average of 33.51 MJ, 25.24 MJ, and 0 MJ of dung cake energy per month. This trend reveals that when the income level of a household increases, their quantity of dung cake energy consumption decreases.

Findings of the Study

The tendency that was found from the study revealed that the consumption of commercial sources of energy of a rural household has a positive relationship with their income level, which is only suitable for electricity and LPG energy consumption. In the case of kerosene energy consumption in rural households, it has a negative relationship with their income level. Another commercial source of petrol and diesel energy consumption for a rural household has an asymmetric relationship with their income level. Therefore, three types of tendencies exist between the income level of the household and the quantity of different forms of commercial energy consumption. On the contrary, the consumption of traditional sources of energy of a rural household has a negative relationship with their income level, which applies to all traditional sources taken into consideration for the analysis.

CONCLUSION

Through the analytical discussion of collected data, we found that among the commercial sources of energy taken into consideration for the study, income and consumption behavior of rural households for electricity and LPG only applies with Keynes' income and consumption behavior (There is a direct and positive relationship between income and consumption behavior of individuals) and, contrary to it, kerosene consumption behavior of rural households has the opposite relationship with income. The diesel and petrol consumption behavior of rural households has an asymmetric relationship with income. The income of rural households has a different relationship with the consumption of each commercial energy source, but it does not have a relationship with the consumption of traditional energy sources. That is, income and consumption behavior of rural households for all traditional sources of energy taken into consideration for the analysis do not apply with Key reassertion and, on the contrary of Keynes' income and consumption behavior, they have an inverse relationship between income and consumption. The reasons behind in these indifferent relationships between income level of rural households and their quantity of different forms of energy consumption is put into further knowledge of the study.

SUGGESTION

The study reveals that as income rises, rural households will stop utilizing non-cleaned (firewood, dunk cake, bran, agricultural residues) energy sources and increase the utilization of cleaned energy sources (electricity and LPG). So, it suggests that the government can reduce indoor air pollution by creating high-income-earning employment opportunities in the rural areas.

REFERENCES

1. Singh, S., Jadhav,P., &Khanna, A. (2020). Economic Sustainability from Fuel wood and Pattern of rural Energy Consumption in Madhya Pradesh.*Global journal of Management & Business Studies.*1(1)
2. Danlami, A.H., Islam, R., &Applanaidu, S. D. (2015). "An analysis of the determinants of household's energy choice: A search for conceptual framework."*International Journal of Energy Economics and Policy,* 5(1), 197-205.
3. Kmari, R., Sethi, N., &Yadav, Y.K.(2011).Domestic Energy Consumption Pattern of Rural Scheduled Caste(SC) Households in Selected District Haryana.*Journal of Dairying Foods & Home Sciences,* 30(2), 134-139.
4. Rao, M. N., & Reddy, B.S. (2007). Variations in energy use by Indian households: An analysis of micro level data. *Energy*, 32(2), 143-153.

Table 1 Energy Consumption According to the Income of the Rural Households (in MJ/month)

S.no	Type of Energy	Monthly Income				
		<7000	7001-10000	10001-13000	>13000	Total
1	Electricity	42257.88 (14)	176160.36 (58.36)	72442.08 (24)	10983.68 (3)	301842 (100)
2	Kerosene	8133 (28.75)	15748 (55.67)	3813 (14.33)	352 (1.24)	28046 (100)
3	LPG	30653.25 (11.59)	174227 (65.90)	47940.35 (18.13)	11534.4 (4.36)	264355 (100)
4	Petrol	15194 (9.40)	115098 (71.23)	27507 (17.02)	3769 (2.33)	161568 (100)
5	Diesel	0 (0)	20894 (37.05)	29747 (52.75)	5751 (10.19)	56392 (100)
6	Dung cake	2740.1 (33.20)	4893.21 (59.50)	599.71 (12.26)	0 (0)	8233.02
7	Agricultural residues	3414.37 (70)	1463.7 (30.00)	0 (0)	0 (0)	4878.07

8	Bran	5758 (51.66)	4258.65 (38.21)	1128.35 (10.12)	0 (0)	11145
9	Firewood	29883.96 (30.88)	61618.08 (63.68)	5253.4 (5.42)	0 (0)	96755.4
	Total	137887.31 (14.77)	574360.6 (61.54)	188577.26 (20.20)	32389.4 3.47	933214.5 (100)

Source: Computed from primary data. Numbers in parenthesis () is percentage

Table.2. Average Energy Consumption According to the Income of the Rural Households (in MJ/month)

S. No	Type of energy	Monthly Income				
		<7000	7001-10000	10001-13000	>13000	Average
1	Electricity	571	696.28	862.40	915	706.88
2	Kerosene	156.40	132.33	95.32	70.4	131.67
3	LPG	519.54	702.52	856.07	961.2	704.94
4	Petrol	323	605	436	314.08	517.84
5	Diesel	0	1160	1062	821.57	1064
6	Dung cake	51.51	33.51	25.24	0	37.08
7	Agricultural residues	94.84	34.85	14.63	0	55.43
8	Bran	106	85.17	62.68	0	89.87
9	Firewood	403.82	296	169.47	0	309.12
10	Average	1863.34	2270.2	2244.96	2699.4	2185

Source: Computed from primary data.

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RESEARCH ARTICLE

Technological Acceptance of Rural Masses Pre and Post COVID-19- A Study of Jammu and Kashmir

Vivek Sharma*

Sr.Assistant Professor, Department of Lifelong Learning, University of Jammu, Jammu and Kashmir, India.

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***Address for Correspondence**
Vivek Sharma,
Sr.Assistant Professor,
Department of Lifelong Learning,
University of Jammu,
Jammu and Kashmir, India.
Email: drviveksharma@jammuuniversity.ac.in

ABSTRACT

This research was driven by the motto of exploring the fondness of Information and communication technology adaptation before and during the COVID19 pandemic in the rural villages of district Samba of Jammu and Kashmir. The emerging questions are what form of innovative applications and to what percentage were these applications used by the villagers before and after Covid-19 pandemic. This study used general survey on convenient basis to contact the educated people living in the rural areas of a particular district of Jammu. Descriptive Statistics was used to find the differences in the usage of information and communication technology. Results revealed a significant difference in the usage of ICT applications by the rural people before and after Covid-19.

Keywords: Telecommunication Industry, Covid-19, Mobile data, Internet users in India, 5G

INTRODUCTION

In the industrialized world, the ICT revolution has impacted every aspect of daily life and has greatly benefited people. For instance, in India, the role of extension workers in supplying agricultural producers with information, education, and help for making decisions has been revitalized. Therefore, the use of ICT might complement traditional agricultural expansion strategies in rural regions not just in India but also in other nations, including Ghana. However, if an organisation or a community is regularly impacted negatively by the introduction of innovative technologies, then the cause of this kind of problem would be the way in which the application and use of technology took place and not the technology itself. Individuals, groups, and organisations all have different observations about the utilization of innovative and original technologies to advance individual effectiveness and

eminence of life (Boateng, 2012). In some cases, technology proves to be a complex field that the rural populace does not always feel comfortable with. For instance, in educational institutions, a rural woman who has never attended school before may feel uneasy using a computer on her own if she is taught how to do so. Therefore, it can be said that even if technology is a complex sector, it will always prove to be beneficial and positive for rural development. Out of the entire population in our nation, 69.8% live in rural villages, and the literacy rate in rural villages throughout different Indian states is considerably lower than 80%. To effectively contact rural residents and provide for their needs is seen to be a bigger difficulty and set of hurdles for government authorities (Govt. of India, 2015). The government of India has taken a number of steps to promote rural communities and has made several efforts to educate rural residents on how to use technology. To meet the needs and demands of rural inhabitants, it has created Common Service Centers in isolated communities. CSCs are viewed as a hub for e-governance services in industries including agriculture, financial inclusion, digital platform services, and health care (Bhuvana & Vasantha, 2020). This study aims to find the difference in the usage of technological gadgets and applications in the rural areas of Samba district of Kashmir Division before and during Covid-19.

Review of Literature

Indian Telecommunication Industry and Covid-19

Today, India has recorded strong growth over the last decade in the world's telecom industry; With 1.16 billion subscribers, India is the second-largest telecoms market. India's mobile economy is growing, according to a research created by the GSM Association (GSMA) and the Boston Consulting Group (BCG). Indian users is one of the highest consumers of data per day, with around 5 hours of daily time spend on smart phones. It will contribute significantly to India's Gross Domestic Product (GDP). This sector is expected to contribute from around 6.5% to 8% of India's GDP in 2022. In 2019, India became the second market in terms of app downloads, surpassing the United States.

Some of the telecom service providers are

Bharti Airtel limited

Headquartered in New Delhi, with operations in 18 countries, Airtel is a dominant player on the international scene. All three of the leading mobile service industries are surpassed by Airtel.

Jio

Reliance The newest company to use exclusively VOLTE to support phone service on its 4G network is Jio. With more than 389 million members as of the most recent update through December 31, 2019, Jio is ranked as the third-largest mobile operator in the world and has the biggest mobile network in India. It is headquartered in Maharashtra,

BSNL (Bharat Sanchar Nigam Limited)

This one is a government company in the telecom industry. Its head office is in New Delhi. For the best communication in India's rural and urban locations, BSNL offers wide network coverage.

Social Media

The usage of social media is a prominent topic of conversation among rural young, who utilise it to develop, invent, disseminate, share, and express their opinions about online communities. The number of individuals using social networking sites has dramatically expanded, and it now makes it possible for young people to stay in touch with friends, family, and other parts of society. Despite the fact that social media is altering how young people communicate with one another on social networking sites. Youths are constantly in contact with those who are more alone and developing new ethnocentrism. Ten years ago, it's possible that rural adolescents only kept in touch with their friends and peer groups when attending college or school events in towns and cities. Rural youth may now be reached via instant messaging, social networks, and online games thanks to the use of numerous social media platforms. Rural adolescents are growing up in a continually linked to the society, and particularly rural youngsters

are always full of optimism for their future lives in order to change the attitudes, perceptions, psychology, culture, and other aspects of society.

Actual Usage (AU)

Actual use is characterized as the behavioral reaction assessed by the person's actual activities. It is the length of time spent utilising the system and the frequency of use (Davis et al., 1989). Researchers have looked at consumers' intentions to use the Internet for shopping. Researchers have built a conceptual model for assessing the actual usage of customers' Internet purchasing using the TAM (Technology Acceptance Model) and TPB (Theory of Planned Behavior) (Hamit, 2012). Senior analysts at business schools' marketing departments have been the focus of studies looking for primary data.

Information and Communication Technology (ICT)

People may use ICTs to identify, customize, and buy and sell products, and help the sector as well as themselves to internationalize by providing tools for producing, managing, and distributing offers internationally. ICTs are swiftly becoming a decisive element for tourism businesses to compete. Improved ICT capabilities, along with reduced equipment sizes and cheaper ICT costs, boosted the reliability, portability, and connectivity of a variety of terminals and applications. ICTs are a strong instrument that may assist the industry's approach and the process by sponsoring and magnifying it. ICT advancements, such as the internet and the web, have aided communication. It has also lowered costs and improved market share.

Digital divide

The disparity in access to and usage of ICT across people, businesses, regions, and nations is known as the "digital divide" at the moment (Pejic *et al.,* 2013). In a democratic society, it is undesirable to have unequal access to chances to meet demands and enhance living circumstances as a result of different approaches to technical tools and services like mobile telephone, computers, and the internet. Since 1995, when the Falling through the Net study by the U.S. Department of Commerce utilised it to explain how different nations had unequal access to developing ICT, this idea has gained popularity (Yu, 2011). In the early research on the topic, the digital divide had two dimensions: one focused on who has access to technology, and the other on the disparity in technical abilities among those who had access.

METHODOLOGY

This study used general survey on convenient basis to contact the educated people living in the rural areas of a particular district of Jammu. A total of 9 villages were selected for the purpose of collecting the data from respondents. Responses of 1100 people living in such villages have been used to measure the trend of ICT usage. Descriptive Statistics was used to find the differences in the usage of information and communication technology in the form of social media and other shopping applications.

RESULTS

Demographic Analysis and Descriptive statistics

Out of the 1100 hundred respondents 700 (63.63%) were males and 400 (36.36%) were females. 824 (75%) respondents are graduates, 165 (15%) respondents are matric pass and 111 (10%) respondents are below matric. With respect to phone usage experience 660 (60%) respondents are using Smartphone from last 9 years, 330 (30%) respondents are using Smartphone from last 5 years and 110 (10%) are using from last 3 years. From Table 2 it is evident that rural areas have seen a surge in the internet usage in the form of various applications whether Social media or Shopping. The results revealed that there is a significant and positive difference in the usage of information and communication technologies by the rural masses before and after covid-19 pandemic paving the way for the slogan ‘Digital India’ by the prime minister of India. As evident from table 2 the use of Facebook has increased by 36.37% from 700 users to

1100 users. Similarly Instagram, YouTube, Snapchat and Email usage has increased by 28%, 19%, 18%, and 15%. Looking at the usage of shopping applications by the rural residents, the usage of Meesho has seen a highest surge with an increase of 45% followed by Amazon (9 %) and Flipkart (9%). From the discussion and results revealed it can be understood that the acceptance of technology by the residents of rural areas has seen a greater surge and thus bringing ease to them by having access to day to day happenings and availabilities in the world. The results are also supported by the report of news9live (2021) which states that the proportion of internet users in rural India has increased. Online media was the second most popular type of media consumed in rural regions in the past six months, after television, according to a survey by media investment firm Group M and data consultant Kantar. Facebook and WhatsApp were determined to be the two most popular social media platforms in rural regions, with 87 and 66 percent usage, respectively, according to the survey, titled "Rural COVID Barometer Report". "By 2025, there would be a greater number of internet users in rural India than in urban India. Given this, the digital ecosystem will need to evolve to address the specific needs of this emerging demography," said Biswapriya Bhattacharjee, executive vice president of Kantar's insights division.

DISCUSSION AND CONCLUSION

This study aimed at exploring the changing attitude of people living in the rural areas about the acceptance of technology. The study was carried out by contacting the respondents living in the different areas/villages of Samba district of Jammu and Kashmir. The study revealed a positive change in the behaviour of rural people towards technology after COVID-19. Technology is not a panacea for all of India's problems. However, it can significantly lessen the frequency and severity of many developmental issues. By enabling more and better services, changing economic activity, and eliminating inequities based on variables like location and history, it can assist enhance quality of life, especially for the poor and disadvantaged. Academic research generally agrees that inadequate rural telecommunication facilities impede rural development, widen regional growth gaps, and eventually harm national economies' competitiveness (Salemink *et al.,* 2017). However, it is also true that, just as youth migration, physical isolation, and a lack of financial means in the case of rural communities, digital inequality is a reflection of earlier social inequalities (Robinson *et al.,* 2015). These phenomena may be significant since many rural areas may not be able to fully utilise this technology even with access to broadband internet, leaving them at a disadvantage against urban areas (Philip & Williams 2019).

Additionally, it has been observed that even in remote areas where broadband access is available, many locals choose not to engage in contemporary online culture owing to a lack of ability or even want to learn new technology. Digital connections are not necessarily used just because someone has access to them. Due to this, it has been assumed that the adoption of digital technology in rural areas is the consequence of the interplay of personal traits like personality, drive, or inventiveness with other contextual traits of a socio-cultural character (Correa & Pavez, 2016). In the present liberalized competitive environment, industry should pay much attention to the external sources of technology. Measures need to be put into practice to upgrade technology. When advancements would take place in technology, then it would contribute efficaciously in bringing about improvements in the livelihoods opportunities of the individuals. The most significant aspect is, when rural artisans, craftsmen, farmers and other individuals will make use of technology in their tasks and activities, and then they would be able to augment productivity. From this context, it has also been inferred that the implementation of digital education programmes aimed at mediating agents who may serve as animators of vulnerable groups is necessary in order to promote their participation in the digital and social spheres.

REFERENCES

1. Bhuvana, M., & Vasantha, S. (2020). Role of Information and Communication Technology (ICT) for rural development through e-governance initiatives. *Int. J. Psychosoc. Rehabil. 24*(08), 2705–2713.

2. Boateng, M. S. (2012). The Role of Information and Communication Technologies in Ghana's Rural Development. Retrieved May 29, 2016 from http://digitalcommons.unl.edu/cgi/viewcontent.cgi?article=2065&context=libphilprac
3. Davis, F., Bagozzi, R., & Warshaw, P. (1989). User acceptance of computer technology: a comparison of two theoretical models. *Manage. Sci. 8,* 982–1003.
4. Government of India: India in Figures (2015). Ministry of Statistics and Program Implementation. Central Statistics Office, Central Statistics Office Research and Publication Unit, New Delhi [Online] (2015). Available: http://www.mospi.gov.in/
5. Hamit Turan, A. (2012). Internet shopping behavior of Turkish customers: comparison of two competing models. *J. Theor. Appl. Electron. Commer. Res. 7*(1), 77–93.
6. News9live. (2021). https://www.news9live.com/india/rural-india-internet-consumption-facebook-whatsapp-kantar-and-groupm-120261
7. Pejic, M., Zoroja, J., & Bosilj, V. (2013). Review of corporate digital divide research: A decadal analysis (2003–2012). *Int. J. Inf. Syst. Proj. Manag., 1*, 41–55.
8. Philip, L. J., & Williams, F. (2019). Remote rural home-based businesses and digital inequalities: Understanding needs and expectations in a digitally underserved community. *J. Rural Stud.,* 68, 306–318
9. Robinson, L., Cotten, S.R., Ono, H., Quan-Haase, A., Mesch, G., Chen, W., Schulz, J., Hale, T., Stern, M. (2015). Digital inequalities and why they matter. *Inf. Commun. Soc.*, *18*, 569–582.
10. Salemink, K., Strijker, D., & Bosworth, G. (2017). Rural development in the digital age: A systematic literature review on unequal ICT availability, adoption, and use in rural areas. *J. Rural Stud., 54,* 360–371.
11. Yu, L. (2011). The divided views of the information and digital divides: A call for integrative theories of information inequality. *J. Inf. Sci., 37*, 660–679.

Table1: Demographic Analysis of respondents

Gender	Male: 700 Female: 400	63.63% 36.36%
Educational Background	Graduates: 824 Matric Pass: 165 Below Matric: 111	75% 15% 10%
Smartphone Usage	9 years: 660 5 years: 330 3 years:110	60% 30% 10%

Table2: Usage of Social Media and other Marketing Applications

Applications	Before Covid-19	During and after Covid-19
Facebook	700 users	1100 users
Instagram	350 users	670 users
Youtube	735 users	950 users
Snapchat	150 users	350 users
Meesho	100 users	600 users
Flipkart	500 users	600 users
Amazon	450 users	550 users
Email	938 users	1100 users

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REVIEW ARTICLE

Herbal Cosmetics: A Review on Herbal Face Pack

Anupriya Sundriyal[1]*, Jasmeen Syan[1], Bhawana Bhatt[2], Yogendr Bahuguna[3] and Chandra Shekher Tailor[4]

[1]PG Research Scholar, Department of Pharmacognosy, Shri Guru Ram Rai University, Patel Nagar, Dehradun, Uttarakhand, India.
[2]Assistant Professor, Department of Pharmacognosy, Shri Guru Ram Rai University, Patel Nagar, Dehradun, Uttarakhand, India.
[3]Associate Professor and Head, Department of Pharmacognosy, Shri Guru Ram Rai University, Patel Nagar, Dehradun, Uttarakhand, India.
[4]Associate Professor, Department of Pharmacognosy, Shri Guru Ram Rai University, Patel Nagar, Dehradun, Uttarakhand 248001, India.

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***Address for Correspondence**
Anupriya Sundriyal,
[1]PG Research Scholar,
Department of Pharmacognosy,
Shri Guru Ram Rai University,
Patel Nagar, Dehradun, Uttarakhand, India.
Email: anupriyasundriyal.25june01@gmail.com

ABSTRACT

In the global market herbal cosmetics have a growing demand. Many cosmetics are being manufactured by the pharmaceutical companies. Herbal cosmetics are the natural cosmetics which have been used since ancient times. Since the herbal cosmetics have lesser side effects than the synthetic ones so they are more prevalent. More than 80% of the world population in dependent upon the herbs. These herbs not only treat the diseases but also are used in the form of cosmetics. Skincare cosmetics is one of the major classifications of the herbal cosmetics. This review article contains the information about the herbs which are used in a face pack. People are using herbs for homemade natural face packs since the ancient era. These natural face packs give smooth and radiant skin and also treats acne, pimples, scars, marks and pigments. They have a non-toxic nature and they reduce the allergic reactions. The aim of this article is to provide information about the herbs which are used in a face pack. These herbs can be used in a combination or can be used a single constituent.

Keywords: Cosmetics, synthetic, allergic, natural, homemade, pharmaceutical

INTRODUCTION

Cosmetics are defined as the products used for the purposes of cleansing, beautifying, promoting attractiveness or alternating the appearance. Different herbs are used for cleaning and beautifying our face. Since ancient times herbs are used for different purposes [1]. The word cosmetic was derived from the Greek word "kosm tikos" meaning having the power and skill in decorating [2]. Health, habits, routine job, climatic conditions and maintenance are the factors on which skin of individual depends. Due to excessive exposure to heat the skin dehydrates during summer and cause wrinkle, freckles, blemishes, pigmentation and sunburns. Cracks, cuts, maceration and infections are caused to skin due to extreme winter which damage our skin [3]. The cosmetic according to the Drugs and Cosmetics Act 1940 is defined as the article intended to be rubbed, poured, sprinkled or sprayed on, introduced into or otherwise applied to the human body or any part thereof for cleansing, beautifying, promoting attractiveness or altering the appearance. The preparations containing phytochemicals from a variety of botanical sources, which influences the functions of skin and provide nutrients necessary for the healthy hair or skin are herbal cosmetics. These cosmetics have lesser side effects than the other cosmetics. The natural herbs and their products are used for their aromatic value in cosmetic preparation. These natural herbs make our skin healthy [3].

Herbal Face Pack

The herbal paste which is applied on face to treat acne, pimple, scars, marks and pigments is known as "mukha lepa" in ayurveda. The process of smearing this herbal mix on face is known as "mukha lepana". This beauty therapy is popular as facial. "Face pack" is the smooth powder which is used for facial application. A good herbal face pack must supply necessary nutrients to skin. It should penetrate the subcutaneous tissues in order to deliver the required nutrients. Different types of skin need different types of herbal face packs. These preparations are applied on the face in the form of liquid or pastes. The face pack is then allowed to dry and set to form film giving tightening, strengthening and cleansing effect to the skin. The face pack is usually left on the skin for five to ten minutes to allow all the water to evaporate. The resulting film thus contracts and hardens and can easily be removed. The colloidal and adsorption clays used in these preparations remove the dirt and grease from the skin of the face while the warmth and tightening effect produced by application of face pack produces the stimulating sensation of a rejuvenated face. Skin debris and deposited dirt gets removed when we remove or peel off the applied face pack [4]. The Natural face packs do contain some vital vitamins that are required for the health and glow of our skin. These substances also prove to be beneficial for our skin in many ways.

The natural face packs are pretty simple to use and less complicated. They help us in increasing the circulation of the blood within the veins of the face. Effects of the facial packs are generally temporary. It should be used 2-3 times a week for regular glow [5]. Nowadays for the oily, normal and dry skin different types of packs are available separately which can be used easily according to the skin type. To increase the fairness and smoothness of the skin face packs must be used regularly. Wrinkles, Pimples, acne and dark circles of the skin are removed by the regular application of the face pack. Face packs which are recommended for oily skin prone to acne, black heads usually control the rate of sebum discharge from sebaceous glands and fight the harmful bacteria present inside acne lesion. Due to the various benefits of herbal face packs over chemical-based packs, herbal face packs are being used on a large scale. They are non-toxic, non-allergic and non-habit forming. They have large shelf life are natural in every aspect. They do not need any preservatives and they can be easily formulated and stored over a larger span of time [6].

Benefits of using Herbal Face Pack [7,8]

1. Make the skin look young and healthy.
2. Provide soothing and relaxing effects on the skin.
3. Regular use of natural face packs improves skin texture and complexion and brings glow to the skin.
4. Wrinkles, fine lines and sagging of skin can be effectively controlled by using natural face packs regularly.
5. The harmful effects of harsh climate and pollution can be effectively tackled with regular use of face packs.

6. Helps in removing uneven skin tone and pigmentation.
7. Nourishes the skin and provides essential nutrients to skin.
8. Reduces scars and marks of the skin.
9. Removes dead cells of the skin.
10. Removes dead skin cells which cause black heads and white heads, cleans and exfoliates the skin.

Procedure to apply a Herbal Face Pack

1.Take the required quantity of herbal powder and mix it with normal water or rose water.
2.Make a thick paste of the powder and apply evenly on the face.
3.Wait for five to ten minutes until it dries.
4.Wash the face with cold water as soon as the face pack dries.

Precautions to be taken while applying Herbal Face Pack[9]

1.Select the face pack according to your skin type. Take opinion of concerned skin expert or a natural skin therapist before applying face pack.
2.The face pack should not be left on face more than 15 to 20 minutes. Formation of wrinkles, sagging of skin and enlargement of open pores can be caused if the face pack is applied for a long period of time.
3.The dried face pack should not be scratched. Before removing dried face pack, water (which is at room temperature) must be sprayed on the face. Roll an ice cube on facial skin after removing the face pack for soothing effect. This helps to close open pores and tighten the skin. It also sooths and tones the skin.
4.Face pack should not be applied on allergic skin.
5.Do not scrub face vigorously as it may result in eruption of pimples and dark spots.
6.Stay away from heat when you have applied face pack.
7.Face pack should not be applied near “eye zone” as the skin around eye is very delicate. The process of removing face pack may damage skin around eyes.

Ideal properties of a Herbal Face Pack [10]

1.It should be non toxic and non-irritating.
2.It should be stable both chemically and physically.
3.It should be free from gritty particles.
4.It should have pleasant odour.

Storage of a Herbal Face Pack

The herbal face pack must be stored in a cool and dry place and in a well closed container and should be kept away from moisture. The herbal face packs are often kept in plastic bags which are well sealed.

Herbs/Plants Used As Face Packs

Manjistha *(Rubia cordifolia)*

Manjistha holds the reputation of a very good skincare herb. It helps one to gain lustre and glow (of the skin) and aids to remove pimples, freckles and discoloration. Its paste should be applied in various skin disorders like itching, black spots on the face, pimples, leukoderma. According to Charaka, Manjistha is varnya (improving the complexion), jvarahara (febrifuge) and visaghna (detoxifier) [11].

Haridra /Turmeric *(Curuma longa)*

Haridra hasanti-allergic and anti-flammatory activity. It is best blood purifier and also helps in wound healing. It has best blood purification action so it is used in all diseases with blood impurities origin. Haridra is rejuvenator of skin and revitalizes skin. It delays the signs of aging like wrinkles. It is used in the face packs due to its antiseptic action. It cures the skin diseases occurring due to blood impurities. The phyto constituents, mainly terpenoids present in it

helps to lighten the skin tone. Turmeric delays the signs of aging like wrinkles, improves skin elasticity. It cures uneven skin tone, pigmentation and dull skin [12].

Rakta chandan / Red Sandalwood *(Santalum album)*

Rakta chandan has curative value in skin allergies. Rakta Chandan powder has cooling and soothing action and it protects the skin against the impact of environmental pollution and keeps the skin cool, fair and healthy. Red Sandalwood is helpful ayurvedic herb with antimicrobial properties. Is used for healing various skin problems and removes scars. Red Sandalwood has an anti-tanning and antiaging property. Red Sandalwood protects the skin against the impact of environmental pollution and keeps the skin cool, fair and healthy. Red Sandalwood is helpful ayurvedic herb with antimicrobial property is used for healing various skin problems and remove scars. Red Sandalwood may have different effects on the skin. Constituents of Red Sandalwood wood may restore and rejuvenate wrinkles skin by several activities. Red Sandalwood has antioxidants, anti-inflammatory, cell regulatory properties. Red Sandalwood has potential to develop advanced skin care products and dermatological treatment products [13].

Lodhra *(Symplocos racemosa)*

Its name lodhra in Sanskrit means “that which makes the body firmer.” Lodhra nourishes the skin and benefits in acne, wrinkles and other health issues related with skin. It lightens skin colour, reduces skin irritation and benefits for acne, wrinkles and other skin related issues. Lodhra is useful in skin diseases requiring purification of the skin[14].

Rose *(Rosa rubiginosa)*

Rose petals powder is rich with the antibacterial properties. Rose petals powder is rich in Vitamin K, C and B. It also has good number of antioxidants. Rose petals powder is rich with the antibacterial properties, due to the positive effects of vitamins K, C and B. It also provides a pleasant smell. Petals of flower are used commercially in perfume industries[15].

Orange *(Citrus sinensis)*

It cures blackheads, cell build up around the pores of the skin. It brightness the face, removes dark circles, dry skin, wrinkles, aging and prevents acne. Orange peel can be used for facial cosmetics. Orange is a folk medicine and it is a good source of Vit C, B, flavonoids and terpenes. As per traditional Chinese it is considered as a symbol of good luck and prosperity. It has Antibacterial, Antifungal and Larvicidal activity. Orange is a citrus fruit which contains different nutritional source such as vitamin C, calcium, potassium and magnesium. It prevents the skin from free radical damage, skin hydration and oxidative stress. It also has instant glow property, prevents acne, blemishes, wrinkles and aging[16].

Tomato *(Solanum lycopersicum)*

Tomato has large amount of antioxidant and vitamin C. Since Tomato acts as a natural bleaching agent, it is widely used for face whitening. It helps in pimple and acne reduction has antiaging effect, reduces oiliness, blackheads and also help wake up the dullest of skin. Tomato may sooth skin inflammation, stimulate collagen production and helps in removing dead skin cells. Tomatoes also helps to shrink large pores and brightens up skin complexion, it contains salicylic sour a common ingredient in acne products. It cleans and exfoliates the skin to remove dead skin cells that can clog pores and white heads or black heads [17].

Potato *(Solanum tuberosum)*

Potatoes contains an enzyme called " Catecholase" which helps to brighten skin and get rid of dark spots. Potato juice is used to lessen the appearance of dark circles. It has antiaging effect and can gently exfoliate skin. Potatoes have essential nutrients, including Potassium and vitamins. Potato powder has antioxidant, antimicrobial and wound healing property [18].

Neem *(Azadirachta indica)*
Neem is anti-inflammatory, antiseptic and highly beneficial for oily and acne prone skin. Its anti-acne effect is due to anti-microbial, anti-inflammatory and anti-oxidant activities of different chemical constituents. "Sarva Roga Nivarini the curer of all ailments" Neem's role as a wonder drug is stressed as far back as 4500 years ago. Some of its health restoring benefits are effective in skin infection, rashes & pimples. It acts as blood purifier for beautiful & healthy skin [19].

Aloe *(Aloe barbadensis)*
Aloe vera rejuvenates skin, hydrates this and keeps skin layer looking fresh all the time. Aloe vera has anti-microbial property rendering it ideal to deal with acne and pimples. Aloe vera is a great moisturizer intended for moisturizing the skin. Aloe vera powder contains several nutrients like glycerin, sodium palmate, sodium carbonate, sodium palm kemelate and sorbitol. It acts as an antibiotic, an astringent, a coagulating agent, a pain inhibitor and a growth stimulator (also called a "wound hormone"), whose function is to accelerate the healing of injured surfaces. It is used for pain relief and healing of 'hemorrhoids, applied externally and internally. It can also be used for the treatment of sunburn, scratch and a cleansing purge for the body or skin. It is an aid to growing new tissue and alleviating the advance of skin cancer caused by the sun [20].

Lemon *(Citrus limon)*
The high content of Vitamin C in lemon helps to lighten the skin tone and remove dark spots caused by skin tan. It prevents the skin from free radical damage, skin hydration and oxidative stress. Lime juice and lemon powder is very beneficial for skin when consumed orally or applied externally. It rejuvenates the skin, keeps it shining, protects it from infections. It also reduces body odour due to presence of a large amount of vitamin-C and Flavonoids, both of which are class-1 anti-oxidants, anti-biotic and disinfectants. When applied externally on skin, its acids scrub out the dead cells, cures dandruff, rashes, bruises etc. and gives you a refreshing bath if its juice or oil is mixed into your bathing water[21].

Green tea *(Camellia sinensis)*
Slows down ageing and reduces skin inflammation and gives skin a healthy glow. Whether applied topically or consumed as a beverage or dietary supplement, green tea is a premiere skin protectant. It protects against direct damage to the cell and moderates' inflammation. Catechins in green tea have antioxidant powers and are twenty times stronger than even vitamin E [22].

Camphor *(Cinnamonu camphora)*
It acts as a counter irritant. Camphor can help in getting rid of rashes and redness when used as topical itch relieving gels. Camphor helps in treating inflammation and is widely used in many lotions, liniments and ointments. There are many pharmaceutical applications for camphor such as topical analgesic, antiseptic, antispasmodic, antipruritic, anti-inflammatory, anti-infective, rubefacient, contraceptive, mild expectorant, nasal decongestant, cough suppressant. Camphor is easily absorbed through the skin and can also be administrated by injection, inhalation and ingestion [23].

Liquorice *(Glycyrriza glabra)*
It is used for the removal of skin pigmentation, prevents sun damage, fades scar and treats wrinkles. Makes the skin look plumper and brighter [24].

Papaya *(Carica papaya)*
It is a popular bleaching agent, antioxidant. Heals chronic skin ulcers and its protease enzyme soothe the irritated skin. The presence of vitamin A helps to restore and rebuild damaged skin. Papaya acts as skin lightening agent. When the Papaya powder is mixed with honey and applied, it can act as soothing agent. It helps in moisturizing the skin [25].

Nutmeg *(Myristica fragrans Houtt)*
Nutmeg has Antimicrobial, Antiseptic, Anti –inflammatory properties. It reduces pigmentation, gently exfoliates the skin, treats oily skin and promotes youthful skin. The nutmeg oil extract is not only beneficial to human skin but also capable of preserving the shelf life of oils within emulsions and cream bases [26].

Almond *(Prunus dulcis)*
Acts as cleanser, scruber and moisturizer. As a rich source of vitamin E it makes the skin soft and smooth. It also helps in reducing fine lines and wrinkles of the face [27].

Cucumber *(Cucumis sativus)*
Hydrates the skin and soothes inflammation, reduces eye wrinkles. Helps combat premature aging and aids acne prone diseases [28].

Tulsi *(Ocimum tenuiflorum)*
Tulsi has anti-bacterial and anti-fungal properties. Fights wrinkles, dark spots, acne and fine lines [29]. Tulsi contains ursolic acid, a compound that prevents wrinkles and helps retain the elasticity prevalent in young faces. Tulsi is widely used in beauty industry and is used as a prime ingredient in herbal cosmetics, including face packs, creams and many other products. Tulsi enhances beauty; many Indian women make it a part of their daily beauty ritual. Applying Tulsi powder removes spots from your face. Leukoderma can be cured by regular application of home made tulsi paste [30]

Saffron *(Crocus sativus)*
It is rich in carotenoid glycosides, mainly containing terpenoids. It lightens the skin tone and provides fair and glowing skin [31]. Prolonged exposure of Saffron face pack to the sun is extremely harmful because it puts the skin in contact with UV rays, known to cause serious lesions. Saffron is known to have anti-sun effects that can protect the skin from harmful UV rays. Saffron reduces the pigment called melanin and it is very effective as a lightening agent for the skin. The formulation containing *C. sativus* extract causes significant depigmentation and anti-rhythmic effect on human skin. In traditional herbal cosmetics uses, saffron can be soaked with a few basil leaves to treat blemishes such as acne. A mixture of soaked saffron strands and virgin coconut oil, or olive oil, and a bit of raw milk is an effective way to exfoliate and improve blood circulation face skin. Saffron is known to reduce a skin condition called erythema, characterized by inflammation, redness or rash. Saffron is rich in antioxidants and is expected to inhibit the expression of markers of inflammation such as tumour necrosis factor and interleukin. An application of the formulation containing 3 % of *C. sativus* extract to human skin may be useful in the management of melanoma [32].

Avocado (*Persea americana*)
Avocado is packed with skin healthy minerals and vitamins such as iron, calcium, potassium, copper, magnesium, vitamin A, E, B and K and unsaturated fats. Avocado helps cure dry weather symptoms. Avocado has nourishing oils which help to smooth wrinkles and improve the elasticity of skin. Avocados are also rich in potassium and fibre and also contain several plant-based nutrients which are beneficial for us [33,34].

Beetroot (*Beta vulgaris*)
Beet roots are rich in vitamins C, A, E, K. They have an important content of B-vitamins (B1 - thiamine, B2 - riboflavin, B3 -niacin, B5 -pantothenic acid, B6 -pyridoxine, B9 -folates and B12-cyancobalamin), as well as folic acid and powerful antioxidants, such as triterpenes, sesquiterpenoids, carotenoids, coumarins, flavonoids and phenolic compounds [35]. A beet root powder face pack protects our face from wrinkles. Beetroot juice with clay and husk like multani mitti and wheat husk is used to keep the skin fair and flawless [36].

Coffee (*Coffea Arabica* Linn)
Skin care happens to be one of the benefits of coffee. Full of antioxidants and anti-ageing properties, coffee is equipped with amazing exploitation abilities that can boost skin health. Applying coffee in the form of face pack reduces skin swelling and puffing by promoting blood circulation [37].

Mung bean (*Vigna radiata* Linn)
Mung bean is one of the plants that is rich in antioxidant compounds. Vitexin and isovitexin have an antioxidant effect. These are flavonoid compounds present in mung bean. Vitexin effectively inhibits UV rays that can stimulate skin cell death [38].

Chick pea (*Cicer arietinum* Linn)
The seed powder of chick pea is used as a face pack. It improves the skin complexion [39].

Tanner's Cassia (*Senna auriculata* Linn)
Flowers of the tree are used as a face pack. It cures itching [39].

Evaluation Parameters For Herbal Face Pack

Organoleptic Properties
The colour, odour, taste and texture of the powder are tested manually.

General powder Characteristics
Microscopy method is used to test the particle size of the powder. Angle of Repose by Funnel method, Bulk Density and Tapped Density by Tapping Method are the tests which are performed to evaluate the flow property of the powder[39].

Physicochemical Evaluation
Total ash and Acid insoluble ash is performed using incinerator, pH is found by using pH meter. Irritancy, stability and washability tests are also performed for the evaluation of a herbal face pack.

CONCLUSION

Face is a parameter that identifies a person and all people try to keep it neat and clean. The herbal formulations are safer to use and have lesser side effects hence these herbs are very useful in cleansing our face. The herbs discussed in the review article have lots of medicinal benefits. These herbs are used in a face pack as a single constituent as well as a poly herbal face pack. These herbs are very beneficial as well as economical. Face pack is a very important cosmetic product and can be easily prepared at home with the help of these herbs. In the present scenario herbs play an important role in all the aspects hence are used widely.

REFERENCES

1. Aglawe SB, Gayke AU, Mindhe SA, Rane VG. Formulation and evaluation of herbal face pack. Int J Pharm Biol Sci. 2018;8:49-52.
2. Shivanand P, Nilam M, Viral D. Herbs play an important role in the field of cosmetics. International Journal of PharmTech Research. 2010;2(1):632-9.
3. Sumit K, Vivek S, Sujata S, Ashish B. Herbal cosmetics: used for skin and hair. Inven. J. 2012 Oct 10;2012:1-7.
4. Yadav N, Yadav R. Preparation and evaluation of herbal face pack. International Journal of Recent Scientific Research. 2015 May;6(5):4334-7.

5. Grace XF, Vijetha RJ, Shanmuganathan S, Chamundeeswari D. Preparation and evaluation of herbal face pack. Adv J Pharm Life Sci Res. 2014;2(3):1-6.
6. Pal RS, Pal Y, Wal P. In-house preparation and standardization of herbal face pack. The Open Dermatology journal. 2017 Oct 31;11(1).
7. Saraf S, Saraf S. Cosmetics a Practical manual, Pharma med Press, 2nd edition, 2005; 126-129.
8. Anilkumar V, Kalyani R, Padmasri B, Prasanth D. In-house preparation, development and evaluation of herbal cosmetics face pack using various natural powders. Journal of Drug Delivery and Therapeutics. 2020 Sep 15;10(5):159-64.
9. Baby AR, Zague V, Maciel CP, Salgado-Santos IM, Kawano Y, Arêas EP, Kaneko TM, Consiglieri VO, Velasco MV. Development of cosmetic mask formulations. Revista Brasileira de Ciências Farmacêuticas. 2004;40:159-61.
10. Chanchal D, Swarnlata S. Herbal photoprotective formulations and their evaluation. The Open Natural Products Journal. 2009 Jun 26;2(1).
11. Meena V, Bhushan S, Chaudhary AK, Resident J. Standardization of Mugdha Lepa: an ayurvedic proprietary herbo-mineral face pack for Acne vulgaris. World J Pharm Res. 2017 Oct 4;6(16):530-9.
12. Pandey S, Hiremath VR, Shashikala K, Gururaj N. SINGLE CASE STUDY-ROLE OF HARIDRADI MUKHALEPA ANUBHUTA YOGA IN COSMETOLOGY.
13. Chaudhari TG, Sneha K, Mukund D, Pallavi J. Role of Lepakalpana for improving Beauty of Skin wsr to Mukhalepa. International Journal of Ayurveda and Pharma Research. 2017 May 24.
14. Parmar S, Pandya DH, Harisha CR, Shukla VJ. PHARMACOGNOSTICAL AND PHARMACEUTICAL EVALUATION OF YAVADILEPA.
15. Nowak R, Olech M, Pecio Ł, Oleszek W, Los R, Malm A, Rzymowska J. Cytotoxic, antioxidant, antimicrobial properties and chemical composition of rose petals. Journal of the Science of Food and Agriculture. 2014 Feb;94(3):560-7.
16. Parle M, Chaturvedi D. Orange: Range of benefits. International Research Journal of Pharmacy. 2012;3(7):59-63.
17. Ijaz N, Durrani AI, Rubab S, Bahadur S. Formulation and characterization of Aloe vera gel and tomato powder containing cream. Acta Ecologica Sinica. 2022 Apr 1;42(2):34-42.
18. Choi SJ, Baeck SW, Choi MH, Jo A, Jang HH, Oh CR, Ahn KJ, An IS, An S, Oh JS. Effects of the face pack containing kaolin, adenosine and plant extracts on the skin improvement. Kor J Aesthet Cosmetol. 2013;11(5):993-1000.
19. Koli DS, Mane AN, Kumbhar VB, Shaha KS. Formulation & evaluation of herbal anti-acne face wash. World J. Pharm. Pharm. Sci. 2016 Apr 15;5(6):2001-200.
20. Rajeswari R, Umadevi M, Rahale CS, Pushpa R, Selvavenkadesh S, Kumar KS, Bhowmik D. Aloe vera: the miracle plant its medicinal and traditional uses in India. Journal of Pharmacognosy and Phytochemistry. 2012 Nov 1;1(4):118-24.
21. Ashawat M, Banchhor M, Saraf S, Saraf S. Herbal Cosmetics:" Trends in Skin Care Formulation". Pharmacognosy Reviews. 2009;3(5):82.
22. Labhade SD, Tomi K. Development and evaluation of bio-transformed fruit face pack. Research Journal of Topical and Cosmetic Sciences. 2015;6(1):44-7.
23. Hamidpour R, Hamidpour S, Hamidpour M, Shahlari M. Camphor (Cinnamomum camphora), a traditional remedy with the history of treating several diseases. Int. J. Case Rep. Images. 2013 Feb 1;4(2):86-9.
24. Bhutkar MK, Shah MM. FORMULATION AND EVOLUTION OF HERBAL ANTIBACTERIAL FACE PACK. Journal of Emerging Technologies and Innovative Research. 2019;6(5).
25. C.K. Kokate, A.P. Purohit, and S.B. Gokhale, Pharmacognosy Pune, India: Nirali Prakashan, 2005; 4: 7-107.
26. Priya R, Anand K, Rasika D. Preparation and evaluation of herbal anti–acne face pack. World J. Pharm. Res. 2017 Mar 31;6:1000-10.
27. SK R, Neelofar Sulatna S, Mohana Priya R, Parameswari CS, Ramana BV, Badarinath AV. FORMULATION AND EVALUATION OF NATURAL HERBAL FACE PACK.
28. Paul BG, Reddy TS, Sailaja AK. Ayurveda Formulation of Herbal Face Pack to Treat Blemishes. Innoriginal: International Journal of Sciences. 2021 Apr 30:30-3.

29. Londhe SS, Joshi AA, Sapkale GN, Bhosale MG. Formulation and evaluation of clay face pack. International Journal of Pharmaceutical Investigation. 2021 Dec 28;11(4):437-40.
30. Chandira KT. Traditional Indian Herbal Plants Tulsi and Its Medicinal Importance. Phytochemistry. 2010 Mar;2(2):103-8.
31. Pal RS, Pal Y, Wal P. In-house preparation and standardization of herbal face pack. The Open Dermatology journal. 2017 Oct 31;11(1).
32. Mzabri I, Addi M, Berrichi A. Traditional and modern uses of saffron (Crocus sativus). Cosmetics. 2019 Oct 25;6(4):63.
33. Zafar J. 5 Effective Remedies to Prepare Your Skin for Winter.
34. Avadiar I, Thanabalan Theeba C, Manimaran G, Peter J, Balakrishnan MK. AVORANGE Facial Mask.
35. Ceclu L, Nistor OV. Red beetroot: Composition and health effects—A review. J. Nutr. Med. Diet Care. 2020;6(1):1-9.
36. Sharma N, Singh P, Gupta SK. A review on role of various medicinal plants in cosmetics and cure health. Current Research in Pharmaceutical Sciences. 2019 Oct 15.
37. Rodrigues F, Palmeira-de-Oliveira A, das Neves J, Sarmento B, Amaral MH, Oliveira MB. Coffee silverskin: a possible valuable cosmetic ingredient. Pharmaceutical Biology. 2015 Mar 4;53(3):386-94.
38. Husni P, Dewi EM. Formulation of peel-off gel mask containing mung bean (Vigna radiata (L.) Wilczek) extract. Indones J Pharm. 2019 Jun 13;1:7-12.
39. Vanithamani M, Mathivani G, Yamuna K, Kaviya S. FORMULATION AND EVALUATION OF TRADITIONAL HERBAL COSMETICS. Agricultural Science: Research and Reviews.:95.
40. Dureja H, Kaushik D, Gupta M, Kumar V, Lather V. Cosmeceuticals: An emerging concept. Indian Journal of Pharmacology. 2005 May 1;37(3):155.

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RESEARCH ARTICLE

Role and Impact Assessment of Mobile Learning: A Study of District Kupwara, North Kashmir

Showkat Ahmad dar[1]* and P. Sakthivel[2]

[1]Research Scholar of Public Administration, Department of Political Science and Public Administration, Annamalai University, Chidambaram, Tamil Nadu, India.
[2]Professor, Department of Political Science and Public Administration, Annamalai University, Chidambaram, Tamil Nadu, India.

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***Address for Correspondence**
Showkat Ahmad dar,
Research Scholar of Public Administration,
Department of Political Science and Public Administration,
Annamalai University, Chidambaram, Tamil Nadu, India.
Email: showkatdar0404@gmail.com

ABSTRACT

Technology changes how, when, and where students learn. Mobile technology makes student-centered learning more compelling than collecting lecture notes. Kashmir is often referred to as "paradise on earth." It's referred to as the "Switzerland of Asia" by Westerners. Kupwara is one of the ten districts in the Kashmir Valley India, and one of its 20 districts. It is the subject of this paper's research. This article examines the function and consequences of mobile technology on students in District Kupwara, as well as the crucial role and challenge it brings to education and other disciplines. Semi-structured interviews and purposive sampling are used in this study, along with a simple tabulation percentage. Results of the study provide assessment of the reported Role and Impact of mobile technology on learning and education at District Kupwara Kashmir. Furthermore, the study adopted SPSS 25.0 for empirical analysis to reach on conclusion.

Keywords: Changes; Learning; lacunas; Mobile; Technology.

INTRODUCTION

In the context of mobile learning, a mobile device is a device that can be used to access educational content while on the move (Park, 2011). Learners can use their mobile devices, such as Smartphone's and laptops as well as computer tablets, for mobile learning. By allowing learners to learn wherever they are and in their own personal context, mobile learning devices allow for meaningful learning, according to (Sharples 2000). As mobile devices have become more widely available and easier to use, they have become an increasingly important part of the mobile learning landscape. Using mobile technology to enhance the learning process is becoming increasingly popular. In light of the rapid advancement of mobile technologies, educational institutions can expect to see a greater role for them in the future. The use of mobile technologies by educational institutions around the world is on the rise, with (Natalya *et al.* 2018) noting that educational multimedia web resources are increasingly being played on mobile devices, as well as educational websites being made more easily accessible to students. It is now necessary for specialists to have an ever-increasing set of knowledge and skills due to the rapid growth in scientific and technological progress, and this has led to increased expectations for the quality of their training. Many academics, including educators and IT professionals, are interested in issues surrounding computer-aided learning.

Professionals in a wide range of fields are becoming more and more important because of the widespread use of information technology. For both work and school, mobile technology has become a must-have. It is also being used to teach students and help them move up in their jobs. Various computer systems and programmes are used in schools to help students learn and test their knowledge. Specialists' education and training can be a lot better when they use mobile learning. As mobile technologies keep getting better, they will be used in the education process to improve the quality of what students learn. Most of the time, schools are using mobile technology to play multimedia learning resources, such as audio and video files, map files, and images. They also make it easy for students to get to learning sites, resources, and dictionaries quickly. Thanks to mobile information technologies, it is possible for students to learn while they are on the go! Students can get information, collaborate with their peers, record their progress, and get feedback from their teachers at any time and from any place they want. They can also get help from their teachers (Ramnath & Kuriakose, 2015). There have been a lot of changes in how people use mobile devices, like how they can use blogs and wikis or social networking sites that use Web 2.0 technologies.

This has made mobile devices more dynamic and more common. A lot of students today think that mobile technology should be an important part of their education. It's important to look into how mobile technology can be used in the classroom, because this could give us a better idea of how 21st-century students will be educated. It was published in 2014 by Yu and Lee. The term "anytime, anywhere learning" refers to the fact that it can be done at any time and in any place. There are a lot of different digital devices and services that all students can use, like computers that are connected to the Internet and mobile computing devices that they can use when and where they need them. People can learn in both formal and informal settings because they don't need to be in the same place at the same time. This changes how we teach (Peters, 2007). Teachers and students alike believe that the educational use of mobile computing devices and mobile applications can have a significant impact on their learning. Even though smart phones have become ubiquitous in our daily lives, integrating mobile technology into educational settings remains a complex and challenging mission requiring new approaches to teaching and learning as well as strategic shifts in organisational priorities and priorities (Traxler & Koole, 2014).

Research Objectives

To explain and analyse the role and impact of mobile technology on learning and education at Kupwara district, Jammu and Kashmir.

MATERIALS AND METHODS

Mobile technology role and impact on learning and education at Kupwara Kashmir was studied using both a survey and a documentary method. Primary and secondary sources of information are used in the research. The data was tabulated using a combination of qualitative and quantitative methods with a Purposive sampling. As a result of the Mobile technology, 300 students from the Kupwara and Handwara College were interviewed to get their thoughts on the role and impact of the incident of mobile technology on learning. Aside from using official government publications and secondary data from books, journal articles, and the internet, the study looked at how mobile technology contributes in learning and to what extent it laid down an impact on students in the process of learning.

Study Area

We chose the District Kupwara, as the study area to investigate role and the impact of the Mobile technology on learning and education. The respondents were drawn from the education field, including up to 12th grade students, Graduates, above graduates, teachers and the Degree college of Kupwara and Handwara, as well as official reports.

Approach with Participants

Small respondents are typically adequate to accomplish immersion to acquire an intensive comprehension of the participants' viewpoints on the role and Impact of mobile technology on learning and education (Guast *et al.,* 2006; Khan, 2020). To acquire essential information, we utilized purposive sampling to enlist and meet with respondents, as well as optional wellsprings of information. The scientists utilized essential information sources to acquire a superior comprehension of the Role and impact of mobile technology on the learning and education in the 21st century. For this reason, Kupwara district were picked. Analysts who knew about the peculiarity being scrutinized broke down the information from the essential review, and significant articulations were separated and coordinated into topics. All respondents were educated regarding the review's motivation and guaranteed that their data would be kept stringently classified and utilized exclusively for research purposes before information assortment. Moreover, appropriate research ethics were noticed and followed during the study research.

Background of The Respondents

The above table 1 reveals that the dominant majority of the respondents i.e. 29.33% per cent of the respondents were graduate. While the 25.6% per cent belong to the above graduates. Further the distribution of respondents with respect to their education. It is revealed that of the total respondents in the sample, 300 i.e. (22.6%) respondents were Upto 12th grade students. Further, the majority of the respondents were teachers 67, i.e. (22.3%). The study's findings on role and Impact of mobile learning at Kupwara Kashmir, as gathered from the participants, are presented in tabular form below. The Study adopted surveys, a questionnaire used to collect the response from 300 target respondents in English, Kashmiri and Urdu language. The respondents were mainly chosen from Kupwara and Handwara colleges. The three point scale has been adopted for finding the result from the respondents viz Y- Yes, N- No and NI: No idea. The Three point **scale** is mentioned below:

DISCUSSION AND FINDINGS

Table. 2. shows the questions to students and teachers about the role and impact of mobile learning in the 21st century in Kupwara Kashmir. The study found that students and teachers in Kupwara district colleges are aware of mobile learning. Mobile learning devices include Smartphone's, tablets, laptops, and digital notebooks. Mobile learning may help students achieve academically. Both students and teachers favoured mobile learning. The students also praised mobile learning as a way to boost motivation. The world of today is becoming increasingly mobile. It is a result of the rapidly evolving digital era. Nothing is flawless. Participants agreed that mobile learning is the most convenient way for students to receive assistance. Our colleges encourage the use of e-learning and mobile technology. However, unlike Tamil Nadu, the state government does not currently offer a free Smartphone's and

Laptops for mobile learning. They believe that by providing us with free Smartphone's and laptops, mobile learning will take off. They stated that many Indian states provide students with free laptops and mobile phones for educational purposes, but we have yet to receive them. Students living below the poverty line shared this view, according to the study. Participants revealed that there are numerous educational apps available online that are rapidly gaining popularity among high school and college students. Additionally, teachers/professors/instructors can study a subject, provide notes and examples, and refer students to these apps as needed. The internet is an excellent source of information for mobile learning. If you are unable to obtain it from one location, try another. If you have any questions about the app while using it, you can easily send feedback and inquiries to the app developer or app Development Company. Mobile learning is available at any time and from any location in the world. Numerous educational applications utilise online quizzes to gauge your progress (daily, weekly or monthly, depending on firm to firm). Students are motivated to improve their scores because the study is presented in such a way. As previously stated, online quizzes are designed to assist you in learning more. Apart from study guides, the internet contains a variety of quizzes, puzzles, and multiple-choice questions that can be used to assess your knowledge and even help you improve your IQ.

The study found many disadvantages of mobile learning such as lack of internet or electricity in rural areas. To enjoy the best mobile learning experience, we must meet all requirements. The study also reveals the truth about mobile learning issues. The participants believe that since the repeal of Article 370 and Covid 19 Pandemic, there is sporadic internet blocking due to armed conflict, which halts mobile learning. Lack of electricity in Kupwara, the remotest district, hampered students' ability to download exam materials such as e-books. After the repeal of Article 370, many students from wealthy families moved to Delhi and other states to continue their education and prepare for various academic and competitive exams and did not affected by any such problem. The majority of participants stated that the repeal of Article 370, which granted Jammu and Kashmir special status and pandemic caused chaos and lawlessness in the state. The student community believes that internet access is a democratic right that should not be restricted for reasons of law and order. Because students are no longer restricted by the traditional classroom, the study concluded that mobile technology in education has an impact on learning. Participants agreed that while technology can make it easier to learn at home, education is still provided by a teacher. Even in online courses, teachers must deliver lectures via videos, tutorials, Skype sessions, zoom, etc. Those online sessions require children to learn computer skills, which are taught by teachers. Finally, a small percentage of participants disagreed with the questionnaire and gave it thumbs down. This was due to poverty and lack of interest in filling out the interview schedule.

CONCLUSIONS AND FUTURE RECOMMENDATIONS

Students' lives are reliant on mobile devices. They expect mobile devices to be embedded in their learning as digital natives. Mobile learning is collaborative and constructivist in nature. It enables ubiquitous learning – anytime, anywhere learning. Furthermore, secondary and post-secondary educational institutions must continually explore new technologies to increase student engagement, retention, and graduation rates. Despite its huge potential and innovative development of mobile technologies, mobile learning is still in its infancy. A theoretical framework and effective instructional design will help integrate mobile learning applications into teaching and learning, benefiting both students and teachers. Teachers and students will no longer be restricted to a specific location and time. Mobile devices and wireless technologies will be used in classrooms and out. Most students are technically and psychologically ready for mobile learning, and new opportunities should be considered to maximise its potential. This task requires educational leaders to organise, researchers and teachers to research and implement mobile learning strategies, forms, and methods into educational processes.

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REFERENCES

1. Aguilar, G. (2021). Will Technology Ever Replace Teachers? Ideas Suplemento, 3(3).
2. Al-Emran, M., Mezhuyev, V., & Kamaludin, A. (2020). Towards a conceptual model for examining the impact of knowledge management factors on mobile learning acceptance. Technology in Society, 61, 101247.
3. Ally, M. (2013). Mobile learning: From research to practice to impact education. Learning and Teaching in Higher Education: Gulf Perspectives, 10(2), 3-12.
4. Ansari, M. S., & Tripathi, A. (2017). An investigation of effectiveness of mobile learning apps in higher education in India. International Journal of Information studies and libraries, 2(1), 33-41.
5. Botha, A., Batchelor, J., Traxler, J., De Waard, I., & Herselman, M. E. (2012). Towards a mobile learning curriculum framework.
6. Carrillo, F. A. G. (2012). Can Technology Completely Replace Human Interaction in Class? World Journal on Educational Technology: Current Issues, 4(3), 153-164.
7. Chesser, H. (2012). Can Technology Replace Teachers? Serving and Accrediting Independent Schools, 1-2.
8. Criollo-C, S., Guerrero-Arias, A., Jaramillo-Alcázar, Á., & Luján-Mora, S. (2021). Mobile learning technologies for education: Benefits and pending issues. Applied Sciences, 11(9), 4111.
9. Dar, M. A., & Bhat, S. A. (2016, September). Evaluation of mobile learning in workplace training. In 2016 International Conference on Advances in Computing, Communications and Informatics (ICACCI) (pp. 1468-1473). IEEE.
10. Elkheir, Z., & Mutalib, A. A. (2015). Mobile learning applications designing concepts and challenges: survey. Research Journal of Applied Sciences, Engineering and Technology, 10(4), 438-442.
11. Hall, O. P., & Smith, D. M. (2011, August). Assessing the role of mobile learning systems in graduate management education. In International Conference on Hybrid Learning (pp. 279-288). Springer, Berlin, Heidelberg.
12. Hassan, M. M., & Mirza, T. (2019). Mobile Learning: An Approach to Improve the Quantity of Educational Attainment in Conflict Hit Areas Like J&K. Journal on Today's Ideas-Tomorrow's Technologies, 7(2), 118-125.
13. Hooper, S., & Rieber, L. P. (1995). Teaching with technology. Teaching: Theory into practice, 2013, 154-170.
14. Kukulska-Hulme, A. (2012). Mobile learning and the future of learning. International HETL Review, 2, 13-18.
15. Langer, L., winters, N., & Stewart, R. (2014, November). Mobile learning for development: Ready to randomise? In International Conference on Mobile and Contextual Learning (pp. 156-167). Springer, Cham.
16. Motiwalla, L. F. (2007). Mobile learning: A framework and evaluation. Computers & education, 49(3), 581-596.
17. Nasution, A., & Siddik, M. (2020, August). IMPACT OF USING MOBILE LEARNING APPLICATIONS IN THE LEARNING PROCESS. In International Conference on Social, Sciences and Information Technology (Vol. 1, No. 1, pp. 37-42).
18. Palla, I. A., & Sheikh, A. (2020). Impact of social media on the academic performance of college students in Kashmir. Information Discovery and Delivery.
19. Pandey, U. C., & Indrakanti, V. (Eds.). (2017). Open and Distance Learning Initiatives for Sustainable Development. IGI Global.

20. Passey, D. (2009). Mobile learning in school contexts: can teachers alone make it happen?. IEEE Transactions on Learning Technologies, 3(1), 68-81.
21. Prokofyeva, N., & Boltunova, V. (2018). The Use of Mobile Technologies in the Educational Process. In BIR Workshops (Vol. 7).
22. Shah, N. H., Noor, A. E., & Akhtar, M. (2021). Role of Mobile Learning in Enhancing Academic Achievement of Students at University Level. Global Educational Studies Review, VI, 6, 213-223.
23. Sharma, D. (2019). Exploring the Potential of Mobile Learning in Distance Higher Education: A Case Study of the University of Jammu, Jammu, and Kashmir.
24. Sharples, M., Arnedillo-Sánchez, I., Milrad, M., & Vavoula, G. (2009). Mobile learning. In Technology-enhanced learning (pp. 233-249). Springer, Dordrecht.
25. Traxler, J., & Bridges, N. (2005). Mobile learning–the ethical and legal challenges. Mobile learning anytime everywhere, 203.

Table1. Demographic profile of the Respondents (Education)

S. No	Variables	Frequency	Percentage
01	Up to 12th class	68	22.6%
02	Graduate	88	29.33%
03	Above Graduate	77	25.6%
04	Teachers	67	22.3%
Total		**300**	**99.83%**

Source: Primary Data

Table.2. Views of respondents on Role and impact of mobile learning at Kupwara Kashmir

S.NO	STATEMENT	Y	N	NI	Total
1.	Do you know anything about Mobile Learning?	211 70.33%	45 15.%	44 14.6%	300 100%
2.	Is Mobile Learning good or bad for students and teachers?	209 69.6%	52 17.33%	39 13%	300 99.93%
3.	Have you face any problem in Mobile Learning?	213 71%	67 15.5%	20 4.3%	300 100%
4.	Does Mobile Learning discard the traditional learning and educational system?	196 65.5%	33 11.3%	71 23.4%	300 100%

Source: Primary Data

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RESEARCH ARTICLE

Prevalence of Musculoskeletal Abnormalities Among School-Going Children in Chennai using the Pediatric Gait, Arms, Legs, Spine (pGALS) Screening Method: A Cross Sectional Study

Janani Ravi[1]* and Arunachalam Ramachandran[2]

[1]Ph.D Research Scholar, Department of Physiotherapy, Madhav University, Rajasthan and Assistant Professor, Meenakshi Faculty of Physiotherapy, Meenakshi Academy of Higher Education and Research, Chennai, Tamil Nadu, India.
[2]Professor and Principal, Department of Physiotherapy, Madhav University, Rajasthan, India.

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***Address for Correspondence**
Janani Ravi,
Ph.D Research Scholar,
Department of Physiotherapy,
Madhav University, Rajasthan and Assistant Professor,
Meenakshi Faculty of Physiotherapy,
Meenakshi Academy of Higher Education and Research,
Chennai, Tamil Nadu, India.
Email: janani15r@gmail.com

ABSTRACT

Prevalence of musculoskeletal disorders or pain was found to range between 55% to 66% in India among 13.2 crore school going children. musculoskeletal pain is the current concern among school going children due to the health effects that it causes. The objective of this study is to find the prevalence of musculoskeletal abnormalities in school going children in Chennai using pediatric Gait, Arms, Legs, Spine (pGALS) screening tool. This was a School based cross sectional study conducted over a period of 5 months covering selected schools in south Chennai. This study was reported as per the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE). The pGALS maneuvers was administered by Physiotherapists specialized in pediatrics to screen for musculoskeletal abnormalities. Children who were positive for abnormal findings during the screening were assessed in detail and referred to the appropriate. Among the total of 538 children, 336 were selected from private and 202 from government schools; 359 (66.8%) were female and 179 (33.2%) were male with a mean age of 11.18 and 11.84 respectively. To conclude, there is a high prevalence of musculoskeletal pain and abnormalities that was observed among school children in Chennai. Hence it is vital to include regular screening program for school children to identify musculoskeletal abnormalities at the earliest to provide early intervention. Thereby, ultimately improving the health of school children.

Keywords: School children, musculoskeletal pain, musculoskeletal abnormalities, prevalence, Chennai

INTRODUCTION

Musculoskeletal Abnormalities (MSA) create a prominent contribution to global burden that substantially leads to disability among children in both the developed and developing countries especially as India. How much of that abnormality leads to burden in children is unclear! There is uncertainty over the total percentage of burden for musculoskeletal abnormality among school children and the proportion due to musculoskeletal pain. It is plausible that the major health effect of musculoskeletal abnormality is shoulder and low back pain and global estimates of this burden are lacking. Musculoskeletal pain is one among the common problem in school going children. these problems are often due to traumatic cause and could be self-limiting in most of the case, but in certain occasion musculoskeletal pain might manifest serious conditions[1]. Fortunately, World Health Organization (WHO) realized the impact of musculoskeletal disorders, and the declared the years 2000-2010 as bone and joint decade. The main focus on the announcement of this bone and joint decade was not just to aim in improving the quality of life of people with musculoskeletal disorders but also to create awareness and identify the population with musculoskeletal disorders and prevent their adverse circumstances.[1,2]. Prevalence of musculoskeletal disorders or pain was found to range between 55% to 66% in India among 13.2 crore school going children. musculoskeletal pain is the current concern among school going children due to the health effects that it causes.

The reason being the extortionate stress on the child's growing musculoskeletal system leading to deleterious consequences in the later years of child's life [3,14,15]. Although there are sufficient research in this area of musculoskeletal abnormalities among school going children, there is a scarce of data on the exact deleterious effects in the long term. According to the literature the aetiology for musculoskeletal abnormalities is often non-progressive and easily identifiable in most of the cases. In children, MSA is not identified until the visit to a paediatrician due to pain, although this is left unnoticed when the child does not complain of pain. not in all cases presence of pain is mandatory to rule in MSA. To identify MSA at the earliest, pGALS (paediatric Gait, Arms, Legs and Spine), screening tool was validated by Foster et al. [4]. Earlier this tool was developed to screen for MSA among children with juvenile idiopathic arthritis (JIA). pGALS was later used to detect disorders of joints and musculoskeletal system among children in various population. In south India, there is minimal to no data in prevalence of musculoskeletal abnormalities among school going children using pGALS. Hence the objective of this study is to find the prevalence of musculoskeletal abnormalities in school going children in south Chennai using pediatric Gait, Arms, Legs, Spine (pGALS) screening tool.

METHODOLOGY

This was a School based cross sectional study conducted over a period of 5 months covering selected schools in south Chennai. This study was reported as per the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) [5]. Using assumption of a single population proportion and Expected prevalence of 55% (based upon previous studies), 98% confidence interval and margin of error 5%, the sample size was calculated to be 538. Hence the study included 538 school-going children between 6 and 17 years old, examined between October 2019 and February 2020. 3 schools were selected for the study through purposive sampling. Administrative approval was obtained from the respective school principals. Parents of children from class III up to class XII (6 -17 years) were informed prior through leaflets in English and vernacular language, which was distributed by class teachers. Informed consent was obtained from parents for children between 6-8 years and verbal and written assent was obtained from children above 9 years in the presence of their class teacher. Conditions that possibly could affect the child's performance and have an effect on pGALS maneuvers such as Children having an obvious trauma, fever on the days of study, congenital deformity, recently diagnosed musculoskeletal injuries, physical limitations to take the test etc were excluded. Screening of children was completely performed within the school premises only.

After the inclusion, children were interviewed with three screening questions. (i) Do you have any pain or stiffness in your joints, muscles or back? (ii) Do you have any difficulty getting yourself dressed without any help? (iii) Do you have any problem going up and down stairs? After the interview, examiners demonstrated the pGALS

maneuvers and children were asked to perform. Prior to the examination for the purpose of familiarity, children were shown a video on pGALS in the classroom. The pGALS maneuvers was administered by Physiotherapists specialized in pediatrics. Children who were positive for abnormal findings during the screening were assessed in detail and referred to the appropriate.

RESULTS

Total of 538 school-going children, aged 6–17 years were screened from 3 schools. Children were given a demonstration of maneuvers in pGALS before the screening. Children detected for an musculoskeletal abnormality were further assessed in detail and referred to the appropriate. The pGALS examination was completed in children, the range of duration for each child varied between 2–5 min.

Participant characteristics

Among the total of 538 children, 336 were selected from private and 202 from government schools; 359 (66.8%) were female and 179 (33.2%) were male with a mean age of 11.18 and 11.84 respectively. The mean age of the participants was 11.4 years (SD 2.7 years). a detail description of participant characteristics is presented in Table 1.

Musculoskeletal Pain and abnormalities

Four hundred and six (n = 456, 84.7%) students have reported to experience musculoskeletal pain and abnormalities. children commonly reported pain in three areas as follows; Back (38.47%), Knee (17.2%) and Hand (13.01%). An overview of the prevalence of pain in all regions is presented in Table 2 and Figure 1. Similarly, children commonly had three abnormalities such as Flat foot (26.2%), kyphosis (31.9%) and scoliosis (34.9%). An overview of the prevalence of musculoskeletal abnormalities is presented in Table 3 and Figure 2.

DISCUSSION

The study aimed to determine the prevalence of musculoskeletal pain and abnormalities among school going children in Chennai. The results showed that back pain and flat foot were the most common musculoskeletal abnormalities among school going children in Chennai, while ankle pain and cubitus valgus were the lowest among the children. a study conducted by Fathi and Rezaei concluded that forward shoulder was one among the common abnormality among school going children [6]. In contrast to this study, Shamsodini *et al.* found that the highest prevelance of musculoskeletal abnormality among school children were in shoulders (37.9%), cervical (28.5%), and hip (17.4%)[7].Akbari *et al*. also showed that 89.7% school going children had abnormality in shoulders[8]. pGALS, the tool that was used to screen musculoskeletal abnormalities was not time consuming and easy to administer. Furthermore, it was easily accepted and found comfortable by the parents and school children. this screening tool has a sensitivity of 97–100 % with a specificity of 98–100%, to detect joint abnormalities. This study found that the prevalence of forward head posture was higher among the children between age group 6-11 years when compared to 12-17 years. rounded shoulders was found to be at a higher prevalence among 12-17 years old children than 6-11 years old children.

cubitus varus was at a higher prevalence rate among children between 6-11 years than cubitus valgus among 12-17 years old. In contrast to the current study, Bueno *et al*. in his study reported that lordosis was more prevalentin children between the age group 8 to 12 years [9], whereas in present study kyphosis was more commonly reported. Astane, in her study, reported that the prevalence of kyphosis was 0.9% among 11 year-old female children[10], in the present study it was reported as 1.4%.In line with the present study, Behruzi*et al*. in her study reported that the prevalence of scoliosis among Araki children between 9-16 yearwas found to be 1.52% [11] and the present study revealed a prevalence percentage of 2.9%. in Ahvaz, Safikhani and Fakorfound that 1.4% was the prevalence of scoliosis in children between 11-15 year[12]. From the above results it is clear that though there a difference in prevalence percentage of musculoskeletal pain and disorders among different countries, still it proves that there a

strong existence of these abnormalities among school going children which should be addressed at the earliest to prevent deliberate complications [13].

CONCLUSION

To conclude, there is a high prevalence of musculoskeletal pain and abnormalities that was observed among school children in Chennai. Hence it is vital to include regular screening program for school children to identify musculoskeletal abnormalities at the earliest to provide early intervention. Thereby, ultimately improving the health of school children.

REFERENCES

1. Murray JL, Lopez AD, eds (1996) The global burden of disease: a comprehensive assessment of mortality and disability from diseases, injuries and risk factor in 1990 and projected to 2020. (WHO, Geneva) .
2. Woolf AD. The bone and joint decade 2000-2010. Ann Rheum Dis 2000;59:81-2. Back to cited text no. 1
3. Sharma R. Epidemiology of Musculoskeletal Conditions in India. New Delhi, India: Department of Physical Medicine and Rehabilitation, Vardhman Mahavir Medical College &Safdarjang Hospital, New Delhi; 2012. p. 171.
4. Foster HE, Kay LJ, Friswell M, Coady D, Myers A. Musculoskeletal screening examination (pGALS) for school-age children based on the Adult GALS Screen. Arthritis Rheum. 2006;55:709–16.
5. Vandenbroucke JP, Von Elm E, Altman DG, Gøtzsche PC, Mulrow CD, Pocock SJ, et al. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. PLoS medicine. 2007;4(10):e297.
6. Fathi M, Rezaei R. Comparison of height anomalies in middle school and high school students. Teaching Phy Edu 2011; 11(1): 46-53.
7. Shamsodini A, Helisaz M, Sobhani V. The frequency of musculoskeletal disorders and associated factors in students of Tehran. J of Qom Univ of Med Sci 2012; 6(2): 31-6.
8. Akbari A, Moodi H, Ghannad R. Prevalence of postural Shoulder Impairments in 10-12 Year Old Students in Primary Schools of Zahedan. BirjandUniv of Med Sci 2010; 15(1): 58-66.
9. Bueno C, Rech R. Postural deviations of students in Southern Brazil. Rev Paul Pediatr 2013; 31(2): 237-42.
10. Astane M. Prevalence of lateral deviation of the spine in children 5-11 years of education areas quad city of Ahvaz. Teaching Phy Edu 1999; 2(3): 11-22.
11. Behruzi AR, Hadi HA, Ghandi AR. The prevalence of scoliosis in pupils aged 9-16 in Arak: 2011-2012. J of Arak Med Univ 2013; 16(70): 10-5.
12. Safikhani Z, Fakor M. Scoliosis status in 15-11 year old secondary school students in Ahwaz. Fayz 2006; 7 (2): 61-64.
13. Mosavigilani S, Sokhangooie Y. Comparison of the prevalence of the spine in the male and female students of Zahedan University of Medical Sciences. J of Olympic. 2003; 20(8): 73-81.
14. Mathew AJ, Chopra A, Thekkemuriyil DV, George E, Goyal V, Nair JB; Trivandrum COPCORD Study Group. Impact of musculoskeletal pain on physical function and health-related quality of life in a Rural community in South India: A WHO-ILAR-COPCORD-BJD India study. Clin Rheumatol2011;30:1491-7.
15. Brackley HM, Stevenson JM. Are children's backpack weight limits enough? A critical review of the relevant literature. Spine (Phila Pa 1976) 2004;29:2184-90.

Table 1: Participant characteristics

Variables	Sample	
	N	%
Total Participants	538	100
Age In Years		
6 - 11 Y	285	52.8
12-17 Y	253	47.02
Gender		
Female	359	66.8
Male	179	33.2
Height (In Cms) As Mean (SD)	143.88	
Weight (In Kg) As Mean (SD)	37.91	

Table 2: Region wise distribution of MusculoSkeletal Pain prevalence

MusculoSkeletal Pain	n	%
Shoulder Pain	40	7.4
Elbow Pain	34	6.3
Wrist Pain	32	5.9
Hand Pain	70	13.01
Back Pain	207	38.47
Knee Pain	93	17.2
Ankle Pain	9	1.6
Foot Pain	22	4.1

Table 3: Prevalence percentage (%) of Musculoskeletal abnormalities

Musculoskeletal Abnormalities	*n*	%
Forward Head Posture	120	22.3
Rounded Shoulder	97	18.02
Cubitus Varus	42	7.8
Cubitus Valgus	18	3.34
Genu Varum	97	18.02
Genu Valgum	81	15.05
Scoliosis	141	26.2
Kyphosis	172	31.9
Flat Foot	188	34.9

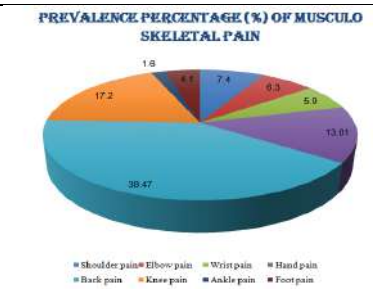

Figure.1:Prevalence Percentage (%) Of MusculoSkeletal Pain

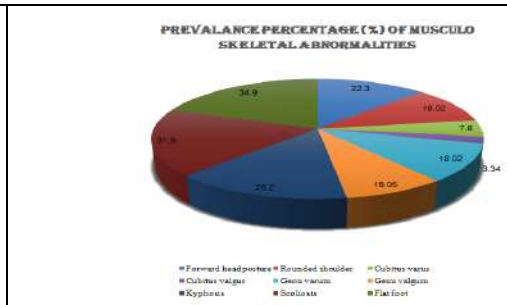

Figure.2: Prevalence Percentage (%) Of MusculoSkeletal Abnormalities

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REVIEW ARTICLE

A Review on New Approaches to Meet The Challenges of Human Envenoming by Snakebites

Faruk Alam[1], Josef Yakin[2]*, Mohidul Islam[2], Biplab Kumar Dey[3], Durgaprasad Kemisetti[1] and Ashim Kumar Sen[4]

[1]Associate Professor, Department of Faculty of Pharmaceutical Science, Assam down town University, Guwahati, Assam, India.
[2]Assistant Professor, Department of Faculty of Pharmaceutical Science, Assam down town University, Guwahati, Assam, India.
[3]Professor Cum Dean, Department of Faculty of Pharmaceutical Science, Assam down town University, Guwahati, Assam, India.
[4]Professor, Department of Pharmacy, Sumandeep Vidyapeeth Deemed to be University, Piparia, Vadodara, Gujarat, India.

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***Address for Correspondence**
Josef Yakin,
Assistant Professor,
Department of Faculty of Pharmaceutical Science,
Assam down town University,
Guwahati, Assam, India.
Email: joseph123yakin@gmail.com

ABSTRACT

Animal venoms have evolved over millions of years for prey capture and defense from predators and rivals. Snake venom is a highly poisonous saliva containing zootoxins that helps in prey immobilization and digestion. Snake venom is infused into the victim by distinctive fangs during bite, whereas certain species can even spit venom to defend themselves. Envenomation of snake is a significant health, economic burden and an estimated 1.8-2.7 million snake bite occurs each year and 81,410-137,880 deaths occurs worldwide. Snakebite deaths and envenomation are largely neglected topics in global health. However, in 2017, the World Health Organization added snakebite envenoming to its priority list of neglected tropical diseases. The World Health Organization's global total was revised after direct estimates of 46,000 annual snakebite deaths in India in 2005. District by district Snakebites are on the rise in India. Snakebite prevalence is found to be notably high in districts located in the southern states of the peninsular Deccan plateau. Snake venom is a mixture of proteins, amino acids, lipids, carbohydrates, metal ions, and other substances that is heterogeneous and complicated and which has been used to generate a number of pharmaceuticals like Tirofiban. Due to the varying toxin compositions injected following a snake bite, they also present one of the most difficult therapeutic targets. In this review, the

multifunctional features of the toxins found in snake venoms, as well as their evolutionary histories, are discussed with the view to identifying novel modes of action and improving snakebite treatments.

Keywords: Snakebite, Venom, Toxicology, Anti-venom, Myotoxins.

INTRODUCTION

Venom is one of the dodgiest poisonous components, especially secreted by an animal's predators and prey and by the both types of animal kingdom that is vertebrates and invertebrates, is delivered through a bite, sting or similar action [1]. Snake venom is a extremely poisonous saliva [2] containing zootoxins that helps in prey immobilization and digestion. Snake venom is infused into the victim by distinctive fangs during bite, whereas certain species can even spit venom [3] to defend themselves. Unfortunately, envenomation of snake is a significant health, economic burden and an estimated 1.8-2.7 million snake bite occurs each year and 81,410-137,880 deaths occurs worldwide [4, 5]. Mostly, Snake bite and death cases are associated with agricultural work, especially in South Southeast Asia, sub-Saharan Africa and central and South America [6, 7]. However, the snake venoms had been in particular originated with inside the Cenozoic Era [8, 9] and they are amongst the very much described of creature toxins, which consist of more than 20 different complex compounds, pharmacologically potent proteins and peptides [10, 11]. The complicated combination of proteins, enzyme and various other components has toxic and lethal properties [3]. The enzyme found in venom performs a critical function in the victim's digestion [12], and certain other constituents are responsible for significant but non-lethal biological properties. Most of the snake venom proteins have significant effects on several biological functions, like coagulation of the blood, blood pressure regulation and nerve or muscle impulses transmission. The venom have been investigated and evolved to be used as pharmacological agent or diagnostic gear and even drugs. A massive wide variety of venom proteins have an effect on the hemostatic system [13] and can have procoagulant, anticoagulant, fibrinolytic or platelet active activities.

Ancrod (Arvin), batroxobin (reptilase) and crotalase are derived from the Malayan pitviper (*Calloselasma rhodostoma*)venom, common lancehead (*Bothrops atrox*) venom and Eastern diamondback rattle snake (*Crotalus adamanteus*) venom respectively and all have been used as defibrino generating agents for variety of clinical conditions such as deep vein thrombosis, myocardial localized necrosis, pulmonary embolus and many others[14]. Venoms with anticoagulant properties are extensively studied for possible medical applications. The drugs Aggrastat (Tirobifan) is a type of antiplatelet medication (glycoprotein IIb/IIIa inhibitors) that was derived from a chemical compound found in venom of the saw-scaled viper (Echiscarinatus) [15] and it is administered to those with unstable angina (Figure 1). A number of snake venoms create a transient condition of depreased blood pressure in envenomed patients. Angiotensin converting enzyme (ACE) inhibitors were produced from a bradykinin potentiating enzyme collected from the snake venom of Brazilian pitviper (*Bothrops jararaca*) and licensed by the FDA in 1979 [16] to treat hypertension and heart disease (Figure 2). They act by inhibiting the alteration of angiotensin-I to angiotensin-II, which is a vasoconstrictor. These inhibitors are now widely prescribed around all over the world and have saved the lives of millions of peoples. ACE inhibitor were produced from a bradykinin-potentiating enzyme, which is derived from the snake venom of Brazilian pitviper (*Bothrops jararaca*) and it is used for the treatment of hypertension and several heart diseases.

RESULTS AND DISCUSSION

Snake Bite Deaths in India

Snakebite deaths and envenomation are largely neglected topics in global health. However, in 2017, the World Health Organization (WHO) added snakebite envenoming to its priority list of neglected tropical diseases (WHO) [17], and in 2019, the WHO launched a snakebite prevention and control strategy, with the goal of halving the

number of deaths and cases of serious disability by 2030 compared to the 2015 baseline (WHO) [18]. Achieving this target will necessitate significant success in India, which accounts for around half of all snakebite deaths worldwide. The WHO's global total was revised after direct estimates of 46,000 per year snakebite deaths occurred in India in 2005[19]. That 2005 Indian reporting was based on analysis of around 123,000 stated autopsy records from Registrar General of India's (RGI) Million Death Study (MDS), one of the world's biggest nationally representative mortality surveys, conducted between 2001 and 2003. For the first time, the MDS has provided cause-specific death patterns for over 600,000 deaths in India between 2001 and 2014. Seasonal and temporal changes in snakebite mortality in India during the last two decades, as well as its spatial distribution, are presented in this paper. We are providing estimated total snakebite deaths for the 20-year period 2000-2019 by age and sex (Table 1).

By extrapolating these annual deaths, deaths from the year 2000 to 2019 were calculated. Outside of the research period, projected annual fatalities in thousands were 54.0 in 2000, 62.3 in 2015, 62.0 in 2016, 61.4 in 2017, 60.3 in 2018, and 59.8 in 2019. Lower and upper uncertainty bounds for estimations are known as lower and upper limits, respectively. The biggest source of uncertainty in our analysis, however, is the cause of death classification, not the demographic totals. As a result, the lower bound was established based on quick agreement between both physicians on the ICD-10 diagnosis for snakebite, while the upper bound was established based on either of two physicians coding as snakebite death. Considerably India accounted for over half of all snakebite deaths worldwide [20]. There is no official method that calculates the sum of snakebite deaths each year. The government, researchers and scientists are not aware of the figures, resulting in large research gaps. Based on the screening of a number of publications and some trustworthy research from 24 states or union territories in India, we detected 87,590 snakebite cases (both fatal and non-fatal) from 2000 to 2019. (Figure 3). Review a year-by-year breakdown of state coverage. According to official government figures, around 1,123 and 1,008 deaths in India were caused by snakebite in 2013 and 2014, respectively [21]. However, a report published in an international journal estimated that between 45,900 and 50,900 people die in India every year, with roughly 97 percent of deaths occurring in rural areas. Andhra Pradesh, Maharashtra, Tamil Nadu, and West Bengal had the most snakebite deaths.

Snakebite deaths were most common when sleeping (30%), playing (30%), and participating in field/outdoor activities (28%) [22,23]. India recorded a surprising 1.2 million death due to snakebite in the past 20-year period from the year 2000 to 2019 with an average of 58,000 deaths every year. Around 70% of these deaths occurred in low altitude and rural regions of a total of eight states such as Madhya Pradesh, Gujarat, Uttar Pradesh, Bihar, Odisha, Jharkhand, Andhra Pradesh (including Telangana) and Rajasthan due to low altitude, rural areas. As per the Union Ministry of Health and Family Welfare, Andhra Pradesh had the second-highest number of deaths due to snakebite in the country behind Odisha in 2019. Telangana is one of the states with the fewest snakebite fatalities. In 2019, there were 3,163 deaths cases reported and 1.6 lakh cases of snakebites were reported across the country. In Andhra Pradesh, 467 snakebites-related deaths have been documented, according to the Health Ministry. With 1,872 deaths, Odisha led the list, followed by West Bengal with 239 deaths. Andhra Pradesh had a sharp increase in mortality, with 117 deaths in 2018 compared to 85 in 2017. The ministry instructed all states to include anti-snake venom serum among essential drugs and to obtain the drugs locally for use to the needy

District wise Incidence of snakebite in India

District by district Snakebites are on the rise in India. Snakebite prevalence is found to be notably high in districts situated in the southern states of the peninsular Deccan plateau, according to a district-level investigation. During the year 2017-2019, districts in Maharashtra (14 districts), West Bengal (12 districts), Andhra Pradesh (9 districts), Tamil Nadu (8 districts), Telangana (2 districts), Odisha (2 districts), and Uttar Pradesh (2 districts) reported the most snakebites in India. During 2018-2019, the number of reported cases of snakebite were maximum in West Bengal's PurbaMedinipur district and Maharashtra's Nashik district, with 4,904 and 4,294 cases reported respectively (Figure 4).Snakebites account for 3% of all fatalities in children between the ages of 5 and 14. Because they preferred traditional therapy from tantriks, vaidyas, and ojhas, 97% of snakebite victims die in rural regions, with 77% of them dying outside of health institutions [24]. These findings should prompt the Ministry of Health to reconsider snakebite as a public health priority and focus its resources where they are most needed. Snakebite is responsible for

3% of all deaths in children aged 5 to 14 years. Ninety seven consistent with cent of the victims of snakebite die in rural regions, 77 in keeping with cent of them outdoor fitness facilities, because they preferred conventional remedy from tantriks, vaidyas and ojhas [1]. These figures need to encourage the Ministry of Health to reconsider the public fitness precedence of snakebite and deploy its resources in which those are maximum wished.

Nature of Snake Venom

Snake venom is a heterogeneous, complex mixture of different chemicals including proteins, amino acids, lipids, carbohydrates, and metal ions. Depending on age and season, a snake's venom can differ significantly between species, within individuals of the same species, and even within the same snake itself. Although there are exceptions, the Elapidae family's venom is mostly neurotoxic and can have some myotoxic effects. The venom of the Viperidae family, on the other hand, is predominantly hemotoxic and myotoxic, with limited (typically mild) neurotoxic effects. The study of venom's components is known as toxicology and it is a topic that is gaining popularity. Snake venom has been used to generate a number of pharmaceuticals. Tirofiban, developed from the saw-scaled viper (Echiscarinatus), and angiotensin converting enzyme inhibitors obtained from the Brazilian viper are two examples (*Bothrops jararaca*). Many more potential venom-derived compounds are now being studied to treat a variety of ailments, including chronic pain and multiple sclerosis. Snake venom differs in composition and effectiveness depending on the species. The venom of Sea snakes and Australian elapids and is said to be among the most effective in the world. Defense, prey neutralisationa, and predigestion are all assumed to be functions of venom. It is injected into prey or attackers via ducts and specialised teeth or fangs from the venom gland. Some snakes species, like vipers, have huge hollow front fangs that are particularly effective at delivering massive amounts of venom, whereas others have poorly formed, ineffectual grooved rear fangs. Envenomation is not always the outcome of venomous snake bites. More than a quarter of snake bites are thought to be 'dry' bites.

Classification of Snake Venom and where they Act

The pharmacological properties of snake venoms are categorized into four main classes of toxin, namely neurotoxins, and cytotoxins [25], which can kill the cells; neurotoxins, which can affect nervous system; myotoxins, which can damage muscles and haemotoxins, which can disrupt blood clotting. The key toxins involved in these properties are PLA2S (Phospholipases), SVMPs (Snake Venom Metalloproteinases), SVSPs (Snake Venom Serine proteinases) and 3FTXs (Three-finger toxins), which are responsible for the various pharmacological effects occurring in snakebite prey in combination or alone. For example, PLA2S and 3FTXs are induced severe neurotoxicity like paralysis and respiratory failure by acting on the pre- or post synaptic junction as antagonist of ion channel and nicotinic or muscrinic receptors [26-28]. Furthermore, PLA2S and 3FTXs along with SVMPs are stimulated to damages the local tissue as a result in swelling, bruising, blistering, nacrosis and systemic effect like hypovolemic shock [29]. In addition, Hemoststic and cardiovascular effects such as hypotension, coagulopathy and hemorrhage are induced by SVSPs and SVMPs [30]. More interesting things is that some PLA2S, SVMPs and SVSPs have capability to trigger the severe pain by modulating pain pathway by activating of ion channels, such as TRPV1 (Type 1 transient receptor potential vanilloid) and acid-sensing ion channel (ASIC) [31]; and or by pain sensitization through inflammatory mediators [32, 33].The inflammation induced by the elapid and viper venoms is widely reported to produce pain or hyperalgesia in human and experimental models [33, 34, 35]. Unfortunately, antivenom and anti-inflammatory therapy do not totally reverse these effects [36, 37]. It's crucial to remember that even little amounts of venom components can have a big impact on toxicity; for example, hyaluronidas from *Crotalus durissus* terrificus represents only 0.23 % protein but increases crotoxin lethality [38]. Figure 5 depicts the primary actions of the enzymatic venom and non-enzymatic venom components. The heterogeneity of venom poses several significant obstacles to the understanding of venom mechanisms. Separating the effects of various venom components in an envenomed animal is the most obvious challenge. Table 2 provides a review of the mostly known venom toxicities, processes, and functional characteristics related to pain, inflammation, bleeding, necrosis, and paralysis.

Diagnostic Tests and Tools

The type of snake venom present in a patient who has been envenomed can only be determined commercially by a single diagnostic test at this time. This test uses antibodies to recognize specific types of venom produced by different species of snakes. There is a demand in some areas and nations for commercial tests that can be used to better guide the choice of anti-venoms to treat patients. Other diagnostic tests that use comparable principles are being used experimentally. However, simple tests and diagnostic tools (algorithms or checklists) can be employed to confirm the presence of essential clinical indication of snakebite envenoming. Which indicate the necessity for quick antivenom treatment and, in some cases, can help distinguish the most possible genus or species of snake responsible for the bite. Spontaneous haemorrhage due to envenoming by some snake species is an important clinical indication for antivenom. A test called the 20 Minute Whole Blood Clotting Test (20WBCT) can help in diagnosis. A clean, dry glass bottle or vial containing 1-2 millilitres of venous blood is placed inverted and the presence or absence of a full clot is recorded after 20 minutes at room temperature. The test result is negative if blood is present, but positive if no clot forms and the blood remains liquid, suggesting the presence of coagulopathy and the need for antivenom treatment (Figure 6).Where this test is used it is essential that it be appropriately standardized using uniform glassware, sample volume and temperature, and validated for accuracy using serial donor samples prior to routine use. By allowing retroactive identification of venom immune types from distinct snake species in pathology samples, diagnostic techniques have the potential to improve snakebite envenoming surveillance. This could help with reporting snakebite envenoming and finding the ideal anti-venom needs for different localities.

Treatment

Snakebite envenoming

Early access to healthcare in a medical setting with staff qualified to identify snakebite envenoming is crucial. This refers to a medical facility that has the fundamental tools required to meet immediate medical treatment, such as administering anti-venom and other adjunct therapies. People who suspected they may have been bitten by a poisonous snake need to be taken to a medical facility immediately. Traditional medicines and other treatments such as wound incision or excision, suction, or application of “black stones” should be avoided. First aid should be applied like as

• Leave the place where the bite happened right away. Use a stick or other object to force the snake to release if it is still clinging. To prevent drowning, victims of sea snakes must be transported to dry land.
• Remove anything that is tightly wrapped around the area of the body that has been bit (e.g.: rings, anklets, bracelets). If the part that was bit swells, this could be harmful.
• Assuage the victim's fears. Non-venomous snakes are responsible for many snake bites. Even yet, most venomous snake bites do not result in immediate death.
• Completely immobilize the subject. To keep the limb motionless, splint it. Carry the victim to a location where transportation to a health facility is available using an improvised stretcher.
• Never use a tight arterial tourniquet.
• Only bites from neurotoxic snakes that do not produce local swelling are suggested for the Australian Pressure Immobilization Bandage (PIB) Method.
• In some circumstances, using a pressure pad to apply pressure to the bite site may be sufficient.
• Traditional first aid methods, herbal medications and other unproven or dangerous first aid methods should be avoided.
• Transport the individual to a medical facility as soon as possible.
• For local pain, paracetamol may be used (which can be severe).
• If vomiting occurs, place the patient in the recovery position on their left side.
• Keep a close eye on the airway and breathing, and be prepared to resuscitate if needed.

Many people die every year while being taken to a health facility while lying flat on their backs with their upper airway occluded by vomit or tongue muscles paralysed. To lessen the risk of this, keep them on their left side with their mouth turned down.

All snakebite cases should be handled by medical facilities as emergencies, with a focus on quickly assessing the patients and starting treatment. Improving the clinical outcomes for the victims of snake bite needs much more than just access to safe antivenoms. Early intravenous access should be obtained, hydration status should be assessed and rectified as necessary, and vital signs should be constantly monitored. The early administration of an adequate dose of effective antivenom to patients with signs of envenoming is crucial. If there isn't any anti-venom on availability, a swift referral to a facility with supplies should be planned and undertaken quickly. If this is not achievable, then symptomatic care should be addressed as necessary, with a focus on maintaining airway patency and breathing, maintaining circulation and controlling bleeding, and treating any local wounds.

Snake Anti-venom in India

Polyvalent antivenom is available in India. Indian antivenom comprises of antibodies against the venom of four species of venomous snakes, which together account for the majority of snakebite deaths in India. For this reason, these snakes are also called the "Big Four". These include the Russell's viper (*Daboia russelii*), Indian Cobra (Najanaja), Common Krait (*Bungarus caeruleus*) and Saw-scaled viper (Echiscarinatus). In order to produce Indian polyvalent antivenom, equine (horse) immunoglobulin fragmentsF(ab')2 are prepared sterilely. Each milliliter of reconstituted antivenom has the potency to neutralize the venom of the following snakes:

- 0.6mg of dried Indian cobra venom
- 0.6mg of dried Russell's viper venom
- 0.45mg of dried saw-scaled viper venom
- 0.45 mg of dried common krait venom

Unfortunately, numbers of other venomous snakes are there in India for which this polyvalent antivenom would not work against. [68].

Side-Effect of Snake Anti-venom

Snake anti-venom reactions are generally rare. However, they may occur in a few susceptible people. The most severe is anaphylaxis, which is a medical emergency that can be life-threatening. Anaphylaxis can be associated with one or more of the following signs and symptoms:

- Rashes and/or itching
- Fever, chills or rigor
- Nausea and vomiting
- Abdominal cramps and diarrhea
- Increased heart rate (tachycardia)
- Low blood pressure (hypotension)
- Spasm of the respiratory tract (bronchospasm)
- Puffy face and neck or other parts of the body (angioedema) resulting from accumulation of fluid under the skin

Anaphylaxis needs to be treated right away since it might develop quickly. The symptoms of anaphylaxis are quickly treated with a medication called epinephrine.

CONCLUSION

Snake venom is one of the most fascinating animal venoms in terms of complexity, evolution, and medicinal potential. Due to the varying toxin compositions injected following a snake bite, they also present one of the most difficult therapeutic targets. The multifunctional approach taken by the major components of their venoms, which includes the use of multidomain proteins and peptides with promiscuous folds (e.g., three-finger fold), as well as the diversity of toxic effects, are unique and have yet to be identified in other animal venoms of such complexity. To develop better snakebite remedies and innovative medications, researchers must first have a better understanding of the evolution, structure-activity connections, and pathogenic mechanisms of these toxins.

REFERENCES

1. S.L. Thornton, Encyclopedia of Toxicology,3rd edit, 2014, ISBN: 978-0-12-386455-0
2. Reptile Venom Research. *Australian Reptile Park. Archived from* the original *on 2 February 2010*. Retrieved 21 December 2010.
3. *BauchotR,* Snakes: A Natural History. *New York City, NY, USA: Sterling Publishing Co., Inc.1994* 194–209. ISBN 978-1-4027-3181-5.
4. Kasturiratne, A., Wickremasinghe, A. R., De Silva, N., Gunawardena, N. K., Pathmeswaran, A., Premaratna, R.,. The global burden of snakebite: a literature analysis and modelling based on regional estimates of envenoming and deaths. PLoS Med,5,2008,e218. doi 10.1371/journal.pmed.0050218
5. Gutierrez, J. M., Calvete, J. J., Habib, A. G., Harrison, R. A., Williams, D. J., and Warrell, D. A., Snakebite envenoming. Nat. Rev. Dis. Primers 3,2017,17079. doi: 10.1038/nrdp.2017.79
6. Warrell DA (1995) Clinical toxicology of snakebite in Asia. In: Meier J, White J, editors. Handbook of clinical toxicology of animal venoms and poisons. Boca Raton (Florida): Taylor and Francis.1995,493–594.
7. Harrison, R. A., Casewell, N. R., Ainsworth, S. A., and Lalloo, D. G., The time is now: a call for action to translate recent momentum on tackling tropical snakebite into sustained benefit for victims. Trans. R. Soc. Trop. Med. Hyg.2019. doi: 10.1093/trstmh/try134. [Epub ahead of print].
8. Fry, B. G., Fromgenome to "venome": molecular origin and evolution of the snake venom proteome inferred from phylogenetic analysis of toxin sequences and related body proteins. Genome Res. 15, 2005, 403–420. doi: 10.1101/gr.3228405
9. Fry, B. G., Vidal, N., Norman, J. A., Vonk, F. J., Scheib, H., Ramjan, S. F. R.,Early evolution of the venom system in lizards and snakes. Nature 439, 2006, 584–588. doi: 10.1038/nature04328
10. Chan, Y. S., Cheung, R. C. F., Xia, L. X., Wong, J. H., Ng, T. B., and Chan, W. Y., Snake venom toxins: toxicity and medicinal applications. Appl. Microbiol. Biotechnol. 100, 2016, 6165–6181. doi: 10.1007/s00253-016-7610-9
11. Casewell, N. R., Wuster, W., Vonk, F. J., Harrison, R. A., and Fry, B. G.,Complex cocktails: the evolutionary novelty of venoms. Trends Ecol. Evol. 28,2013 219–229. doi: 10.1016/j.tree.2012.10.020
12. *Bottrall JL, Madaras F, Biven CD, Venning MG, MirtschinPJ,*Proteolytic activity of Elapid and Viperid Snake venoms and its implication to digestion. *Journal of Venom Research. 1 (3):2010,18–28.* PMC 3086185. PMID 21544178.
13. Jr. Markland FS, Snake venom fibrinogenolytic and fibrinolytic enzymes: an updated inventory. Registry of Exogenous Hemostatic Factors of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis. ThrombHaemost 1998; 79(3),1998,668-674.PMID: 9531060
14. Markland FS, Snake venoms and the hemostatic system. Toxicon,36(12),1998, 1749-1800.DOI: 10.1016/s0041-0101(98)00126-3
15. G D Hartman, M S Egbertson, W Halczenko, W L Laswell, M E Duggan, R L Smith, A M Naylor, P D Manno, R J Lynch, G Zhang. Non-peptide fibrinogen receptor antagonists. 1. Discovery and design of exosite inhibitors. J.MedChem,35(24),1992,4640-2DOI: 10.1021/jm00102a020
16. D W Cushman, H S Cheung, E F Sabo, B Rubin, M A Ondetti, Development of specific inhibitors of angiotensin I converting enzyme (kininase II).Fed Proc. 38(13),1979,2778-82. PMID: 228989
17. World Health Organization (WHO). 2019. Neglected tropical diseases. https://www.who.int/neglected_diseases/diseases/en/ [Accessed August 1, 2019].
18. World Health Organization (WHO).,Snakebite envenoming - A strategy for prevention and control. WHO Neglected tropical diseases/Snakebites reference number: 9789241515641. 2019. WHO Press. https://apps.who.int/iris/bitstream/handle/10665/324838/9789241515641-eng.pdf?ua=1 [Accessed August 1, 2019].
19. Mohapatra B, Warrell DA, Suraweera W, Bhatia P, Dhingra N, Jotkar RM, Rodriguez PS, Mishra K, Whitaker R, Jha P, Million Death Study Collaborators, Snakebite mortality in India: a nationally representative mortality survey. PLOS Neglected Tropical Diseases 5, 2011,e1018. DOI: https://doi.org/10.1371/journal.pntd.0001018, PMID: 21532748

20. Kasturiratne A, Wickremasinghe AR, de Silva N, et al. The global burden of snakebite: a literature analysis and modelling based on regional estimates of envenoming and deaths. PLoS Med,5(11), 2008,e218. https://doi.org/10.1371/journal.pmed. 0050218.
21. Pandey K. Half the global snake bite deaths happen in India. Why are we not prepared? Down To Earth. Published https://www.downtoearth.org.in/news/wildlifebiodiversity/serpentine-problem-58396; 2017, Accessed date: 20 June 2019
22. Whitaker R. Snakebite in India today. Neurol India,63(3),2015,300–303. https://doi. org/10.4103/0028-3886.158155.
23. Alirol E, Sharma SK, Bawaskar HS, Kuch U, Chappuis F. Snake bite in south Asia: a review. PLoS Neglected Trop Dis,4(1),2010,e603. https://doi.org/10.1371/journal. pntd.0000603
24. David A. Warrell, José María Gutiérrez, Juan J. Calvete, David Williams, New approaches & technologies of venomics to meet the challenge of human envenoming by snakebites in India, Indian J Med Res, 138(1), 2013, 38-59.PMID: 24056555
25. WHO, Guidelines for the Prevention and Clinical Management of Snakebite in Africa. WHO.2010
26. Casewell, N.R., Wuster, W., Vonk, F.J., Harrison, R.A., and Fry, B.G., Complex cocktails: the evolutionary novelty of venoms. Trends EcolEvol28,2013, 219-229
27. Harris, J.B., and Scott-Davey, T., Secreted phospholipases A2 of snake venoms: Effects on the peripheral neuromuscular system with comments on the role of phospholipases A2 in disorders of the CNS and their uses in industry. Toxins 5,2013, 2533-2571
28. Tsetlin, V.I., Three-finger snake neurotoxins and Ly6 proteins targeting nicotinicacetylcholine receptors: pharmacological tools and endogenous modulators. Trends inPharmacological Sciences 36,2015, 109-123.
29. Rivel, M., Solano, D., Herrera, M., Vargas, M., Villalta, M., Segura, A., Arias, A.S., Leon, G.,and Gutierrez, J.M., Pathogenesis of dermonecrosis induced by venom of the spitting cobra, Najanigricollis: An experimental study in mice. Toxicon119,2016, 171-179.
30. Slagboom, J., Kool, J., Harrison, R.A., and Casewell, N.R., Haemotoxic snake venoms:their functional activity, impact on snakebite victims and pharmaceutical promise. British Journal of Haematology177, 2017,947-959.
31. Zhang, C.C., Medzihradszky, K.F., Sanchez, E.E., Basbaum, A.I., and Julius, D., Lys49 myotoxin from the Brazilian lancehead pit viper elicits pain through regulated ATP release. Proceedings of the National Academy of Sciences of the United States of America 114, 2017, E2524-E2532
32. Zambelli, V.O., Picolo, G., Fernandes, C.a.H., Fontes, M.R.M., and Cury, Y. Secreted phospholipases A2 from animal venoms in pain and analgesia. Toxins (Basel) 9.2017
33. Mamede, C.C., De Sousa, B.B., Pereira, D.F., Matias, M.S., De Queiroz, M.R., De Morais,.C., Vieira, S.A., Stanziola, L., and De Oliveira, F., Comparative analysis oflocal effects caused by Bothropsalternatusand Bothropsmoojenisnake venoms: enzymatic contributions and inflammatory modulations. Toxicon117, 2016, 37-45.
34. Bucaretchi, F., Capitani, E.M., Vieira, R.J., Rodrigues, C.K., Zannin, M., Da Silva, N.J., Jr.,Casais-E-Silva, L.L., and Hyslop, S., Coral snake bites (Micrurus spp.) in Brazil:a review of literature reports. ClinToxicol (Phila) 54, 2016, 222-234
35. Kleggetveit, I.P., Skulberg, P.K., and Jorum, E., Complex regional pain syndrome following viper-bite. Scand J Pain 10,2016, 15-18.
36. Ferraz, C.R., Calixto-Campos, C., Manchope, M.F., Casagrande, R., Clissa, P.B., Baldo, C., and Verri, W.A., Jr., Jararhagin-induced mechanical hyperalgesia depends on TNF-α, IL-1β and NFκB in mice. Toxicon103, 2015,119-128
37. Hifumi, T., Sakai, A., Kondo, Y., Yamamoto, A., Morine, N., Ato, M., Shibayama, K., Umezawa, K., Kiriu, N., Kato, H., Koido, Y., Inoue, J., Kawakita, K., and Kuroda, Y., Venomous snake bites: clinical diagnosis and treatment. J Intensive Care 3,2015, 16.
38. Bordon, K.C.; Perino, M.G.; Giglio, J.R.; Arantes, E.C. Isolation, enzymatic characterization and antiedematogenic activity of the first reported rattlesnake hyaluronidase from Crotalusdurissusterrificus venom. Biochimie2012, 94, 2740–2748.
39. Gutierrez, J.M.; Lomonte, B. Phospholipases A2: Unveiling the secrets of a functionally versatile group of snake venom toxins. Toxicon2013, 62, 27–39.

40. Bohlen C.J., Chesler, A.T., Sharif-Naeini, R., Medzihradszky, K. F., Zhou, S., King, D.Sanchez, E.E., Burlingame, A.L., Basbaum, A.I., and Julius, D. A heteromeric Texas coral snake toxin targets acid-sensing ion channels to produce pain. Nature 479, 2011, 410-414.
41. Honore P, Kage K, Mikusa J, Watt AT, Johnston JF, Wyatt JR, Faltynek CR, Jarvis MF, Lynch K. Analgesic profile of intrathecal P2X(3) antisense oligonucleotide treatment in chronic inflammatory and neuropathic pain states in rats. Pain,99(1-2):2002,11-9. doi: 10.1016/s0304-3959(02)00032-5. PMID: 12237180.
42. Bernardes C.P, Menaldo, D.L, Mamede, C.C.N., Zoccal, K.F., Cintra, A.C.O., Faccioli, L.H., Stanziola, L., De Oliveira, F., and Sampaio, S.V., Evaluation of the local inflammatory events induced by BpirMP, a metalloproteinase from Bothropspirajaivenom. Molecular Immunology 68,2015, 456-464.
43. De Toni, L.G.B., Menaldo, D.L., Cintra, A.C.O., Figueiredo, M.J., De Souza, A.R., Maximiano,W.M.A., Jamur, M.C., Souza, G.E.P., and Sampaio, S.V., Inflammatory mediators involved in the paw edema and hyperalgesia induced by Batroxase, metalloproteinase isolated from Bothropsatroxsnake venom. International Immunopharmacology28,2015, 199-207.
44. Ferraz, C.R., Calixto-Campos, C., Manchope, M.F., Casagrande, R., Clissa, P.B., Baldo, C.,andVerri, W.A., Jr., Jararhagin-induced mechanical hyperalgesia depends on TNF-α, IL-1β and NFκB in mice. Toxicon103,2015, 119-128
45. Menaldo, D.L., Bernardes, C.P., Pereira, J.C., Silveira, D.S., Mamede, C.C., Stanziola, L.,Oliveira, F., Pereira-Crott, L.S., Faccioli, L.H., and Sampaio, S.V., Effects of two serine proteases from Bothropspirajaisnake venom on the complement system and the inflammatory response. IntImmunopharmacol15,2013, 764-771.
46. Diochot, S., Baron, A., Salinas, M., Douguet, D., Scarzello, S., Dabert-Gay, A.S., Debayle, D.,Friend, V., Alloui, A., Lazdunski, M., and Lingueglia, E., Black mamba venom peptides target acid-sensing ion channels to abolish pain. Nature 490, 2012,552-555
47. Camargo, E.A., Ferreira, T., Ribela, M.T., De Nucci, G., Landucci, E.C., and Antunes, E., Role of substance P and bradykinin in acute pancreatitis induced by secretory phospholipase A2. Pancreas 37, 2008, 50-55.
48. Costa, S.K.P., Camargo, E.A., and Antunes, E., Inflammatory action of secretory phospholipases A2 from snake venoms. Toxins and Drug Discovery,2017, 35-52.
49. Casais-E-Silva, L.L., Teixeira, C.F.P., Lebrun, I., Lomonte, B., Alape-Giron, A., and Gutierrez,J.M., Lemnitoxin, the major component of Micruruslemniscatuscoral snake venom, is a myotoxic and pro-inflammatory phospholipase A2. Toxicology Letters 257,2016, 60-71.
50. Laing, G.D., Clissa, P.B., Theakston, R.D., Moura-Da-Silva, A.M., and Taylor, M.J., Inflammatory pathogenesis of snake venom metalloproteinase-induced skin necrosis.Eur J Immunol33, 2003, 3458-3463.
51. Zychar, B.C., Dale, C.S., Demarchi, D.S., and Goncalves, L.R., Contribution of metalloproteases, serine proteases and phospholipases A2 to the inflammatory reaction induced by Bothropsjararaca crude venom in mice. Toxicon55,2010, 227-234.
52. Teixeira, S.S., Silveira, L.B., Da Silva, F.M., Marchi-Salvador, D.P., Silva, F.P., Jr., Izidoro,L.F., Fuly, A.L., Juliano, M.A., Dos Santos, C.R., Murakami, M.T., Sampaio, S.V., Da Silva, S.L., and Soares, A.M., Molecular characterization of an acidic phospholipase A2 from Bothropspirajaisnake venom: synthetic C-terminal peptide identifies its antiplatelet region. Arch Toxicol85,2011, 1219-1233
53. Escalante, T., Rucavado, A., Fox, J.W., and Gutierrez, J.M., Key events in microvascular damage induced by snake venom hemorrhagic metalloproteinases. J Proteomics 74,2011, 1781-1794.
54. Ainsworth, S., Slagboom, J., Alomran, N., Pla, D., Alhamdi, Y., King, S.I., Bolton, F.M.,Gutiérrez, J.M., Vonk, F.J., and Toh, C.-H. The paraspecificneutralisation of snake venom induced coagulopathy by antivenoms. Communications Biology 1,2018, 34.
55. Kamiguti, A.S., Platelets as targets of snake venom metalloproteinases. Toxicon45, 2005, 1041-1049.
56. Kini, R.M, The intriguing world of prothrombin activators from snake venom. Toxicon45,2005,1133-1145.
57. Serrano, S.M.T., The long road of research on snake venom serine proteinases. Toxicon62, 2013, 19-26.
58. Girish, V.M., and Kini, R.M.,Exactin: A specific inhibitor of Factor X activation byextrinsictenase complex from the venom of Hemachatushaemachatus. Sci Rep 6,2016,32036.

59. Mcdowell, R.S., Dennis, M.S., Louie, A., Shuster, M., Mulkerrin, M.G., and Lazarus, R.A., Mambin, a potent glycoprotein IIb-IIIa antagonist and platelet aggregation inhibitor structurally related to the short neurotoxins. Biochemistry 31, 1992,4766-4772.
60. Gutierrez, J.M., and Ownby, C.L.,Skeletal muscle degeneration induced by venom phospholipases A2: insights into the mechanisms of local and systemic myotoxicity.Toxicon42,2003, 915-931.
61. Harris, J.B., and Maltin, C.A., Myotoxic activity of the crude venom and the principal neurotoxin, taipoxin, of the Australian taipan, Oxyuranusscutellatus. Br J Pharmacol, 76,1982, 61-75.
62. Ownby, C.L., Fletcher, J.E., and Colberg, T.R., Cardiotoxin 1 from cobra (Najanajaatra) venom causes necrosis of skeletal muscle in vivo. Toxicon31,1993, 697-709.
63. Sribar, J., Oberckal, J., and Krizaj, I.,Understanding the molecular mechanism underlying the presynaptic toxicity of secreted phospholipases A2: an update. Toxicon, 89,2014,9-16.
64. Gutierrez, J.M., Calvete, J.J., Habib, A.G., Harrison, R.A., Williams, D.J., and Warrell, D.A.,Snakebite envenoming. Nat Rev Dis Primers 3, 2017,17079.
65. Brust, A., Sunagar, K., Undheim, E.A., Vetter, I., Yang, D.C., Casewell, N.R., Jackson, T.N.,Koludarov, I., Alewood, P.F., Hodgson, W.C., Lewis, R.J., King, G.F., Antunes, A.,Hendrikx, I., and Fry, B.G.,Differential evolution and neofunctionalization of snake venom metalloprotease domains. Mol Cell Proteomics 12,2013,651-663.
66. Marquer, C., Fruchart-Gaillard, C., Letellier, G., Marcon, E., Mourier, G., Zinn-Justin, S.,Menez, A., Servent, D., and Gilquin, B.,Structural model of ligand-G protein coupled receptor (GPCR) complex based on experimental double mutant cycle data: MT7 snake toxin bound to dimeric hM1 muscarinic receptor. J BiolChem286,2011, 31661-31675.
67. Yang DC, Deuis JR, Dashevsky D, et al. The Snake with the Scorpion's Sting: Novel Three-Finger Toxin Sodium Channel Activators from the Venom of the Long-Glanded Blue Coral Snake (Calliophisbivirgatus). Toxins,Oct;8(10), 2016 ,E303. DOI: 10.3390/toxins8100303.
68. David A Warrell , José Maria Gutiérrez, Juan J Calvete, David Williams New approaches & technologies of venomics to meet the challenge of human envenoming by snakebites in India, Indian J Med Res.,138(1), 2013,38-59.PMID: 24056555**,** PMCID: PMC3767246

Table 1: Snake bite deaths in thousands by age and sex

Age range	Male			Female			Both		
	Total	Upper limit	Lower limit	Total	Upper limit	Lower limit	Total	Upper limit	Lower limit
0-14 years	149	154	134	176	180	160	325	334	294
15-29 years	109	111	102	88	82	89	197	199	184
30-69 years	290	303	269	253	260	232	543	564	501
70 years or more	54	60	45	48	50	44	102	110	89
All Ages	602	626	551	565	578	518	1167	1068	1204

Table 2: Major toxins and their mechanisms and functional properties associated to pain, inflammation, hemorrhage, necrosis and paralysis

Pro.	Toxin group			
	PLA2s	SVMP	SVSP	3FTX
Pain	Acute pain through ASIC1 activation [40]; Inflammatory pain, thermal hypersalgesia and mechanical Allodynia [41]; Excitation of sensory neurons [40, 41]	Inflammatory hyperalgesia [42, 43, 44]	Mild mechanical Hyperalgesia [45]	Analgesic effect through inhibition of ASIC channels [46]

Infla.	Neurogenic inflammation [47]; Non-neurogenic Inflammation [48] ; Endematogenic and pro-inflammatory [48,49]	Endematogenic activity independent of proinflammatory mediators [50]	Leucocyte Migration [45]; Mild edema[51]	Not described
Hem &Coa	Inhibits platelet Aggregation [52]	Cleavage of basement membrane of capillary vessels and endothelial cells adhesion proteins [53]; Procoagulant through activation of prothrombin and Factor X[54]; Inhibition of platelet aggregation [55]	Procoagulant through activation of prothrombin and factors VII and X [56]; Anti-coagulant through activation of Protein C and thrombin-like enzymatic activity [57].	Inhibits platelet aggregation [58]; Inhibits Factor X[59]
Nec.	Phospholipase activity [60] ; Myotoxic [61]	Dermonecrotic Activity dependent on TNF signaling [50]	Not described	Cytotoxins induce necrosis in skeletal muscle [62]
Para	Pre-synaptic toxin, block of neuromuscular transmission leading to muscle paralysis[63, 64]	Potential paralysis through inhibition of α-7 neuronal AChR by the cysteine-rich and disintregin- like domains complex [65]	Not described	Pre- and postsynaptic toxin, block of neuromuscular transmission through modulation of nAChR, AChE, NaV1.4 and L-type calcium channels [66,67]

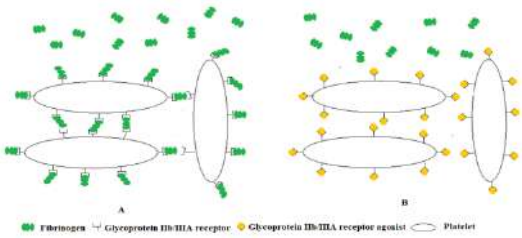

Figure.1: Mechanisms of action of Aggrastat, an anticoagulant derived from the chemical compound found in the venom of the Indian saw-scaled viper (Echiscannatus). A: The development of fibrinogen bridges between the glycoprotein IIb/IIIa receptors. B: Aggrastat, a glycoprotein IIb/IIIa receptor antagonist.

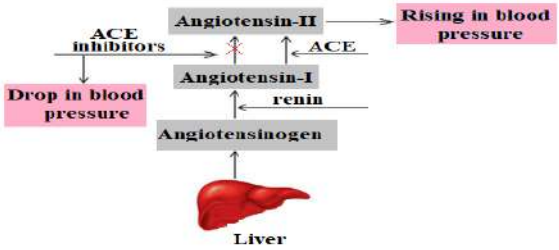

Figure.2: Mechanism of action of ACE inhibitors

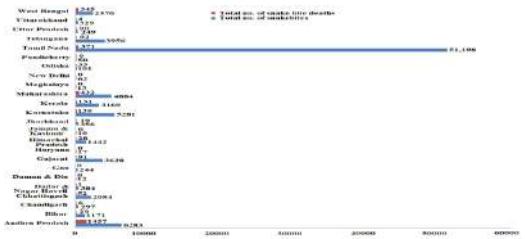

Figure.3: Review summary of state coverage by years

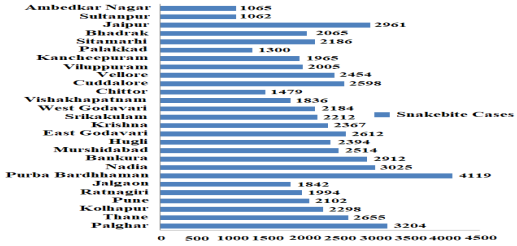

Figure.4:District wise prevalence of snakebite(India, From 2017-19)

Figure 5: Classification of Snake venom[39].

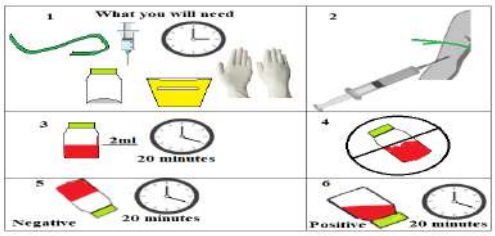

Figure 6: Twenty minutes whole blood clotting test (For diagnosis of coagulopathy following snakebite envenoming)

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RESEARCH ARTICLE

Fuzzy Hybridization for Classification - A Comparative Study using Clinical Data Set

S. Poongothai and S. Leoni Sharmila*

Assistant Professor, Department of Mathematics, SRMIST, Ramapuram, Chennai, Tamil Nadu, India.

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***Address for Correspondence**
S. Leoni Sharmila
Assistant Professor,
Department of Mathematics,
SRMIST, Ramapuram, Chennai,
Tamil Nadu, India.
Email: leonishs@ srmist.edu.in

ABSTRACT

Data mining holds great promise that can reduce the mortality ratio in the healthcare sector. This helps health systems to use the data and analytics routinely to recognize inefficiencies and best practices to optimize treatment and reduce costs. For this work, hybrid intelligent system predicts and achieves high classification performances. Two hybrid methods such as Fuzzy Neural Network (FNN) using min-max method and simplified Fuzzy Art Map (SFAM) applied on Diabetes, thoracic, Hepatitis, thyroid, and liver datasets taken form UCI Repoaisitory. The results are analyzed and compared with Fuzzy Logic method using grid partition, Artificial Neural Network using Multilayer Perceptron (MLP).

Keywords: Fuzzy Logic, Neural Network, FNN, SFAM.

INTRODUCTION

Data mining has been used extensively in the medical domain such as, DNA, genetics, medicine, and biomedicine in recent years. This technology aims to greatly enhance the efficiency in the field of disease detection, prevention and treatment. Data mining operates on the information already collected in the medical domain, and finds the best possible solution by analyzing and defining the recurrent patterns or trends in past data. Past experience in medical science plays a crucial role in diagnosing every new situation. It is a crucial role in the medical field from which patients can be properly diagnosed and treated. It has been noted in the past that the mortality ratio is that tremendously, this can also be minimized by using recent techniques available [1]. Data mining in the medical sciences is growing with great scope in better diagnosis and outcomes. Early works explained about the various Machine Learning methods, techniques and their classification rates in different medical datasets. This chapter explains about hybrid technique especially Fuzzy hybridization. Fuzzy is integrated with Artificial Neural Network in order to improve the accuracy level and classification rate. Hybrid system is one which combines one or more

Machine Learning techniques such as Fuzzy Logic, NNs, GAs, and expert systems to guarantee its efficacy under a wide variety of health problems. Each intelligent technique have unique computational properties that render them appropriate for particular issues and not others.

In an instance, as NNs are good at identifying model, they are poor at describing how their conclusion are made. Fuzzy Logic systems are good at explaining their choices, which can argue with imprecise knowledge, yet they can not immediately learn the laws they use those decisions to make. These restrictions had been a key driver of growth to sophisticated hybrid systems when two or more strategies are combined in such a way that overcomes disadvantages of similar methods [2]. While considering the varied complexity of application domains, hybrid systems are also essential. With positive implementations in medical diagnosis, need for Intelligent Hybrid systems is rapidly increasing. Although Fuzzy Logic offers a method of inference under cognitive instability, computational NNs offer promising benefits like understanding, adaptation, fault tolerance, parallelism, and generalization. In order that allow program that cope with cognitive uncertainty more like humans, the principle of Fuzzy Logic may be integrated into the NNs [3].

LITERATURE REVIEW

As just a major ingredient in soft computing, Fuzzy NN (FNN) is an intelligent hybrid model with the ability to learn and intelligently process the information. In [4,5] Fuzzy neurons and some systematic results on FNNs were first proposed by McCulloch-Pitts in mid-1970s that's where when interest faltered in NNs. These novel Neural Networks had therefore not attracted attention until 1987 by Kosko B. developed a blurred associative memory (FAM) to manage smart knowledge by inserting certain blurred operators into associative memory networks [6]. Research on NNs increased dramatically in the early 1980s due to work of Hopfield J. J. [7]. The FNN models also caught the attention of many scholars. A lot of new ideas have been created, including creative architecture and training rules and models on FNN's. Throughout action, FNN's found it useful in many areas of use, for example device modeling [8, 9], system reliability analysis [10] pattern recognition [11], and technology skills and so forth.

Simplified Fuzzy Art Map (SFAM)

Simplified fuzzy art map paradigm blends Fuzzy Logic with Adaptive Resonance Theory (ART) NNs. The network is supervised and can perform iterative learning. This network has the advantage of reducing the training time relative to other NNs. Simplified fuzzy art map is made of dual layers: one input and one output sheet. Simplified fuzzy art map network block diagram, the primary design is shown in Fig. 6.2. The data must be fixed in the network to a range from 0 to 1. Thus a appropriate standardization attribute must be chosen so that no information comes outside the acceptable range. Information is normalized by compliment coder, and the fuzzy compliment is also provided per each value. Then, this input (I) is passed to the input layer. Weights (w) check the input layer from each of the op nodes, rendering the weights top down [11]. Training starts with only one hidden node and the corresponding weights are set equal to the first record and prediction is set to the first record class. When a new class is discovered creating a new list. The node, whose weights best match the current input, provides the forecast given, the level of match approaches the threshold value of vigilance. If this prediction is correct, the weights of this successful node are set to this input. If this is not accomplished by an incorrect prediction or vigilance level, a new node with weights is generated and forecast equals this record [10]. Network is said to reach a resonance state if the value of the Network feature approaches the threshold of vigilance. Network are said to be mismatch, reset, if the parameter of vigilance exceeds the value of the corresponding function. Once the network is equipped, all op nodes compute activation feature with respect to input by moving input pattern into coder and then input layer. The winner is the node that has the maximum activation function, is selected The input tier is determined by assigning the highest-activated node group as max (Tj). The label layer contains the titles of the (m) groups that should be identified by the system[10]. For its completeness the training algorithm is now defined. The complement vector $\bar{C}$ describes the absence of each attribute for the given *ip* vector *c* of *d* functions.

$$\bar{c} = 1 - c \quad (1)$$

The internal *ip* vector *I* complement code is then *2d* dimension.

$$I = (c, \bar{c}) = (c_1, c_2, ..., c_d, \bar{c}_1, \bar{c}_2, ..., \bar{c}_d) \quad (2)$$

The activation and matching functions shall be defined as

$$T_j = \frac{|I \wedge w_j|}{\alpha + |w_j|} \quad (3)$$

$$M = \frac{|I \wedge W_j|}{|I|} \quad (4)$$

Wj is present template values (weight vector) associated with *op* nodes *j* and α, this is a minor value near zero. The resonant domain templates updates are defined as an assignment declaration.

$$W_j = (1 - \beta) W_j + \beta |I \wedge W_j| \quad (5)$$

where β is the learning rate, $0 \le \beta \le 1$. The operator $|I \wedge W_j - \sum \min(I, W_j)|$ used in (5.4) and (5.5) describe the Fuzzy AND feature which assumes positive, standardized *ips* values [10].

Fuzzy Min-Max Method

The FMM classification system is developed utilizing Fuzzy hyperbox sets. Hyper box, specified solely by its lowest and highest points. With respect to these hyperbox, the membership function is defined as min-max position, this explains the extent to which the pattern fits within the hyperbox. A cubic unit K^n is described as the spectrum of membership values here between 0 and 1. To an *ip* model of n-dimensions,a pattern found in the hyperbox has the membership quality of one and are described as follows. :

$$F_j = \{X, M_j, N_j, f(X, M_j, N_j)\} \quad \forall X \in K^n \quad (6)$$

where *Mj* and *Nj* are the ones relevant min and max points.

Implementing the above condition of a fuzzy hyperbox set consisting of the cumalative fuzzy set to classify *P* th pattern class, *Lp*, is

$$L_p = \bigcup_{j \in P} F_j \quad (7)$$

In which *P* is the index set to class *p* associated hyperboxes. Most of the computation is Concerned with the position and fine tuning of class boundaries, is an essential property of this method. The system of learning is, FMM enables the overlapping of same class hyperboxes and prevents overlapping of unusual classes. The membership function of *j*th hyper box *fj* (*Aa*) , $0 \le f_j(Aa) \le 1$, computes the degree to which the a^{th} *ip* pattern Oa falls exterior to hyper box F_j. It can be used as a metric to see how much each factor is superior (or lesser) than the peak (or least) value together with the dimension that lies outside the hyperbox's min-max boundaries. Still, when *fj (Aa)* reaches 1 shows that the point in the hyperbox should be more "protected" . The purpose that fulfills all of those parameters are the amount of the average of breach of max point, and the average amount of breach of min point. The membership function is:

$$f_j(O_a) = \frac{1}{2n} \sum_{i=1}^{n} [\max(0, 1 - \max(0, \gamma \min(1, o_{ai} - n_{ji}))) + \max(0, 1 - \max(0, \gamma \min(1, m_{ji} - o_{ai})))] \quad (8)$$

where, $O_a = (o_{a1}, o_{a2}, ..., o_{an}) \in K^n$ is the ath *ip* pattern, $M_j = (w_1, w_2, ..., w_{jn})$ is the least

point for F_i, $N_j = (n_1, n_2, ..., n_{jn})$ gives the max point of F_i, and γ is the sensitivity variable that regulates time when the membership values decline when the gap from *Oa* to F_i rises.FMM layout is a network of three levels, given in Figure 2. The initial layer is the *ip* layer which is identical to the measurements of protocol template as *ip* nodes. *op* level has nodes identical to class numbers. Hidden layer is known as hyperbox layer, every nodule contains a complicated series of hyperboxes, in which the links from the ip layer to the hidden layer are considered the min-max points. The hidden transfer layer feature is the hyperbox membership function that is described in [8]. The least and highest points respectively, are represented as matrices M and N. Binary values contained in matrix U are the relations between secret layer and op layer nodes.

EXPERIMENTAL RESULTS AND DISCUSSIONS

For this work, medical data sets such as Liver, Thyroid and Hepatitis are used form UCI Machine Learning repository. (i)Liver isorders data contains a total 345 male patient instances which is classified into 2 classes namely, normal and diseased. Out of 345, 200 are normal cases and 145 are diseased cases.(ii) Thyroid classification data set consists of 215 samples out this 150 patients does not have the disease. 30 patients have hyper thyroid and 35 have hypo thyroid. There are 6 inputs out of which the first attribute has to be classified. All attributes are continuous. (iii) Hepatitis data set consists of 155 data with 20 input variables along with class variable. For classification, the data is split into 30% & 70% for training and testing purpose. Results are discussed below:

Performance of FNN

Table I, presents a detailed performance of classification rate of various medical data using FNN method for five data sets such as liver, thyroid, hepatitis, thoracic and diabetes. It is noticed that liver as an accuracy of 74.2%, thyroid has 97%, hepatitis has 89%, of accuracy rates.

Performance of SFAM

Table 6.10 explains a detailed performance of classification rate of various medical data using simplified fuzzy art map method for three data sets such as liver, thyroid, hepatitis. It is noticed that liver as an accuracy of 89.4%, thyroid has 94%, and hepatitis has 94.5%.

CONCLUSION

Fuzzy Logic and artificial Neural Networks are an actively growing research area of soft computing, and have demonstrated their usefulness in many complex issues. Nevertheless, using a mere one of them, these challenges can not be addressed successfully, because each has its own shortcomings and disadvantages. Such constraints were the driving force behind the development of smart hybrid systems, where two or more soft computing resources are merged to transcend the limited power of each particular strategy. In fact, work into hybrid structures is an attempt to establish the next century of intelligent systems. FNN is appropriate for diagnosis of clinical diseases, and numerous successful examples have been found at home and abroad. Therefore, this work brings the Fuzzy Neural Network technology to the medical diagnosis and applies it to various medical evidence for diagnosis of disease.

REFERENCES

1. Divya, T. and Sonali, A. (2013). "A Survey on Data Mining Approaches for Healthcare", International Journal of Bio-Science and Bio-Technology, 5, pp. 241– 66.
2. Aliev, R.A. (2001). "Genetic Algorithm-Based Learning of Fuzzy Neural Network, Part 1: Feed- Forward Fuzzy Neural Networks", Fuzzy Sets and Systems, 118, pp. 351–358.

3. Blanco, A., Delgado, M. and Requena, I. (1995). "Identification of Relational Equations by Fuzzy Neural Networks", Fuzzy Sets and Systems, 71, pp. 215–226.
4. Buckley, J.J. and Hayashi, Y. (1994). "Can Fuzzy Neural Nets Approximate Continuous Fuzzy Functions", Fuzzy Sets and Systems, 61, pp. 43–51.
5. Lee, S.C. and Lee, E.T. (1975). "Fuzzy Neural Networks", Math. Biosci., 23, pp. 151–177.
6. Lee, S.C. and Lee, E.T. (1974). "Fuzzy Sets and Neural Networks", J. Cybernetics, 4, pp. 83–103.
7. Kosko, B. (1987). "Fuzzy Associative Memories", in: Kandel A. (Ed.) Fuzzy Expert Systems, Addison- Weley, Reading, MA.
8. Hopfield, J.J. (1982). Neural Networks and Physical Systems with Emergent Collective Computational Abilities, Proc. Nat. Acad. Sci., 13, pp. 2254–2258.
9. Chung, F.-L. and Duan, J.-C. (2000). "On Multistage Fuzzy Neural Network Modeling", IEEE Trans, on Fuzzy Systems, 8, pp. 125–142.
10. Venkatesan, P. and Suresh, M.L. (2009). "Classification of Renal Failure Using Simplified Fuzzy Adaptive Resonance Theory Map," IJCSNS International Journal of Computer Science and Network Security, 9, pp. 129–134.
11. Carpenter, G.A., Grossberg, S. and Rosen, D.B. (1991). "Fuzzy ART: An Adaptive Resonance Algorithm for Rapid, Stable Classification of Analog Patterns", Proc. of Internal Joint Conf. on Neural Networks, 2, pp. 411–416.

Table I - Perfomance Of Fnn For Various Medical Data Sets

	Liver	Thyroid	Hepatitis
Accuracy	**74%**	**97%**	**89%**
Sensitivity	74%	96.7%	89%
Specificity	74%	96%	88.8%
F measure	73.6%	96.7%	88.9%
ROC	0.717	0.946	0.961
Error	25%	3%	10.8%

Table II -Perfomance Of Sfam For Various Medical Data Sets

	Liver	Thyroid	Hepatitis
Accuracy	**89%**	**94%**	**94.5%**
Sensitivity	97.5%	100%	100%
Specificity	84.7%	92%	94%
F measure	86.9%	95.8%	40%
Error	10.6%	6.1%	5%

Table III - Comparative Study Of Fuzzy Logic, Nn and Fnn Technique

Data Set	Fuzzy Logic	NN	FNN	SFAM
Liver	67%	73.7%	74%	**89%**
Thyroid	84%	93%	**97%**	94%
Hepatitis	82%	88.6%	89%	**94.5%**

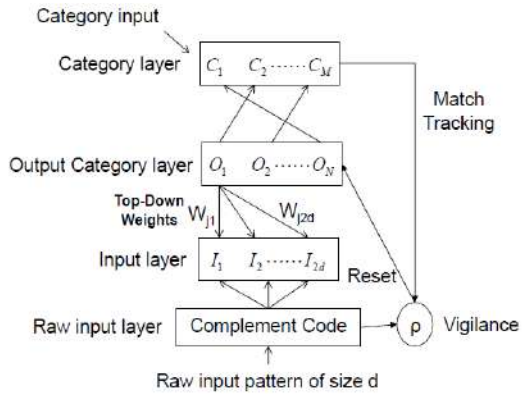

Figure I - Architecture of SFAM Model

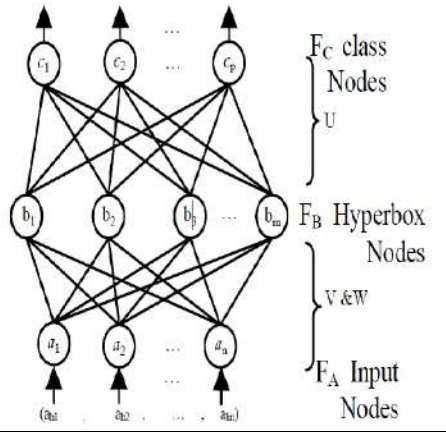

Fig. II: A Three layer FMM network

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RESEARCH ARTICLE

Effect of Weed Management Pracices on Growth and Yield of Direct Seeded Rice

M.P. Priyadharshini[1]*, A. Balasubramanian[2], R.Gobi[2] and S.Sathiyamurthi[3]

[1]Research Scholar, Department of Agronomy, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India.
[2]Assistant Professor, Department of Agronomy, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India.
[3]Assistant Professor, Department of Soil Science and Agricultural Chemistry, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India.

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***Address for Correspondence**
M.P. Priyadharshini,
Research Scholar,
Department of Agronomy,
Faculty of Agriculture,
Annamalai University,
Annamalai Nagar,
Chidambaram, Tamil Nadu, India.
Email: smp.surabi97@gmail.com

ABSTRACT

Field investigation was carried out at Vadipatti village, Nilakottai Taluk, Madurai District during Kuruvai (July – Nov) 2021 with rice variety NLR 34449, to study the effect of integrated weed management practices on growth and yield parameters in direct seeded rice. The experiment consisted of nine treatments laid out in randomized block design with three replications. The treatment involves pre-emergence herbicide *viz.,* Pretilachlor, early post emergence herbicide *viz.,* Pyrazosulfuron- ethyl and post emergence herbicide *viz.,* 2, 4-D Sodium Salt and hand weeding at 20 and 40 DAS. All the treatments significantly influenced the crop biometrics like growth, yield components of direct seeded rice. Growth components *viz.,* plant height, number of tillers m^{-2}, LAI, crop DMP and yield parameters *viz.*, number of panicles m^{-2}, number of filled grains^{-1}, thousand grain weight were registered with the application of Pretilachlor 50% EC @ 0.75 kg a.i. ha^{-1} on 3 DAS fb Pyrazosulfuron- ethyl 10% WP @ 200 g a.i. ha^{-1} on 15 DAS with efficient for weed control in direct seeded rice.

Keywords: Direct seeded rice, IWM, Pretilachlor, Pyrazosulfuron- ethyl, yield.

INTRODUCTION

Rice (*Oryza sativa* L.) is the most important and extensively grown crop in tropical and subtropical regions of the world. In India, rice is grown in an area of 43.66 million hectares having an annual production of 118.87 million tonnes with a productivity of 4.08 t ha^{-1}(DES, 2021). Direct seeding of rice refers to the process of establishing the crop from seeds sown in the field rather than by transplanting seedlings from the nursery. Weeds are most severe and widespread biological constrains to crop production in India and alone cause 33 per cent of losses out of total losses due to pests (Yakadri*et al.,* 2016). In direct seeded rice, high infestation causes grain yield losses up to 90 per cent. Weeds pose a serious threat by competing for nutrients, light, space and moisture just from the time of emergence and throughout the growing season. A weed free period for the first 25 to 45 DAS is required to avoid any loss in the yield in direct seeded rice. Weeds can reduce the grain yield of dry-direct seeded rice by 75.8 per cent, wet-direct seeded rice by 70.6 per cent and transplanted rice by 62.6 per cent (Singh *et al.*, 2021). The sequential application of a pre-emergence herbicide followed by early post emergence and /or post emergence herbicide can provide effective weed control in direct seeded rice, if supplemented with some other weed management strategies like hand weeding, mechanical weeding (Yadav *et al.,* 2018b). Considering the above facts, an attempt has been made in the present study to evaluate the efficacy of herbicides *viz.,* Pretilachlor 50% EC @ 0.75 kg a.i. ha^{-1} , Pyrazosulfuron- ethyl 10% WP @ 200 g a.i. ha^{-1}, 2, 4-D Sodium Salt @ 1 kg a.i. ha^{-1} on control of weeds in direct seeded rice.

MATERIALS AND METHODS

Field experiment was conducted at the Vadipatti village, Madurai district, Tamil Nadu is situated at 10° 05′ North latitude, 77° 98′East longitude and at an altitude of 81 m above the MSL. The soil of the experimental field is sandy loam in texture which is high in available N and K. This research was laid out in randomized block design with nine treatments and three replications by using the rice variety NLR 34449. The treatments comprised of Unweeded control (T_1), weed free check (T_2), two hand weeding on 20 DAS and 40 DAS (T_3), pre-emergence application of Pretilachlor 50% EC @ 0.75 kg a.i. ha^{-1} on 3 DAS fb hand weeding on 40 DAS (T_4), pre-emergence application of Pretilachlor 50% EC @ 0.75 kg a.i. ha^{-1} on 3 DAS fb post emergence application of 2, 4-D Sodium Salt @ 1 kg a.i. ha^{-1} on 35 DAS (T_5), early post emergence application of Pyrazosulfuron- ethyl 10% WP @ 200 g a.i. ha^{-1} on 15 DAS fb post emergence application of 2, 4-D Sodium Salt @ 1 kg a.i. ha^{-1} on 35 DAS (T_6), pre-emergence application of Pretilachlor 50% EC @ 0.75 kg a.i. ha^{-1} on 3 DAS fb early post emergence application of Pyrazosulfuron- ethyl 10% WP @ 200 g a.i. ha^{-1} on 15 DAS (T_7), hand weeding on 20 DAS fb post emergence application of 2, 4-D Sodium Salt @ 1 kg a.i. ha^{-1} on 35 DAS (T_8), early post emergence application of Pyrazosulfuron- ethyl 10% WP @ 200g a.i. ha^{-1} on 15 DAS fb hand weeding on 40 DAS (T_9).

RESULT AND DISCUSSION

Growth parameters

Growth parameters like plant height, number of effective tillers m^{-2}, leaf area index, crop dry matter production were recorded higher with the application of Pretilachlor 50% EC @ 0.75 kg a.i. ha^{-1} on 3 DAS followed by Pyrazosulfuron-ethyl 10% WP @ 200 g a.i. ha^{-1} on 15 DAS (T_7) are presented in table 1. This might be due to better weed control throughout the growth stages of rice and better availability of all resources *viz.,* light, moisture, space and nutrients to rice. This was in conformity with the findings of Haldar*et al.* (2021). In unweeded control, the weeds were allowed to grow uninterruptedly throughout the crop growth period. It resulted in maximum crop weed competition for resources since beginning resulting in minimum height of rice plants. This observation was in accordance with the report of Mukherjee (2020)**.**Higher leaf area index was due to effective weed control which facilitated the crop to absorb more nutrients from soil and produce more photosynthates through expansion of leaf area. This findings was coincides with Yadav *et al.* (2018a)

Yield and yield parameters

Application of Pretilachlor 50% EC @ 0.75 kg a.i. ha^{-1} on 3 DAS followed by Pyrazosulfuron- ethyl 10% WP @ 200 g a.i. ha^{-1} on 15 DAS (T_7) recorded higher grain and straw yield which was effective than other treatments due to more number of panicles m^{-2}, number of filled grains $panicle^{-1}$, thousand grain weight as presented in table 2. Effective and timely weed management reduced the density as well as dry weight of weeds consequently resulting into increased number of panicles m^{-2}, number of filled grains $panicle^{-1}$ and finally the yield. The results were in line with the findings of Teja *et al.* (2016). Weed free situation at early stage favoured the vigorous growth of seedlings, without any crop weed competition and sustained nutrient availability leads to better uptake of N, P and K by the crop might have contributed to synchronous tillering and spikelet formation leading to higher number of panicles m^{-2} and higher number of filled grains $panicle^{-1}$. By reducing the weed density it becomes stable environment for direct seeded rice which has enhanced the uptake of essential nutrients and translocation of photosynthates from the source to sink which influenced the yield attributes positively. This was in conformity with the findings of Suryakala*et al.* (2019). In direct seeded rice yield and yield attributes were tremendously increased due to timely control of weeds in critical period of crop weed competition has enhanced the availability of nutrients to the crop and also increase the crop yield with timely application of the broad spectrum herbicide combination. The similar results reported by Rao *et al.* (2019).Lower grain and straw yield was recorded in unweeded control. It might be due to lesser number of productive tillers and filled grain $panicle^{-1}$ and lesser uptake of NPK by the crop. This was in conformity with the findings of Venkatesh *et al.* (2021).

CONCLUSION

Based on the results of the study, it can be concluded that the pre-emergence application of Pretilachlor 50% EC @ 0.75 kg a.i. ha^{-1} on3 DAS followed by early post emergence application of Pyrazosulfuron- ethyl 10% WP @200 g a.i. ha^{-1} on 15 DAS (T_7) effectively reduced the infestation of weeds and favoured the crop growth parameters, yield attributes, yield of rice and gave the higher net return and benefit cost ratio in direct seeded rice.

REFERENCES

1. Directorate of Economics and Statistics, Government of Tamilnadu, 2020 – 2021.
2. Haldar, P., A. K. Thakur, M. Kumar, G. P. Ayam and D. P. Singh. 2021. Effect of Different Scented Rice Variety, Planting Geometry and Weed Management Practices on Yield Parameters and Yield at Southern Bastar of Chhattisgarh. Int. J. Curr. Microbiol. App. Sci, 10(01): 102-112.
3. Mukherjee, D. 2020 Effect of different crop establishment and weed control options on yield and nutrient uptake in rice.Ann. Agric. Res. New Series Vol. 41 (3): 266-275.
4. Rao, S., J. H. Kumar, M. Venkataramulu and P. R. Reddy. 2019. Evaluation of different herbicides in direct seeded rice (Oryza sativa L.). Int. J. Curr. Microbiol. App. Sci., 8(12): 790-798.
5. Singh, A., R. S. Singh, M. Kumar, V. K. Pandey, V. Singh and A. K. S .Shahi. 2021. Effect of weed management practices on weed flora, growth and yield of direct seeded rice (*Oryza sativa* L.). J. Pharmacogn. Phytochem., SP 10(1):138-142.
6. Suryakala, P., G. Murugan, M. Saravanaperumal, K. Suseendran and P. Stalin. 2019. Effect of weed management practices with new generation herbicides in transplanted rice**.** J. Pharmacogn. Phytochem., 8(3): 3913-3915.
7. Teja, K. C., B. Duary and S. Dash. 2016. Sole and combined application of herbicides on composite weed flora of transplanted rice. Indian J. Weed Sci., 48(3):254–258.
8. Venkatesh, B., Y. S. Parameswari, M. Madhavi and T. R. Prakash. 2021. Performance of herbicides and herbicide mixtures on weed control in transplanted rice. Indian J. Weed Sci., 53(2): 179-181.
9. Yadav, D. B., A. Yadav, S. S. Punia, N. Singh and A. Duhan. 2018a. Pretilachlor+ Pyrazosulfuron-ethyl (ready-mix) against complex weed flora in transplanted rice and its residual effects. Indian J. Weed Sci., 50(3): 257–261.
10. Yadav, R. I., M. K. Singh, R. K. Singh and M. Nagargade. 2018b. Planting methods and weed management to improve yield in dry-seeded rice. Indian J. Weed Sci., 50(1): 22-26.
11. Yakadri, M., M. Madhavi, T. Ramprakash and L. Rani. 2016. Herbicide combinations for control of complex weed flora in transplanted rice. Indian J. Weed Sci., 48(2): 155–157.

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RESEARCH ARTICLE

Synthesis and Characterization of Co-Polymer Poly (NIPAM) - Acrylamide Micro Gel

Sunita Gaikwad-Govilkar[1], Aniket A Garud[2] and RPS Rathore[3]*

[1]KBHSS Trust's Institute of Pharmacy, Malegaon, Nashik, Maharashtra, India.
[2]Rasiklal M. Dhariwal Institute of Pharmaceutical Education and Research Chinchwad, Pune, Maharashtra, India.
[3]Bhupal Nobles University, Udaipur (Rajasthan), India.

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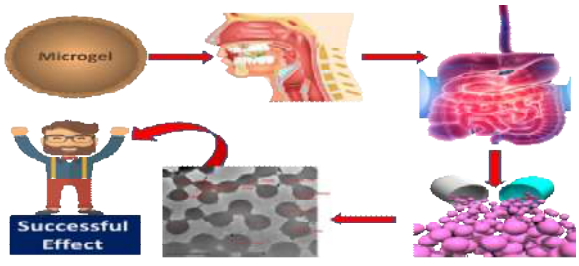
***Address for Correspondence**
RPS Rathore,
Bhupal Nobles University,
Udaipur (Rajasthan), India.
Email: rajendrapalrathore@yahoo.com

ABSTRACT

The PNIPAM (Poly N-isopropyl acrylamide) Microgels are thermosensitive & at room temperature they are in swollen state but as temperature increases, they get shrunk. By incorporating hydrophobic or hydrophilic co-monomer the properties of PNIPAM can be tuned. In the present work, the monodispersed polyN-isopropyl acrylamide (NIPAM) micro gel was prepared by SFEP (surfactant-free emulsion polymerisation method. A co-polymer microgel of poly (N-isopropyl acrylamide / acrylamide) was also prepared in different ratios and at different injection times by the same method. The characterization of microgel was done by DLS and TEM. In DLS it was observed that the particle size ranged from 240nm to 911nm. The hydrodynamic diameter obtained from TEM was correlated with the results of DLS. The polydispersity index was > 0.1, which indicated that synthesized microgels were monodispersed in nature. By using these properties of these microgels it can be used in many aspects like drug delivery vehicle, Protective for biodegradable drugs and proteins.

Keywords: proteins, vehicle, drugs, temperature, NIPAM, PNIPAM



INTRODUCTION

Microgels are colloidal dispersions of the gel formed from a network of microscopic filaments of polymer. Baker first introduced the term "microgel" [1].They also have known as a colloidal dispersion of gel particles. Microgel is referred to as intramolecular and intermolecular cross-linked polymers. Microgels are called 'smart' materials as it shows swollen and deswollen properties with changes in pH and temperature. eg. poly (NIPAM). They show complex structures. Staudinger and Husemann were the first to produce microgels using a suspension polymerization technique in 1935. The name microgel, on the other hand, was not coined until 1949. A microgel is a small particle of cross-linked polymer chains (0.1 to 1000 m) with the ability to expand and contain a large amount of excellent solvent (e.g. water) [2.3,4]. Microgels- Polyelectrolyte has soft particles due to their ability to osmotically de-swell. Microgels behave like soft Brownian particles in dilute suspensions. They form pastes when closely packed. The flow properties of suspensions and pastes are common. They can be tuned as per the requirement of the rheological behavior which is desired [5]. Colloidal microgels can absorb solvated materials into particles for example they act like micro sponges at a given set of conditions and then rapidly undergo conformational changes and release solvated material following environmental changes (such as pH, temperature, ionic strength, electric field), (Figure 1) [6].

Microgels are broadly used in the Pharmaceutical, Biotechnological, and Medical fields. There are a variety of applications for Microgel particles such as diagnostics (assays), biological analysis and research, bioseparation, NMR imagining Microsystems, cosmetics, drug delivery systems, and targeting [7,8]. Fast response to potential stimuli is essential for most applications. A Microgel shows a fast kinetic response. As poly (NIPAM) Microgel particles are 'smart materials' they show the dramatic change in volume with response to external stimuli such as pH, temperature, and ionic strength [7,9]. The Microgels are hydrophilic below the VPTT (Volume phase transition temperature), they can incorporate a large amount of protein-based drugs to ensure maximum biocompatibility. Thus Microgels have potential importance in the oral drug delivery system [10]. Adding copolymer to poly (NIPAM) can lower the lower critical solution temperature to temperatures around human body temperatures, which can make it an excellent candidate for drug delivery systems [11]. "Thermally on-off switching of polymers for drug permeation and release", will help to encapsulate the drug molecule [12].

MATERIALS AND METHODS

Method: Surfactant free emulsion polymerisation (SFEP) method to prepare a poly(NIPAM)\Co-AAmmicrogel.
Chemicals: N-isopropyl acrylamide (NIPAM), N, N'-Methylenebisacrylamide (BA), di-Potassium peroxodisulphate (KPS), and acrylamide (AAm). All Chemicals used were of Analytical grade. The characterization of Microgel particles was done using DLS. TEM was used for size determination of Microgel particles and Hot air oven was used for dry weight analysis of microgel after dialysis.

Procedure
All the ingredients were weighed according to Table 1. The setup of the reaction was done as shown in Figure 2. KPS was added to a reaction vessel containing 800ml of distilled water & was kept on a magnetic stirrer. The flow of Nitrogen gas was maintained throughout the reaction. The temperature was set to 70°C &the rotation speed was set around 300rpm. NIPAM & BA were dissolved in 200mL of distilled water in a beaker. When the temperature reached around 70°C, NIPAM and BA mixture was immediately added into the reaction vessel & heated for approx 6 hrs. Then dialysis of microgel was done for a week. Different concentrations of poly(NIPAM) microgels were prepared using different ratios of monomer NIPAM and co-polymer Acrylamide, while the weight of cross linker BA and initiator KPS were kept constant.

RESULT AND DISCUSSION

Microgel particle size: It was observed that with an increase in the concentration of acrylamide the hydrodynamic diameter of the particle was also increased at 25ºC and 50ºC. At 25ºC the microgel particles were in swollen state and they show the hydrodynamic diameters range from 478-510nm but for 70:30% P-NIPAM-Co-AAm the diameter was higher which was 609nm. That means a small amount of acrylamide addition did not affect P-NIPAM particle size. But as the amount of acrylamide increased, there was an increase in diameter. At 25ºC, the acrylamide amount shows the same results on particle size for all samples. But at 50ºC, the microgel particles were in a collapsed state, as the temperature was increased and size was reduced to approximately 240nm and for sample 70:30%, it was 427nm.

Dry weight analysis

Percent yield of all microgels was above 90%, while for the 70:30% sample A it was only 56%. The percentage yield obtained was good for all microgel samples except from 70:30% sample A.

CONCLUSION

Particle size of PNIPAM was increased by copolymerizing with Acrylamide. The incorporation of Acrylamide increases the volume-phase transition temperature (VPTT). The particle dispersion was stable at room temperature and as temperature was raised particles get aggregated. TEM results show that PNIPAM-co- microgel samples are spherical in shape. The polydispersity index was > 0.1, which indicated that synthesized microgels were monodispersed in nature.

REFERENCES

1. W.O Baker, Microgel, A New Macromolecule.Ind, Eng.Chem. 41 (1949) 511.
2. H.Staudinger, E.Husemeann, ÜberhochpolymereVerbindungen, 116. Mitteil.:Über das begrenztquellbare Poly-styrol.Ber. 68(1935).
3. Lopez, V.C. et al, Swelling of radiation cross-linked Acrylamide-based microgels and their potential applications, Radiat.Phys. Chem, 72, 111, 2005.
4. Spherical Microgel-Colloids - Hard Spheres from Soft Matter E. Bartsch* , S. Kirsch#, P. Lindner§, T. Scherer, S. StölkenInstitutfürPhysikalischeChemie der Universität Mainz, Jakob- Welder-Weg 15, D-55099 Mainz, Germany.
5. Cloitre M, Borrega R, Monti F, Leibler L. Structure and flow of polyelectrolyte microgels: from suspensions to glasses. ComptesRendus Phys. 2003;4:221–230.
6. Pelton R. Temperature-sensitive aqueous microgels. Adv. Colloid Interface Sci. 2000, 85, 1–33.
7. Saunders BR, Vincent B. Microgel particles as model colloids: theory, properties and applications. Adv. Colloid Interface Sci. 1999;80:1–25.
8. Kausar N, Chowdhry BZ, Snowden MJ (2008) Microgels from smart polymers. In: Galaev I, Mattiasson B (eds) Smart polymers: applications in biotechnology and biomedicine (2nd). CRC, Boca Raton, pp 137–175.
9. Gracia, L. H and Snowden,M.J., Preparation, properties and applications of colloidal microgels, Handbook of Industrial Water Soluble Polymer, William, P., BlackED, Blackwell, Oxford, U.K., 2006.
10. Pelton., Unresolved issues in the preparation and characterization of thermoresponsiveMicrogelsMacromolSymp (2004) 207:57–65.
11. AT. Okano; Y.H. Bae; H. Jacobs; S.W. Kim. Thermally on-off switching polymers for drug permeation and release.,Journal of Controlled Release.,Volume 11, Issues 1–3, January 1990, Pages 255-265.
12. Arijit Gandhi Abhijit Paul et al., Studies on thermoresponsive polymers: Phase behaviour, drug delivery and biomedical applications. Asian Journal of Pharmaceutical Sciences,Volume 10, Issue 2, April 2015, Pages 99-107.

Table 1: Weight of Ingredients used for Synthesis of Different Concentrations of Microgels.

Sr. No.	Name of Sample	Weight of monomer (g)	Weight of co-monomer (g)	Weight of cross-linker (g)	Weight of initiator (g)	Ratio of monomer & co-monomer (W/w %)	Acrylamide Injection time (duration)
		NIPAM	Acrylamide	BA	KPS		
1	Poly (NIPAM)	5.0	-	0.6	0.5	100:0	-
2	Poly (NIPAM)-CO-AAM	4.5	0.5	0.6	0.5	90:10	1hr.
3	Poly (NIPAM)-CO-AAM	4.25	0.75	0.6	0.5	85:15	Non-injection
4	Poly (NIPAM)-CO-AAM	4.25	0.75	0.6	0.5	85:15	1hr.14 min.
5	Poly (NIPAM)-CO-AAM	4.00	1.00	0.6	0.5	80:20	1hr.10min.
6	Poly (NIPAM)-CO-AAM A	3.5	1.50	0.6	0.5	70:30	1hr.25min.
7	Poly (NIPAM)-CO-AAM B	3.5	1.50	0.6	0.5	70:30	1hr.2min.
8	Poly (NIPAM)-CO-AAM C	3.5	1.50	0.6	0.5	70:30	1hr.

Table 2: Correlate size from DLS with TEM images.

Sr No.	Percentage of P-NIPAM (%)	Percentage of Acrylamide (%)	DLS Size in collapsed state at 50^{0}C(nm)	Size from TEM Measurement (nm)
1	100	0	240±2	252±6
2	90	10	256±1	266±10
3	85 (Direct)	15	584±3	601±9
4	85 (Inject)	15	250±2	263±5
5	80	20	255±1	269±3
6	70 (Sample A)	30	427±1	459±7
7	70 (Sample B)	30	330±3	260±10
8	70 (Sample C)	30	908±4	257±15

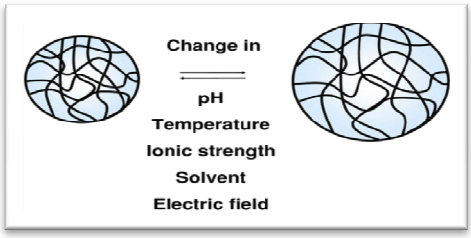


Figure 1: Change in microgel size in response to various environmental changes.

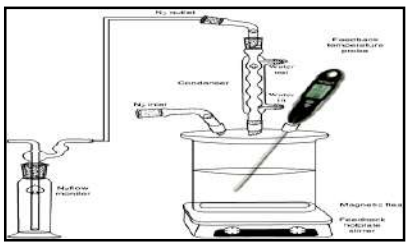


Figure2.The setup of reaction vessel used for synthesis of microgel (Snowden, 2010).

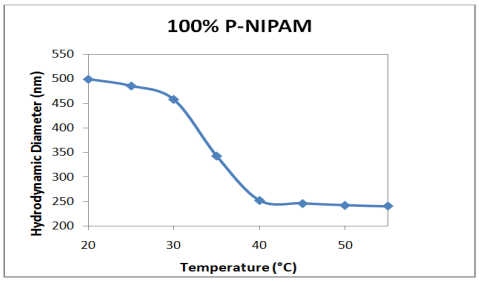


Figure 3: Hydrodynamic diameter of 100%P-NIPAM microgel as function of temperature.

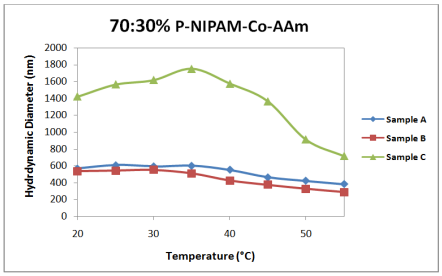
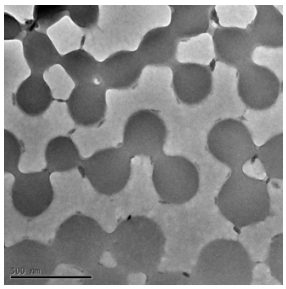


Figure 4: Hydrodynamic diameter of 70:30% (A, B, C) P-NIPAM microgel as a function of temperature.



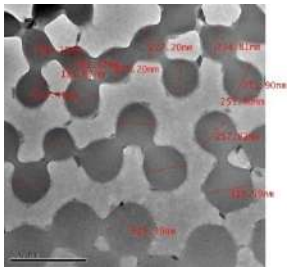
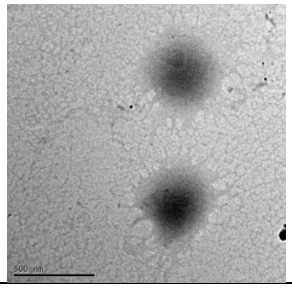


Figure 5.100% PNIPAM Microgel TEM Images. Average Size of particle : 251±6 nm



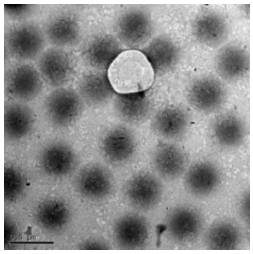


Figure 6.70:30% Sample 'A' Microgel TEM Images. Average Size: 450±7 nm

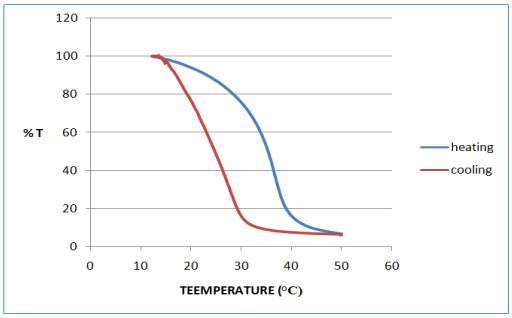


Figure 7: Graph of temperature (ºC) vs % transmittance (T) of 100% NIPAM.

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RESEARCH ARTICLE

Low Power Co-Design of Algorithms and Hardware in Computing Systems: Embedded Systems

Nilamani Ganesan[1]* and T.Muthumanickam[2]

[1]Research Scholar, Department of Electronics and Communication Engineering, Vinayaka Mission's Research Foundation - Deemed to be University, Salem, Tamil Nadu, India.
[2]Professor and Head, Department of Electronics and Communication Engineering, Vinayaka Mission's Kirupananda Variyar Engineering College, Vinayaka Mission's Research Foundation -Deemed to be University, Tamil Nadu, Salem, India.

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***Address for Correspondence**
Nilamani Ganesan,
Research Scholar,
Department of Electronics and Communication Engineering,
Vinayaka Mission's Research Foundation - Deemed to be University,
Salem, Tamil Nadu, India.

ABSTRACT

The need for embedded systems in software that manages sensitive or confidential data has drawn designers' attention to the security features of this type of system. This work presents a design flow that facilitates the development and evaluation of various solutions for the hardware implementation of authenticated cyphers and their incorporation as accelerating peripherals in embedded systems for various application cases using the C programmes and HDL descriptions available in the repositories of the CAESAR Competition for Authenticated Encryption: Security, Applicability, and Robustness. The mentioned methods can be used to any of the proposals submitted to the CAESAR Competition; however they have only been applied to three cyphers that were finalists in the several categories created in the competition. The hardware platform for all the designs has been a Zybo-Z7 development board with a Zynq-7000 device from Xilinx, which blends programmable logic from the 7-Series FPGAs with a dual-core Cortex-A9 ARM processor system. The implementation of the cypher, its conversion into an IP module, and its integration in an embedded system have all been carried out using the Vivado environment, which has been used to carry out the various stages of synthesis and verification required

Keywords: Low Power , Computing Systems ,Embedded , FPGA , Hardware , software,

INTRODUCTION

Power Reduction of Embedded Systems Although on Partial Alteration

The general purpose processor and microcontroller are the main components of the vast field of embedded systems. The modern FPGA offers a framework that can accommodate CPU and bespoke logic needs. Design professionals have the opportunity to shorten the lifecycle of chip creation thanks to reconfigurable technology. We are possible to use Field Programmable Gate Array (FPGA) devices in numerous designs as reconfigurable embedded processors thanks to new emerging features in FPGA, including improvements in time delays and cost per unit device. New Electronic-Design-Automation (EDA) tools enable us to build Systems-on-a-Chip prototypes quickly and with great maturity. The development cycle is significantly shortened by using soft-core components like processors and peripherals in hardware-software co-design environments. Microcontrollers currently outperform FPGAs in terms of power. This work presents a novel power management technique that uses on-the-fly partial reconfiguration to enable the usage of FPGA in low power embedded systems. Significant power savings are seen and presented here.
Traditionally low gate count programmable devices are now able to accommodate significant portions of digital system logic. Today's digital designers have the option of using the embedded system controller only as an FPGA device. Digital designers now have a lot of freedom to create unique digital designs utilising FPGA and HDLs thanks to the accessibility of high-density, low-cost FPGA devices [1].

FPGA devices have developed from their predecessors, which used glue logic, into a device that today has a wide range of integrated digital components (memory, multipliers, transceivers and many more).Over time, FPGA device density has increased, and at the same time, its price has decreased, making it economically feasible to employ in a variety of applications. In order to implement complicated digital logic, modern FPGAs have thousands of look up tables (LUTs) and function fields (FFs).FPGA-based systems are becoming more popular because, as illustrated in Fig. 1, they combine digital logic design, processors, and communication interface on a single chip. In FPGA devices, the technology that enables hardware reconfigurability also comes at a cost. SRAM-based interconnect switches are used by the majority of FPGAs to provide reconfigurability. The cost is measured in terms of the power used by the FPGA. Embedded System on FPGA, Fig. 1.FPGA designs are constrained in their ability to enter mainstream low-power applications due to the large power consumption of FPGA devices. We want to decrease power consumption without significantly reducing performance or requiring a larger chip area in order to successfully increase the range of FPGA application areas. The two main components of overall power dissipation are static and dynamic. Leakage currents have a significant impact on static power, which becomes more significant with deep submicron technologies. The switching activity that determines dynamic power dissipation directly relates to the system or block clock rate. A well-known and effective method to reduce dynamic power in ASIC and controller-based architectures is clock gating or selective clocking. Xilinx FPGAs now come with a brand-new functionality called on-the-fly partial reconfiguration. By enabling designers to modify a section of the FPGA while the rest of the design is still operational, the innovation improves reconfigurability even further.

Applications where it is necessary to have the flexibility to modify parts of a design without having to entirely redesign the entire device can benefit from partial reconfiguration [2].Utilizing these features may result in a decrease in the number of devices, a reduction in power usage, and a more effective use of the board space that is available.[3] is an example of how on-the-fly partial reconfiguration can effectively cut down static power dissipation. The new use of on-the-fly partial reconfiguration for clock switching to cut the dynamic power in embedded systems using FPGA is discussed in this research. This work makes a contribution by demonstrating the viability of on-the-fly partial reconfiguration in the context of low power embedded systems on programmable chip design. Real-time video filter application created on the Virtex-4 is used as a case study to demonstrate the theory. The article, which covers FPGA technology and power usage in FPGA, is organised as follows. On-the-fly partial reconfiguration in clock switching

Embedded System Hardware

This chapter will cover the hardware needed for information processing, storage, and communication as well as the "cyphy-interface," or interface between the physical world and information processing. Covering the cyphy-interface is essential because of CPS. Due to their effect on performance, timing characteristics, power consumption, safety, and security; additional hardware components must be covered. We will present circuits for the cyphy sampling, interface's digitalization, and reverse process of physical values. The sampling theorem and its effects will be discussed. We will discuss effective hardware for information processing, including field programmable gate arrays, multi-core systems, general-purpose computing on graphics processors, and digital signal processors (FPGAs).We shall describe the memory hierarchy as it is employed in embedded systems with regard to information storage. We'll also go through whether and how to use the current communication technologies. Electrical energy is needed for electronic information processing. The generation (e.g., harvesting), storage, and effective use of electrical energy in embedded systems, as well as battery and energy consumption models, are therefore covered in this chapter. A survey of the difficulties supporting security in hardware concludes this chapter.

Hardware designs

Hardware designs are frequently recycled, either as actual hardware parts or as intellectual property (IP).The platform-based design methodology's core principle is the reuse of existing hard- and software components .This concept is regarded as a crucial technique for controlling the embedded systems' increasing complexity. We will now go over some of the fundamentals of embedded system hardware in accordance with the requirement to take into account available hardware components and with the design information flow depicted in Fig. 2 .Compared to personal computers, embedded system hardware is far less standardised. It is impossible to give a thorough over view of every type of hardware component due to the enormous variety of embedded system hardware. Nevertheless, we'll make an effort to give a brief overview of some of the crucial parts that are included in most systems. Hardware is often employed in a loop in cyber-physical systems, particularly in control systems .This loop will be used to organise how the chapter's components are presented. Sensors are used in this (control) loop to provide data about the physical environment. Sensors often produce unbroken sequences of analogue readings. We shall focus on information processing in this book, which involves digital computers processing discrete sequences of values. Two types of circuits sample-and-hold circuits and analog-to-digital converters perform appropriate conversions (ADCs).Information can be processed digitally after such conversion. The generated results can be seen and used to use actuators to manipulate the physical environment. Since many actuators are analogue actuators, it might also be necessary to convert digital to analogue signals. We'll examine how this conversion can be carried out directly using DACs or inadvertently through pulse-width modulation (PWM).We presume that we need electrical energy since electronic information processing is the norm today. There must be a source of this energy. We may need to store energy, for example, in rechargeable batteries or capacitors, if our energy source does not provide energy continuously. Much of the electrical energy used by the system will be transformed into thermal energy while it is operating (heat).It could be essential to drain the system of its heat energy. Evidently, this type is suitable for control applications. It can be used as a first-order approximation for other applications. Following the layout. we'll go over the crucial hardware elements of embedded and cyber-physical systems in the sections that follow.

RESULT

EMBEDDED INPUT AND OUTPUT: PHYSICAL AND VIRTUAL WORLD INTERFACE SENSORS

The cyphy-essential interface's components are the sensors. There are sensors for almost every physical quantity. There are sensors for things like weight, speed, acceleration, voltage, electrical current, and temperature. The creation of sensors can make use of a wide range of physical effects .Examples include photoelectric effects and the law of induction, which produces voltages in an electric field. Chemical compound sensors are also available .A significant portion of the advancement in developing smart systems can be ascribed to the design of a wide variety of sensors in recent years. The Fig 3a and 3b construction of sensor networks, a crucial component of the Internet of Things, has been made possible by the accessibility of sensors. We are unable to fully describe this subset of cyber-

Physical hardware technology and can only provide representative examples

• Sensors that measure acceleration

The depicts a tiny sensor made with micro system technology. There is a little bulk inside the sensor. The mass will be moved from its normal position when it is accelerated, which will alter the resistance of the tiny wires that are attached to the mass. The robust inertial measurement units (IMUs) come with acceleration sensors. They capture up to six degrees of freedom, including location (x, y, and z) and orientation (roll, pitch, and yaw), and they have gyros and accelerometers .They are contained in aeroplanes, cars, robots, and other devices in order to give inertial navigation.

• Image sensors

Charge-coupled devices (CCDs) and CMOS sensors are the two main types of image sensors. Light sensor arrays are employed in both scenarios. The design of CMOS sensor arrays is comparable to that of conventional memories in that a random address can be used to access a single pixel. CMOS sensors employ conventional CMOS integrated circuit technology. As a result, logic circuits and sensors can coexist on the same chip. As a result, so-called smart sensors can undergo some pre-processing on the sensor chip. The only standard supply voltage needed by CMOS sensors is one, and connection is generally simple. As a result, CMOS-based sensors may be affordable.CCD technology, on the other hand, is tailored for optical applications. Charges must be moved from one pixel to the next in CCD technology before they can be read out at an array boundary. CCDs got their name from this sequential charge transfer as well. Interface design is more difficult for CCD sensors. Several limitations, which change as technology advances, must be taken into consideration while choosing the best image sensor. The initial image supremacy of CCDs has been called into doubt since the image quality of CMOS sensors has improved recently. Therefore, both CCD and CMOS sensors are capable of producing images of high quality. For cameras with live view modes or video recording capabilities, CMOS sensors are preferred due to their quicker readout speed . Additionally, CMOS sensors are recommended for inexpensive devices and when designing smart sensors. CCDs are still employed in areas like scientific image acquisition, despite the fact that several CCD application areas have vanished.

• Biometric sensors

The necessity to protect portable and detachable equipment as well as the demand for tighter security standards has boosted interest in authentication. The shortcomings of password-based security, such as password theft and loss, have drawn attention to biometric sensors and biological authentication. The goal of biometric authentication is to determine whether or not a particular individual is who they say they are. Face recognition, fingerprint sensors, and iris scans are examples of biometric authentication techniques. False acceptance and rejection are both fundamental issues with biometric authentication exact matches are not possible, in contrast to password-based authentication.

• Projects involving artificial eyes have attracted a lot of attention. While some initiatives directly affect the eyes, others indirectly contribute to eyesight. For instance, the Dobelle Institute conducted research using a camera connected to a computer that directly contacted the brain with electrical pulses .More lately, many have favoured the less invasive translation of visuals into sounds.

• Radio frequency identification (RFID)

An RFID tag's response to radio frequency signals serves as the foundation for the technology .An integrated circuit and an antenna make up the tag, which identifies itself to RFID scanners. Depending on the type of tag, the maximum distance between tags and readers may vary. The technology is a crucial enabler for the Internet of Things and is used to identify things, animals, or people.

• Automotive sensors

Modern automobiles come equipped with a lot of sensors. This includes sensors for the weather, tyre pressure, collisions; etc. The main objective is to make the atmosphere, as well as the passengers, comfortable and safe. Other common sensors include Hall Effect sensors, thermal sensors, engine control sensors, and many more.

Power usage and future work

We suggest time-correlated measurements of memory accesses and power usage for multi-modal anomaly identification in embedded systems. One-class support vector machine (SVM) and isolation forest classifiers are trained using time series of processor power usage and memory accesses between L2 cache and memory bus under known-good conditions. These side channels can detect anomalies in addition to the main channels. The technology successfully detects anomalies in tests using a high-fidelity CPU emulator. Index Terms: Support vector machine, memory access, power consumption, and cyber security (SVM).

Observations

Since there are no specific test locations or provisions on board for measuring the power consumed by the FPGA alone, the power consumption of the FPGA is approximated to be that of the complete ML-402 board. The circuit measures the voltage and current across the input terminals of the board and is external, allowing us to determine the amount of dc power used. Two distinct frequencies for Microblaze, 100 and 32 MHz, which were dynamically produced and loaded via ICAP setting the DCM core, were measured for power consumption under a voltage of 5 V. Avg .Power saving percentage and dynamic power dissipation (W)
CLK = 33MHz Power (W) CLK = 100MHz Power (W), saving 13.27, 9.06 7.85 %

HW/SW co-design techniques

In this study, we examine how computing systems, from embedded devices to data enters, use algorithm-hardware co design techniques. We suggest HW/SW co-design techniques for embedded systems under uncertainty at the level of embedded systems. In order to decrease energy consumption at both the server and data enter levels, we developed algorithm-hardware co-design at the data enter level. We intend to use the special qualities of deep learning tasks operating in the cloud in the future to optimise energy consumption while enhancing utilisation and latency in cloud computing.

CONCLUSION

By scaling the clock on the FPGA, this research demonstrates the efficient use of on-the-fly partial reconfiguration to lower dynamic power consumption in embedded system design. Adaptive video filter case study using fly PR system module is shown. As a result, the configuration interface and processing core are already present on the FPGA. To alter the DCM's multiply and divide properties, a difference-based bit file is produced. The clock frequency of the block is altered during partial bit file download dependent on the level of system activity required. The amount of dynamic power dissipation decreases as the clock rate changes. In the case shown, a 13.27% reduction in dynamic power is seen without degrading system performance or using more hardware resources. Although a real-time video filtering application is shown, the technique is general and can be used with the same advantages by other embedded systems using FPGAs that support partial reconfiguration. Utilizing the technique results in significant dynamic power dissipation cost savings. However, the application and design have an impact on the real savings. Future work will focus on reducing partial reconfiguration time because it also affects power dissipation.

REFERENCES

1. M. Meerwein, C. Baumgartner, T. Wieja and W. Glauert, "Embedded systems verification with FGPA-enhanced in-circuit emulator," Proceedings 13th International Symposium on System Synthesis, 2000, pp. 143-148, doi: 10.1109/ISSS.2000.874041.
2. Huang Lei, "Design of embedded data acquisition system based on FPGA," 2009 2nd IEEE International Conference on Computer Science and Information Technology, 2009, pp. 530-534, doi: 10.1109/ICCSIT.2009.5234478.

3. T. Wu, J. Ye and J. Lu, "Hardware Implementation of SM2 ECC Protocols on FPGAs," 2021 IEEE 5th Information Technology,Networking,Electronic and Automation Control Conference (ITNEC), 2021, pp. 33-37, doi: 10.1109/ITNEC52019.2021.9586889.
4. J. Wu and J. -L. Wang, "A Real-Time Embedded Platform for Mixed Energy-Criticality Systems," 2021 7th International Conference on Applied System Innovation (ICASI), 2021, pp. 58-62, doi: 10.1109/ICASI52993.2021.9568416.
5. M. -G. Unguritu and T. -C. Nichiţelea, "Adaptive Real-Time Operating System in Automotive Multicore Embedded Systems," 2021 25th International Conference on System Theory, Control and Computing (ICSTCC), 2021, pp. 150-153, doi: 10.1109/ICSTCC52150.2021.9607172.
6. S. Masood, S. A. Khan and A. Hassan, "Simulating Synchronization Issues on a Multiprocessor Embedded System for Testing," 2021 IEEE International Conference on Information Communication and Software Engineering (ICICSE), 2021, pp. 299-302, doi: 10.1109/ICICSE52190.2021.9404126.
7. Agirre, "Safe and secure software updates on high-performance embedded systems," 2020 50th Annual IEEE/IFIP International Conference on Dependable Systems and Networks Workshops (DSN-W), 2020, pp. 68-69, doi: 10.1109/DSN-W50199.2020.00021.

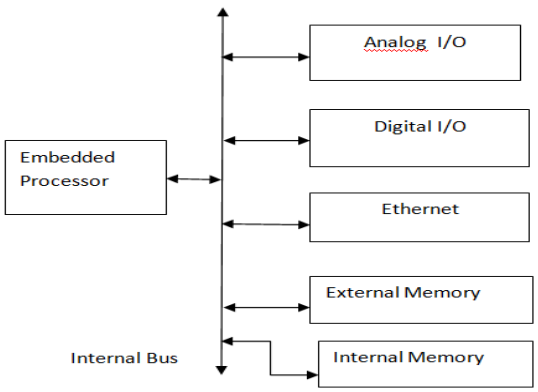

Fig.1 Embedded System on FPGA.

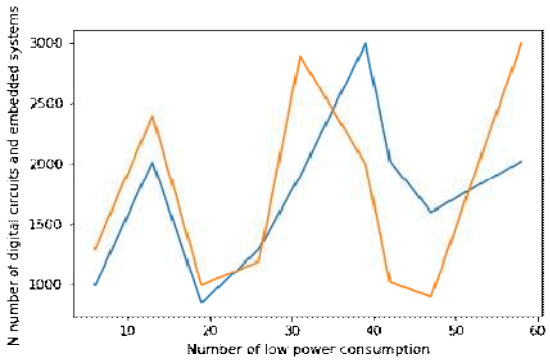

Fig. 2 Application Testing of Low Power consumption Sensor

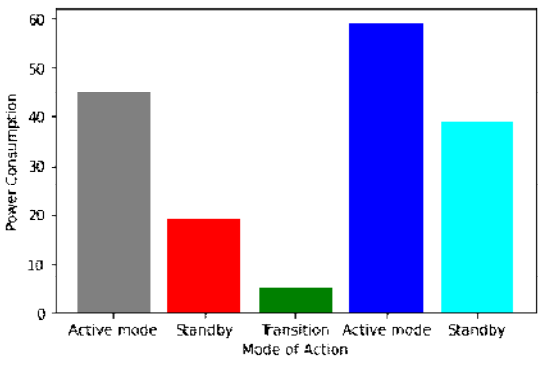

Fig. 3a Low power Mode electronics

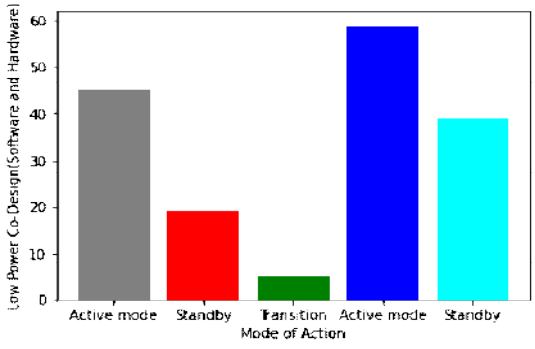

Fig. 3b Low power Mode electronics

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RESEARCH ARTICLE

Formulation, Evaluation, and Optimization of Silymarin-Quercetin Loaded Nanoparticles

Senthila S[1]*, P. Manoj Kumar[2] and P.Venkatesan[3]

[1]Professor and HoD, Department of Pharmaceutics, The Dale View College of Pharmacy and Research Centre, Trivandrum, Kerala India
[2]Professor, Department of Pharmaceutical Chemistry, Lisie College of Pharmacy, Kochi, Kerala, India
[3]Associate Professor, Department of Pharmacy, Annamalai University Chidambaram, Tamil Nadu, India

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***Address for Correspondence**
Senthila S
Professor and HoD,
Department of Pharmaceutics,
The Dale View College of Pharmacy and Research Centre,
Trivandrum, Kerala India
Email: senthilasivakami@gmail.com

ABSTRACT

Formulation technique when coupled with chemometrics tools, experimental designs and Derringer's desirability function provide a holistic view of the formulation process. The use of a desirability function appeared to be a useful approach for handling the problem of multiple responses in this case. This approach allows the formulator to generate mathematical models for the objective responses in relation to various formulation factors. Formulations of Silymarin-Quercetin loaded prolonged release nanoparticles were prepared by spontaneous emulsification solvent diffusion method and optimized using response surface methodology by fitting a second order Model to the response data. The model was found to be adequate for describing the relationships between formulation variables and individual response variables, as well as the relationships between formulation variables and the overall desirability (0.940).The prepared prolonged release Silymarin-Quercetin loaded nanoparticles were characterized. The nanoparticles were found to have suitable physio-chemical properties, desired drug entrapment efficiency, and drug release. FT-IR studies ruled out the compatibility of the drug with excipients in the physical mixture. This was further confirmed by the DSC results. SEM image revealed that the nanoparticles have smooth surface and spherical shape. Good entrapment efficiency was achieved by employing the global optimization technique. The *in-vitro* release pattern of the nanoparticle was observed to be in biphasic manner characterized by a burst effect followed by a slow release. The drug release profile of optimized nanoparticle formulation showed 92.75 % drug release at the end of 12th hour. Formulations released an amount of 22.34% in the first one hour which reflected the significant amount of Silymarin adsorbed on to surface of the nanoparticles. The correlation coefficient value R^2 is

taken into account to decide upon the relevance of the model which will best describe the extent of fit. Based on the R^2 values, Peppa's model was found to be a perfect fit with R^2 value of 0.999 for the optimized nanoparticle formulation.

Keywords: Derringer's desirability function, Silymarin-Quercetin loaded prolonged release nanoparticles, spontaneous emulsification solvent diffusion method, response surface methodology, second order Model.

INTRODUCTION

Optimization through a trial-and-error approach for making a good quality formulation will be a time-consuming, expensive challenging phase. Therefore, the use of systematic experimental design along with mathematical optimization is time and cost-effective [1]. Response surface methodology has identified tools for attain some objectives such as to determine and quantify the relationship between the formulation's response and the independent variables and to find the settings of these formulation variables that produce the best response values. The procedure encloses designing a set of experiments. Response surface methodology (RSM) has proven to be a useful tool for achieving both objectives [2]. In this study, we used RSM to design and optimize a prolonged-release formulation of Silymarin-Quercetin loaded nanoparticles. During optimization of pharmaceutical formulations, such as the Silymarin-Quercetin nanoparticles, usually, several response variables are to be optimized. Some of these variables are to be maximized and some of them are to be minimized. In many cases, these responses are competing with each other, i.e., improving one response may have an opposite effect on another, which further complicates the situation. Various methods can be used to overcome this problem. Another approach to solving the problem of multiple responses is through the use of a desirability function that combines all the responses into one measurement [5]. In this study, we have investigated the effect of formulation variables of prolonged-release Silymarin-Quercetin nanoparticles on several response variables, and subsequently, we have utilized response surface methodology [6]to optimize the formulation after constructing a desirability function that combines four response variables. The central composite design was applied to optimize the formulation of Silymarin-Quercetin loaded prolonged-release nanoparticles.

MATERIALS AND METHODS

Chemicals

The Silymarin and D-alpha-tocopheryl poly (ethylene glycol) 1000 succinate (TPGS) was purchased from Sigma Aldrich, India, Quercetin was purchased from Sisco Research Laboratories, Mumbai, Poly Lactic-co-Glycolic Acid (PLGA) (75:25) was obtained as a gift sample from Hindustan latex Limited (HLL), Trivandrum. Ethanol (Analytical grade) was purchased from SD Fine, Nashik, India. Deionized water was used throughout the experiment.

Software

The Experimental design, data analysis, and desirability function calculations were performed with Stat-Ease Design expert 13 trial version.

Preparation of Silymarin-Quercetin loaded PLGA-TPGS Nanoparticles by Spontaneous emulsification solvent diffusion method

Nanoparticles were prepared using the spontaneous emulsification solvent diffusion method [3,4].Drug and polymer were added into the mixture of DCM/ Ethanol (1:1) 20 mL each and stirred for 15 minutes to ensure that all material was dissolved. This solution of the organic phase was slowly poured into an aqueous solution containing an emulsifier using a high-speed homogenizer at 14000 RPM for 5-25 minutes. Stirring continued for the evaporation of the internal phase. The polymeric nanoparticles were then precipitated and the nanoparticles were separated by

using a centrifuge at 10000 RPM for 15 minutes and washed trice with deionized water. Then the suspension was freeze-dried at -80°C to obtain the fine powder, which is then kept at desiccator.

Characterisation methods includes Yield of nanoparticles

An equal quantity of drugs (50mg of each drug) and the polymer was taken for the preparation. Thus, the formed nanoparticles were weighed. The percentage yield was obtained as the percentage ratio between the practical and theoretical yield. Particle size analysis- Average particle diameter and size distribution of nanoparticles were determined by laser diffractometry using a Mastersizer 2000 (Malvern Instruments, Malvern, UK.Entrapment efficiency- The freshly prepared nanoparticles dispersion (5 mL containing 100 mg of Silymarin and 100mg Quercetin) was centrifuged at 10,000 RPM for 20 minutes using ultracentrifuge. The amount of un-incorporated drug was measured for Quercetin and Silymarin at 257 nm & 288 nmrespectively using UV spectrophotometer. *In-vitro* release studies- The release of Silymarin and Quercetin from entrapped in NPs, were determined by dialysis tube diffusion technique (Dialysis membrane-3500Da, MEDIA, Mumbai). Drug-Excipient Compatibility studies **- FT-IR spectroscopy was performed on Fourier transform infrared spectrophotometer.** Surface morphology- Scanning electron microscopy was conducted for the optimised formulation. *Zeta potential*- The Zeta potential of Silymarin-Quercetin loaded NPs was measured by Malvern Zetasizer Nano series-ZS[7].*In-vitro* release kinetics-The *in-vitro* release pharmacokinetics parameters were calculated by using Mathcad 15 software (trial version).Differential Scanning Calorimetry (DSC) analysis-The thermal properties of Silymarin-Quercetin loaded PLGA nanoparticles were investigated by Differential Scanning Calorimetry [9] X-ray diffraction analysis *(XRD)*-The sample of the nanoparticles was subjected to XRD analysis.

Optimization of individual responses by central composite design

Twenty formulations of Silymarin-Quercetin loaded PLGA nanoparticles were prepared as per experimental design methodology and evaluation of significant responses such as % yield, particle size, % entrapment Efficiency, and % drug release were studied.

Nanoparticles yield

The perturbation graph of %yield is shown in figure 1. It indicates that factor Drug-polymer ratio (A) is more influencing than the other factors amount of TPGS (B) and homogenization time (C) respectively. This perturbation graph gives the significant factor among all three factors used in the study with respect to the response by showing more deviation from the central point. To identify the effect of each factor with other factors on the % yield, an Interaction plot can be used. While increasing the amount of TPGS and Drug-Polymer ratio, the % yield of the nanoparticles is getting more. The positive interaction between the amount of TPGS and the Drug-Polymer ratio is shown in the interaction plot (Figure 2).The predicted Three-dimensional response surface methodology graph for % yield was shown in figure3. The 3D response plot indicates that when increasing the amount of TPGS, the % yield also increases.

Particle size analysis

The perturbation graph of particle size is shown in figure 4. It indicates that factor Drug-polymer ratio(A) is more influencing than the other factors amount of TPGS (B) and homogenization time (C) respectively. It confirms that factors Drug-polymer ratio and the amount of TPGS are playing a key role in the particle size of nanoparticles. To identify the effect of each factor with other factors among all three factors on particle size, an interaction plot can be used. The positive interaction between the and Drug-Polymer ratio and amount of TPGS is shown in the interaction plot (Figure 5). The predicted three-dimensional response surface methodology graph of particle size was shown in figure 6. when the Drug-Polymer ratio and the amount of TPGS are increasing, the particle size also increases. It can be confirmed by a 3D response surface plot.

Entrapment efficiency

The perturbation graph of %entrapment efficiency is shown in figure 7. It indicates that factor Drug-polymer ratio (A) is more influencing than the other factors amount of TPGS (B) and homogenization time (C) respectively. To

identify the effect of each factor on entrapment efficiency, an interaction plot can be used. The interaction plot (Figure 8) shows a positive interaction can be identified by the fixed solid line which is seen above the central point. The predicted three-dimensional response surface methodology graph was shown in figure 9. As the Drug-polymer ratio and the amount of TPGS increase, the entrapment efficiency also increases.

***In-vitro* drug release**

This perturbation graph and Interaction plot gives the significant factor among all three factors used in the study with respect to the response by showing more deviation from the central point**.**

Optimization Of Individual Response Values for the Experiments Performed As Per Design Matrix

The central composite design was applied to optimize the formulation of Silymarin-Quercetin loaded prolonged-release nanoparticles. In this work, the important formulation factors were selected and optimized by a central composite design experiment. From preliminary experiments, the key factors selected for the optimization process were Drug-Polymer ratio (A) and Amount of TPGS (B) homogenization time (C) shows the levels of each factor studied for finding out the optimum values and responses.

Model fitting

The evaluation results of each formulation in terms of the individual responses were calculated. The results of the applied statistical tests indicated that all four response variables measured in this study showed good fitting to the second-order model. The fitting equations that resulted after model simplification are given in table 2. The results of the applied statistical tests show that all four response variables such as adjusted R^2 is >0.8, the Model P-value is <0.05, % C.V is <10%, and adequate precision is >4. All these values are within the limits, which indicates a good fitting to the second-order model.

Global optimization of formulation using derringer's desirability function-
Multi-criteria decision making

In the present study, to optimize three responses with different targets, Derringer's desirability function, was used. Derringer's desirability function, D, is defined as the geometric mean, weighted, or otherwise, of the individual desirability functions. The expression that defines Derringer's desirability function is (RyadAmdoun*et al.,* 2018)

$$D= [d_1^{p1} X d_2^{p2} X d_3^{p3} X \ldots\ldots\ldots X d_n^{pn}]^{1/n}$$

where p^1 is the weight of the response, n is the number of responses and di is the individual desirability function of each response obtained from the transformation of the individual response of each experiment. The scale of the individual desirability function ranges between di = 0, for a completely undesired response, to di = 1 for a fully desired response. Weights can range from 0.1 to 10. Weights lower than 1 give less emphasis to the goal, whereas weights greater than 1 give more emphasis to the goal (in both cases, di varies in a non-linear way while approaching the desired value). But with a weight of 1, di varies linearly. In the present report, we chose weights equal to 1 for all six responses. A value of D different to zero implies that all responses are in a desirable range simultaneously and consequently, for a value of D close to 1, the combination of the different criteria is globally optimal, so as the response values are near target values.

Validation of predicted models

The criteria for the optimization of each response are shown in table 8.Criteria have been proposed for selecting an optimum experimental condition for the formulation of Silymarin-Quercetin loaded prolonged-release nanoparticles. As can be seen under the criteria, three responses % yield, % entrapment efficiency, and % drug release were maximized, to get high yield Silymarin-Quercetin loaded prolonged-release nanoparticles with good entrapment efficiency. The response surface obtained for the global desirability function is presented in figure 17**.**The coordinates producing the maximum desirability value (D=0.940) were Drug-Polymer ratio 1:3, amount of TPGS 191.5mg, and homogenization time 15.7 minutes. The predicted response values corresponding to the latter value of D were: % yield = 83.49, particle size = 124.10 nm, entrapment efficiency (% w/w) = 86.47 and % drug release (t_{12} hour) = 92.09%.

Results of Characterization of Optimized Nanoformulation

Particle size

The average particle size of nanoparticles was found to be 125.7nm (Figure 15). It was observed the size of the nanoparticle was increased with an increase in the concentration of the polymer and drug whereas the size of the nanoparticles was decreased within increasing with homogenization time.

Entrapment efficiency

The percent drug entrapment and percent free drug for the prepared nanoparticles was determined. The optimized nanoparticles of Silymarin and Quercetin were analyzed for the drug entrapment. The results are shown in table 4.All the analysis was carried out in triplicate and the average was taken.

***In-vitro* drug release**

The *in-vitro* drug release was studied and the cumulative percent drug release was calculated using the calibration curve in phosphate buffer pH 7.4 at λ max of 288 nm for Silymarin and 257 nm for Quercetin. The cumulative percentage drug release from the Silymarin-Quercetin nanoparticles was shown in figure 16. A better release profile was shown by both Silymarin and Quercetin.

Other characterization parameters for the Silymarin-Quercetin optimized nanoformulation

Drug-excipient compatibility studies

The IR spectrum of pure Silymarin, Quercetin, PLGA, and physical mixtures of Drug-Polymer in the ratio 1:1 was taken and compared. The FT-IR spectra were shown from figure 20 to figure 22. On comparison of the individual spectra of the pure sample with that of physical mixtures, no prominent difference in the spectrums was seen. Thus, it was concluded that there are no major degenerative interactions. Hence the excipients could be used safely to formulate the nanoparticles.**FT-IR spectra of optimized formulation (Figure 17) is compared with individual spectra of drugs and excipients showed no degenerative changes, from the position of FT-IR absorption peaks which confirms that the drug and excipients are compatible with each other in the final formulation.**

Surface morphology

The surface morphology of the prepared nanoparticles was determined. The scanning electron microscopic photographs of the prepared nanoparticles were shown in figure 19& figure 20.The results of surface morphological studies of the nanoparticles revealed a smooth surface and the prepared nanoparticles were spherical to near-spherical for all the formulations analyzed. The nanoparticle produced by the spontaneous emulsification solvent diffusion method showed a perfect sphere.

Zeta Potential for optimized Silymarin-Quercetin loaded nanoparticles

Zeta potential is the measure of the surface charge of nanoparticles prepared. The measurement itself is particle electrophoresis, the particle velocity is determined via the doppler shift of the laser light scattered by the moving particles. The field strength applied was 20 V/cm. The electrophoretic mobility was converted to the zeta potential in mV, yielding the ZP is -53.8mV (Figure 18). The surface charge of a particle in turn indicates how stable is our particle in the suspension system (higher the surface charge higher is repulsion between particles). Hence it is mandatory to measure the zeta potential of prepared particles. Nanoparticles with Zeta Potential values greater than +25 mV or less than -25 mV typically have high degrees of stability. Hence the Optimised formulation shows high degrees of stability.

***In-vitro* curve fits for various release systems for optimized Silymarin-Quercetin loaded nanoparticles**

In-vitro drug release data of optimized formulations were fitted into zero-order, first-order, Higuchi equation, and Korsmeyer-Peppas equation. The results of *in-vitro* dissolution studies obtained for the formulations were plotted in 4 models. To know the drug release from the formulations, the *in-vitro* drug release data were subjected to fit into Zero-order, first-order, Higuchi, Korsmeyer-Peppas equations, to ascertain the mechanism of drug release. In a comparison of R^2 values of zero order and first order, it was observed that the R^2 values of the first order were higher

than that of zero-order plots. And this indicates that the drug release from the formulations, more likely to follow first-order kinetics, and the R^2 values of first-order kinetics were found to be close to unity and the curve fits for various release systems was shown in table 5.The correlation coefficient value R^2 is taken into account to decide upon the relevance of the model/curve fit which will best describe the extent of fit. According to Pappas fit, the release of the drug is decided upon the diffusion of the polymeric matrix and the drug release is governed by a variation of Fick's law of diffusion. Peppas n value obtained for Silymarin is 1.118 and for Quercetin is 1.094. The factors which control this are the diffusion coefficient and permeability coefficient of the polymer at a constant temperature. The prepared Silymarin-Quercetin nanoparticles release the drug by a diffusion process which releases the drug from the polymeric matrix based upon the extent diffusion, erosion of polymeric matrix and subsequent domain separation of the drug due to diffusion might also be a possibility.

Differential scanning calorimetry (DSC) analysis

DSC analysis was performed to find out the physical nature of the drugs, silymarin-Quercetin entrapped in PLGA nanoparticles and also ensures the drug-excipient compatibility. Individual thermo grams of pure drug, polymer, and drug-loaded nanoparticles (optimized formulation) were performed and the thermo grams of DSC are shown in figure 21.The thermo grams showed the characteristic exothermic peeks of the drug at the melting point 161℃. This confirmed that the drug and excipients were compatible with each other. Figure 28 shows the DSC thermo gram of the final nanoformulation. A sharp symmetric melting endothermic peak indicates the compatibility of the Drug and the excipients in the final formulation.

X-ray powder diffraction analysis (XRD)

XRD analysis was performed to find out the physical nature of the drugs, Silymarin and Quercetin entrapped in, Poly Lactic-co-Glycolic Acid Nanoparticles. The XRD patterns of drug silymarin and quercetin produced a characteristic peek when analysed in bulk powder form. The XRD pattern of the samples was shown in figure 22.This result indicated that the drug present in the polymeric nanoformulation developed is either dispersed/entrapped molecularly or present in an amorphous form in the polymer matrix.

SUMMARY

Silymarin-Quercetin loaded prolonged-release nanoformulation was optimized using response surface methodology by fitting a second-order model to the response data. The model was found to be satisfactory for describing the relationships between formulation variables and individual responses as well as the relationships between formulation variables and the maximum overall desirability (0.940). The optimization method enabled us to predict the values of response variables and overall desirability within the experimental range. The optimum nanoparticles prepared in the study were spherical with a size range of 125.7nm with entrapment efficiency 87.02 % and % drug release 92.75%. A good agreement between the predicted and experimental values were observed. Optimum desirability of Silymarin-Quercetin loaded prolonged-release nanoparticle was achieved at high homogenization time and Drug-Polymer ratio and intermediate levels in the amount of TPGS.

REFERENCES

1. Javed S, Kohli K, Ali M. Reassessing bioavailability of silymarin. Altern Med Rev. 2011 Sep;16(3):239-49.
2. Saminathan C, Venkatesan P, Madhusudhan S. Selection of Better Method for the Preparation of Nanoparticles by Applying Analytic Hierarchy Process. Rese Jour of Pharm and Technol. 2019;12(11):5320.
3. Zhang Z, Feng S. Nanoparticles of poly(lactide)/vitamin E TPGS copolymer for cancer chemotherapy: Synthesis, formulation, characterization and in vitro drug release. Biomaterials. 2006 Jan;27(2):262-70.
4. Parasuraman S, Anand David A, Arulmoli R. Overviews of biological importance of quercetin: A bioactive flavonoid. Phcog Rev. 2016;10(20):84.
5. Kreuter J: Nanoparticles, in: J. Kreuter. Colloidal Drug Delivery Systems, Marcel Dekker, New York, 1994; 219342.

6. Senthil Kumar C, Mohan.S Fabrication, characterization and evaluation of hepatoprotective activity drug loaded flavono nanoparticle delivery system J Phyto.2015; 4(2): 90-96.
7. Wissing S, Kayser O, Müller R. Solid lipid nanoparticles for parenteral drug delivery. Advanced Drug Delivery Reviews. 2004 May;56(9):1257-72.
8. Saiyyad IM, Bhambere D, Kshirsagar S. Formulation and Optimization of Silymarin Loaded PLGA Nanoparticle for liver targeting. Asian Jour Pharm and Technol. 2017;7(4):209.
9. Esmaeili F, Atyabi F, Dinarvand R. Preparation of PLGA nanoparticles using TPGS in the spontaneous emulsification solvent diffusion method. Journal of Experimental Nanoscience. 2007 Sep;2(3):183-92.
10. SamerBayda, Muhammad Adeel, TizianoTuccinardi, Marco Cordani, FlavioRizzolio. The History of Nanoscience and Nanotechnology: From Chemical–Physical Applications to Nanomedicine. Molecules. Jan;2020:1-15

Table 1. Selected critical parameters for the study to perform the optimization

Factor	Name	Units	Minimum	Maximum
A	Drug-Polymer Ratio	mg	1:1	1:5
B	Amount of TPGS	mg	100	300
C	Homogenization time	Minutes	5	25

Table 2: Reduced response models[a] and statistical parameters obtained from ANOVA (after backward elimination)

Response	Regression model	Adjusted R^2	Model P-Value	%C.V.	Adequate precision
Particle Size	83.57+1.72A+0.1850B+0.2421C-0.2950AB+0.3325AC-0.6500BC-1.15A^2-1.26B^2-0.5304C^2	0.8088	0.0006	1.20	9.0176
% Yield	+124.86+14.50A+3.74B+0.7009C-5.07AB+1.63AC-4.35BC +11.02A^2 +5.10B^2+4.60C^2	0.8726	0.0001	4.55	13.7527
% Entrapment efficiency	86.46+0.8889A-0.4377B +0.0624C-0.2988AB-0.1713AC-0.4313BC-0.2070A^2+0.3268B^2-0.2795C^2	0.8168	0.0005	0.5283	11.9917
% Drug release	92.06-0.2901A-0.1820B+ 0.1168C-0.0475AB +0.0650AC-0.1400BC-0.9141A^2-0.4898B^2-0.3785C^2	0.8290	0.0004	0.4552	10.5101

[a] Only significant coefficients with P < 0.05 are included. Factors are in coded levels.

Note: A, B, and C represent the formulation variables drug to polymer ratio, amount of TPGS (mg), Homogenization time (minutes) respectively.

Table 3 Criteria for the Global optimization of the individual responses

Response	Lower limit	Upper limit	Criteria	
			Goal	Importance
% Yield	77.16	84.24	Maximize	3
Particle size(nm)	120.34	172.35	Minimize	3
% Entrapment efficiency	84.21	88.68	Maximize	3
% Drug release	89.25	92.04	Maximize	3

Table 4: The comparison of experimental and predictive values of different objective functions under optimal conditions

Optimum conditions	Drug: polymer ratio	Amount of TPGS (mg)	Homogenization time (minutes)	% Yield	Particle Size (nm)	% Entrapment efficiency	% Drug release
1	Desirability value(D) =0.940						
	1:3	191.5	15.7				
Predictive				83.49	124.10	86.47	92.09
Experimental				83.46	125.7	87.02	92.75

Table 5: *In-vitro* curve fits for various release systems for optimized Silymarin-Quercetin loaded nanoparticles

#	Equation	Silymarin R^2	Quercetin R^2
01	Zero Order	0.976	0.974
02	First Order	0.920	0.900
03	Higuchi Model	0.967	0.962
04	Korsmeyer-Peppas Model	0.999	0.998

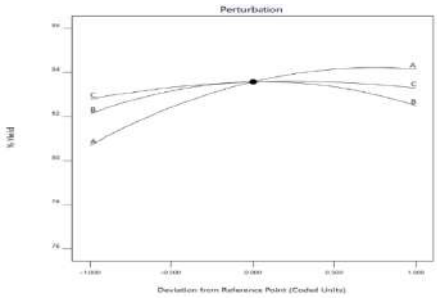

Figure 1. Perturbation graph-% yield

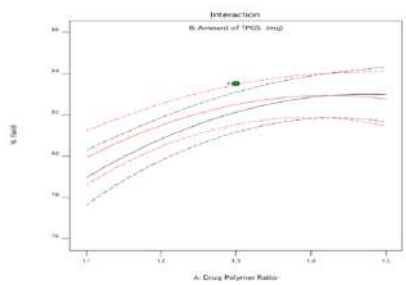

Figure 2. Interaction plot-% yield

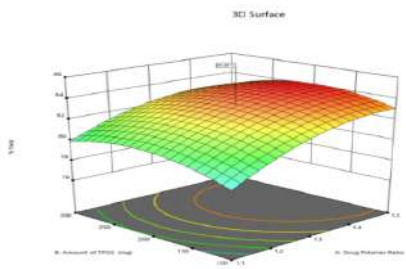

Figure 3. Predicted 3D Response surfaces plot-% yield

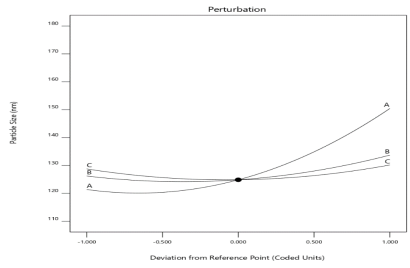

Figure 4. Perturbation Graph-Particle size

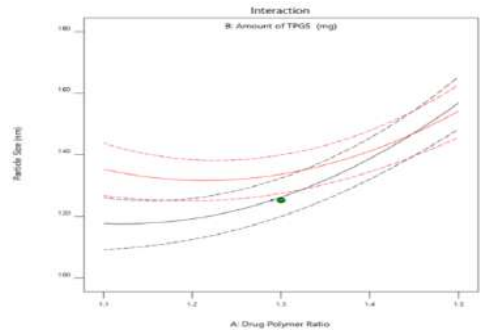

Figure 5. Interaction Plot-Particle size

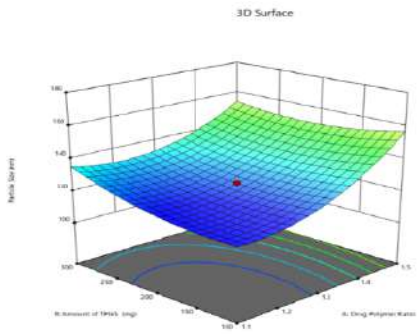

Figure 6. Predicted 3D response Surface graph-Particle size

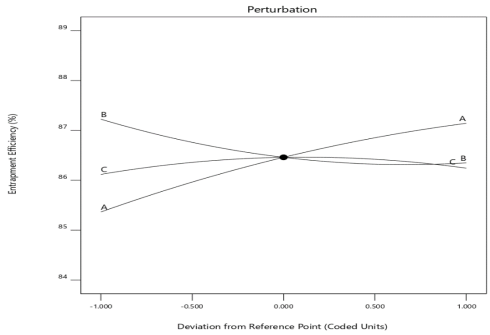

Figure 7. Perturbation plot-% entrapment efficiency

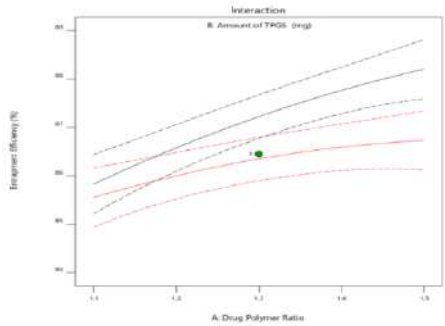

Figure 8. Interaction Plot-% entrapment efficiency

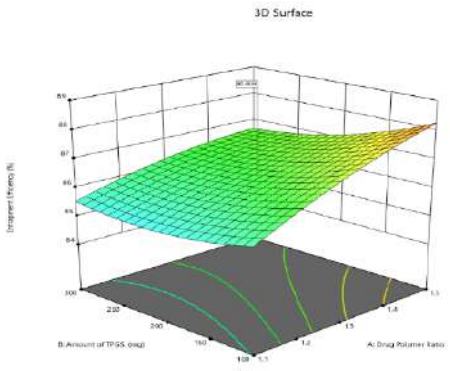

Figure 9. Predicted 3D response surface graph-% entrapment efficiency

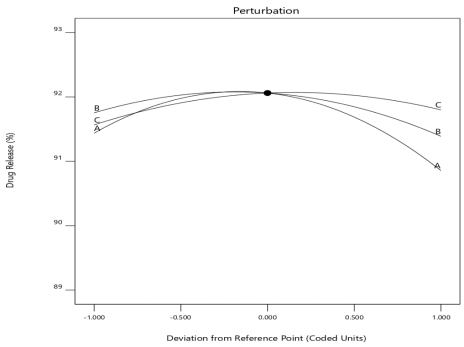

Figure 10. Perturbation graph-% Drug release

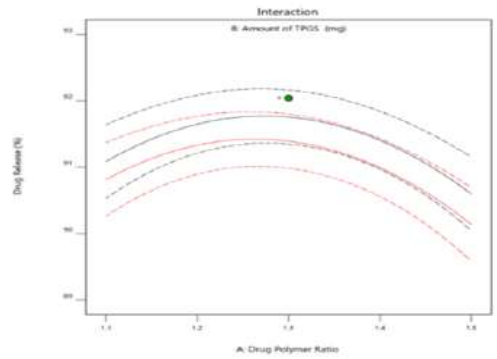

Figure 11. Interaction Plot-% drug release

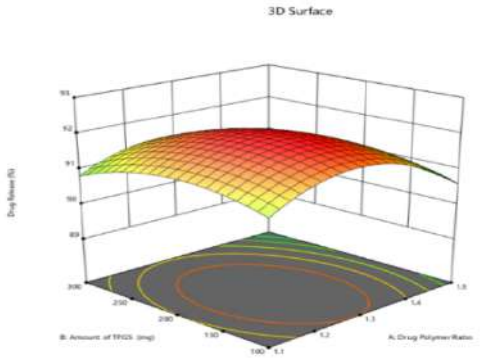

Figure 12. Predicted 3D response surface graph-% Drug release

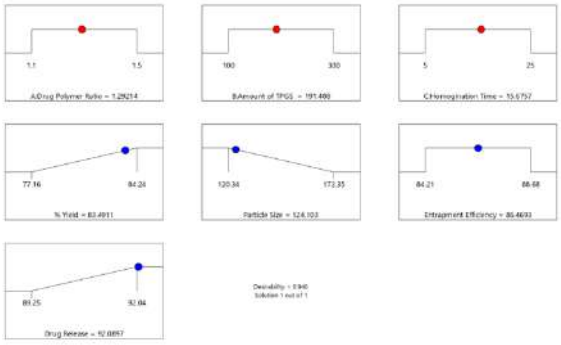

Figure 13. Ramps for optimal formulation condition and corresponding predicted responses

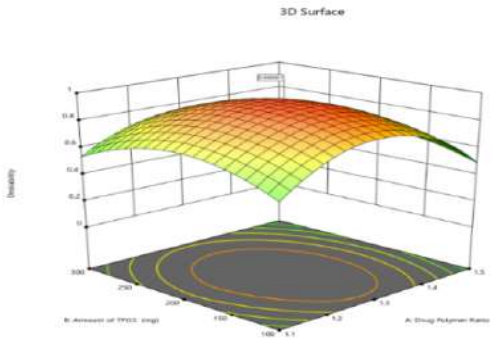

Figure 14. 3D response surface graph of the overall desirability function D

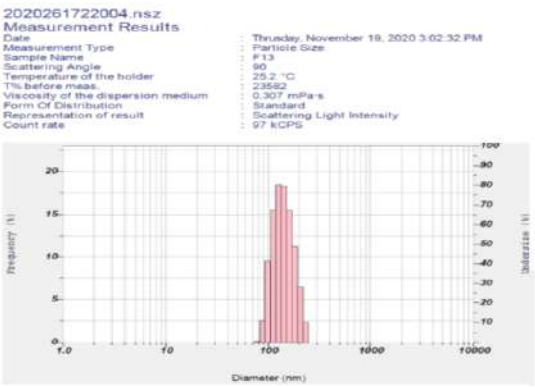

Figure 15. Particle size of optimized nanoparticles

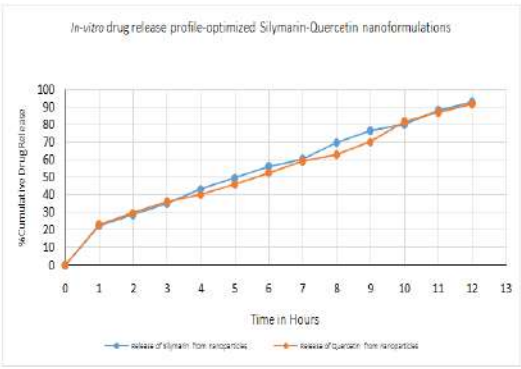

Figure 16. *In-vitro* drug release profile-optimized formulation

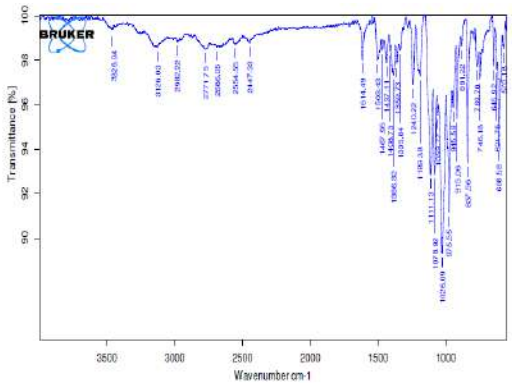

Figure 17. FT-IR Spectrum of optimized formulation

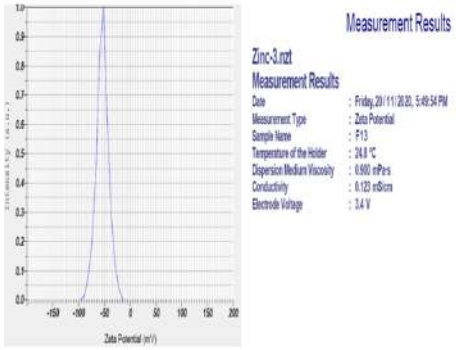

Figure 18. Zeta potential of optimized formulation

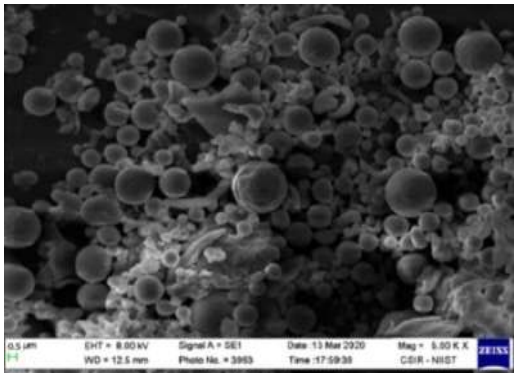

Figure 19. Scanning electron microscopic photographs of optimized formulation

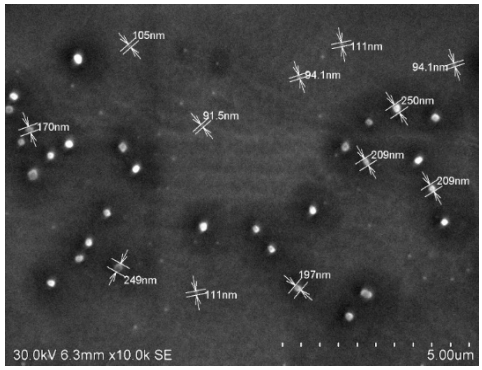

Figure 20. Scanning electron microscopic photographs of optimized formulation with dimensions

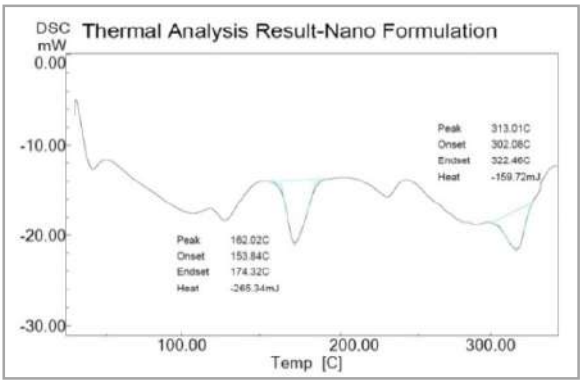

Figure 21. DSC thermo gram of Optimized formulation

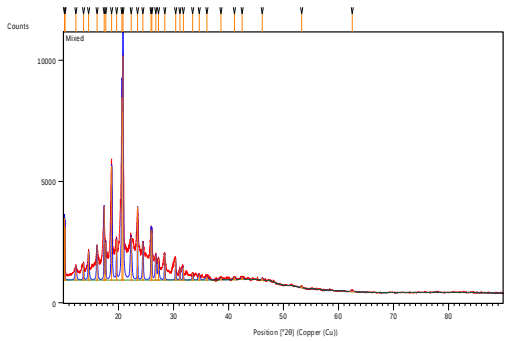

Figure 22. X-Ray Diffract gram of Optimized formulation

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RESEARCH ARTICLE

Early Prediction of Breast Cancer using Machine Learning Classifiers with WBCD

A.Malarvizhi[1]* and A.Nagappan[2]

[1]Research Scholar and Assistant Professor of Electronics and Communication Engineering, Vinayaka Mission's Kirupananda Variyar Engineering College, Vinayaka Mission's Research Foundation (Deemed to be University), Salem, Tamil Nadu, India.
[2]Director-Innovation, Incubation and Entrepreneurship, Vinayaka Mission's Research Foundation (Deemed to be University), Salem, Tamil Nadu, India.

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***Address for Correspondence**
A.Malarvizhi,
Research Scholar and Assistant Professor of Electronics and Communication Engineering,
Vinayaka Mission's Kirupananda Variyar Engineering College,
Vinayaka Mission's Research Foundation (Deemed to be University),
Salem, Tamil Nadu, India.

ABSTRACT

Breast cancer is a major public health concern confirms more than 2 million women diagnosed each year reflecting the vast majority of newly diagnosed cancer cases and related deaths. However, In its early stage, there is a chance of recovery rate is high. In this early stage of detection and diagnosis of breast cancer, Mammography is one of the most reliable techniques and reduces the death rate. The Wisconsin Breast Cancer Dataset contains hundreds of samples and features and it has been used for cancer research. This research paper aims by developing a simple machine learning model in breast cancer diagnosis and prognosis to review python and machine learning (ML) algorithms. From this Wisconsin Breast cancer data set, ML also can detects critical features. Data set is loaded first and fed to various classifiers like Stochastic Gradient Decent Support Vector Machine (SVM), K-Nearest Neighbours, Naïve Bayes, Forest and Tree methods. All classifiers accuracy are evaluated and time taken to build the model. Forest and Tree methods yields the deep predictions and obtains the best model yielding high and accurate results. Other Methods obtained less accuracy in comparison with Random Forest.

Keywords: Breast Cancer, Classifiers, Machine Learning, Python, Wisconsin Breast Cancer Data Set.

INTRODUCTION

When healthy cells in the breast change and grow out of control, they form a tumor, which is a mass or sheet of cells. Tumors can be malignant or noncancerous. A malignant tumor is one that has the potential to grow and spread to other regions of the body. The term "benign tumor" refers to a tumor that can develop but not spread. When breast cancer invades nearby organs or other parts of the body, it spreads. The term "benign tumor" refers to a tumor that can grow but not spread. Breast cancer spreads when it invades adjacent organs or other parts of the body, or when breast cancer cells travel through blood vessels and lymph vessels to other parts of the body. This is referred to as a metastasis. Breast cancer kills the most women between the ages of 40 and 55, and it is the second leading cause of death among Women [1].The mortality rate has decreased significantly as a result of increased emphasis on diagnostic techniques and effective treatment [2]. A lump or thickening compared to surrounding breast tissue, changes in breast size, shape, or appearance, changes in skin such as dimpling, appearance of inverted nipple, redness on skin over breast, or peeling, scaling, crusting of the pigmented area around areola or breast skin are all signs and symptoms of breast cancer [3]. Medical diagnosis is gradually increasing the use of a classifier system, taking symptoms into account. Without a doubt, evaluating a patient's dataset by an expert decision is important, but systems and artificial intelligence techniques improve diagnosis to a greater extent.

It will not only reduce the risk of error, but it will also examine the medical report thoroughly in a short amount of time. Machine learning techniques have been widely used in healthcare systems for decades. With the introduction of new technologies, it has become easier to obtain and store large amounts of data, such as electronic patient records [4]. It is impossible to handle and analyse complex data sets without the aid of technology, especially in complex data interrogation. The technology-driven healthcare system is a valuable asset. It aids professionals in accurately diagnosing patients and providing more meaningful benchmarks. Machine learning is now handling some complex manual work in the health industry, such as text and voice recognition for health professional responses [5-6].Several data mining and machine learning techniques for breast cancer detection and classification have been developed and tested over the last few decades [7-9]. It can be divided into three stages viz. preprocessing, feature extraction and, classification. Preprocessing of mammography films helps to improve visibility and intensity distribution of peripheral region. There are several methods to assist preprocessing. Feature extraction is next stage in detection of breast cancer as it helps in differentiating benign tumor from malignant. After that, segmentation is used to extract image properties of the breast, such as smoothness, depth, regularity, and coarseness [10].

The stage of classification is a complex optimization problem, and researchers have used a variety of machine learning techniques to solve classification problems because the veracity of machine learning technology is promising. Machine learning is becoming increasingly important in today's world, and it will eventually be integrated into services. Machine learning, on the other hand, is a field that is still unexplored in many ways, with many barriers and often requiring expert knowledge. The following sections will provide a literature review as well as a detailed explanation of the methodology used to detect breast cancer using Python. Variable data quality results affect the classification result when looking for the best and most accurate algorithm classification result. As a result, a benchmark dataset of Wisconsin breast cancer diagnosis (WBCD) is used in the application [11-12] to test the machine learning technique in a dataset. There are also other breast cancer benchmark datasets [13],such as Wisconsin Breast Cancer (Diagnostics) (WBCD) [14], which is used in this paper. The use of machine learning algorithms to detect cancer in humans is explained in this paper.

Literature Review

The authors conducted a thorough literature study to examine state-of-the-art machine learning algorithms for prediction and classification. Many research on breast cancer datasets have been conducted, and the majority of them have sufficient classification accuracy [15,16]. To categorise a Wisconsin breast cancer dataset, Aruna *et al* [16] employed nave Bayes, support vector machine, and decision trees, with the best result coming from the support vector machine (SVM) with an accuracy score of 96.99 percent. [17] Using a Wisconsin breast cancer dataset with

naive Bayes, SVM, neural networks, and decision tree approaches, Chaurasia *et al* compared the performance of supervised learning classifiers. The study found that SVM produced the highest accurate findings, with a score of 96.84 percent. Asri *et al.* [18] examined the same data and compared the performance of SVM, decision tree (c4.5), nave Bayes, and k-nearest Neighbours machine learning methods. The study compared the accuracy, precision, sensitivity, and specificity of each algorithm in order to categorise data in terms of efficiency and effectiveness. The experimental results revealed that SVM had the best score, with a 97.13 percent accuracy. With 202,932 patient records, Delen *et al.* [19] investigated the prediction of breast cancer data. The dataset was separated into two groups: those who survived (93,273) and those who did not (109,659), and then the nave Bays, neural network, and c4.5 decision tree methods were used. The results revealed that the c4.5 decision tree performed better than the other strategies. To find the best results for classifying the Diabetic illness dataset, Ou *et al.* [20] compared nave Bayes, decision trees, and random trees.

With a score of 76.3 percent, naive Bayes was determined to be the best classifier in this investigation. Srinivas *et al.* [21] used medical data like as age, sex, blood pressure, and blood sugar to train a dependency augmented nave Bayes classifier and a nave creedal classifier to predict heart attacks. According to the findings, nave Bayes produced better outcomes. Clinical data from medical intensive care units was used by Bernal *et al.* [22]. Logistic regression, neural networks, decision trees, and k-nearest neighbours were used to estimate the decrease in patients inside the hospital over the course of 24 hours. Among the training data, logistic regression and the k-nearest neighbour (KNN)-5 methodology yielded the highest accuracy ratings. Bernal [22] pointed out that in order to improve accuracy, parameters must be chosen rather than the algorithm. Wang *et al*. [23] used data mining algorithms on multiple records to discover the best technique to predict breast cancer. Support vector machine (SVM), artificial neural network (ANN), nave Bays classifier, and AdaBoost Tree were all used. The goal of reducing the feature space was addressed, and then Principle Component Analysis (PCA) was used to achieve that goal. They employed two datasets for the evaluation of the models' performance: the Wisconsin Breast Cancer Database (1991) and Wisconsin Diagnostic Breast Cancer (1995) [24].They gave a thorough analysis of the models and test errors. Williams *et al*. [25] used data mining classification algorithms to conduct research on breast cancer risk prediction. Breast cancer is the most prevalent cancer kind among Nigerian women. To detect breast cancer earlier, few services are available. As a result, they needed to find a reliable approach to predict breast cancer.

The J48 decision trees and nave Bays were two data mining approaches used in their investigation. The biggest challenge with breast cancer, according to Nithya *et al.* [26], is categorising the breast tumour. Breast cancer has been detected and characterised using computer-assisted diagnosis (CAD).Their main goal was to use data mining techniques to improve breast cancer prediction. Bagging, multiboot, random subspace, support vector machine-sequential minimal optimization (SVM-SMO), and multilayer perceptron were used to improve the classification performance of nave Bays. Breast cancer biopsy predictions with a mammographic diagnosis were investigated by Oyewola *et al*. [27]. They used classifications such as logistic regression (LR), linear discriminant analysis (LDA), quadratic discriminant analysis (QDA), random forest (FR), and support vector machine (SVM) in their research.SVM, LR, multilayer perceptron, KNN, softmax regression, SVM, and Gated Recurrent Unit (GRU) SVM techniques were employed by Agarap [28].The most reliable result was obtained from a multilayer perceptron with an accuracy score of 99.4 percent .Westerdijk [29] studied several machine learning techniques for the prediction of breast cancer cells. She tested the performance of the models by looking at their accuracies, sensitivities, and specificities. Compared accuracy scores of LR, random forest, SVM, neural network, and ensemble models .

Breast cancer prediction should be improved with the accuracy score. Vard *et al.* [30] investigated a reliable method for predicting eight types of cancer, including breast cancer, lung cancer, and ovarian cancer. They used Particle Swarm Optimization to normalise datasets and statistical feature selection methods to separate features on a normalised dataset in their research. Kourou *et al.* [31] investigated the classification of cancer patients' risk groups as low or high. To present a model for cancer risks or patient outcomes, ANN, Bayesian networks (BNs), SVM, and decision tree (DT) techniques were used. According to Pratiwi [32], breast cancer is the leading cause of death in women. To diagnose breast cancer, machine learning techniques were preferred. Pratiwi used Java to create

intelligent breast cancer prediction, demonstrating that all functionalities worked properly and quickly. Shukla *et al*. [33] investigated a robust data analysis model that could be applied to breast cancer datasets. The survivability of patients and tumours was factored into the model. They identified using the Surveillance, Epidemiology, and End Results (SEER) programme, and clustered using the Self-Organizing Map (SOM) and Density-Based Spatial Clustering of Applications with Noise (DBSCAN).

Machine Learning- Breast Cancer Diagnostic Data Set

The methodology seeks to assess the most beneficial feature in prediction of malignant and benign tumor. This may help to visualise general trend in selecting appropriate model. With the use of python, the goal is to distinguish benign and aggressive breast cancer tumors. The use of Stochastic Gradient Decent (SGD), Support Vector Machine (SVM), K-Nearest Neighbours (KNN), and Nave Bayes (Nave Bayes) Forest and Tree methods (RF,DT) is highlighted.

SVM Learning with Stochastic Gradient Descent (SGD) Optimization

The support vector machine classification technique with hinge loss function was implemented using SGD. One of the most common optimization methods used in deep learning and machine learning techniques is gradient descent. Instead of picking batch data as in batch gradient descent, stochastic gradient descent [34] selects random samples from a dataset. The training data set is randomly shuffled via stochastic gradient descent optimization. It calculates gradient and updates weights.

Support Vector Machine

SVM stands for Simple Vector Machine. For classification and regression, it is a Supervised Machine Learning algorithm. The data set is presented as an n-dimensional graph in the SVM method (where n is no. of instances in dataset).It clearly distinguishes and contrasts various aspects in the hyperplane. It's utilised in handwriting digital recognition, picture recognition, and face recognition, Bioinformatics among other things.

Naïve Bayes

Based on Bayes Theorem, Navies Bayes is a statically and probabilistically classifier. Each attribute's characteristic operates independently of the others. It's a classification method that was created to categorise high-dimensional datasets. The probability of an event is computed as follows.

$$P(y|X) = \frac{P(X|y)P(y)}{P(X)}$$

K-Nearest Neighbor Classification

KNN [35] maintains all of the training data and uses a similarity measure to classify the query data. The number of nearest Neighbours to be included in the voting procedures is referred to as k in KNNs. Feature similarity is used by KNNs.KNN parameter tuning is done by selecting a suitable value of k to improve performance.

Random Decision Forest

The bootstrapping algorithm with decision tree (CART) model is comparable to the random decision forest. By selecting a random subset S of training samples, the random decision forest tries to create k distinct decision trees [37].It produces the entire Iterative Dichotomiser 3 (ID3) [38] tree without any pruning. The mean of each prediction is used to produce a final prediction. Random decision trees can easily interpret and deal with irrelevant attributes. They're small and can deal with lacking info.

Random Decision Tree

One of the most often used supervised machine learning algorithms for graphical depiction of all possible answers is the random decision tree [36]. The decisions are simple to understand and are based on certain conditions. It detects and selects the most important characteristics that aid classification**.**

METHODOLOGY

The screening mammography goal is to find recognised proof [3] of breast malignant development at earlier stages. Despite the existence of screening programmes all over the world, the high rate of false positives and false negatives has an impact on mammography interpretation. This research proposes an artificial intelligence (AI) framework capable of surpassing human experts in the prediction of breast cancer progression. Artificial intelligence algorithms of many forms have lately been introduced. The most prevalent methods are classified as supervised learning. To develop a novel model for predicting breast cancer at an earlier stage in the future, a vast data store of patient records with symptoms and diagnoses in hospitals and clinics will be maintained. Using vast data bases, researchers can construct more categorization models, which will enhance accuracy. In comparison to prior methods, machine learning technologies make finding accuracy a straight forward operation.

Dataset

This work makes use of the Wisconsin Breast Cancer Diagnostics (WBCD) dataset from the UCI Machine Learning Repository [14].It was invented by Dr. William H. Wolberg of the University of Wisconsin Hospital in Madison, Wisconsin, USA. The data contains 569 patients with 32 different characteristics. These attributes were divided into 32 columns in the dataset. Table 1. The attribute informations are

RESULTS AND DISCUSSION

The WBCD dataset, which is freely available, was examined (it can be downloaded from the UCI repository [42]).We'll load the data collection and print some basic information using Pandas. We can count how many diagnoses are malignant (M) and how many are benign (B) after loading the data set in Python (B).There are 569 diagnoses in the data, 357 of which are malignant and 212 of which are benign. Breast cancer is predicted using AI-ML algorithms. When compared to other classifiers, Random Forest and Decision Trees classifiers accurately predict 569 breast cancer cases.

CONCLUSION

In this paper, it was discovered that even with a smaller number of characteristics, equivalent accuracy can be reached for breast cancer prediction. Only the features selected by a certain feature selection methodology will be used as input for the machine learning classifier, reducing the model's computational complexity. As a result, a future study will look at whether the number of features to choose is affected by characteristics such as data set, standard deviation, correlation, and so on. Furthermore, as a future step, it is proposed to compare and demonstrate the effectiveness of presented algorithms using hybrid machine learning classifiers based on deep learning and extreme learning classifiers. Furthermore, it was suggested that for feature reductions and smooth cancer identification from biological databases, a nature-inspired algorithm be used.

REFERENCES

1. http:// www.imaginis.com/breasthealth/breast_cancer.asp, Last Accessed August 2007.
2. West D, R., & West V et.al. (2005). Ensemble strategies for a medical diagnosis decision support system: A breast cancer diagnosis application. European Journal of Operational Research(162), 532 551
3. https://www.mayoclinic.org/diseases-conditions/breast-cancer/symptoms-causes/syc- 20352470
4. Pavlopoulos.et.al, A.N. Designing and implementing the transition to a fully digital hospital. IEEE Trans. Inf. Technol. Biomed. 1999, 3, 6 19.
5. Barracliffe *et.al*, A pilot study of breast cancer

Patients: Can machine learning predict healthcare professionals responses to patient emotions? In Proceedings of the International Conference on Bioinformatics and Computational Biology, March 2017;pp.101-106.

6. Birkett *et al.,* Towards objective and reproducible study of patient –doctor interaction: Automatic text analysis based VR-CODES annotation of Consultation transcripts. In proceedings of the IEEE Engineering in Medice and Biology Soceity Conference,11-15 July2017; pp.2638-2641.
7. A.J.Cruz *et.al.,* Applications of machine learning in cancer prediction and prognosis, Cancer Informatics,vol.2.pp59-77,2006.
8. Valvano *et.al.,* Convolutional neural networks for the segmentation of micro calcification in mammography imaging, Journal of Healthcare Engineering 2019.
9. Akay et.al., Support vector machines combined with feature election for breast cancer diagnosis, Expert systems with Applications,vol.36,3240-3247,2009.
10. Salembier *et.al.,* Binary partition tree as an efficient representation for image processing, segmentation and information retrival, IEEE Transactions on image processing, vol 9,561-576,2009.
11. Mangasarian *et.al.,* W.H. Pattern recognition via linear programming: Theory and application to medical diagnosis. In Large-Scale Numerical Optimization; SIAM: Philadelphia, PA, USA, 1990; pp. 22 31.
12. Wolberg et.al., Multisurface method of pattern separation for medical diagnosis applied to breast cytology. Proc. Natl. Acad. Sci. USA 1990, 87, 9193 9196.
13. Sharma et.al Machine learning approaches for breast cancer diagnosis and prognosis. In Proceedings of the International Conference on Soft Computing and Its Engineering Applications, Changa, India, 1 2 December 2017.
14. .https://www.kaggle.com/uciml/breast-cancer-wisconsin-data
15. Aalaei *et.al*., S. Feature selection using genetic algorithm for breast cancer. 16 Computational and Mathematical Methods in Medicine diagnosis: An experiment on three different datasets. Iran. J. Basic Med. Sci. 2016, 19, 476.
16. Aruna *et.al.,* Knowledge based analysis of various statistical tools in detecting breast cancer. Comput. Sci. Inf. Technol. 2011, 2, 37–45.
17. Chaurasia *et.al.,* Data mining techniques: To predict and resolve breast cancer survivability. Int. J. Comput. Sci. Mob. Comput. 2014, 3, 10–22.
18. Asri *et.al.,* Using machine learning algorithms for breast cancer risk prediction and diagnosis. Procedia Comput. Sci. 2016, 83, 1064–1069.
19. Delen *et.al.,* Predicting breast cancer survivability: A comparison of three data mining methods. Artif. Intell. Med. 2005, 34, 113–127.
20. Qu, Z *et.al.,* Predicting diabetes mellitus with machine learning techniques. Front. Genet. 2011, 9, 515.
21. Srinivas, K. Analysis of coronary heart disease and prediction of heart attack in coal mining regions using data mining techniques. In Proceedings of the 5th International Conference on Computer Science & Education, Hefei, China, 24–27 August 2010; pp. 1344–1349.
22. Bernal, J.L.; Cummins, S.; Gasparrini, A. Interrupted time series regression for the evaluation of public health interventions: A tutorial. Int. J. Epidemiol. 2017, 46, 348–355.
23. Wang *et.al.,* Breast cancer prediction using data mining method. In Proceedings of the 2015 Industrial and Systems Engineering Research Conference, Nashville, TN, USA, 30 May–2 June 2015.
24. Wolberg *et.al.,* Wisconsin Breast Cancer Database; University of Wisconsin Hospitals: Madison, WI, USA, 1991.
25. Williams *et.al.,* Risk prediction models for colorectal cancer in people with symptoms: A systematic review. BMC Gastroenterol. 2016, 16, 63.
26. Nithya *et.al.,* Classification of normal and abnormal patterns in digital mammograms for diagnosis of breast cancer. Int. J. Comput. Appl. 2011, 28, 0975–8887.
27. Oyewola *et.al.,* M. Using five machine learning for breast cancer biopsy predictions based on mammographic diagnosis. Int. J. Eng. Technol. IJET 2017, 2, 142–145.
28. Agarap, A.F.M. On breast cancer detection: An application of machine learning algorithms on the wisconsin diagnostic dataset. In Proceedings of the 2nd International Conference on Machine Learning and Soft Computing, Phuoc Island, Vietnam, 2–4 February 2018; pp. 5–9.
29. Westerdijk, L. Predicting Malignant Tumor Cells in Breasts; Master Business Analytics; Vrije Universiteit Amsterdam: Amsterdam, The Netherlands, 2018.

30. Vard *et.al.,* An optimized framework for cancer prediction using immunosignature. J. Med. Signals Sens. 2018, 8, 161.
31. Kourou *et.al.,* Machine learning applications in cancer prognosis and prediction. Comput. Struct. Biotechnol. J. 2015, 13, 8–17.
32. Pratiwi, P.S. Development of intelligent breast cancer prediction using extreme learning machine in Java. Int. J. Comput. Commun. Instrum. Eng. 2016, 3.
33. Shukla *et.al.,* Breast cancer data analysis for survivability studies and prediction. Comput. Methods Programs Biomed. 2018, 155, 199–208.
34. Bottou, L. Large-scale machine learning with stochastic gradient descent. In *Proceedings of COMPSTAT'2010*; Springer: Berlin/Heidelberg, Germany, 2010; pp. 177–186.
35. Nazir, S.; Yousaf, M.H.; Velastin, S.A. Evaluating a bag-of-visual features approach using spatio-temporal features for action recognition. *Comput. Electr. Eng.* 2018, *72*, 660–669.
36. Rokach, L. Decision forest: Twenty years of research. *Inf. Fusion* 2016, *27*, 111–125.
37. Daho, M.E.H.; Chikh, M.A. Combining bootstrapping samples, random subspaces and random forests to build classifiers. *J. Med. Imaging Health Inform.* 2015, *5*, 539–544.
38. Khedr, A.E.; Idrees, A.M.; El Seddawy, A.I. Enhancing Iterative Dichotomiser 3 algorithm for classification decision tree. *Wiley Interdiscip. Rev. Data Min. Knowl. Discov.* 2016, *6*, 70–79.

Table 1. The attribute informations are

NO	No of Attributes
1	ID number
2	Diagnosis (M = malignant, B = benign)
3	32 Ten real-valued features are computed for each cell nucleus
a) radius (mean of distances from center to points on the perimeter)	
b) texture (standard deviation of gray-scale values`)	
c) perimeter	
d) area	
e) smoothness (local variation in radius lengths)	
f) compactness (perimeter^2 / area - 1.0)	
g) concavity (severity of concave portions of the contour)	
h) concave points (number of concave portions of the contour)	
i) symmetry	
j) fractal dimension ("coastline approximation" - 1	
The mean, standard error and "worst" or largest (mean of the three largest values) of these features were computed for each image, resulting in 30 features. For instance, field 3 is Mean Radius, field 13 is Radius SE, field 23 is Worst Radius.	

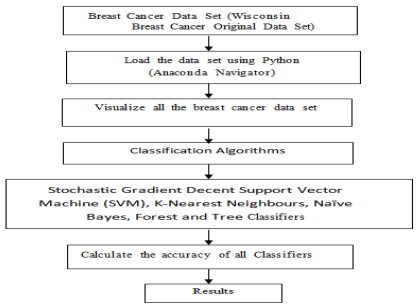
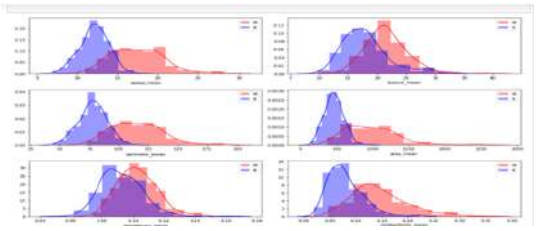


Figure 1.Methodology of breast cancer data classification

	id	diagnosis	radius_mean	texture_mean	perimeter_mean	area_mean	smoothness_mean	compactness_mean	con
0	842302	M	17.99	10.38	122.8	1001.0	0.11840	0.27760	
1	842517	M	20.57	17.77	132.9	1326.0	0.08474	0.07864	
2	84300903	M	19.69	21.25	130.0	1203.0	0.10960	0.15990	

3 rows × 33 columns

Figure 2.First three rows of loaded data



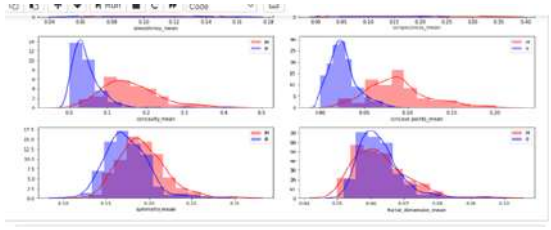


Figure 3. Shows the Converting the diagnosis value of M and B to a numerical value where M (Malignant) = 1 and B (Benign) = 0

	accuracy_all	accuracy_selection	diff_accuracy	cvs_all	cvs_selection	diff_cvs
SGD	0.763158	0.701754	-0.061404	0.785732	0.834731	0.048999
SVC	0.921053	0.921053	0.000000	0.885841	0.885841	0.000000
NuSVC	0.921053	0.921053	0.000000	0.852445	0.854200	0.001754
LinearSVC	0.649123	0.842105	0.192982	0.843689	0.747151	-0.096538
KNeighbors	0.938596	0.921053	-0.017544	0.885779	0.882270	-0.003509
GaussianNB	0.947368	0.947368	0.000000	0.913895	0.908663	-0.005232
RandomForest	0.956140	0.938596	-0.017544	0.940258	0.919190	-0.021068
ExtraTrees	0.956140	0.938596	-0.017544	0.940242	0.919190	-0.021053
DecisionTree	0.947368	0.938596	-0.008772	0.913911	0.910356	-0.003555

Figure 4. Accuracy of Stochastic Gradient Decent(SGD), Support Vector Machine (SVM),K-Nearest Neighbours (KNN), Naïve Bayes (NB), Forest and Tree methods (RF,DT) Classifiers in Jupiter notebook Using Python

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RESEARCH ARTICLE

Evaluation of Dose Deposited Inside Patient Body Other than PTV using Different IMRT Beams in the Treatment Plan of Brain, Head and Neck, and Pelvic Cases

Bhavya Shree P[1] , Shanmukhappa B. Kaginelli[2]* and Rajesh. R[3]

[1]Assistant Professor, Division of Medical Physics, School of Life Sciences, JSS AHER, Mysore, Karnataka, India

[2]Associate Professor, Division of Medical Physics School of Life Sciences, JSS AHER, Mysore, Karnataka, India

[3]Sr. Medical Physicist, Narayana Multispecialty, Hospital, Mysore, Karnataka, India

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***Address for Correspondence**
Shanmukhappa B. Kaginelli
Associate Professor,
Division of Medical Physics School of Life Sciences,
JSS AHER, Mysore, Karnataka, India
Email: drsbkaginelli@jssuni.edu.in

ABSTRACT

The goal of the study is to identify, how accurately we are using treatment planning system for the treatment of patient tumor volume while minimizing dose to the surrounding healthy tissues. Also, we come across the evaluation of dose deposition inside patient body other than PTV. In case of Intensity modulated radiation therapy (IMRT) using treatment planning system (TPS) according to tumor sites, fields or beams are selected to get minimum dose to nearby normal tissues. For analyzing the TPS plan in DVH with respect to Patient body dose deposited and other volumes dose deposited, we considered 9 patients as total in which 3patients for each of brain, head and neck & pelvis cases. We can understand the difference in dose deposition for different fields of 3 cases. The data are taken through the treatment planning system by analyzing and evaluating the DVH of each patient[10].

Keywords: Linear accelerating machine (Elekta), TPS, PTV, CT, MV EPID imaging, Tumor and Iso-Centre

INTRODUCTION

Cancer is known as the deadliest disease and is characterized by the growth of aberrant cells that divide uncontrolled, have the capacity to invade healthy bodily tissue, and cause tissue destruction.[11] Changes (mutations) to the DNA within cells are what lead to cancer. A cell's DNA is organized into numerous distinct genes,

each of which carries a set of instructions directing the cell performance of certain tasks as well as its growth and division. Incorrect instructions can make a cell cease functioning normally and even give it the chance to develop cancer. Radiation therapy is a highly targeted medical procedure that hits the cancer wherever it may be in the body with pinpoint accuracy. As a result, the majority of the body other organs and tissues are protected while the cancer cells are eliminated or reduced in number. Radiation therapy relieves symptoms like pain and enhances the quality of life for many people, accounting for 40% of all cancer cures worldwide.

Radiation therapy

Radiation therapy damages cancer cells' DNA, which either kills or stunts cancer cells' growth when administered in high quantities. Cancer cells that can no longer divide due to DNA damage do so or pass away. The body breaks down and expels the damaged cells once they pass away. Radiation treatment affects malignancies differently depending on the type of malignancy. A cancer's radio-sensitivity explains how it reacts to radiation[8].Modest radiation dosages quickly kill cancer cells that are highly radio-sensitive. High-energy particles or waves, such as x-rays, gamma rays, electron beams, or protons, are used in radiation therapy to kill or harm cancer cells. Radiation therapy is often a local treatment, in contrast to chemotherapy and other cancer-fighting medications that are taken orally or administered intravenously and typically expose the entire body [14].This indicates that it is often directed towards and only has an impact on the affected bodily area. Radiation therapies are designed to kill cancer cells while causing the least amount of harm to neighbouring healthy cells. A malignant tumour can be reduced in size or completely removed by radiation therapy, which will prevent or decrease the growth of the tumour.

IMRT (Intensity Modulated Radiation Therapy)

IMRT is defined as a dose delivery by treatment plans that are optimized using technique of forward (or) inverse planning for treatment delivery with modulated beams using collimators (or) MLC shaping by modes of step/shoot (static multi-leaf collimator-SMLC) or sliding window (dynamic multi-leaf collimator- DMLC). Techniques of inverse planning included signed compensators for tissue irregularity & missing tissues[07].Inverse planning for treatment delivery with modulated beams employing collimators, MLC shaping by modes of step/shoot (static multi-leaf collimator, SMLC), or sliding window optimization techniques are used to give dose in IMRT (dynamic multi-leaf collimator - DMLC). Inverse planning methods included signed compensators for uneven tissue and missing tissues.

Treatment planning system used in IMRT planning

Treatment planning involves a number of steps, such as diagnosing the patient, tumour staging, image acquisition for treatment planning, the localization of tumour and healthy tissue volumes, the placement of the best beam, and simulation and optimization of the treatment. In order to maximize tumour control and minimize consequences to normal tissue, external beam radiation therapy uses computerized treatment planning systems (TPS) to determine beam shapes and dose distributions. Prior to the 1970s, treatment planning was typically done by manually manipulating standard isodose charts onto patient body contours that were produced by direct tracing or lead-wire representation, and it heavily depended on an experienced dosimetrist's careful selection of the beam weight and wedging[10].The simultaneous development of computerised tomography and the availability of affordable computing power starting in the 1970s gave rise to CT-based computerised treatment planning, which allowed users to see dose distributions superimposed directly onto patients' axial anatomy. Modern TPS can now display three-dimensional representations of human anatomy, tumour targets, and even dosage distributions. The visuals, calculation, and optimization features of modern systems have seen the most substantial developments in treatment planning hardware and software throughout time [13].

Integral dose

Energy is absorbed when a photon beam penetrates a patient, not just in the tumour but also along the beam's path and from scattered radiation that enters healthy tissues. The term imparted energy or integral dose refers to the entire energy that the patient has absorbed. The perfect beam theoretically would only provide a focused dose to the tumour, doing no harm to surrounding tissue. An alternative optimization strategy would be to deliver a focused dose to the tumour volume while minimising the integral dose. The energy absorbed by a mass is only the sum of the

dose and the mass of the medium, according to the definition of dose (J/kg)..[14]Due to the fact that the dose varies with depth, the total energy is just its integration over the depth as$\Sigma = \int D\,x.\,P.\,A.\,d\,x$. Where D x is the dose at depth, ϱ is the density of tissue, A is the area of the beam and dx is the depth having adose of D x. For a single beam, the variation of dose as a function of depth can be approximated by theexponential term as

$D\,x = DO\,e^{-\mu X}$

Substituting the function into equation and integrate, we have

$\Sigma = D\,o\,A\,\varrho/\,\mu(1 - e^{-\mu d})$

Where d is the patient thickness. If d1/2 represents the depth of the 50% isodose surface, linear attenuation coefficient is related to the half-value thickness as μ=0.693/d1/2, we have

$\Sigma = 1.44\,DO.A.\,\varrho\,(1 - e^{-.693d/d1/2})$

The above expression does not take into account the geometric spread of thebeam. If this effect is taken into account the expression for the total energy imparted or integral dose is

$\Sigma = \int D\,x.\,P.\,A.\,d\,x$

Where D x is the dose at depth, ϱ is the density of tissue, A is the area of the beam and dx is the depth having adose of D x. For a single beam, the variation of dose as a function of depth can be approximated by theexponential term as

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$\Sigma = 1.44\,DO.A.\,\varrho\,(1 - e^{-.693d/d1/2})$

The above expression does not take into account the geometric spread of thebeam. If this effect is taken into account the expression for the total energy imparted or integral dose is

$\Sigma = 1.44\,DO.A.\,\varrho.\,(1 - e^{-.693d/d1/2})\,(1 + 2.88d1/2/\,SSD)$

Where SSD is the source-to-skin distance. The above equation can be simplified by assuming that d >>d1/2, i.e., the patientthickness d is much larger than the half-value thickness of the beam. Under such condition as with low energy beam, theexponential term approaches zero,

$\Sigma = 1.44\,DO.A.\,\varrho.\,(1 + 2.88d1/2/\,SSD)$

The concept of integral dose can be used to evaluate different plans of treatment. An optimal plan reflects maximum dosedelivery to the tumor and minimal irradiation of normal tissue. A means of quantifying a plan is to define a figure of meritfor evaluation as: F=energy imparted to target volume/total energy imparted to patient.

Recurrent Tumors

A cancer recurrence occurs when some cancer cells persist despite greatest efforts to eradicate them; these cells may proliferate and cause symptoms in the area where the cancer first appeared or they may spread to another area of the body[01]. Regional recurrence denotes that the tumour has spread to nearby lymph nodes or tissues where the cancer first manifested itself. The term "distant recurrence" refers to the tumour spreading to tissues and organs that are far from the original area. It is known as metastasis or metastatic cancer when the tumour or cancer cells invade other body parts or important organs and spread to a distant location [01].

MATERIALS AND METHODS

Intensity-modulated radiation therapy (IMRT) is an advanced mode of high-precision radiotherapy that uses computer-controlled linear accelerators to deliver precise radiation doses to a malignant tumor or specific areas within the tumor. IMRT is provided in two treatment phases: planning and delivery. Certain services should not be billed when they are performed as part of developing an IMRT plan. IMRT has a potential to minimize doses to surrounding normal or critical structures and can be safely delivered with a minimum risk of side effects. However, both fraction time and exposure of normal tissue to low doses are significantly higher for IMRT than in conventional radiotherapy. Precise dose adjustment to the tumor geometry is usually achieved with combinations of several intensity-modulated fields distributed among different beam directions. Dose distribution calculation and optimization processes are highly computerized. Typically, IMRT dose maps are inversely planned using dose and volume constraints, as well as priority factors for each structures which define acceptable and penalized dose ranges.

Treatment Planning System Used for study:

CMS Xio (form – 4.71) Elekta 's xio gives a vigorous arranging framework to molecule treatment medicines. for precision plans and smooth work processes. Xio extensive arranging work process apparatuses give quick contouring, combination, virtual recreation, arranging and survey instruments in one. With 2D, 3D and IMRT ability, xio can deal with photon and electron arranging utilizing numerous calculations. Xio joined with MOSAIQ offers both an incorporated oncology data framework and devoted treatment arranging programming for molecule treatment with a solitary patient record.

Patient Selection

In the present study, 9 patients were selected out of which 03 are with Pelvis cases (involving Ca cervix, Ca Rectum, Ca Prostate, Ca Bladder and Ca Anal canal), 03 with Head and Neck case (Ca tongue, Ca hard pallet, Ca soft pallet, Ca Nasopharynx, Ca tonsils) and 03 with Brain case (all cases of brain tumors).

Planning Criteria:

Brain Site.

3 patients are selected for brain cases with planned fields of 5 ,6 & 8 beams:

- Patient A , Prescribed dose – 60Gy
- Patient B , Prescribed dose – 60Gy
- Patient C , Prescribed dose – 60Gy

Dose constraints : 95% of PTV should get 95% of prescribed dose for PTV -60

- Eyes (right & left)- maximum dose less than 5000cGy
- Optic nerves(right & left)-maximum dose less than 5400cGy
- Eye lens(right & left)- maximum dose less than 2500cGy
- Brainstem(right & left)- maximum dose less than 5400cGy
- Chiasma (right & left)- maximum dose less than 540cGy

Head and Neck Site

3 patients are selected for head & neck cases with planned fields of 5 ,7 & 9 beams:

- Patient A , Prescribed dose – 70Gy,63GY,56Gy
- Patient B , Prescribed dose – 70Gy,63GY
- Patient C , Prescribed dose – 70Gy ,56Gy

Dose constraints : 95% of PTV should get 95% of prescribed dose for both PTV -60 & PTV -50

- Spine-maximum dose less than 4700Gy.
- Brainstem- maximum dose less than 5400Gy.
- Parotid -50% volume maximum dose less than 3000Gy.
- Mandible- maximum dose less than 7000Gy.

Pelvis Site

3 patients are selected for pelvis cases with planned fields of 5 ,7 & 9 beams:

- Patient A , Prescribed dose – 55.8Gy,50Gy
- Patient B , Prescribed dose – 55.8Gy,50Gy
- Patient C , Prescribed dose – 55.8Gy,50Gy

Dose constraints : 95% of PTV should get 95% of prescribed dose for both PTV -60 & PTV -50

- Rectum-50% volume should get less than 50Gy
- Bladder-50% volume should get less than 65Gy
- Femoral head-40% volume should get less than 40Gy
- Bowel-200cc volume should get less than 45Gy

Data Analysis Method

Dose Volume Histogram is an effective tool which is being employed to evaluate each plans nature. After dose calculation of desired beam angle and desired optimization parameters, isodose distribution is generated along with DVH graph. Isodose line tells us the percentage of dose been deposited at various depth which will parallelly gives the information of the volume covering the area with that dose. Also DVH graph is plotted by the system which gives more additional information for each organs. From there we evaluate the dose been received by the body or patient structure and other OARs.

RESULTS AND DISCUSSION

The role of IMRT in radiotherapy is to deliver high dose to tumor from different directions with beams of non-uniform fluencies. Conventional radiation therapy techniques employ uniform radiation beams to destroy tumor cells in the body of a patient. While tissues outside the radiation field can be protected by shaping the radiation beam to encompass the tumor, a column of tissue is irradiated indiscriminately from the beam's entrance to exit. Using the fact that not all tissues respond in same way to radiation, IMRT varies the intensity of the radiation across the region. Once the treatment plan is generated, the computer sends it for delivery of the treatment to the radiation machine. Here we consider 9 patients, in which 3 patients with brain cases, 3 with head and neck cases ,3 patients with pelvis cases. By evaluating their treatment plan in IMRT should understand dose deposition in body other than PTV for different number of beams. The table includes the data collected from DVH for different IMRT fields. Accuracy of dose delivery to tumor and deposition on PTV and body can be demonstrated using this table. The above tables reveal that the DVH data obtained by TPS planning calculation for pelvis, head & neck, and brain cases shows the patient dose contributed by the number of beam angles in IMRT planning. The obtained results can be inferred that due to increase beam number there is gradual increase in patient dose being deposited in the body apart from PTVs and OARs.

CONCLUSION

IMRT patient treatment planning is evaluated with interest of patient dose being deposited and being compared with that of the other structures and Volumes. The different beams considered for each case of brain, head & neck and pelvis cases showed the accuracy level of dose deposition on tumor as well as body. From DVH analysis and then by tables we could find out that by increasing the fields or beams number the precision of dose deposition towards the tumor is more and to PTV the volume is increasing. But simultaneously it increases the patient dose dumping. From this work we can state that the using beams for IMRT TPS planning need to be justified, only when site or the case warrants for more net benefit to the patient then it can be considered. This would be the situation for any tumor site being involved.

REFERENCES

1. Radiation-induced second cancers: the impact of 3D-CRT and IMRT. Eric J Hall, D.Sc. Cheng-Shie Wuu, Ph.D. International Journal of Radiation Oncology.Volume 56, Issue 1, P83-88, May 01, 2003.
2. Wu B, Ricchetti F, Sanguineti G, et al. Data-driven approach to generating achievable dose-volume histogram objectives in intensity-modulated radiotherapy planning. *Int J Radiat Oncol Biol Phys*. 2011; 79: 1241– 1247.
3. Yuan L, Ge Y, Lee WR, Yin FF, Kirkpatrick JP, Wu QJ. Quantitative analysis of the factors which affect the interpatient organ-at-risk dose sparing variation in IMRT plans. *Med Phys*. 2012; 39: 6868– 6878.
4. Fried DV, Chera BS, Das SK. Assessment of PlanIQ feasibility DVH for head and neck treatment planning. *J Appl Clin Med Phys*. 2017; 18: 245– 250.
5. Journal of Applied Clinical Medical physics. Evaluation of auto planning in IMRT and VMAT for Head & Neck Cancer 04 July 2019https://doi.org/10.1002/acm2.12652
6. Prescribing, recording, and reporting photon beam therapy. Bethesda, MD, USA: ICRU Publications; International Commission on Radiation Units and Measurements; 1993. ICRU Report 50.http://www.icru.org/home/reports/prescribing-recording-and-reporting-photon-beam-therapyreport-50.
7. International Commission of Radiation Units and Measurements. ICRU report 83: Prescribing, Recording, and Reporting Photon-Beam Intensity-Modulated Radiation Therapy (IMRT). J ICRU. 2010; 10(1):1–106. ISSN 1473-6691 http://jicru.oxfordjournals.org/content/10/1.toc.
8. Bentzen SM, Constine LS, Deasy JO, Eisbruch A, Jackson A, Marks LB, et al. Quantitative analyses of normal tissue effects in the clinic (quantec): an introduction to the scientific issues. Int J Radiat Oncol Biol Phys. 2010; 76(3):3–9.
9. Miften MM, Das SK, Su M, Marks LB. A dose-volume based tool for evaluating and ranking imrt treatment plans. J Appl Clin Med Phys. 2004; 5(4):1–14. http://www.ncbi.nlm.nih.gov/pubmed/15738916.
10. Pyakuryal A, Myint WK, Gopalakrishnan M, Jang S, Logemann JA, Mittal BB. A computational tool for the efficient analysis of dose-volume histograms for radiation therapy treatment plans. J Appl Clin Med Phys/American College of Medical Physics. 2010; 11(1):3013.
11. Niemierko A, Goitein M. Modeling of normal tissue response to radiation: the critical volume model. Int J Radiat Oncol Biol Phys. 1993; 25(1):135–45.
12. Kehwar T. Analytical approach to estimate normal tissue complication probability using best fit of normal tissue tolerance doses into the ntcp equation of the linear quadratic model. J Cancer Res Ther. 2005; 1(3):168–79. http://www.ncbi.nlm.nih.gov/pubmed/17998649.
13. Webb S. Intensity-Modulated Radiation Therapy, Series in Medical Physics and Biomedical Engineering. Bristol (UK): CRC Press; 2001. https://www.crcpress.com/Intensity-Modulated-Radiation-Therapy/Webb/9780750306997.
14. Alfonso J, Buttazzo G, García-Archilla B, Herrero M, Núñez L. Selecting radiotherapy dose distributions by means of constrained optimization problems. Bull Math Biol. 2014; 76(5):1017–44.
15. Lyman JT. Complication probability as assessed from dose-volume histograms. Radiat Res. 1985; 104(2s):13–19.

Table 1 : Dose deposition on body and PTV volume of 3 patients with different fields of brain cases

pat study	IMRT 5 FIELD					IMRT 6 FIELD					IMRT 8 FIELD				
	Prescribed	PTV		body		prescribed	PTV		body		prescribed	PTV		body	
		dose	%vol	dose	%vol		dose	%vol	dose	%vol		dose	%vol	dose	%vol
pat A	60Gy	6420 (107%)	0.7	4500	5.4	60Gy	6420(107%	0.06	4500	5.4	60Gy	6420(107%)	0.54	4500	6
		5700(95%)	98.1	3500	10.38		5700(95%)	97.7	3500	10.82		5700(95%)	97.7	3500	12
				2500	20.5				2500	20.36				2500	22
				1000	33.2				1000	34.87				1000	37
pat B	60Gy	6420(107%)	0.11	4500	6.23	60Gy	6420(107%)	0.5	4500	6.38	60Gy	6420(107%)	0.17	4500	6.5
		5700(95%)	98	3500	12.14		5700(95%)	98.3	3500	12.53		5700(95%)	98.1	3500	13
				2500	24.67				2500	24.17				2500	24
				1000	38.58				1000	40.95				1000	42
pat C	60Gy	6420(107%)	0.93	4500	2.54	60Gy	6420(107%)	0.47	4500	2.73	60Gy	6420(107%)	0.87	4500	3
		5700(95%)	98.2	3500	5.11		5700(95%)	98.38	3500	5.54		5700(95%)	98.5	3500	5.6
				2500	10.45				2500	10.59				2500	11
				1000	19.65				1000	21.39				1000	22

Table 2: dose deposition on body and PTV volume of 3 patients with different fields of Head and Neck cases

Pat study	IMRT 5 FIELD					IMRT 7 FIELD					IMRT 9 FIELD				
	Prescribed	PTV		body		prescribed	PTV		body		prescribed	PTV		body	
		dose	%vol	dose	%vol		dose	%vol	dose	%vol		dose	%vol	dose	%vol
pat A	70Gy	7490(107%)	0.88	4500	8.76	70Gy	7490(107%)	0.84	4500	8.59	70Gy	7490(107%)	0.24	4500	8.84
		6650(95%)	94	3500	14.56		6650(95%)	94.7	3500	14.25		6650(95%)	94.44	3500	14.6
				2500	21.44				2500	22.23				2500	21.35
				1000	30.07				1000	32.12				1000	31.73
	63Gy	5985(95%)	96.22			63Gy	5985(95%)	95.57			63Gy	5985(95%)	97.22		
	56Gy	5320(95%)	98.5			56Gy	5320(95%)	98.9			56Gy	5320(95%)	98.7		
pat B	70Gy	7490(107%	0.26	4500	6.79	70Gy	7490(107%)	1.18	4500	6.99	70Gy	7490(107%)	0.45	4500	6.9
		6650(95%)	96.07	3500	12.96		6650(95%)	98.09	3500	13.29		6650(95%)	98.88	3500	12.89
				2500	21.34				2500	21.6				2500	20.02
				1000	35				1000	40.59				1000	41.03
	63Gy	5985(95%)	95.1			63Gy	5985(95%)	96.16			63Gy	5985(95%)	96.78		
Pat C	70Gy	7490(107%)	0.91	4500	4.63	70Gy	7490(107%)	0.46	4500	4.64	70Gy	7490(107%)	0.6	4500	5.44

Table 3: dose deposition on body and PTV volume of 3 patients with different fields of pelvis cases

Pat study	IMRT 5 FIELD					IMRT 7 FIELD					IMRT 9 FIELD				
	Prescribed	PTV		body		prescribed	PTV		body		prescribed	PTV		body	
		dose	%vol	dose	%vol		dose	%vol	dose	%vol		dose	%vol	dose	%vol
patient A	55.8Gy	5970(107%)	0.17	4500	3.11	55.8Gy	5970(107%)	0.72	4500	3.2	55.8Gy	5970(107%)	0.89	4500	3.34
		5301(95%)	97.9	3500	11.05		5301(95%)	98.2	3500	11.73		5301(95%)	98.8	3500	11.46
				2500	31.3				2500	26.27				2500	28.49
				1000	54.17				1000	59.55				1000	62.97
	50Gy	4750(95%)	98.01			50Gy	4750(95%)	98.08			50Gy	4750(95%)	98.89		
patient B	55.8Gy	5970(107%)	0.22	4500	7.92	55.8Gy	5970(107%)	0.26	4500	7.26	55.8Gy	5970(107%)	0.1	4500	7.57
		5301(95%)	99.82	3500	17.2		5301(95%)	98.2	3500	17.87		5301(95%)	98.9	3500	17.75
				2500	33.35				2500	32.53				2500	33.72
				1000	55.59				1000	59.62				1000	62.03
	50Gy	4750(95%)	98			50Gy	4750(95%)	98.7			50Gy	4750(95%)	98.59		
patient C	55.8Gy	5970(107%)	0.4	4500	7.12	55.8Gy	5970(107%)	0.63	4500	6.45	55.8Gy	5970(107%)	0.5	4500	5.94
		5301(95%)	96.5	3500	15.92		5301(95%)	98.05	3500	14.35		5301(95%)	97.8	3500	13.63
				2500	26.03				2500	26.8				2500	27.78
				1000	45.47				1000	49.63				1000	51.7
	50Gy	4750(95%)	97.84			50Gy	4750(95%)	97.54			50Gy	4750(95%)	98.9		

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RESEARCH ARTICLE

Role of Chitosan on Inhibition of Oxidative Browning and Enhancement of SolasodineContent in *Solanum xanthocarpum* Schrad and Wendl

Mounika Karupusamy[1], V.S Hansiya[1], P. Umamaheswari[1], Anju Byju[1], J.Aarthi[1] and Geetha Natesan[2]*

[1]Research Scholar, Department of Botany, Bharathiar University, Coimbatore, Tamil Nadu, India.
[2]Professor, Department of Botany, Bharathiar University, Coimbatore, Tamil Nadu, India.

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***Address for Correspondence**
Geetha Natesan,
Professor,
Department of Botany,
Bharathiar University,
Coimbatore, Tamil Nadu, India.
Email: ngeethaptc@gmail.com

ABSTRACT

Oxidative browning is severe problem in plant tissue culture caused by the oxidation of phenolic compounds. It results in reduced growth, lower rates of regeneration and can ultimately lead to tissue death. The present studyis aimed to evaluate the *in vitro* chitosan application on inhibition of oxidative browning and enhancement of solasodine content of *S. xanthocarpum. In vitro* grown leaf segments were cultured on MS media supplemented with various concentrations of 2,4-D, NAA and IAA. After one month, suspension culture was established using MS liquid medium containing 5.0 mg/l IAA with various concentrations of chitosan. Aqueous suspension cells extract was prepared after one month and subjected to qualitative screening of phenols and alkaloids. Using spectrophotometer, total phenolic content was determined quantitatively and solasodine content was estimated using HPLC.A Factorial experiment in Complete Randomized Design (CRD) consisting of 15 treatments with three replications was followed for tissue culture experiments. Data were subjected to analysis of variance and the differences between means were analysed by Duncan's multiple range test. Callus tissue browning was observed more after third weeks of callus culture compared to first and second weeks of culture. Chitosantreated cell suspension cultures showed less browning and more solasodine content compared to control. This observation was confirmed by qualitative, quantitative and HPLC analyses. This investigation proves chitosan efficiency for inhibition of oxidative browning and enhancement of solasodine content in *S. xanthocarpum*

Keywords: Oxidative browning; phenolic compounds; *in vitro*; chitosan; solasodine

INTRODUCTION

Solanum xanthocarpum Shrad and Wendl. is one of the members of the Dasamula (ten roots) of the Ayurveda (Mohan , 2007). In English it iscalled as yellow berried nightshade and in Hindi as kateli. Its synonyms are *S. virginiannum* L. and S. surratense Burm .f. and belongs to the family Solanaceae. The plant is used in cough, bronchial asthma (Govindan *et.al,* 2004), chest pain, leprosy, skin diseases, scabies, in wound healing(Kumar *et.al,*2010)and cardiac diseases (Krayer and Briggs,1950).The plant extract owns antipyretic, anthelmintic, carminative, stomachic, febrifuge, laxative, rejuvenating and aphrodisiac properties. Stems, flowers and fruits are bitter, carminative. Root decoction used as febrifuge, diuretic and an expectorant (Vadnere *et.al,* 2008). Leaves are used in muscle pain when applied locally and its juice in rheumatism while mixed with black pepper (Sharma *et.al* ,2010). Dried fruit extract of the species possesses anti-inflammatory activity (Anwikar and Bhitre ,2010). Solasodine is the principal constituent of *S. xanthocarpum*, a steroidal glycoalkaloid, an N-analogue of diosgenin and used as the starting material for the synthesis of steroid hormones like corticosteroids, anabolic steroids, etc(Mann ,1978). Oxidative browning is a common problem in plant tissue culture; resulting in reduced growth(Krishana *et.al,*2008), (Uchendu *et.al*,2011) lower rates of regeneration or recalcitrance (Laukkanen *et.al,*2000),(Aliyu,2005),(Parthasarathy,2005) and can ultimately lead to cell/tissue/plant death (Toth *et.al,*1994), (Panaia *et.al,*2000),(Tabiyeh *et.al,*2005). The occurrence of browning varies among species, cultivars, and the physiological state of the plant/tissue but in many cases severely restricts plant growth and development. The underlying cause of tissue browning is the accumulation and subsequent oxidation of phenolic compounds in the tissue and culture media. Tissue browning results from the accumulation and subsequent oxidation of phenolic compounds, it is intimately linked to phenylalanine ammonia lyase (PAL) activity (Laukkanen *et.al,*2000),(Toth *et.al*,1994).

PAL is the first dedicated enzyme in the phenylpropanoid pathway and converts phenylalanine into trans-cinnamic acid, providing the substrate for further synthesis of phenolic compound(Dixon and Paiva ,1995). Due to the omnipresent nature and severe consequences of tissue browning, a substantial amount of research has gone into developing methods to prevent and/or ameliorate it (Bhat and chandel,1991),(Dalal *et.al,*1992),(Madhusudhanam and Rahiman,2000), (Tang *et.al,* 2004),(Tang *et.al,*2004),(Thomas,2008). Several improvements have been made towards reducing oxidative browning by altering environmental conditions used in tissue culture. For example, tissues cultured in the dark often display lower levels of browning than those grown in the light (Laine and David,1991),(Ochoa-Alejo and Ramirez-Malagon 2001) Altering the basic media composition and the type/concentration of plant growth regulators can also reduce the degree of browning. Pre-treating explants or amending culture media with compounds specifically selected to reduce tissue browning is also often employed. Most of these treatments/amendments can be divided into two general categories: (A) antioxidants such as ascorbic acid, melatonin, or citric acid, that reduce oxidative stress and prevent oxidation of phenolic compounds, (B) adsorbants that bind phenolic compounds rendering them less toxic such as activated charcoal or PVPP. These approaches are often combined with frequent sub-cultures to reduce exposure (Shukla *et.al,* 2012) although in some species frequent subculture exacerbates the problem, presumably by further stressing the explants(Zeraatpishe *et.al,*2011).

Chitosan is a natural biodegradable polymer and chemically it is a linear unbranched polymer of β-1, 4-D-glucosamine. It is obtained from chitin, a co-polymer of N-acetyl-D-glucosamine and D-glucosamine constituting the main component of the exoskeleton of arthropods. Its non-hazardous, biocompatible, antibacterial and biodegradable properties have led to significant research towards biomedical applications such as drug delivery, tissue engineering, wound-healing dressing etc.(Ali and Ahmed ,2018) and post- harvest applications such as delayed discolouration associated with reduced enzyme activities of polyphenol oxidase, peroxidase, catalase, phenylalanine ammonia lyase and laccase as well as lower total phenolic content(Eissa,2007). To our knowledge, there are no reports on the effects of chitosan on prevention of oxidative browning in leaf derived suspension culture of *S. xanthocarpum* and also reduction of total phenolic content coupled with enhanced production of solasodine

content. Therefore, the present study was aimed to evaluate the in vitro chitosan application on inhibition of oxidative browning and enhancement of solasodine content of *S. xanthocarpum.*

MATERIALS AND METHODS

Chemicals

MS basal medium powder, growth regulators such as 2,4-D, NAA and IAA were purchased from Sigma Aldrich and used for in vitro studies. Solasodine was also procured from the same company and used in HPLC analysis. All the reagents used in this work were of analytical grade.

Preparation of MS medium

In the present study, MS medium (Murashige and Skoog ,1962)was prepared using MS macro and micro nutrients, vitamins, iron source, sucrose and distilled water. The pH was adjusted to 5.8 and solidified with 0.8% (w/v) agar and was sterilized by autoclaving at 121°C and 15 psi for 20 min.

Plant material collection and raising of aseptic seedlings

Viable seeds from the ripe berries of *S. xanthocarpum* were collected and used for rising of aseptic seedlings under in vitro condition. The seeds were first washed thoroughly in running tape water with 40% teepol for 10 min with continuous swirling followed by thorough wash with tap water to remove detergent completely. Then, the seeds were washed with 70% ethanol for 1 min under laminar hood and rinsed 5-6 times with sterile distilled water to remove any ethanol present on the surface. After sterilization, the seeds were inoculated onto MS medium.

Culture maintenance

After inoculation, cultures were maintained in the culture room under a daily photoperiod of 16/8 (light/dark) provided by cool white fluorescent light (3000 lux) at 25±2°C.

Callus induction using in vitro derived leaves

Leaf segments (0.5-1.0 cm) were excised from in vitro grown seedlings and cultured in tubes containing different types of auxins such as 2,4-D (2,4-Dichlorophenoxy acetic acid), NAA (1-Naphthalene acetic acid) and IAA (Indole-3-acetic acid) at various concentrations (1.0, 2.0, 3.0, 4.0 and 5.0 mg/L) separately. All different types of callus induction media were incubated in a culture room at 25 ± 2oC and exposed to 16/8 h day photoperiod-controlled automatically from the white cool light of fluorescent lamps for four weeks.

Establishment of cell suspension culture with different concentrations of chitosan

Various concentrations of chitosan (100, 200, 300, 400 and 500 mg/L) were taken and dissolved in 0.1 M glacial acetic acid and TPP. The reaction mixture was stirred continuously overnight to dissolve properly. The dissolved chitosan solution was mixed with MS liquid medium containing IAA. The pH of the solution was adjusted to 5.8 with NaOH or HCl before autoclaving. For initiation of suspension culture, the medium type showing maximum callus induction percentage was selected. For this, portions of 30-day-old friable callus (0.5 g FW) were inoculated into 125 mL Erlenmeyer flasks containing 25 mL of MS liquid medium containing 5.0 mg/l IAA and various concentrations of chitosan. The inoculated flasks were placed on a rotary orbital shaker at 110 rpm and incubated at 25 ± 2 °C for a photoperiod of 16 h with white fluorescent light (60 μmol m-2s −1).

Preparation of aqueous suspension cells extract

After one month, chitosan treated suspension cells were collected separately and weighed and ground finely in mortar and pestle using one ml of distilled water. The mixture was centrifuged at 12,000 rpm for 15 minutes. The supernatants were collected and stored at 4oC for experimental analysis.

Qualitative phytochemical analysis

Qualitative phytochemical screening for phenols (Sofowora,1993) and alkaloids (Harbone ,2005)was carried out using aqueous suspension cells extracts.

Quantitative phytochemical analysis

Total phenolic content of aqueous suspension cells extracts was determined quantitatively using spectrophotometer as described (Singleton and Rossi,1965),(Gulcin *et.al* ,2013).

Estimation of solasodine content by HPLC

Solasodine content was quantified in aqueous suspension cells extracts using Varian HPLC system (SHIMADZU LC 20 PDA) by adopting the method (Rasheeduz and Parisa,2015).

Preparation of mobile phase

The mobile phase was prepared by mixing methanol and water buffered with 20 mM phosphate buffer and 0.5% OPA (Fluoraldehyde O-Phthaldialdehyde- amino acid derivatization agent) was added in the ratio of 65:35 (pH – 3.5). The mobile phase was degassed by sonication and filtered through vacuum filtration assembly (0.45 µm membrane filter) just before the HPLC analysis.

Preparation of stock and standard Dilution

Standard stock solution of solasodine (100 µg/mL) was prepared as follows: 3 mg of standard was weighed accurately and dissolved in 30 mL of methanol (HPLC grade) and the solution was stored at a temperature of 4oC protected from light. Working standard solutions were obtained freshly by diluting the stock solutions in methanol during analysis. The dilutions were prepared by serial dilution method.

Preparation of samples

One mL of aqueous suspension cells extracts was subjected to hydrolysis with 1 M HCl under reflux for three hours so as to remove the sugar residues. The hydrolysed material was cooled and the residue was dissolved in methanol and final volume made upto 10 mL and filtered through 0.2 µm membrane filter (Gelman Science, India) and analyzed by HPLC.

Statistical analysis

Observations were recorded for four weeks after culture response. The culture responses were expressed in terms of the percentage of explants forming callus. The experiments were arranged in a factorial completely randomized design. Fifteen treatments with three replications for tissue culture experiments and one sample with three replicates for biochemical estimations and HPLC analysis were used. The data were analysed using standard ANOVA procedures. The differences between the means were determined by Duncan's multiple range test using the SPSS statistical package (version 17.0).

RESULTS AND DISCUSSION

***In vitro* rising of seedlings and establishment of callus from *in vitro* derived leaves**

After incubation of seeds on MS basal medium, seeds became swollen and germination occurred within the first week of culture (Figure. 1 A). Ten days old in vitro grown leaf material was used for callus induction. The leaf segments began to produce callus seven days after inoculation. Callus induction frequencies were significantly influenced by the concentration of the specific plant growth regulator. Among three different growth regulators such as 2, 4-D, NAA and IAA at various concentrations such as 1.0, 2.0, 3.0, 4.0 and 5.0 mg/L tried, MS +5.0 mg/l IAA provided the maximum callus induction percentage (98%) with Fresh weight (17.6±2.5) (Table 1). Callus tissue browning was observed more after third weeks of culture compared to first and second weeks of culture (Figure. 1A-B). This calli browning was observed in all the three growth regulators supplemented MS media. In the present

investigation, initially callus was induced from in vitro grown leaf of *S. xanthocarpum* using MS media supplemented with various concentrations (1.0, 2.0, 3.0, 4.0and 5.0 mg/L) of auxins such as 2,4-D, NAA and IAA. Callus induction was observed within seven days of culture in all the media with different growth hormones. Analysis of variance showed an interaction ($p<0.05$) among types of auxin and their concentrations used in MS media (Table 1). Among three hormones, IAA produced maximum percentage of callus (98% with 17.6±2.5 FW) at the end of four weeks. Auxin has a wide variety of effects on plant growth and morphogenesis. Indole-3-acetic acid (IAA), a natural auxin of higher plants, is involved in regulating cell elongation, cell division and differentiation(Dietz et.al,1990)

Establishment of cell suspension culture and preparation of suspension cells extract

For initiation of suspension culture, MS liquid medium containing 5.0 mg/l IAA and various concentrations of chitosan (100, 200, 300, 400 and 500 mg/L) were used. After one month, aqueous suspension cells extract was prepared and subjected to qualitative screening of phenols and alkaloids.

Qualitative screening of phenols and alkaloids

Chitosan treated cell suspension cell extracts showed positive results for both phenol and alkaloids during qualitative phytochemical analysis. However, the intensity of colour was found decreased for phenol and increased for alkaloid with the increase of chitosan concentration (Table 2). Plants are the prime source of medicinally important compounds. Many plant products are used as pharmaceuticals, pigments, herbicides, etc. Phytochemically, *S.xanthocarpum* contains a number of phytoconstituents including alkaloids, sterols, saponins, flavonoids and their glycosides and carbohydrates, fatty acids, amino acids etc. Solasodine functions as an important intermediate in synthesis of steroidal hormones (Street *et.al,* 1977)and is a potential alternative to diosgenin as a precursor in synthesis of steroidal hormons (Macek,1989). Plant in vitro culture is an attractive technology for enhancing secondary metabolites that are either difficult to synthesize chemically or produced in limited quantities in wild plants (Kolew *et al*,2008)

Quantitative estimation of total phenolic content

The dose response of chitosan demonstrated that tissue browning declined in a concentration dependent manner up to 500 mg/L chitosan. The same result was reflected in total phenol content of the sample extracts. The addition of chitosan into the suspension medium resulted in significant reductions in total phenolic content (Table 3 and Figure 2. A-F). During callus induction, browning of callus tissue was observed within second week of culture. Browning was found more after third weeks of culture (fig. 1 B and C). This calli browning was observed in all the three growth regulators supplemented MS media which may be due to secretion of phenolic compounds from the cut surface of the explants which accumulate in the medium. Phenolic secretion is caused by injuries during the isolation of explants and the oxidation of these phenolic compounds on cut surfaces can cause necrosis, which hinders nutrient uptake and results in the death of explants(Erland and Mahmoud,2014),(Yildirium and Turker ,2014).

Polyphenol oxidase (PPO) enzyme is thought to play a key role in the oxidation of phenolic compounds(Larson,1988) other oxidative enzymes, such as phenylalanine ammonia lyase (PAL) and peroxidase (POD) also affect the oxidation of phenolic substances at cut surfaces of explants(Andersone and Ievinsh,2002),(Tabiyeh *et.al*,2006).(Yu *et .al* 1992,)studied the polyphenol oxidase expression in glandular trichomes of various solanaceae members. To reduce the effects of PPO and POD on phenolic compound oxidation and the subsequent effects of oxidized phenolic compounds on tissue culture, a number of culture manipulations have been developed. These include the initial culture of explants in darkness (Da *et.al*,2015)or under low temperature (Nguyen *et.al*,2007), (Rather *et.al*,2011). pretreatment of explants with antioxidants(Ahmad *et.al,* 2016)and the culture of explants on medium supplemented with adsorbents and antioxidants (Kdenoticova *et.al*,2013), (Meziani *et.al*,2016).Jones and Saxena (Jones and Saena, 2013) indicated that inhibiting phenylpropanoid biosynthesis with aminoindane-2-phosphonic acid (AIP), a competitive phenylalanine ammonia lyase inhibitor, is an effective approach to reduce tissue browning in Artemisia annua culture medium. (Irshad *et .al* 2017).reported the influence of anti-browning additives on phenolic secretion and callus formation frequency in various explants of *Abelmoschus esculentus*. Incorporation of activated charcoal (0.25%) significantly reduced the browning and improved the establishment of cultures as compared to other anti-

browning agents such as citric acid and ascorbic acid during nodal explants culture of pomegranate cv. Bhagva (Patel *et.al,*2018)

Determination of solasodine content by HPLC

HPLC analysis revealed significant accumulation of solasodine accumulation in cells suspension culture extracts treated with various concentrations of chitosan. The experimental results were calculated as mentioned (Yogananth *et.al,*2009). The solasodine content was found increased with increase of chitosan concentrations In the present study, various concentrations of chitosan were added into the suspension medium. All the samples extracts prepared after 30 days culture, showed positive test for both phenol and alkaloids. However, the intensity of colour was found declined for phenol test whereas it was found increased for alkaloid test with respect to presence of chitosan concentrations in the cell suspension culture. These results clearly show that suspension cells are rich with alkaloid and phenolic contents. However, the phenolic content was begun to reduce and alkaloid content was begun to increase when the concentrations of chitosan increased in the suspension medium (fig. 2 A-F). In fact, tissue browning was found to decline in a dose dependent manner up to 500 mg/l chitosan. The decrease of phenol and increase of alkaloid were also reflected as colour intensity during their qualitative screening (Table 2). The reduced level of total phenolic content was confirmed by spectrophotometrically. Maximum level of total phenolics was observed in the suspension culture supplemented with 100 mg/L chitosan i.e 38.07±0.31 mg GAE/g FW and minimum level was found in the medium containing 500 mg/L chitosan i.e 0.07±0.19 mg GAE/g FW (Table 3). Chitosan delayed the increase in polyphenol oxidase (PPO) activity and partially inhibited the increase in peroxidase activity (Caro and Jous,2018), (Zhang and Quantick ,1997).

A-HPLC chromatogram shows peaks for standard- solosodine; B- HPLC chromatogram shows peaks for cell suspension treated with 100 mg/L chitosan; C- HPLC chromatogram shows peak for cell suspension treated with 200 mg/L chitosan; D- HPLC chromatogram shows peak for cell suspension treated with 300 mg/L chitosan; E- HPLC chromatogram shows peak for cell suspension treated with 400 mg/L chitosan; F- HPLC chromatogram shows peak for cell suspension treated with 300 mg/L chitosan. According to the USP and ICH guidelines, there are various parameters to validate the reproducibility of the method of HPLC *viz.* the effectiveness, the limit of detection (LOD), the limit of quantitation (LOQ), the linearity, the precision and the accuracy(Seal,2016). In the present study, in order to ascertain the linearity, the stock solution of the standard solasodine (1 mg/mL) was diluted to five different concentrations (10, 20, 30, 40, 50 $\mu g/mL$) which were fed individually in triplicate to the HPLC system and the calibration curve so obtainedby plotting peak area versus concentration for each sample where the square of the correlation coefficient R2 > 0.99 is indicative of the measure of linearity. Peak areas were identified by comparison of retention times (RT) and photo diode array spectral characteristics with standard. The chromatogram of standard solasodine showed 3.813 retention time. The chromatographic peaks of sample extracts were found to be varied. The plant samples treated with 100 and 200 mg/L chitosan showed 2.858 RT with 37.134 mg/100 g FW and 2.115 RT with 47.530 mg/100 g FW, respectively. But plant samples treated with 300, 400 and 500 mg/L chitosan had 3.777 RT with 78.913 mg/100 g FW, 3.744 RT with 78.913 mg/100 g FW and 3.803 RT with 93.777 mg/100 g FW,respectively (Table 4 and fig. 3 A-F).

From HPLC analysis, it is very clear that the solasodine content was found increased with increase of chitosan concentration and found decreased with the increase of phenolic content. At 100 and 200 mg/L chitosan treated cells showed low amount of solasodine. It may be due to more phenolic secretion which is not absorbed by the given dose of chitosan effectively. But at higher concentrations of chitosan used ie. 300, 400 and 500 mg/L, the liberated phenolic content was effectively absorbed by chitosan. As a result, the solasodine content was increased and showed RT with concentration on par with standard. In order to prevent oxidative browning of litchi (*Litchi chinensis*) fruit, chitosan coating was used (Jiang *et.al,*2005), (Joas *et.al,*2005)

CONCLUSION

Conclusively, suspension cells of *S.xanthocarpum* showed reduction of tissue browning and optimal production of solasodine content with various concentrations of chitosan application. The mechanism of reducing oxidative tissue browning by using chitosan should be studied in detail. Regardless, inhibiting the production of phenolic compounds and optimal production of desired secondary metabolite in cell suspension culture with the incorporation of chitosan could have a wide application in systems where oxidative browning restricts the development of *in vitro* culture techniques for efficient secondary metabolites production.

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Conflict of interest

Authors declare no conflict of interest.

REFERENCES

1. Mohan L, Sharma P and Srivastava CN.2007. Comparative efficacy of *Solanum xanthocarpum* extracts alone and in combination with a synthetic pyrethroid, cypermethrin, against malaria vector *Anopheles stephensi*, *Southeast Asian Journal of Tropical Medicine and Public health.,* 38(2):256.
2. Govindan S, Viswanathan S, Vijayasekaran V and Alagappan R.2004. Further studies on the clinical efficacy of *Solanum xanthocarpum* and *Solanum trilobatum* in bronchial asthma. Phytotherapy Research: *An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*.,18(10):805-9.
3. Kumar N, Prakash D and Kumar P.2010. Wound healing activity of *Solanum xanthocarpum*Schrad. &Wendl. Fruits, *Indian Journal of Natural Products and Resources*., 1(4): 470-475.
4. Krayer O and Briggs LH.1950. Studies on solanum alkaloids: II. The anti-accelerator cardiac action ofSolasodine and some of its derivatives. *British Journal of Pharmacology and Chemotherapy* .,5(4):517.
5. Vadnere GP, Gaud RS and Singhai AK.2008. Evaluation of anti-asthmatic property of *Solanum xanthocarpum*flower extracts, *Pharmacologyonline*.,1:.513-22.
6. Sharma N, Sharma AK and Zafar R. Kantikari.2010. A prickly medicinal weed~ ecosensorium. *Journal of Phytological Research*.,9(1):13-7.
7. Anwikar S and Bhitre M 2010.Study of the synergistic anti-inflammatory activity of *Solanumxanthocarpum*Schrad and Wendl and *Cassia fistula* Linn. *International Journal of Ayurveda researchbv*.,1(3):167.
8. Mann JD, Production of solasodine for pharmaceutical industry1978. In Advances in Agronomy N. C. Brady (Ed.),*Academic Press, London*.,207-245.
9. Krishna H, Sairam RK, Singh SK, Patel VB and Sharma RR 2008. Mango explant browning: Effect ofontogenic age, mycorrhization and pre-treatments,*Scientia Horticulturae*.,118(2):132-8.
10. Uchendu EE, Paliyath G, Brown DC and Saxena PK, 2011.In vitro propagation of North American ginseng (*Panaxquinquefolius* L.),*In Vitro Cellular & Developmental Biology-Plant*.,47(6):710-8.
11. Laukkanen H,Rautiainen L, Taulavuori E andHohtola A. 2000.Changes in cellular structures and enzymatic activities during browning of Scots pine callus derived from mature buds. *Tree Physiology*.,20(7):467-75.
12. Aliyu OM, 2005.Application of tissue culture to cashew (*Anacardiumoccidentale* L.) breeding: An appraisal., *African Journal of Biotechnology*.,4(13).
13. Parthasarathy VA 2005.High tech propagation of horticultural crops-accent on recalcitrance. InRecent trends in horticultural biotechnology, Vol. I and II. ICAE national symposium on biotechnological interventions for improvement of horticultural crops: issues and strategies, Vellanikkara, Kerala, India,10-12, 85-91: *New India Publishing Agency*

14. Toth K, Haapala T and Hohtola A.1994. Alleviation of browning in oak explants by chemical pretreatments,*BiologiaPlantarum*.,36(4):511-7.
15. Panaia M,Senaratna T, Bunn E, Dixon KW and Sivasithamparam K, 2000.Micropropagation of the critically endangered Western Australian species, *Symonanthusbancroftii* (F. Muell.) L. Haegi (Solanaceae),*Plant cell, tissue and organ culture*.,63(1):23-9.
16. Tabiyeh DT, Bernard F and Shacker H 2005.Investigation of glutathione, salicylic acid and GA3 effects on browning in *Pistaciavera* shoot tips culture,*In IV International Symposium on Pistachios and Almonds*.,726: 201-04.
17. Dixon RA and Paiva NL,1995.Stress-induced phenylpropanoid metabolism,*The plant cell*.,7(7):1085.
18. Bhat SR and Chandel KP.1991. A novel technique to overcome browning in tissue culture. *Plant Cell Reports*.,10(6):358-61.
19. Dalal MA, Sharma BB and Rao MS.1992. Studies on stock plant treatment and initiation culture mode in control of oxidative browning in in vitro cultures of grapevine,*Scientia Horticulturae*.,1:51(1-2):35-41.
20. Madhusudhanan K and Rahiman BA.2000. The effect of activated charcoal supplemented media to browning of in vitro cultures of Piper species,*BiologiaPlantarum*.,43(2):297-9.
21. Tang W, Harris LC, Outhavong V and Newton RJ.2004. Antioxidants enhance in vitro plant regeneration by inhibiting the accumulation of peroxidase in Virginia pine(*Pinusvirginiana*Mill.),*Plant Cell Reports*.,22(12):871-7.
22. Tang W, Newton RJ and Outhavong V 2004. Exogenously added polyamines recover browningtissues into normal callus cultures and improve plant regeneration in pine, *PhysiologiaPlantarum*.,122(3):386-95.
23. Thomas TD 2008. The role of activated charcoal in plant tissue culture, *Biotechnology Advances*.,1;26(6):618-31.
24. Lainé E and David A 1991. Regeneration of plants from leaf explants of micropropagated clonal *Eucalyptus grandis*,*Plant Cell Reports*.,13(8):473-6.
25. Ochoa-Alejo N and Ramirez-MalagonR 2001. *In vitro* chili pepper biotechnology, *In vitro Cellular & Developmental Biology-Plant*.,37(6):701-29.
26. Shukla MR, Jones AM, Sullivan JA, Liu C, Gosling S and Saxena PK .2012. *In vitro* conservation of American elm (*Ulmusamericana*): potential role of auxin metabolism in sustained plant proliferation,*Canadian Journal of Forest Research*.,42(4):686-97.
27. Zeraatpishe A, Oryan S, Bagheri MH, Pilevarian AA and Malekirad AA 2011 Effects of *Melissa officinalis* L. on oxidative status and DNA damage in subjects exposed to long-term low-dose ionizing radiation,*Toxicology and Industrial Health*.,27(3):205-12.
28. Ali A and Ahmed S 2018. A review on chitosan and its nanocomposites in drug delivery,*International Journal of Biological Macromolecules*., 109:273-86.
29. Eissa HA 2007. Effect of chitosan coating on shelf life and quality of fresh cut mushroom, *Journal of Food Quality*., 30(5):623-45.
30. Murashige T and Skoog F 1962 .A revised medium for rapid growth and bio assays with tobacco tissue culture, *PhysiologiaPlantarum*.,15(3):473-97.
31. Sofowora 1993.A Medicinal Plants and Traditional Medicinal in Africa, Screening Plants for Bioactive Agents 2nd (Ed.), *Sunshine House, Ibadan, Nigeria: Spectrum Books Ltd*.
32. Harborne JB.2005. Phytochemical methods – A guide to modern techniques of plant analysis, New Delhi, *Springer Pvt. Ltd.*
33. Singleton VL and Rossi JA 1965.Colorimetry of total phenolics with phosphomolybdic- phosphotungstic acid reagents, *American Journal of Enology and Viticulture*., 16(3):144-58.
34. Gülçin İ, Büyükokuroğlu ME, Oktay M and Küfrevioğlu Öİ.2003. Antioxidant and analgesic activities of turpentine of *Pinusnigra*Arn. subsp. *pallsiana* (Lamb.) Holmboe,*Journal of Ethnopharmacology*.,86(1):51-8.
35. Rasheeduz Z and Parisa H, Quantification of solasodine in callus and suspension cultures of *Solanum xanthocarpum*Schrad. AndWendl by RP-HPL.2015. *International Journal of Pharmacognosy and Phytochemical Research*.,7:206-14.
36. Dietz A, Kutschera U and Ray PM 1990. Auxin enhancement of mRNAs in epidermis and internal tissues of the pea stem and its significance for control of elongation, *Plant Physiology*.,(2):432-8.

37. Street HE, Butcher DN and Staba EJ.1977. Cell Culture and Secondary Products. *In Applied and Fundamental Aspects of Plant Cell, Tissue, and Organ Culture: Springer, Berlin, Heidelberg.*
38. Macek TE,*Solanum aviculare*Forst.,*Solanum laciniatum* Ait.(Poroporo)1989:*In vitro* culture and the production of solasodine, *In Medicinal and Aromatic Plants II: Springer, Berlin, Heidelberg*.
39. Kolewe ME, Gaurav Vand Roberts SC.2008. Pharmaceutically active natural product synthesis and supply via plant cell culture technology, *Molecular Pharmaceutics* 5(2):243-56.
40. Erland LA and Mahmoud SS, An efficient method for regeneration of lavandin (*Lavandula* x intermedia cv.'Grosso').2014. *In Vitro Cellular & Developmental Biology-Plant*.,50(5):646-54.
41. Yildirim AB and Turker AU, .2014. Effects of regeneration enhancers on micropropagation of*Fragariavesca* L. and phenolic content comparison of field-grown and *in vitro*-grown plant materials by liquid chromatography-electrospray tandem massspectrometry (LC–ESI-MS/MS), *Scientia Horticulturae*.,169:169-78.
42. Larson RA.1988. The antioxidants of higher plants, *Phytochemistry* .,27(4):969-78.
43. Andersone U and Ievinsh G.2002. Changes of morphogenic competence in mature *Pinussylvestris* L.buds *in vitro*, *Annals of Botany*., 1;90(2):293-8.
44. Tabiyeh D, Bernard F and Shacker H .2006. Investigation of glutathione, salicylic acid and GA3effects on browning in *Pistaciavera* shoot tips culture, *ActaHorticulturae*.,726 (726) : 201-204.
45. Yu H, Kowalski SP and Steffens JC.,1992. Comparison of polyphenol oxidase expression in glandulartrichomes of *Solanum* and *Lycopersicon* species, *Plant Physiology*.,100(4):1885-90.
46. Da Silva AL, Gollo AL, Brondani GE, Horbach MA and Oliveira LS.2015. Micropropagation of *Eucalyptus saligna* Sm. from cotyledonary nodes, *Pakistan Journal of Botany*.,47(1):311-8.
47. Nguyen TV, Thu TT, Claeys M and Angenon G.2007. *Agrobacterium*-mediated transformation of sorghum (*Sorghum bicolor* (L.) Moench) using an improved *in vitro* regeneration system,*Plant cell, tissue and organ culture*.,91(2):155-64.
48. Rather ZA, Nazki IT, Peer FA, Mir MA and Hussain G.2011. Minimizing medium browning during *in vitro* cultures of herbaceous peony (*Paeonialactiflora* Pall.), *Indian Journal of Horticulture*.,68(4):529-34.
49. Ahmad I, Jaskani MJ, Nafees M, Ashraf I and Qureshi R.2016. Control of media browning in micro propagation of guava (*Psidiumguajava* L.),*Pakistan Journal of Botany*.,48(2):713-6.
50. Klenotičová H, Smýkalová I, Švábová L and Griga M.2013. Resolving browning during the establishment of explant cultures in *Viciafaba* L. for genetic transformation. *ActaUniversitatis AgriculturaetSilviculturaeMendelianaeBrunensis*.,61(5):1279-88.
51. Meziani R, Jaiti F, Mazri MA, Hassani A and Salem SB.2016. Organogenesis of *Phoenix dactylifera* L. cv. Mejhoul: Influences of natural and synthetic compounds on tissue browning, and analysis of protein concentrations and peroxidase activity in explants, *Scientia Horticulturae*.,204:145-52.
52. Jones AM and Saxena PK, Inhibition of phenylpropanoid biosynthesis in *Artemisia annua* L.2013.: a novel approach to reduce oxidative browning in plant tissue culture,*PloS one*.,8(10):e76802.
53. Irshad M, He B, Liu S, Mitra S, Debnath B, Li M, Rizwan HM and Qiu D.2017. *In vitro* regeneration of *Abelmoschusesculentus* L. Cv. Wufu: Influence of anti-browning additives on phenolic secretion and callus formation frequency in explants, *Horticulture, Environment, and Biotechnology*.,58(5):503-13.
54. Patel A, Patil G, Mankad M and Subhash N.2018. Optimization of surface sterilization and manipulation of *in vitro* conditions for reduced browning in pomegranate (*Punicagranatum* L.) varietyBhagava*, International Journal of Chemical Studies*.,6:23-8.
55. Caro Y and Joas J.2005. Postharvest control of litchi pericarp browning (cv. KwaiMi) by combined treatments of chitosan and organic acids: II. Effect of the initial water content of pericarp,*Postharvest Biology and Technology*.,38(2):137-44.
56. Yogananth N, Bhakyaraj R, Chanthuru A, Parvathi S and Palanivel S, Comparative Analysis of Solasodine from *in vitro* and *in vivo* Cultures of *Solanum nigrum* Linn.2009. *Kathmandu University Journal of Science, Engineering and Technology*.,5(1):99-103.
57. Zhang D and Quantick PC.1997. Effects of chitosan coating on enzymatic browning and decay duringpostharvest storage of litchi (*Litchi chinensis*Sonn.) fruit,*Postharvest Biology and Technology*.,12(2):195-202.

58. Seal T.2016. Quantitative HPLC analysis of phenolic acids, flavonoids and ascorbic acid in four different solvent extracts of two wild edible leaves, *Sonchusarvensis* and *Oenanthelinearis* of North- Eastern region in India, *Journal of Applied Pharmaceutical Science*.,6(2):157-66.
59. Jiang Y and Li J, Jiang W 2005.Effects of chitosan coating on shelf life of cold-stored litchi fruit at ambient temperature,*LWT-Food Science and Technology*.,38(7):757-61.
60. Joas J, Caro Y, Ducamp MN and ReynesM.2005. Postharvest control of pericarp browning of litchi fruit (*Litchi chinensis*Sonn cv KwaïMi) by treatment with chitosan and organic acids: I. Effect of pH and pericarp dehydration, *Postharvest Biology and Technology*. 38(2):128-36.

Table 1. Effect of different growth regulators on callus induction from leaf explants of S. xanthocarpum

Growth hormones used(mg/L)	Percentage ofresponse for callusing	Fresh weightof callus (g)
2,4-D		2.6 ± 1.1^{c}
1.0	45	4.0 ± 1.0^{b}
2.0	43	4.5 ± 1.0^{b}
3.0	49	7.0 ± 1.0^{a}
4.0	34	7.3 ± 2.0^{a}
5.0	50	
NAA		2.0 ± 1.0^{c}
1.0	55	1.1 ± 1.5^{c}
2.0	50	4.0 ± 1.0^{b}
3.0	59	4.3 ± 2.0^{b}
4.0	60	6.0 ± 1.0^{a}
5.0	63	
IAA	78	4.3 ± 1.1^{c}
1.0	80	7.0 ± 1.0^{c}
2.0	85	8.0 ± 1.0^{b}
3.0	90	10.0 ± 1.0^{b}
4.0	98	17.6 ± 2.5^{a}
5.0		

Values are the means of three experiments. Mean values within a column followed by the same letter are not significantly different (P< 0.05).

Table 2. Qualitative phytochemical screening for phenols and alkaloids

Chitosan Concentration(mg/L)	Name of the phytochemical	Colour intensity
100	**Alkaloids**	+
200		+
300		+
		+
400		+
		+
		+
500		+
		+
		+

100	**Phenols**	+ + +
200		+ + +
300		+ +
400		+
500		+

Symbols denotes +: present, ++: present at moderate intensity, +++: present at higher intensity

Table 3. Quantitative estimation of total phenolic content of suspension cells extracts treated with various concentrations of chitosan

Chitosan Concentration(mg/L)	Total Phenolic Content(mg GAE/g FW)
Control (without chitosan)	148.32 ± 7.39^{a}
100	38.87 ± 0.31^{a}
200	22.12 ± 0.40^{b}
300	3.03 ± 2.76^{c}
400	1.02 ± 0.25^{d}
500	0.07 ± 0.19^{d}

Values are the means of three experiments. Mean values within a column followed by the same letter are not significantly different (P< 0.05).

Table 4. Retention time and concentration of solasodine content of suspension cells extracts treated with various concentrations of chitosan

Chitosan concentrationmg/L	Retention time(min)	Solasodinecontent (mg /100 g FW)
Standard	3.813	100.00 ± 1.20^{a}
100	2.858	37.13 ± 0.21^{e}
200	2.115	47.53 ± 0.11^{d}
300	3.777	78.91 ± 1.40^{c}
400	3.744	81.73 ± 0.22^{b}
500	3.803	93.78 ± 0.11^{a}

Values are the means of three experiments. Mean values within a column followed by the same letter are not significantly different (P< 0.05).

Figure 1. A. *In vitro* grown seedling of *S. xanthocarpum*; B. Two week grown calli; C. Four weeks grown calli.

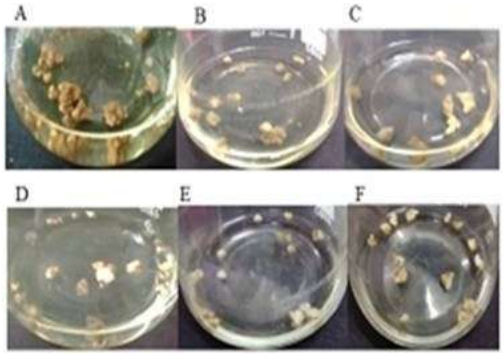

Figure 2. A-F Cell suspension culture treated with various concentrations of chitosan.
A- Cell suspension culture without chitosan;
B-Cell suspension treated with 100 mg/L *Chitosan; C- Cell suspension treated with 200 mg/L chitosan; D- Cell suspension treated with 300 mg/L chitosan; E- Cell suspension treated with 400 mg/L chitosan; F- Cell suspension treated with 500 mg/L chitosan.*

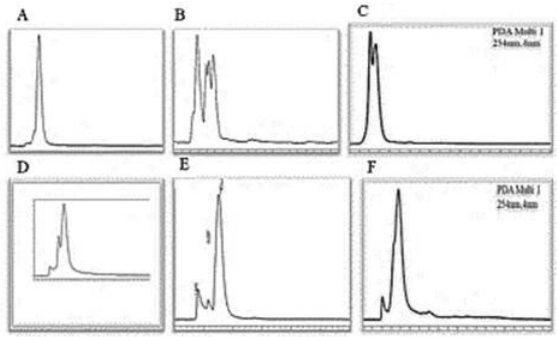

Figure 3 A-F: HPLC chromatograms show peaks for solosodine content in suspension cells treated with various concentration of chitosan.

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RESEARCH ARTICLE

Effect of Yogic Practices on Blood Glucose and Low Density Lipoprotein among Geriatric Men

N.Elumalai[1] and S.Murugesan[2]*

[1]Ph.D Scholar (Full-time), Faculty of Yoga Sciences and Therapy, Meenakshi Academy of Higher Education and Research (Deemed to be University), No.12, Vembuliamman Koil Street, West K . K Nagar, Chennai, Tamil Nadu, India.
[2]Professor and Head, Faculty of Yoga Sciences and Therapy, Meenakshi Academy of Higher Education and Research (Deemed to be University), No.12, Vembuliamman Koil Street, West K . K Nagar, Chennai, Tamil Nadu, India.

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***Address for Correspondence**
S.Murugesan
Professor and Head,
Faculty of Yoga Sciences and Therapy,
Meenakshi Academy of Higher Education and Research (Deemed to be University),
No.12, Vembuliamman Koil Street,
West K . K Nagar, Chennai, Tamil Nadu, India
Email: elumalain1955@gmail.com

ABSTRACT

The study's primary purpose was to determine whether or not elderly male yogis would show any significant differences in fasting blood sugar and low-density lipoprotein levels. Fifteen males in their 65s and 75s were randomly recruited from the population of Chennai for the experimental research of random groups. It was postulated that geriatrics who engaged in Yogic practices would show markedly different blood sugar (fasting) and low-density lipoprotein (LDL) than the control group. Before beginning the training program, both groups (A and B) took a pretest on the opted-for dependent variables. Yogic techniques were administered to Group A, whereas the Control Group (no therapy other than active rest) served as the comparison. After eight weeks of experimentation, groups (A and B) were retested on the same dependent variables. Significant differences between the experimental and control groups were determined using analysis of covariance (ANCOVA). At the 0.05 significance level, the findings indicated that senior males who engaged in Yogic practices had lower blood sugar levels (fasting) and low-density lipoprotein. As a senior, I have found that yoga practices help me keep my blood sugar (Fasting) and low-density lipoprotein in check.

Keywords: Yogic Practices, Fasting Blood Sugar, Low-Density Lipoprotein, Geriatric men.

INTRODUCTION

According to the most widely accepted definition, geriatrics are those who are 60 years of age and older. It was anticipated that a good number of the systems would decline as a natural result of having reached this age. The great majority of geriatric problems are truly caused by a combination of factors, some of which may be age-related or disease-related changes in the way the system of balance works. The care of aged patients is known as geriatric medicine. This is a population that spans a wide range of ages, making it challenging to classify. When a person enters their 70s, 75s, or 80s, they often no longer need the services of a geriatrician since their health has stabilised. Gerontology is the study of the many different aspects of ageing, and the name gerontology refers to this academic area. The contributions that older men provide to society, such as care giving, community leadership, and the labour force, are very important in many different ways. Although the vast majority of men over the age of 65 enjoy excellent mental health, a significant number are at risk for developing mental illnesses, neurological diseases, or drug use problems. In addition, many are at risk for developing other health issues such as diabetes, hearing loss, and osteoarthritis. It is also more usual for persons of advanced age to suffer from many health problems at the same time. The average age of the world's population is steadily creeping upward. Between the years 2015 and 2050, it is anticipated that the proportion of the world's elderly would almost double, from around 12% to 22%. This is comparable to an increase of more than 900 million people over the age of 60 throughout the globe, which would bring the total population of people over the age of 60 to more than 2 billion. It is essential that the one-of-a-kind issues about the elderly's bodily and mental health be taken into account. In India, there has been a significant rise in the number of senior males, and the nation is seeing a rapid transition in its demographic makeup as a whole.

Headache difficulties account for about 4% of all impairments (disability adjusted life years) in those aged 60 and older, but mental and neurological diseases account for over 20% of all impairments (disability adjusted life years). It is predicted that these illnesses cause a loss of 17.4 million years of life in the elderly (YLDs). Around five percent and seven percent, respectively, of the world's senior population are afflicted with dementia and depression, two of the most prevalent forms of mental and neurological disorder that are associated with ageing. This is particularly true for people who suffer from anxiety disorders (3.8 percent), drug use concerns (almost 1 percent), and despair. People who have reached the age of 60 or older account for about one quarter of all deaths caused by self-harm (3.8 percent). It is very uncommon for elderly people who struggle with drug abuse difficulties to be undiagnosed or get the incorrect diagnosis.

The reason for doing the research

The goal of this research was to examine the relationship between yoga practises and fasting blood sugar and low-density lipoprotein levels in men of advanced age.

HYPOTHESIS

The elderly male control group was anticipated to have higher levels of fasting blood sugar and low-density lipoprotein than the yoga group.

DELIMITATIONS

Thirty elderly men, all between the ages of 65 and 75, were the only participants in the research. Only males above the age of 65 who are residents of Chennai were included in the analysis. Yogic techniques were the only ones included as an independent variable in the research. Specifically, only fasting blood sugar and low density lipoprotein were included as dependent variables.

REVIEW OF RELATTED LITERATURE

The reason Bijlani, R. (2005) undertook the research was to examine the short-term effects of a brief lifestyle intervention based on yoga on some of the biochemical markers of risk for cardiovascular disease and diabetes mellitus. By using a pre-post design, we were able to take measurements of the relevant variables on Day 1 and Day

10 of the intervention. The research was conducted as part of our Integral Health Clinic's ongoing operational research (IHC). The IHC is a community health centre that offers 8-day yoga-based lifestyle change programmes for the treatment and prevention of chronic illness. Every other week throughout the academic year, a new class will begin. This research relies on information from 98 people (20-74 years old; 67 men, 31 women) who participated in one of our programmes. The participants had a wide range of health problems, including hypertension, heart disease, diabetes, and others. Asanas (postures), Pranayama (breathing exercises), Relaxation Techniques, Group Support, Individualized Advice, Lectures, and Films on the Philosophy of Yoga and the Role of Yoga in Everyday Life, Meditation, Stress Management, Nutrition, and Disease Education were all part of the intervention. Fasting plasma glucose and serum lipoprotein profile were used as the end measures. Fasting blood samples were collected on the first and final days of class to identify these factors. On the final day of the course, HDL cholesterol was considerably greater, whereas fasting plasma glucose, serum total cholesterol, LDL cholesterol, very-LDL cholesterol, the ratio of total cholesterol to HDL cholesterol, and total triglycerides were all reduced. Hyperglycemic and hypercholesterolemic patients had more pronounced alterations. Data suggests that beneficial metabolic benefits may be shown after just 9 days of participation in a stress management and lifestyle change education programme.

Authors: Pal A., Srivastava (2011) Consistent yoga practise and self-discipline were investigated for their potential to help CAD patients lose weight and lower their blood cholesterol levels. One hundred seventy (170) patients with coronary artery disease (CAD) of both sexes were randomly recruited from the Department of Cardiology for this research. Eighty-five (85) participants were randomly assigned to one of two groups: the yoga group or the non-yoga group. One hundred fifty-four (154) participants out of the total of 170 finished the whole research. At the Department of Physiology at CSMMU UP Lucknow, the yoga intervention lasted for six months, during which time participants practised for 35-40 minutes five days a week. Measurements of body fat and estimates of lipid profiles were taken before and six months after the yogic intervention in the yoga group, although neither group did any yoga. Changes in body mass index (p 0.04), fat percentage (p 0.0002), fat-free mass (p 0.04), systolic blood pressure (p 0.002), diastolic blood pressure (p 0.009), heart rate (p 0.0001), total cholesterol (p 0.0001), triglycerides (p 0.0001), high-density lipoprotein (HDL) (p 0.0001), and Patients with heart disease or hypertension may benefit from frequent yoga practise since it lowers their blood pressure and cholesterol levels. Therefore, the yogic practises examined here are beneficial for those with heart problems.

MATERIALS AND METHODS

Random group experimental investigation was conducted on 60 elderly males in the age range of 65 to 75 from the city of Chennai. The sample size was decreased to 30 by further random selection. Group 1 received yoga instruction, while Group 2 served as the control. Fasting Blood Sugar and LDL Cholesterol are the outcomes of interest. Random group experimental design was utilised. Asana, Pranayama, Meditation, Mudra, etc., are all forms of yoga that may be used to bring about balance and harmony in one's physical, mental, psychic, and spiritual selves. Six mornings a week, for a maximum of an hour, the experimental groups trained for eight weeks. A statistical method called analysis of co-variance (ANCOVA) was employed to identify significant differences between the groups. The significance threshold is 0.05 percent.

YOGIC PRACTICES

1. Loosening the joints.
2. Surya Namaskar
3. Asanas
   - Trikonasana
   - Vriksasana
   - Janu sirsasana
   - Paschimotanasana
   - Utthanapadasana

- Ardhahalasana
- Sethubandhasana
- Bhujangasana
- Shalabasana
- Naukasana

4. Pranayama
- Anulomvilom
- Kapalapathi
- Ujjai

5. Yoga Nidra

RESULTS AND DISCUSSIONS

Results from the pre-test were insignificant at the 0.05 level since the F value of 1.02 achieved was less than the needed F value of 4.2. It is demonstrated that the pre-test randomization was fair and that there were no statistically significant differences between the groups. The post-test scores analysis indicated a considerable difference between the groups, as the achieved F value of 36.49 was larger than the necessary F value of 4.21. This demonstrated statistically significant variations in the participants' post-test averages. Adjusted mean scores were determined, and statistical analysis was performed based on the differences in test results between the groups before and after therapy. Expert opinions, such as those of Pal, A., Srivastava, corroborate the conclusions as mentioned above (2011) The following table provides a visual representation of the mean values of the Experimental group and the Control group on LDL before and after the experiment, as well as after the adjustment. For the pre-test scores, the resulting F value of 0.49 was lower than the necessary F value of 4.2 for significance at the 0.05 level. This demonstrated that the randomization during the pre-test was fair and that there were no significant differences between the groups. The post-test scores study indicated a statistically significant difference between the groups, as the resulting F value of 41.58 was more than the minimum necessary F value of 4.21. This demonstrated that there were statistically significant variations in the participants' post-test averages. Adjusted mean scores were determined and statistical analysis was performed based on the differences in test results between the groups before and after therapy.The effects of yoga on participants' fasting blood sugar were measured using the specified biochemical variables, and the findings showed a statistically significant difference in favour of group 1. The hypothesis was therefore accepted with a degree of confidence of 0.05. Bijlani, R., an expert, also provides evidence supporting the conclusion above (2005).

THE HYPOTHESIS: A DISCUSSION

Males of retirement age would show a significant disparity between the yoga group and the control group regarding fasting blood sugar and low-density lipoprotein. At the 0.05 level of significance, the results of the tables suggested that yoga practises decreased biochemical variables such as blood sugar. In contrast, fasting and low-density lipoprotein connected to yogic practises among the elderly.

CONCLUSION

Among older males, yoga practices reduce biochemical variables such as blood sugar while fasting and low-density lipoprotein, according to the study's findings. Yogic practices might, thus, be beneficial to men of a certain age.

REFERENCES

1. Bijlani, R. L., Vempati, R. P., Yadav, R. K., Ray, R. B., Gupta, V., Sharma, R., ... & Mahapatra, S. C. (2005). A brief but comprehensive lifestyle education program based on yoga reduces risk factors for cardiovascular disease and diabetes mellitus. Journal of Alternative & Complementary Medicine, 11(2), 267-274.
2. Pal, A., Srivastava, N., Tiwari, S., Verma, N. S., Narain, V. S., Agrawal, G. G., ... & Kumar, K. (2011). Effect of yogic practices on lipid profile and body fat composition in patients of coronary artery disease. Complementary Therapies in Medicine, 19(3), 122-127.
3. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3576750/
4. https://www.sciencedirect.com/science/article/abs/pii/S1744388119309715
5. www.google scholar .com

Table 1. Analysis of covariance of the means of two experimental groups and the control group on LDL(mg/dl)

Test	Experimental group	Control Group	Source of Variation	Degrees of Freedom	Sum of Squares	Mean Sum of Squares	F-Ratio
Pre	116.07	111.33	**Between**	1	168.03	168.03	**1.02**
			Within	28	4602.27	164.37	
Post	90.00	113.67	**Between**	1	4200.83	4200.83	**36.49***
			Within	28	3223.33	115.12	
Adjusted Post	88.53	115.14	**Between**	1	5123.56	5123.56	**95.80***
			Within	27	1443.96	53.48	

* Significant at 0.05 level of confidence. (Table F ratio at 0.05 level, of confidence for df 1 and 28 = 4.2, 1 and 27 = 4.21)
The ordered adjusted means on LDL were presented through bar diagram for better understanding of the results of this study in Figure - 2.

Table 2. Computation of Mean and Analysis of Covariance of Blood Sugar (Fasting) of Experimental and Control Group

Test	Experimental Group	Control group	Source of variance	Df	Sum of square	Mean square	F
Pre-test mean	131.93	133.13	Between	1	10.80	10.80	0.49
			Within	28	620.67	22.17	
Post-test mean	122.53	132.13	Between	1	691.20	691.20	41.58*
			Within	28	465.47	16.62	
Adjusted mean	122.88	131.78	Between	1	583.86	583.86	62.06*
			Within	37	254.02	9.41	

* Significant at 0.05 level of confidence. (The table value required for significance at 0.05 with df 1and 28 and 1 and 27 are 4.2 and 4.21 respectively)

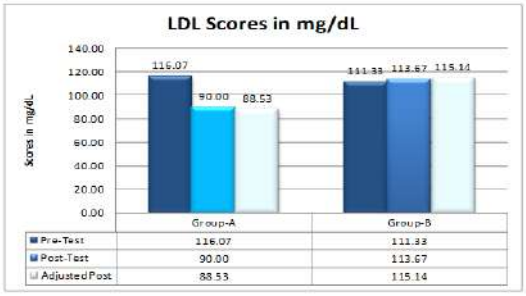

Fig. 1: bar diagram showing the mean differences among the groups on low density lipo protein (*LDL*) *(mg/dl)*

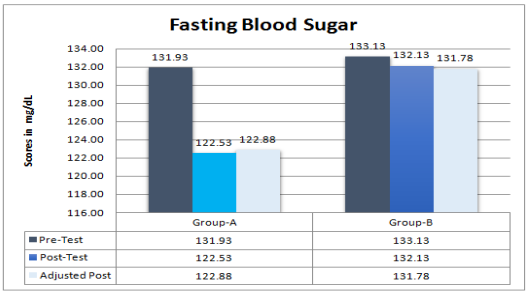

Fig 2: bar diagram showing the mean differences among the Groups on blood sugar (fasting)*(mg/dl)*

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RESEARCH ARTICLE

An Efficient Network Intrusion Detection System Model for Preventing Black hole attacks and Forging Attacks in MANETs

V.Deepa[1]* and N.Radha[2]

[1]Assistant Professor, PSGR Krishnammal College for Women, Coimbatore, Tamil Nadu, India.
[2]Associate Professor, PSGR Krishnammal College for Women, Coimbatore, Tamil Nadu, India.

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***Address for Correspondence**
V.Deepa,
Assistant Professor,
PSGR Krishnammal College for Women,
Coimbatore, Tamil Nadu, India.

ABSTRACT

MANET is a collection of mobile nodes which are connected to each other using wireless technology without any central communication control. As this network not having a fixed topology, and lack of infrastructure vulnerability occurs this leads to various kinds of attacks. Among all active attacks, most prominent attack in MANET is black hole attack. In this paper we proposed a security measure to prevent black hole attack and forging attack. In this paper black hole attack and forging attack are simulated in Network Simulator (NS2). The chosen attributes should be capable of illustrating network activity and emphasize the difference between normal and abnormal network activity. Each training instance contains summary statistics of network activity for the specified interval utilizing all the features as well as the type of attack carried out during this interval. A training dataset is built for each sampling interval time. The proposed Deep Learning model is enacted in a system with resource rich system and is trained using gathered dataset. Then, the created model utilized in cluster header for discovering malicious nodes that prevent black hole attack and forging attack in MANET.

Keywords: Black hole attacks, Forging attack, Deep Learning Model, Packet Transmission rate, cost function, miscellaneous node.

INTRODUCTION

Mobile Ad hoc Networks MANET, are made up of autonomous mobile nodes with a dynamic topology and no fixed infrastructure. Nodes in MANET can enter and exit the network at any time [1]. Network nodes can serve as hosts or routers. Nodes are communicating with each other by sending broadcasts message packets among them and thus form a network. MANETs are appropriate for a variety of applications because to their simplicity and versatility,

including emergency rescue operations, battlefield communication, and vehicle communication. In MANET due to the various constraints like noise, interference conditions the resources are restricted to some extent. Here are no authorization facilities because of any fixed network structure. Each and every node will act as both host and router. So here is lack of physical security, as a result the MANETs are very much prone to attacks .In MANETs the nodes that are not participated in particular broadcasting will be termed as sleeping nodes. Due to the sleeping nodes data transfer will be delayed. Wireless networks have different security requirements and concerns. The following are the issues that arise because of the characteristics and nature of wireless networks. (1) Inadequate infrastructure: Centralized, integrated structures like routers are not always present in wireless networks. Since all network nodes must function together, their security solutions are typically decentralized, distributed, and dependent on this [5]. (2) Using a wireless link: Unlike wired networks, wireless networks do not have common defense lines. Attackers don't require physical access to the link to target any node from any angle. (3) Multihopping: The nodes themselves serve as routers in the majority of wireless routing schemes, Therefore, it is impossible to trust every node for a task like routing. Due to these security issues, providing a secured environment in the MANET is a challenging concern. Nowadays most of the researchers focusing on developing an efficient intrusion Detection Systems. Intrusion Detection Systems in MANETs can be based on two categories.(i) Anomaly Detection(ii) Misuse detection. Anomaly detection means observing the patterns of behaviors of users and compared with the data taken. If any derivation from the base then it is consider as intrusion. Similarly in Misuse detection the system maintains a record of an established pattern of attacks and contrasts it with the information it has recorded. Each matching pattern is considered an incursion, and the appropriate action is taken.

Attacks in MANET

Attacks in MANETS are categorized as Active and Passive attacks. The attack in which the authorized node alters or destroys the data that is to be communicated in the network is called as Active attack [3]. The attack in which unauthorized node gets the data without disturbing the network operation is called as passive attack. In the network layer of MANET Flooding attacks, Grey Hole Attack, Black Hole Attack, Message Tampering, Byzantine attack are the possible attacks. In this paper the nature of black hole attack and forging attack and security measures are taken to prevent these attacks have been discussed.

Black hole attack

The Black hole attack comes under the category of Denial of Service (DOS) Attack, due to this attack the network's performance will be affected. A Black hole node is malicious, and it can drop the packets or forward them to an unknown or inexistent node [4]. Sending a false RREP promoting the quickest route to a destination is the initial step in the Black hole attack. After the malicious or black hole node establishes the connection, the source node then begins to transfer the data packets. The third and final step is when the black hole node starts discarding packets instead of forwarding them to their destinations. The black hole attack [3] can bypass the AODV protocol as once the source sends the routing request, the malicious node replies with the most significant sequence number (which indicates that route is fresh and valid) immediately without referring to its routing table so when the source receives the routing reply it chooses that path considering that routing discovery process was completed ignoring all routing replies from other destinations and started forwarding the data to the malicious node which in turn controls the direction of the traffic so that it can drop or deliver it somewhere as below in.

Forging attack

In this attack, the attacker can inject control packets using a constructed node's identity meanwhile keeping silent in order to interfere with the network's performance [8]. As a consequence, it cannot be recognized using the fundamental Sybil attack detection approaches, which rely on the simultaneous operation of the attacker and the fake identity. Every time the link breaks, the attacker also keeps altering the false identity.

Related Works

Prabhakar Reddy B Bhaskar Reddy B Dhananjaya B [1] proposed the AODV routing protocol with built-in security to counter black hole attack in MANET. This study promotes the use of cryptographic verification and threshold

evaluation in the OSPFV [for wireless LANs] protocol integration with built-in security. In this research work, two protocols, the postulated AODV-BS protocol and the black hole attack protocol are simulated on several MANET models, and two additional network metrics are employed. In order to determine the outcome, the network packet delivery ratio, the normalized out of routing overhead use and the network delay are determined. Khyati Mewada et [al] recommended [2] Mitigation of Black Hole Attack in AODV Protocol .In this work they have Enhance black hole AODV approach in that approach Leader nodes are employed for black hole attack detection . Leader nodes will broadcast a block message containing the id of the malicious node to all of their neighbors if packet loss exceeds a threshold of 10 (the threshold). The protocols are implemented in NS 2 tool and the parameters throughput and packet delivery ratios are measured.

Mai Mostafa Gaber, Marianne A. Azer [3], Black hole Attack effect on MANETs' Performance", recommended, the impact of the Black hole attack on MANETs using the AODV routing protocol is the main topic of this research. They compared the effect of the AODV routing system with and without the Black hole attack on single and multiple connections in random mobility. Packet Dropping Ratio, Routing Overhead, Throughput, and Packet Delivery Ratio were the measures they used to gauge network performance. Nan Kan ,Elhadi M Shak Shuki *et al* proposed Detecting Forged Acknowledgements in MANETs by examine the performance of the Digital Signature Algorithm (DSA) in MANET and integrate it into the EAACK scheme in this work. The goal of this work is to propose EAACK2, an enhanced version of EAACK that performs better when false misbehavior and partial dropping are present. Cyber Pulse++, a machine learning (ML)-based security framework that analyses gathered network statistics in real-time to detect aberrant path performance on network links using a pre-trained ML repository, was projected by Rasool *et al*. for release in 2021 [7].The IEAACK safe detection method for packet-dropping attacks was suggested in MANETs by Ms. Preeti Sharanvijay Chikkshetty *et al*. They looked into a brand-new MANET protocol called IDS-based EAACk, which comprises of ACK, S-ACK, and MRA to address every issue with the Watchdog approach in MANET IDS. An intrusion detection system based on fuzzy logic was proposed by B.V.R. Reddy and Ruchi Makni [12] to detect MANET attacks. They developed a strong IDS system, as well as a number of protocol features and fuzzy rules, to defend the network.

Proposed Method

This research work contributes the analyzing mechanism of Black hole attack and Forging attacks in MANET, and proposed a methodology to prevent those attacks. This research work has been divided in to three phases. In first phase 100 nodes are arranged in X and Y axis of NS2 environment. In the second phase the nodes are formed as clusters and cluster head has been created for each cluster. In the third phase Black hole attack and Forging attacks in MANETs is simulated using Network Simulation tool (NS2)and the attributes of each nodes are noted.

Experiment Analyses

In the Network Simulation tool (NS2) for these work 100 nodes are positioned on a 1000m flat plane. The nodes are positioned on the X and Y axis.

Node placements in network-Sample

```
set t 0
$node(0) set X_ [expr 80+rand()*1]
$node(0) set Y_ [expr 80+rand()*1]
$node(0) set Z_ 0
```

Thus the 100 nodes are placed on the plane. Radio propagation employs the Two Ray Ground model. Nodes move with an erratic 10 m/s speed. 802.11 are used for the Media Access Control protocol. Each node's communication range is 250 meters. After placing all the nodes, source node and destination nodes are selected randomly and path between the source node and destination nodes are calculated by searching the neighbor nodes.

```
puts "\n Neighbour node calculation"
```

```
foreacha $nodelist {
setneighbour($a) {}
for {set b 0} {$b<$val(nn)} {incrb} {
if {$a!=$b} {
set x1      [$node($a) set X_]
set y1      [$node($a) set Y_]
set x2      [$node($b) set X_]
set y2      [$node($b) set Y_]
set x       [expr ($x1-$x2)]
set y       [expr ($y1-$y2)]
set z       [expr (sqrt(($x*$x)+($y*$y)))]
#puts "Distance between node($i) and node($j) is $dis"
if {$z<=$dis} {
lappend neigh($a) $b
}
}
}
puts "Neighour nodes of Node $a is $neigh($a)"
setnlen($a) [llength $neigh($a)]
```

Thus the network has been created .Later the nodes are formed in to clusters and for each cluster, cluster head has been created by using cost functions. The cluster nodes will monitors their cluster members and within their members and if any miscellaneous nodes detected it will be removed from the path. The ad hoc routing protocol Ad hoc On Demand Distance Vector (AODV) is used in MANET for providing routing between the source and destination node and coding with Python. After making this arrangements in Network Simulator tool(NS2) environment, black hole attack and forging attack are simulated. A malicious node advertises fraudulent routing information in a black hole attack, which results in packets being received without being forwarded but instead being dropped. A malicious black hole node receiving an RREQ packet and sending an RREP (RouteREPly) packet to the target without first confirming that the node has a path to the target was the situation we simulated in the black hole attack[9]. As a result, the black hole node always responds first to an RREQ packet and discards any packets it receives in return. Additionally, the malicious-black hole node drops all RREP and data packets it receives if they are meant for other nodes. A simulated RERR (RouteERRor)[12] packet forging attack involves every malicious node altering and broadcasting (to a particular target) an RERR packet every 100 msec, which repeatedly causes links to fail thus the forging attacks are simulated.

After simulating these attacks, feature vectors are selected this will be used in the classification. The chosen features should be able to succinctly represent network activity, while differentiating between ‘‘normal’’ and ‘‘abnormal’’ activity. Thus the dataset has been created. The created datasets have been grouped in the following class labels, such as bandwidth, residual energy, traffic, hop count, Roundtrip Time, Total No. of packets dropped, Total No. of packets received, and Total No. of packets in communication. By using these attributes one training dataset was produced for each sampling interval. By considering these parameters and for each sampling interval time (5, 10, 15, 30 s) we have created one training dataset, Each training dataset was created by running different simulations with duration 100ms for different network mobility and varying numbers of malicious nodes (5, 15, 25). A similar procedure was followed in order to produce the testing datasets. The testing datasets were created using a similar procedure.

The Enhanced Deep Correlated Hierarchical Model, which is made up of the CCNN and BiLSTM algorithms, is then implemented using the resulting datasets. Cross Correlation layer h t=C(h (t-1)h t+b a) is used in Convolutional Neural Networks to extract spatial data, while BiLSTM is used in enhanced deep hierarchical models. To extract temporal features, use the forward and backward LSTMH t=[h t back ward, h t forward] formula. The output layer's

classification function, known as Softmax, learns and categorizes attack features. In the beginning, the FGSM approach uses the derived dataset to synthesize an adversarial sample. For each input in the dataset, this approach does a one-step gradient update along the gradient's significant direction. Subsequently, the back propagation algorithm prevailed. The prominence of each input feature is then determined by computing the gradient of the model prediction with respect to a specific input using the back propagation technique guided interpreters. The idea is that a feature's importance to the prediction is higher when the gradient magnitude is larger. The trust value rule was used after the dataset had been processed to identify the malicious node in the cluster.

The model will clearly learn the attributes of each and every node by applying the above mentioned parameters value and analyze which node is normal and which one is malicious. For example if the value of No of packets dropped is greater than the No of packet forwarded then that node will be suspected [7]. If the value of forwarded packet is zero then this attribute will referred to black hole attack. By analyzing the parameter value of hop count, residual energy ,no of packets in communication the black hole attack and forging attacks can be detected and also the malicious nodes on the particular path can be identified. Transfer Learning is a brand-new training algorithm that we recommend using it to train derived datasets and learn the principles of network intrusions from a base dataset. For the development and evaluation of the TL-MANET datasets, we construct an experimental platform. The outcome of our experiment revealed that the model is effective at finding fraudulent nodes, eradicating them, and then creating a new secure path and transferring data. Thus, it has been determined that adopting the suggested network intrusion detection system will result in great network performance and the prevention of attacks.

CONCLUSION AND FUTURE SCOPE

The main purpose of this paper is to provide an efficient method to detect a black hole attack and forging attack in MANET. The widespread dispersion of nodes and open medium make MANET vulnerable to malicious intruders. In this scenario, designing successful intrusion-detection systems is essential to defending MANET against threats [9]. For the goal of detecting and mitigating black hole attack and forging attack in MANET, an excellent Network Intrusion Detection System is recommended in this research. Attacks are simulated and attributes are learned to distinguish between normal and above-normal network activity in order to learn and train the deep learning model. The proposed Deep Learning model is enacted in a system with plenty of resource and is trained using retrieved dataset. Then, the created model applied in the cluster header for detecting malicious nodes that stops threats in MANET exhibited higher harmful-behavior-detection rates in some conditions while having minimal impact on the network performance. Future work on this project could include detecting and stopping unknown attacks in MANET and improving the environment's security.

REFERENCES

1. Prabhakar Reddy B Bhaskar Reddy B Dhananjaya B," The AODV routing protocol with built-in security to counter blackhole attack in MANET", MaterialToday Publishing, Vole 50,Part 5,page: 1152-1158.
2. KhyatiMewada et[al] , " Mitigation of Black Hole Attack in Aodv Protocol", International Journal of Research in Engineering and Technology, EISSN: 2319-1163.
3. Mai MostafaGaber, Marianne A. Azer[3]," Blackhole Attack effect on MANETs' Performance", International Mobile ,Intelligent and Ubiquitious Computing Conference 2022,IEEE.
4. Y. a. R. F. Ebazadeh, A reliable and secure method for network-layer attack discovery and elimination in mobile ad-hoc networks based on a probabilistic threshold vol. 5, no. 1, p. 183, 2020.
5. R. M. R. a. O. S. Khalladi, "A new efficient approach for detecting single and multiple black hole attacks," The Journal of Supercomputing, vol. 1, p. 19, 2021.
6. V. S. R. D. D. S. Rani P, " Mitigation of black hole attacks using firefly and artificial neural network," Neural Computing and Applications, vol. 1, p. 7, 2022.

7. Rasool, Raihan& Ahmed, Khandakar & Anwar, Zahid& Wang, Hua& Ashraf, Usman&Rafiq, Wajid. (2021). CyberPulse++: A Machine Learning Based Security Framework for Detecting Link Flooding Attacks in Software Defined Networks. International Journal of Intelligent Systems. 2021. 1-28. 10.1002/int.22442.
8. R Makani, BVR Reddy "Designing of Fuzzy Logic-Based Intrusion Detection System (FIDS) for Detection of Blackhole Attack in AODV for MANETs", Cyber Security and Digital Forensics, 2022 – Springe
9. N. D. T. a. E.-N. H. Tran Hoang Hai, "Performance Evaluation of Aodv and Aomdv Routing Protocols Under Collaborative Blackhole and wormhole Attacks," in Advances in Computer Science and Ubiquitous Computing, Springer, Singapore, 2021.
10. MsPreetiSharanvijayChikkshetty, Prof Abhijit V. Mophare, Prof Amarnath Shivan and Chadchankar, "IEAACK-Secure Detection System for Packet-Dropping Attack in Manets, Published 2015
11. Nan Kan ,Elhadi M ShakShuki et[al] proposed Detecting Forged Acknowledgements in MANETs, International Conference on Advanced Information Networking and Application,2011
12. R Makani, BVR Reddy "Designing of Fuzzy Logic-Based Intrusion Detection System (FIDS) for Detection of Blackhole Attack in AODV for MANETs", Cyber Security and Digital Forensics, 2022 – Springe
13. Singh, P.K., Sharma, G.: An efficient prevention of black hole problem in AODV routing protocol in MANET. In: 2012 IEEE 11th International Conference on Trust, Security and Privacy in Computing and Communications (TrustCom), pp. 902–906. IEEE (2012).
14. C. Panos, C. Xenakis, P. Kotzias and I. Stavrakakis, "A specification-based intrusion detection engine for infrastructure-less networks," Computer Communications, 54, 67-83, 2014.

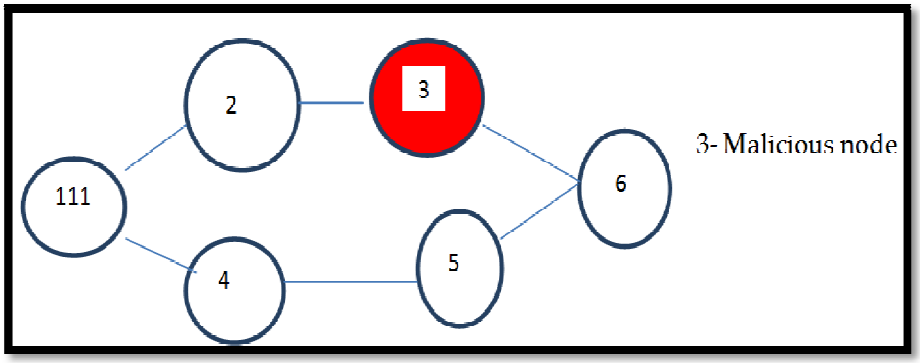

Figure 1: Blackhole attack

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RESEARCH ARTICLE

Pell Labeling of Certain Classes of Graphs

J. Rashmi Kumar[1]* and N. Ramya[2]

[1]Assistant Professor, Department of Mathematics, Bharath Institute of Higher Education and Research, Chennai, Tamil Nadu, India.
[2]Associate Professor, Department of Mathematics, Bharath Institute of Higher Education and Research, Chennai, Tamil Nadu, India.

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***Address for Correspondence**
J. Rashmi Kumar,
Assistant Professor,
Department of Mathematics,
Bharath Institute of Higher Education and Research,
Chennai, Tamil Nadu, India.
Email: rashmilenny@gmail.com

ABSTRACT

In this paper we develop SD of the edges of the star $k_{1,n}$, the graph ($k_{1,n}^{(1)}$, $k_{1,n}^{(2)}$) ,two copies of SD of the edges of the star $k_{1,n}$, theta graph T_{α} , Bull graph, Moser spindle graph, Herschel graph are pell graph.

Keywords: Pell graph , star $k_{1,n}$, Theta graph , Bull graph. AMS classification : 05C78.

INTRODUCTION

We examine graphs that are simple, undirected and finite .We follow Gallian[1] and Hararay [2] for all expression and notation. Shiama [3] introduced pell labeling. Celin mary[7], Avudainayaki, Selvam[8] and Sriram [9] have investigated for certain graphs. In this paper we discus spell labeling for few graphs.

1.1.Preliminaries

The basic concepts of pell labeling, star graph (SG), Subdivision (SD), Duplication, Theta graph, Moser spindle graph (MS), Herschel graph (HG), Bull graph (BG) are referred [3],[13], [17], [4], [5], [16], [12].

Theorem 2.1: The SD of edges of the star $k_{1,n}$ acknowledges pell labeling.
Proof : Let G = (V,$\hat{E}$) be a graph acquired by the SD of edges of the star graph $k_{1,n}$ with the vertex set V(G) = { v,u_j ,w_j / 1≤j≤n } , edge set $\hat{E}$(G) = { vw_j , $w_j u_j$ / 1≤j≤n }.

$|V(G)| = 2n+1$ and $|\hat{E}(G)| = 2n$ .Vertex labeling is established as

$f(v) = 0$

$f(u_j) = 2j$, $1 \le j \le n$

$f(w_j) = 2j-1$, $1 \le j \le n$

For the above the labeling of the edge $f^* : \hat{E}(G) \to N$ is bounded by $f^*(uv) = f(u) + 2f(v)$ for all uv belongs to $\hat{E}(G)$ is given by

$f^*(vw_j) = 4j-2$

$f^*(w_ju_j) = 6j-1$

The entire 2n edges are distinct.

Theorem 2.2 : The graph ($k_{1,n}^{(1)}$, $k_{1,n}^{(2)}$) admits pell labeling.

Proof: Regard the graph G with vertex set u_i ,v_i ,u,v for $1 \le I \le n$,

the edge set as{wu, wv, uu_i ,vv_i / $1 \le j \le n$ } .

Obviously , $|V(G)| = 2m+3$ and $|\hat{E}(G)| = 2m+2$. Define a mapping g:V→{0,1,2……..p-1} as

$g(w) = 0$

$g(u) = 1$

$g(v) = 2$

$g(v_j) = 2j+2$

$g(u_j) = 2j+1$

The labeling of edges $f^* : \hat{E}(G) \to N$ is determined by $f^*(uv) = f(u) + 2f(v)$ for all uv€$\hat{E}$(G) are all distinct

$f^*(wu) = 2$

$f^*(wv) = 4$

$f^*(uu_i) = 4j+3$

$f^*(vv_i) = 4j+6$

Therefore the graph ($k_{1,n}^{(1)}$, $k_{1,n}^{(2)}$) admits pell labeling.

Theorem 2.3: Two copies of SD of edges of the star $k_{1,n}$ is pell graph.

Proof : Consider the graph G be the SD of the edges ($k_{1,n}^{(1)}$, $k_{1,n}^{(2)}$) admits pell labeling. Let w , u, v , u_j , v_j, u_j' , v_j' ,w' , w'' be vertex set of G and edge set $\hat{E} = \hat{E}_1 \cup \hat{E}_2 \cup \hat{E}_3$,

where

$\hat{E}_1$ = { ww' , ww'' , uw' , vw'' }

$\hat{E}_2$ = { uu_j' ,vv_j' / $1 \le j \le n$ }

$\hat{E}_3$ = { $u_j'u_j$, $v_j'v_j$/ $1 \le j \le n$ }

Determine the vertex valued for f : v →{ 0,1,……p-1} as

$f(w) = 0$

$f(u) = 1$

$f(v) = 2$

$f(u_j) = 4j+1$, $1 \le j \le n$

$f(v_j) = 4j+2$, $1 \le j \le n$

$f(u_j') = 4j-1$

$f(v_j') = 4j$

$f(w') = f(u_n) + 2$

$f(w'') = f(v_n) + 2$

and the edge labeling $f^* : \hat{E} \to N$ is set outas

$f^*(ww') = 2[f(u_n) + 2]$

$f^*(ww'') = 2[f(v_n) + 2]$

$f^*(uw') = 2\,f(u_n) + 5$

$f^*(vw'') = 2\,f(v_n) + 6$

$f^*(uu_j') = 8j-1$

$f^*(u_j'u_j) = 12i+1$

$f^*(vv_j) = 8i+2$
$f^*(v_j'v_j) = 12i+4$
Since $\hat{E}_1 \neq \hat{E}_2 \neq \hat{E}_3$, G admits pell labeling.

Theorem 2.4 : The theta graph T_α admits pell labeling.
Proof : Let T_α be the graph with centre v_0 and w_1, w_2 , ……..w_6 be the vertices and
{ w_jw_{j+1} , w_0w_1, w_0w_4 , w_1w_6 / $1 \leq j \leq 5$ } be the edges of the theta graph T_α .
The vertex function of T_α defined by $f : v \rightarrow \{0,1,\ldots.6\}$ as
$f(w_j) = j-1$, $1 \leq j \leq 5$
$f(w_0) = 6$
The edge labeling are
$f^*(w_jw_{j+1}) = 3j-1$, $1 \leq j \leq 5$
$f^*(w_0w_1) = 2\, f(w_0)$
$f^*(w_0w_4) = 12$
$f^*(w_1w_6) = 10$
The edge labeling $f^*\hat{E} \rightarrow N$ specified by $f^*(yz) = f(y) +2f(z)$, yz€$\hat{E}$ are all distinct.

Theorem 2.5 : BG(C_3) is pell graph.
Proof : Let BG(C_3) graph has 5 vertices and 5 edges . Define the vertex valued function as follows :
$f(v_i) = i-1$, $1 \leq i \leq 5$
and the edge labeling $f^* : \hat{E} \rightarrow N$ is bounded as
$f^*(v_iv_{i+1}) = 3i-1$, $1 \leq i \leq 4$
$f^*(v_2v_4) = 7$
The entire 5 edge labelings are distinct.

Theorem 2.6 : The duplication of the pendant vertex by the edge of BG(C_3) admits pell labeling.
Proof : Let G have vertex set $V(G) = \{ v_j , v_1' , v_1'' , v_5' , v_5'' / 1 \leq j \leq 5 \}$,
$\hat{E}(G) = \{ v_jv_{j+1} , v_1v_1' , v_1v_1'' , v_1'v_1'' , v_5v_5' , v_5v_5'' , v_5'v_5'' , v_2v_4 / 1 \leq j \leq 4 \}$
$|V(G)| = 9$ & $|\hat{E}(G)| = 11$
Specify f: $V(G) \rightarrow \{0,1,\ldots..8\}$ as
$f(v_j) = j-1$, $1 \leq j \leq 5$
$f(v_1') = 5$
$f(v_1'') = 7$
$f(v_5') = 6$
$f(v_5'') = 8$
and f^*yields the labeling of edged as
$f^*(v_jv_{j+1}) = 3j-1$
$f^*(v_1v_1') = 10$
$f^*(v_2v_4) = 7$
$f^*(v_1'v_1'') = 19$
$f^*(v_1v_1'') = 14$
$f^*(v_5v_5') = 16$
$f^*(v_5'v_5'') = 22$
$f^*(v_5v_5'') = 20$
All the edge labeling in $\hat{E}(G)$ are distinct.

Theorem2.7 : The MG is a Pell labeling graph
Proof : The MG has 7 vertices and 11 edges . Let u_k be the vertex set for $0 \leq k \leq 6$. Vertex function f and edge function f^* is established as
$f(u_k) = k$, $0 \leq k \leq n-1$

$f^*(u_0u_k) \equiv 0(\text{mod } 2)$ k = 1,2,5,6
$f^*(u_6u_k) \equiv 0(\text{mod } 2)$ k = 4,5
$f^*(u_3u_4) = 11$
$f^*(u_4u_5) = 13$
$f^*(u_2u_3) = 8$
$f^*(u_1u_2) = 5$
$f^*(u_1u_3) = 7$
Hence the theorem.

Theorem 2.8 : The HG satisfies Pell Labeling
Proof : Consider the HG with 11 vertices and 18 edges. Let v_j be the vertex set for $0 \le j \le 10$. Then the vertex and edge function f and f^* are respectively defined as
$f(v_j) = j$, $0 \le j \le 10$
$f^*(v_0v_j) \equiv 0(\text{mod } 2)$, j = 1,2,3
$f^*(v_jv_{j+3}) \equiv 0(\text{mod } 9)$, j = 1,4,7
$f^*(v_jv_{j+3}) \equiv 0(\text{mod } 3)$, j = 3,6
$f^*(v_2v_j) \equiv 0(\text{mod } 2)$, j = 4,6
$f^*(v_1v_5) = 11$
$f^*(v_3v_5) = 13$
$f^*(v_4v_8) = 20$
$f^*(v_5v_7) = 19$
$f^*(v_5v_9) = 23$
$f^*(v_6v_8) = 22$
$f^*(v_8v_{10}) = 28$
$f^*(v_9v_{10}) = 29$.
Hence the theorem is proved.

CONCLUSION

In this work we proved that the SD of the edges of the star $k_{1,n}$, the graph ($k_{1,n}^{(1)}$, $k_{1,n}^{(2)}$) , two copies of SD of the edges of the star $k_{1,n}$. Theta graph and DG are pell graph. One can also explore the exclusive application of pell labeling in real life problems. They provide a larger scope for strong research in this area.

REFERENCES

1. J.A.Gallian , "A dynamic survey of graph labeling" , The electronics journal of combinatories , 17(2010) , #DS6.
2. F. Harary ,"Graph theory" , Narosa publication House reading , New Delhi , 1998.
3. J.Shiama , "Pell labeling for some graphs" , Arian journal of current Engineering and Maths 2(2013) , 267-272.
4. K.Bhuvaneswari , P.Jagadeeswari, " Square difference for some path union and duplication of graphs " , IJERT ,ISSN : 2278- 0108, vol 8 , Issue 10 , oct 2019.
5. G.Subashini , K.Bhuvaneswari , K. Manimekalai , "Square difference labeling of theta graphs " , IJERT , ISSN : 2278-0108, vol 8 , Issue 9, Sep 2019.
6. J.Shiama , "pell labelling for some graphs" , Asian Journal of current Engineering and maths , 2(4)(2013).
7. V.Celin Mary et al., "Some Graph Labeling on Inflated Graphs of Triangular snake and Alternate Triangular snake Graph" ,Amals of pure and Applied mathematics, vol 14 , No.3, 2017, 547-554.
8. R.Avudainayaki and B. Selvam, "Harmonious and pell Labeling for some extended Duplicate Graph" , International Journal of scientific Research in mathematical and statistical sciences ., vol.5 , Issue – 6 , pp. 152-156 , Dec-2018.

9. S.Sriram et al.,"pell Labeling of Joins of square of path graph" , International journal of Engineering and Advanced Technology , ISSN : 2249-8958 , vol.9 , Issue 153 , Dec 2019.
10. P.Indira , B.Selvam andK.Thirusangu, "Pell labeling and mean square sum labeling for the extended duplicate graph of quadrilateral snake " , Advances and applications in mathematical sciences,Vol 20 , Issue 9, July 2021, Pages 1709-1718.
11. A.Rosa, On certain valuations of the vertices of the graph, Theory of graphs (International symposium, Rome , July 1966) , Gordon and Breech , N.Y. and Dunodparis(1967) , 349-355.
12. P.Jagadeeswai, "Considering Square difference labeling for validating Theta graphs of dynamic machinaries" , International journal of Innovative Technology and Exploring Engineering, ISSN : 2278-3075, vol-9, Issue-2S4 dec2019.
13. J.JebaJesintha, K.Ezhilarasi, Hilda, "International journal of pure and applied mathematics", vol 101, No 6, 2015, 949-956.
14. P. Jagadeeswari, K. Ramanathan and K. Manimekalai, "Square Difference Labeling for pyramid graph and its related graphs", International journal of Mathematics And its Applications, 6(1), 91–96 (2018).
15. G.V.Ghodasara, Mitesh.J.Patel, "Some bistar related square sum graph", International journal of mathematics Trends and Technology (IJMTT) – vol 47 no.3, July 2017, ISSN 2231-5373.
16. Dr.A.MydeenBibi, M.Malathi, "Equality labeling on special graphs " , International journal of modern trends in Engineering and Research, vol 5 , Issue 04 (Apr 2018) ISSN(online) 2349-9745 ISSN(print) 2393-8161.
17. V.Annamma, N. Hameeda Begum, "Prime labeling of some star related mirror graphs" , International journal of mathematics and computer science, 15(2020) no.4, 1207-1211.

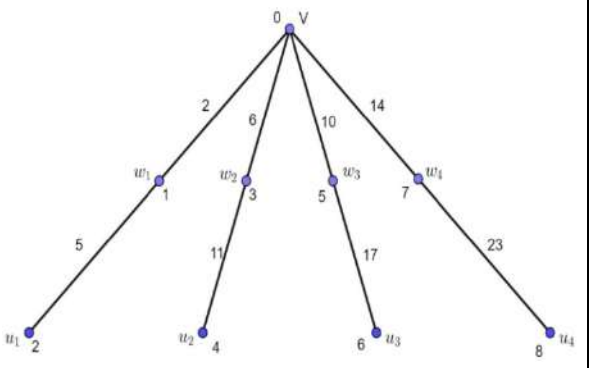

Figure 1. SD of the edges of the star K(1,4)

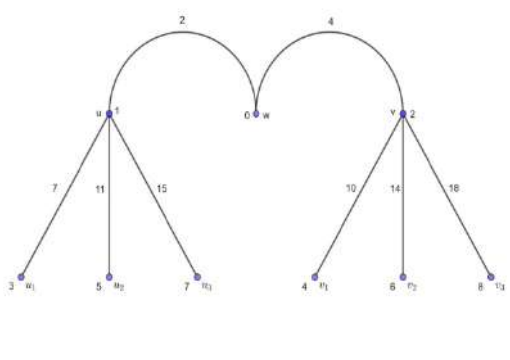

Figure 2. ($K_{1,3}^{(1)}$, $K_{1,3}^{(2)}$)

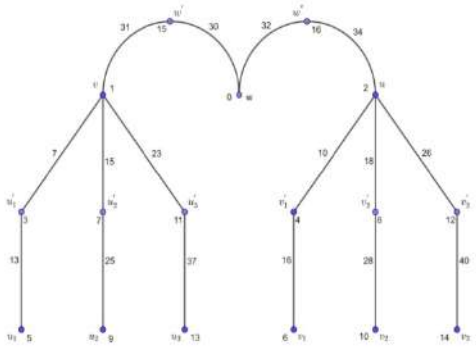

Figure 3.Two copies of SD of the edges of the star $k_{1,3}$

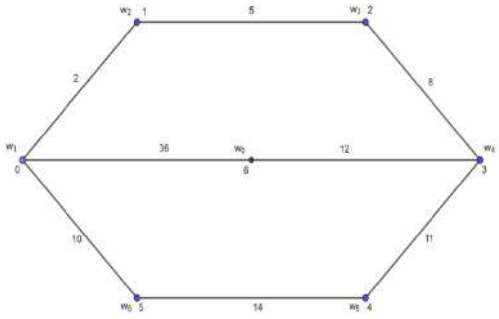

Figure 4. T_α

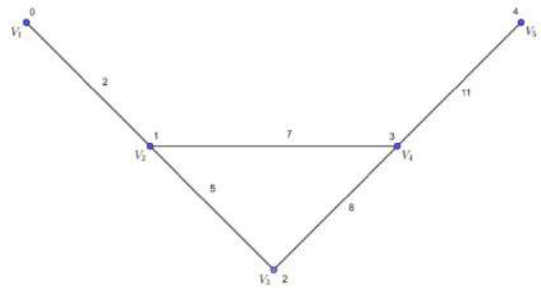

Figure 5. BG (c_3)

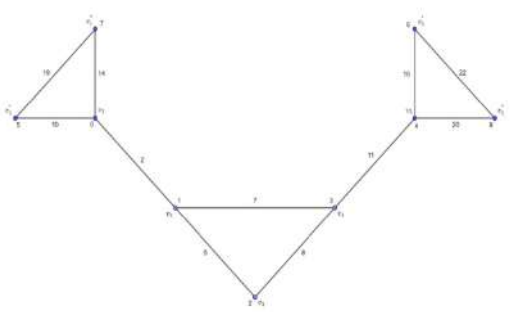

Figure 6. : The duplication of the pendant vertex by the edge of BG (C_3)

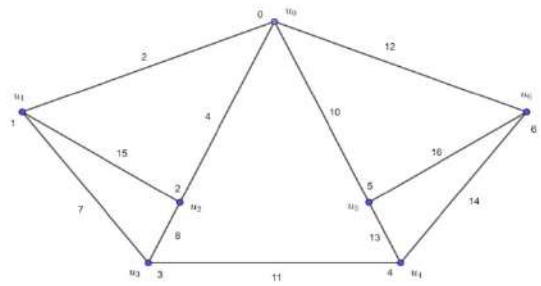

Figure 7. MG

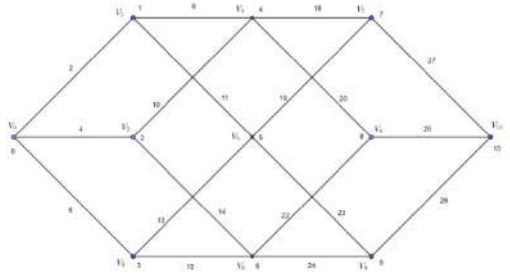

Figure 8.HG

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RESEARCH ARTICLE

Factors Influencing Farmers Participation in Groundwater market in Cuddalore District of Tamil Nadu

R.Bala[1]*, R.Venkataraman[2] and S.Ravichandran[3]

[1]Research Scholar, Department of Agricultural Economics, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India.
[2]Professor, Department of Agricultural Economics, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India.
[3]Associate Professor, Department of Agricultural Economics, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India.

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***Address for Correspondence**
R.Bala,
Research Scholar,
Department of Agricultural Economics,
Annamalai University,
Annamalai Nagar,
Chidambaram, Tamil Nadu, India.
Email: balarameshbabu2011@gmail.com

ABSTRACT

Water markets in India are unorganized and informal in structure formed by farmer's initiatives. Groundwater markets are nothing but facilitating the sale of surplus/extra water for money or in kind with mutual cooperation of buyers and sellers. The small and marginal farmers with fragmented land holding are not able to make such investment so they rely on groundwater markets for their irrigation. These markets are important in areas which depend heavily on groundwater for irrigation. They enable the farmers to access the irrigation water at a minimal cost. Thus, converts irrigation water as an economic resource based on the degree of scarcity. The Groundwater market participation was influenced by various factors such as education, fragmented landholdings, farm size etc. Seller may got ancillary revenue from the transaction of water market. Buyer who were lack of water lifting devices were benefitted from water market participation. Thus, the water market participation benefitted both the buyers and sellers categories. This study aims to analyse the various factors / determinants that influences farmer's participation in water markets in Cuddalore district. Farmer's decisions to participate in water markets are heavily influenced by their education. The probability of purchasing groundwater decreases as farm size increases. The farmers with high hp power motors have a greater chance of selling groundwater to others.

Keywords: Groundwater, water market, market participation, education, farm size.

INTRODUCTION

Water is essential not only for the survival of all living beings but also for socio-economic development of households, communities and nations all over the world. Water plays a significant role in agriculture and food production. Out of the freshwater resources, 70% of water is used for agricultural purpose. Both the Surface and groundwater resources are utilized for agricultural purpose. Due to the failure of monsoons, drought and famine the dependence of farmers on groundwater resources were increased [1]. Groundwater markets are nothing but facilitating the sale of surplus / extra water for money or in kind with mutual cooperation of buyers and sellers. Water markets expanded mainly due to the spontaneous development and expansion of tube well irrigation. But this type of arrangement require huge initial investment for the installation of pumps needed for lifting of groundwater for irrigation. The small and marginal farmers with fragmented land holding are not able to make such investment so they rely on groundwater markets for their irrigation. Thus, converts irrigation water as an economic resource based on the degree of scarcity. Water pricing is used as a control measure for groundwater extraction. The prices are fixed based on the demand and supply of water. There are different types of water pricing method which are all framed on area, time and volume basis. Water markets in India are unorganized and informal in structure formed by farmer's initiatives [2]. The transaction arrangement is through mutual cooperation between buyers and sellers. Water rights in these markets are not clearly defined. In addition, water markets have promoted water-use efficiency by allocating this resource to high-value uses [3,4]. As a result, water markets appear to be helpful to society in a variety of ways. The elements that determine farmer's participation in groundwater markets are investigated in this article. The findings are expected to aid policymakers in developing measures to address barriers related to farmers' involvement in the water market.

MATERIALS AND METHODS

Location

The study was conducted in Annagramam and Cuddalore blocks of Cuddalore district in Tamil Nadu. The purposive selection was made for the reason that the number of tube wells and area irrigated under the groundwater were the highest in this district. In terms of groundwater extraction Annagramam block falls under semi-critical (70-90 per cent of draft) category and Cuddalore falls under over – exploited (more than 100 per cent draft) category as per the stage of groundwater development status.

Data Collection

A sample size of 200 groundwater-dependent farmers were equally allocated between the two selected blocks as 100 each. In each block two villages where agricultural activities where hectic were purposively selected and the sample size of 100 was distributed as probability proportion to number of tube wells. The sample was later post stratified as: self-users, water sellers and water buyers for further analysis.

Tools of Data Analysis

The buying and selling of groundwater being dichotomous dependent variables, their determinants were assessed using the logit model. The model postulated that P_i, the probability that i^{th} farmer would sell or buy groundwater was a function of an index variable Z_i, summarizing a set of the explanatory variables. In fact, Z_i was equal to the logarithm of the odds ratio, i.e., the ratio of probability that a farmer would sell or buy groundwater to the probability that he would not sell or buy groundwater and it could be estimated as a linear function of explanatory variables (X_{ki}). These could be mathematically expressed as:

$$P_i = F(Z_i) = F(X_i) = \frac{1}{1+e^{-zi}}$$

$$Z_i = \ln\left\{\frac{pi}{1-pi}\right\} = \alpha + \sum_{k=1}^{M} \beta_i X_{ki}$$

Where,
X_{ki} = The k^{th} explanatory variable of the i^{th} farmer
i = 1, 2,..., N, where, N was the number of farmers
k = 1, 2,..., M, where, M was the total number of explanatory variables
a = constant and
b = An unknown parameter

With the specification of logit model above, it is hypothesized that the probability of a farmer buying or selling groundwater depends on the total operational holding in acres (X_{1i}), Number of land fragments per farm (X_{2i}), Gross cropped area under sugarcane/banana cultivation in percent (X_{3i}), education of the farmer (X_{4i}), Installed horsepower of the water lifting device / irrigation cost (X_{5i}), Agricultural credit availability (X_{6i}). The indexed variables Z_i indicating whether a farmer bought and sell groundwater or not, is expressed as a linear function of the above listed variables as

$$Z_i = a + b_1X_{1i} + b_2X_{2i} + b_3X_{3i} + b_4X_{4i} + b_5X_{5i} + b_6X_{6i} + U_i$$

$$Z_i = \theta_0 + \theta_1(\text{AREA OWN}) + \theta_2(\text{FRAGMENT}) + \theta_3(\text{PGCASC/PGCABC}) + \theta_4(\text{EDUCATION}) + \theta_5(\text{HPWLD}) + \theta_6(\text{AGLCA}) + U_i$$

Where,

Z_i = Binary variable. (1 = if participated in water markets, zero otherwise)

U_i = Disturbance term.

AREA OWN = Land owned (in acres)

FRAGMENT = Number of fragments per farm

PGCASC = Gross cropped area under sugarcane/banana cultivation in percent

EDUCATION = Years of schooling

*HPWLD/IC = Installed horsepower of the water lifting device / irrigation cost

AGLCA = Agricultural credit availability

Except AGLCA all other variables are continuous. AGLCA takes a value of 1 if the farmers get the credit, zero otherwise. *HPWLD for sellers and IC for buyers.

RESULT AND DISCUSSION

Literacy Rate in the Selected Blocks

The literacy rate was higher in cuddalore block (73.17%) compared to that of annnagramam block (66.97%). The rural literacy rate was greater in annagramam block but actually the population was higher in rural areas of cuddalore block. The female literacy rate was higher in cuddalore block(68.08%). The gap between male and female literacy rate was greater in annagramam block (14%) compared to that of cuddalore block(10.21%).

Land Holding Particulars of the Selected Blocks

The land holding particulars of Cuddalore and Annagramam blocks are presented in the Table 2. The size of holdings of the selected blocks are classified under 3 categories namely marginal and small farmers, medium farmers and big farmers. It could be observed from table that more than 90 per cent of the farmers in both Cuddalore and Annagramam block were small and marginal farmers. However the proposition of small and marginal farmers was marginally higher in Annagramam block and the proposition of big farmers was found higher in Cuddalore block.

Structure of Water Market

The farm size distribution is presented in Table 3. It could be observed from the table that the category of small and marginal famers were higher in both the blocks followed by medium and big farmers. Most of the water buyers belonged to small and marginal farmer category and in the case of sellers they belonged to medium and big farmer category. From the table it could be observed that the marginal farmers are lacking access to their own groundwater

devices due to lack of financial resources. Overall it was observed that 62 per cent of sample farmers in cuddalore block and 70 per cent of sample farmer's annagramam block were participated in ground water market activity either as buyer or seller. Most of the farmers in seller category belong to big farmer's category.

Determinants of Groundwater Selling

The logit regression analysis presented in table 4 revealed that the farm size, number of fragments, education, and hp motor power were the most important elements influencing farmers' water selling decisions. The probability of selling increased by 0.06 times in Cuddalore block and 0.08 times in Annagramam block for every unit increase in farm size. The next contributing element was the number of fragmented landholdings; farmers with fewer fragmented landholdings had a higher chance of selling groundwater in Cuddalore block. The education coefficient was predicted to be 0.08 in Cuddalore block and -0.011 times in Annagramam block. This means that a farmer's decision to sell is heavily influenced by his or her education. The farmers with high hp power motors have a greater chance of selling groundwater to others.

Determinants of Groundwater Buying

From table 5 farmer's water buying activity was influenced by variables such as farm size, number of fragments, education, and irrigation cost on the buyers' side. The probability of purchasing groundwater decreased as farm size increased, with a one-unit increase in farm size lowering the probability by 0.047 percent in Cuddalore block and 0.005 percent in Annagramam block. The increase in the number of land fragments have a greater impact on water buying. Farmer's decisions to participate in water markets are heavily influenced by their education. Farmer's gross revenue has been influenced by irrigation costs. Irrigation cost plays a major role in farmer's decision to participate in water markets.

CONCLUSION

Small and marginal farmers were found to be majority in the research area. Overall it was observed that 62 percent of sample farmers in cuddalore block and 70 percent of sample farmer's Annagramam block were participated in ground water market either as buyer or seller. Seller may got ancillary revenue from the transaction of water market. Buyer who were lack of water lifting devices were benefitted from water market participation. Thus, the water market participation benefitted both the buyers and sellers categories and also made the resource equally distributed among the market participants. The probability of selling increased by 0.06 times in Cuddalore block and 0.08 times in Annagramam block for every unit increase in farm size. The education coefficient was predicted to be 0.08 in Cuddalore block and -0.011 times in Annagramam block that concluded farmer's decision strongly influenced by education. The probability of purchasing groundwater decreased as farm size increased, with a one-unit increase in farm size lowering the probability by 0.047 percent in Cuddalore block and 0.005 percent in Annagramam block. The increase in the number of land fragments have a greater impact on water buying. A proper legal system should be put in place to protect the rights of both buyers and sellers in water markets, as well as to prevent over-exploitation of our country's available water resources. Human factors such as trust, faith and willingness to help play an important role in the establishment of a water market in India.

REFERENCES

1. Giordano Mark (2009) "Global Groundwater? Issues and solutions"*Annual Review of Environment and Resources,* 34[1]: 153-178.
2. Kajisa, K. and Sakurai, T. (2005) "Efficiency and equity in groundwater markets: The case ofMadhya Pradesh, India". *Environment and Development Economics*, 10:801-819.
3. Nagaraj, N., Suvarna Kumar, A.H. and Chandrakanth, M.G. (2005) "Economic analysis of groundwater markets in central dry zone of Karnataka". *IWMI-Tata Water Policy Research* Highlight No.13, available at http:// purl.umn.edu/53071

4. Manjunathaa A V, Anikb A R, Speelmanc S and Nuppenaud E A (2014) "Farmers'participation in informal groundwater market in hard rock areas of peninsular India". *Agri Econ Res Rev* 27: 45-54.
5. Meinzen-Dick R (1996) "Groundwater Markets in Pakistan: Participation and Productivity". IFPRI (Mimeo), Washington, DC.
6. Molle, F., Shah, T and Barker, R (2003) "The groundswell of pumps: multilevel impacts of asilent revolution". *ICID Asian Regional Workshop on Sustainable Development of Water Resources and Management and Operation of Participatory Irrigation Organisations*. 10-12 November, Taipei, Taiwan.
7. Mukherji A, Shah T (2004) "Socio-ecology of groundwater irrigation in Asia: an overview of issues and evidence". *IAH Hydrogeology Selected Papers*, Balkema.
8. Saleth R M (1996). "Water Institutions in India: Economic, Law and Policy". *Commonwealth Publishers, New Delhi.*
9. Sharma, P. and Sharma, R.C. (2006) "Factors determining farmers' decision for buying irrigation water: Study of groundwater markets in Rajasthan". *Agricultural Economics Research Review*, 19:39-56.
10. Vasanth, P.Gandhi and Vaibhav Bhamoriya (2011). "Groundwater Irrigation in India-Growth, Challenges and Risk". *India Infrastructure Report (2011)*. 97-110.

Table 1. Literacy Rate of the Selected Blocks

Sector	Cuddalore Block			Annagramam Block		
	Male	Female	Total	Male	Female	Total
Rural	83353 (42.29)	68985 (34.77)	152338 (38.52)	47542 (49.10)	37191 (38.37)	84733 (43.73)
Urban	70949 (36.00)	66082 (33.31)	137031 (34.65)	24145 (24.93)	20889 (21.55)	45034 (23.24)
Total literate population	154302 (78.29)	135067 (68.08)	289369 (73.17)	71687 (74.03)	58080 (59.92)	129767 (66.97)
Total block Population	197071 (100.00)	198366 (100.00)	395437 (100.00)	96817 (100.00)	96921 (100.00)	193738 (100.00)

Source : Statistical Hand Book of Cuddalore and Annagramam Block, Block Statistical office, Cuddalore

Table.2 Size of Land holdings of the Selected Blocks

S. No	Size of the Farm (in ha)	Cuddalore Block		Annagramam Block	
		Number	Area (ha)	Number	Area (ha)
1	Small and Marginal farmers (<2 ha)	15175 (90.18)	7788.56 (53.86)	11587 (91.67)	6294.185 (62.53)
2	Medium farmers (2-5ha)	1260 (7.48)	3753.015 (25.95)	898 (7.10)	2602.275 (25.85)
3	Big farmers (>5 ha)	391 (2.32)	2917.325 (20.17)	154 (1.21)	1168.365 (11.60)
4	Total	16826 (100.00)	14458.90 (100.00)	12639 (100.00)	10064.825 (100.00)

Source : Statistical Hand Book of Cuddalore and Annagramam Block, Block Statistical office, Cuddalore

Table 3. Structure of Water Market

Land holdings	Self user	Buyer	Self user+seller	Self user+Buyer	Total
Cuddalore block					
Marginal farmer	16(42)	14(100)	-	3(18.75)	33(33)
Small farmers	8(21)	-	2(6.25)	13(81.25)	23(23)
Medium farmers	10(26.31)	-	26(81.25)	-	36(44)
Big farmers	4(10.52)	-	4(12.50)	-	8(8)

Total	38(38)	14(14)	32(32)	16(16)	100(100)
Annagramam block					
Marginal	3(10)	28(84.84)	-	2(50)	33(33)
Small farmers	19(63.33)	5(15.15)	12(36.36)	2(50)	38(38)
Medium farmers	6(20)	-	18(54.54)	-	24(24)
Big farmers	2(6.66)	-	3(9.09)	-	5(5)
Total	30(30)	33(33)	33(33)	4(4)	100(100)

Table 4. Logistic regression analysis of factors affecting selling of groundwater

Variables	Cuddalore Block		Annagramam Block	
	Co-Efficient	Standard error	Co-Efficient	Standard error
AREA OWN	0.067*	0.044	0.084*	0.055
FRAGEMNT	-0.088*	0.063	-0.122	0.098
PGCASC	-0.128	0.080	0.197	0.122
EDUCATION	0.008**	0.005	0.011*	0.008
HPWLD/IC	0.043**	0.009	0.068*	0.013
AGLCA	-0.054	0.087	-0.109	0.091
Intercept	1.050	0.553	0.135	0.726

*** = 1% level of significance, ** = 5% level of significance, * = 10% level of significance

Table 5. Logistic regression analysis of factors affecting buying of groundwater

Variables	Cuddalore Block		Annagramam Block	
	Co-Efficient	Standard error	Co-Efficient	Standard error
AREA OWN	-0.047**	0.051	-0.005***	0.036
FRAGEMNT	0.035**	0.093	0.054**	0.094
PGCASC	0.053	0.122	-0.118	0.115
EDUCATION	-0.007***	0.004	0.001***	0.004
HPWLD/IC	-0.050**	0.014	-0.064*	0.007
AGLCA	0.084	0.096	-0.014	0.063
Intercept	0.638	0.426	0.961	0.379

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RESEARCH ARTICLE

Breeding Details of Six Waterbird Species at the Vaduvur Lake, Tamil Nadu, India

Varadharajan Gokula*

Associate Professor and Head, PG and Research Department of Zoology, National College, (Affiliated to Bharathidasan University), Tiruchirappalli, Tamil Nadu, India

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***Address for Correspondence**
Varadharajan Gokula*
Associate Professor and Head,
PG and Research Department of Zoology,
National College, (Affiliated to Bharathidasan University),
Tiruchirappalli, Tamil Nadu, India
Email: gokulae@yahoo.com

ABSTRACT

Breeding details of Asian Openbill *Anastomus oscitans,* Black-crowned Night Heron *Nycticorax nycticorax,* Indian Pond Heron *Ardeola grayii,* Grey Heron *Ardea cinerea,* Little Egret *Egretta garzetta,* and Little Cormorant *Phalacrocorax niger* were studied in Vaduvur Lake, Tamil Nadu, India. All these six species of birds showed their peak breeding activities during the months of November and December and their breeding activities declined afterwards. Birds with longer breeding cycles (large sized birds) remained till April while birds with shorter breeding cycle (small to medium sized) left the lake much earlier. In total, 41 nests of Asian Openbill, 39 nests of Black-crowned Night Heron, 45 nests of Indian Pond Heron, 28 nests of Grey Heron, 65 nests of Little Egret, and 35 nests of Little Cormorant were studied to understand the breeding of these bird species. The clutch size varied from 2 to 4 eggs in Indian Pond Heron, 2 to 6 eggs in Little Cormorant and 3 to 5 eggs in Grey Heron, Little Egret, Asian Openbill and Black-crowned Night Heron. The incubation period varied from 24 to 26 days in Asian Openbill and Black-crowned Night Heron, 18 to 25 days in Indian Pond Heron, 23 to 29 days in Grey Heron, 19 to 23 days in Little Egret and 14 to 21 days in Little Cormorant. The nestling period varied from 17 to 29 days in Little Cormorant, 19 to 23 days in Little Egret, 21 to 26 days in Indian Pond Heron, 28 to 32 days in Grey Heron, 29 to 36 days in Black-crowned Night Heron and 30 to 34 days in Asian Openbill.

Keywords: nest, wetland, nestling, incubation

INTRODUCTION

Species of storks, herons, cormorants, and egrets nest colonially, displaying spatio-temporal clumping of nests, with colonies sometimes comprising thousands of nests [1]. Due to their bio-indicator capacity at wetlands [2,3], any environmental changes would induce variability in the distribution, habitat use and reproductive parameters of these birds. However, majority of the water birds in India are poorly studied and thus envisaging their ecological status due to eco-detrimental developmental activities is often complex. Only some long-term and detailed studies on the biology of Cattle Egret [4], Asian Openbill *Aanastomus oscitans*, [5], *Ciconiiformes* [6], and colonial nesting birds [7] are available in India. Reference [8] compiled information on the heronries across India. Since there exists very little information on the breeding status of Asian Openbill *Aanastomus oscitans* Boddaert (1783), Black-crowned Night Heron *Nycticorax nycticorax* Linnaeus (1758), Indian Pond Heron *Ardeola grayii* Sykes (1832), Grey Heron *Ardea cinerea* Linnaeus (1758), Little Egret *Egretta garzetta* Linnaeus (1766) and Little Cormorant *Phalacrocorax niger* Vieillot (1817), the present study collected information to establish base line data on the breeding of these species in Vaduvur Lake, Tamil Nadu, India.

STUDY AREA

The Vaduvur Lake (declared as a protected area by the forest department in July 1999) is situated between 10^0 42' 19" N and 79^0 18' 53" E and spreading over c.128 ha (1.28 sq.km) in Tiruvarur District, Tamil Nadu, India. Vegetation of the lake consists of *Prosopis chilensis, Azadirachta indica,* and *Tamarindus indica,* including planting of *Acacia nilotica* by the forest department under the Sanctuary Management Program. Ten mounds, measuring a height of 1.5 metres running to a length of 50 metres and 10 metres in breadth, have been formed inside the Vaduvur Lake for the birds to nest when water is available. Reference [9] reported the occurrence of 118 species of birds belonging to 87 genera, 48 families and 18 orders. The Vennaru River is the main source of water in addition to northeast monsoon. The lake is surrounded by a large bund (bank) around the southern side, while on the northern portion; the bund is short as the elevation serves as natural bund. These bunds help in holding the water upto an average depth of c.2.5metre in the lake. Vaduvur Thenpathi and Vaduvoor Vadapathi are the two villages situated around the lake. The lake has also been identified as one of the Important Bird Areas (IBA: IN-TN-28) of India by Indian Bird Conservation Network. However, no comprehensive attempt has been made so far to study this wetland or its dependent avifaunal species. The average maximum temperature is about 34.7^0C and the average minimum temperature is 30^0C (Figure 1). The maximum temperature reaches as high as 39.40^0C and the minimum falls down to 20.30^0C. Winds blow from various quarters towards the end of May. The Southwest Monsoon sets in during April (the strongest in June) and continues till September. Northeast monsoon starts during the later part of the month of October and blows till the earlier part of the month of January. The average annual rainfall of 1306.38 mm spread over c.53 rainy days. It contributes about 60% of the total annual rainfall. The Southwest monsoon rains from June to September and summer rains from March to May accounts equally for the rest of the annual rainfall. Since the area is represented by Cauvery Deltaic Zone, alluvium is the predominant soil type. Paddy is the main crop cultivated around the wetlands and it is grown three times in a year.

METHODS

Census was carried out from a elevated vantage point during early morning (0500-0900hrs) and evening (1630-1800hrs) hours using total count method using a pair of binocular (vanguard 10X40), and Telescope (Audubon 80x) to know the abundance of Asian Openbill, Black-crowned Night Heron, Indian Pond Heron, Grey Heron, Little Egret and Little Cormorant utilizing the Vaduvur Lake. Breeding of the above water birds was studied by monitoring activities of the breeding birds. Information on date of nest construction, date of eggs laid, number of eggs laid, incubation period, eggs hatched and those failed to hatch, were recorded in order to document the breeding. Only completely visible nests along with their contents (eggs/chicks), built in the outermost trees in clusters found inside the lake, were chosen for the present study of the breeding biology of the aforementioned species. The breeding information of six species of birds was collected from September 2010 to February 2012 covering two breeding

seasons. In general, incubation can start as soon as the first egg is laid, in which case eggs will hatch asynchronously (one or two days apart). Incubation can also start when the clutch is completed, in which case the eggs hatch synchronously, or at some point in between. For altricial species, the nestling period is typically the time spent in the nest, although birds may be dependent on their parents for a much longer period out of the nest (in some cases several weeks). In the present study, the nestling period was considered the time between the hatching of eggs and the independence of the chicks, i.e., fledging (leaving) the nest.

RESULTS

During the study period (September 2010 to February 2012), a maximum of 92 (in December 2010 and Jan 2011) Asian Openbills, 120 (in Jan 2012) Black-crowned Night Herons, 202 (in December 2011 and Jan 2012) Indian Pond Herons, 19 (in December 2011) Grey Herons, 45 (in Jan 2012) Little Egret, and 232 (in December 2010 and Jan 2011) Little Egrets were recorded in Vaduvur Lake (Figure 2). In total, 41 nests of Asian Open bill, 39 nests of Black-crowned Night-heron, 45 nests of Indian Pond Heron, 28 nests of Grey Heron, 65 nests of Little Egret, and 35 nests of Little Cormorant were studied from September 2010 to February 2012 covering two breeding seasons to understand the breeding phenology of those bird species. All the six species of birds showed their peak breeding activities during the months of November and December and their breeding activities declined afterwards (Table 1). The clutch size of Asian Openbill varied from 3 to 5 eggs (Table 2). Incubation period varied from 24 to 26 days while nestling period varied from 30 to 34 days (Table 2). The clutch size of Black-crowned Night Heron varied from 3 to 5 eggs with a mean of 3.9. The incubation period varied from 24-26 days (mean 24.92) while nestling period varied from 29 to 36 days (Table 2). The Indian Pond Heron laid 2 to 4 eggs. The incubation period varied from 18 to 25 days (mean 21.76) and the nestling period varied from 21 to 26 days (mean 22.27). The clutch size of Grey Heron varied from 3 to 5 eggs with a mean of 4.11 eggs (Table 2). The incubation period varied from 23 to 29 days (mean 25.71) while nestling period varied from 28 to 32 days (mean 29.29). The clutch size of the Little Egret varied from 3 to 5 with a mean of 4.22 (Table 2). The incubation period varied from 19 to 23 days (mean 20.54) while nestling period varied from 15 to 21 days (mean 17.62 days). The clutch size of the Little Cormorant varied from two to six eggs. The incubation period varied from 14 to 21 days in the present study. The nestling period varied from 17 to 29 days .

DISCUSSION

Birds start arriving largely in the month of November and remain until the month of March with a peak congregation in the months of Late November to early January in Vaduvur. Birds with longer breeding cycles (large sized birds) remain till April while birds with shorter breeding cycle (small to medium sized) leave the lake much earlier. Moreover, November and December are the rainy months in Tamil Nadu, and thus Vaduvur is with sufficient water to support water birds. In Tamil Nadu, summer starts from the month of April and continues up to June and thus majority of the wetlands experience semi/full dryness which could attract less number of birds. These fluctuations increase and decrease the area of wetland and water level, which in turn influences the variation in the diversity wetland, associated birds through altering the prey base as suggested by Gokula [10]. This could be the reason for getting maximum number of birds in November and December and declining numbers afterwards. The nesting seasonality of birds in these wetlands was similar to other heronries occurring in Tamil Nadu described by Subramanya [8] and Gokula [10]. Birds of this wetland nest during the north-east monsoon season as majority of the water birds of other wetlands in Tamil Nadu [8]. The Vaduvur Lake is also a nesting site for the 6 aforementioned species, similar to other heronries in Tamil Nadu. Subramanya [8] reported heronries with as many as 17 species of birds nesting, in Tamil Nadu. Such difference may be attributed to factors *viz*., the age of the colony, site fidelity, availability of adequate nesting substrate and the quality of protection the nesting sites [8,10]. Disappearance of certain nesting species of birds over the years are also known to have occurred. The Spot-billed Pelican *Pelicanus philippensis* is not known to nest in this lake in recent years, while local people found it nesting until 2007. In addition to above said factors, such changing nesting patterns with some of the species may be linked to its movement pattern within the state. However, lack of protection and continued disturbance of the nesting activity of birds would

completely purge any nesting colony within a short period as Tamil Nadu has already witnessed 39 nesting sites over the decades [8]. The availability of nesting substrate would encourage an increase in the number of species and the total number of birds in Vaduvur over time. Ali and Ripley [11] state that the breeding season of storks is highly dependent on the monsoon and related water conditions, which trigger the abundance of food. This study also shows a strong relation between the monsoon and the nesting activity of Asian Openbill. Similar breeding details for the Asian Openbill were reported elsewhere [12,13,14,15]. The breeding phenology of Black-crowned Night-Heron observed in Vaduvur was similar to Durmuş and Adizel [16]. The clutch size of Indian Pond Heron varied from 2 to 4 eggs as reported by Ali and Ripley [11]. However, 4-6 was most common in Burma [17] and 1-4 in captivity [18]. The incubation period varied from 18 to 25 days (mean 21.76) and the nestling period varied from 21 to 26 days (mean 22.27). Reference [18] also reported an incubation period ranging from 21 to 24 days, the average being 23.02 days. Blaker [19] also reported similar incubation and nestling period. Similar breeding phenology of Grey Heron was reported elsewhere [20, 21]. The clutch size of Little egret is highly variable among studies.

The present mean clutch size (4.2) fell within the range of mean sizes from comparable studies. For breeding sites, it is reported 4.9 in Spain [22], 4.8 in Serros [20], 4.7 in Greece [23], 4.3 and 4.1 in the Axios Delta, Greece [24,25], 3.22 in Uttar Pradesh, India [26] and 3.17 in Turkey [27]. The clutch size of four was the most frequent in the present study (43.5% of 23 nests) followed by 3 (34.8%) for clutch size 5. In India, 39% of nests of Little Egrets contained more than 4 eggs, 39% with 3 eggs and 22% with 1 or 2 eggs [26]. However, clutch size in birds is often dependent on the age of parents: younger parents lay fewer eggs [28, 29]. Little Egret clutch size also depends on the quality of diet and the female's body condition [30]. Many studies remain to be undertaken on these matters. The breeding of the Little Cormorant began in November and continued till February similar to observations by Whistler [31], Smythies [17], Ali & Ripley [11], Begum [32], and Siriwannichkul [33]. The slight variations in the above studies may be attributed to the changes in the rainy seasons among southern India, Sri Lanka and Thailand. The clutch size varied from two to six eggs (Table 2) as reported elsewhere [34, 31, 35, 11, 32, 33, 36]. The incubation period varied from 14 to 21 days in the present study. However, Siriwannichkul [33], Perrins [37], and Begum [32] reported 22-26 days. The nestling period varied from 17 to 29 days after hatching in the present study while Siriwannichkul [33] and Begum [32] reported 35 to 42 and 39 to 45 days respectively. The numerical differences in the incubation and nestling period of little cormorant among studies may be attributed to the sample size involved.

CONCLUSION

The Asian Openbill, Black-crowned Night Heron, Indian Pond Heron, Grey Heron, Little Egret, and Little Cormorant showed their peak breeding activities during the months of November and December. Their breeding activities showed a declining trend after the month of December. The numerical differences, in the clutch size, incubation period, and nestling period of these six species of birds, found between the present study and the earlier reports may be attributed to the differences in the sample size involved, age of the parent, quality of diet, female's body condition and other geographical conditions.

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REFERENCES

1. Burger, J. A model for evolution of mixed species colonies of Ciconiiformes. *Quarterly Review of Biology.* 1981; (56): 143–167.
2. Temple, S.A. and J.A. Wiens Bird populations and environment changes: can birds be bioindicators?. *Am. Birds.*

1989; (43): 260-270.

3. Kushlan, J.A. Colonial waterbirds as bioindicators of environmental change. Colon. Waterbirds; 1993; (16): 223-251.
4. Singh, N. and Sodhi, N. S.. Heronries and the breeding population density of the Cattle Egret Bubulcus ibis coromandus (Boddaert): during 1985, in Tehsil Kharar of the Ropar district (Punjab). *Pavo.* 1986; (23): 77–84.
5. Mukhopadhyay, A. Some observations on the biology of the open bill stork, Anastomus oscitans (Boddaert), in southern Bengal. *J. Bombay nat. Hist. Soc.,* 1980; (77): 133-137.
6. Parasharya, B. M., & R.M.Naik Ciconiiform birds breeding in Bhavnagar City, Gujarat. A study of their nesting and plea for conservation. Pp. 429–445. 1990; In: Daniel, J. C. & Serrao, J. S. (eds): *Conservation in Developing countries: Problems and Prospects*. Bombay: Oxford University Press.
7. Sivasubramanian, C. Ecological investigation on the piscivorous birds in Keoladeo National Park, Bharatpur. Ph.D. dissertation, Saurashtra University, Rajkot 1992.
8. Subramanya, S. Distribution, status and conservation of Indian heronries. *J. Bombay Nat. Hist. Soc.* 1996; 93(3): 459–486.
9. Gokula, V. & P Ananth Raj. Birds of Vaduvoor Bird Sanctuary, Tamilnadu, India. *Zoo's Print* 2011; XXVI: No 6: 20-24.
10. Gokula, V. Status of distribution and potential breeding and foraging sites of Spot-billed Pelican in Tamil Nadu, India *J. Sci. Trans. Environ. Technov*. 2011; 5 (2):59-69.
11. Ali, S. & S.D. Ripley . *Compact handbook of the birds of India and Pakistan together with those of Bangladesh, Nepal, Bhutan and Sri Lanka.* 2nd ed. Delhi: Oxford University Press. 1987.
12. Datta, T. Behavioural Ecology of Feeding, Reproduction in Open-Billed Stork (Anastomus oscitans) (Boddaert) at the Raiganj Wildlife Sanctuary Raiganj, West Dinajpur, India. Ph.D Theses, North Bengal University, India, 1992.
13. Johnson, M. Feeding and breeding biology of Openbilled Stork in Andhra Pradesh. Ph.D. Thesis, Osmania University, Hyderabad,1992.
14. Pramanik, A.K., K.B. Santra., & C.K. Manna Seasonal abundance and factors influencing the population of Asian open-billed stork (Anastomus oscitans) in the Raiganj wildlife sanctuary, West Bengal, India. *Asian journal of conservation biology*,2014; 3(1): 28-37.
15. Pramanik, A.K., K.B. Santra, & C.K. Manna Nest-building Behaviour of the Asian Open Billed Stork Anastomus oscitans, in the Kulik Bird Sanctuary, Raiganj, India *Our Nature* 2009; 7: 39-47.
16. Durmuş, A. & O. Adizel Breeding ecology of night heron (*Nycticorax nycticorax* linne,1758) in the Lake Van Basin, Turkey, *The Journal of Animal & Plant Sciences*, 2010; 20(2): 73-78.
17. Smythies, B. E. *The Birds of Burma*. Oliver & Boyd, Edinburgh, UK, 1953.
18. Yesmin, R., R.K.Hague., Nazrul., & N. Haque The breeding biology of the pond heron (*Ardeola grayii* Sykes) in captivity. *Tiger Paper* 2001; 28 (1):15-18.
19. Blaker, D. Behavior of the Cattle Egret. *Ostrich.* 1969; (40): 74-129.
20. Cramp, S. & K.E.L. Simmons *The Birds of the Western Palearctic*, Vol. 1. Oxford University Press, Oxford, UK, 1977.
21. Owen, D. F. . The nesting success of the heron Ardea cinera in relation to the availability of food. *Proceedings of the Zoological Society of London* 1960; (133): 597-617.
22. Inoue, Y. The process of a synchronous hatching and sibling competition in the Little Egret Egretta garzetta. *Colonial Waterbirds* 1985; (8): 1 12.
23. Tsahalidis E. Biology and ecological behaviour of Little Egret *Egretta garzetta* in artificial Lake Kerkini, Serros, Greece. Unpublished Ph.D. thesis, Aristotelian University of Thessaloniki, Greece. 1990.
24. Kazantzidis, S., Hafner H. & V.Goutner Comparative breeding ecology of Little Egret *Egretta garzetta* in the Axios Delta (Greece) and the Camargue (France). *Reve Ecologie (Terée et Vie)*: 1996; (49): 53 62.
25. Kazantzidis, S., V.Goutner., M.Pyrovetsi., & A. Sinsis. Comparative nest site selection and breeding success in sympatric ardeids, Black- crowned Night Heron *Nycticorax nycticorax* and of Little Egret *Egretta garzetta* in the Axios Delta, Macidonia, Greece. *Colonial Waterbirds*; 1997; (20): 505 517.
26. Hilaluddin, J.N. Shah, & T.A. Shawl Nest site selection and breeding success by Cattle Egret and Little Egret in Amroha, Uttar Pradesh, India. Waterbirds 2003; 26(4): 444 448.
27. Uzun A., Uzun B. and Kopij G..Year-to-year variation in clutch size and breeding success of the Little Egret,

Egretta garzetta Linnaeus, 1766, in Turkey. *Zoology in the Middle East* 45: 35-40.

28. Klomp, H. 1970.The determinants of clutch size in birds. *Ardea*: 2008; (58): 1 124.
29. Coulson, J.C. & J.M.Porter. Reproductive success of the Kittiwake Gull Rissa tridactyla: the role of clutch size, chick growth rates and parental quality. *Ibis* 1985; (127): 450 466.
30. Hafner, H. E.. Didner Species account on the Squacco Heron Ardeola ralloides. *B.W.P. Update* 1997; 1: 166-174.
31. Whistler, H.. *Popular Handbook of Indian Birds*. 4th edition. Gurney and Jackson, London, 1949
32. Begum, A.R. The breeding biology and daily activities of little cormorant, *Phalacrocorax niger* (Vieillot). M.Sc. Thesis. Dept. of Zoology, University of Dhaka, Dhaka; 1997, Pp. xi+121 (unpublished).
33. Siriwannichkul, O. Food Habits and Breeding Biology of Little Cormorant (*Phalacrocorax niger*). Kasetsart Univ. Bangkok. Thailand, 1981.
34. Jerdon, T.C.. *The Birds of India*. Vol. 11, part 1. The Military Orphan Press, Calcatta: 1862; xiv+535pp.
35. Thomson, A.L. *A New Dictionary of Birds*. Thomas Nelson and Sons, London, 1965.
36. Barnes, H.E. *The Birds of India* (A guide to Indian ornithology). Vol. 2.Cosmo Publications, New Delhi; 1981; xiii+737pp.
37. Perrins, C.M. The Illustrated Encyclopedia of Birds: The Definitive Guide to Birds of The World. International Council for Bird Preservation, Marshall editions developments Ltd. Great Britain, 1990.

Table 1. Nesting activity recorded for Asian Openbill, Black-crowned Night Heron, Indian Pond Heron, Grey Heron, Little Egret, and Little Cormorant breeding in Vaduvur Lake (darkest area indicates the peak nesting activity)

S.No	Bird Species	Vaduvur Lake											
		Months											
		January	February	March	April	May	June	July	August	September	October	November	December
1	Asian Openbill *Anastomus oscitans.*												
2	Black-crowned Night Heron *Nycticorax nycticorax*												
3	Indian Pond-heron *Ardeola grayii*												
4	Grey Heron *Ardea cinerea*												
5	Little Egret *Egretta garzetta*												
6	Little Cormorant *Phalacrocorax niger*												

Table 2. Breeding details of Asian Open bill, Black-crowned Night Heron, Indian Pond Heron, Grey Heron, Little Egret, and Little Cormorant: a comparison with previous studies

Bird Species	Breeding details	(range, mean and SD)
Asian Openbill *Anastomus oscitans* (n=41)	Number of Eggs	3-5, 4.2±0.68
	Incubation period	24-26, 25.24±0.86
	Nestling period	30-34, 32±1.4
Black-crowned Night Heron *Nycticorax nycticorax* (n=39)	Number of Eggs	3-5, 3.9±0.64
	Incubation period	24-26, 24.92±0.77
	Nestling period	29-36, 31.92±1.8
Indian Pond-heron *Ardeola grayii* (n=45)	Number of Eggs	2-4, 3.51±0.59
	Incubation period	18-25, 21.76±1.65
	Nestling period	21-26, 22.7±1.25
Grey Heron *Ardea cinerea* (n=28)	Number of Eggs	3-5, 4.11±0.74,
	Incubation period	23-29, 25.71±1.44
	Nestling period	28-32,29.29±1.12
Little Egret *Egretta garzetta* (n=65)	Number of Eggs	3-5, 4.22±0.62
	Incubation period	19-23, 20.54±1.19
	Nestling period	15-21, 17.62±1.52
Little Cormorant *Phalacrocorax niger* (n=35)	Number of Eggs	2-6, 3.29±0.96
	Incubation period	14-21, 18.37±1.96
	Nestling period	17-29, 24.94±4.04

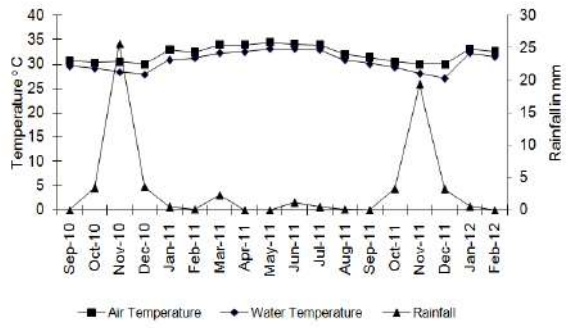

Figure 1. Mean air and water temperature, and mean rainfall recorded in the study area

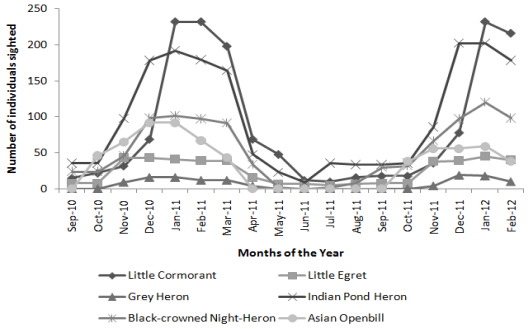

Figure 2 . Abundance of Asian Openbill, Black-crowned Night Heron, Indian Pond Heron, Grey Heron, Little Egret, and Little Cormorant during various months in Vaduvur Lake, Tamil Nadu, India

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RESEARCH ARTICLE

Fruit Classification and Disease Detection using Deep Convolutional Neural Network

Monisha R[1]*, Tamilselvan KS[2] and Yogeswari P[1]

[1]Research Scholar, Department of Electronics and Communication Engineering, KPR Institute of Engineering and Technology, Coimbatore, Tamil Nadu, India.
[2]Professor, Department of Electronics and Communication Engineering, KPR Institute of Engineering and Technology, Coimbatore, Tamil Nadu, India.

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***Address for Correspondence**
Monisha R,
Research Scholar,
Department of Electronics and Communication Engineering,
KPR Institute of Engineering and Technology,
Coimbatore, Tamil Nadu, India.
Email: 21ftec004@kpriet.ac.in

ABSTRACT

Horticulture currently makes up around 25% of the agricultural sector and has a significant reference in the fruit industry. To achieve good yield, effective growth and enhanced field culture must be integrated. Farmers require a reliable monitoring system to achieve this. Fruit disease is difficult for farmers to detect and identify, let alone discover its cause. Additionally, due to frequent changes in climatic conditions and environmental factors, fruits are more vulnerable to infection throughout production. The prior method of fruit disease detection required a lot of time and didn't reveal the illness's type. The farmer can identify the type of disease, acquire recommendations for prevention, and identify the disease using the suggested fruit disease detection system. The captured images are improved using image processing techniques. The system was then taught to classify and characterize the fruit and its disease using a deep Convolutional Neural network. This system will benefit farmers across India.

Keywords: Convolutional Neural Network, Deep Neural Network, Disease detection, Horticulture.

INTRODUCTION

India is a country where agriculture is crucial to the economy of the nation. To support the world's expanding population, agricultural products face a constant struggle. Increasing the output of the products depends critically on the resistance of agricultural products to illnesses. Biotic stressors and hostile environmental and meteorological conditions are faced by plants and trees [1]-[4]. Farmers employ a variety of strategies to address these issues, including pesticide, fertilizer, irrigation practice, etc. Fruit farming gives people food to eat and allows them to experience a variety of flavors. Ability to detect fruit and plant diseases is one application of image processing in particular. In the conventional approach, professionals use their unaided eyes to find plant illness. This method necessitates constant observation, which comes at a considerable cost to large farms. Additionally, timely plant disease control reduces product losses and the need for chemical poisons, which reduces the contamination of underground sources [2]. In an apple, there is no connection between an illness and cork spot [3], which is a physiological condition of the tree. The ailment renders the fruit unsightly on the outside and gives its interior a corky texture. It generally appears in fruit with a low calcium content. In some situations, it might also be brought on by a potassium, magnesium, or calcium imbalance. Although the fruit is technically edible, it will be difficult to sell it because it can taste bitter. Customers of a small commercial orchard could worry that the apples are mold-contaminated. The outermost portion of the fruit's flesh will develop small green depressions or dimples as the first indication that something is wrong. This process usually begins in the first few weeks of summer and can go on as the fruit develops and enlarges. The tiny spots eventually enlarge and turn corky and discolored. The spots could be as big as half an inch. Apple scab (*Venturia inaequalis*), a devastating disease of apples, affects both leaves and fruit. The fungal illness results in pale yellow or olive green patches on the exterior of the leaves. The lower surface may develop patches that are dark and velvety. The leaves of plants that have been severely diseased may fall off before the summer has fully arrived. One of the most dangerous illnesses that affects mature bananas is anthracnose [5], which is brought on by the *Colletotrichum* species. Black, depressed lesions with spore clusters or acervuli inside the lesion are signs of anthracnose.

Infection on the banana typically begins during fruit development but does not show symptoms until the fruit is mature; symptoms frequently appear during storage and marketing (Prusky & Plumbley 1992). When banana fruits are damaged by scratches during handling and shipping, anthracnose worsens and the fruit becomes unsellable. Fruit speckle [6] is a fungal infection that can be problematic from October to May, just like leaf speckle. Fruit speckle, in contrast to leaf speckle, affects just the fruit and not the leaves of the developing plant. It grows on dead banana leaves in the plantation where it survives. When it's rainy or humid outside, the fungus's spores are released into the air and land on fruit, where they quickly develop into dark brown or black spots that are 2-4 mm in diameter. All maturational phases of the bunches may have the illness. The bacterium *Xanthomonas citri* is the source of the citrus disease known as citrus canker citri. Canker has severe negative impact on citrus trees' vitality, causing leaves and fruit to fall off before their time even though it is not hazardous to humans. Canker-infected fruit can still be eaten, but its marketability as fresh fruit is diminished. The plant-pathogenic fungus Diaporthe citri (anamorph: *Phomopsis citri*) is responsible for citrus melanose [8]. The fungus rarely affects the pulp, although it can cause severe fruit rind defects. On leaves, the tiny, elevated, black lesions are frequently encircled by yellow haloes and might deform the leaf. The illness causes a surface imperfection on the fruit that won't likely influence the output of processed fruit overall, but causes external blemishes that lower the value of fruit meant for the fresh market. On foliage, this disease typically has negligible economic impact.

Numerous citrus species are affected by citrus melanose, which is present in many of the world's citrus-growing regions. When the tissues grow and stretch over an extended period of rainy or humid weather, it affects immature leaves and fruits of several citrus species or variations. The signs of this widespread fungal illness range from tiny lesions that resemble scabs or patches to damage patterns known as star melanose, mud cake, etc. This is one of the citrus fruit diseases that is most frequently seen worldwide. DNN, particularly CNN [7], has generated astonishing advances in picture categorization and detection precision with advancement of technologies along with the rise in

computing power. With the use of deep learning [9], a computer model may learn to carry out categorization tasks using images, text, or voice. Deep learning algorithms are capable of excellent categorization accuracy, frequently beating humans. Multi-layer neural network designs are used to train the models. Deep learning techniques have recently been achieved in the agricultural area and have been successfully applied in numerous real-world applications. Short overview of the related existing works is provided in Section II. The proposed methodology, which is built on a Deep CNN architecture, is presented in Section III. The results obtained are discussed in Section 4. Finally, in Section 5, conclusion and future work are made.

RELATED WORK

An approach of separating apples into rotten and good apples was put out by Singh *et al*. [10]. Based on discrete wavelet transform, correlation matrix for gray level, Texture Energy, and many other features, they extract texture features. After that, the apples were sorted using SVM, logistic regression, k-means clustering, linear discriminant; Support Vector Machine was shown to be the best classifier with a performance of 98.9%. A method for detection of apple leaf diseases that obtained a 98.54% accuracy rate was also suggested by Baranwal *et al.* [11]. In order to detect disease in apple leaves, Jiang *et al*. [12] suggested CNN. They enhanced CNN to diagnose diseases in real time from picture datasets that included complex real photos and laboratory images. The images were first enhanced and annotated. Then, a deep CNN is suggested and assessed using a withhold-out test, with their suggested strategy achieving a performance of 78.80%. With high precision, training effectiveness, and universality, the authors of a different study [13] developed a mathematical formula depending on a neural learning approach to identify diseases in plants. Even in a complicated environment, leaves could be found and located using a region proposal network (RPN). The transfer learning model is then adjusted using the segmented leaf images from the RPN model. Utilizing plant illnesses like black rot, rusts, and plaque diseases, their model's output was assessed. On the test dataset, accuracy of 83.57% is attained. 5 convolutional layers, batch normalization layers, max pooling layers, and dense layers were the result of the model created by Shrestha *et al.* [14].

Nearly 58 million training parameters were available in all. The model was trained using 15 distinct plant infections in photos, and the dataset's CA was 88.8%. They provided a deep learning related end-to-end recognition technique in [15] that extracts and categorizes six different apple leaf diseases. Their suggested method has a 97.18% accuracy rate. According to Gargade *et al*. [16], KNN and SVM can be used to identify leaf diseases and deficiencies. Their suggested method had a 99.5% accuracy rate. [17] defines the integration of CNN, LSTM and RNN for fruit image categorization. In this method, CNN and RNN are used to create sequential labels and discriminative characteristics. LSTM offers a justification by incorporating a storage cell for encoding training at each classification interval. It has been determined from empirical findings that the proposed classification method yields effective results. To improve, segment, recognise, and categorize fruit photos, Type-II Fuzzy, TLBO (Teacher-Learner Based Optimization), deep learning Convolution Neural Network (CNN), Recurrent Neural Network (RNN), and Long Short-Term Memory (LSTM) applications were proposed in [18]. This task uses CNN, RNN, and LSTM deep learning models to classify fruits using selected best-fit and derived attributes. Preliminary evidence suggests that the suggested approach has good accuracy and produces accurate quantitative analysis results. An eight-class dataset for dates was produced in [19], for training the suggested model. In order to improve accuracy, the suggested model has been preprocessed using a variety of methods, including image augmentation, decaying model checkpointing, learning rate, and hybrid weight adjustment. The outcomes demonstrate that the proposed model, which is based on the MobileNetV2 architecture, has 99% accuracy.

The suggested method was contrasted with its contemporaries already in use. The outcomes demonstrate the suggested approach does a better job overall than its alternatives. Computer vision & machine learning techniques [20] have been used to streamline the fruits quality check & classify all in separate groups. Transfer Learning is a well-liked method for creating a fruit classifier that can categorize fruits into various classes or attributes. The experiment uses two datasets of fruits. The pre-trained model Dense Net is also partly unfrozen and re-trained for increasing the quality of fruit classification. The findings indicate that this model has a fruit categorization accuracy of 99.61%. Images of many types of fruit have been recognised using the VGG16 [21] algorithm. Next, the fruit data

set which includes 6 classes also developed for network model training and validation performances. A deep CNN was developed to recognise six different types of fruits from images of a collection of fruits. Indicating the practicality of this concept, the ratio reached 100%. A clear way to simple fruit categorization is provided by the strategy of including genuine learning models in the process of training them using sizable, freely accessible image data sets. In this study, a machine learning-based method for categorizing and recognising six different fruits using a dataset of 2677 photos is provided.

METHODOLOGY

The study is to classify the fruits (apple, banana, and orange) and determine the diseases that affect them. Pre-trained Learning algorithms will be adjusted to categorize two diseases for each fruit in order to achieve this. We have around 1490 images in the dataset we have developed. The VGG19 and ResNet (proposed deep CNN) pre-trained models will be examined. The performance of the classification could be hampered by the variation in sample sizes within classes. Adam optimizer is used to increase the efficiency of classification. Fig 2 shows the outline of the overall methodology used in this study. Images were sourced and collected to form the

Tri-fruit dataset. This dataset contains the following:
1.fresh images of apples, bananas and oranges.
2.apple scab and cork spot affected images of apples.
3.anthracnose symptoms and fruit speckle affected images of bananas.
4.citrus canker and melanose affected images of oranges.

The training specifics are as follows:
1.EPOCHS=75
2.Learning rate= 1e-3

After which the image size is reduced and dimensions are normalized in relevance to our requirements. By looping over the dataset, we label each image and update the label path. For training the data, we use two models namely VGG19 and ResNet. VGG19 is a convolutional neural network architecture from Visual Geometry Group, Department of Engineering Science, University of Oxford. It is 19 layers deep (16 Convolutional layers and 3 Fully Connected layers). It takes in an image input size of 224×224. The model is used for image classification and has about 1000 classes. ResNet, also known as residual Networks [22]-[23], has 50 layers with parameters upto 23 million. They are applicable for computer vision applications. A better model known as ResNetV2 was built following the initial release of ResNet [24]. The layer arrangement in the residual block is the main distinction between ResNet and ResNet V2. Before the 2D convolutional layer in ResNetV2, we use a batch normalization with a Relu layer. The optimizer method played a key role in training the deep classification algorithm by repeatedly adjusting the network's layers' parameters. In place of the conventional stochastic gradient descent method, Adam [25] is an optimization technique that may be used to update weights of the neural network iteratively depending on input data. Adam is aware of the advantages of RMSProp and AdaGrad. Adam uses the average of the second moments of the gradients in addition to the average of the first moments, which is how RMSProp adjusts the parameter learning rates (the uncentered variance). The parameters beta 1 and beta 2 regulate the decay rates of these moving averages, and the algorithm specifically creates an exponential moving average of the gradient and the squared gradient.

RESULTS AND DISCUSSION

We divided the dataset into two categories namely training and testing. The training set has 80% (1192) and the testing set has 20% (298). Python 3.7.2 version shell was used to implement the methodology. For the VGG19 architecture, the learning rate with the Adam was less compared with the ResNet architecture. As for the classification accuracy VGG19 showed 0.91 for fresh fruits and 0.846 for the fruits with diseases. The ResNet model

showed 0.982 for fresh fruits and 0.964 for the fruits with diseases. Table I shows the Classification Accuracy for every fruit (apple, banana and orange). They include both the fresh fruits and disease infected fruits. This performance result is based on the fact of combining the proposed Deep CNN model with appropriate optimization algorithm. Adam optimizer performed well for VGG19 and ResNet. Fig 2 is an apple with cork spot defect and was classified with 95.02% accuracy. The model predicts the probability for all the fruit categories. Fig 3 shows a fresh banana. Here the CA is measured to be 100% and the chances of it having any infection is negligible.

CONCLUSION AND FUTURE WORK

In this study, we categorize fruits and fruit-related diseases in detail. According to the analysis, the automated method of disease detection in fruits like apples, oranges, and bananas using CNN can be very effective. Noise might cause distortion in the images that are being processed. In this instance, a denoising mechanism is developed. A few of the typical ailments that affect apples, oranges, and bananas are listed here. Utilizing image processing techniques like pre processing, extraction, and others, it is possible to identify a fruit's ailment at an early stage. The architecture used here can classify the fresh fruits with 99.2% accuracy and the disease identification for all the fruits has about 96.46% accuracy. The Adam optimizer was found to be efficient for the specified architecture. It can be inferred that larger the dataset, larger will be the classification accuracy. The work can be extended to other fruits and vegetables. Also there are possibilities to incorporate other optimization techniques like Gradient descent, etc. appropriately in order to improve the classification rate. In the future, the work can be extended using transfer learning.

REFERENCES

1. Gimenez E, Salinas M, and Manzano-Agugliaro F. Worldwide research on plant defense against biotic stresses as improvement for sustainable agriculture. Sustainability 2018;10:391.
2. Thapa R, Snavely N, Belongie S, and Khan A. The plant pathology 2020 challenge dataset to classify foliar disease of apples. arXiv preprint arXiv:2004.11958, 2020.
3. Tomar A, Gupta G, Salehi W, Vanipriya CH, Kumar N, Sharma B. A Review on Leaf-Based Plant Disease Detection Systems Using Machine Learning. Recent Innovations in Computing. 2022;297-303.
4. Alsayed A, Alsabei A, Arif M. Classification of apple tree leaves diseases using deep learning methods. International Journal of Computer Science & Network Security. 2021;21(7):324-30.
5. Zakaria L, Sahak S, Zakaria M, Salleh B. Characterisation of colletotrichum species associated with anthracnose of banana. Trop Life Sci Res. 2009 Dec;20(2):119-25. PMID: 24575184; PMCID: PMC3819051.
6. https://barmac.com.au/problem/fruit-speckle/#:~:text=Like%20leaf%20speckle%2C%20fruit%20speckle,leaves%20of%20the%20growing%20plant.
7. Jiang H, Li X, Safara F. IoT-based agriculture: Deep learning in detecting apple fruit diseases. Microprocessors and Microsystems. 2021 Aug 14:104321.
8. Gopal K, Lakshmi LM, Sarada G, Nagalakshmi T, Sankar TG, Gopi V, Ramana KT. Citrus melanose (Diaporthe citri Wolf): A review. Int. J. Curr. Microbiol. App. Sci. 2014;3(4):113-24.
9. I. Goodfellow, Y. Bengio, and A. Courville, Deep learning. MIT press, 2016.
10. Singh S, Singh NP. Machine learning-based classification of good and rotten apple. InRecent trends in communication, computing, and electronics 2019 (pp. 377-386). Springer, Singapore.
11. Baranwal S, Khandelwal S, Arora A. Deep learning convolutional neural network for apple leaves disease detection. InProceedings of International Conference on Sustainable Computing in Science, Technology and Management (SUSCOM), Amity University Rajasthan, Jaipur-India 2019 Feb 26.
12. Jiang P, Chen Y, Liu B, He D, Liang C. Real-time detection of apple leaf diseases using deep learning approach based on improved convolutional neural networks. IEEE Access. 2019 May 6;7:59069-80.
13. Guo Y, Zhang J, Yin C, Hu X, Zou Y, Xue Z, Wang W. Plant disease identification based on deep learning algorithm in smart farming. Discrete Dynamics in Nature and Society. 2020 Aug 18;2020.

14. Shrestha G, Das M, Dey N. Plant disease detection using CNN. In2020 IEEE Applied Signal Processing Conference (ASPCON) 2020 Oct 7 (pp. 109-113). IEEE.
15. Khan AI, Quadri SM, Banday S. Deep learning for apple diseases: classification and identification. arXiv preprint arXiv:2007.02980. 2020 Jul 6.
16. Gargade A, Khandekar S. Custard apple leaf parameter analysis, leaf diseases, and nutritional deficiencies detection using machine learning. In Advances in Signal and Data Processing 2021 (pp. 57-74). Springer, Singapore.
17. Gill HS, Khehra BS. An integrated approach using CNN-RNN-LSTM for classification of fruit images. Materials Today: Proceedings. 2022 Jan 1;51:591-5.
18. Gill HS, Murugesan G, Khehra BS, Sajja GS, Gupta G, Bhatt A. Fruit recognition from images using deep learning applications. Multimedia Tools and Applications. 2022 Apr 18:1-22.
19. Albarrak K, Gulzar Y, Hamid Y, Mehmood A, Soomro AB. A Deep Learning-Based Model for Date Fruit Classification. Sustainability. 2022 Jan;14(10):6339.
20. Verma R, Verma AK. Fruit Classification Using Deep Convolutional Neural Network and Transfer Learning. InInternational Conference on Emerging Technologies in Computer Engineering 2022 (pp. 290-301). Springer, Cham.
21. Abu-Jamie TN, Abu-Naser SS, Alkahlout MA, Aish MA. Six Fruits Classification Using Deep Learning.
22. Leelaruji T, Teerakawanich N. Short term prediction of solar irradiance fluctuation using image processing with ResNet. In2020 8th International Electrical Engineering Congress (iEECON) 2020 Mar 4 (pp. 1-4). IEEE.
23. Sarwinda D, Paradisa RH, Bustamam A, Anggia P. Deep learning in image classification using residual network (ResNet) variants for detection of colorectal cancer. Procedia Computer Science. 2021 Jan 1;179:423-31.
24. He K, Zhang X, Ren S, Sun J. Deep residual learning for image recognition. InProceedings of the IEEE conference on computer vision and pattern recognition 2016 (pp. 770-778).
25. https://machinelearningmastery.com/adam-optimization-algorithm-for-deep-learning/
26. Rohith G, Kumar LS. Design of Deep Convolution Neural Networks for categorical signature classification of raw panchromatic satellite images. Multimedia Tools and Applications. 2022 Mar 30:1-38.
27. Proceedings of the International Conference on Artificial Intelligence and Computer Vision (AICV2020), Springer Science and Business Media LLC, 2020.

Table.1. Performance of each fruit for the selected models.

Fruits	VGG19	ResNet
Apple	95.02	99.33
Banana	78.36	94
Orange	80.5	96.05

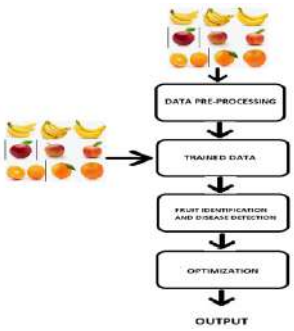

Figure 1. Block Diagram of the proposed methodology.

Figure 2. Apple with cork spot

Figure 3. Fresh banana

Figure 4. Orange with Melanose

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RESEARCH ARTICLE

A New Generalization of Two Parameter Sujatha Distribution with Applications using Lung Cancer Data

R.Sivaranjani[1], P.Arumugam[2] and E.Susiganeshkumar[3]

[1]Research Scholar, Department of Statistics, Annamalai University, Annamalai Nagar, Chidambaram, Cuddalore, Tamil Nadu, India.
[2]Associate Professor, Department of Statistics, Annamalai University, Annamalai Nagar, Chidambaram, Cuddalore, Tamil Nadu, India.
[3]Assistant Professor and Head, Department of Statistics, Periyar Government Arts College, Cuddalore, Tamil Nadu, India.

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***Address for Correspondence**
R.Sivaranjani,
Research Scholar,
Department of Statistics,
Annamalai University,
Annamalai Nagar,
Chidambaram, Cuddalore,
Tamil Nadu, India.
Email: sivaranjani160692@gmail.com

ABSTRACT

In the present paper, we have used a length biased version to execute the new model of two parameter Sujatha distribution called as length biased two parameter Sujatha distribution. The newly proposed distribution has been discussed with several statistical properties including its moments, harmonic mean, survival function, hazard rate function, order statistics, bonferroni and Lorenz curves. Its parameters have also been estimated by using the technique of maximum likelihood estimation and also its Fisher's information matrix have been discussed. Finally, a real life-time data set has been used to examine the usefulness of newly proposed distribution.

Keywords: Weighted distribution, Two parameter Sujatha distribution, Order statistics, Survival analysis, Maximum likelihood estimation.

INTRODUCTION

The concept namely weighted distributions arise when the observations generated from a stochastic process are not given equal chances of being recorded, instead they are recorded according to some weight function. Fisher (1934) introduced the concept of weighted distributions to model the ascertainment biases which were later formalized in a unifying theory by Rao (1965) for problems where the observations fall in non-experimental, non-replicated and non-random manner. The weighted distributions are applied in various research areas related to reliability, biomedicine, ecology, meta analysis, analysis of family data, analysis of intervention data and other areas for the development of proper statistical models. The weighted technique plays a very prominent role in distribution theory by adding an extra parameter to the existing classical distribution, because the classical distribution may not provide the best fit to lifetime data. The weighted distribution reduces to length biased distribution when the weight function considers only the length of the units of interest. Length biased distribution is a special case of weighted distribution. More generally, when the sampling mechanism selects units with probability proportional to measure of the unit size, resulting distribution is called size-biased. The concept of length biased sampling was introduced by Cox (1969) and Zelen (1974). The concept of length biased was originally introduced by Cox (1962) in the context of renewal theory and it arises in many forestry applications as well as other environmental, econometric, and biomedical sampling problems. The concept of length biased may also have large applications in various biomedical area such as family history and disease, survival analysis and intermediate events. Much work has been done to characterize the relationships between original distributions and their length biased versions.

Kadim and Hussein (2014) discussed on length biased weighted exponential and Rayleigh distribution with application. Rather and Ozel (2021) presented the length biased power Lindley distribution with properties and its applications. Ahmed *et al*. (2013) studied the size biased generalized beta distribution of first kind. Das and Roy (2011) obtained the length biased weighted generalized Rayleigh distribution. Elfattah *et al. (*2021) discussed on the length biased Burr-XII distribution with properties and applications. Saghir *et al*. (2016) studied the length-biased weighted exponentiated inverted weibull distribution. Mustafa and Khan (2022) executed the length biased powered inverse Rayleigh distribution with applications. Mudasir and Ahmad (2018) studied length biased Nakagami distribution. Reyad (2017) discussed on the length-biased weighted frechet distribution with properties and estimation. Subramanian and Shenbagaraja (2020) studied length biased quasi Sujatha distribution with properties and applications. Recently, Ganaie and Rajagopalan (2021) presented the length biased power quasi Lindley distribution with properties and applications which shows more reliable and flexible than the classical distribution.

The two parameter Sujatha distribution is a newly introduced lifetime model proposed by Tesfay and Shanker (2018) and the size-biased Lindley distribution and Sujatha distribution are particular cases of the proposed two parameter sujatha distribution. Its different statistical properties including shapes of density function for varying values of parameters, coefficient of variation, skewness, kurtosis, index of dispersion, hazard rate function, mean residual life function, stochastic ordering, mean deviation, bonferroni and lorenz curves, and stress-strength reliability have been discussed. Its parameters have also been estimated through method of moments and method of maximum likelihood estimation. Shanker (2016) discussed on one parameter Sujatha distribution with applications, derive its various statistical properties and estimate its parameters by method of moments and method of maximum likelihood estimation.

Length Biased Two Parameter Sujatha (LBTPS) Distribution

The probability density function of two parameter Sujatha (TPS) distribution is given by

$$f(x;\theta,\alpha)=\frac{\theta^3}{\alpha\theta^2+\theta+2}\left(\alpha+x+x^2\right)e^{-\theta x};\ x>0,\theta>0,\alpha\geq 0 \qquad (1)$$

and the cumulative distribution function of two parameter Sujatha distribution is given by

$$F(x;\theta,\alpha)=1-\left(1+\frac{\theta x(\theta x+\theta+2)}{\alpha\theta^2+\theta+2}\right)e^{-\theta x};\ x>0,\theta>0,\alpha\geq 0 \qquad (2)$$

Suppose X is a random variable of non-negative condition has probability density function $f(x)$ .Let $w(x)$ be its non-negative weight function, then the probability density function of weighted random variable X_w is given by

$$f_w(x)=\frac{w(x)f(x)}{E(w(x))},\quad x>0.$$

Where $w(x)$ be its non - negative weight function and $E(w(x))=\int w(x)f(x)dx<\infty$.

In this paper, we have to obtain the length biased version of two parameter Sujatha distribution. We have considered the weight function as *w(x) = x* to obtain the length biased two parameter Sujatha distribution. Then, the probability density function of length biased distribution is given by

$$f_l(x)=\frac{x f(x)}{E(x)} \qquad (3)$$

Where $E(x)=\int_0^\infty x f(x)dx$

$$E(x)=\frac{\alpha\theta^2+2\theta+6}{\theta(\alpha\theta^2+\theta+2)} \qquad (4)$$

By substituting equations (1) and (4) in equation (3), we will obtain the probability density function of length biased two parameter Sujatha distribution as

$$f_l(x)=\frac{\theta^4}{\alpha\theta^2+2\theta+6}x\left(\alpha+x+x^2\right)e^{-\theta x} \qquad (5)$$

and cumulative distribution function of length biased two parameter Sujatha distribution can be obtained as

$$F_l(x)=\int_0^x f_l(x)dx$$

$$F_l(x)=\int_0^x\frac{\theta^4}{\alpha\theta^2+2\theta+6}x\left(\alpha+x+x^2\right)e^{-\theta x}dx$$

$$F_l(x)=\frac{1}{\alpha\theta^2+2\theta+6}\int_0^x x\theta^4\left(\alpha+x+x^2\right)e^{-\theta x}dx$$

$$F_l(x)=\frac{1}{\alpha\theta^2+2\theta+6}\left(\alpha\theta^4\int_0^x xe^{-\theta x}dx+\theta^4\int_0^x x^2e^{-\theta x}dx+\theta^4\int_0^x x^3e^{-\theta x}dx\right) \qquad (6)$$

Put $\theta x=t \quad\Rightarrow\theta dx=dt \quad\Rightarrow dx=\frac{dt}{\theta}$, when $x\to x, t\to\theta x$ and when $x\to 0, t\to 0$

Also $x=\frac{t}{\theta}$

After simplifying above equation (6), we will obtain the cumulative distribution function of length biased two parameter Sujatha distribution as

$$F_l(x) = \frac{1}{\alpha\theta^2 + 2\theta + 6}\left(\alpha\theta^2\gamma(2,\theta x) + \theta\gamma(3,\theta x) + \gamma(4,\theta x)\right) \quad (7)$$

Survival Analysis

In this section, we will discuss the survival function, hazard rate and Reverse hazard rate functions of the proposed length biased two parameter Sujatha distribution.

Survival function

The survival function is also known as reliability function and the survival function of length biased two parameter Sujatha distribution is given by

$$S(x) = 1 - F_l(x)$$

$$S(x) = 1 - \frac{1}{\alpha\theta^2 + 2\theta + 6}\left(\alpha\theta^2\gamma(2,\theta x) + \theta\gamma(3,\theta x) + \gamma(4,\theta x)\right)$$

Hazard function

The hazard function is also known as hazard rate or failure rate or force of mortality and is given by

$$h(x) = \frac{f_l(x)}{1 - F_l(x)}$$

$$h(x) = \frac{x\theta^4(\alpha + x + x^2)e^{-\theta x}}{(\alpha\theta^2 + 2\theta + 6) - (\alpha\theta^2\gamma(2,\theta x) + \theta\gamma(3,\theta x) + \gamma(4,\theta x))}$$

Reverse hazard function

The reverse hazard function is given by

$$h_r(x) = \frac{f_l(x)}{F_l(x)}$$

$$h_r(x) = \frac{x\theta^4(\alpha + x + x^2)e^{-\theta x}}{(\alpha\theta^2\gamma(2,\theta x) + \theta\gamma(3,\theta x) + \gamma(4,\theta x))}$$

Structural Properties

In this section, we will obtain various statistical properties of Length biased two parameter Sujatha distribution including its moments, harmonic mean, moment generating function and characteristic function.

Moments

Let X be a random variable following length biased two parameter Sujatha distribution with parameters θ and α, then the rth order moment $E(X^r)$ is obtained as

$$E(X^r) = \mu_r' = \int_0^\infty x^r f_l(x)dx$$

$$E(X^r) = \mu_r' = \int_0^\infty x^r \frac{\theta^4}{\alpha\theta^2 + 2\theta + 6}x(\alpha + x + x^2)e^{-\theta x}dx$$

$$E(X^r) = \mu_r' = \frac{\theta^4}{\alpha\theta^2 + 2\theta + 6} \int_0^\infty x^{r+1}(\alpha + x + x^2)e^{-\theta x}dx$$

$$E(X^r) = \mu_r' = \frac{\theta^4}{\alpha\theta^2 + 2\theta + 6}\left(\alpha\int_0^\infty x^{(r+2)-1}e^{-\theta x}dx + \int_0^\infty x^{(r+3)-1}e^{-\theta x}dx + \int_0^\infty x^{(r+4)-1}e^{-\theta x}dx \right) \quad (8)$$

After simplification, equation (8) becomes

$$E(X^r) = \mu_r' = \frac{\alpha\theta^2\Gamma(r+2) + \theta\Gamma(r+3) + \Gamma(r+4)}{\theta^r(\alpha\theta^2 + 2\theta + 6)} \quad (9)$$

Putting r = 1, 2, 3 and 4 in equation (9), we will obtain the first four moments of length biased two parameter Sujatha distribution.

$$E(X) = \mu_1' = \frac{2\alpha\theta^2 + 6\theta + 24}{\theta(\alpha\theta^2 + 2\theta + 6)}$$

$$E(X^2) = \mu_2' = \frac{6\alpha\theta^2 + 24\theta + 120}{\theta^2(\alpha\theta^2 + 2\theta + 6)}$$

$$E(X^3) = \mu_3' = \frac{24\alpha\theta^2 + 120\theta + 720}{\theta^3(\alpha\theta^2 + 2\theta + 6)}$$

$$E(X^4) = \mu_4' = \frac{120\alpha\theta^2 + 720\theta + 5040}{\theta^4(\alpha\theta^2 + 2\theta + 6)}$$

$$\text{Variance} = \frac{6\alpha\theta^2 + 24\theta + 120}{\theta^2(\alpha\theta^2 + 2\theta + 6)} - \left(\frac{2\alpha\theta^2 + 6\theta + 24}{\theta(\alpha\theta^2 + 2\theta + 6)} \right)^2$$

$$S.D(\sigma) = \sqrt{\frac{6\alpha\theta^2 + 24\theta + 120}{\theta^2(\alpha\theta^2 + 2\theta + 6)} - \left(\frac{2\alpha\theta^2 + 6\theta + 24}{\theta(\alpha\theta^2 + 2\theta + 6)} \right)^2}$$

Harmonic mean

The harmonic mean for the proposed model can be obtained as

$$H.M = E\left(\frac{1}{x}\right) = \int_0^\infty \frac{1}{x} f_l(x)dx$$

$$H.M = \int_0^\infty \frac{\theta^4}{\alpha\theta^2 + 2\theta + 6}(\alpha + x + x^2)e^{-\theta x}dx$$

$$H.M = \frac{\theta^4}{\alpha\theta^2 + 2\theta + 6}\left(\alpha\int_0^\infty x^{(2)-2}e^{-\theta x}dx + \int_0^\infty x^{(2)-1}e^{-\theta x}dx + \int_0^\infty x^{(3)-1}e^{-\theta x}dx \right)$$

After simplification of above equation, we obtain

$$H.M = \frac{\theta}{\alpha\theta^2 + 2\theta + 6}\left(\alpha\theta + \theta + 2\right)$$

Moment Generating Function and characteristic function

Let X be a random variable following LBTPS distribution, then the moment generating function of X can be obtained as

$$M_X(t) = E\left(e^{tx}\right) = \int_0^\infty e^{tx} f_l(x)dx$$

$$M_X(t) = \int_0^\infty \left(1 + tx + \frac{(tx)^2}{2!} + \ldots.\right) f_l(x)dx$$

$$M_X(t) = \int_0^\infty \sum_{j=0}^\infty \frac{t^j}{j!} x^j f_l(x)dx$$

$$M_X(t) = \sum_{j=0}^\infty \frac{t^j}{j!}\mu_j'$$

$$M_X(t) = \sum_{j=0}^\infty \frac{t^j}{j!}\left(\frac{\alpha\theta^2\Gamma(j+2) + \theta\Gamma(j+3) + \Gamma(j+4)}{\theta^j(\alpha\theta^2 + 2\theta + 6)}\right)$$

$$M_X(t) = \frac{1}{(\alpha\theta^2 + 2\theta + 6)}\sum_{j=0}^\infty \frac{t^j}{j!\theta^j}\left(\alpha\theta^2\Gamma(j+2) + \theta\Gamma(j+3) + \Gamma(j+4)\right)$$

Similarly, the characteristic function can be obtained as

$$\varphi_x(t) = M_X(it)$$

$$M_X(it) = \frac{1}{(\alpha\theta^2 + 2\theta + 6)}\sum_{j=0}^\infty \frac{it^j}{j!\theta^j}\left(\alpha\theta^2\Gamma(j+2) + \theta\Gamma(j+3) + \Gamma(j+4)\right)$$

Order Statistics

Order statistics have large applications in the field of applied and statistical sciences especially in modeling auctions, car races and insurance policies. Let X(1), X(2) ,…, X(n) denotes the order statistics of a random sample X1, X2,…, Xn drawn from a continuous distribution with probability density function fx(x) and cumulative distribution function FX(x), then the probability density function of rth order statistics X(r) is given by

$$f_{x(r)}(x) = \frac{n!}{(r-1)!(n-r)!} f_X(x)\left(F_X(x)\right)^{r-1}\left(1 - F_X(x)\right)^{n-r} \qquad (10)$$

Using equations (5) and (7) in equation (10), we will obtain the probability density function of r^{th} order statistics of length biased two parameter Sujatha distribution as

$$f_{x(r)}(x)=\frac{n!}{(r-1)!(n-r)!}\left(\frac{\theta^4}{\alpha\theta^2+2\theta+6}x(\alpha+x+x^2)e^{-\theta x}\right)$$
$$\times\left(\frac{1}{\alpha\theta^2+2\theta+6}\left(\alpha\theta^2\gamma(2,\theta x)+\theta\gamma(3,\theta x)+\gamma(4,\theta x)\right)\right)^{r-1}$$
$$\times\left(1-\frac{1}{\alpha\theta^2+2\theta+6}\left(\alpha\theta^2\gamma(2,\theta x)+\theta\gamma(3,\theta x)+\gamma(4,\theta x)\right)\right)^{n-r}$$

Therefore, the probability density function of higher order statistic X(n) of length biased two parameter Sujatha distribution can be obtained as

$$f_{x(n)}(x)=\frac{n\theta^4}{\alpha\theta^2+2\theta+6}x(\alpha+x+x^2)e^{-\theta x}\left(\frac{1}{\alpha\theta^2+2\theta+6}\left(\alpha\theta^2\gamma(2,\theta x)+\theta\gamma(3,\theta x)+\gamma(4,\theta x)\right)\right)^{n-1}$$

and probability density function of first order statistic X(1) of length biased two parameter Sujatha distribution can be obtained as

$$f_{x(1)}(x)=\frac{n\theta^4}{\alpha\theta^2+2\theta+6}x(\alpha+x+x^2)e^{-\theta x}\left(1-\frac{1}{\alpha\theta^2+2\theta+6}\left(\alpha\theta^2\gamma(2,\theta x)+\theta\gamma(3,\theta x)+\gamma(4,\theta x)\right)\right)^{n-1}$$

Test for Length biasedness of Length Biased Two Parameter Sujatha Distribution

Let X1, X2, ……., Xn be a random sample of size n drawn from the length biased two parameter Sujatha distribution. We set up the hypothesis for testing.

$$H_o: f(x)=f(x;\theta,\alpha) \quad \text{against} \quad H_1: f(x)=f_l(x;\theta,\alpha)$$

In order to test, whether the random sample of size n comes from the two parameter Sujatha distribution or length biased two parameter Sujatha distribution, the following test statistic is used.

$$\Delta=\frac{L_1}{L_o}=\prod_{i=1}^{n}\frac{f_l(x;\theta,\alpha)}{f(x;\theta,\alpha)}$$

$$\Delta=\frac{L_1}{L_o}=\prod_{i=1}^{n}\left(\frac{x_i\,\theta(\alpha\theta^2+\theta+2)}{\alpha\theta^2+2\theta+6}\right)$$

$$\Delta=\frac{L_1}{L_o}=\left(\frac{\theta(\alpha\theta^2+\theta+2)}{\alpha\theta^2+2\theta+6}\right)^n\prod_{i=1}^{n}x_i$$

We should reject the null hypothesis, if

$$\Delta=\left(\frac{\theta(\alpha\theta^2+\theta+2)}{\alpha\theta^2+2\theta+6}\right)^n\prod_{i=1}^{n}x_i>k$$

Obviously, we also reject the null hypothesis where

$$\Delta^*=\prod_{i=1}^{n}x_i>k\left(\frac{\alpha\theta^2+2\theta+6}{\theta(\alpha\theta^2+\theta+2)}\right)^n$$

$$\Delta^* = \prod_{i=1}^{n} x_i > k^*, \text{Where } k^* = k\left(\frac{\alpha\theta^2 + 2\theta + 6}{\theta(\alpha\theta^2 + \theta + 2)}\right)^n$$

For large sample of size n, 2log Δ is distributed as chi-square distribution with one degree of freedom and also chi-square distribution is used for getting p value. Thus we refuse to accept the null hypothesis, when the probability value is given by

$p\left(\Delta^* > \gamma^*\right)$, Where $\gamma^* = \prod_{i=1}^{n} x_i$ is less than a specified level of significance and $\prod_{i=1}^{n} x_i$. is the observed value of the statistic Δ^*.

Bonferroni and Lorenz Curves

The Bonferroni and Lorenz curves are oldest classical curves used to measure the distribution of inequality in income or wealth and are also known as income distribution curves. The bonferroni and Lorenz curves are defined as

$$B(p) = \frac{1}{p\mu_1'}\int_0^q x f_l(x)dx$$

and $$L(p) = \frac{1}{\mu_1'}\int_0^q x f_l(x)dx$$

Where $\mu_1' = E(X) = \frac{2\alpha\theta^2 + 6\theta + 24}{\theta(\alpha\theta^2 + 2\theta + 6)}$ and $q = F^{-1}(p)$

$$B(p) = \frac{\theta(\alpha\theta^2 + 2\theta + 6)}{p(2\alpha\theta^2 + 6\theta + 24)}\int_0^q \frac{\theta^4}{\alpha\theta^2 + 2\theta + 6} x^2(\alpha + x + x^2)e^{-\theta x}dx$$

$$B(p) = \frac{\theta^5}{p(2\alpha\theta^2 + 6\theta + 24)}\int_0^q x^2(\alpha + x + x^2)e^{-\theta x}dx$$

$$B(p) = \frac{\theta^5}{p(2\alpha\theta^2 + 6\theta + 24)}\left(\alpha\int_0^q x^{3-1}e^{-\theta x}dx + \int_0^q x^{4-1}e^{-\theta x}dx + \int_0^q x^{5-1}e^{-\theta x}dx\right)$$

After simplification, we obtain

$$B(p) = \frac{\theta^5}{p(2\alpha\theta^2 + 6\theta + 24)}\left(\alpha\gamma(3, \theta q) + \gamma(4, \theta q) + \gamma(5, \theta q)\right)$$

$$L(p) = \frac{\theta^5}{(2\alpha\theta^2 + 6\theta + 24)}\left(\alpha\gamma(3, \theta q) + \gamma(4, \theta q) + \gamma(5, \theta q)\right)$$

Maximum Likelihood Estimation and Fisher's Information Matrix

In this section, we will discuss the parameter estimation of length biased two parameter Sujatha distribution by using the technique of maximum likelihood estimation and also obtain its Fisher's information matrix. Let $X_1, X_2, \ldots, X_n$ be a random sample of size n from the length biased two parameter Sujatha distribution, then the likelihood function can be written as:

$$L(x)=\prod_{i=1}^{n} f_l(x)$$

$$L(x)=\prod_{i=1}^{n}\left(\frac{\theta^4}{\alpha\theta^2+2\theta+6}x_i(\alpha+x_i+x_i^{\,2})e^{-\theta x_i}\right)$$

$$L(x)=\frac{\theta^{4n}}{(\alpha\theta^2+2\theta+6)^n}\prod_{i=1}^{n}\left(x_i(\alpha+x_i+x_i^{\,2})e^{-\theta x_i}\right)$$

The log likelihood function is given by

$$\log L=4n\log\theta-n\log(\alpha\theta^2+2\theta+6)+\sum_{i=1}^{n}\log x_i+\sum_{i=1}^{n}\log(\alpha+x_i+x_i^{\,2})-\theta\sum_{i=1}^{n}x_i \qquad (11)$$

Now differentiating the log likelihood equation (11) with respect to parameters θ and α. We must satisfy the normal equations

$$\frac{\partial\log L}{\partial\theta}=\frac{4n}{\theta}-n\left(\frac{2\alpha\theta+2}{\alpha\theta^2+2\theta+6}\right)-\sum_{i=1}^{n}x_i=0$$

$$\frac{\partial\log L}{\partial\alpha}=-n\left(\frac{\theta^2}{\alpha\theta^2+2\theta+6}\right)+\sum_{i=1}^{n}\left(\frac{1}{(\alpha+x_i+x_i^{\,2})}\right)=0$$

Because of the complicated form of above likelihood equations, algebraically it is very difficult to solve these system of non-linear equations. Therefore, we use R and wolfram mathematics for estimating the required parameters of the proposed distribution.

For the purpose of obtaining the confidence interval, we use the asymptotic normality results. We have that if $\hat{\gamma}=(\hat{\theta},\ \hat{\alpha})$ denotes the MLE of $\gamma=(\theta,\ \alpha)$. We can state the result as follows:

$\sqrt{n}(\hat{\gamma}-\gamma)\rightarrow N_2(0,\ I^{-1}(\gamma))$

Where $I^{-1}(\gamma)$ is Fisher's information matrix.i.e.,

$$I(\gamma)=-\frac{1}{n}\begin{pmatrix}E\left(\frac{\partial^2\log L}{\partial\theta^2}\right) & E\left(\frac{\partial^2\log L}{\partial\theta\,\partial\alpha}\right)\\ E\left(\frac{\partial^2\log L}{\partial\alpha\,\partial\theta}\right) & E\left(\frac{\partial^2\log L}{\partial\alpha^2}\right)\end{pmatrix}$$

Here we define

$$E\left(\frac{\partial^2\log L}{\partial\theta^2}\right)=-\frac{4n}{\theta^2}-n\left(\frac{2\alpha(\alpha\theta^2+2\theta+6)-(2\alpha\theta+2)(2\alpha\theta+2)}{(\alpha\theta^2+2\theta+6)^2}\right)$$

$$E\left(\frac{\partial^2\log L}{\partial\alpha^2}\right)=n\left(\frac{\theta^4}{(\alpha\theta^2+2\theta+6)^2}\right)-\sum_{i=1}^{n}\left(\frac{1}{(\alpha+x_i+x_i^{\,2})^2}\right)$$

$$E\left(\frac{\partial^2 \log L}{\partial\theta\,\partial\alpha}\right) = -n\left(\frac{2\theta(\alpha\theta^2+2\theta+6)-(2\alpha\theta+2)\theta^2}{(\alpha\theta^2+2\theta+6)^2}\right)$$

Since γ being unknown, we estimate $I^{-1}(\gamma)$ by $I^{-1}(\hat{\gamma})$ and this can be used to obtain asymptotic confidence intervals for θ and α

Application

In this section, we have fitted a real data set in length biased two parameter sujatha distribution to discuss its goodness of fit and the fit has been compared over two parameter Sujatha, Sujatha, exponential and Lindley distributions. The real life data set is given below as:

The real life time data set represents the survival times (in months) of lung cancer patients is the non-censored data reported from Pena (2002).This data was recently used by L. S. Diab and E. S. El-Atfy (2017) in Paper "A moment inequality for overall decreasing life class of life distributions with hypothesis testing applications" and the data set is given below in table 1. In order to estimate the model comparison criterion values, the unknown parameters are also estimated through R software. In order to compare the length biased two parameter Sujatha distribution with two parameter Sujatha, Sujatha, exponential and Lindley distributions, we employ the criterion values AIC (Akaike Information Criterion), BIC (Bayesian Information Criterion), AICC (Akaike Information Criterion Corrected) and -2logL. The better distribution is which corresponds to lesser values of AIC, BIC, AICC and -2logL. For the calculation of criterion values AIC, BIC, AICC and -2logL, following formulas are used:

$$AIC = 2k - 2\log L, \qquad BIC = k\log n - 2\log L \qquad \text{and} \qquad AICC = AIC + \frac{2k(k+1)}{n-k-1}$$

Where k is the number of parameters in the statistical model, n is sample size and –2logL is the maximized value of log-likelihood function under the considered model. From table 2 given above, it has been observed from the results that the length biased two parameter Sujatha distribution have the lesser AIC, BIC, AICC and -2logL values as compared to two parameter Sujatha, Sujatha, exponential and Lindley distributions. Hence, it can be concluded that the length biased two parameter Sujatha distribution leads to a better fit over two parameter Sujatha, Sujatha, exponential and Lindley distributions.

CONCLUSION

The present paper deals with the new distribution namely length biased two parameter Sujatha distribution. The proposed new distribution is generated by using the length biased technique. Its various statistical structures including its mean, variance, harmonic mean, moment generating function, characteristic function, survival function, hazard rate function, order statistics, Bonferroni and Lorenz curves have been discussed. Its parameters have also been estimated through the technique of maximum likelihood estimation. Finally, the newly proposed length biased two parameter Sujatha distribution has been demonstrated with a real life data set to discuss its goodness of fit and the fit has been found good in comparison with two parameter Sujatha, Sujatha, exponential and Lindley distributions.

REFERENCES

1. Abd-Elfattah, A. M., Mahdy, M. and Ismail, G. (2021). Length biased Burr- XII distribution: properties and application. *International Journal of Sciences: Basic and Applied Research* (*IJSBAR*), 56(1), 222-244.
2. Ahmed, A., Mir, K. A. and Reshi, J. A. (2013). On size biased generalized beta distribution of first kind. *IOSR Journal of Mathematics* (*IOSR-JM*), 5(2), 41-48.

3. Al-Kadim, K. A. and Hussein, N. A. (2014). New proposed Length-Biased Weighted Exponential and Rayleigh distribution with application. *Mathematical Theory and Modeling*, 4(7), 137-152.
4. Cox, D. R. (1962). "Renewal theory", *Barnes and Noble*, *New York*.
5. Cox, D. R. (1969). Some sampling problems in technology, In New Development in Survey Sampling, Johnson, N. L. and Smith, H., Jr .(eds.) *New York Wiley- Interscience*, 506-527.
6. Das, K. K. and Roy, T. D. (2011). Applicability of length biased weighted generalized Rayleigh distribution. *Pelagia Research Library*, 2(4), 320-327.
7. Diab, L. S. and El-Atfy, E. S. (2017). "A Moment inequality for overall decreasing life class of life distributions with hypothesis testing applications". *International Journal of Mathematics and Statistics Invention*, 5(1), 62-71.
8. Fisher, R. A. (1934). The effects of methods of ascertainment upon the estimation of frequencies. *Annals of Eugenics*, 6, 13-25.
9. Ganaie, R. A. and Rajagopalan, V. (2021). A new extension of power quasi Lindley distribution with properties and applications of the life-time data. *International Journal of Statistics and Reliability Engineering*, 8(1), 171-183.
10. Mudasir, S. and Ahmad, S. P. (2018). Characterization and estimation of the length biased Nakagami distribution. *Pak.j.stat.oper.res*., 14(3), 697-715.
11. Mustafa, A. and Khan, M. I. (2022). The length-biased powered inverse Rayleigh distribution with applications. *J. Appl. Math. & Informatics*, 40(1-2), 1-13.
12. Rao, C. R. (1965). On discrete distributions arising out of method of ascertainment, in classical and Contagious Discrete, G.P. Patiled; *Pergamum Press and Statistical publishing Society, Calcutta.* 320-332.
13. Rather, A. A. and Ozel, G. (2021). A new length-biased power Lindley distribution with properties and its applications. *Journal of Statistics and Management Systems*, 25(1), 23-42.
14. Reyad, H. M., Hashish, A. M., Othman, S. A. and Allam, S. A. (2017). The length-biased weighted frechet distribution: properties and estimation. *International Journal of Statistics and Applied Mathematics*, 3(1), 189-200.
15. R core team. R version 3.5.3 (2019): A language and environment for statistical computing. R Foundation for statistical computing, Vienna, Austria.URL https:// www.R-project. Org/.
16. Saghir, A., Tazeem, S. and Ahmad, I. (2016). The length-biased weighted exponentiated inverted Weibull distribution. *Cogent Mathematics*, 3(1), 1-18.
17. Shanker, R. (2016). Sujatha distribution and its applications. *Statistics in Transition new Series*, 17(3), 391-410.
18. Subramanian, C. and Shenbagaraja, R. (2020). Length biased quasi Sujatha distribution with properties and applications to bladder cancer data. *Journal of Information and Computational Science*, 10(1), 493-506.
19. Tesfay, M. and Shanker, R. (2018). A two-parameter Sujatha distribution. *Biometrics & Biostatistics International Journal*, 7(3), 188-197.
20. Zelen, M. (1974). Problems in cell kinetic and the early detection of disease, in Reliability and Biometry, F. Proschan & R.J. Sering, eds, *SIAM, Philadelphia*, 701-706.

Table 1: Data regarding survival times (in months) for lung cancer patients reported by Diab and Atfy (2017)

0.99	1.28	1.77	1.97	2.17	2.63	2.66	2.76	2.79	2.86
2.99	3.06	3.15	3.45	3.71	3.75	3.81	4.11	4.27	4.34
4.40	4.63	4.73	4.93	4.93	5.03	5.16	5.17	5.49	5.68
5.72	5.85	5.98	8.15	8.62	8.48	8.61	9.46	9.53	10.05
10.15	10.94	10.94	11.24	11.63	12.26	12.65	12.78	13.18	13.47
13.96	14.88	15.05	15.31	16.13	16.46	17.45	17.61	18.20	18.37
19.06	20.70	22.54	23.36						

Table 2: Comparison of fitted distributions

Distribution	MLE	S.E	-2logL	AIC	BIC	AICC
Length Biased Two Parameter Sujatha	$\hat{\alpha} = 122.37035237$ $\hat{\theta} = 0.30883130$	$\hat{\alpha} = 152.10371139$ $\hat{\theta} = 0.05169433$	388.3382	392.3382	396.656	392.5349
Two Parameter Sujatha	$\hat{\alpha} = 0.51599723$ $\hat{\theta} = 0.32358928$	$\hat{\alpha} = 2.51912217$ $\hat{\theta} = 0.03182261$	392.2026	396.2026	400.5203	396.3993
Sujatha	$\hat{\theta} = 0.31959653$	$\hat{\theta} = 0.02292163$	392.2343	394.2343	397.3932	394.2988
Exponential	$\hat{\theta} = 0.11481347$	$\hat{\theta} = 0.01435059$	405.0524	407.0524	409.2113	407.1169
Lindley	$\hat{\theta} = 0.20971909$	$\hat{\theta} = 0.01867716$	392.0752	394.0752	398.234	394.1397

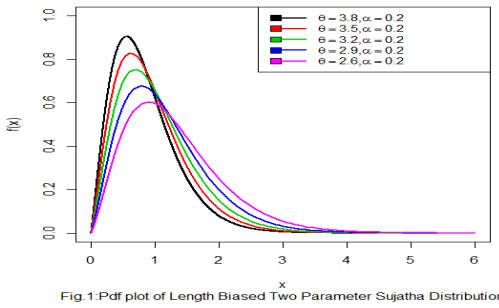

Fig.1:Pdf plot of Length Biased Two Parameter Sujatha Distribution

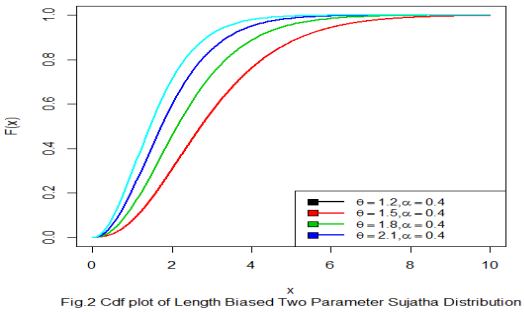

Fig.2 Cdf plot of Length Biased Two Parameter Sujatha Distribution

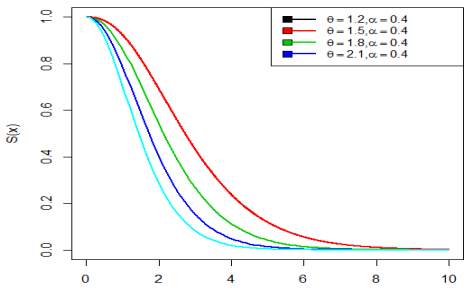

Fig.3:Survival plot of Length Biased Two Parameter Sujatha Distribution

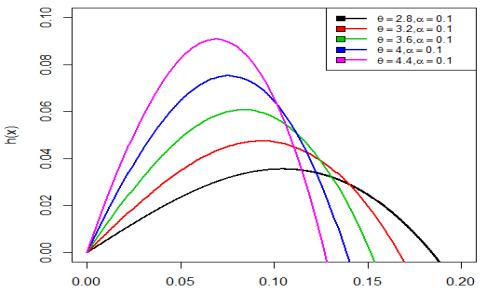

Fig.4:Hazard plot of Length Biased Two Parameter Sujatha Distribution

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RESEARCH ARTICLE

Effective Prediction of Breast Cancer through the Hormone Receptors using Novel Extreme Gradient Boosting Multi-Layer Perceptron Deep Learning

S.Saranya[1] and S.Sasikala[2]*

[1]Research Scholar and Assistant Professor, Department of Computer Applications, Hindusthan College of Arts and Science, Affiliated to Bharathiar University, Coimbatore, Tamil Nadu, India.
[2]Head and Associate Professor, Department of Computer Science with Cognitive Systems Hindusthan College of Arts and Science, Affiliated to Bharathiar University, Coimbatore, Tamil Nadu, India.

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***Address for Correspondence**
S.Sasikala
Head and Associate Professor,
Department of Computer Science with Cognitive Systems,
Hindusthan College of Arts and Science,
Affiliated to Bharathiar University,
Coimbatore, Tamil Nadu, India.
Email: iamsasikalamohit@gmail.com

ABSTRACT

Machine learning plays a significant role in prediction and early diagnosis of the disease. Breast cancer prediction can be done through the deep learning techniques. This paper proposed a novel method for the prediction of the breast cancer using the extreme gradient boosting and multilayer perceptron. Healthcare data mining is one of the conspicuous exploration fields in the current situation with the fast improvement of innovation and information. It is hard to deal with the immense measure of information of the patients. It is more vital to deal with this information through deep learning. There are a great deal of techniques for the treatment of numerous disease across the world. Deep learning is an arising approach that aides in forecast, finding of a sickness. This paper portrays the prediction of breast cancer using the deep learning. The role of the hormone receptors in breast cancer is investigated in this paper. The dataset deployed for the paper is Hormone Receptor dataset [1]. Experimental setup, results and discussion is given in the sections of the paper. Survival analysis is carried out for empirical dataset using the Log-Rank test. Results are tabulated. Results are compared with the base multilayer perceptron, radial bias network and Bayes Net classifiers. The proposed method gives maximum accuracy 96% for classification of the disease among the other methods. Results are promising and proven to have the efficacy in breast cancer prediction. Results using the proposed deep learning method seems to be promising.

Keywords: Breast Cancer, Hormonal Receptors, Multilayer Perceptron, Deep learning, Gradient Boosting.

INTRODUCTION

Breast cancer is the abnormal growth of the cancerous cells in the breast area. Breast cancer growth emerges in the coating cells (epithelium) of the pipes (85%) or lobules (15%) in the glandular tissue of the breast. At first, the harmful development is restricted to the pipe or lobule ("in situ") where it by and large causes no indications and has insignificant potential for spread (metastasis). Over the long haul, these in situ (stage 0) malignant growths might advance and attack the encompassing breast tissue (intrusive Breast disease) then, at that point, spread to the close by lymph hubs (territorial metastasis) or to different organs in the body (far off metastasis). Assuming that a lady passes on from Breast disease, it is a direct result of far reaching metastasis. Breast malignancy growth treatment can be exceptionally mandatory, particularly when the infection is recognized early. Therapy of malignant growth regularly comprises of a mix of careful expulsion, radiation treatment and medicine (hormonal treatment, chemotherapy and additionally designated organic treatment) to treat the infinitesimal disease that has spread from the breast cancer through the blood. Such therapy, which can forestall disease development and spread, subsequently saves lives.

Social Relevance of the Research

In 2020, there were 2.3 million women determined to have malignant breast cancer growth and 685 000 demise internationally. As of the finish of 2020, there were 7.8 million ladies alive who were determined to have malignant breast growth in the beyond 5 years, making it the world's most pervasive disease. There are more Disability Adjusted life years (DALYs) by ladies to breast disease internationally than some other kind of malignant growth. Breast cancer disease happens in each nation of the world in ladies at whatever stage in life later pubescence yet with expanding rates in later life.

Hormone Receptors in the Breast Cancer

Clinical therapies for breast tumors, which might be given before surgery is called the neo-adjuvant technique or later is called adjuvant technique, depends on the natural sub typing of the diseases. Malignant growth that express the Estragon Receptor (ER) as well as Progesterone Receptor (PR) are probably going to react to endocrine (chemical) treatments like tamoxifen or aromatase inhibitors. These medications are taken orally for 5-10 years, and decrease the shot at repeat of these chemical positive malignant growths by almost half. Endocrine treatments can cause indications of menopause however are for the most part all around endured. Tumors that don't communicate ER or PR are "chemical receptor negative" and should be treated with chemotherapy except if the malignant growth is tiny. The chemotherapy regimens accessible today are extremely compelling in lessening the odds of malignant growth spread or repeat and are by and large given as short term treatment. Chemotherapy for breast malignant growth for the most part doesn't need clinic affirmation without any entanglements. Breast tumors may freely over express cell called the HER-2/neuoncogene, which may be termed as triple positive. These "HER-2 positive" diseases are manageable to treatment with designated organic specialists. These natural specialists are exceptionally successful yet additionally extravagant, on the grounds that they are antibodies rather than synthetic substances. At the point when designated organic treatments are given, they are joined with chemotherapy to make them viable at killing malignant growth cells.

Related Literature

Naik *et.al.* (2020) deployed the deep learning based model for the investigation of the hormone receptors role in breast cancer through learning deep neural networks using multiple instances and it will predict the estragon receptors. The proposed method gives the area under curve value of 0.89 for training set and 0.92 for the test set using the 10 fold cross validation [2]. Alakwaa *et. al.* (2018) proposed feed forward neural networks for the estimation of the estragon receptors for predicting the breast cancer mass. They used the metabolomics data. They compared the proposed feed forward network against the machine learning techniques including the random forest, support vector machine, linear discriminant analysis, and boosted models. They reported maximum accuracy of 93% for the deep learning method.

They also focus on the protein assimilation and retention and ATP-restricting tape carrier's pathways are likewise affirmed in coordinated investigation among metabolomics and quality articulation information in these examples. Their profound learning technique shows benefits for metabolomics based bosom malignant growth ER status grouping, with both the most noteworthy forecast exactness and better disclosure of disease. They instigated the reception of feed-forward networks based profound learning technique in the metabolomics in breast cancer [3]. Ribelles *et. al.* (2021) proposed technique based on the machine learning methods for the prediction of the breast cancer to opt for the front line treatment in advanced stage of the breast cancer based on the hormone receptor. Using machine learning strategies, they created prescient models for right on time and late movement to front-line therapy of HR positive and HER2 negative metastatic breast malignant growth, likewise finding that NLP-based machine learning models are better compared to traditional models dependent on simulated information [4] .

Kim *et. al.* (2022) proposed the trans-membrane receptors on malignant growth cell surfaces can uncover biophysical highlights of the disease cells, in this manner giving a technique to portraying malignant growth cell aggregates. While ordinary examination of receptor movements in the cell film generally depends on the mean-squared relocation plots, much data is lost while delivering these plots from the directions. They utilize profound figuring out how to characterize breast cancer disease cell types dependent on the directions of epidermal development factor receptor (EGFR). They used neural networks prepared on the EGFR movements obtained from six breast disease cell lines of differing obtrusiveness and receptor status [5]. Pironet *et. al.* (2021) deployed machine learning method for breast cancer biomarkers from the pathology reports. Different kinds of numeric elements are registered from north of 1,300 physically clarified reports connected to breast cancers analysed in 2014. They deployed various standard machine learning techniques and achieved maximum of 92% of accuracy [6]. Shamai *et. al.* (2019) proposed method based on hormonal abnormality assessment of tissue microarrays among the breast cancer patients using the machine leaning methods [7]. Li *et. al.* (2021) proposed method based on the assessment of neo-adjuvant chemotherapy through the breast image, using the deep learning methods [8]. Abhang and Lopez (2018) proposed the Health belief model based on psychology to predict the health behaviour. They analyse various behaviour for preventing an array of diseases. They adopted iteration based method for period of time and suggest self-examination and health belief model from social dimension [9]. Arjun *et. al.* (2012) perform comprehensive review of breast cancer based on the graphical data. They studied the types and causes along with different stages, signs and symptoms. They also analysed the treatments for the breast cancer including chemotherapy, radiotherapy, surgery and nutraceuticals based treatments [10].

Lavanya *et. al.* (2014) performed a Questionnaire based survey among the patients in the Erode cancer centre and among them breast cancer patients undergo maximum stress up to 70% among all other types of cancers among women [11]. Ravindra *et. al.* (2011) perform analysis on SERM selective estrogen receptor modulators in various aspects of the breast cancer. They also conducted a comprehensive study of the estrogen receptors chemical reactions in the breast cancer [12]. Dange *et. al.* (2017) analysed the diagnosis stage of the breast cancer demographically. They also made a comprehensive review on the treatment options and various factors influencing the breast cancer including the hormone receptor [13]. Sandip *et. al.* (2020) analysed the HER2 Human epidermal growth factor receptor influence on the breast cancer and they also studied the anti-breast cancer drug Niratinib maleate [14]. Sampoornam (2014) analysed the role of bio markers including proteomic, genetic, glycomic, epigenetic and imaging biomarkers. The way in which how they can be interpreted through non-invasive methods including bio fluids like blood and serum are instigated [15]. Geetha *et. al* (2017) carried out FACT-B Questionnaire based method for assessing the quality of life among the breast cancer patients [16]. Akshay *et. al.* (2020) studied the role of anticancer action of different leaf extracts of psidiumguajava in accordance to the breast cancer cell line (MCF-7) [17]. Kalpanapriya and Saravanan (2017) proposed wavelet transform based ultrasonic imaging based method for predicting the breast cancer [18].

MATERIALS AND METHODS

Materials

The hormone receptors dataset is deployed for the research [1]. The dataset contains the subject ID, Date extraction of the data. The demographics information including the age and race of the patient is in the dataset. The pre-treatment information including the ER positive – Estrogen Positive, PgR Positive indicating Progesterone positive status, HR positive indicating the hormone receptor status, HER2 positive specifies the triple positive status, HR_HER2_CATEGORY representing the severity category, HR_HER2_STATUS status of the pre-treatment, BilateralCa representing the presence of the bilateral breast cancer before the neo-adjuvant therapy, Laterality indicating the index of the tumour laterality.

Methods of Predicting Hormonal Receptor Value and Need of Hormonal Therapy with Neo-Adjuvant Chemotherapy for Breast Cancer

Machine Learning is the logical investigation of factual models and calculations that PC frameworks use to play out an assignment without unequivocal guidelines. Deep Learning is a new field that possesses the a lot more extensive field of Machine Learning. Deep Learning is generally popular for its neural organizations like Recurrent Neural Networks, Convolutional Neural Networks, and Deep Belief Networks. While other AI calculations utilize measurable investigation strategies for design acknowledgment, Deep learning is designed according to the neurons of the human cerebrum. They are designed according to the construction and working of the human brain functioning. It needs to understand how the sensory system in the human body works. Perception realize that the sensory system is developed of neurons. These neurons can get a handle on data that is sent to body and brain. These neurons can learn data over the long haul. Deep learning based method XGBMLP – Extreme Gradient Boosting incorporated in the Multi-Layer Perceptron is proposed for the research.

Need of the XGBMLP in Breast Cancer Prediction

Machine learning plays a significant role in prediction and early diagnosis of the disease. Breast cancer prediction can be done through the deep learning techniques. This paper proposed a novel method for the prediction of the breast cancer using the extreme gradient boosting and multilayer perceptron. Healthcare data mining is one of the conspicuous exploration fields in the current situation with the fast improvement of innovation and information. It is hard to deal with the immense measure of information of the patients. It is more vital to deal with this information through deep learning. There are a great deal of techniques for the treatment of numerous disease across the world. Deep learning is an arising approach that aides in forecast, finding of a sickness. This paper portrays the prediction of breast cancer using the deep learning. The role of the hormone receptors in breast cancer is investigated in this paper. The dataset deployed for the paper is Hormone Receptor dataset [1]. Experimental setup, results and discussion is given in the sections of the paper. Survival analysis is carried out for empirical dataset using the Log-Rank test. Results are tabulated. Results using the proposed deep learning method seems to be promising.

Survival study

An empirical data of 20% is considered for the survival analysis. The two attributes considered are Neo-adjuvant chemotherapy, which is carried out for the reduction of the cancer severity before surgery. Another factor is the Hormone Therapy (HT) based on the hormone receptor levels.

The proposed hypothesis is as follows

H0: Hormone Therapy Combined with Neo-Adjuvant Chemotherapy does not have impact in the cancer treatment

H1: Hormone Therapy Combined with Neo-Adjuvant Chemotherapy have significant impact in the cancer treatment

The log rank test is carried out for the proposed hypothesis. Log rank test is the survival evaluation test among two set of groups for a particular treatment or drug usage. It is a non-parametric test commonly used for the substantiating the efficiency of a new treatment. Consider two group of patients for a specific two group of treatments. The treatment and control variables are to be considered for the evaluation. Consider 1, 2, …j which

represents events observed in the two groups. Now assume N1j and N2j as the subjects at risk at the initial period j. assume O1j and O2j as the observed events at the group. Consider the metrics as:
Nj = N1j + N2j and Oj = O1j + O2j

Now calculate variance, V as follows

$V_{ij} = E_{ij}\left(\frac{N_j - O_j}{N_j}\right).\left(\frac{N_j - N_{ij}}{N_j - 1}\right)$ and

Log rank statistic as

$$Z = \frac{\sum_1^j O_{ij} - E_{ij}}{\sqrt{\sum_1^j V_{ij}}} \xrightarrow{d} N(0,1)$$

For the hypothesis the calculations are deployed and the result achieved is as follows
For the hypothesis the calculations are deployed and the result achieved is as follows
Log Rank statistic: 4.392861

Degrees of freedom : 1 This is the number of groups, minus 1
p-value: 0.036090

The obtained p-value below a pre-set critical value such as 0.05, indicates rejection of the omnibus null hypothesis, that there is difference in survival rates of the groups. The alternate hypothesis that one or more of the groups has a different survival rate is accepted.

H1: Hormone Therapy Combined with Neo-Adjuvant Chemotherapy have significant impact in the cancer treatment
→ Hypothesis Accepted
Hence the efficacy of the hormone receptors is substantiated through the hypothesis using the log rank test. Now the dataset has the aforementioned hormone receptors and it is deployed in the novel proposed algorithm. It is explicated in the subsequent sections of the paper.

Nuances of the Proposed Algorithm XGBMLP

The proposed strategy XGBMLP uses the optimization extreme gradient boosting helping to get surmising of the highlights from the XGB trees. It makes the computation for the each layer of the neural organization through weight updating though XGB trees, result of the significant features. The proposed technique can be diagrammatically addressed as follows. For the specific neural network hidden layer the gradient boosting optimizations can be used for effective weight updating. The proposed algorithm is given in Figure 3. The steps are exemplified using the samples. Experiments are carried out with the Intel core i7, 64 bit operating system, 32 GB RAM with Windows 10 environment. The PYCHARM console is used for the implementation which uses the PANDAS, SCIPY, NUMPY, SKLEARN and KERAS API. Tensor flow is used for the neural network configuration settings The parameter setting are as follows Table.1. The proposed algorithm is implemented and the results are exemplified for two different epochs 100 and 50. The graph simlated for 100 epochs is given in the Figure 4.

DISCUSSION

The XGB part of the proposed algorithm will help in order to reveal the feature significance. In healthcare predictions accuracy is the key parameter, hence it may reveal the information of the patient diagnosis result to commence the treatment. Early prognosis of the disease may save the patient life and to decide the type of the treatment to be given. The algorithm is presented, along with the experimental setup and results are proven to show

the efficacy. Figure 4 shows the epoch wise Accuracy and Loss of the proposed algorithm. And Figure 5 portrays the accuracy comparison of the proposed algorithm. Results are compared with the base multilayer perceptron, radial bias network and Bayes Net classifiers. The proposed method gives maximum accuracy 96% for classification of the disease among the other methods. Results are promising and proven to have the efficacy in breast cancer prediction.

CONCLUSION

Hormonal receptors provides key insights for the breast cancer prediction. They also support in the assessment of the severity of the disease. The hormone therapy can be carried out by indicators of the hormone receptors including the estrogen, progesterone and HER positive status. This research utilizes the hormone levels and presence of the receptors in the patient. If the indication is identified in earlier then the hormone therapy can be combined with the Neo-Adjuvant Chemotherapy for the patient to reduce the tumor size and severity of the disease. This research proposed a novel method based on the deep learning, XGBMLP. Deep learning models are proven to give promising results in the disease prediction. This research proposes the novel hybrid model names extreme gradient boosting incorporated in the multi-layer perceptron. Results are compared with the base multilayer perceptron, radial bias network and Bayes Net classifiers. The proposed method gives maximum accuracy 96% for classification of the disease among the other methods. Results are promising and proven to have the efficacy in breast cancer prediction.

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REFERENCES

1. Esserman, Laura J *et al.* "Pathologic complete response predicts recurrence-free survival more effectively by cancer subset: results from the I-SPY 1 TRIAL--CALGB 150007/150012, ACRIN 6657." Journal of clinical oncology :official journal of the American Society of Clinical Oncology vol. 30,26 (2012): 3242-9. doi:10.1200/JCO.2011.39.2779
2. Naik, N., Madani, A., Esteva, A. et al. Deep learning-enabled breast cancer hormonal receptor status determination from base-level H&E stains. Nat Commun 11, 5727 (2020). https://doi.org/10.1038/s41467-020-19334-3
3. Alakwaa, F. M., Chaudhary, K., & Garmire, L. X. (2018). Deep Learning Accurately Predicts Estrogen Receptor Status in Breast Cancer Metabolomics Data. Journal of proteome research, 17(1), 337–347. https://doi.org/10.1021/acs.jproteome.7b00595
4. Ribelles, N., Jerez, J. M., Rodriguez-Brazzarola, P., Jimenez, B., Diaz-Redondo, T., Mesa, H., Marquez, A., Sanchez-Muñoz, A., Pajares, B., Carabantes, F., Bermejo, M. J., Villar, E., Dominguez-Recio, M. E., Saez, E., Galvez, L., Godoy, A., Franco, L., Ruiz-Medina, S., Lopez, I., & Alba, E. (2021). Machine learning and natural language processing (NLP) approach to predict early progression to first-line treatment in real-world hormone receptor-positive (HR+)/HER2-negative advanced breast cancer patients. European journal of cancer (Oxford, England : 1990), 144, 224–231. https://doi.org/10.1016/j.ejca.2020.11.030
5. Mirae Kim, Soonwoo Hong, Thomas E Yankeelov, Hsin-Chih Yeh, Yen-Liang Liu, Deep learning-based classification of breast cancer cells using transmembrane receptor dynamics, Bioinformatics, Volume 38, Issue 1, 1 January 2022, Pages 243–249, https://doi.org/10.1093/bioinformatics/btab581
6. Pironet A, Poirel HA, Tambuyzer T, De Schutter H, van Walle L, Mattheijssens J, Henau K, Van Eycken L and Van Damme N (2021) Machine Learning-Based Extraction of Breast Cancer Receptor Status From Bilingual Free-Text Pathology Reports. Front. Digit. Health 3:692077. doi: 10.3389/fdgth.2021.692077

7. Shamai G, Binenbaum Y, Slossberg R, Duek I, Gil Z, Kimmel R. Artificial Intelligence Algorithms to Assess Hormonal Status From Tissue Microarrays in Patients With Breast Cancer. JAMA Netw Open. 2019;2(7):e197700. doi:10.1001/jamanetworkopen.2019.7700
8. Li, F., Yang, Y., Wei, Y., He, P., Chen, J., Zheng, Z., & Bu, H. (2021). Deep learning-based predictive biomarker of pathological complete response to neoadjuvant chemotherapy from histological images in breast cancer. Journal of translational medicine, 19(1), 348. https://doi.org/10.1186/s12967-021-03020-z
9. Nayna Abhang, Joe Lopez. Health Belief Model for Social Marketing of Breast Self-Examination – A Review of Literature. Asian Journal of Management. 2018; 9(1):493-499.
10. Arjun Patidar, S.C.Shivhare, Umesh Ateneriya, Sonu Choudhary. A Comprehensive Review on Breast Cancer. Asian J. Nur. Edu. & Research 2(1): Jan.-March 2012; Page 28-32.
11. S. Lavanya, Nalini Jeyavantha Santha, Gowri Sethu. A descriptive study to assess the level of stress among women with selected type of cancer in Erode Cancer Centre at Erode. Asian J. Nur. Edu. & Research 4(3): July-Sept., 2014; Page 321-324.
12. Ravindra B. Saudagar, Nachiket S. Dighe, Deepak S. Musmade , Vinayak M. Gaware, D. A. Jain. SERM's in Treatment of Breast Cancer. Asian J. Pharm. Res. 1(4): Oct. - Dec. 2011; Page 81-86.
13. V. N. Dange, S. J. Shid, C.S. Magdum, S.K. Mohite. A Review on Breast cancer: An Overview. Asian J. Pharm. Res. 2017; 7(1): 49-51.
14. Sandip S. Kshirsager, Dr. Siraj N. Shaikh, Narendra B. Patil, Ketan B. Patil. Novel Molecule of Protein Tyrosine Kinase Enzyme Inhibitor in Treatment of Breast Cancer: Neratinib Maleate. Asian J. Res. Pharm. Sci. 2020; 10(2):100-102.
15. Sampoornam W. Nurse Researcher's Role in Breast Cancer Biomarkers. Int. J. Adv. Nur. Management 2(1):Jan. - Mar., 2014; Page 46-47.
16. M. Geetha, P. Padmavathi, K. Menaka. Effectiveness of Multi Interventional Package on Quality of Life among Breast Cancer Patients Receiving Chemotherapy Drugs – Pilot Study. Int. J. Adv. Nur. Management. 2017; 5(2):164-168.
17. Akshay R. Yadav, Shrinivas K. Mohite. Anticancer Activity of Psidium guajava Leaf Extracts on Breast Cancer Cell Line. Res. J. Pharma. Dosage Forms and Tech.2020; 12(4):298-300.
18. D. Kalpanapriya, M. Saravanan. Detection of Breast Cancer by Ultrasonic Image using Wavelet Transforms. Research J. Pharm. and Tech. 2017; 10(3): 839-842.

Table.1.Extreme Gradient Boosted

Layer (type)	Output Shape	Param #
dense (Dense)	(None, 60)	1860
dense_1 (Dense)	(None, 60)	3660
dense_2 (Dense)	(None, 110)	6710
dense_3 (Dense)	(None, 400)	44400
dense_4 (Dense)	(None, 150)	60150
dense_5(Dense)	(None, 1)	151

Total params: 116,931
Trainable params: 116,931
Non-trainable params: 0

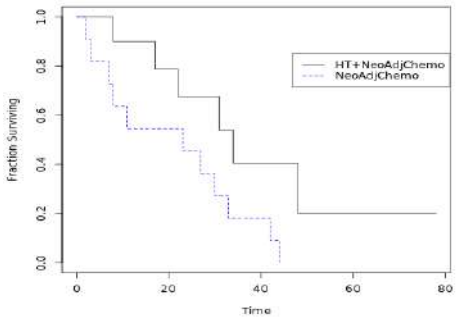


Figure 1. Survival Curve obtained from Log-Rank Statistic for the HT+Neo-Adjuvant Chemotherapy

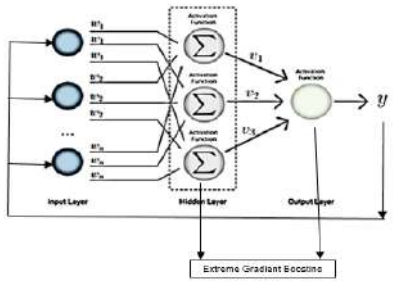
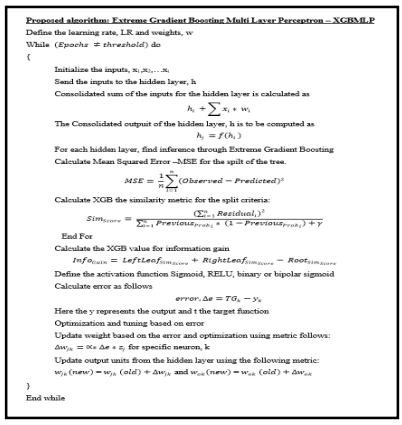


Figure 2. Working Method of the Proposed XGBMLP



Proposed algorithm: Extreme Gradient Boosting Multi Layer Perceptron – XGBMLP

Define the learning rate, LR and weights, w

While $(Epochs \neq threshold)$ do

{

Initialize the inputs, $x_1, x_2, \ldots x_i$

Send the inputs to the hidden layer, h

Consolidated sum of the inputs for the hidden layer is calculated as

$$h_i + \sum x_i * w_i$$

The Consolidated output of the hidden layer, h is to be computed as

$$h_i = f(h_i)$$

For each hidden layer, find inference through Extreme Gradient Boosting

Calculate Mean Squared Error –MSE for the spilt of the tree.

$$MSE = \frac{1}{n}\sum_{i=1}^{n}(Observed - Predicted)^2$$

Calculate XGB the similarity metric for the split criteria:

$$Sim_{score} = \frac{(\sum_{i=1} Residual_i)^2}{\sum_{i=1} Previous_{Prob_i} * (1 - Previous_{Prob_i}) + \gamma}$$

End For

Calculate the XGB value for information gain

$$Info_{Gain} = LeftLeaf_{Sim_{score}} + RightLeaf_{Sim_{score}} - Root_{Sim_{score}}$$

Define the activation function Sigmoid, RELU, binary or bipolar sigmoid

Calculate error as follows

$$error.\Delta e = TG_i - y_i$$

Here the y represents the output and t the target function

Optimization and tuning based on error

Update weight based on the error and optimization using metric follows:

$\Delta w_{ik} = \propto * \Delta e * x_i$ for specific neuron, k

Update output units from the hidden layer using the following metric:

$w_{ik}(new) = w_{ik}(old) + \Delta w_{ik}$ and $w_{ok}(new) = w_{ok}(old) + \Delta w_{ok}$

}

End while

Figure 3. Proposed Algorithm XGBMLP

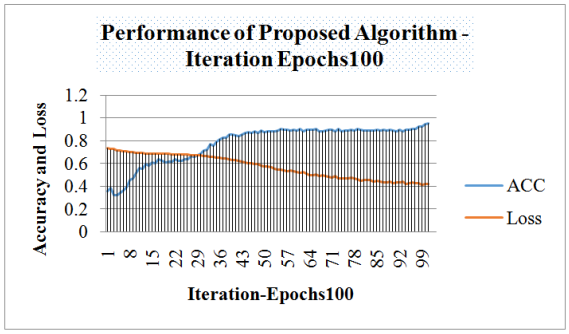


Figure 4. Epoch wise Accuracy and Loss of the proposed algorithm

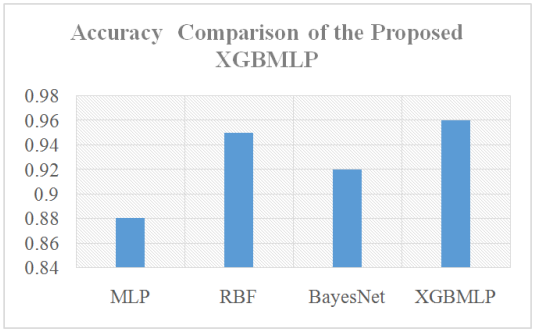


Figure 5. Performance comparison of the proposed algorithm

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RESEARCH ARTICLE

Analysis of Teaching of Foreign Language by Native and Non-Native Speaker Teachers

Tanuja Yadav*

Associate Professor, Department of Languages, Literatures and Cultural Studies, Manipal University Jaipur, Jaipur, Rajasthan, India.

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***Address for Correspondence**
Tanuja Yadav,
Associate Professor,
Department of Languages,
Literatures and Cultural Studies,
Manipal University Jaipur,
Jaipur, Rajasthan, India.
Email: tanuja.yadav@jaipur.manipal.edu

ABSTRACT

The knowledge of foreign language is playing an important role in the education for the millennials and Gen Z. Although the technology has already facilitated the learning and teaching in the various domains, but the teaching of the foreign language by the teacher acts as a catalyst for the learner. The teacher motivates the learner to practice the language through the role playing which increases the immersion and involvement of the learner in the foreign language environment. Moreover, the culture integration is an inevitable part for the learning of the foreign language. There is always a belief that Native Speaker Teacher is more effective in teaching the foreign language than the Non-native speaker teacher. This paper will explore the different aspects of the teaching of foreign language by the native and Non-native speaker teacher. The native speaker is deeply immersed into one's own language and culture that he /she communicates in his/her language in a most convenient manner than the non-native speaker teacher. The ease of teaching the language develops the confidence in the teacher and the learner as well. As a result, the learner gets the vast knowledge of the language even beyond their requirement of the language for the particular level. While the Non -native speaker teacher enhances the learning of the foreign language among the learners in a more convenient and adaptable manner. The teaching strategies of the non-native speaker teacher is enriched with his/her own learning experiences. The non-native speaker teacher understands the problems of the learners more easily. It's true that the non-native speaker teachers cannot be at par with the language knowledge of the native speaker teachers but the benefit of learning and then teaching the foreign language for the non-native teacher makes the teacher skillful for the use of the appropriate strategy of teaching the foreign language to his/her learners.

Keywords: Native and Non-native speaker, learning, teaching, foreign language.

INTRODUCTION

There is always a controversy related to the native and non native teachers in the foreign language class. According to Cook (1999), the native speakers are the one who have the first language in their childhood while the non native acquires the same language not as the first language but as the second or the foreign language. The cultural integration is the foremost aspect of the learning and teaching of the foreign language. Thus, the native speaker teacher responds immediately to the learners at various situations of language classroom. The experiences of the non-native speaker teacher as a student and a teacher facilitate the teaching of the language in class. The native speaker teachers are more confident in front of the learners, but the non-native teachers are sometimes stuck with their own questions, or the question posed by the learner. At that moment they need time to understand and verify their own answer and reply to the learner. On the contrary, it has been found that the learners are more confident, comfortable, and free to ask the questions to the non-native teachers than the native teachers. The non native teachers facilitate the understanding of the language learning in the most appropriate manner. They use the teaching and learning techniques that are easy to conduct and as the learner also belongs to their own culture, it becomes easy for both of them to understand each other during the teaching and learning process. The learners are more comfortable to share their problems with the non native teachers. Although the native teachers are considered more competent, the non native teachers use different learning strategies to enhance the competencies of the learners.

METHODOLOGY

The paper focuses on the qualitative descriptive analysis of the preferences of the native and non native teachers. The analysis revisits the perceptions for the good language teachers for the foreign languages.

Native and Non Native Speaker Teacher and Foreign Language Teaching

There are various perceptions regarding the preferences related to the native and non native teachers among the learners of the foreign language. The cultural background of the learners plays a pivotal role in the preferences of the language teachers. Besides this, the teaching experience and the language background of the teacher also laid emphasis on the teaching and learning of the foreign language. These teachers develop various pedagogical strategies to enhance the learning and motivation among the learners. The non-native teachers have the benefit of sharing their experiences as learners as well as teachers to strengthen the learning of the foreign language. Moreover, the Non native teachers working as the foreign language teachers are also the role model and inspiration for the learners to be the future professor. Lee (2005:8), along with Kubota (2004); Maum (2002) and Medgyes (1992) defines the native speaker as: "the individual acquired the language in early childhood and maintains the use of the language, the individual has intuitive knowledge of the language, the individual is able to produce fluent, spontaneous discourse, the individual is communicatively competent and able to communicate within different social settings, the individual identifies with or is identified by a language community, and the individual does not have a foreign accent."

Although there is no difference in the native and non native speaker teachers of the target language but the institutions and the learners are slightly biased towards the native speaker teachers. It could be the prejudice that only native teachers are better than the non-native speaker teachers. The non native teachers are suffering from the discrimination not only in terms of teaching on the part of students but also administration and the parents. The stakeholders are judging the native and non-native teachers on the basis of their perceptions, prejudice and preconceived notions. The reality lies with the performance of the teachers and the learners of the foreign language. The non -native teachers are less confident in the pronunciation of the foreign language and the learners always try to question the competency of the non native teachers in the foreign language. On the contrary, the stakeholders are confident for the language knowledge of the native speaker teachers. The pedagogical skills that play the important role in the teaching and learning of the foreign language is positioned at the second step by the stakeholders. The

very first preference is always given to the native speaker teacher. The "Native speaker fallacy" of Phillipson (1992) unfolds the preference of the learners for the Native Speaker teacher in the foreign language class.

The native teachers work best with the learners of higher level where the mastery of the language and native proficiency is required along with the learning of the culture, society, language and literature of the target language is required. It has also been found through the researches that the preference has been given to the native speaker teacher with the level of the language learning. At advanced level, the higher level of acquisition of target language is required to increase the conversational and communicative skills. Thus the native teachers who are skilled with the plethora of the vocabulary in the target language are found suitable for the learners of higher level. But it does not mean that the non-native teachers donot have the skills of teaching the learners of the higher level. The non-native teachers who are trained from time to time by the native professional trainers and are working with the target language by updating themselves with the literature, language, communication skills, culture and general affaires of the society of the target language, are equally the good teachers. Besides this, the non native teachers are the best for the learners at the basic level of the language where the learner needs the bilingual method and the implicit approach with their common understanding of the non native teachers. Besides this, Widdowson (1994), states that: "real proficiency is when you are able to take possession of the language, turn it to your advantage, and make it real for you" (p. 384). The most important characteristic of the foreign language teacher is her/his pedagogical skills used in the class for the motivation of the learners. The non native speaker teacher connects with the learners more easily than the native speaker teachers due to the sharing of the culture and first language.

The non native teachers are able to understand the problems faced by the learners more easily that the non native and they respond fast to the needs of the learners. They are aware of the barriers and challenges faced by them when they learned the target, thus facilitating the learning of the target language. It has been found that non native teachers find themselves low in front of the native teachers due to their low proficiency in communication skills. Thus, they should focus more on their oral proficiency and pronunciation. Farrell (2015) states that it is not the teacher's ethnicity, mother tongue or culture that define them as a good or a bad teacher. The local teachers accent on the performance of the oral proficiency of the target foreign language learners is also one of the various factors that accentuates on the requirement of the native language teachers. The nonnative teachers spent more time on the grammar of the foreign language in comparison to the pronunciation while the native teacher is confident with her his first language, so he/she focuses on the pronunciation than on the grammar. The nonnative teacher understands the problems faced by the foreign language learners faster than the native teacher and she /he can resolve the problems by using the common first language in the most appropriate manner. The native teachers are unable to catch the problem faced by the learners due to the difference in the language of the native teacher and the learners.
The learners in the class of the native teachers are benefitted by the oral language skills as well as the cultural information of the target foreign language country. They provide the recent information regarding the culture and the language. Rubrecht (2006) states that the learners who favor the native teachers, have the desire to enter into the target foreign language and want to interact with the native speakers.

The native teachers are creative and informative about the culture of the target language. They are the best motivator for the enhancement of the communication skills of the learners in the foreign language. The native teachers motivate the learners to communicate in the target language and the learners have no choice than speaking in the target language which is the most important step of learning the foreign language while the non native are master of the grammar of the foreign language and understands the learners and their problems. They are the facilitators to their problems in the grammar of the foreign language. The non native speaker teachers are more empathetic to their learners as they possess the similar foreign language learning experiences. They are aware of the strategies to use that can work the best for their learners. These are the major strengths of the Non native speaker teachers. Ellis (2002 as cited in Moussu & Llurda, 2008) defines that good language teachers are those who have had experience with the acquisition and the use of a new language besides being good at linguistics, pedagogy and methodology so that they can understand the processes and experiences that their students will have with learning their new language.

Thus, the professional development trainings and the language workshops should be conducted to enrich the teaching and learning strategies of the non native teachers. Moreover the teachers should join the associations of professional body that will provide them the platform to share their experiences and enrich their knowledge. The associations of teachers of foreign or target languages conduct the training programs as well as they offer the opportunities for the foreign language teachers to enhance their professionalism and raise their self-confidence.

CONCLUSION

The effectiveness of the learning and teaching comes out from the pedagogical skills employed by the teachers in the class of foreign language. Both the native and non native teachers have the weaknesses and strength in their teaching. Thus, we cannot name any one of them as an ideal teacher. It is their pedagogical strategies that motivate the learners and facilitate their learning of the foreign language. The development of the four skills of the learners is the proof of the effective teaching and the teacher. If an importance is given to the difference in the native and non native teaching style on the basis of the first language and second or foreign language as their domain then it is futile. The good command of the language, the teaching skills and the attitude or the personal qualities of the teacher are considered to be the most effective characteristics of the foreign language teacher. Thus, one should not follow the pre-conceived notion that Native speaker teachers are best for foreign language teaching. The foreign language teaching should depend on the pedagogical skills employed by the non native and native teachers for the effective and motivating teaching.

REFERENCES

1. Cook, V. (1999). Going beyond the native speaker in language teaching. TESOL Quarterly, 33(2), 185-209. http://dx.doi.org/10.2307/3587717
2. Kubota, M. (2004). Native speaker: A unitary fantasy of a diverse reality. The Language Teacher, 28(1), 3-30
3. Ellis, E. (2002). Teaching from experience: A new perspective on the non-native teacher in adult ESL. Australian Review of Applied Linguistics, 25(1), 71-107.
4. Farrell, T.S.C. (2015). It's Not Who You Are! It's How You Teach! Critical Competencies Associated with Effective Teaching. *RELC Journal, 46(*1), 79-88. https://doi.org/10.1177/0033688214568096
5. Lee, J. (2005). The native speaker: An achievable model? Asian EFL Journal, 7(2).
6. Maum, R. (2002). Nonnative- English-Speaking teachers in the English teaching profession. ERIC Digest. Retrieved April 2, 2011, from http://www.eric.ed.gov/PDFS/ED470982.pdf.
7. Medgyes, P. (1992). Native or non-native: Who's worth more? ELT Journal, 46(4), 340-349. http://dx.doi.org/10.1093/elt/46.4.340
8. Medgyes, P. (1994). The non-native teacher. London: Macmillan.
9. Phillipson, R. (1992). Linguistic imperialism. Oxford: Oxford University Press.
10. Rubrecht, B.G. (2006). Expanding the Gardnerian model of motivation. In K. Bradford-Watts (Ed.), JALT2006 conference proceedings. Tokyo: JALT.
11. Widdowson, H. G. (1994). The ownership of English. TESOL Quarterly, 28(2), 377-389. http://dx.doi.org/10.2307/3587438

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REVIEW ARTICLE

Recent Updates on Green Synthesis of Pyrazole Derivatives: A Review

Manojmouli.C[1], Sai Bharath.V.M[2], Munazilla Khaleel[2], T. Y. Pasha[3], B.Ramesh[4] and K.Naga Prashant[1]*

[1]Assistant Professor, Department of Pharmaceutical Chemistry, Sri Adichunchanagiri College of Pharmacy, Adichunchanagiri University, B. G. Nagara, Karnataka, India.
[2]M.Pharm, Department of Pharmaceutical Chemistry, Sri Adichunchanagiri College of Pharmacy, Adichunchanagiri University, B. G. Nagara, Karnataka, India.
[3]HoD and Professor, Department of Pharmaceutical Chemistry, Sri Adichunchanagiri College of Pharmacy, Adichunchanagiri University, B. G. Nagara, Karnataka, India.
[4]Principal, Department of Pharmaceutical Chemistry, Sri Adichunchanagiri College of Pharmacy, Adichunchanagiri University, B. G. Nagara, Karnataka, India.

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***Address for Correspondence**
K.Naga Prashant,
Assistant Professor,
Department of Pharmaceutical Chemistry,
Sri Adichunchanagiri College of Pharmacy,
Adichunchanagiri University,
B. G. Nagara, Karnataka, India.

ABSTRACT

Substituted pyrazoles are one of the important classes of heterocyclic compounds because they can serve as unique and versatile pharmacophores in drug design and discovery. They are very well known for diversified biological activities like antibacterial, antifungal, anti-tuberculosis anti-inflammatory, antioxidant anticancer, analgesic, etc. As a consequence, a wide range of green methods are available for the green synthesis of substituted pyrazole derivatives. The current review deals with the green method of synthesizing pyrazole through condensation of 1, 3 diketones/ ketones/ aldehydes/ carboxylates/ malononitrile/ Hydroxyl compound/ aniline with hydrazines/ semicarbazides and also summarized the various recent updates on different methods for green synthesis of pyrazole derivatives.

Keywords: anticancer, pyrazoles, green, synthesis, drug, antifungal, anti-tuberculosis

INTRODUCTION

Greener synthesis is necessary for organic chemistry, where the use of toxic chemicals and solvents in several industrial processes causes significant environmental harm. Green technologies are widely used in the pharmaceutical industry and medicinal chemistry, particularly for the administration of drugs and the fight against tropical and other illnesses [1]. Conventional techniques of organic synthesis typically require considerable heating

times and laborious apparatus setup, increasing process costs and requiring large amounts of solvents and reagents. In addition to the environmental issues brought on by their usage and disposal of trash, these procedures also present several health and safety issues for employees. Green Chemistry strives to employ less harmful solvents, cut down on the number of steps in synthetic processes, and eliminate waste to the greatest extent conceivable [2]. Pyrazoles are heterocyclic molecules with two nitrogen atoms in a five-membered parent ring that plays a key role in medicinal chemistry. They include numerous substituted pyrazoles and their derivatives.

Green synthesis

From 1, 3 Diketone

Kakhki RM *et al.* synthesized the pyrazole derivatives (c) by condensation of various phenyl hydrazines derivatives (a) with ethyl acetoacetate (b) in presence of water by used the green nanocatalyst ZnO modified nanoclinoptilolite [3]. Barge M *et al.* described the efficient green synthesis of pyrazole derivatives(c) by stirring the acetylacetone (a), hydrazines or hydrazides (b) in the presence of 5cm^3 of 50% aqueous sodium-p-toluenesulfonate (NaPTS) solution for a few minutes at room temperature [4]. Marandi A *et al.* described a method for one-pot multicomponent green synthesis of pyrazole-fused isocoumarins derivatives (c) by stirring, a mixture of ninhydrin(a), 1-benzylidene 2-phenylhydrazine (b), and Fe_3O_4@apple seed starch-In(III) as green catalyst under solvent-free conditions (Fig.33) for 10 minutes at 100 degrees Celsius [5]. Sadjadi S *et al.* described an effective method for the green synthesis of pyrazole derivatives (f) through Cascade Reaction by heating the mixture of hydrazine hydrate (b)ethyl acetoacetate (a), followed by the addition of other components including Naphthalen-2-ol (d) or Naphthalen-1-ol (e), benzaldehyde (c), and HPA-IL/CDNS at 80 °C [6].

From 1, 3 diketones, malononitrile, and aldehyde

Ahmadzadeh M *et al.* described the green synthesis of Bispyrano [2, 3-c] pyrazole derivatives (e) by stirring the equimolar mixture of terepthalaldehyde or isophthaladehyde (d), hydrazine hydrate, or phenylhydrazine (c), β-ketoester (a), and malononitrile (b) in the presence of recycled and environmental friendly catalyst, copper (II) anchored on amine-modified montmorillonite K10{MMT-[$(CH_2)_3$-NH=CHPy]-Cu(II)} under reflux condition for the accurate time [7]. Nguyen HT et al. described a method for green synthesis of pyrano [2, 3-c] pyrazole scaffolds (e) by using AC-SO_3H / [CholineCl] $[Urea]_2$ as a green catalyst. A mixture of ethyl acetoacetate (b), phenylhydrazine (c), and [CholineCl] $[Urea]_2$ was stirred at room temperature until a homogenous solution was obtained. Then, aldehyde (a), malononitrile (d), and AC-SO_3H catalyst were added, and the mixture was stirred continuously for 60 minutes at room temperature [8]. Maddila S *et al.* described the green synthesis of pyrano [2,3-c] pyrazoles(e) by irradiating an equimolar mixture of ethyl acetoacetate (d), hydrazine hydrate (b), malononitrile (c), aromatic aldehyde (a), and Mn-doped ZrO_2 catalystunder ultrasound at 40 kHz at room temperature for 10 min [9]. Kangani M *et al.* described the green synthesis of 1,4- dihydropyrano [2,3-c]pyrazole (d) derivatives from aromatic aldehydes (a), malononitrile (d), ethyl acetoacetate(b), and hydrazine monohydrate (c) in presence of saccharose as a green catalyst under thermal solvent-free conditions [10].

From Aldehyde and Malononitrile

Ali El-Remaily MA *et al*. synthesized the Polysubstituted Pyrazole-4-Carbonitrile Derivatives (d) under ultrasonic irradiation conditions utilizing a Pd (II) catalyst. A model reaction in this context was a simple one-pot condensation process for three components of aryl aldehyde(a), malononitrile (b), and phenylhydrazine (c) [11]. M'Hamed MO *et al.* described the synthesis of highly purified 5-Oxo-pyrazolidine derivatives by stirring an equimolar amount of benzaldehyde (a), phenylhydrazine (b), and malonic acid (c) in stainless steel vials with 31.80 g of stainless-steel balls was placed in a SPEX 8000 mixer [12]. Kiyani H *et al.* described the green synthesis of 5-aminopyrazole-4-carbonitriles (d) by stirring the equimolar amount of aryl/heteroaryl aldehyde (a), phenylhydrazine (b), malononitrile (c) and Sodium ascorbate (SA) as a safe and green catalyst in a mixture of ethanol: water at 50°C [13].

From Ketones

Paveglio GC *et al*. described the green synthesis pyrazole derivatives (c) via Acid-catalyzed cyclocondensation by milling enaminone(a), hydrazine hydrochloride (b), and 4-Methylbenzene-1-sulfonic acid(p-TSA) as a green catalyst

in 25mlof steel milling beaker equipped with 10mm of 5 steel milling balls for 3 min [14]. Tayde DT *et al*. described a technique for the highly efficient and green synthesis of 4, 5-dihydro-1, 3, 5-triphenyl-1H-pyrazole derivatives (c) by condensing equimolar mixture of substituted chalcone (a), phenylhydrazine hydrate(b) and green catalyst, mesoporous SiO_2-Al_2O_3 nanosized mixed metal oxide (MMO's) for 60 min in EtOH [15]. Khatab TK *et al.* reported the green synthesis of 5-amino-pyrazole derivatives (c) by stirring equimolar mixture of ketene S, N –acetals (a) and hydrazine hydrate (b)and V_2O_5 / SiO_2 as heterogeneous catalyst under solvent-free conditions at 70-80^oC [16]. Jaiswal D *et al.* has reported a effective method for the green synthesis of multisubstituted pyrazole derivatives (c) by stirring the equimolar quantities of chalcones (a) and phenyl hydrazines/hydrazine (b) and sarcosine as a recyclable organocatalyst in glycerol at 50^oC [17]. Acharya AP *et al.* described an environmental-friendly synthesis of novel indeno-pyrazoles (c) by condensing an equimolar mixture of α, β- unsaturated Het/Ar aldehyde (a), and phenylhydrazine (b) for 3-4 hours in the presence of polyethylene glycol-400 (PEG-400) as a green catalyst [18].

From Aldehydes

Mishra AD *et al*. synthesized apyrazole derivative (d) by subjecting the equimolar mixture of hydrazines (a) and aryl aldehyde (b) to microwave irradiation. Then resulted propanoyl hydrazone (c) and formic acid were adsorbed in acidic alumina and then subjected to microwave irradiation at an interval of 30 sec for 4-7 minutes [19].

From carboxylates

Soltanzadeh Z *et al.* synthesized the pyrazole derivatives by mixing a well ground mixture of 1, 2-dibenzoylhydrazines, or 4-phenylbutazone(a), dialkyl acetylene dicarboxylates (b), isocyanides (c), and Tetra-N-butylammonium bromide, at room temperature by green method under solvent-free conditions [20]. Tabarsaei N *et al.* described a catalyst-free green synthesis of novel pyrazole derivatives (f) with good yield by stirring equimolar mixture of isoquinoline (a), activated acetylenic compounds (b) in water. After 10 minutes, the alkyl bromide (c) and triphenylphosphine (d) mixture were added to the prior mixture, which had been mixed under ultrasonic irradiation in water for 20 minutes. After 20 minutes, hydrazine was added to the mixture and stirred for 10 minutes under ultrasonic irradiation [21].

From 1, 3 diketones and Malononitrile

Hojati SF *et al.* described a ultrasound assisted Green method for the Synthesis of Spiro Indoline-3,4/-pyrano[2,3-c]pyrazoles (e) by sonicating an equimolar amount of ethyl 3-oxobutanoate (a) and hydrazine hydrate or phenylhydrazine (b) in H_2O for 5 min at 25^0 C. Then, at room temperature, isatin (c) and malononitrile (d)were added to the reaction mixture, irradiated with ultrasound for the period specified [22].

From Malononitrile

Ding Y *et al.* reported the green method for the synthesis of 5-amino-1-methyl-3-phenyl-1H-pyrazole-4-carbonitrile derivatives (c) through a visible-light photo-redox catalysis (VLPC) promoted reaction of methylhydrazine (a), 2-benzylidenemalononitrile (b) and methanolin presence of $Ru^{II}(bpy)_3Cl_26H_2O$ catalystat 25°C [23]. Sagir H *et al.* described an efficient method for the green synthesis of 5-amino-pyrazole-4-carbonitrile (d) by visible-light-Photoredoxation of phenylhydrazine(a), 2-Fluorophenyl isothiocyanate (b), and Cyanoacetonitrile (c) in the presence of Eosin [24].

From Hydroxyl compounds

Cardinale L *et al.* described the green synthesis of 1, 5-diaryl pyrazoles (c)from arenediazoniums (b) and arylcyclopropanols (a) via photo-catalysis in the presence of Tris(bipyridine)ruthenium(II) ($[Ru(bpy)_3]^{2+}$)catalyst under blue-light [25].

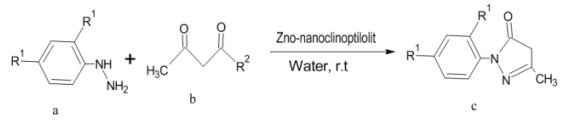
CONCLUSION

After an extensive literature survey, the authors can conclude that pyrazoles are very important scaffold with variety of pharmacological activities. Therefore, synthesizing the pyrazole derivatives with various modifications is of great interest to researchers. Designing new pyrazole derivatives followed by their synthesis using various available conventional methods is resulting in delivering many hazardous wastes to the planet. Converting to greener methods is the most important task for the researchers in order to make a green globe. The authors had also summarized various greener methods reported for the synthesis of pyrazole derivatives.

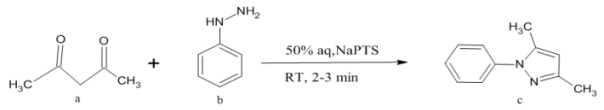
REFERENCES

1. Kharissova O v., Kharisov BI, González CMO, Méndez YP, López I. Greener synthesis of chemical compounds and materials. Vol. 6, Royal Society Open Science. Royal Society Publishing; 2019.
2. Singh Grewal A. Microwave assisted synthesis: a green chemistry approach Therapeutic targeting of type 2 diabetes View project [Internet]. Available from: https://www.researchgate.net/publication/261872979
3. Mohammadzadeh Kakhki R, Karimian A, Hasan-nejad H, Ahsani F. Zinc Oxide–Nanoclinoptilolite as a Superior Catalyst for Visible Photo-Oxidation of Dyes and Green Synthesis of Pyrazole Derivatives. Journal of Inorganic and Organometallic Polymers and Materials. 2019 Jul 1;29(4):1358–67.
4. Barge M, Kamble S, Kumbhar A, Rashinkar G, Salunkhe R. Hydrotrope: Green and rapid approach for the catalyst-free synthesis of pyrazole derivatives. Monatshefte fur Chemie. 2013 Aug;144(8):1213–8.
5. Marandi A, Nasiri E, Koukabi N, Seidi F. The Fe3O4@apple seed starch core-shell structure decorated In(III): A green biocatalyst for the one-pot multicomponent synthesis of pyrazole-fused isocoumarins derivatives under solvent-free conditions. International Journal of Biological Macromolecules. 2021 Nov 1;190:61–71.
6. Sadjadi S, Heravi MM, Daraie M. A novel hybrid catalytic system based on immobilization of phosphomolybdic acid on ionic liquid decorated cyclodextrin-nanosponges: Efficient catalyst for the green synthesis of benzochromeno-pyrazole through cascade reaction: Triply green. Journal of Molecular Liquids. 2017 Apr 1;231:98–105.
7. Ahmadzadeh M, Sadeghi M, Safari J. Copper(II) Anchored on Amine-Functionalized MMT: A Highly Efficient Catalytic System for the One-Pot Synthesis of Bispyrano[2,3-c]pyrazole Derivatives. Journal of Chemistry. 2021;2021.
8. Nguyen HT, Le T van, Tran PH. AC-SO3H/[CholineCl][Urea]2as a green catalytic system for the synthesis of pyrano [2,3-c]pyrazole scaffolds. Journal of Environmental Chemical Engineering. 2021 Jun 1;9(3).
9. Maddila S, Gorle S, Shabalala S, Oyetade O, Maddila SN, Lavanya P, et al. Ultrasound mediated green synthesis of pyrano[2,3-c]pyrazoles by using Mn doped ZrO2. Arabian Journal of Chemistry. 2019 Jul 1;12(5):671–9.
10. Kangani M, Hazeri N, Maghsoodlou MT, Khandan-Barani K, Kheyrollahi M, Nezhadshahrokhabadi F. Green procedure for the synthesis of 1,4-dihydropyrano[2,3-c]pyrazoles using saccharose. Journal of the Iranian Chemical Society. 2015;12(1):47–50.
11. Ali El-Remaily MAEAA, El-Dabea T, Alsawat M, Mahmoud MHH, Alfi AA, El-Metwaly N, et al. Development of New Thiazole Complexes as Powerful Catalysts for Synthesis of Pyrazole-4-Carbonitrile Derivatives under Ultrasonic Irradiation Condition Supported by DFT Studies. ACS Omega. 2021 Aug 17;6(32):21071–86.
12. M'Hamed MO, Alduaij OK. Green and effective one-pot synthesis of 5-oxo-pyrazolidine and 5-amino-2,3-dihydro-1H-pyrazole derivatives through ball milling under catalyst-free and solvent-free conditions. Asian Journal of Chemistry. 2016;28(3):543–7.
13. Kiyani H, Bamdad M. Sodium ascorbate as an expedient catalyst for green synthesis of polysubstituted 5-aminopyrazole-4-carbonitriles and 6-amino-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitriles. Research on Chemical Intermediates. 2018 Apr 1;44(4):2761–78.
14. Paveglio GC, Longhi K, Moreira DN, München TS, Tier AZ, Gindri IM, et al. How mechanical and chemical features affect the green synthesis of 1 H -pyrazoles in a ball mill. In: ACS Sustainable Chemistry and Engineering. American Chemical Society; 2014. p. 1895–901.

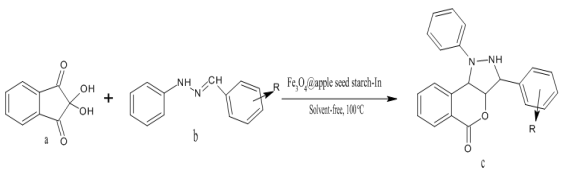
15. Tayde D, Lande M. Mesoporous SiO2-Al2O3: An Efficient Catalyst for Synthesis of 4,5-dihydro-1,3,5-triphenyl-1H-pyrazole. J Chem Lett. 2021;2:25–32.
16. Khatab TK, Hassan AS, Hafez TS. V 2 O 5 /SiO 2 as an efficient catalyst in the synthesis of 5-amino-pyrazole derivatives under solvent free condition. Bull Chem Soc Ethiop. 2019;33(1):135–42.
17. Jaiswal D, Tiwari J, Singh S, Sharma AK, Singh J, Singh J. Sarcosine as a Novel and Recyclable Organocatalyst: A Greener Approach Towards the Synthesis of Multisubstituted Pyrazole Derivatives. Current Organocatalysis. 2018 Aug 23;5(3):229–38.
18. Acharya AP, Kamble RD, Hese S v, Kadam SN, Gacche RN, Dawane BS. Eco-friendly synthesis of novel indeno-pyrazole derivatives and their in-vitro antimicrobial screening [Internet]. Vol. 7, Org. Commun. 2014. Available from: www.acgpubs.org/OC/index.htm
19. Mishra AD. Microwave-Induced Synthesis of Some Novel Fungicidal Pyrazole Derivatives. Vol. 30, J. Nepal Chem. Soc.
20. Soltanzadeh Z, Imanzadeh G, Noroozi-Pesyan N, Şahin E. Green synthesis of pyrazole systems under solvent-free conditions. Green Chemistry Letters and Reviews. 2017 Jul 3;10(3):148–53.
21. Tabarsaei N, Hamedani NF, Shafiee S, Khandan S, Hossaini Z. Catalyst-free green synthesis and study of antioxidant activity of new pyrazole derivatives. Journal of Heterocyclic Chemistry. 2020 Jul 1;57(7):2945–54.
22. Hojati SF, Mohamadi S. Ultrasound Promoted Green Synthesis of Spiro Indoline-3,4′-pyrano[2,3-c]pyrazoles. Organic Preparations and Procedures International. 2020 Jul 3;52(4):304–10.
23. Ding Y, Zhang T, Chen QY, Zhu C. Visible-light photocatalytic aerobic annulation for the green synthesis of pyrazoles. Organic Letters. 2016 Sep 2;18(17):4206–9.
24. Sagir H, Rai P, Ibad A, Ibad F, Siddiqui IR. Visible-light-photoredox catalytic C[sbnd]C, C[sbnd]N bond formation: Synthesis of pyrazole derivatives via radical ions. Catalysis Communications. 2017;100:153–6.
25. Cardinale L, Neumeier M, Majek M, Jacobi Von Wangelin A. Aryl Pyrazoles from Photocatalytic Cycloadditions of Arenediazonium. Organic Letters. 2020 Sep 18;22(18):7219–24.



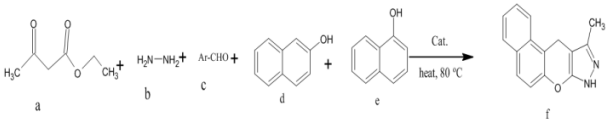
Scheme 1: Scheme for green synthesis of some pyrazole derivatives using ZnO–nanoclinoptilolite



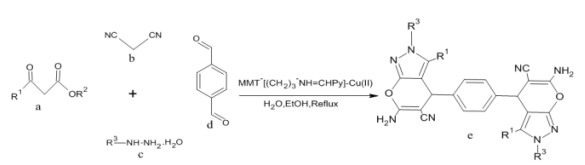
Scheme 2: Scheme for green synthesis of pyrazole derivatives by using hydrotrope (NaPTS).



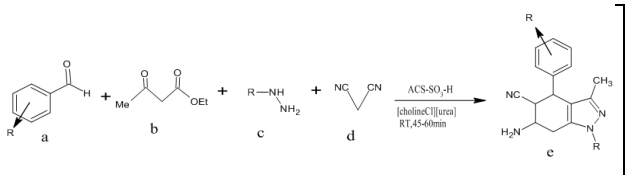
Scheme 3: Scheme for green synthesis of pyrazole-fused isocoumarins derivatives by used Fe_3O_4@apple seed starch-In (III) as the catalyst.



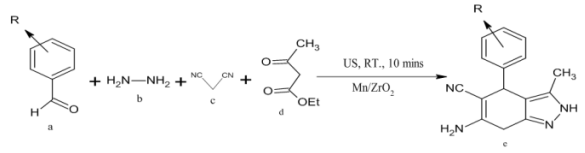
Scheme 4: Scheme for green synthesis of benzochromeno-pyrazole through Cascade Reaction.



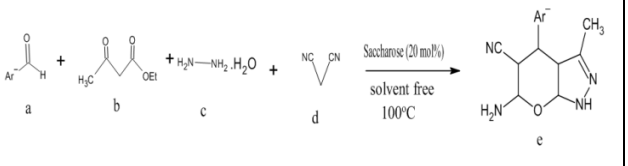
Scheme 5: Scheme for one-pot green synthesis of Bispyrano [2, 3-c] pyrazole Derivatives.



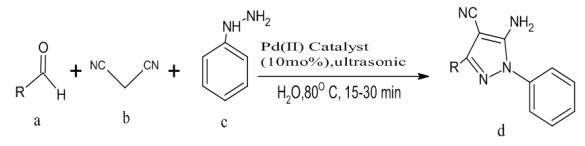
Scheme 6: Scheme for green synthesis of [2, 3-c] pyrazole scaffolds by using AC-SO_3H/ [CholineCl][Urea]$_2$ as a green catalyst.



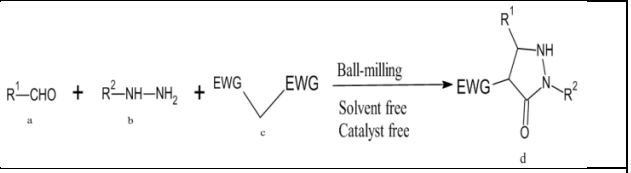
Scheme 7: Scheme for the green synthesis of pyrano [2, 3-c] pyrazoles by using Mn doped ZrO_2 under ultrasound.



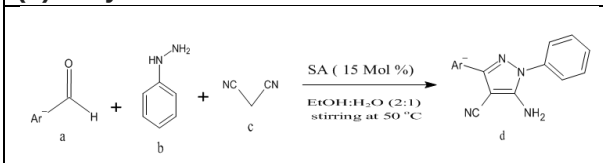
Scheme 8: Scheme for green synthesis of 1, 4-dihydropyrano [2, 3-c] pyrazoles using saccharose



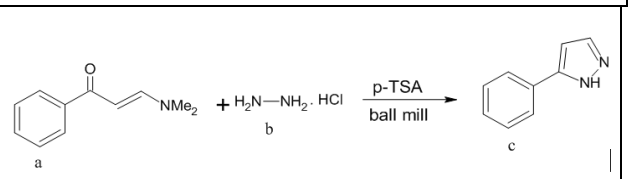
Scheme 9: Scheme for green synthesis of Polysubstituted Pyrazole-4-Carbonitrile Derivatives under ultrasonic irradiation conditions utilizing a Pd (II) catalyst



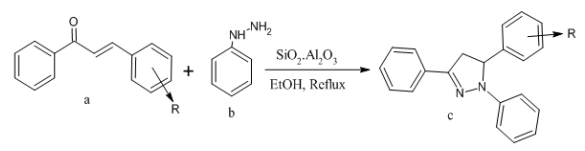
Scheme 10: Scheme for green and effective synthesis of 5-oxo-pyrazolidine derivatives through ball milling under catalyst-free and solvent-free conditions.



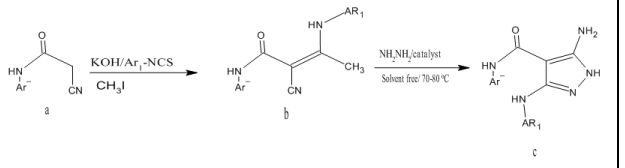
Scheme 11: Scheme for green synthesis of 5-aminopyrazole-4-carbonitriles.



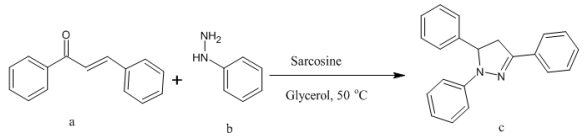
Scheme 12: Scheme for the green synthesis pyrazole derivatives (c) via Acid-catalyzed cyclocondensation



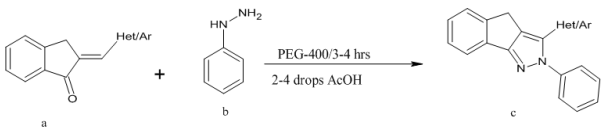
Scheme 13: scheme for the synthesis of 4, 5-dihydro-1, 3, 5-triphenyl-1H-pyrazole derivatives by condensation



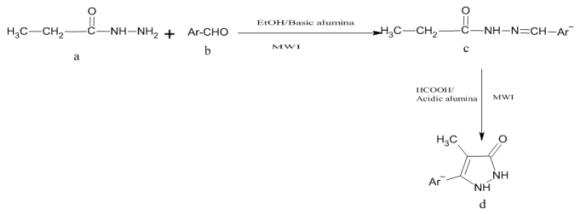
Scheme 14: Scheme green synthesis of 5-amino-pyrazole derivatives (c) under solvent-free conditions.



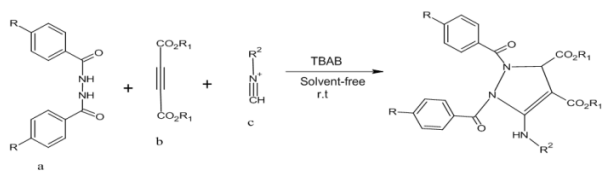
Scheme 15: Scheme for green synthesis of multisubstituted pyrazole derivatives by using Sarcosine as a novel and recyclable organocatalyst



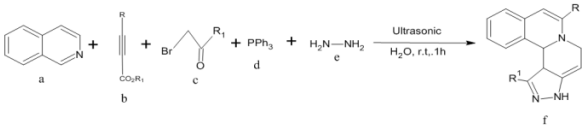
Scheme 16: Scheme for environmental-friendly synthesis of novel indeno-pyrazole derivatives by using PEG-400 as a green catalyst.



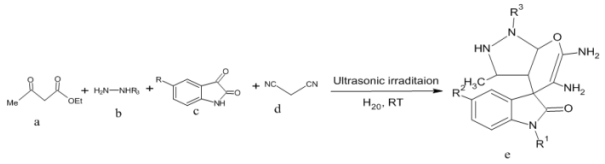
Scheme 17: Scheme for the synthesis of pyrazole derivatives by microwave irradiation.



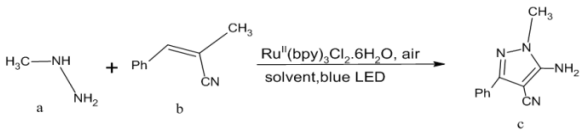
Scheme 18: Scheme for green synthesis of pyrazole in the presence of Tetra-N-butylammonium bromide.



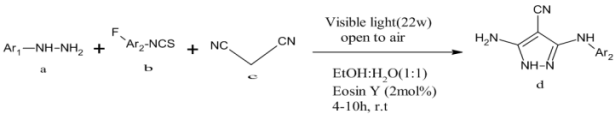
Scheme 19: Scheme for catalyst-free green synthesis of pyrazole derivatives.



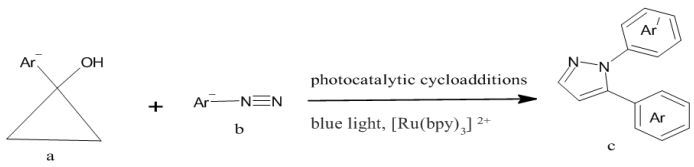
Scheme 20: Scheme for Green Synthesis of Spiro Indoline-3, 4-pyrano [2, 3-c]pyrazole by sonicating.



Scheme 21: Scheme for green synthesis of pyrazole derivatives by visible-Light photocatalytic aerobic Annulation.



Scheme 22: Scheme for green synthesis of pyrazole derivatives by Visible-Light-Photoredoxation viaradical ions.



Scheme 23: Scheme for the synthesis of diaryl Pyrazoles by Photo catalytic Cycloadditions of Arenediazonium.

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RESEARCH ARTICLE

Anticancer Activity of *Cassia auriculata* Against Human Breast Cancer MCF-7: An *In-vitro* and *In-silico* Molecular Docking Approach

M. Muneeswaran[1], S. Gowri[2]*, M. Koteeswaran[1] and M. Porkodi[3]

[1]Ph. D Research Scholar, Department of Biochemistry, Dr. N.G.P. Arts and Science College, Coimbatore, Tamil Nadu, India

[2]Professor and Head, Department of Biochemistry, Dr. N.G.P. Arts and Science College, Coimbatore, Tamil Nadu, India.

[3]Ph.D Research Scholar, Department of Biochemistry, Annamalai University, Annamalai Nagar, Chidambaram 608002, Tamil Nadu, India.

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***Address for Correspondence**
S. Gowri
Professor and Head,
Department of Biochemistry,
Dr. N.G.P. Arts and Science College,
Coimbatore, Tamil Nadu, India.
Email: drgowri@drngpasc.ac.in

ABSTRACT

Breast cancer is a growing health issue globally and accounts as a second most cause of mortality. Natural products have been a fundamental of health care for long. Plants derived natural products have gained considerable attention over synthetic medicines, since they are safe and non-toxic. *Cassia auriculata* commonly known as Tanner's *Cassia* is an important medicinal shrub used in traditional systems of medicine. In this present research we investigated the anticancer activity of *C. auriculata* extract and its bioactive compounds in an in-vitro and *In-silico* approach against human breast cancer cellline MCF-7. *C. auriculata* extract exhibited significant toxicity to MCF-7 cells with an IC_{50} value of 38.0 µg/ml. Following that, morphological alterations related with apoptosis were observed using acridine orange and ethidium bromide staining (AO/EtBr) with various doses of *C. auriculata* leaf methanolic extract. The *C. auriculata* extract induced nuclear fragmentation assays analysis on MCF-7 cells with fluorescence microscopy by DAPI staining, propidium iodide staining and also inhibited cell proliferation in MCF-7 cells. In the MCF-7cell line, the cytotoxic efficacy of *C. auriculata* leaf methanolic extract was shown to be dose dependant manner. Using florescence analysis, the effect of *C. auriculata* leaf methanolic extract on Cell cycle analysis against breast cancer cells by flow cytometry with was studied. Docking result showed that flavone have the potential to inhibit Bax, BCL-2, Caspase-3, Caspase-9, and p53 protein that are expressed more during Breast Cancer.

Keywords: Cytotoxicity; MCF-7 breast cancer cell line; Molecular docking; *Cassia auriculata*

INTRODUCTION

Cancer was the second leading cause of death globally in 2012 and was responsible for 8.2 million deaths which increased to 9.6 million in 2018. Globally, about 1 in 6 deaths is due to cancer and it is projected to be 23.6 million new cases with 12 million deaths per year by 2030 [1]. According to world health organization (WHO), breast cancer is a growing health issue globally and is the second most cause of mortality. Approximately 1 in 10 women is diagnosed with breast cancer at some stage of life [2]. As per the International Agency for Research on Cancer (IARC) and Globocan 2018 data, breast cancer accounted for 0.62 million deaths besides 2.08 million new cases (approximately 11.6% of all types of cancer recorded) [3]. If the current trends are to be believed, the mortality troll is expected to rise to a nerve-racking high of 6.99 million by 2040 [4]. According to epidemiological reports, the incidence of breast cancer is continuously rising in the developing and developed countries [5].Although an extensive research effort has been made to understand breast cancer and find new ways to combat it. The primary female sex hormones, estrogens, can stimulate the development, proliferation, migration, and survival of target cells, as well as the development of secondary sexual characteristics. Estrogen exerts its biological effect by binding to α and β estrogen receptor (ER), which are members of the super family nuclear receptor transcription factors that are characterized by highly conserved DNA-and ligand binding domains. These nuclear receptors are important for the control of gene expression. The mutation of this gene produces abnormal version of the estrogen receptor or mutated protein also called ER+, the expression of which results a breast cancer in female [1].

Nonetheless, the available treatments mainly include chemotherapy, involved the use of cytotoxic agents. However, the role of chemotherapy is still uncertain. Although it has shown effective in some cases, but its usage is often followed by risk factors or side effects that may vary from short term to life threatening [6]. The main reason of side effects associated with chemotherapy is the lack of specificity of drugs for the cancer cells [7]. Chemotherapy, the currently preferred method, used along with surgical procedures have severe side effects that can reduce patients' quality of life emphasizing the need to develop novel, direct and integrative therapies. Such developments can help improve disease outcomes and patients' quality of life [8].Natural products are an extremely promising strategy for chemoprevention to block the development of cancer in human. Many natural plants have furnished modern medicine with the drugs that are used in cancer therapy as cytotoxic agents. Investigations from basic research are confirming that many chemopreventive dietary compounds are active at molecular levels [9] [10]. Since many years, plant extracts have been used as traditional remedies to treat a various diseases including cancer [11]. *Cassia auriculata* commonly known as Tanner's Cassia is an important medicinal shrub used in traditional systems of medicine. It holds a very prestigious position in Ayurveda and Siddha systems of medicine.

It also growing wild in Central Provinces and Western peninsula and cultivated in other parts of India. It is valuable as a tanning material and as a green manure crop. The plant has been reported to possess antipyretic, hepatoprotective, antidiabetic, antiperoxidative and antihyperglyceamic and microbicidal activity [12].Since cancer is a complex and multiple gene-related disease, the development of multi-targeted anticancer drugs to improve therapeutic efficacy and reduce drug resistance is currently the focus of promising research. In this present research we investigated the anticancer activity of *C. auriculata* extract and its bioactive compounds in an in-vitro and *In-silico* approach against human breast cancer cell line MCF-7. Potential in-vitro cytotoxic and antiproliferative effect of *C. auriculata* extract in MCF-7 human breast cancer cells. Following that, morphological alterations related with apoptosis were observed using acridine orange and ethidium bromide staining (AO/EtBr) with various doses of *C. auriculata* leaf methanolic extract. The *C. auriculata* extract induced nuclear fragmentation assays analysis on MCF-7 cells with fluorescence microscopy by DAPI staining, propidium iodide staining and also inhibited cell proliferation in MCF-7 cells. In the MCF-7cell line, the cytotoxic efficacy of *C. auriculata* leaf methanolic extract. Using florescence analysis, the effect of *C. auriculata* leaf methanolic extract on Cell cycle analysis against breast cancer cells by flow cytometry with was studied. Moreover we investigated the *In-silico* molecular docking studies that flavone have the potential to inhibit Bax, BCL-2, Caspase-3, Caspase-9 and p53 protein.

MATERIALS AND METHODS

Cell culture maintenance

The National Centre for Cell Science (NCCS) in Pune, India, purchase the human breast cancer cells (MCF-7). The cells were kept in Dulbecco modified eagle medium (DMEM), which was supplemented with balanced salt solution and 2mM L-glutamine and contained 1.5 g/L glucose, 1.5 g/L Na2CO3, essential amino acids, and bovine serum. Penicillin and streptomycin concentrations (100 IU/100 g) were adapted to 1mL/L. The cells were cultured at 37°C in 5 % CO_2 atmosphere. The cells were exposed to various ratios of *C. auriculata* (25, 50, or 100 μg/mL) for around 2hrs before being exposed to 6-OHDA (100 M) for 24 hrs.

Evaluation of cytotoxicity

Cell cytotoxicity assay [MTT, Hi-Media) was conducted to assess inhibitory concentration (IC_{50}) value. The MCF-7 cells had been allowed to develop in 96-six well plate (1×10^4cells/well) for 48hr to obtain 80% cell confluency. The culture medium replaced with *C. auriculata* containing fresh medium at various concentrations (25, 50, or 100 μg/mL) and similarly they were treated with MCF-7 cells accompanied by means of incubation for 48hr. Consequently, observed via removal of culture medium and addition of 100μL solution of MTT mixed with the wells and subsequently incubated at 37°C for 4hr, and also accompanied by supernatant elimination. After that, each well was supplied with 50 μL of DMSO and incubated for 15 min to disperse the formazan crystals. The optical density (OD) of the MCF-7 cells at 570 nm was calculated using a Biotek multimode plate reader (USA).

% cell viability = OD of experimental sample/OD of experimental control ×100

% cytotoxicity = 100 – % cell viability

Morphological Study

The MCF-7 breast cancer cells ($1x10^5$) were treated for 24hrs with various doses of *C. auriculata* extract (25 μg/mL, 50 μg/mL and 100 μg/mL) then fixed in an acetic acid: ethanol (1:3; v/v). The cover slips were carefully put on glass slides for the morphologic investigation. Each experimental group had three monolayers micrographic. The morphological changes of the cells were examined using Nikon (Japan) bright field inverted light microscopy at 20X magnifications.

Cell cycle analysis

For cell cycle study, breast cancer cell MCF-7 ($1x10^5$) was maintained in a 6-well plate for 48hrs with different dosages of *C. auriculata* extract. The cells were then isolated and incubated in 500 mL of PBS solution with 25 μg propidium iodide (PI) and 50g RNase for 30 min at room temperature. BD Facsverse USA used flow cytometry to analyze the samples.

Retrieval of the protein

The crystal structure of breast cancer proteins Bax, BCL-2, Caspase-3, Caspase-9 and p53 was downloaded from Protein data bank.

Selection of Compound

The PubChem Database (http://pubchem.ncbi.nlm.nih.gov/) contains chemical compounds with information provided to depict structure. Filtering and searching of chemical structure are provided with its calculated properties and descriptors. Flavone (CID: 10680) (Figure 1) was retrieved from PubChem database in 3d.sdf format. The compound 3d.sdf format was converted into the .pdb format using Open Babel 2.3.1 96 software In graphical representation of flavone compound used version 9.3 (2012) Schrodinger. This compound was used for docking analysis.

Molecular docking studies

The molecular docking studies has been carried out using Glide module available on version 5.8 (2012) Schrodinger suite for comparing and analyzing the molecular interaction pattern between breast cancer proteins Bax, BCL-2, Caspase-3, Caspase-9 and p53 receptor with Flavone. The ligand was docked with the target protein using Glide module of Schrödinger suite.

Data Analysis

All analyses have been carried out using the SPSS statistical software version 16.0 edition. Probity analysis was used to calculate IC_{50} for cell apoptosis and maximum inhibition data [13]. The cell viability collected data were analyzed using ANOVA.

RESULTS AND DISCUSSION

Cytotoxic Assay

The effect of *C. auriculata* extract on MCF-7 cells by using the MTT assays.

The cytotoxicity effects on MCF-7 human breast cancer with MTT bioassay in a 24-hours examination was presented in (Fig. 2) and the IC_{50} value is shown in Table 1. Figure 2 show that *C. auriculata* extract can inhibit the growth of MCF-7 human breast cancer at certain doses, which demonstrated that IC_{50} values were, respectively 38 µg/ml. Cancerous cell lines were estimated by MTT assay. Similarly, the methanol and acetone extract leaf of *Cucumis sativus* has shown anticancer activity on MCF-7 cancer cell lines. Among the tested extracts, methanolic extract was found to have potent cytotoxicity against cancerous cell lines with MCF-7 values ranging IC_{50} 15.6 ± 1.3 [14]. The aromatic plants are a rich source of compounds with anticancer properties and deliver less harmfulness in normal cells. In this way, expanding consideration has been put on recognizing novel anticancer medication medicines from regular sources [15] [16] [17].

Morphological Study

Figure 3 shows MCF-7 cells morphological changes during 24 hr of treatment with the *C. auriculata* extract at 38 µg/ml (IC_{50} concentration). Treatment affects the shape of MCF-7 cells in a portion subordinate way. Enhanced *C. auriculata* extract increased cytotoxicity. This extract had incited cell shrinkage, adjusting of the cell and reducing the number of feasible cells. These progressions demonstrate that *C. auriculata* extract actuated apoptosis in MCF-7 cells (Fig 3 b, c & a; d). While the untreated control cells on the other hand, had not shown any significant effect (Fig 3a). Similarly, [18] *Helicteres isora* fruit extracts have anti proliferative action against human lung cancer cells, which is similar to our findings. Furthermore, [19] *Solanum anguivi* leaves inhibited the proliferation of HepG-2 and MCF-7 cancer cells.

AO/EtBr staining for fluorescence microscopy investigation of nuclear fragmentation

The effect of the *C. auriculata* extract apoptogenic activity on cancer cells was studied using fluorescence microscopy. No aggregation of *C. auriculata* extract was shown during the experiment because most of the *C. auriculata* extract was dispersed at all these low concentrations. The induction of apoptosis in MCF-7 cells following treatment with the IC_{50} values of *C. auriculata* extract was measured using fluorescence microscopy stained by acridine orange/ethidium bromide (AO/EtBr).These findings revealed that live cells fluoresced green, while dead cells fluoresced acridine orange. Untreated control cells showed a high number of live cells (Fig. 4 (a)). MCF-7 cells treated with *C. auriculata* extract, on the other hand, exhibited more cellular damage and bodies with nuclear shrinkage, membrane blabbing, and nucleus degradation as orangish bodies (Fig. 4). (b, c, & d). Dual fluorescent stain acridine orange (AO) and ethidium bromide (EtBr) clearly indicated that most of our treated cells were in early apoptosis with the greenish yellow fluorescence nuclei in contrast, to control where we observed uniformly green colour cells without fluorescence. AO/EtBr stains viable cells uniformly green, early apoptosis cells could be recognized by greenish yellow colour fluorescence, late apoptosis cells show orange colour fluorescence and necrotic cells display orange to red colour fluorescence nuclei without chromatin fragmentation [20].

Fluorescence microscopic analysis of nuclear fragmentation - DAPI Staining

Further more, we evaluated the *C. auriculata* extract using the DAPI staining method. Figure 4 shows a fluorescence microscopic image of cells labelled through DAPI after 24 hrs in the presence and absence of *C. auriculata* extract. Figure 5 (a) shows that there were no significant changes in the cells, whereas Bright signals were observed in *C. auriculata* extract treated cells (Fig. 5 b, c, & d), representative the structure of shortened chromatins and nuclear fragmentations in MCF-7 cells. According to the results of the fluorescence microscopic study, the *C. auriculata* extract could be used as an efficient cancer therapy agent. Evaluation of apoptosis is crucial to differentiate it from necrosis. Nuclear fragmentation is one of the characteristic features of apoptotic mode of cell death. We used DAPI, a fluorescent DNA-binding agent, staining to observe cell death and cellular morphological changes involved in apoptosis [21] [22].

Cell cycle evaluation

Flow cytometry was used to determine the stage of the cell cycle in which the cells were arrested. The cells in the control group (untreated group) were uniformly dispersed in the G0-G1, S, and G2M phases. *C. auriculata* extract concentrations of 25, 50, and 100 µg/ml were applied to breast cancer cells. Cells were arrested in G2M phase for doses of 25µg/ml and 50µg/ml, whereas cells were arrested at S phase for values of 100 µg/ml. This meant that increasing the concentration prevented breast cancer cells from synthesizing as they were exposed with *C. auriculata* extract, as shown in figure 6. The observation of cancerous cell-death is a bioassay model that indicates the potential of the fraction to inhibit the progression of cancer. The progression of cell proliferation is halted by the arrest of the cell division cycle at one of the checkpoints (G0/G1, S or G2/M phases) in the cell division cycle. The arrest is mainly triggered by the irreparable or repairable damage in the cell's DNA. In case of an irreparable DNA damage, the cell death pathways are triggered. The cell death could be either apoptotic or necrotic [23] [24].

Molecular docking study

Molecular docking analysis was performed for breast cancer proteins Bax, BCL-2, Caspase-3, Caspase-9 and p53 protein with flavone compound. The best interaction was determined based on the lowest binding energy value, lowest ligand efficiency and number of hydrogen bonds between receptor and ligand. In our results, flavone compound showed highest G Score, D Score and binding energy with breast cancer proteins Bax, BCL-2, Caspase-3, Caspase-9 and p53 (Table. 2). The graphical representation of the interaction of breast cancer proteins Bax, BCL-2, Caspase-3, Caspase-9 and p53 protein with flavone compound is shown in Fig 7 to and 11.

CONCLUSION

The findings of present study revealed that the aquous extract of *C. auriculata* could be used as a potential alternative for development of bioactive leads in the treatment of cancer. The IC_{50} values clearly indicated, the anticancer activity of aquous extract of *C. auriculata* is high in-comparison with MCF-7 cell line. Accordingly, we presume that *C. auriculata* bioactive part can possibly be utilized as a disease treatment specialist.

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REFERENCES

1. Sibuh BZ, Khanna S, Taneja P, Sarkar P, Taneja NK. Molecular docking, synthesis and anticancer activity of thiosemicarbazone derivatives against MCF-7 human breast cancer cell line. Life Sciences. (2021) 273:119305.

2. Caffarel MM, Pensa S, Wickenden JA, Watson CJ. Molecular biology of breast cancer. Chichester. John Wiley & Sons, Ltd., UK, (2016) pp. 1–9.
3. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2018 Nov; 68(6):394-424.
4. Ferlay J, Colombet M, Soerjomataram I, Dyba T, Randi G, Bettio M, Gavin A, Visser O, Bray F. Cancer incidence and mortality patterns in Europe: Estimates for 40 countries and 25 major cancers in 2018. European journal of cancer. 2018 Nov 1: 103:356-87.
5. Rice S, Whitehead SA. Phytoestrogens and breast cancer–promoters or protectors?. Endocrine-Related Cancer. 2006 Dec 1; 13(4):995-1015.
6. Middleton JD, Stover DG, Hai T. Chemotherapy-exacerbated breast cancer metastasis: a paradox explainable by dysregulated adaptive-response. International journal of molecular sciences. 2018 Oct 26; 19(11):3333.
7. Weinberg SE, Chandel NS. Targeting mitochondria metabolism for cancer therapy. Nature chemical biology. 2015 Jan; 11(1):9-15.
8. Siegel SE, Stock W, Johnson RH, Advani A, Muffly L, Douer D, Reed D, Lewis M, Freyer DR, Shah B, Luger S. Pediatric-inspired treatment regimens for adolescents and young adults with philadelphia chromosome–negative acute lymphoblastic leukemia: A review. JAMA oncology. 2018 May 1; 4 (5):725-34.
9. Russo R, Catani L, Cianchi A, Tazzari S, Langner J. High quality superconducting niobium films produced by an ultra-high vacuum cathodic arc. Superconductor Science and Technology. 2005 Jun 3; 18 (7):L41.
10. Pan B, Xing B. Adsorption mechanisms of organic chemicals on carbon nanotubes. Environmental science & technology. 2008 Dec 15; 42 (24):9005-13.
11. Farshori NN, Al-Sheddi ES, Al-Oqail MM, Musarrat J, Al-Khedhairy AA, Siddiqui MA. Anticancer activity of *Petroselinum sativum* seed extracts on MCF-7 human breast cancer cells. Asian Pacific Journal of Cancer Prevention. 2013; 14(10):5719-23.
12. Murugan T, Wins JA, Murugan M. Antimicrobial activity and phytochemical constituents of leaf extracts of *Cassia auriculata*. Indian journal of pharmaceutical sciences. 2013 Jan; 75(1):122.
13. Finney DJ. Statistical aspects of monitoring for dangers in drug therapy. Methods of information in medicine. 1971; 10(01):1-8.
14. Tuama AA, Mohammed AA. Phytochemical screening and in vitro antibacterial and anticancer activities of the aqueous extract of Cucumis sativus. Saudi journal of biological sciences. 2019 Mar 1;26(3):600-4.
15. Mukherjee P, Ahmad A, Mandal D, Senapati S, Sainkar SR, Khan MI, Parishcha R, Ajaykumar PV, Alam M, Kumar R, Sastry M. Fungus-mediated synthesis of silver nanoparticles and their immobilization in the mycelial matrix: a novel biological approach to nanoparticle synthesis. Nano letters. 2001 Oct 10;1(10):515-9.
16. Wang D, Feng Y, Liu J, Yan J, Wang M, Sasaki JI, Lu C. Black garlic (Allium sativum) extracts enhance the immune system. Medicinal and Aromatic Plant Science and Biotechnology. 2010;4(1):37-40.
17. Desai AG, Qazi GN, Ganju RK, El-Tamer M, Singh J, Saxena AK, Bedi YS, Taneja SC, Bhat HK. Medicinal plants and cancer chemoprevention. Current drug metabolism. 2008 Sep 1;9(7):581-91.
18. Raaman N, Balasubramanian K. Antioxidant and anticancer activity of Helicteres isora dried fruit solvent extracts. J Acad Indus Res. 2012;1(3):148-52.
19. Gandhiappan J, Rengasamy R. Antiproliferative activity of Solanum anguivi against cancer cell lines. Der Pharmacia Lettre. 2012;4(3):875-80.
20. Syed FQ, Elkady AI, Mohammed FA, Mirza MB, Hakeem KR, Alkarim S. Chloroform fraction of Foeniculum vulgare induced ROS mediated, mitochondria-caspase-dependent apoptotic pathway in MCF-7, human breast cancer cell line. Journal of ethnopharmacology. 2018 May 23;218: 16-26.
21. Kasibhatla S, Amarante-Mendes GP, Finucane D, Brunner T, Bossy-Wetzel E, Green DR. Acridine orange/ethidium bromide (AO/EB) staining to detect apoptosis. Cold Spring Harbor Protocols. 2006 Aug 1;2006(3):pdb-rot4493.
22. Pajaniradje S, Mohankumar K, Pamidimukkala R, Subramanian S, Rajagopalan R. Antiproliferative and apoptotic effects of Sesbania grandiflora leaves in human cancer cells. BioMed research international. 2014 Oct;2014.

23. Masoko P, Gololo SS, Mokgotho MP, Eloff JN, Howard RL, Mampuru LJ. Evaluation of the antioxidant, antibacterial, and antiproliferative activities of the acetone extract of the roots of Senna italica (Fabaceae). African Journal of Traditional, Complementary and Alternative Medicines. 2010;7(2).
24. Tagne RS, Telefo BP, Nyemb JN, Yemele DM, Njina SN, Goka SM, Lienou LL, Kamdje AH, Moundipa PF, Farooq AD. Anticancer and antioxidant activities of methanol extracts and fractions of some Cameroonian medicinal plants. Asian Pacific journal of tropical medicine. 2014 Sep 1;7:S442-7.

Table 1: Cytotoxicity activity of *C. auriculata* extract (µg/mL)

Sample	MCF-7	HEK
C. auriculata	38.0±0.7	Insignificant toxicity

IC_{50} values of respective sample (at 24hrs)

Table 2: Binding affinity of flavone with proteins

ligand	Protein	Docking score (kcal/mol)	Ligand efficiency	Inhibition constant (µM)	Intermolecular energy	VDW-H Bond Desolvation Energy	Electrostatic energy
Flavone	Bax,	-5.06	-0.3	195.27	-5.36	-5.41	0.05
	BCL-2,	-4.86	-0.29	274.78	-5.16	-5.1	-0.06
	Caspase-3,	-5.9	-0.35	47.22	-6.2	-6.19	-0.01
	Caspase-9	-5.13	-0.3	174.93	-5.42	-5.41	-0.01
	p53	-5.4	-0.32	109.85	-5.7	-5.7	0.01

Figure 1: Flavone compound

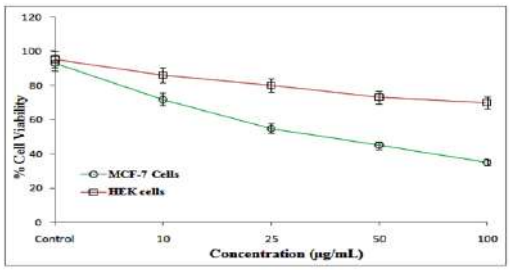

Figure 2: MTT cytotoxicity analysis: *In vitro* cell viability of breast cancer cells after treatment with *C. auriculata*

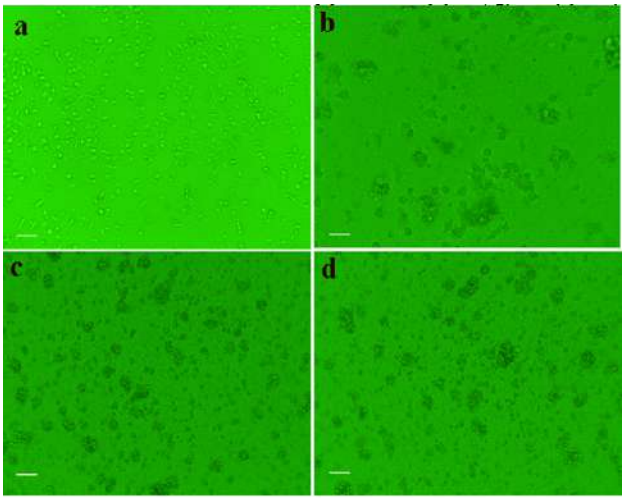

Figure 3: The morphological changes in MCF-7 cells during 24hrs of treatment with the *C. auriculata* IC_{50} concentration (a) Control, (b) 25µg/mL (c) 50 µg/mL (d) 100 µg/mL.

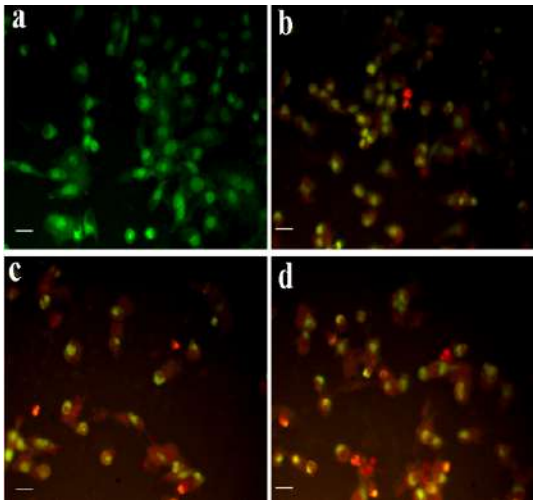

Figure 4: AO/EtBr staining for fluorescence microscopy investigation of nuclear fragmentation (a) Control, (b) 25µg/mL (c) 50 µg/mL (d) 100 µg/mL

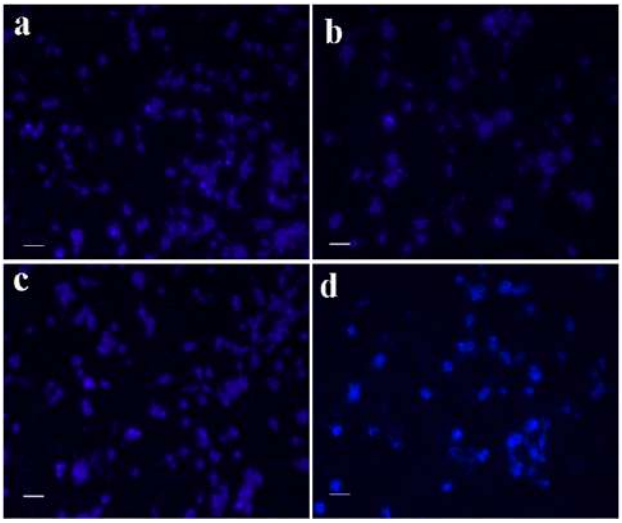

Figure 5: Fluorescence microscopic analysis of nuclear fragmentation - DAPI Staining

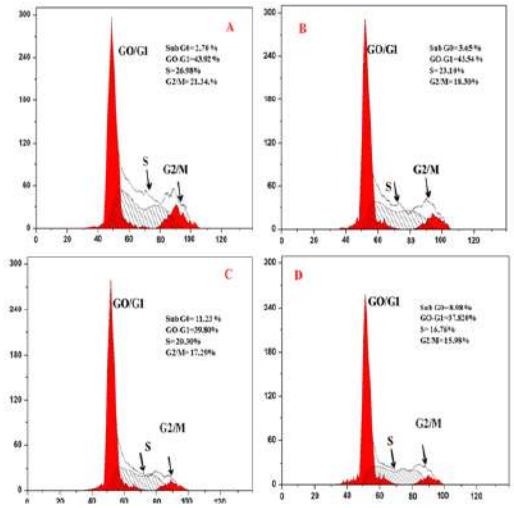

Figure 6: Cell cycle analysis determined using flow cytometry (a) Control, (b) 25µg/mL (c) 50 µg/mL (d) 100 µg/mL

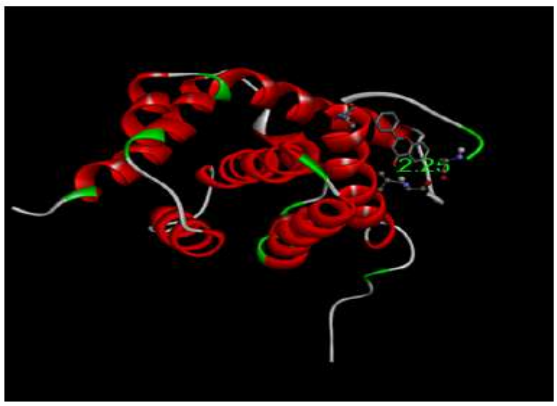

Figure 7: 3D interaction of Bax protein with Flavone

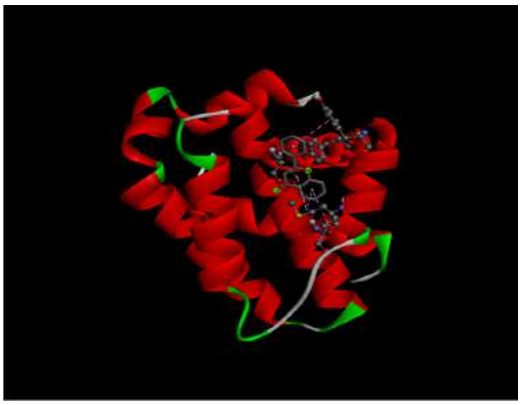

Figure 8: 3D interaction of BCL-2 protein with Flavone

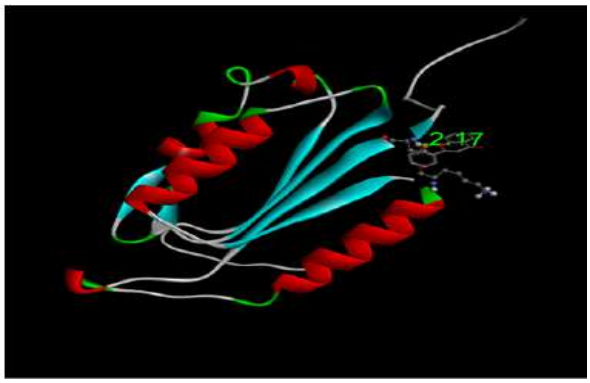

Figure 9: 3D interaction of Caspase-3 protein with Flavone

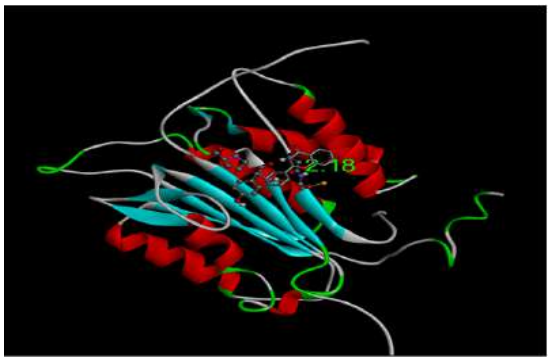

Figure 10: 3D interaction of Caspase-9 protein with Flavone

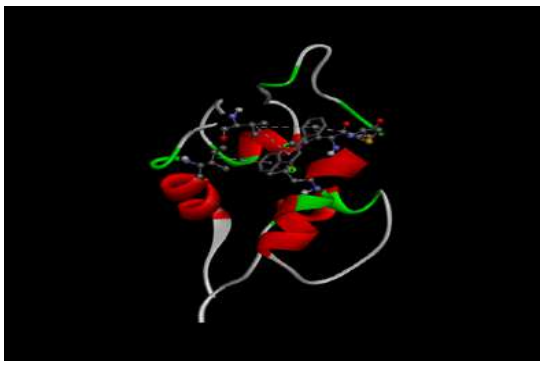

Figure 11: 3D interaction of p53 protein with Flavone

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RESEARCH ARTICLE

Studies on Phytochemical and α- Amylase Inhibition Activity from *Clitoria ternatea* Leaves Collected from Chennai

Karthick.A[1], Priyadurairaj.D[2], Vijayalakshmi.R[3], Abinash.R[1] and Jegan.G[4]*

[1]II Year Msc Student, Department of Biotechnology, Dr. M.G.R. Educational and Research Institute University, Maduravoyal, Chennai, Tamil Nadu, India.
[2]Assistant Professor, Department of Biotechnology, Dr. M.G.R. Education and Research Institute, University, Maduravoyal, Chennai, Tamil Nadu, India.
[3]Department of Biotechnology, Apollo Arts and Science College, Poonamallee, Chennai, Tamil Nadu, India.
[4]Assistant Professor, Department of Biotechnology, Apollo Arts and Science College, Poonamallee, Chennai, Tamil Nadu, India.

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***Address for Correspondence**
Jegan.G,
Assistant Professor,
Department of Biotechnology,
Apollo Arts and Science College,
Poonamallee, Chennai, Tamil Nadu, India.
Email: jeganbgl@gmail.com

ABSTRACT

Diabetes is the group of diseases that results in too much of sugar present in the blood, its means high blood glucose. The report of Centers for Disease Control (CDC) global health there will be half billion will have diabetes in 2040. Therefore, in this study focused on Antidiabetic activity using plant extraction. The blue flowered plant leaves of *Clitoria ternatea* were collected from different places of Sri Ramachandra Institute of Higher Education and Research at Porur, Chennai, Tamil Nadu, India. The results of phytochemicals showed that the ethanol extract contains flavonoids, saponins, Tanins, Phenols, Cardiac glycosides, steroids, Terpenoids, Quinones and Proteins. However, the hot distilled water extract contains flavonoids, Tanins, Phenols, Cardiac glycosides, steroids, Terpenoids, Quinones and Proteins. The Alkaloids were absence in both the extraction. The major difference between the both extractions confirmed that the absence of saponins in the hot distilled water extract when compared to ethanol extract. The percentage of the α-amylase inhibition activity by the ethanolic extracts of *Clitoria ternatea* leaves was studied in a different concentration and also same concentration of standard Acarbose

was prepared for comparison. The ethanol extraction of the *Clitoria ternatea* leaves was showed the activity of α-amylase inhibition at the range of moderate when compared to standard.

Keywords: Diabetic**,** *Clitoria ternatea,* Leaves, Phytochemicals, α-amylase inhibition

INTRODUCTION

Diabetes is the group of diseases that results in too much of sugar present in our blood cells, its means high blood glucose. This is commonly known as high blood sugar or doesn't make the enough insulin in the body. In today population there are 415 million people in worldwide are living with diabetes. The report of Centers for Disease Control (CDC) global health there will be half billion will have diabetes in 2040. Insulin is a hormone which made by the pancreas, which helps glucose from food to get into our cells to be used as energy form. When our body not being able to produce insulin or use its effect leads to rise of glucose level in our blood commonly known as hyperglycaemia disease. The WHO (World Health Organization) as reported in 2014, 8.5% of adults aged 18 years and older had diabetes. Diabetes was the direct cause of 1.5 million death and 48% of death happened due to diabetes in 2019, also occurred before the age of 70 years. There are two main types of diabetes; type1 and type 2 diabetes. In type 1 diabetes we cannot make any of insulin in our body but in type 2 diabetes insulin are cannot work effectively, or our body doesn't produce enough of it. All type of diabetes shows that glucose cannot get into our cells properly, so it begins to build up in our blood cells and too much of glucose in our blood and resulting leads to diabetes symptoms. The treatment of Diabetic mellitus (DM) is based on parenteral insulin and oral antidiabetic drugs. The Oral hypoglycemic agents including sulphonylureas, biguanides, and other drugs like acarbose but these drugs have serious side effects and deleterious contraindications (K. A. Wadkar, *et al.,* 2008).

Currently some of inhibitors in clinical use are acarbose and miglitol which inhibit glycosidases such as a-glucosidase and α-amylase while others such as voglibose inhibit a-glucosidase. Although, many of these types of synthetic hypoglycemic agents have their limitations, are non-specific, produce serious side effects and resulting fail to elevate diabetic complications. The main side effects of these inhibitors are causes gastrointestinal problems such as abdominal discomfort, bloating, flatulence and diarrhea (Cheng, *et al*., 2005). Therefore, the search for new pharmacologically active agents obtained from natural sources such as medicinal plants or their extracts can lead to potent and specific inhibitors for α-amylase activity (Tarling, *et al*., 2008).Herbal medicines are getting more importance in the treatment of diabetes as they are free from side effects and less expensive when compared to synthetic hypoglycemic agents (Grover, *et al*., 2002; Mukherjee, *et al*., 2006). In India, indigenous herbal remedies such as Ayurveda and other Indian traditional medicine have been used plant since ancient times in treatment of diabetes (Babu, *et al*., 2006). The hypoglycemic properties were studied from nearly 200 plants species (K. Karthic, *et al*., 2009). The most common and effective antidiabetic medicinal plants are origin from India including the plant *Clitoria ternatea*.

The plant *Clitoria ternatea* is wildly common to known as butterfuly-pea and cordofan-pea belong to the family of fabaceae and it has been used traditionally in Ayurvedic medicine for various health issues (Thakur *et al*.2018). All parts of the *Clitoria ternate*a (leaf, root, flowers and shoot) are herb used in medicine especially the leaves are very rich in antioxidant and anti-diabetes property. Since this as a multipurpose plant, this has been used in traditional system of medicine, herbal tea, cover crop, green manure, animal feed, and nitrogen fixation crop also it use as a weed purpose (Chakraborthy G S *et al*, 2018). The phytochemical composition of *Clitoria ternatea* leaves were contains Alkaloids, reducing sugar , flavonoids , steroids , glycosides and some other compounds which are responsible for prevention from neurodegenerative disease and mainly very effectively controls the diabetes mellitus and sweating excessive in body.(Chakaraborthy, *et al*. 2018). Therefore, In the present investigation the isolation of phytochemicals

and analysis of alpha amylase inhibition activity was carried out using the plant *Clitoria ternatea* leaves. This is the best way for the selected herbal drugs to use in the treatment of diabetes due to presence of bioactive compounds.

MATERIALS AND METHODS

Sample Collection

Plant materials

The blue flowered plant leaves of *Clitoria ternatea* were collected from different places of Sri Ramachandra Institute of Higher Education and Research (SRIHER) at Porur, Chennai, Tamil Nadu, India. The collection was made in the month of February 2022. The collected plants were photographed and the figure 1 shows the plants of blue flowered *Clitoria ternatea.* The wet weight of collected *Clitoria ternatea* leaves was noted. The leaves were transported to the laboratory and washed it to remove undesirable compounds using tap water and distilled water. Leaves of *Clitoria ternatea* were shade dried at room temperature (Fig: 2) for one week and stored in air tight containers. The dried leaves were pulverized by grinding using mortar and pestle and dry weight was measured.

Preparation of extract

In the experiment two different solvent were used namely Ethanol and Hot Distilled water for the extraction of phytochemicals from the *Clitoria ternatea* leaf powder. The extractions were made using the 20 g of *Clitoria ternatea* leaf powder with 250 mL of two different solvents such as Ethanol and Hot distilled water separately for 72 h (3 Days) at room temperature. Finally the extracts were collected, filtered through muslin cloth, then through What man filter paper and evaporated by rotary evaporator at 40ºC and stored in air tight container at 4°C for further experiments.

Qualitative phytochemical analysis

The ethanol and hot distilled water solvents extractions were subjected to qualitative phytochemical analysis by the method given by Harborne J.B., 1973.

Test for alkaloids

Mayer's Test

To the extract, 2 ml of mayer's reagent was added; formation of reddish brown precipitate indicates the presence of alkaloids.

Test for saponins

To 1 ml of the extract, 5 ml of water was added and the tube was shaken vigorously. Copious lather formation indicates the presence of saponins.

Test for tannins

To the extract, ferric chloride was added, formation of a dark blue or greenish black color showed the presence of tannins.

Test for cardiac glycosides

Keller-Kiliani test

To 1ml of the extracts, 2 ml of glacial acetic acid containing a drop of $FeCl_3$. Equal volume of conc. H_2SO_4 was added from the sides of the tube. A brown colour ring indicates the presence of cardiac glycosides.

Test for flavonoids

Alkaline reagent test

Extract was treated with 10% NaOH solution; formation of intense yellow colour indicates presence of flavonoid.

Test for phenols

Lead acetate test: The extract was taken; 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicated the presence of phenolic compounds.

Test for steroids

1 ml extract was dissolved in 10 ml of chloroform & equal volume of concentrated H_2SO_4 was added from the side of test tube. The upper layer turns red and H_2SO_4 layer showed yellow with green fluorescence .This indicates the presence of steroid.

Test for terpenoids

Salkowski test

5 ml of extract was mixed in 2 ml of chloroform, and concentrated sulphuric acid was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

Test for Quinones

The extracts were treated separately with Alc. KOH solution. Appearance of colors ranging from red to blue indicates the presence of quinones.

Test for Proteins

Ninhydrin test

The extract was taken and few drops of freshly prepared Ninhydrin reagent was added and heated. The appearance of pink or purple colour indicates that the presence of proteins, peptides or amino acids.

Alpha-amylase inhibition assay of *Clitoria ternatea* leaves

Previous reports confirmed that the ethanol extraction was good source for the extraction of bioactive compounds. Therefore only the ethanol extraction was used for the study of Alpha-amylase inhibition activity except Hot water extraction. The α-amylase inhibitory activity of the ethanol extraction of *Clitoria ternatea* leaf powder was carried out according to the standard method with minor modification (Unuofin *et al.,* 2018). 100 µL of α-amylase solution (0.1 mg/mL) was mixed with different concentrations (10, 20, 40, 80, 160, and 320 µg/mL) of ethanol extraction, reference standard (Acarbose) and control (without standard/ ethanol extraction) and pre-incubated at 37 °C for 15 min. Then, 100 µL of starch solution was added to initiate reaction and incubation was done at 37 °C for 60 min., then 10 µL of 1 M HCl and 100 µL of iodine reagent were added to the test tubes. The absorbance of the mixture was measured at 565 nm. α-amylase inhibitory activity was measured using the formula,

% Inhibition = [(OD of test - OD of control)/OD of test] x 100

RESULT AND DISCUSSION

In this study the *Clitoria ternatea* plant leaves were collected from inside of Sri Ramachandra Institute of Higher Education and Research (SRIHER), Porur, Chennai, Tamil Nadu, India. The collected leaves were washed with sterilized water to removed dust particles. The wet and dry weight of the collected *Clitoria ternatea* leaves were measured and which showed 135g of wet leaves given 53g of dry leaf powder (Table 1 and Fig: 3).

Leaf powder preparation

The dried leaves were fine powdered with the help of mortar and pestle for extraction. The figure 4 shows the *Clitoria ternatea* leaf powder. This powder was used to extract crude for further analysis using solvents. The crude extract will be good based on the fine powdered sample.

Crude Extraction

The crude was extracted from the *Clitoria ternatea* leaf powder using the ethanol and hot distilled water (aqueous) for 72 hours (3 Days). These two crude extracts were subjected to phytochemical analysis for identification. The results were confirmed that the presence of bioactive compounds (Fig: 5 and 6).

Phytochemical screening

The phytochemical studies of those prepared two crude extracts namely Ethanol and Hot distilled water extraction of *Clitoria ternatea* leaves were analyzed on the method of Harborne J.B., 1973. The results of phytochemicals showed that the ethanol extract contains flavonoids, saponins, Tanins, Phenols, Cardiac glycosides, steroids, Terpenoids, Quinones and Proteins. However, the hot distilled water extract contains flavonoids, Tanins, Phenols, Cardiac glycosides, steroids, Terpenoids, Quinones and Proteins. The Alkaloids were absence in both the extraction. The major difference between the both extractions confirmed that the absence of saponins in the hot distilled water extract when compared to Ethonolic extract (Table 2: and Fig 5 & 6).

Inhibition of Alpha-amylase activity by using *Clitoria ternatea* leaves

The α-amylase inhibitory activity of the leaves of *Clitoria ternatea* was investigated. The percentage of the α-amylase inhibition activity by the ethanolic extracts of *Clitoria ternatea* leaves was studied in a different concentration range from 10, 20, 40, 80, 160, and 320 µg/mL and also same concentration of standard (Acarbose) was prepared for comparison. The results were showed the α-amylase inhibition activity percentages by ethanol crude extract and standard at different concentration and the results were showing in the table 3 and 4 and figure 7. In which the IC_{50} value of the given ethanol extraction and the reference standard (Acarbose) were found to be 22.08 µg/mL and 1.12 µg/mL, respectively (Table: 5 and Fig 8). The ethanol extraction of the *Clitoria ternatea* leaves was showed the activity of Alpha-amylase inhibition at the range of moderate when compared to standard.

DISCUSSION

The plant *Clitoria ternatea* leaves were collected from Sri Ramachandra Institute of Higher Education and Research (SRIHER) at porur, Chennai, Tamil Nadu, India. The leaves were subjected to wash to remove undesirable compounds using tap water and distilled water. The wet and dry weight was measured and stored into an air tight container for further experiment. The powdered leaf was used for the extraction of phytochemicals using of ethanol and hot distilled water and ethanol extraction crude was analyzed the α-amylase inhibition activity. In many years, there has been increasing the search for natural products having potent bioactive compounds due to low toxicity, possess ability to oxidize fats, control appetite, regulate levels of hormones related to obesity and inhibit digestive enzymes involved in the absorption of carbohydrates and lipids (Cho *et al*., 2010; Rains *et al*., 2011) when compared to synthetic compounds. Phytochemicals are compounds which are produced by plants through primary or secondary metabolism. They are found in all parts of the plants such as leaf, fruits, vegetables, grains, beans etc. The previous reports have been confirming that the curative potential of several botanicals against chronic diseases like cancer, diabetes, inflammation, stroke, aging etc. (Kalita *et al*., 2012). Moreover various types of plant parts contain different types of phytochemicals and which are responsible for various pharmacological activities. The *Clitoria ternatea* plant is a perennial herb with several medicinal properties. In the year 1950s the studies of *C. ternatea* were elucidate its active constituents, phytochemical composition and pharmacological activities (Grindley *et al*., 1954; Kulshreshtha and Khare, 1967; Morita *et al*., 1976; Piala *et al*., 1962).

The *Clitoria ternatea* plant consist of a compound anthocyanins termed “ternatins” which render *C. ternatea* flowers with their vivid blue color, which was first isolated in the year 1985 (Saito *et al*., 1985). The Leaf extract of *Clitoria ternatea* is consisting of various bioactive compounds such as Alkaloids, reducing sugars, flavonoids, steroids, glycosides and these compounds were used for the prevention of neurodegenerative diseases and diabetes mellitus and also control excessive sweating (Nadkarni, 1992; Scalbert, et al., 2005). Therefore, in this investigation the blue flower *Clitoria ternatea* leaf was used for the extraction of phytochemicals. The ethanol extract of *Clitoria ternatea* plant

confirms the presence of various phytochemicals such as terpenoid, flavonoid, tannin and steroid which may act as antioxidant (Rai Kiranmai S, 2010). This research was carried out for the extraction of phytochemicals using two solvents namely ethanol and hot distilled water and which confirmed that presents of flavonoids, saponins, Tanins, Phenols, Cardiac glycosides, steroids, Terpenoids, Quinones and Proteins. The Alkaloids were absence in both the extraction. The major difference between the both extractions confirmed that the absence of saponins in the hot distilled water extract when compared to Ethonolic extract. The anti-hyperglycemic activities are found in many Ethnobotanical plant extractions which are used for the treatment of diabetes in Ayurveda. These plant extractions are used for the preparation of different modern medicines. In recent studies the leaf extracts of *C. ternatea* has been showed potential to the treatment of antidiabetic (Chusak *et al*., 2018b; Kavitha, 2018). Therefore, in this study the traditional medicinal plant of blue flower *Clitoria ternatea* was used for the extraction of bioactive compounds to treat diabetes. An important enzyme called α-amylase is that hydrolyses the α bonds of large α linked polysaccharide such as starch and glycogen to yield disaccharides like maltose which will further hydrolyze by α-glucosidase to yield monosaccharides like glucose. A many number of studies have been reported that some of medicinal plants possess the enzymes α- amylase, α-glucosidase and lipase inhibitory activities (Shirwaikar *et al*., 2005; Ortiz-Andrade *et al*., 2007).

In the present investigation the ethanolic extract of *Clitoria ternatea* leaf was investigated for the inhibition of α-amylase activity at different concentration (10, 20, 40, 80, 160, and 320 µg/mL). The acarbose standard drug was used for comparison to the plant leaf extract from *Clitoria ternatea*. The inhibitors of α amylases which are bind to α bond of polysachharide and stop the breakdown of polysaccharide into mono and disaccharide. Phenolic compounds and flavonoids which are bind covalently to alpha-amylase and change its activity due to the ability to form quinones or lactones that the react with nucleophilic groups on the enzyme molecule (S. Oyedemi, *et al*., 2013). The compound Flavonoids are hydroxylated phenolic compounds which contain a benzo-γ-pyrone structure which are mostly present in plants in response to microbial infections (S. Rohn, *et al*., 2010). These are the some compounds which induce more effectively inhibiting alpha-amylase activity based on the ability to form quinone with the 4-oxopyrane structure of the enzyme (J.-S. Kim, *et al*., 2000). The α-amylase inhibitory effect of ethanol extracts of *Clitoria ternatea* leaf was analyzed and the result of this research was showed moderate α-amylase inhibitory activity when compared to standard acarbose.

Many number of factors like degree of ripeness at the time of harvest, processing, environmental factors, and storage affect the polyphenol content of plants, which might be responsible for variation in the IC_{50} value of plants (Manach C *et al.,* 2004). The IC_{50} value of the given ethanol extraction and the reference standard (acarbose) were found to be 22.08 µg/mL and 1.12 µg/mL, respectively. The value of IC_{50} was showed good results in ethanol extraction but not like standard acarbose. Finally the result is concluding that the ethanol extraction of the *Clitoria ternatea* leaves was showed the activity of alpha-amylase inhibition.

CONCLUSION

In this study, the ethanol extract of blue flowered leaves of *C. ternatea* were evaluated for the presence of phytochemicals and α-amylase activity. The presence of various bioactive compounds indicates that these leaves extracts compounds can be used as therapeutic drugs for Antidiabetic, Antioxidant and Antibacterial activity. This study confirmed that the ethanol extraction of the *Clitoria ternatea* leaves was showed the activity of alpha-amylase inhibition. These plant extractions and methods are simple, eco-friendly and cost effective when compare to synthetic drug.

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REFERENCES

1. Anupsingh vijaysinghThakur, SonuAmbwani, Tanuj Kumar Ambwani, A.H. Ahmad and Dharmendra Singh Rawat ; Thakur *et al*., 2018.
2. Babu PA, Suneetha G, Boddepalli R, Lakshmi VV, Rani TS, Rambabu Y, Srinivas K: A database of 389 medicinal plants for diabetes. Bioinformation 2006, 1(4):130-171.
3. Chakraborthy GS ,Kumaar V ,Gupta S, Kumar A, Gautam N, Kumari L; Phytochemcial and pharmacological aspects of *Clitoria ternatea* -A review 2018(http://www.japsr.in).
4. Cheng AYY, Fantus IG: Oral antihyperglycemic therapy for type 2 diabetes mellitus. Canadian Medicinal Association Journal 2005, 172(2):213-226.
5. Chusak, C., Thilavech, T., Henry, C. J., and Adisakwattana, S. (2018b). Acute effect of *Clitoria ternatea* flower beverage on glycemic response and antioxidant capacity in healthy subjects: a randomized crossover trial. BMC Complement. Altern. Med. 18:6. doi: 10.1186/s12906-017-2075-7.
6. Grindley, D. N., Burden, E. H. W. J., and Akour, A. A. (1954). The seed oils of *Clitoria ternatea* and Entada phaseoloides. J. Sci. Food Agric. 5, 278–280. doi: 10.1002/jsfa.2740050605.
7. Grover JK, Yadav S, Vats V: Medicinal plants of India with anti-diabetic potential. J Ethanopharmacol 2002, 81:81-100.
8. Harborne JB (1973). Methods of plant analysis. In: Phytochemical Methods. Chapman and Hall, London
9. J.-S. Kim, C.-S. Kwon, and K. H. Son, "Inhibition of Alpha glucosidase and Amylase by Luteolin, a Flavonoid," *Bioscience, Biotechnology and Biochemistry*, vol. 64, no. 11, pp. 2458– 461, 2000.
10. K. A. Wadkar, C. S.Magdum, S. S. Patil, and N. S. Naikwade, "Anti-diabetic potential and Indian medicinal plants," *Journal of Herbal Medicine and Toxicology*, vol. 2, no. 1, pp. 45–50, 2008.
11. K. Karthic, K. S. Kirthiram, S. Sadasivam, B. Thayumanavan, and T. Palvannan, "Identification of α amylase inhibitors from *Syzygium cumini* Linn seeds," *Indian Journal of Experimental Biology*, vol. 46, no. 9, pp. 677–680, 2008.
12. Kalita, S., Kumar, G., Karthik, L., and Rao, K.V.B. (2012). A review on medicinal properties of lantana camara linn. Res. J. Pharm. Tech. 5, 711–715.
13. Kavitha, R. (2018). Biochemical studies on the effect of ethanolic extracts of Trichosanthes dioica and *Clitoria ternatea* in streptozotocin induced male Wistar rats. Int. J. Pharm. Sci. Res. 9, 4682–4689. doi: 10.13040/ijpsr.0975-8232. 9(11).4682-89.
14. Kulshreshtha, D. K., and Khare, M. P. (1967). Chemical investigation of the seeds of *Clitoria ternatea* 'Linn'. Curr. Sci. 36, 124–125.
15. Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L. Polyphenols: food sources and bioavailability. Am J Clin Nutr. 2004;79(5):727–47.
16. Morita, N., Ariwasa, M., Nagase, M., Hsu, H.-Y., and Chen, Y.-P. (1976). Studies on the constituents of formosan leguminosae: the constituents in the leaves of *Clitoria ternatea* L. Yakugaku Zasshi 97, 649–653. doi: 10.1248/yakushi1947.97. 6_649.
17. Mukherjee PK, Maiti K, Mukherjee K, Houghton PJ: Leads from Indian medicinal plants with hypoglycemic potentials. J Ethnopharmacol 2006, 106(1):1-28.
18. Nadkarni AK. Indian Materia Medica. 3rd Ed., Bombay; Popular Prakashan 1992: Vol-I, pp. 354.
19. Ortiz-Andrade, R.R., Ramirez-Avila, G., Garcia-Jimenez, S. Villlobos-Molina, R. and EstradaSoto, S. (2007) α-Glucosidase inhibitory activity of the methanolic extract from Tournefortia hartwegiana: An anti-hyperglycemic agent. Journal of Ethnopharmacology 109: 48-53.
20. Piala, J. J., Madissoo, H., and Rubin, B. (1962). Diuretic activity of roots of *Clitoria ternatea* L. in dogs. Experientia 18:89. doi: 10.1007/BF02138275

21. Rai Kiranmai S., Neurogenic Potential of *Clitoria ternatea* Aqueous Root Extract–A Basis for Enhancing Learning and Memory, World Academy of Science, Engineering and Technology 70 2010,237-240
22. S. Oyedemi, T. Koekemoer, G. Bradley, M. Van De Venter, and A. Afolayan, "In vitro anti-hyperglycemia properties of the aqueous stem bark extract from *Strychnos henningsii* (Gilg)," *International Journal of Diabetes in Developing Countries*, vol. 33, no. 2, pp. 120–127, 2013.
23. Saito, N., Abe, K., Honda, T., Timberlake, C. F., and Bridle, P. (1985). Acylated delphinidin glucosides and flavonols from *Clitoria ternatea* . Phytochemistry 24, 1583–1586.
24. Scalbert A, Manach C, Morand C, Remesy C and Jimenez L. Dietary polyphenols and the prevention of diseases. Critical Review on Food Science and Nutrition. 2005; 45: 287–306.
25. Shirwaikar A, Rajendran K, Punitha ISR (2005): Antidiabetic activity of alcoholic stem extract of Coscinium fenestratum in STZ-Nicotinamide induced type 2 diabetic rats. J Ethnopharmacol 97: 369–374.
26. Tarling CA, Woods K, Zhang R, Brastianos HC, Brayer GD, Andersen RJ, Withers SG: The Search for Novel Human Pancreatic α-Amylase Inhibitors: High-Throughput Screening of Terrestrial and Marine Natural Product Extracts. ChemBioChem 2008, 9:433-438.
27. Unuofin, J.O., Otunola, G.A. and Afolayan, A.J., 2018. In vitro α-amylase, α-glucosidase, lipase inhibitory and cytotoxic activities of tuber extracts of Kedrostis africana (L.) Cogn. *Heliyon*, 4(9), p.e00810.
28. WHO classification of diabetes mellitus 2019 (https://www.who.int/health-topics/diabetes).

Table 1: The Wet and Dry weight of the *Clitoria ternatea* leaves

Wet weight (g)	Dry weight (g)
135	53

Table 2: The table shows the presence and absence of Phytochemicals extracted from the *Clitoria ternatea* leaves

Solvents	Alkaloids	Flavonoids	Saponins	Tannins	Phenols	Cardiac glycosides	Steroids	Terpenoids	Quinones	Proteins
Ethanol	-	+	+	+	+	+	+	+	+	+
Hot Distilled Water	-	+	-	+	+	+	+	+	+	+

Table 3: Percentage of Alpha-amylase Inhibition activity by Standard (Acarbose)

Sample	Conc.(µg)	% of Inhibition
Acarbose (Standard)	10	61.64772929
	20	80.51434824
	40	88.58281866
	80	92.37123319
	160	95.0462363
	320	96.44080629

Table 4: Percentage of Alpha-amylase Inhibition activity by Ethanolic extract of *Clitoria ternatea*

Sample	Conc.(µg)	% of Inhibition
Ethanol Extraction	10	12.95728833
	20	55.47238329
	40	76.14556104
	80	92.68059638
	160	94.38993377
	320	95.88495897

Table 5: The IC_{50} value of the Standard (Acarbose) and the Ethanol extraction of *Clitoria ternatea*

Sample	IC_{50} value of *Clitoria ternatea*
Acarbose	1.12
Ethanol	22.08

Fig. 1: The image shows the blue flowered *Clitoria ternatea*

Fig. 2: The Dry leaves of the *Clitoria ternatea*

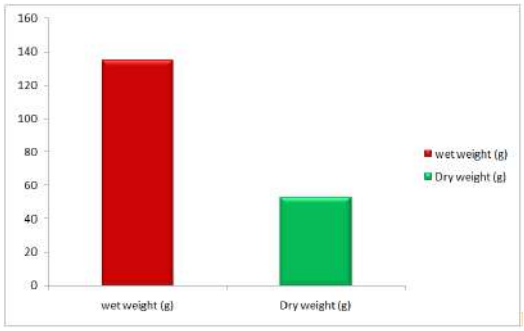

Fig. 3: Wet and Dry weight of the *Clitoria ternatea* leaves

Fig. 4: The powder of *Clitoria ternatea* leaves

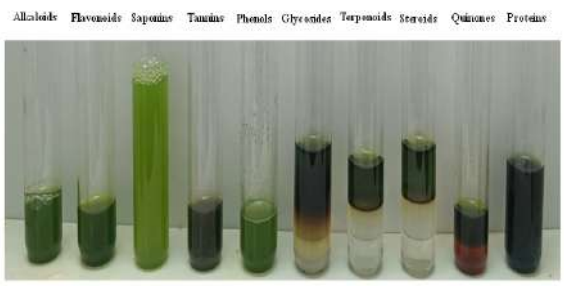

Fig. 5: The image shows the presence of Phytochemicals from the Ethanol extraction

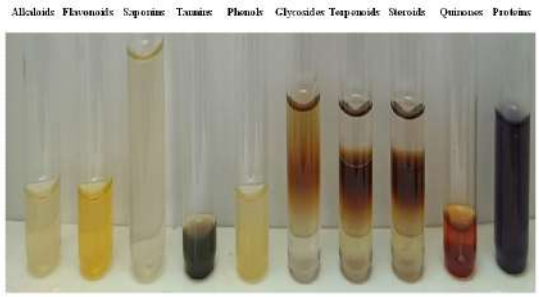

Fig. 6: The image shows the presence of Phytochemicals from the Hot distilled water extraction (Aqueous)

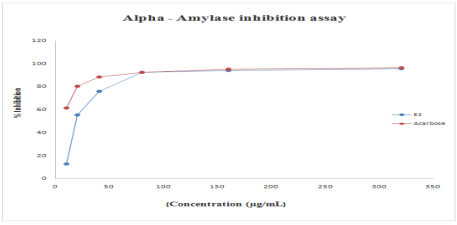

Fig. 7: The graph shows the percentage (%) of α-amylase inhibitory activity of the Acarbose (Standard) and ethanolic crude extract of *Clitoria ternatea*

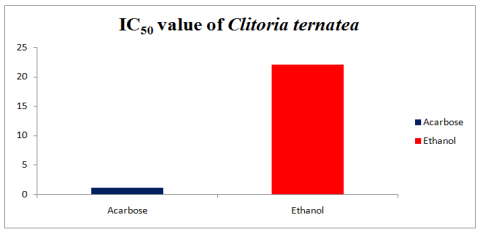

Fig. 8: IC_{50} value of the Standard (Acarbose) and the Ethanol extraction of *Clitoria ternatea*

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RESEARCH ARTICLE

Modal Operators on Einstein Operations of Pythagorean Fuzzy Matrix

M.Ramakrishnan[1]* and S.Sriram[2]

[1]Research Scholar, Department of Mathematics, Annamalai University, Annamalai Nagar, Chidambaram, Cuddalore, Tamil Nadu, India.
[2]Head and Professor, Department of Mathematics, Annamalai University, Annamalai Nagar, Chidambaram, Cuddalore, Tamil Nadu, India.

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***Address for Correspondence**
M.Ramakrishnan,
Research Scholar,
Department of Mathematics,
Annamalai University,
Annamalai Nagar,
Chidambaram, Cuddalore,
Tamil Nadu, India.
Email: mrkmaths85@gmail.com

ABSTRACT

In this paper, we propose two modal operators, which are analogous to Modal Operators necessity and possibility on Einstein Operations of Pythagorean Fuzzy Matrix. Some new relations are established and Some relations are procured with numerical verifications.

Keywords: Modal Operator, Einstein Sum and Einstein Product, Pythagorean Fuzzy Matrix, Einstein Operations of Pythagorean Fuzzy Matrix, Modal operators of Einstein operations Pythagorean Fuzzy Matrix.

INTRODUCTION

The fuzzy set was introduced by zadeh [17], he discussed membership degree. The fuzzy Matrices was established by Thomason [13], who described the convergence of powers of fuzzy matrix. As a stretching concept of fuzzy set, the intuitionistic fuzzy set(IFS) is debated membership degree and non-membership degree was expanded by Atanassov [1,2]. Intuitionistic fuzzy Matrices(IFMs) were presented by Pal [12] and developed Intuitionistic Fuzzy Determinant, min-max, max-min, a solved equality between IFMs[4,7,8].Sriram was proposed in the IFMs [3,5,6,9] and established Modal Operators in Intuitionistic Fuzzy Matrices. Yager[14,15,16] introduced the Pythagorean fuzzy set (PFS) and proved relations for PFS. The PFS satisfying the condition that the square sum of its membership degree and nonmember ship degree. Silambarasan and Sriram [11] established Pythagorean fuzzy matrix(PFM)and

discussed algebraic operations for Pythagorean fuzzy matrices. Also they defined modal operators for Pythagorean fuzzy matrix and proved their algebraic properties.

Preliminaries

In 2018, Silambarasan and Sriram [11] defined Pythagorean Fuzzy matrix and its basic properties.

Definition 2.1 [11] A Pythagorean fuzzy matrix is a pair $U = (\langle u_{lm\mu}, u_{lmv}\rangle)$ of non negative real numbers $u_{lm\mu}, u_{lmv} \in [0,1]$ satisfying $0 \leq u_{lm\mu}^2 + u_{lmv}^2 \leq 1$, for all l, m.

Definition 2.2[11] Given two Pythagorean Fuzzy matrices U and V of same size, the algebraic operations are defined as follows:

$(i)\ U \oplus_S V = \left(\langle \sqrt{u_{lm\mu}^2 + v_{lm\mu}^2 - u_{lm\mu}^2 v_{lm\mu}^2}, u_{lmv} v_{lmv}\rangle\right)$,

$(ii)\ U \odot_M V = \left(\langle u_{lm\mu} v_{lm\mu}, \sqrt{u_{lmv}^2 + v_{lmv}^2 - u_{lmv}^2 v_{lmv}^2}\rangle\right)$

Where $+, -$ and $\cdot$ are real numbers addition, subtraction and multiplication respectively.

Also,

$(iii)\ U \vee V = (\langle \max(u_{lm\mu}, v_{lm\mu}), \min(u_{lmv}, v_{lmv})\rangle)$

$(iv)\ U \wedge V = (\langle \min(u_{lm\mu}, v_{lm\mu}), \max(u_{lmv}, v_{lmv})\rangle)$

$(v) U^C = (\langle u_{lmv}, u_{lm\mu}\rangle)$

Definition 2.3[10]

Let A PFM is defined as $U = (\langle u_{lm\mu}, u_{lmv}\rangle)$ where $u_{lm\mu}, u_{lmv} \in [0,1]$ are respectively the measure of positive, negative membership of u_{lm} for $l = 1,2,\ldots x$ and $m = 1,2,\ldots y$ satisfying $0 \leq u_{lm\mu} + u_{lmv} \leq 1$.

Let $U = (u_{lm\mu}, u_{lmv})$ *and* $V = (v_{lm\mu}, v_{lmv})$ be two Einstein Operations on Pythagorean Fuzzy Matrices (EPFMs) of same size, then,

$$(i)\ U \oplus_S V = \left(\sqrt{\frac{(u_{lm\mu})^2 + (v_{lm\mu})^2}{1 + (u_{lm\mu})^2 (v_{lm\mu})^2}}, \frac{u_{lmv} v_{lmv}}{\sqrt{1 + (1 - (u_{lmv})^2)(1 - (v_{lmv})^2)}}\right)$$

$$(ii)\ U \odot_M V = \left(\frac{u_{lm\mu} v_{lm\mu}}{\sqrt{1 + \left(1 - (u_{lm\mu})^2\right)\left(1 - (v_{lm\mu})^2\right)}}, \sqrt{\frac{(u_{lmv})^2 + (v_{lmv})^2}{1 + (u_{lmv})^2 (v_{lmv})^2}}\right)$$

In general,

$$nU = \left(\sqrt{\frac{\left(1 + (u_{lm\mu})^2\right)^n - \left(1 - (u_{lm\mu})^2\right)^n}{\left(1 + (u_{lm\mu})^2\right)^n + \left(1 - (u_{lm\mu})^2\right)^n}}, \frac{\sqrt{2}(u_{lmv})^n}{\sqrt{(2 - (u_{lmv})^2)^n + ((u_{lmv})^2)^n}}\right)$$

$$U^n = \left(\frac{\sqrt{2}(u_{lm\mu})^n}{\sqrt{\left(2 - (u_{lm\mu})^2\right)^n + \left((u_{lm\mu})^2\right)^n}}, \sqrt{\frac{(1 + (u_{lmv})^2)^n - (1 - (u_{lmv})^2)^n}{(1 + (u_{lmv})^2)^n + (1 - (u_{lmv})^2)^n}}\right)$$

Modal operators of Pythagorean Fuzzy Matrix

Silambarasan and Sriram [11] introduced the modal operators of Pythagorean Fuzzy matrices and studied their properties with respect to the algebraic operations. In this section, we study the algebraic properties of modal operators with respect to the Einstein operations.

The modal operators of an PFM, □ (Necessity) and ◊ (Possibility) of a PFM U are defined in the following way:

$$\Box U = \left(\langle u_{lm\mu}, \sqrt{1-(u_{lm\mu})^2}\rangle\right) \text{ and } \Diamond U = \left(\langle\sqrt{1-(u_{lmv})^2}, u_{lmv}\rangle\right)$$

Theorem 3.1

Let $U = \left(\langle u_{lm\mu}, u_{lmv}\rangle\right)$ *and* $V = \left(\langle v_{lm\mu}, v_{lmv}\rangle\right)$ be two PFMs of same size, then

$(\boldsymbol{i})\Box(U \oplus_S V) = \Box U \oplus_S \Box V$

$(\boldsymbol{ii})\ \Diamond(U \oplus_S V) = \Diamond U \oplus_S \Diamond V$

$(\boldsymbol{iii})\Box(U \odot_M V) = \Box U \odot_M \Box V$

$(\boldsymbol{iv})\ \Diamond(U \odot_M V) = \Diamond U \odot_M \Diamond V$

Proof:

$$(i)\ \mathbf{L.H.S} = \Box U \oplus_S \Box V = \left(u_{lm\mu}, \sqrt{1-(u_{lm\mu})^2}\right) \oplus_S \left(v_{lm\mu}, \sqrt{1-(v_{lm\mu})^2}\right)$$

$$= \left(\sqrt{\frac{(u_{lm\mu})^2+(v_{lm\mu})^2}{1+(u_{lm\mu})^2(v_{lm\mu})^2}}, \frac{\sqrt{1-(u_{lm\mu})^2}\sqrt{1-(v_{lm\mu})^2}}{\sqrt{1+(u_{lm\mu})^2(v_{lm\mu})^2}}\right)$$

$$= \left(\sqrt{\frac{(u_{lm\mu})^2+(v_{lm\mu})^2}{1+(u_{lm\mu})^2(v_{lm\mu})^2}}, \frac{\sqrt{1-(u_{lm\mu})^2-(v_{lm\mu})^2+(u_{lm\mu})^2(v_{lm\mu})^2}}{\sqrt{1+(u_{lm\mu})^2(v_{lm\mu})^2}}\right)$$

$$= \left(\sqrt{\frac{(u_{lm\mu})^2+(v_{lm\mu})^2}{1+(u_{lm\mu})^2(v_{lm\mu})^2}}, \sqrt{1-\left(\sqrt{\frac{(u_{lm\mu})^2+(v_{lm\mu})^2}{1+(u_{lm\mu})^2(v_{lm\mu})^2}}\right)^2}\right)$$

$= \Box(U \oplus_S V) = \mathbf{R.H.S}.$

$$(iv)\ \mathbf{L.H.S} = \Diamond U \odot_M \Diamond V = \left(\sqrt{1-(u_{lmv})^2}, u_{lmv}\right) \odot_M \left(\sqrt{1-(u_{lmv})^2}, u_{lmv}\right)$$

$$= \left(\frac{\sqrt{1-(u_{lmv})^2}\sqrt{1-(u_{lmv})^2}}{\sqrt{1+(u_{lmv})^2(u_{lmv})^2}}, \sqrt{\frac{(u_{lmv})^2+(v_{lmv})^2}{1+(u_{lmv})^2(v_{lmv})^2}}\right)$$

$$= \left(\frac{\sqrt{1-(u_{lmv})^2-(u_{lmv})^2+(u_{lmv})^2(u_{lmv})^2}}{\sqrt{1+(u_{lmv})^2(u_{lmv})^2}}, \sqrt{\frac{(u_{lmv})^2+(v_{lmv})^2}{1+(u_{lmv})^2(v_{lmv})^2}}\right)$$

$$= \left(\sqrt{1 - \left(\sqrt{\frac{(u_{lmv})^2 + (v_{lmv})^2}{1 + (u_{lmv})^2 (v_{lmv})^2}} \right)^2}, \sqrt{\frac{(u_{lmv})^2 + (v_{lmv})^2}{1 + (u_{lmv})^2 (v_{lmv})^2}} \right)$$

$= \Diamond\, U \odot_M \Diamond\, V = \mathbf{R}.\mathbf{H}.\mathbf{S}.$
Thus $(i), (iv)$ holds.
$(ii), (iii)$ It can be proved similarly.

Theorem 3.2
Let $U = \left(\langle u_{lm\mu}, u_{lmv} \rangle\right)$ be a PFM of same size, then
$(\boldsymbol{i}) \Box(nU) = n(\Box U)$
$(\boldsymbol{ii}) \Diamond (nU) = n(\Diamond\, U)$
$(\boldsymbol{iii}) \Box(U^n) = (\Box U)^n$
$(\boldsymbol{iv}) \Diamond (U^n) = (\Diamond\, U)^n$

Proof:

$$(i)\ \mathbf{L}.\mathbf{H}.\mathbf{S} = \Box(nU) = \Box\left(\sqrt{\frac{\left(1 + \left(u_{lm\mu}\right)^2\right)^n - \left(1 - \left(u_{lm\mu}\right)^2\right)^n}{\left(1 + \left(u_{lm\mu}\right)^2\right)^n + \left(1 - \left(u_{lm\mu}\right)^2\right)^n}}, \frac{\sqrt{2}(u_{lmv})^n}{\sqrt{(2 - (u_{lmv})^2)^n + ((u_{lmv})^2)^n}} \right)$$

$$= \left(\sqrt{\frac{\left(1 + \left(u_{lm\mu}\right)^2\right)^n - \left(1 - \left(u_{lm\mu}\right)^2\right)^n}{\left(1 + \left(u_{lm\mu}\right)^2\right)^n + \left(1 - \left(u_{lm\mu}\right)^2\right)^n}}, \sqrt{1 - \left(\frac{\left(1 + \left(u_{lm\mu}\right)^2\right)^n - \left(1 - \left(u_{lm\mu}\right)^2\right)^n}{\left(1 + \left(u_{lm\mu}\right)^2\right)^n + \left(1 - \left(u_{lm\mu}\right)^2\right)^n} \right)} \right)$$

$$= \left(\sqrt{\frac{\left(1 + \left(u_{lm\mu}\right)^2\right)^n - \left(1 - \left(u_{lm\mu}\right)^2\right)^n}{\left(1 + \left(u_{lm\mu}\right)^2\right)^n + \left(1 - \left(u_{lm\mu}\right)^2\right)^n}}, \frac{\sqrt{2}\left(\sqrt{1 - \left(u_{lm\mu}\right)^2}\right)^n}{\sqrt{\left(2 - \left(\sqrt{1 - \left(u_{lm\mu}\right)^2}\right)^2\right)^n + \left(\left(\sqrt{1 - \left(u_{lm\mu}\right)^2}\right)^2\right)^n}} \right)$$

$$= n\left(u_{lm\mu}, \sqrt{1 - \left(u_{lm\mu}\right)^2}\right) = n(\Box U) = \mathbf{R}.\mathbf{H}.\mathbf{S}$$

$(\boldsymbol{iv})\mathbf{L}.\mathbf{H}.\mathbf{S} = \Diamond\, (U^n)$

$$= \Diamond \left(\frac{\sqrt{2}(u_{lm\mu})^n}{\sqrt{\left(2 - \left(u_{lm\mu}\right)^2\right)^n + \left(\left(u_{lm\mu}\right)^2\right)^n}}, \sqrt{\frac{(1 + (u_{lmv})^2)^n - (1 - (u_{lmv})^2)^n}{(1 + (u_{lmv})^2)^n + (1 - (u_{lmv})^2)^n}} \right)$$

$$= \left(\sqrt{1 - \left(\sqrt{\frac{(1 + (u_{lmv})^2)^n - (1 - (u_{lmv})^2)^n}{(1 + (u_{lmv})^2)^n + (1 - (u_{lmv})^2)^n}} \right)^2}, \sqrt{\frac{(1 + (u_{lmv})^2)^n - (1 - (u_{lmv})^2)^n}{(1 + (u_{lmv})^2)^n + (1 - (u_{lmv})^2)^n}} \right)$$

$$= \left(\frac{\sqrt{2}\left(\sqrt{1-(u_{lmv})^2}\right)^n}{\sqrt{\left(2-\left(\sqrt{1-(u_{lmv})^2}\right)^2\right)^n + \left(\left(\sqrt{1-(u_{lmv})^2}\right)^2\right)^n}}, \sqrt{\frac{(1+(u_{lmv})^2)^n - (1-(u_{lmv})^2)^n}{(1+u_{lmv}{}^2)^n + (1-(u_{lmv})^2)^n}} \right)$$

$$= \left(\sqrt{1-(u_{lmv})^2}, u_{lmv}\right)^n = (\Diamond U)^n = \mathbf{R.H.S}$$

Thus $(i), (iv)$ holds.

$(ii), (iii)$ It can be proved similarly.

Theorem 3.3

Let $U = \left(\langle u_{lm\mu}, u_{lmv}\rangle\right)$ be a PFM of same size, then

$(\boldsymbol{i})\left(\Box(\Box U^C)\right)^C = \Diamond U$

$(\boldsymbol{ii})\left(\Box(\Diamond U^C)\right)^C = \Box U$

$(\boldsymbol{iii})\left(\Diamond(\Box U^C)\right)^C = \Diamond U$

$(\boldsymbol{iv})\left(\Diamond(\Diamond U^C)\right)^C = \Box U$

Proof:

$(i)\mathbf{L.H.S} = \left(\Box(\Box U^C)\right)^C$

$$= \left(\left(u_{lmv}, \sqrt{1-(u_{lmv})^2}\right)\right)^C = \left(u_{lmv}, \sqrt{1-(u_{lmv})^2}\right)^C = \left(\sqrt{1-(u_{lmv})^2}, u_{lmv}\right)$$

$= \Diamond U = \mathbf{R.H.S}$

$(\boldsymbol{iv})\mathbf{L.H.S} = \left(\Diamond(\Diamond U^C)\right)^C$

$$= \left(\Diamond\left(\sqrt{1-(u_{lm\mu})^2}, u_{lm\mu}\right)\right)^C = \left(\sqrt{1-(u_{lm\mu})^2}, u_{lm\mu}\right)^C = \left(u_{lm\mu}, \sqrt{1-(u_{lm\mu})^2}\right)$$

$= \Box U = \mathbf{R.H.S}$

Thus $(i), (iv)$ holds.

$(ii), (iii)$ It can be proved similarly.

We can prove the following corollaries using the above theorems

Corollary 3.4

Let $U = \left(\langle u_{lm\mu}, u_{lmv}\rangle\right)$ be a PFM of same size, then

$(\boldsymbol{i})(\Box U^C)^C = \Diamond U$

$(\boldsymbol{ii})(\Diamond U^C)^C = \Box U$

Corollary 3.5

Let $U = \left(\langle u_{lm\mu}, u_{lmv}\rangle\right)$ be a PFM of same size, then

$(\boldsymbol{i})(\Box U^C)^C = \left(\Box(\Box U^C)\right)^C = \left(\Diamond(\Box U^C)\right)^C$

$(\boldsymbol{ii})(\Diamond U^C)^C = \left(\Box(\Diamond U^C)\right)^C = \left(\Diamond(\Diamond U^C)\right)^C$

Corollary 3.6

Let $U = (\langle u_{lm\mu}, u_{lmv}\rangle)$be a PFM of same size, then

(i) $\square(\square\mathcal{U}) = \square\mathcal{U} = \Diamond(\square\mathcal{U})$

(ii) $\square(\Diamond\mathcal{U}) = \Diamond\mathcal{U} = \Diamond(\Diamond\mathcal{U})$

Theorem 3.7

Let $U = (\langle u_{lm\mu}, u_{lmv}\rangle)$ *and* $V = (\langle v_{lm\mu}, v_{lmv}\rangle)$ be two PFMs of same size, then

(i) $\left(\square(U^C \oplus_S V^C)\right)^C = \Diamond U \odot_M \Diamond V$

(ii) $\left(\square(U^C \odot_M V^C)\right)^C = \Diamond U \oplus_S \Diamond V$

(iii) $\left(\Diamond(U^C \oplus_S V^C)\right)^C = \square U \odot_M \square V$

(iv) $\left(\Diamond(U^C \odot_M V^C)\right)^C = \square U \oplus_S \square V$

Proof:

(i) **L.H.S** $= \left(\square(U^C \oplus_S V^C)\right)^C$

$$= \left(\square\left(\sqrt{\frac{(u_{lmv})^2 + (v_{lmv})^2}{1 + (u_{lmv})^2(v_{lmv})^2}}, \frac{u_{lm\mu}v_{lm\mu}}{\sqrt{1 + \left(1 - (u_{lm\mu})^2\right)\left(1 - (v_{lm\mu})^2\right)}}\right)\right)^C$$

$$= \left(\sqrt{\frac{(u_{lmv})^2 + (v_{lmv})^2}{1 + (u_{lmv})^2(v_{lmv})^2}}, \sqrt{1 - \left(\sqrt{\frac{(u_{lmv})^2 + (v_{lmv})^2}{1 + (u_{lmv})^2(v_{lmv})^2}}\right)^2}\right)^C$$

$$= \left(\sqrt{\frac{(u_{lmv})^2 + (v_{lmv})^2}{1 + (u_{lmv})^2(v_{lmv})^2}}, \sqrt{\frac{(1 - (u_{lmv})^2)(1 - (v_{lmv})^2)}{1 + (u_{lmv})^2(v_{lmv})^2}}\right)^C$$

$$= \left(\sqrt{\frac{(1 - (u_{lmv})^2)(1 - (v_{lmv})^2)}{1 + (u_{lmv})^2(v_{lmv})^2}}, \sqrt{\frac{(u_{lmv})^2 + (v_{lmv})^2}{1 + (u_{lmv})^2(v_{lmv})^2}}\right)$$

$$= \left(\sqrt{1 - (u_{lmv})^2}, u_{lmv}\right) \odot_M \left(\sqrt{1 - (v_{lmv})^2}, v_{lmv}\right) = \Diamond U \odot_M \Diamond V = \textbf{R.H.S}$$

(iv) **L.H.S**$= \left(\Diamond(U^C \odot_M V^C)\right)^C$

$$= \left(\Diamond\left(\frac{u_{lmv}v_{lmv}}{\sqrt{1 + (1 - (u_{lmv})^2)(1 - (v_{lmv})^2)}}, \sqrt{\frac{(u_{lm\mu})^2 + (v_{lm\mu})^2}{1 + (u_{lm\mu})^2(v_{lm\mu})^2}}\right)\right)^C$$

$$= \left(\sqrt{1 - \left(\sqrt{\frac{(u_{lm\mu})^2 + (v_{lm\mu})^2}{1 + (u_{lm\mu})^2(v_{lm\mu})^2}}\right)^2}, \sqrt{\frac{(u_{lm\mu})^2 + (v_{lm\mu})^2}{1 + (u_{lm\mu})^2(v_{lm\mu})^2}}\right)^C$$

$$= \left(\sqrt{\frac{\left(1-\left(u_{lm\mu}\right)^2\right)\left(1-\left(v_{lm\mu}\right)^2\right)}{1+\left(u_{lm\mu}\right)^2\left(v_{lm\mu}\right)^2}}, \sqrt{\frac{\left(u_{lm\mu}\right)^2+\left(v_{lm\mu}\right)^2}{1+\left(u_{lm\mu}\right)^2\left(v_{lm\mu}\right)^2}} \right)^C$$

$$= \left(\sqrt{\frac{\left(u_{lm\mu}\right)^2 + \left(v_{lm\mu}\right)^2}{1 + \left(u_{lm\mu}\right)^2\left(v_{lm\mu}\right)^2}}, \sqrt{\frac{\left(1 - \left(u_{lm\mu}\right)^2\right)\left(1 - \left(v_{lm\mu}\right)^2\right)}{1 + \left(u_{lm\mu}\right)^2\left(v_{lm\mu}\right)^2}} \right)$$

$$= \left(u_{lm\mu}, \sqrt{1 - \left(u_{lm\mu}\right)^2}\right) \oplus_S \left(v_{lm\mu}, \sqrt{1 - \left(v_{lm\mu}\right)^2}\right)$$

$= \square U \oplus_S \square V = \mathbf{R.H.S}$

Thus $(i), (iv)$ holds.
$(ii), (iii)$ It can be proved similarly.

Theorem 3.8

Let $U = \left(\langle u_{lm\mu}, u_{lmv}\rangle\right)$ *and* $V = \left(\langle v_{lm\mu}, v_{lmv}\rangle\right)$ be two PFMs of same size, then

$(\boldsymbol{i})$ $\square(U^C \vee V^C) = \square U^C \vee \square V^C$

$(\boldsymbol{iii})$ $\square(U^C \wedge V^C) = \square U^C \wedge \square V^C$

$(\boldsymbol{iii})$ $\Diamond (U^C \vee V^C) = \Diamond U^C \vee \Diamond V^C$

$(\boldsymbol{iv})$ $\Diamond (U^C \wedge V^C) = \Diamond U^C \wedge \Diamond V^C$

Proof:

$(\boldsymbol{i})$ $\mathbf{L.H.S} = \square(U^C \vee V^C)$

$= \square\left(\max(u_{lmv}, v_{lmv}), \min(u_{lm\mu}, v_{lm\mu})\right)$

$= \left(\max(u_{lmv}, v_{lmv}), \sqrt{1 - (\max(u_{lmv}, v_{lmv}))^2}\right)$

$= \left(\max(u_{lmv}, v_{lmv}), \min\left(\sqrt{1 - (u_{lmv})^2}, \sqrt{1 - (v_{lmv})^2}\right)\right)$

$= \left(\square(u_{lmv}, u_{lm\mu})\right) \vee \left(\square(v_{lmv}, v_{lm\mu})\right)$

$= \square U^C \vee \square V^C = \mathbf{R.H.S}$

$(\boldsymbol{iv})$ $\mathbf{L.H.S} = \Diamond (U^C \wedge V^C)$

$= \Diamond \left(\min(u_{lmv}, v_{lmv}), \max(u_{lm\mu}, v_{lm\mu})\right)$

$= \left(\sqrt{1 - \left(\max(u_{lm\mu}, v_{lm\mu})\right)^2}, \max(u_{lm\mu}, v_{lm\mu})\right)$

$= \left(\min\left(\sqrt{1 - \left(u_{lm\mu}\right)^2}, \sqrt{1 - \left(v_{lm\mu}\right)^2}\right), \max(u_{lm\mu}, v_{lm\mu})\right)$

$= \left(\Diamond (u_{lmv}, u_{lm\mu})\right) \wedge \left(\Diamond (v_{lmv}, v_{lm\mu})\right)$

$= \Diamond U^C \wedge \Diamond V^C = \mathbf{R.H.S}$

Thus $(i), (iv)$ holds.
$(ii), (iii)$ It can be proved similarly.
We can prove the following corollaries using the above theorems

Corollary 3.9
Let $U = \left(\langle u_{lm\mu}, u_{lmv}\rangle\right) and\, V = \left(\langle v_{lm\mu}, v_{lmv}\rangle\right)$ be two EPFMs of same size, then
$(\boldsymbol{i})$ $\Box(U^C \vee V^C) = \Box U^C \vee \Box V^C = (\Diamond U)^C \vee (\Diamond V)^C$

$(\boldsymbol{ii})$ $\Box(U^C \wedge V^C) = \Box U^C \wedge \Box V^C = (\Diamond U)^C \wedge (\Diamond V)^C$

$(\boldsymbol{iii})$ $\Diamond (U^C \vee V^C) = \Diamond U^C \vee \Diamond V^C = (\Box U)^C \vee (\Box V)^C$

$(\boldsymbol{iv})$ $\Diamond (U^C \wedge V^C) = \Diamond U^C \wedge \Diamond V^C = (\Box U)^C \wedge (\Box V)^C$

Lemma3.10
Let $U = \left(\langle u_{lm\mu}, u_{lmv}\rangle\right) and\, V = \left(\langle v_{lm\mu}, v_{lmv}\rangle\right)$ be two EPFMs of same size, then
$(\boldsymbol{i})$ $\Box(U \vee V) = \Box U \vee \Box V$

$(\boldsymbol{ii})$ $\Box(U \wedge V) = \Box U \wedge \Box V$

$(\boldsymbol{iii})$ $\Diamond (U \vee V) = \Diamond U \vee \Diamond V$

$(\boldsymbol{iv})$ $\Diamond (U \wedge V) = \Diamond U \wedge \Diamond V$

Numerical Verifications
Example 4.1
Let $U \in PFS(x)$ for $x = \{e, f, g, h\}$. Suppose,

$$U = \begin{bmatrix} e(0.8,0.3) & f(0.6,0.5) \\ g(0.5,0.7) & h(0.4,0.6) \end{bmatrix} \text{ and } V = \begin{bmatrix} e(0.9,0.2) & f(0.7,0.4) \\ g(0.8,0.5) & h(0.3,0.1) \end{bmatrix}$$

Then we have,

$$\Box U = \begin{bmatrix} e(0.8,0.6) & f(0.6,0.8) \\ g(0.5,0.866025403) & h(0.4,0.916515139) \end{bmatrix}$$

$$\Box V = \begin{bmatrix} e(0.9,0.435889894) & f(0.7,0.714142842) \\ g(0.8,0.6) & h(0.3,0.953939201) \end{bmatrix}$$

$$\Diamond U = \begin{bmatrix} e(0.953939201,0.3) & f(0.866025403,0.5) \\ g(0.714142842,0.7) & h(0.8,0.6) \end{bmatrix}$$

$$\Diamond V = \begin{bmatrix} e(0.979795897,0.2) & f(0.916515139,0.4) \\ g(0.866025403,0.5) & h(0.994987437,0.1) \end{bmatrix}$$

$(\boldsymbol{i})$ $\Box(U \oplus_S V) = \Box U \oplus_S \Box V$

$$= \begin{bmatrix} e(0.977216752,0.212243774) & f(0.850025501, 0.526741537) \\ g(0.875923158,0.482450641) & h(0.496438419,0.868071941) \end{bmatrix}$$

$(\boldsymbol{ii})$ $\Diamond (U \oplus_S V) = \Diamond U \oplus_S \Diamond V$

$$= \begin{bmatrix} e(0.99903882,0.043834172) & f(0.987653847,0.156652093) \\ g(0.954668741,0.297670278) & h(0.998897531,0.046943823) \end{bmatrix}$$

$(\boldsymbol{iii})$ $\Box(U \odot_M V) = \Box U \odot_M \Box V$

$= \begin{bmatrix} e(0.696571267,0.717487609) & f(0.364680073,0.931132881) \\ g(0.354942603,0.934888093) & h(0.090340553,0.995910932) \end{bmatrix}$

$(\boldsymbol{iv})\ \Diamond\,(U \odot_M V) = \Diamond U \odot_M \Diamond V$

$= \begin{bmatrix} e(0.932987846,0.359907875) & f(0.778311782,0.627877989) \\ g(0.583744155,0.811937658) & h(0.794561024,0.607184303) \end{bmatrix}$

$(\boldsymbol{v})\ \Box(\Box U) = \begin{bmatrix} e(0.8,0.6) & f(0.6,0.8) \\ g(0.5,0.866025403) & h(0.4,0.916515139) \end{bmatrix} = \Box U = \Diamond(\Box U)$

$(\boldsymbol{vi})(\Diamond U) = \begin{bmatrix} e(0.953939201,0.3) & f(0.866025403,0.5) \\ g(0.714142842,0.7) & h(0.8,0.6) \end{bmatrix} = \Diamond U = \Diamond(\Diamond U)$

Example 4.2

Let $U \in PFS(x)$ for $x = \{e, f, g, h\}$. Suppose,

$U^C = \begin{bmatrix} e(0.3,0.8) & f(0.5,0.6) \\ g(0.7,0.5) & h(0.6,0.4) \end{bmatrix}$ and $V^C = \begin{bmatrix} e(0.2,0.9) & f(0.4,0.7) \\ g(0.5,0.8) & h(0.1,0.3) \end{bmatrix}$

Then we have,

$(\boldsymbol{i})(\Box(U^C \oplus_S V^C))^C = \begin{bmatrix} e(0.932987846,0.359907875) & f(0.778311782,0.627877989) \\ g(0.583744155,0.811937658) & h(0.794561024,0.607184303) \end{bmatrix} = \Diamond U \odot_M \Diamond V$

$(\boldsymbol{ii})(\Box(U^C \odot_M V^C))^C = \begin{bmatrix} e(0.99903882,0.043834172) & f(0.987653847,0.156652093) \\ g(0.954668741,0.297670278) & h(0.998897531,0.046943823) \end{bmatrix} = \Diamond U \oplus_S \Diamond V$

$(\boldsymbol{iii})\left(\Diamond\,(U^C \oplus_S V^C)\right)^C = \begin{bmatrix} e(0.696571267,0.717487609) & f(0.364680073,0.931132881) \\ g(0.354942603,0.934888093) & h(0.090340553,0.995910932) \end{bmatrix} = \Box U \odot_M \Box V$

$(\boldsymbol{iv})\left(\Diamond\,(U^C \odot_M V^C)\right)^C = \begin{bmatrix} e(0.977216752,0.212243774) & f(0.850025501,0.526741537) \\ g(0.875923158,0.482450641) & h(0.496438419,0.868071941) \end{bmatrix} = \Box U \oplus_S \Box V$

Example 4.3

Let $U \in PFS(x)$ for $x = \{e, f, g, h\}$. Suppose,

$U^C = \begin{bmatrix} e(0.3,0.8) & f(0.5,0.6) \\ g(0.7,0.5) & h(0.6,0.4) \end{bmatrix}$ and $V^C = \begin{bmatrix} e(0.2,0.9) & f(0.4,0.7) \\ g(0.5,0.8) & h(0.1,0.3) \end{bmatrix}$

Then we have,

$(\boldsymbol{i})(\Box U^C)^C = \begin{bmatrix} e(0.953939201,0.3) & f(0.866025403,0.5) \\ g(0.714142842,0.7) & h(0.8,0.6) \end{bmatrix} = \Diamond U$

$(\boldsymbol{ii})(\Diamond U^C)^C = \begin{bmatrix} e(0.8,0.6) & f(0.6,0.8) \\ g(0.5,0.866025403) & h(0.4,0.916515139) \end{bmatrix} = \Box U$

$(\boldsymbol{iii})\left(\Box(\Box U^C)\right)^C = \begin{bmatrix} e(0.953939201,0.3) & f(0.866025403,0.5) \\ g(0.714142842,0.7) & h(0.8,0.6) \end{bmatrix} = \Diamond U$

$(\boldsymbol{iv})\left(\Box(\Diamond U^C)\right)^C = \begin{bmatrix} e(0.8,0.6) & f(0.6,0.8) \\ g(0.5,0.866025403) & h(0.4,0.916515139) \end{bmatrix} = \Box U$

$(\boldsymbol{v})\left(\Diamond\,(\Box U^C)\right)^C = \begin{bmatrix} e(0.953939201,0.3) & f(0.866025403,0.5) \\ g(0.714142842,0.7) & h(0.8,0.6) \end{bmatrix} = \Diamond U$

$(\boldsymbol{vi})\left(\Diamond\left(\Diamond U^C\right)\right)^C = \begin{bmatrix} e(0.8,0.6) & f(0.6,0.8) \\ g(0.5,0.866025403) & h(0.4,0.916515139) \end{bmatrix} = \Box U$

$(\boldsymbol{i})(\Box U^C)^C = \left(\Box(\Box U^C)\right)^C = \left(\Diamond(\Box U^C)\right)^C = \begin{bmatrix} e(0.953939201,0.3) & f(0.866025403,0.5) \\ g(0.714142842,0.7) & h(0.8,0.6) \end{bmatrix} = \Diamond U$

$(\boldsymbol{ii})(\Diamond U^C)^C = \left(\Box(\Diamond U^C)\right)^C = \left(\Diamond(\Diamond U^C)\right)^C \begin{bmatrix} e(0.8,0.6) & f(0.6,0.8) \\ g(0.5,0.866025403) & h(0.4,0.916515139) \end{bmatrix} = \Box U$

Example 4.4

Let $U \in PFS(x)$ for $x = \{e, f, g, h\}$. Suppose,

$U = \begin{bmatrix} e(0.8,0.3) & f(0.6,0.5) \\ g(0.5,0.7) & h(0.4,0.6) \end{bmatrix}$ and $U = \begin{bmatrix} e(0.9,0.2) & f(0.7,0.4) \\ g(0.8,0.5) & h(0.3,0.1) \end{bmatrix}$

$U^C = \begin{bmatrix} e(0.3,0.8) & f(0.5,0.6) \\ g(0.7,0.5) & h(0.6,0.4) \end{bmatrix}$ and $V^C = \begin{bmatrix} e(0.2,0.9) & f(0.4,0.7) \\ g(0.5,0.8) & h(0.1,0.3) \end{bmatrix}$

Then we have,

$(U \vee V) = \begin{bmatrix} e(0.9,0.2) & f(0.7,0.4) \\ g(0.8,0.5) & h(0.6,0.3) \end{bmatrix}$

$(U \wedge V) = \begin{bmatrix} e(0.8,0.3) & f(0.6,0.5) \\ g(0.5,0.7) & h(0.1,0.4) \end{bmatrix}$

$(U^C \vee V^C) = \begin{bmatrix} e(0.3,0.8) & f(0.5,0.6) \\ g(0.7,0.5) & h(0.4,0.1) \end{bmatrix}$

$(U^C \wedge V^C) = \begin{bmatrix} e(0.2,0.9) & f(0.4,0.7) \\ g(0.5,0.8) & h(0.3,0.6) \end{bmatrix}$

$(\boldsymbol{i})\ \Box(U \vee V) = \begin{bmatrix} e(0.9,0.435889894) & f(0.7,0.714142842) \\ g(0.8,0.6) & h(0.6,0.8) \end{bmatrix} = \Box U \vee \Box V = \left(\Diamond (U \vee V)^{\boldsymbol{c}}\right)^{\boldsymbol{c}}$

$(\boldsymbol{ii})\Box(U \wedge V) = \begin{bmatrix} e(0.8,0.6) & f(0.6,0.8) \\ g(0.5,0.866025403) & h(0.1,0.994987437) \end{bmatrix} = \Box U \wedge \Box V = \left(\Diamond (U \wedge V)^{\boldsymbol{c}}\right)^{\boldsymbol{c}}$

$(\boldsymbol{iii})\ \Diamond (U \vee V) = \begin{bmatrix} e(0.979795897,0.2) & f(0.916515139,0.4) \\ g(0.866025403,0.5) & h(0.953939201,0.3) \end{bmatrix} = \Diamond U \vee \Diamond V = \left(\Box (U \vee V)^{\boldsymbol{c}}\right)^{\boldsymbol{c}}$

$(\boldsymbol{iv})\ \Diamond (U \wedge V) = \begin{bmatrix} e(0.953939201,0.3) & f(0.866025403,0.5) \\ g(0.714142842,0.7) & h(0.916515139,0.4) \end{bmatrix} = \Diamond U \wedge \Diamond V = \left(\Box (U \wedge V)^{\boldsymbol{c}}\right)^{\boldsymbol{c}}$

$(\boldsymbol{v})\ \Box(U^C \vee V^C) = \begin{bmatrix} e(0.3,0.953939201) & f(0.5,0.866025403) \\ g(0.7,0.714142842) & h(0.4,0.916515139) \end{bmatrix} = \Box U^C \vee \Box V^C = (\Diamond U)^C \vee (\Diamond V)^C$

$(\boldsymbol{vi})(U^C \wedge V^C) = \begin{bmatrix} e(0.2,0.979795897) & f(0.4,0.916515139) \\ g(0.5,0.866025403) & h(0.3,0.953939201) \end{bmatrix} = \Box U^C \wedge \Box V^C = (\Diamond U)^C \wedge (\Diamond V)^C$

$(\boldsymbol{vii})\ \Diamond (U^C \vee V^C) = \begin{bmatrix} e(0.6,0.8) & f(0.8,0.6) \\ g(0.866025403,0.5) & h(0.994987437,0.1) \end{bmatrix} = \Diamond U^C \vee \Diamond V^C = (\Box U)^C \vee (\Box V)^C$

$(\boldsymbol{viii})\ \Diamond\ (U^C \wedge V^C) = \begin{bmatrix} e(0.435889894,0.9) & f(0.714142842,0.7) \\ g(0.6,0.8) & h(0.8,0.6) \end{bmatrix} = \Diamond\ U^C \wedge \Diamond\ V^C = (\Box U)^C \wedge (\Box V)^C$

CONCLUSION

In this paper, we proposed two modal operators, which are analogous to Modal Operators necessity and possibility on Einstein Operations of Pythagorean Fuzzy Matrix, which provide a good complement to the existing operations on PFMs. The properties of these operations are investigated and Some relations are solved with numerical verifications.

REFERENCES

1. K. Atanassov, Intuitionistic fuzzy set, *Fuzzy Sets and Systems*, 20 (1986) 87-96.
2. K. Atanassov, More on Intuitionistic fuzzy set, *Fuzzy Sets and Systems*, 33 (1989) 37-46.
3. J. Boobalan and S. Sriram, Arithmetic operations on intuitionistic fuzzy matrices, Proceedings of the International Conference On Mathematical Sciences, Sathyabama University (2014) 484- 487.
4. S. K. Khan and M. Pal, "Some operations on Intuitionistic Fuzzy Matrices", ActaCiencialndica, 32(2006) 515-524.
5. T. Muthuraji, S. Sriram, P. Murugadas, Decomposition of Intuitionistic fuzzy matrices, Fuzzy Information and Engineering, Vol.8(3) (2016) 345-354.
6. P. Murugadas, S.Sriram, T.Muthuraji, "Modal operators in intuitionistic fuzzy matrices", International Journal of Computer Application, 90(17)(2014) 1-4.
7. M. Pal, Intuitionistic fuzzy determinant, Vidyasagar University Journal of Physical Sciences, Vol.7 (2001) 87-93.
8. Pal M, Khan S.K, Shyamal A.K., "Intuitionistic fuzzy matrices", Notes on Intuitionistic Fuzzy sets. 2002; 8(2): 51-62.
9. M. Ramakrishnan and S.Sriram, Two new operations on intuitionistic fuzzy matrices, AIP Conference Proceedings 2177, 020080 (2019),
10. T.M. Selvarajan, R.S. Ramya, "Einstein Operations on Pythagorean Fuzzy Matrices", Advances in Mathematics: Scientific Journal 8 (2019), no.3, 634–640.
11. Silambaran. I., Sriram. S. (2018) "Algebraic operations on Pythagorean Fuzzy matrices", Mathematical Sciences International Research Journal, Vol.7(2), 406 -414
12. A.K. Shyamal and M. Pal, "Distances between intuitionistic fuzzy matrices", V.U.J. Physical Sciences, 8 (2002) 81-91.
13. M.G. Thomason, Convergence of powers of a fuzzy matrix, Journal of Mathematical Analysis and Applications, Vol.57 (2) (1977) 476-480.
14. R. R. Yager, "Pythagorean fuzzy subsets". In: Proc Joint IFSA World Congress and NAFIPS Annual Meeting, Edmonton, Canada, (2013) 57-61. 23.
15. R. R. Yager, "Pythagorean membership grades in multi-criteria decision making", IEEE Transactions on Fuzzy Systems, 22 (2014) 958-965.
16. R. R. Yager and A. M. Abbasov, "Pythagorean membership grades, complex numbers, and decision making", International Journal of Intelligent Systems, 28 (2013) 436-452. 24.
17. L.A. Zadeh, Fuzzy sets, Journal of Information and control 8 (1965) 338-353.

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REVIEW ARTICLE

Deprotection Reactions using Cerium (IV) Ammonium Nitrate in Organic Synthesis: A Short Review

Mrityunjoy Datta*

Assistant Professor of Chemistry, Sarojini Naidu College for Women, 30 Jessore Road, Kolkata, India

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***Address for Correspondence**
Mrityunjoy Datta
Assistant Professor of Chemistry,
Sarojini Naidu College for Women,
30 Jessore Road, Kolkata, India
Email: mdattap@rediffmail.com

ABSTRACT

Ceric ammonium nitrate (CAN) with its low toxicity, inexpensive, air and moisture stable properties has attracted organic scientists by widespread applications in organic synthesis. Particularly, high reduction potential, Lewis acid nature and especially its role as a single-electron transfer oxidant make this reagent very much attractive towards organic chemists. With these advantages, this review summarizes the genesis and development of a general and selective deprotection methodology using cerium(IV) ammonium nitrate (CAN) in organic synthesis.

Keywords: Ceric ammonium nitrate (CAN), organic synthesis, deprotection, protection, oxidant.

INTRODUCTION

The application of lanthanide reagents in organic chemistry is receiving increased attention in recent years. A considerable number of efficient and selective synthetic protocols have been developed by the effective utilization of these reagents. Many of these synthetic transformations are based on cerium(IV) – promoted reactions, particularly those involving cerium(IV) ammonium nitrate (CAN). The versatile use of CAN, $(NH_4)_2Ce(NO_3)_6$, in organic chemistry as a one-electron oxidant [1]. May be attributed to its large reduction potential value (+1.61 V vs normal hydrogen electrode) and thus makes Ce(IV) a very efficient oxidizing reagent as compared to other metal ions. The most common oxidation state of lanthanides are +3 state whereas cerium shows +3 and +4 oxidation state of which Ce^{+4} has the extra stability due to presence of its vacant f shell. Electronic configuration of cerium in its ground state is $[Xe]4f^26s^2$ ([Xe] = configuration of xenon). Thus electronic configuration of Ce^{+3} and Ce^{+4} ions are $[Xe]4f^1$ and $[Xe]4f^0$ respectively. Because of its large positive charge, the similarity between Ce(IV) and an electrophlic reagent in their reactions towards organic substrates is quite expected. CAN has the additional advantages like low toxicity, inexpensive, reasonably soluble in many organic media, air stable, easily handled and thus allowing for a considerable degree of experimental simplicity. Investigations in a number of laboratories, have shown that cerium(IV) ammonium nitrate (CAN) is a convenient and excellent reagent for effecting a wide array of synthetic

transformations [2]. Since CAN is capable of effecting only one-electron changes, it is being increasing used for radical coupling reactions, particularly for C-C bond formation reactions [2c]. A review [2e] focusing on deprotection methodoloy using cerium(IV) ammonium nitrate as catalyst has also been included in the literature. The purpose of the present review is to provide a comprehensive account of the deprotection reactions in synthetic organic chemistry using CAN.

RESULTS AND DISCUSSION

It is true when studying multistep total synthesis to achieve a target molecule, protection and deprotection methodologies are often unavoidable [3]. There are so many examples in the literature where interesting synthetic targets could not be attained, either because the protective groups that were employed stubbornly resisted cleavage or the deprotection conditions that were required were too harsh for advanced, sensitive synthetic intermediates. As a result, a number of new methods for the cleavage of commonly employed protective groups appear regularly in the literature [4]. Catalytic deprotection of a range of functionalized tetrahydropyranyl (THP) and tetrahydrofuranyl (THF) ethers **1** has efficiently been performed to the corresponding alcohols **2** under neutral conditions using as little as 3 mol % CAN in MeCN / borate buffer (pH=8) (Scheme 1)[5]. It is noteworthy that under these conditions, no oxidation of benzylic alcohols, aromatic and even aliphatic sulfides is observed. Although a strong oxidant, the Ce (IV) reagent behaves solely as a powerful and highly selective Lewis acid in this case. Moreover, HNO_3/Silica gel supported CAN [6] has been utilized for the oxidative deprotection of benzylic tetrahydropyranyl ethers under solvent-free conditions using microwave irradiation. The reagent system, CAN / pyridine at pH 4.4 has been utilized as demasking agent for primary and (in some cases) secondary acetonides, benzylidenes, and tetrahydropyranyl ethers [7]. Under these conditions, Lewis acidity of cerium was found to play a prominent role in the selective deprotection of several acetonides **3** in the presence of other commonly used hydroxyl protecting groups(Scheme 2).
In 2007, CAN catalyzed chemoselective deprotection of acetonides 4 (Scheme 3) achieved by Roy group in excellent yields at room temperature [8]. It is noteworthy to mention that different sensitive functional groups such as OMs, OCH_2Ph, OTBDMS, double bond and triple bonds were remained unaffected under the reaction conditions. Not only acetonides but also cyclohexyl protected 1,2-diols 5 and 6 were also cleaved smoothly to furnish **7** and 8 respectively in good yields under same reaction conditions (Scheme 4).

In 2002, the same group also developed mild, efficient and chemoselective method for the construction of geminal diacetates 10 from both aromatic and aliphatic aldehydes 9 using acetic anhydride in the presence of a catalytic amount of CAN without using any solvent [9]. The ketones remained unaffected under the reaction conditions. The diacetates 10 were deprotected to the corresponding aldehydes 11 in excellent yields (86-91%) by treatment with CAN (10 mol%) in the presence of water in acetonitrile at 70°C for 4 to 7 hours (Scheme 5). It is noteworthy that the phenolic acetates remain untouched under the reaction conditions.In 2005, cleavage of acetals 12 to 4-oxo-4*H*-1-benzopyran-3-carbaldehydes 13 observed by Shindalkar group in aqueous acetonitrile (1:1) at 70 °C with catalytic amount of CAN in good yields (Scheme 6) [10]. CAN has also been used as a powerful reagent for the efficient and rapid deprotection of cyclic acetals and ketals 14 to the corresponding carbonyl compounds 15 (Scheme 7) [11]. Reaction of N-BOC amino acids 16 with CAN in an alcohol as the solvent at room temperature resulted in the esterification of N-BOC amino acids 17 with retention of BOC group [12]. When the reaction was conducted at reflux temperature, esterification was accompanied with simultaneous removal of the BOC group yielded 18. Both reactions gave the desired products in good yields (Scheme 8). In 2000, Hwu et al. reported that CAN adsorbed on silica gel (SiO_2/CAN) efficiently removed the Tr (trityl), MMTr (monomethoxytrityl), DMTr (dimethoxytrityl), *tert*-butyldimethylsilyl (TBDMS) (Table 1), and triisopropylsilyl (TIPS) (Table 2) functionalities from a variety of polyprotected ribonucleoside substrates 19 and 20, rapidly converting them to the parent monodeprotected alcohols [13]. Comparison of the results obtained from detritylation in the presence and in the absence of silica gel as solid support suggests that the above reactions were much faster than those performed with CAN alone. In this regard, quite interesting is the comparison of activity of CAN and CAN/SiO_2 with the substrate 21. The Tr group was selectively removed by treatment of 21 with CAN, giving 22, whereas the isopropylidene group was cleaved when

CAN/SiO_2 was utilized, generating product 23 (Scheme 9). Deprotection of tritylated amines 24 to the corresponding amines 25 has been demonstrated by Sinha group in 2011 with 20 mol % ceric ammonium nitrate, 10 equiv of acetic acid and 15 equiv of water in dichloromethane at room temperature (Scheme 10) [14]. This method also worked well in morpholino nucleosides. In 2001, Gordon group observed that benzyloxyaniline linker 26 that uses CAN as a cleavage reagent [15] has been developed for the synthesis and release of secondary amides, including β-lactams. Cleavage of the linker 27 with CAN occurred rapidly (< 0.5 h) in mild conditions (aq. CH_3CN, rt) and compounds 28, 29 and 30 of high purity (typically > 90%) were obtained after a simple extraction and filtration protocol (Scheme 11). In 2000, Davies et al. described that tertiary amines containing one or more N-benzyl protecting groups 31 with aqueous CAN resulted N-debenzylation to gave the corresponding secondary amines 32 with complete chemoselectivity (Scheme 12) [16]. Jarrahpour et.al reported in 2008 that silica gel supported ceric ammonium nitrate (CAN–SiO_2) as an efficient reagent for N-dearylation of *N*-(*p*-methoxyphenyl) and *N*-(*p*-ethoxyphenyl) groups from 2-azitidinones 33, commonly known as β-lactams in solutions and 'on column' reactions with good to excellent yields (Scheme 13) [17]. It is noteworthy that in the 'on-column' method, both N-dearylation and purification were happened at the same time. The same group also observed in 2012 that treatment of N-alkoxyphenyl-β-lactams such as *N*-(2,4-dimethoxyphenyl)-β-lactams 34 and *N*-(3,4-dimethoxyphenyl)-β-lactams 35 with 'on column type B' (10% CAN-SiO_2, 15 min) afforded the pure corresponding NH-β-lactams in excellent yields (80-85%) (Scheme 14) [18]. The resulted benzoquinone derivatives were trapped in the trapping zone (Na_2SO_3-SiO_2).

The type B column was packed from the bottom: a little silica gel (~1 cm), 10% SiO_2-Na_2SO_3, 10% CAN-SiO_2, and a little silica gel. 10% CAN-SiO_2 zone is called *N*-dearylation zone and 10% SiO_2-Na_2SO_3 is called trapping zone. 2-Azitidinones 36 containing the 4-methoxynaphthyl group on N1 position which is very similar to 4-methoxyphenyl group was cleaved to the corresponding β-lactams and naphthaquinone by 'on column type B' (Scheme 15). It has been found that 4-methoxynaphthyl group was oxidized better than the 4-methoxyphenyl group.In addition, N-dearylation of N-(alkoxybenzyl)-2-azitidinons like *N*-(*p*-methoxybenzyl)-β -lactams 37 using ceric ammonium nitrate in aqueous acetonitrile has also been reported [19] along with on column technology [17]. *p*-Methoxybenzaldehyde is the byproduct of this reaction (Scheme 16). In 2019, chemoselective removal of p-methoxybenzyl (PMB) group in the presence of the p-methylbenzyl (MBn) group 38 from the corresponding alcohols observed using CAN in acetonitrile-water by Ikeuchi et al. (Scheme 17) [20]. It was noted that the reaction was sluggish for the deprotection of MBn group alone. The authors also found selective oxidative deprotection of 2-naphthylmethyl (NAP) group in compound 39 bearing the Mbn group. Highly selective deprotected compound 40 was obtained along with 43% overall yield of 40 and 41 using 3.3 equivalent of CAN (Scheme 18).

CONCLUSION

The use of CAN for the deprotection of commonly employed protecting groups no longer appears to be a synthetic curiosity. Rather, it has become a powerful methodology bearing a number of advantages over standard procedures. Selectivity and the simplicity of the experimental procedures using CAN is very much attractive to the organic chemist, particularly in the total synthesis of complex and sensitive natural products. The low cost and air stability of CAN may make it a useful alternative to the expensive, hygroscopic lanthanide triflates. I hope that this review will serve to stimulate research in this fascinating and very useful area of organic synthesis.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

REFERENCES

1. T. L. Ho, "Ceric Ion Oxidation in Organic Chemistry," *Synthesis*, no. 6, pp. 347-354, 1973.
2. For reviews on CAN-mediated reactions, see: (a) T. L. Ho, "Organic Synthesis by Oxidation with Metal Compounds," Plenum Press, New York, 1986 and references therein. (b) T. Imamoto, "Lanthanide Reagents in Organic Synthesis," Academic Press, London, p 119, 1994. (c) V. Nair, J .Mathew and J. Prabhakaran, "Carbon–carbon bond forming reactions mediated by cerium(IV) reagents," *Chemical Society Reviews*, vol. 26, no. 2, pp. 127-132, 1997. (d) J. R. Hwu and K. Y. King, "Versatile reagent ceric ammonium nitrate in modern chemical synthesis," *Current Science*, vol. 81, no. 8, pp. 1043-1053, 2001. (e) N. Maulide, J.-C. Vanherck, A. Gautier and I. E. Markó, "Mild and Neutral Deprotections Catalyzed by Cerium(IV) Ammonium Nitrate," *Accounts of Chemical Reserch*, vol. 40, no. 6, pp. 381-392, 2007.
3. For beautiful illustrations of the skillful use of protecting groups in synthesis, see: (a) K. C. Nicolaou and E. J. Sorensen, "Classics in Total Synthesis," VCH: Weinheim, Germany, 1996. (b) K. C. Nicolaou and S. A. Snyder, "Classics in Total Synthesis II. VCH: Weinheim, Germany, 2003.
4. (a) P. J. Kocienski, "Protecting Groups," Georg Thieme: New York, 1994. (b) T. W. Greene and P. G. M. Wuts, "Protecting Groups in Organic Chemistry," Wiley: New York, 1991.
5. I. E. Markó, A. Ates, B. Augustyns, A. Gautier, Y. Quesnel, L. Turet and M. Wiaux, "Remarkable deprotection of THP and THF ethers catalysed by cerium ammonium nitrate (CAN) under neutral conditions," *Tetrahedron Letters*, vol. 40, no. 30, pp. 5613-5616, 1999.
6. M. M. Heravia, P. Kazemian, H. A. Oskooie and M. Ghassemzadeh, "HNO_3/Silica gel supported CAN; oxidative deprotection of benzylic tetrahydropyranyl ethers under solvent-free conditions using microwaves," *Journal of Chemical Reserch*, pp. 105-106, 2005.
7. G. Barone, E. Bedini, A. Iadonisi, E. Manzo and M. Parrilli, "Ceric Ammonium Nitrate/Pyridine: A Mild Reagent for the Selective Deprotection of Cyclic Acetals and Ketals in the Presence of Acid Labile Protecting Groups," *Synlett*, no. 10, pp. 1645-1648, 2002.
8. S. Jana and S. C. Roy, "Ce(IV) ammonium nitrate catalyzed chemoselective deprotection of acetonides, *Indian Journal of Chemistry*, vol. 46B, pp. 707-709, 2007.
9. S. C. Roy and B. Banerjee, "A Mild and Efficient Method for the Chemoselective Synthesis of Acylals from Aldehydes and their Deprotections Catalysed by Ceric Ammonium Nitrate," *Synlett,* no. 10, pp. 1677-1678, 2002.
10. S. S. Shindalkar, B. R. Madje, R. V. Hangarge and M. S. Shingare, "Ceric ammonium nitrate catalyzed efficient and chemoselective method for protection and deprotection of 4-oxo-4*H*-1-benzopyran-3-carbaldehydes," *Indian Journal of Chemistry*, vol. 44B, pp. 2409-2411, 2005.
11. (a) A. Ates, A. Gautier, J. M. Plancher, Y. Quesnel and I. E. Markó, "Remarkably Efficient Deprotection of Cyclic Acetals and Ketals, *Tetrahedron Letters*, vol. 40, no. 9, pp. 1799-1802, 1999. (b) V. Nair, L. G. Nair, L. Balagopal and R. Rajan, "An exceedingly mild and efficient CAN mediated method for the deprotection of acetals," *Indian Journal of Chemistry*, vol. 38B, pp. 1234-1236, 1999.
12. A. Kuttan, S. Nowshudin and M. N. A. Rao, "Ceric ammonium nitrate (CAN) mediated esterification of *N*-Boc amino acids allows either retention or removal of the *N*-Boc group," *Tetrahedron Letters*, vol. 45, no. 12, pp. 2663-2665, 2004.
13. J. R. Hwu, M. L. Jain, F. Y. Tsai, S. C. Tsay, A. Balakumar and G. H. Hakimelahi, "Ceric Ammonium Nitrate on Silica Gel for Efficient and Selective Removal of Trityl and Silyl Groups," *Journal of Organic Chemistry*, vol. 65, no. 17, pp. 5077-5088, 2000.
14. S. Pattanayak and S. Sinha, "Ceric ammonium nitrate-mediated detritylation of tritylated amines," *Tetrahedron Letters,* vol. 52, no. 1, pp. 34–37, 2011.
15. K. H. Gordon and S. Balasubramanian, "Exploring a Benzyloxyaniline Linker Utilizing Ceric Ammonium Nitrate (CAN) as a Cleavage Reagent: Solid-Phase Synthesis of N-Unsubstituted β-Lactams and Secondary Amides," *Organic Letters*, vol. 3, no. 1, pp. 53-56, 2001.

16. S. D. Bull, S. G. Davies, G. Fenton, A. W. Mulvaney, R. S. Prasad and A. D. Smith, "Chemoselective oxidative debenzylation of tertiary *N*-benzyl amines," Chemical Communications, no. 5, pp. 337-338, 2000.
17. A. Jarrahpour and M. Zarei, "From Solution-Phase to 'On-Column' N-Dearylation of b-Lactams by Silica-Supported Ceric Ammonium Nitrate (CAN–SiO_2)," *Synlett*, no. 3, pp. 381–385, 2008.
18. M. Zarei, A. Jarrahpour, E. Ebrahimi, M. Aye and S. A. T. Badrabady, "On-column N-dearylation of 2-azetidinones by silica-supported ceric ammonium nitrate," *Tetrahedron*, vol. 68, no. 27-28, pp. 5505-5512, 2012.
19. (a) N. Boyer, P. Gloanec, G. D. Nanteuil, P. Jubault and J. C. Quirion, "Synthesis of α,α-Difluoro-β-amino Esters or *gem*-Difluoro-β-lactams as Potential Metallocarboxypeptidase Inhibitors," *Europian Journal of Organic Chemistry*, no. 25, pp. 4277-4295, 2008. (b) H. Ishibashi, C. Kameoka, H. Iriyama, K. Kodama, T. Sato and M. Ikeda, "Sulfur-Directed Regioselective Radical Cyclization Leading to .beta.-Lactams: Formal Synthesis of (.+-.)-PS-5 and (+)-Thienamycin. *Journal of Organic Chemistry*, vol. 60, no. 5, pp. 1276-1284, 1995. (c) X. G Li, M. Lahitie, M. Paivio and L. T. Kanerva, "Enantioselective acylation of alcohols with fluorinated β-phenyl-β-lactams in the presence of *Burkholderia cepacia* lipase," *Tetrahedron: Asymmetry*, vol. 18, no. 13, pp. 1567-1573, 2007.
20. K. Ikeuchi, K. Murasawa, K. Ohara and H. Yamada, "*p*-Methylbenzyl Group: Oxidative Removal and Orthogonal Alcohol Deprotection," *Organic Letters*, vol. 21, no. 17, pp. 6638–6642, 2019.

Table 1:Selective removal of TBDMS group in polyprotected compounds

	R^1	R^2	B	Yield (%)
19a	H	$SiMe_2Bu^t$	adenin-9-yl	87
19b	$SiMe_2Bu^t$	H	adenin-9-yl	85
19c	$SiMe_2Bu^t$	$SiMe_2Bu^t$	adenin-9-yl	85
19d	$SiMe_2Bu^t$	$SiMe_2Bu^t$	uracil-1-yl	93
19e	$SiMe_2Bu^t$	$SiMe_2Bu^t$	cytosin-1-yl	95

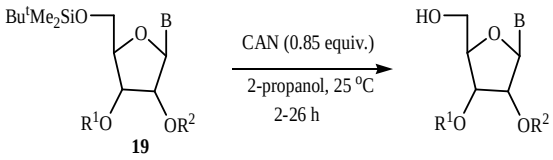
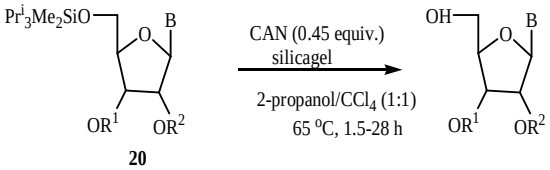
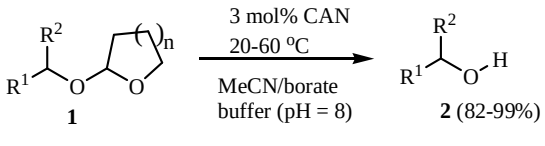


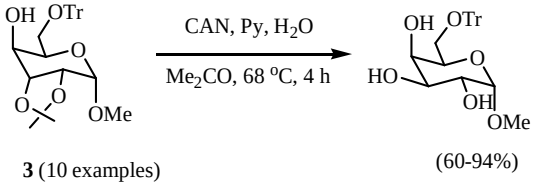
Table 2: Selective removal of TIPS group in polyprotected compounds

	R^1	R^2	B	Yield (%)
20a	$SiPr^i_3$	$SiPr^i_3$	uracil-1-yl	87
20b	H	$SiPr^i_3$	uracil-1-yl	91
20c	$SiPr^i_3$	H	uracil-1-yl	85
20d	H	$SiPr^i_3$	cytosine-1-yl	80
20e	$SiPr^i_3$	H	cytosine-1-yl	83

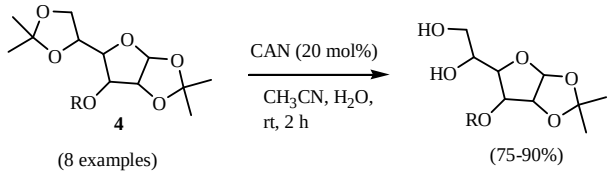




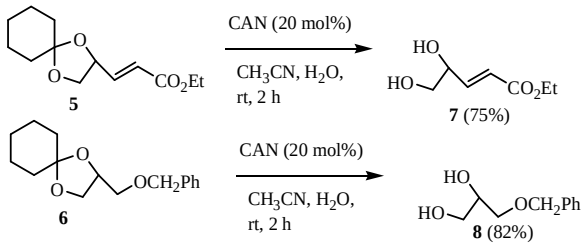
Scheme 1: Deprotection of THP and THF ethers.



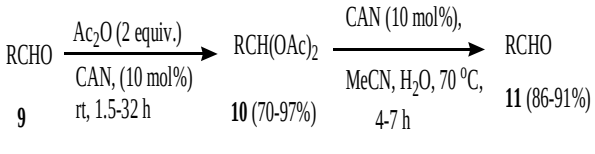
Scheme 2: Cleavage of acetonides using CAN/Py system.



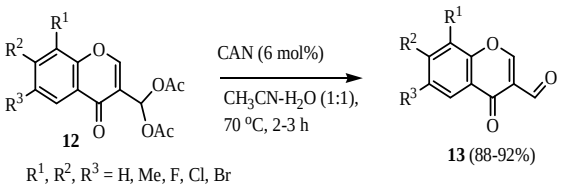
Scheme 3: CAN catalyzed chemoselective deprotection of acetonides



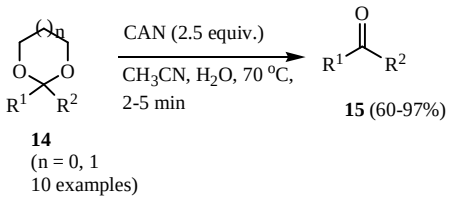
Scheme 4: Chemoselective deprotection of 1,2-diols catalyzed by CAN



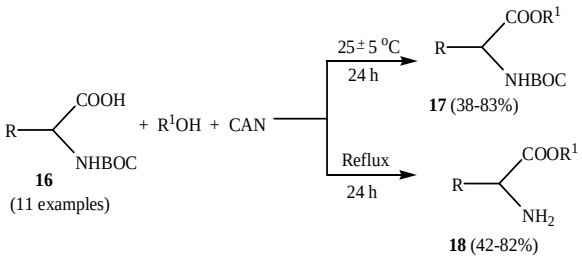
Scheme 5: Protection-deprotection of aldehydes using CAN.



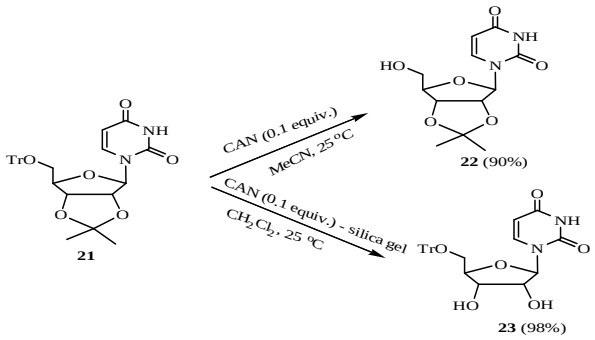
Scheme 6: CAN catalyzed cleavage of acetals.



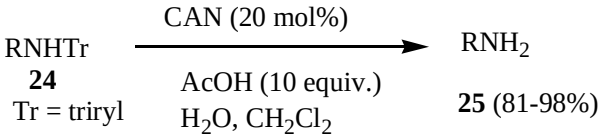
Scheme 7: Deprotection of cyclic acetals and ketals.



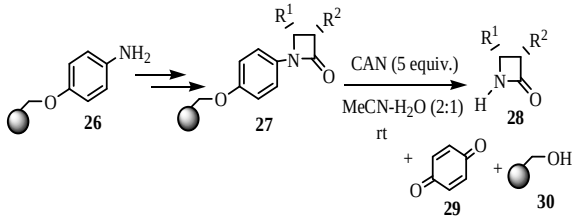
Scheme 8: Effect of CAN with BOC protected amino acids.



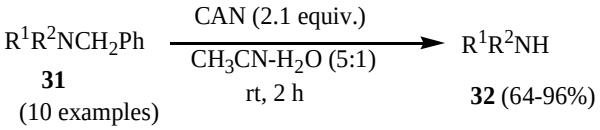
Scheme 9: Selective deprotection of trityl and isopropylidine group.



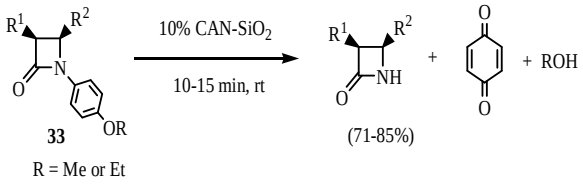
Scheme 10: Deprotection of tritylated amines.



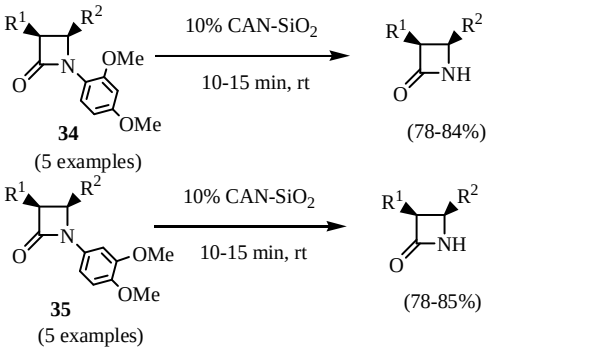
Scheme 11: Cleavage of benzyloxyaniline linker from β-lactams.



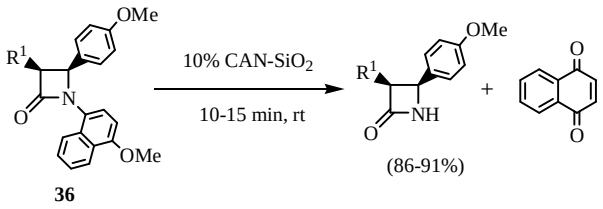
Scheme 12: Deprotection of N-benzyl group to amines.



Scheme 13: CAN-silica N-dearylation of β-lactams.



Scheme14: N-dearylation of β-lactams 34 and 35 with CAN-SiO2.



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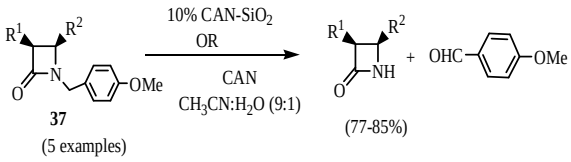
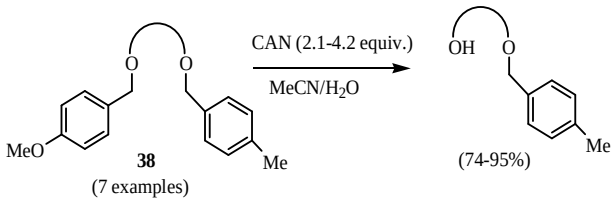
Scheme 15: Removal of 4-methoxynaphthyl group from N1 position of β-lactams by 'on column type B'



37

(5 examples)

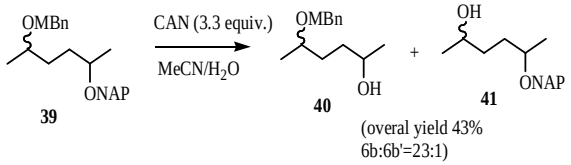
Scheme16: N-dearylation of *N*-(*p*-methoxybenzyl)-β –lactams 37



38

(7 examples)

Scheme 17: Selective PMB deprotection in the presence of the MBn group.



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Scheme 18. Selective NAP deprotection

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RESEARCH ARTICLE

A Few Kind of Continuous Functions in Binary Topological Spaces

P. Sathishmohan[1], K. Lavanya[2], V. Rajendran[1] and K. Rajalakshmi[3]

[1]Assistant Professor, Department of Mathematics, Kongunadu Arts and Science College (Autonomous), Coimbatore, Tamil Nadu, India.
[2]Research scholar, Department of Mathematics, Kongunadu Arts and Science College (Autonomous), Coimbatore, Tamil Nadu, India.
[3]Assistant Professor, Department of Science and Humanities, Sri Krishna College of Engineering and Technology, Coimbatore, Tamil Nadu, India.

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***Address for Correspondence**
P. Sathishmohan,
Assistant Professor,
Department of Mathematics,
Kongunadu Arts and Science College (Autonomous),
Coimbatore, Tamil Nadu, India.
Email: iiscsathish@yahoo.co.in

ABSTRACT

The aim of this paper is to introduce, the conception of binary generalized semi continuous and binary semi generalized continuous functions in binary topological spaces. Also, we define and study totally binary generalized semi continuous, strongly binary generalized semi continuous functions in binary topological spaces. Further, we have given an appropriate example to understand the abstract concepts clearly.

Keywords: b-gs-continuous, b-sg-continuous, totally b-gs-continuous, and strongly b-gs-continuous.

Mathematics Subject Classification: 54A05, 54C05, 54A99.

INTRODUCTION

In 2011, Nithyanantha Jothi and Thangavelu [1,4,2] introduced binary topology from X to Y. The authors introduced and investigated the concepts of binary closed, binary closure, binary interior, binary continuity, base and sub base of binary topological spaces. In a binary topological space $(X,Y,\mathcal{M})$ a set (A,B) is said to be binary semi open if there exists a binary open set (U,V) such that $(U,V) \subseteq (A,B) \subseteq$ b-cl(U,V), where b-cl(U,V) denotes the binary closure of (U,V) in (X,Y). The complement of a binary semi open set is called binary semi closed. A subset (A,B) of (X,Y) is said to be generalized binary closed if $\text{b-cl}(A,B) \subseteq (U,V)$ whenever $(A,B) \subseteq (U,V)$ and (U,V) is binary open. The

complement of generalized binary closed set is called generalized binary open. Recently, Sathishmohan et.al. [5] introduced and studied the concept of binary generalized semi closed sets and binary semi generalized closed sets, Consequently they[6]conceptualized binary generalized semi (binary semi generalized)-closure and binary generalized semi(binary semi generalized)-interior of a sets in binary topological spaces. The purpose of this paper is to introduce b-gs-continuous, b-sg-continuous in binary topological spaces. Also, we define and study totally binary generalized semi continuous, strongly binary generalized semi continuous functions in binary topological spaces and study some of their basic properties.

Definition 2.11. [3], Let f : Z ⟶ X × Y be a function. Let A ⊆ X and B ⊆ Y, we define
f^{-1}(A,B) = {z ∈ Z : f(z) = (x, y) ∈ (A, B)}.

Definition 2.12. Let (Z, τ) be a topological space and (X,Y,$\mathcal{M}$) be a binary topological space. Then the map
f : Z ⟶ X × Y is called
(1) binary continuous (generalized binary continuous) [3], if f^{-1}(A,B) is open (generalized open) in Z for every binary open set (A,B) in (X,Y,$\mathcal{M}$).
(2) binary semi continuous [4], if f^{-1}(A,B) is semi open in Z for every binary open set (A,B) in (X,Y,$\mathcal{M}$).
(3) totally binary continuous (totally binary semi continuous, strongly binary continuous, strongly binary semi continuous) [4], if f^{-1} (A,B) is clopen (semi clopen) in Z for every binary open set (binary set) (A,B) in (X,Y,$\mathcal{M}$).

b-gs(b-sg)-continuous functions

In this section, we initiate a new type of binary continuous functions in binary topological spaces called binary generalized semi continuous and binary semi generalized continuous functions and study some of their characterizations.

Definition 3.1. Let ($\mathcal{L}$,τ) be a topological space and ($\mathcal{K}$,$\mathcal{P}$,$\mathcal{M}$) be a binary topological space. Then the map $\mathcal{Q}$: $\mathcal{L}$⟶$\mathcal{K}$×$\mathcal{P}$ is called

- binary generalized semi continuous (briefly, b-gs-continuous), if $\mathcal{Q}^{-1}$($\mathcal{F}$,$\mathcal{G}$) is generalized semi open in $\mathcal{L}$ for every binary open set ($\mathcal{F}$,$\mathcal{G}$) in ($\mathcal{K}$,$\mathcal{P}$,$\mathcal{M}$).
- binary semi generalized continuous (briefly, b-sg-continuous), if $\mathcal{Q}^{-1}$($\mathcal{F}$,$\mathcal{G}$) is semi generalized open in $\mathcal{L}$ for every binary open set ($\mathcal{F}$,$\mathcal{G}$) in ($\mathcal{K}$,$\mathcal{P}$,$\mathcal{M}$).

Theorem 3.2.

1.Let $\mathcal{Q}$: $\mathcal{L}$⟶$\mathcal{K}$×$\mathcal{P}$ be binary continuous. Then $\mathcal{Q}$ is b-gs(b-sg)-continuous.
2.Let $\mathcal{Q}$: $\mathcal{L}$⟶$\mathcal{K}$×$\mathcal{P}$ be gb-continuous. Then $\mathcal{Q}$ is b-gs-continuous.
3.Let $\mathcal{Q}$: $\mathcal{L}$⟶$\mathcal{K}$×$\mathcal{P}$ be binary semi continuous. Then $\mathcal{Q}$ is b-gs-continuous.
4.Let $\mathcal{Q}$: $\mathcal{L}$⟶$\mathcal{K}$×$\mathcal{P}$ be binary semi continuous. Then $\mathcal{Q}$ is b-sg-continuous.

Proof: Let ($\mathcal{F}$,$\mathcal{G}$) be a binary open set in ($\mathcal{K}$,$\mathcal{P}$,$\mathcal{M}$). Since $\mathcal{Q}$ is binary continuous, we have $\mathcal{Q}^{-1}$($\mathcal{F}$,$\mathcal{G}$) is open in $\mathcal{L}$. Since every open set is gs(sg)-open in $\mathcal{L}$, then $\mathcal{Q}^{-1}$($\mathcal{F}$,$\mathcal{G}$) is gs(sg)-open in $\mathcal{L}$. Hence $\mathcal{Q}$ is b-gs(b-sg)-continuous.
Proof of (2) to (4) is obvious.
The converse of the above theorem need not be true as seen from the subsequent example.

Example 3.3. Let $\mathcal{K}$ = {α,β}, $\mathcal{P}$ = {α,β,γ} and $\mathcal{L}$ = {1,2,3}. Clearly $\mathcal{M}$ = {(∅,∅),({α}{α,β}), ($\mathcal{K}$,$\mathcal{P}$)} is a binary topology form $\mathcal{K}$ to $\mathcal{P}$ and τ = {∅, $\mathcal{L}$,{1}} is a topology on $\mathcal{L}$. Then closed subset in $\mathcal{L}$ are ∅,{2,3} and gs-open subset in $\mathcal{L}$ are ∅,$\mathcal{L}$,{1},{2},{3},{1,2},{1,3}. Define $\mathcal{Q}$: $\mathcal{L}$⟶$\mathcal{K}$×$\mathcal{P}$ by $\mathcal{Q}$(1) = ({α},{α,β}) = $\mathcal{Q}$(2) and $\mathcal{Q}$(3) = ({α},{α}), clearly $\mathcal{Q}$ is b-gs(b-sg)-continuous but not binary continuous. For, $\mathcal{Q}^{-1}$(∅,∅) = ∅, $\mathcal{Q}^{-1}$($\mathcal{K}$,$\mathcal{P}$) = $\mathcal{L}$ and $\mathcal{Q}^{-1}$({α},{α,β}) = {1,2} which is gs(sg)-open in $\mathcal{L}$ but not a open set in $\mathcal{L}$.

Example 3.4. from 3.3 Let τ = {∅, $\mathcal{L}$,{1},{1,3}} is a topology on $\mathcal{L}$. Then gs-open subset in $\mathcal{L}$ are ∅, $\mathcal{L}$,{1},{3},{1,2},{1,3}. Define $\mathcal{Q}$: $\mathcal{L}$⟶$\mathcal{K}$×$\mathcal{P}$ by $\mathcal{Q}$(1) = ({α},{α,β}) = $\mathcal{Q}$(2) and $\mathcal{Q}$(3) = ({α},{α}), clearly $\mathcal{Q}$ is b-gs-continuous but not generalized

binary continuous. For, $\mathcal{Q}^{-1}(\emptyset,\emptyset) = \emptyset$, $\mathcal{Q}^{-1}(\mathcal{K},\mathcal{P}) = \mathcal{L}$ and $\mathcal{Q}^{-1}(\{\alpha\},\{\alpha,\beta\}) = \{1,2\}$ which is gs-open in $\mathcal{L}$ but not a g-open set in $\mathcal{L}$.

Example 3.5. from 3.3 Define $\mathcal{Q} : \mathcal{L}\longrightarrow\mathcal{K}\times\mathcal{P}$ by $\mathcal{Q}(2) = (\{\alpha\},\{\alpha,\beta\})$ and $\mathcal{Q}(1) = (\{\alpha\},\{\alpha\}) = \mathcal{Q}(3)$, clearly $\mathcal{Q}$ is b-gs-continuous but not generalized binary continuous. For, $\mathcal{Q}^{-1}(\emptyset,\emptyset) = \emptyset$, $\mathcal{Q}^{-1}(\mathcal{K},\mathcal{P}) = \mathcal{L}$ and $\mathcal{Q}^{-1}(\{\alpha\},\{\alpha,\beta\}) = \{2\}$ which is gs-open in $\mathcal{L}$ but not a semi open set in $\mathcal{L}$.

Example 3.6. from 3.3 Let $\tau = \{\emptyset, \mathcal{L},\{2\},\{1,3\}\}$ is a topology on $\mathcal{L}$. Then sg-open subset in $\mathcal{L}$ are P(X). Define $\mathcal{Q} : \mathcal{L}\longrightarrow\mathcal{K}\times\mathcal{P}$ by $\mathcal{Q}(1) = (\{\alpha\},\{\alpha,\beta\}) = \mathcal{Q}(2)$ and $\mathcal{Q}(3) = (\{\alpha\},\{\alpha\})$, clearly $\mathcal{Q}$ is b-gs-continuous but not gb-continuous. For, $\mathcal{Q}^{-1}(\emptyset,\emptyset) = \emptyset$, $\mathcal{Q}^{-1}(\mathcal{K},\mathcal{P}) = \mathcal{L}$ and $\mathcal{Q}^{-1}(\{\alpha\},\{\alpha,\beta\}) = \{1,2\}$ which is sg-open in $\mathcal{L}$ but not a semi open set in $\mathcal{L}$.

Theorem 3.7. Let $\mathcal{Q} : \mathcal{L}\longrightarrow\mathcal{K}\times\mathcal{P}$ be a function such that $\mathcal{L} - \mathcal{Q}^{-1}(\mathcal{F},\mathcal{G}) = \mathcal{Q}^{-1}(\mathcal{K}\text{-}\mathcal{F}, \mathcal{P}\text{-}\mathcal{G})$ for all $\mathcal{F} \subseteq \mathcal{K}$ and $\mathcal{G} \subseteq \mathcal{P}$. Then $\mathcal{Q}$ is b-gs-continuous if and only if $\mathcal{Q}^{-1}(\mathcal{F}, \mathcal{G})$ is gs-closed in $\mathcal{L}$ for all b-gs-closed sets $(\mathcal{F}, \mathcal{G})$ in $(\mathcal{K},\mathcal{P},\mathcal{M})$.

Proof: Assume that f is b-gs-continuous. Let $(\mathcal{F},\mathcal{G})$ be a b-gs-closed set in $(\mathcal{K},\mathcal{P},\mathcal{M})$. Therefore, $(\mathcal{K}\text{-}\mathcal{F}, \mathcal{P}\text{-}\mathcal{G})$ is b-gs-open set. Since $\mathcal{Q}$ is b-gs-continuous, we have $\mathcal{Q}^{-1}(\mathcal{K}\text{-}\mathcal{F}, \mathcal{P}\text{-}\mathcal{G})$ is gs-open in $\mathcal{L}$. Therefore, $\mathcal{L} - \mathcal{Q}^{-1}(\mathcal{F},\mathcal{G})$is gs-open in $\mathcal{L}$. This shows that $\mathcal{Q}^{-1}(\mathcal{F},\mathcal{G})$ is gs-closed in $\mathcal{L}$.
Conversely, assume that if $\mathcal{Q}^{-1}(\mathcal{F},\mathcal{G})$ is gs-closed in $\mathcal{L}$ for all b-gs-closed set $(\mathcal{F},\mathcal{G})$ in $(\mathcal{K},\mathcal{P},\mathcal{M})$. Let $(\mathcal{F},\mathcal{G})$ be a b-gs-open set $(\mathcal{K},\mathcal{P},\mathcal{M})$. We have $(\mathcal{K}\text{-}\mathcal{F}, \mathcal{P}\text{-}\mathcal{G})$ is b-gs-closed set in $(\mathcal{K},\mathcal{P},\mathcal{M})$. Therefore, by our assumption $\mathcal{Q}^{-1}(\mathcal{K}\text{-}\mathcal{F}, \mathcal{P}\text{-}\mathcal{G})$ is gs-closed in $\mathcal{L}$. Thus, $\mathcal{L} - \mathcal{Q}^{-1}(\mathcal{F},\mathcal{G})$ is gs-closed in $\mathcal{L}$. This shows that $\mathcal{Q}^{-1}(\mathcal{F},\mathcal{G})$ is gs-open in $\mathcal{L}$ that implies $\mathcal{Q}$ is b-gs-continuous.

Theorem 3.5. $\mathcal{Q} : \mathcal{L}\longrightarrow\mathcal{K}\times\mathcal{P}$ is a b-gs-continuous function if and only if for every $z \in \mathcal{L}$ and for every binary open set $(\mathcal{F}, \mathcal{G})$ with $\mathcal{Q}(z) \in (\mathcal{F}, \mathcal{G})$ there is gs-open set $U \subseteq \mathcal{L}$ such that $\mathcal{Q}(U) \subseteq (\mathcal{F}, \mathcal{G})$.
Proof: Consider, $\mathcal{Q} : \mathcal{L}\longrightarrow\mathcal{K}\times\mathcal{P}$ is a b-gs-continuous. Let $(\mathcal{F}, \mathcal{G})$ be a binary open set with $\mathcal{Q}(z) = (x,y) \in (\mathcal{F}, \mathcal{G})$. Then $z \in \mathcal{Q}^{-1}(\mathcal{F}, \mathcal{G})$. Take $U = \mathcal{Q}^{-1}(\mathcal{F}, \mathcal{G})$. Then U is gs-open in $\mathcal{L}$ with $z \in U$. Also $\mathcal{Q}(U) = \{\mathcal{Q}(u) : u \in U\} \subseteq (\mathcal{F}, \mathcal{G})$.
Conversely, we assume that for all $z \in \mathcal{L}$ and for every binary open set $(\mathcal{F}, \mathcal{G})$ with $\mathcal{Q}(z) \in (\mathcal{F}, \mathcal{G})$ there exists a gs-open set U in $\mathcal{L}$ with $z \in U$, $\mathcal{Q}(U) \subseteq (\mathcal{F}, \mathcal{G})$. Let $(\mathcal{F}, \mathcal{G})$ be a binary open set. To show that $f^{-1}(\mathcal{F}, \mathcal{G})$ is gs-open in $\mathcal{L}$. Let $u \in \mathcal{Q}^{-1}(\mathcal{F}, \mathcal{G})$. Then $\mathcal{Q}(u) \in (\mathcal{F}, \mathcal{G})$. By our assumption there exists a gs-open set U with $\mathcal{Q}(U) \subseteq (\mathcal{F}, \mathcal{G})$. Therefore, $\mathcal{Q}^{-1}(\mathcal{Q}(U)) \subseteq \mathcal{Q}^{-1}(\mathcal{F}, \mathcal{G})$. That is $U \subseteq \mathcal{Q}^{-1}(\mathcal{F}, \mathcal{G})$. This shows that for each $u \in \mathcal{Q}^{-1}(\mathcal{F}, \mathcal{G})$ there is gs-open set U containing u such that $U \subseteq \mathcal{Q}^{-1}(\mathcal{F}, \mathcal{G})$ that implies $\mathcal{Q}^{-1}(\mathcal{F}, \mathcal{G})$ is a union of gs-open sets in $\mathcal{L}$. This proves that $\mathcal{Q}^{-1}(\mathcal{F}, \mathcal{G})$ is gs-open in $\mathcal{L}$ that implies $\mathcal{Q}$ is b-gs-continuous.

Theorem 3.6. $\mathcal{Q} : \mathcal{L}\longrightarrow\mathcal{K}\times\mathcal{P}$ is a b-gs-continuous function if and only if for every $\mathcal{F} \subseteq \mathcal{K}$ and $\mathcal{G} \subseteq \mathcal{P}$, $\mathcal{Q}^{-1}(\text{b-gs-int}(\mathcal{F}, \mathcal{G})) \subseteq \text{gs-int}(\mathcal{Q}^{-1}(\mathcal{F}, \mathcal{G}))$.
Proof: Suppose $\mathcal{Q} : \mathcal{L}\longrightarrow\mathcal{K}\times\mathcal{P}$ is a b-gs-continuous function. Let $\mathcal{F} \subseteq \mathcal{K}$ and $\mathcal{G} \subseteq \mathcal{P}$, b-gs-int$(\mathcal{F}, \mathcal{G})$ is b-gs-open in $(\mathcal{K}, \mathcal{P},\mathcal{M})$ and contained in $(\mathcal{F}, \mathcal{G})$. Therefore $\mathcal{Q}^{-1}$(b-gs-int$(\mathcal{F}, \mathcal{G})$) is gs-open in $\mathcal{L}$. Now, b-gs-int$(\mathcal{F}, \mathcal{G}) \subseteq (\mathcal{F}, \mathcal{G})$
$\Longrightarrow \mathcal{Q}^{-1}(\text{b-gs-int}(\mathcal{F}, \mathcal{G})) \subseteq \mathcal{Q}^{-1}(\mathcal{F}, \mathcal{G})$
$\Longrightarrow \text{gs-int}(\mathcal{Q}^{-1}(\text{b-gs-int}(\mathcal{F}, \mathcal{G}))) \subseteq \text{gs-int}(\mathcal{Q}^{-1}(\mathcal{F}, \mathcal{G}))$
$\Longrightarrow \mathcal{Q}^{-1}(\text{b-gs-int}(\mathcal{F}, \mathcal{G})) \subseteq \text{gs-int}(\mathcal{Q}^{-1}(\mathcal{F}, \mathcal{G}))$.
Conversely, assume that $\mathcal{Q}^{-1}(\text{b-gs-int}(\mathcal{F}, \mathcal{G})) \subseteq \text{gs-int}(\mathcal{Q}^{-1}(\mathcal{F}, \mathcal{G}))$ for every $\mathcal{F} \subseteq \mathcal{K}$ and $\mathcal{G} \subseteq \mathcal{P}$. Let $(\mathcal{F}, \mathcal{G})$ is a b-gs-open set. Then b-gs-int$(\mathcal{F}, \mathcal{G}) = (\mathcal{F}, \mathcal{G})$. Therefore, $\mathcal{Q}^{-1}(\mathcal{F}, \mathcal{G}) = \mathcal{Q}^{-1}(\text{b-gs-int}(\mathcal{F}, \mathcal{G})) \subseteq \text{gs-int}(\mathcal{Q}^{-1}(\mathcal{F}, \mathcal{G}))$. Therefore, gs-int$(\mathcal{Q}^{-1}(\mathcal{F}, \mathcal{G}))$ is gs-open in $\mathcal{L}$. Hence $\mathcal{Q}$ is b-gs-continuous.

Totally Binary Generalized Semi Continuous Functions

In this section we define and study the concept of totally binary generalized semi continuous functions and we obtain some of their characterizations.

Definition 4.1. Let $(\mathcal{L},\tau)$ be a topological space and $(\mathcal{K},\mathcal{P},\mathcal{M})$ be a binary topological space. Then the map $\mathcal{Q} : \mathcal{L}\longrightarrow\mathcal{K}\times\mathcal{P}$ is called

- totally generalized binary continuous(briefly, totally gb-continuous), if $\mathcal{Q}^{-1}(\mathcal{F},\mathcal{G})$ is generalized clopen in $\mathcal{L}$ for every binary open set $(\mathcal{F},\mathcal{G})$ in $(\mathcal{K},\mathcal{P},\mathcal{M})$.
- totally binary generalized semi continuous(briefly, totally b-gs-continuous), if $\mathcal{Q}^{-1}(\mathcal{F},\mathcal{G})$ is generalized semi clopen in $\mathcal{L}$ for every binary open set $(\mathcal{F},\mathcal{G})$ in $(\mathcal{K},\mathcal{P},\mathcal{M})$.

Theorem 4.2.

1.Let $\mathcal{Q} : \mathcal{L}\longrightarrow\mathcal{K}\times\mathcal{P}$ be totally binary continuous. Then $\mathcal{Q}$ is totally gb-continuous.
2.Let $\mathcal{Q} : \mathcal{L}\longrightarrow\mathcal{K}\times\mathcal{P}$ be totally binary continuous. Then $\mathcal{Q}$ is b-gs-continuous.
3.Let $\mathcal{Q} : \mathcal{L}\longrightarrow\mathcal{K}\times\mathcal{P}$ be totally b-gs-continuous. Then $\mathcal{Q}$ is b-gs-continuous.
4.Let $\mathcal{Q} : \mathcal{L}\longrightarrow\mathcal{K}\times\mathcal{P}$ be totally binary continuous. Then $\mathcal{Q}$ is totally b-gs-continuous.
5.Let $\mathcal{Q} : \mathcal{L}\longrightarrow\mathcal{K}\times\mathcal{P}$ be totally binary semi continuous. Then $\mathcal{Q}$ is totally b-gs-continuous.
6.Let $\mathcal{Q} : \mathcal{L}\longrightarrow\mathcal{K}\times\mathcal{P}$ be totally gb-continuous. Then $\mathcal{Q}$ is totally b-gs-continuous.

Proof: Let $(\mathcal{F},\mathcal{G})$ be a binary open set in $(\mathcal{K},\mathcal{P},\mathcal{M})$. Since $\mathcal{Q}$ is totally binary continuous, we have $\mathcal{Q}^{-1}(\mathcal{F},\mathcal{G})$ is both open and closed in $\mathcal{L}$. Since every open set is g-open and every closed set is g-closed in $\mathcal{L}$, then $\mathcal{Q}^{-1}(\mathcal{F},\mathcal{G})$ is both g-open and g-closed in $\mathcal{L}$. Hence $\mathcal{Q}$ is totally gb-continuous.
Proof of (2) to (6) is obvious.
The converse of the above theorem need not be true as seen from the subsequent example.

Example 4.3. Let $\mathcal{K} = \{\alpha,\beta\}$, $\mathcal{P} = \{\alpha,\beta,\gamma\}$ and $\mathcal{L}$ = {1,2,3}. Clearly $\mathcal{M}$ = {(∅,∅),({β}{α,γ}), $(\mathcal{K},\mathcal{P})$ } is a binary topology form $\mathcal{K}$ to $\mathcal{P}$ and $\tau = \{\emptyset,\mathcal{L},\{1\},\{2,3\}\}$ is a topology on $\mathcal{L}$. Then closed subset in $\mathcal{L}$ are ∅,$\mathcal{L}$,{2,3},{1}. Hence the clopen sets in $\mathcal{L}$ are ∅,$\mathcal{L}$,{2,3},{1}. Now g-open subsets in $\mathcal{L}$ are P($\mathcal{L}$). Thus the g-clopen sets are P($\mathcal{L}$). Define $\mathcal{Q} : \mathcal{L}\longrightarrow\mathcal{K}\times\mathcal{P}$ by $\mathcal{Q}(1)$ = ({β},{α,γ}) = $\mathcal{Q}(2)$ and $\mathcal{Q}(3)$ = ({α},{γ}), For, $\mathcal{Q}^{-1}(\emptyset,\emptyset) = \emptyset$, $\mathcal{Q}^{-1}(\mathcal{K},\mathcal{P}) = \mathcal{L}$ and $\mathcal{Q}^{-1}(\{\beta\},\{\alpha,\gamma\}) = \{1,2\}$ which is g-clopen in $\mathcal{L}$ and hence $\mathcal{Q}$ is totally gb-continuous, but $\mathcal{Q}$ is not totally binary continuous, since {1,2} is not a clopen set in $\mathcal{L}$.

Example 4.4. Form 4.3 which is gs-open in $\mathcal{L}$ and hence $\mathcal{Q}$ is bgs-continuous, but $\mathcal{Q}$ is not totally binary continuous, since {1,2} is not a clopen set in $\mathcal{L}$.

Example 4.5. Form 4.3 $\tau = \{\emptyset,\mathcal{L},\{1\}\}$ is a topology on $\mathcal{L}$. Then closed subset in $\mathcal{L}$ are ∅,$\mathcal{L}$,{2,3}. Hence the clopen sets in $\mathcal{L}$ are ∅,$\mathcal{L}$. Define $\mathcal{Q} : \mathcal{L}\longrightarrow\mathcal{K}\times\mathcal{P}$ by $\mathcal{Q}(1)$ = ({β},{α,γ}) and $\mathcal{Q}(2)$ = ({α},{γ}) = $\mathcal{Q}(3)$, For, $\mathcal{Q}^{-1}(\emptyset,\emptyset) = \emptyset$, $\mathcal{Q}^{-1}(\mathcal{K},\mathcal{P}) = \mathcal{L}$ and $\mathcal{Q}^{-1}(\{\beta\},\{\alpha,\gamma\}) = \{1\}$ which is gs-open in $\mathcal{L}$ and hence $\mathcal{Q}$ is b-gs-continuous, but $\mathcal{Q}$ is not totally b-gs-continuous, since {1} is not a gs-clopen set in $\mathcal{L}$.

Example 4.6. Form 4.3 which is gs-clopen in $\mathcal{L}$ and hence $\mathcal{Q}$ is totally b-gs-continuous, but $\mathcal{Q}$ is not totally binary continuous, since {1,2} is not a clopen in $\mathcal{L}$.

Example 4.7. Form 4.6 But $\mathcal{Q}$ is not totally binary semi continuous, since {1,2} is not a semi clopen in $\mathcal{L}$.

Example 4.8. Form 4.3 $\tau = \{\emptyset,\mathcal{L},\{1\},\{1,3\}\}$ is a topology on $\mathcal{L}$. Then closed subset in $\mathcal{L}$ are ∅,$\mathcal{L}$,{2},{2,3}. Hence the g-clopen sets in $\mathcal{L}$ are ∅,$\mathcal{L}$. Now gs-open subsets in $\mathcal{L}$ are ∅, $\mathcal{L}$,{1},{3},{1,3},{1,2}. Thus the gs-clopen sets are ∅, $\mathcal{L}$,{3},{1,2}. Which is gs- clopen in $\mathcal{L}$ and hence $\mathcal{Q}$ is totally b-gs-continuous, but $\mathcal{Q}$ is not totally gb-continuous, since {1,2} is not a g-clopen in $\mathcal{L}$.

Theorem 4.9. A function $\mathcal{Q} : \mathcal{L}\longrightarrow\mathcal{K}\times\mathcal{P}$ from a topological spaces $\mathcal{L}$ into binary topological spaces $(\mathcal{K},\mathcal{P},\mathcal{M})$ is totally binary generalized semi continuous if and only if the inverse image of every binary closed in $(\mathcal{K},\mathcal{P},\mathcal{M})$ is generalized semi clopen in $\mathcal{L}$.

Proof: Suppose $\mathcal{Q} : \mathcal{L}\longrightarrow\mathcal{K}\times\mathcal{P}$ is totally b-gs-continuous. Let $(\mathcal{H},\mathcal{J})$ be a binary closed in $(\mathcal{K},\mathcal{P},\mathcal{M})$. Then $(\mathcal{H},\mathcal{J})^c$ is binary open. Since $\mathcal{Q}$ is totally b-gs-continuous. $\mathcal{Q}^{-1}((\mathcal{H},\mathcal{J})^c)$ is gs-clopen in $\mathcal{L}$. But $\mathcal{Q}^{-1}((\mathcal{H},\mathcal{J})^c) = \mathcal{L} - \mathcal{Q}^{-1}(\mathcal{H},\mathcal{J})$ and so $\mathcal{Q}^{-1}(\mathcal{H},\mathcal{J})$ is gs-clopen in $\mathcal{L}$.

Conversely, suppose inverse image of every binary closed subset of $(\mathcal{K},\mathcal{P},\mathcal{M})$ is gs-clopen in $\mathcal{L}$. Let $(\mathcal{R},\mathcal{S})$ be a binary open. Then $(\mathcal{R},\mathcal{S})^c$ is binary closed, By hypothesis $\mathcal{Q}^{-1}((\mathcal{R},\mathcal{S})^c)$ is gs-clopen in $\mathcal{L}$, that is $\mathcal{Q}^{-1}(\mathcal{R},\mathcal{S})$ is gs-clopen in $\mathcal{L}$. Therefore, f is totally b-gs-continuous.

Theorem 4.10. Let $\mathcal{Q} : \mathcal{L}\longrightarrow\mathcal{K}\times\mathcal{P}$ be a function, $(\mathcal{L},\tau)$ be a topological space and $(\mathcal{K},\mathcal{P},\mathcal{M})$ be a binary topological spaces. Then the following are equivalent.
(1) $\mathcal{Q}$ is totally b-gs-continuous
(2) for every $z \in \mathcal{L}$ and for every binary open set $(\mathcal{F},\mathcal{G})$ with $\mathcal{Q}(z) \in (\mathcal{F},\mathcal{G})$ there is a gs- clopen set $U \subseteq \mathcal{L}$ such that $f(U) \subseteq (\mathcal{F},\mathcal{G})$.

Proof
(1)⟶(2) Suppose $\mathcal{Q} : \mathcal{L}\longrightarrow\mathcal{K}\times\mathcal{P}$ is a totally b-gs-continuous and $(\mathcal{F},\mathcal{G})$ be a binary open set with $\mathcal{Q}(z) = (x,y) \in (\mathcal{F},\mathcal{G})$ such that $z \in \mathcal{Q}^{-1}(\mathcal{F},\mathcal{G})$. Since $\mathcal{Q}$ is totally b-gs-continuous, $z \in \mathcal{Q}^{-1}(\mathcal{F},\mathcal{G})$ is gs-clopen in $\mathcal{L}$. Let $U = z \in \mathcal{Q}^{-1}(\mathcal{F},\mathcal{G})$ then U is gs-clopen in $\mathcal{L}$ and $z \in U$. Also $\mathcal{Q}(U) = \{\mathcal{Q}(u) : u \in U\} \subseteq (\mathcal{F},\mathcal{G})$. This implies $\mathcal{Q}(U) \subseteq (\mathcal{F},\mathcal{G})$.
(2)⟶(1) We assume that for all $z \in \mathcal{L}$ and for every binary set $(\mathcal{F},\mathcal{G})$ in $(\mathcal{K},\mathcal{P},\mathcal{M})$. Let $z \in \mathcal{Q}^{-1}(\mathcal{F},\mathcal{G})$ be a any arbitrary point. This implies $\mathcal{Q}(z) \in (\mathcal{F},\mathcal{G})$ therefore by (2) there is a gs-clopen set U in z with $z \in U$, $\mathcal{Q}(U) \subseteq (\mathcal{F},\mathcal{G})$, which implies $u \in \mathcal{Q}^{-1}(\mathcal{F},\mathcal{G})$ is a gs-clopen neighbourhood of z. Since z is arbitrary, it implies $\mathcal{Q}^{-1}(\mathcal{F},\mathcal{G})$ is a gs-clopen neighbourhood of each of its points. This proves that $\mathcal{Q}^{-1}(\mathcal{F},\mathcal{G})$ is gs-clopen in $\mathcal{L}$ that implies $\mathcal{Q}$ is totally b-gs-continuous.

Strongly Binary Generalized Semi Continuous Functions
This section is devoted to introduce a new class of binary functions known as strongly binary generalized semi continuous functions and to study some of their characterizations.

Definition 5.1. Let $(\mathcal{L},\tau)$ be a topological space and $(\mathcal{K},\mathcal{P},\mathcal{M})$ be a binary topological space. Then the map $\mathcal{Q} : \mathcal{L}\longrightarrow\mathcal{K}\times\mathcal{P}$ is called

- strongly generalized binary continuous(briefly, strongly gb-continuous), if $\mathcal{Q}^{-1}(\mathcal{F},\mathcal{G})$ is generalized clopen in $\mathcal{L}$ for every binary set $(\mathcal{F},\mathcal{G})$ in $(\mathcal{K},\mathcal{P},\mathcal{M})$.
- strongly binary generalized semi continuous(briefly, strongly b-gs-continuous) if $\mathcal{Q}^{-1}(\mathcal{F},\mathcal{G})$ is generalized semi clopen in $\mathcal{L}$ for every binary set $(\mathcal{F},\mathcal{G})$ in $(\mathcal{K},\mathcal{P},\mathcal{M})$.

Theorem 5.2. A function $\mathcal{Q} : \mathcal{L}\longrightarrow\mathcal{K}\times\mathcal{P}$ the following hold
(1) Every strongly binary continuous is (strongly generalized binary, binary generalized semi, strongly binary generalized semi) continuous.
(2) Every strongly binary semi continuous is strongly binary generalized semi continuous.
(3) Every strongly generalized binary continuous is strongly binary generalized semi continuous.
(4) Every strongly binary generalized semi continuous is binary generalized semi continuous.

Proof: Let $(\mathcal{F},\mathcal{G})$ be a binary set in $(\mathcal{K},\mathcal{P},\mathcal{M})$. Since $\mathcal{Q}$ is strongly binary continuous, we have $\mathcal{Q}^{-1}(\mathcal{F},\mathcal{G})$ is both open and closed in $\mathcal{L}$. Since every open set is g-open and every closed set is g-closed in $\mathcal{L}$, then $\mathcal{Q}^{-1}(\mathcal{F},\mathcal{G})$ is both g-open and g-closed in $\mathcal{L}$. Hence $\mathcal{Q}$ is strongly generalized binary continuous.
Proof of (2) to (4) is obvious.
The converse of the above theorems need not be true as seen from the subsequent example.

Example 5.3. Let $\mathcal{K} = \{\alpha,\beta\}$, $\mathcal{P} = \{\alpha,\beta,\gamma\}$ and $\mathcal{L} = \{1,2,3\}$. Clearly $\mathcal{M} = \{(\emptyset,\emptyset),(\{\alpha\}\{\beta,\gamma\}), (\mathcal{K},\mathcal{P})\}$ is a binary topology form $\mathcal{K}$ to $\mathcal{P}$ and $\tau = \{\emptyset,\mathcal{L},\{1\},\{2,3\}\}$ is a topology on $\mathcal{L}$. Then closed subset in $\mathcal{L}$ are $\emptyset,\mathcal{L},\{2,3\},\{1\}$. Hence the clopen sets in $\mathcal{L}$ are $\emptyset,\{1\},\{2,3\},\mathcal{L}$. Now g-open subset in $\mathcal{L}$ are $P(\mathcal{L})$. Thus the g-clopen sets are $P(\mathcal{L})$. Define $\mathcal{Q} : \mathcal{L}\longrightarrow\mathcal{K}\times\mathcal{P}$ by $f(3) = (\{\alpha\},\{\beta,\gamma\})$ and $f(1) = (\{\alpha\},\{\alpha\}) = f(2)$, clearly $\mathcal{Q}$ is b-sg-continuous. For, $f^{-1}(\emptyset,\emptyset) = \emptyset$, $f^{-1}(\emptyset,\{\alpha\}) = \emptyset$, $f^{-1}(\emptyset,\{\beta\}) = \emptyset$, $f^{-1}(\emptyset,\{\gamma\}) = \emptyset$, $f^{-1}(\emptyset,\{\alpha,\beta\}) = \emptyset$, $f^{-1}(\emptyset,\{\alpha,\gamma\}) = \emptyset$, $f^{-1}(\emptyset,\{\beta,\gamma\}) = \emptyset$, $f^{-1}(\emptyset,\mathcal{P}) = \emptyset$, $f^{-1}(\{\alpha\},\emptyset) = \emptyset$, $f^{-1}(\{\alpha\},\{\alpha\}) = \{1,2\}$, $f^{-1}(\{\alpha\},\{\beta\}) = \emptyset$, $f^{-1}(\{\alpha\},\{\gamma\}) = \emptyset$, $f^{-1}(\{\alpha\},\{\alpha,\beta\}) = \{1,2\}$, $f^{-1}(\{\alpha\},\{\alpha,\gamma\}) = \{1,2\}$, $f^{-1}(\{\alpha\},\{\beta\gamma\}) = \{3\}$, $f^{-1}(\{\alpha\},\mathcal{P}) = \mathcal{L}$,

$f^{-1}(\{\beta\},\emptyset) = \emptyset$, $f^{-1}(\{\beta\},\{\alpha\}) = \emptyset$, $f^{-1}(\{\beta\},\{\beta\}) = \emptyset$, $f^{-1}(\{\beta\},\{\gamma\}) = \emptyset$, $f^{-1}(\{\beta\},\{\alpha,\beta\}) = \emptyset$, $f^{-1}(\{\beta\},\{\alpha,\gamma\}) = \emptyset$, $f^{-1}(\{\beta\},\{\beta\gamma\}) = \{2\}$, $f^{-1}(\{\beta\},\mathcal{P}) = \{2\}$, $f^{-1}(\mathcal{K},\emptyset) = \emptyset$, $f^{-1}(\mathcal{K},\{\alpha\}) = \{1,2\}$, $f^{-1}(\mathcal{K},\{\beta\}) = \emptyset$, $f^{-1}(\mathcal{K},\{\gamma\}) = \emptyset$, $f^{-1}(\mathcal{K},\{\alpha,\beta\}) = \{1,2\}$, $f^{-1}(\mathcal{K},\{\alpha,\gamma\}) = \emptyset$, $f^{-1}(\mathcal{K},\{\beta,\gamma\}) = \{3\}$, $f^{-1}(\mathcal{K},\mathcal{P}) = \mathcal{L}$, This gives inverse image of every binary sets in $(\mathcal{K},\mathcal{P},\mathcal{M})$ is generalized clopen in $\mathcal{L}$. Hence $\mathcal{Q}$ is (strongly generalized binary, binary generalized semi, strongly binary generalized semi) continuous. But $\mathcal{Q}$ is not strongly binary continuous, since {1} is not a clopen, in $\mathcal{L}$.

Example 5.4. From 5.3 Hence $\mathcal{Q}$ is strongly b-gs-continuous, But $\mathcal{Q}$ is not strongly bs-continuous, since {1,3} is not a semi clopen in $\mathcal{L}$.

Example 5.5. From 5.3 $\tau = \{\emptyset,\mathcal{L},\{1\},\{1,3\}\}$ is a topology on $\mathcal{L}$. Hence $\mathcal{Q}$ is strongly b-gs-continuous, But $\mathcal{Q}$ is not strongly gb-continuous, since {1,2} is not a g-clopen in $\mathcal{L}$.

Example 5.6. From 5.3 $\tau = \{\emptyset,\mathcal{L},\{2\},\{1,3\}\}$ is a topology on $\mathcal{L}$. Hence $\mathcal{Q}$ is b-gs-continuous, but $\mathcal{Q}$ is not strongly b-gs-continuous, since {1} is not a clopen in $\mathcal{L}$.

CONCLUSION

In this paper, we had introduced and studied the concept of b-gs (b-sg)-continuous functions. Likewise, totally binary generalized semi continuous and strongly binary generalized semi continuous in binary topological spaces and interrogate some of their characterizations. Additionally, we capture certain important results; such as totally binary generalized semi continuous is binary generalized semi continuous and strongly binary generalized semi continuous is binary generalized semi continuous. This lead to achieve further results in binary topological spaces.

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REFERENCES

1. Nithyanantha Jothi. S and Thangavelu. P, Topology between two sets, Journal of Mathematical Sciences and Computer Applications, 1(3), 2011, 95-107.
2. Nithyanantha Jothi. S and Thangavelu. P, Generalized binary closed sets in binary Topological spaces, Ultra Scientist Of Physical Sciences, 26(1)A, 2014, 25-30.
3. Nithyanantha Jothi. S, Binary Semi Continuous functions in Binary Topological Spaces, International Journal of Mathematics Trends and Technology, 49(2), 2017, 152-155.
4. Sathishmohan. P, Lavanya. K and Mehar sudha. U, On b-gs-closed and b-sg-closed sets in Binary Topological spaces, Strad Research, 8(3), 2021, 20-24.
5. Sathishmohan. P, Rajendran. V, Lavanya. K and Rajalakshmi. K, On b-gs(b-sg)-closure and b-gs(b-sg)-interior of a sets in Binary Topological spaces, Gedrag & Organisatie Review, 34(1), 2021, 359-366.

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REVIEW ARTICLE

A Review on Malpractices in Regulatory Submission in India

Shantanu Thakre[1], Rohit Gahilod[2] and Balamuralidhara. V*[3]

[1]Research Student, Department of Pharmaceutics, Pharmaceutical Regulatory Affairs Group, JSS College of Pharmacy, JSS Academy of Higher Education and Research, Mysuru, Karnataka, India
[2]Department of Pharmaceutics, Pharmaceutical Regulatory Affairs Group JSS College of Pharmacy, JSS Academy of Higher Education and Research, Mysuru, Karnataka, India
[3]Head and Associate Professor, Department of Pharmaceutics, JSS College of Pharmacy, JSS Academy of Higher Education and Research, S.S.Nagar, Mysuru, Karnataka, India.

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***Address for Correspondence**
Balamuralidhara. V
Head and Associate Professor,
Department of Pharmaceutics, JSS College of Pharmacy,
JSS Academy of Higher Education and Research,
S.S.Nagar, Mysuru, Karnataka, India.
Email: baligowda@jssuni.edu.in

ABSTRACT

India got a ton of criticism a couple of years ago after a report uncovered misbehaviour in the working of the Central Drugs Standards Control Organization (CDSCO). The report expressed that CDSCO approved some drugs without conducting clinical trials and also notice that there was no scientific proof to show that these medications are truly viable and safe for Indian patients. No move had been initiated after the Mashelkar report (2003) was distributed portraying the necessary foundation at the CDSCO. Malpractices occur when a regulatory professional neglects to provide appropriate data to the regulatory authority. The significance of this article is, that when the 59th report of the Parliamentary Standing Committee on Health and Family Welfare (2012) was published and recommended the importance of conducting clinical trials, India's regulatory framework becomes globalized because of the strict regulation and extensive safety. The Indian economy turns into the world's third-biggest with regard to purchasing power parity (PPP) and the eleventh largest in terms of conventional Gross Domestic Product (GDP).

Keywords: Malpractices, Regulatory submission, malpractices in a regulatory submission, Negligence.

INTRODUCTION

Malpractice, often known as professional negligence, is defined as an instance of professional negligence or incompetence[1]. After a report showed malpractices in the working of the Central Drugs Standards Control Organization (CDSCO), India has been heavily criticized in the last years. The parliamentary standing committee on health and family welfare had slammed the CDSCO for the inaccuracies in its findings. Other difficulties, on the other hand, must be addressed to close the gaps. At the CDSCO, less than half of the 327 approved positions were filled at the time the report was released. In addition, there were only 846 drug inspectors instead of the requisite 1,349. More astonishingly, the minimum academic requirements for the position of DCGI are a Bachelor of Pharmacy (B.Pharm) degree, which is well below international norms. Many of these problems about the CDSCO's working and decisions are raised in the 59threport of the parliamentary standing board on the CDSCO's functioning[2]. Every government is responsible for ensuring that its citizens have access to safe, effective, and high-quality medication. As a result, it enacts special regulations to govern all aspects of pharmaceuticals, including their manufacturing, sale, distribution, import, and human clinical research. Since independence, India's regulatory framework has expanded to include fresh issues such as import, clinical research, and adverse drug reaction checking. The principles mentioned in publications released by the Ministry of Chemicals and Fertilizers and the Department of Chemicals and Petrochemicals underpin the Indian drug regulatory system [3].

Malpractices and Recommendations

There has been widespread worry about fake, counterfeit, and inferior pharmaceuticals across the country. The Indian Supreme Court, the National Human Rights Commission, and Members of Parliament have all expressed worry about the nation's medication control framework being modernized. Although the Rules have been revised from time to time, the D & C act has not been thoroughly examined since its commencement. The Indian government established multiple committees that investigated the issues and provided numerous suggestions in the past. The government has implemented some of these recommendations, but the basic concerns have yet to be rectified. Coming a decade when Mashelkar's report was published in 2003 [4].

Mashelkar Report (2003)

The Indian government formed an Expert Committee, chaired by Dr. R.A. Mashelkar, to look into all areas of the regulatory infrastructure as well as the scope, what's more, the issue of deceptive/unsatisfactory medications in the country [5].

Recommendations

Suggest another construction for the Drug Regulatory System in the nation including the setting up of a National Drug Authority

The Committee resolved that the country's administrative moves are generally due to:

- State and Central medication control foundation is inadequate or non-existent
- Deficient testing abilities
- lack of drug inspectors
- implementation irregularity
- a lack of especially instructed workforce for indicated administrative regions
- The shortfall of an information bank and exact data isn't accessible [6].

The Committee proposes that the State Drug Control Organizations be strengthened as soon as possible with qualified and trained personnel and enough funding. The specific recommendations are as follows:

- State legislatures ought to further develop their states' medication guideline frameworks. In a few states, where the heft of assembling and deals units are arranged, there is a need to build how many Drug Inspectors.
- State enforcement personnel's capability and skill should be continually improved through sufficient training in specialized regulatory review and examination areas.

- State legislatures ought to give satisfactory cash to transportation and test buys for the state DRA office and field officials
- Structured systems should be established to allow interstate regulatory authorities to exchange information and gain a better understanding of the methods used in different jurisdictions. This would aid in the harmonization of enforcement practices and greater uniformity [7].

The following are the exact actions that are advised for State Drug Control Organizations:

- Increment the labor supply, foundation, specialized abilities, and monetary assets accessible to the State Drug Control Organization.
- Set up insight and lawful cells under the authority of senior nodal officials who have been prepared. The state government should establish an effective system for providing quick police assistance to these officers.
- Establish an effective surveillance system to keep an eye on suspects. Watchers should be employed, and hidden monies for intelligence activities might be made accessible.
- Set up an effective communication network for communicating and sharing information in cases of interstate drug trafficking.
- Request that the government creates certain courts for the expedited trial of bogus drug cases.
- Set up a reasonable testing research facility by the need to inspect thought tests quickly.
- Examine the sources of drug purchases and the quality of drugs kept on hand by healthcare professionals and institutions.
- Give a complimentary number to public protests, data, and different necessities
- The licensing criterion for drug sales should be carefully enforced [8].

Malpractice (2012)

The Drug Controller General of India (DGCI) supported 2,167 medications between January 2001 and November 30, 2010, without directing clinical trials on Indian patients, as per a concentrate on the working of the Central Drugs Standard Control Organization (CDSCO), an office under the Union wellbeing service that controls drug showcasing in the country [9].

The report on the working of the Central Drug Standard Control Organization (CDSCO)

- The Committee mentioned data [sponsors; preapproval Phase III clinical trials; abroad administrative status in the United States, Canada, the United Kingdom, Australia, and the European Union; signs; names of specialists reached, and PSURs] to dissect new prescription endorsements, on account of 42 drugs picked indiscriminately from CDSCO's rundown of new items posted on its site.
- From 2004 to August 31, 2010, 38 medications were supported; one medication had been endorsed before in 2001[10].
- 3 medications were already been approved in the mid-1990s. Between January 2001 and November 30, 2010, the DCGI approved 2,167 medicines. Subsequently, the example size for arbitrary review was under 2%.
- The Ministry was unable to produce papers for three medications (pefloxacin, lomefloxacin, and sparfloxacin) out of 42 randomly selected for review due to non-traceable files.
- All of these medications were licensed at separate times and in different years, raising questions about whether their removal was intentional. Surprisingly, all three incidents involved problematic pharmaceuticals; 1 was never promoted in the United States, Canada, the United Kingdom, Australia, or different countries with advanced administrative frameworks, while the other 2 were at last gotten rid of. Each of the three drugs was as of now accessible in India.
- It is impossible to verify whether manufacturers are adhering to the approval requirements, such as indications, dosage, contraindications, and precautions.
- It's additionally difficult to stay aware of current changes in item monographs and security data, endangering patients. Significant enhancements in the well-being profile of the 2 medications sold in the United States yet later eliminated from the market were incorporated into the solution suggestions, including Black Box Warnings (intended to stand out to genuine unfriendly impacts) [11].

- The Committee discovered the following shortcomings in 39 medications for which information was available: On account of 11 medications (28%), Phase III clinical preliminaries commanded by Rules were not led. These medications were
  (i) Everolimus by Novartis
  (ii) Colistimethateby Cipla
  (iii) Exemestane by Pharmacia
  (iv) Buclizine by UCB
  (v) Pemetrexidby Eli Lilly
  (vi) Aliskirenby Novartis
  (vii)Pentosan by West Coast
  (viii) Ambrisentanby GlaxoSmithKline
  (ix) Ademetionine by Akums
  (x) Pirfenidone by Ciplaand
  (xi) FDC of Pregabalin, Methylcobalamin, Alpha Lipoic Acid, Pyridoxine & Folic Acid by Theon.
- Clinical trials for two medications (Sanofi's Dronedarone and Novartis' Aliskiran) were done on just twenty-one and forty-six people, separately, despite the administrative necessity of something like a hundred individuals.
- Trials were undertaken at only two hospitals in one case (Irsogladine of Macleods), despite the regulatory need of 3 to 4.
- Not only were no obligatory Phase III clinical trials undertaken, but no expert opinion was sought in the case of four medications (10%) (Novartis' Everolimus; UCB's Buclizine; Eli Lilly's Pemetexid; and FDC's Pregabalin with other agents). CDSCO's non-medical staff decided to approve these medications entirely on their own[12].
- During oral declaration, when gotten some information about the reasoning for approving new drugs without clinical preliminaries, the Health Secretary asserted that endorsement of new medications without Phase-III clinical preliminaries in the "public interest" was finished with specialized direction.
- The Ministry defended its choice to postpone the need for neighborhood clinical trials for the assembling/import of imaginative drugs by asserting that the D and C Rules don't demonstrate when a clinical preliminary exclusion may be allowed because of "public interest"[13].
- The Drug Control General of India (DCGI), on the other hand, has the authority to reduce, postpone, or eliminate toxicological and clinical data requirements for pharmaceuticals intended to treat life-threatening or serious disorders, as well as diseases with particular importance to the Indian health situation. Before reaching a choice, it was additionally guaranteed that the situation with the administrative endorsement of the given medication in different nations, as well as the assessment of clinical experts in the applicable field, were counseled[14]. Moreover, before acquiring showcasing approval, candidates should show post-marketing observing information.
- The expectation of the individuals who drafted the Act and Rules seems to have been to eliminate a little window open for new medications to enter without going through trials in genuine crises like an episode of a formerly obscure sickness (e.g., SAARS, Bird Flu, or Swine Flu) in which there may not be sufficient opportunity to test new medications and no other choice except for to proceed with a potentially dangerous course of action. The thirty-three medications were generally not named crisis medicines. Moreover, a few medications have been accessible in different business sectors for a long time, permitting adequate time for preliminaries in India. A few occurrences are as per the following [15].

a) Novartis' Daptomycin (Cubicin) was licensed in India on 28 January 2008, after being released worldwide on September 13, 2003. There was no urgency in approving the medicine without clinical studies.

b) Eli Lilly's Pemetrexed (Alimta) was approved in the United States on 5-2-2004. It was endorsed by DCGI without trials on June 28, 2006, following a two-year stand-by. Despite the fact that there was a sizable amount of chance to embrace Phase III examinations in India, the organization was given special treatment.

c) Merk Sharp and Dhome'sRaltegravir (Isentress) Despite the fact that there was adequate opportunity to embrace mandatory clinical trials, were delivered abroad on October 12, 2007, and authorized in India on January 27, 2010, without leading clinical trials[16].

- Such irregular approvals save drug companies money and effort while endangering Indian patients. DCGI approves one medicine without trials every month on average. By any stretch of the imagination, this cannot be in the public interest. Furthermore, it was noted that in such circumstances,
    I. professional advice is sought, and
    II. Post-Marketing Surveillance Data is required.
- In any case, a survey of DCGI's endorsement information uncovers that well-qualified assessment was looked for in just five of the thirty-three such far removed endorsements.
- In terms of Post-Marketing Surveillance data, the Ministry neglected to convey even one of the four medications approved without trials that were randomly chosen.
- The commission presumes that there is "no logical proof" that these medicines are genuinely valuable and safe for Indian patients subsequent to surveying the information on each medication. Without trials, the service couldn't offer observation information on the prescriptions authorized[17].

Recommendations

- The Committee proposes that, when approving Phase III clinical trials, the DCGI ensure that, subject to facility availability, such trials are disseminated around the country to include patients from a variety of ethnic origins and provide a truly representative sample.
- Moreover, given the pervasiveness of exceptional clinical universities and huge medical clinics in many pieces of the nation today, instead of private facilities, preliminaries ought to be directed in exceptional clinical schools and enormous clinics with nonstop crisis administrations to deal with surprising extreme incidental effects and ability in research.
- The Ministry should order DCGI to conduct an investigation and take necessary measures against the official(s) who provided the interested party authorization to select and receive expert opinion, and then authorized the medicine.
- An information bank of all marked drug items alongside their ingredients ought to be transferred to the CDSCO site and routinely refreshed [16].

CASE STUDY: **[18].**

Case Name: Prashant Reddy v/s Ministry of Health & Family Welfare

At: Central Information Commission, Baba Gangnath Marg, Munirka, New Delhi – 110067

Appellant: Mr.Prashant Reddy, RTI applicant

Respondent: Mr. R. G. Singh, CPIO and Mr. Abhishek Chawardol, Drugs Inspector, Mr. Sushanta Sarkar, CPIO & ADC (I), Mr. A. K. Pradhan, FAA & DDC (I), and Mr. R. K. Singh, Legal Consultant, CDSCO in person

Case no: CIC/MH & FW/A/2018/159460-BJ

Date of Hearing: 12.05.2020

Date of Decision: 26.05.2020

Facts of the case

- Because of the remarks made by the Parliamentary Standing Committee on Health and Family Welfare in its 59th Report headed by Dr. T. M. Mohapatra, the Appellant documented an RTI (Right To Information) application looking for duplicates of the reports and proposals of the Office Order gave by the DCGI on March 26, 2013, as well as survey processes utilized by CDSCO in endorsing new medications and clinical preliminaries.
- In a letter dated 30.05.2018, the CPIO (Centre Public Information Officer), RTI Cell (O/o the DCG-I) stated that Dr. T. M. Mohapatra's Committee report was not promptly accessible and thus declined to deliver data. Under section 6(3) of the RTI Act, 2005, the CPIO forwarded his application to the M/o H&FW. The Appellant addressed the FAA after being dissatisfied with the CPIO's response. According to information gathered from the concerned division of CDSCO, the report presented by Prof. T. M. Mohapatra Committee was not available, according to the FAA's (First Appellant Authorities) ruling of 04.07.2018.

Case Details

- The Appellant restated the terms of his RTI (Right To Information) application, alleging that all he wanted was a copy of the Mohapatra Committee Report, which the Respondent falsely refused because it was untraceable. However, after waiting two years after receiving the notice of immediate hearing, the Respondent (DCG-I, RTI Cell) emailed a copy of the Mohapatra Committee on May 11, 2020, at 9:28 PM, which was neither signed nor certified, indicating their deception.
- The true reasons for the Respondent's suppression of a copy of the report, according to the Appellant, were that the Mohapatra committee brought up serious failures by the DCGI office in the clearance of new products under the D& C Act. Many of these blunders are criminal. The appellant was also misled by the Respondent (Drug Regulation Section, M/o Health and Family Welfare).
- The CPIO (Centre Public Information Officer) noted in its June 21, 2018 reply that it did not have a copy of the Mohapatra Committee Report after Respondent No. 1 transferred the case to it. Dr. Shailendra Kumar, Director, Ministry of Health, was appointed to the committee based on the report's content. As a result, a copy of the report was required by the Ministry of Health. The Appellant proceeded to express that the DCGI's office hoped to have a well-established issue with missing records. The Parliamentary Standing Committee on Health and Family Welfare made several observations about missing files at the DCGI office in its 59th report.
- The Appellant cited a paragraph of the same report to back up his claim. Similarly, the Mohapatra Committee has expressed concern about the DCGI's office's badly maintained records and missing papers. It was also claimed that the Commission has previously stated that a missing file constitutes a crime under the Public Records Act of 1993 and that if a file goes missing, a legal investigation must be launched. The Appellant asked for time to prepare a detailed written submission outlining his arguments and citing relevant case law.

Contention of Respondent

- In its reply, **the Respondent (Drugs Regulation Section, Ministry of Health and Family Welfare)** repeated the response of CPIO/FAA and claimed that the application was submitted to the Ministry of Health and Family Welfare of the RTI Act, 2005 because the information asked was not available to them. It was additionally featured that the office has given a careful activity taken report on the suggestions made in the Report of the Department Related Parliamentary/ Standing Committee about the functioning of CDSCO on multiple occasions in its responses to Parliamentary Questions.
- While indicating that the Appellant received an appropriate response, the Respondent requested that the Appellant submit a detailed written contribution via email by May 15, 2020, to comment on the aforementioned submission.
- In its answer, the Respondent (CDSCO, RTI Cell) stated that the documents sought by the Appellant were not previously kept or accessible to them. After receiving the Commission's notice of hearing, they approached Prof T.M. Mohapatra personally, and he gave them a copy of the "Report of the Committee Constituted to Review the Procedures and Practices Followed by CDSCO for Granting Approval and Clinical Trials on Certain Drugs," which was directed to the Appellant in the form it was made available to them.
- During the hearing, the Respondent highlighted that they presented all of the documents that they had on hand. It was also promised that the Appellant would receive a certified copy of the papers in compliance with the RTI Act of 2005.
-

Observation by Commission

- Following the issuance of the notification of the hearing, the Commission noted that the Respondent CDSCO furnished a copy of the Mohapatra Committee Report that was not certified by the RTI Act, 2005.
- The Commission concluded that the CPIO must respond to information seekers in a clear, logical, and exact manner, as required by the RTI Act of 2005 and other court decisions on the topic. Section 7 [8] I of the RTI Act, 2005 additionally provides that if a request for information disclosure is denied, the CPIO must report the reasons for the denial.
- The Commission also stated that the CPIO shall make every effort to provide maximum support to RTI applicants to ensure the flow of information.

- The Commission concluded that the Respondent Public Authority needed to build a solid record-keeping system and evaluate its effectiveness regularly.
- The Commission expressed that intentional revelation of all data that ought to be shown in the public area ought to be the standard, with exceptions made for members of the public who need information. The RTI Act's cherished goal of open government can only be accomplished if all government agencies follow proactive divulgence guidelines. Sec 4[2] of the RTI Act requires each open position to propose however much data to people in general as could reasonably be expected routinely through different method for correspondence, including the Internet, with the goal that people, in general, doesn't need to utilize the RTI Act.

Order

- That's what the Commission noticed, considering the current realities of the case and the remarks made by the two players, an answer was given to the Appellant following its intercession.
- The Commission, then again, communicated grave worry about the record-keeping philosophy utilized by DCGI/CDSCO after a significant report connecting with a survey of CDSCO's techniques and practices for conceding endorsement and directing clinical trials on specific medications disappeared from their office and must be acquired from the creator after the Commission got notice of hearing.
- Despite the fact that the Public Authority's weaknesses were likewise brought to the consideration of the Parliamentary Standing Committee. The Public Authority's objective and activities ought to constantly be above board with regards to conceding endorsements through an extensive and objective technique. The Commission empowers the Secretary, Ministry of Health and Family Welfare, Government of India, to examine what is happening prior to making any further move completely.
- Within 30 days of receipt of this ruling, the Commission directs the Respondent (CDSCO) to deliver a certified copy of the information provided to the Appellant through a letter dated 11.05.2020.
- Furthermore, taking into account the observations made in the preceding paragraphs, the Commission advises the Respondent, without commenting on the merits of the case, to take quick strides to smooth out the course of digitization of records within the Public Authority so that RTI applications/First Appeals are handled promptly.
- The Commission likewise requires public authorities to distribute their reports and other related papers in the public area on their drive to assist the overall population.

Current drug regulatory procedure

The Central Drugs Standard Control Organization (CDSCO) under the Ministry of Health and Family Welfare (MoH and FW) establishes guidelines for guaranteeing the safety, efficacy, and quality of drugs, beauty care products, diagnostics, and gadgets in India[19]. It also oversees medication imports and approves manufacturing licenses, as well as regulates the market approval of new drugs and clinical trial criteria. The National Pharmaceutical Pricing Authority (NPPA), which is part of the Ministry of Chemicals and Fertilizers' Department of Chemicals and Petrochemicals, sets or on the other hand modifies drug prices, keeps track of production, exports, and imports, and enforces and monitors medicine availability, as well as providing advice to parliament on related issues [20].

CONCLUSION

In our view, the Indian drug regulatory system had various issues related to the approval of drugs and submission of data to the regulatory authority. As of now, both Central and state legislatures control the Indian drug industry. While the state regulatory authorities are liable for directing the manufacturing, sales, and import of medications, the public controller endorses new medications and clinical trials, controls the import of medications, and furthermore arranges among the state bodies.

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REFERENCES

1. Bal BS. An Introduction to Medical Malpractice in the United States. Clin Orthop Relat Res. 2009;467(2):339–47.
2. Health ministry forms committee to investigate charges by parliamentary standing committee [Internet]. [cited 2022 May 23]. Available from: https://www.downtoearth.org.in/news/health-ministry-forms-committee-to-investigate-charges-by-parliamentary-standing-committee-38155
3. Scott ER, Wallsten RL. A look to the future: Pharmacogenomics and data technologies of today and tomorrow. Pharmacogenomics Challenges Oppor Ther Implement. 2018 Jan 1;271–88.
4. Remember the Mashelkar panel report? - The Economic Times [Internet]. [cited 2022 May 23]. Available from: https://economictimes.indiatimes.com/news/company/corporate-trends/remember-the-mashelkar-panel-report/articleshow/2237588.cms?from=mdr
5. Kadam AB, Maigetter K, Jeffery R, Mistry NF, Weiss MG, Pollock AM. Correcting India's Chronic Shortage of Drug Inspectors to Ensure the Production and Distribution of Safe, High-Quality Medicines. Int J Heal Policy Manag [Internet]. 2016 [cited 2022 May 23];5(9):535. Available from: /pmc/articles/PMC5010656/
6. Drug regulatory bodies in India have few inspectors and no records [Internet]. [cited 2022 May 23]. Available from: https://scroll.in/article/943829/few-inspectors-no-records-drug-regulation-in-india-is-hobbled-by-a-number-of-many-problems
7. Biswas P, Biswas AR. Setting standards for proactive pharmacovigilance in India: The way forward. Indian J Pharmacol [Internet]. 2007 May 1 [cited 2022 May 23];39(3):124. Available from: https://www.ijp-online.com/article.asp?issn=0253-7613;year=2007;volume=39;issue=3;spage=124;epage=128;aulast=Biswas
8. Raine J, Wise L, Blackburn S, Eichler HG, Breckenridge A. European perspective on risk management and drug safety. Clin Pharmacol Ther [Internet]. 2011 May [cited 2022 May 23];89(5):650–4. Available from: https://pubmed.ncbi.nlm.nih.gov/21512526/
9. Clinical trial deaths rise in '12, drug makers say not at fault | Business Standard News [Internet]. [cited 2022 May 23]. Available from: https://www.business-standard.com/article/companies/clinical-trial-deaths-rise-in-12-drug-makers-say-not-at-fault-113011000105_1.html
10. India's drug regulation system in a total shambles - India News [Internet]. [cited 2022 May 23]. Available from: https://www.indiatoday.in/india/north/story/drug-regulation-system-in-india-is-in-a-shambles-dcgi-101563-2012-05-09
11. Taylor M, Kirkham R. Health Data, Public Interest, and Surveillance for Non-health-Related Purposes. 2021 Dec 9;8:93–118.
12. Bhaduri S, Kipgen T. 'New Drugs' Approvals in India: An Institutional Perspective: https://doi.org/101177/0971721818762931 [Internet]. 2018 Apr 27 [cited 2022 May 23];23(3):444–62. Available from: https://journals.sagepub.com/doi/full/10.1177/0971721818762931
13. Chowdhury N, Joshi P, Patnaik A, Saraswathy B. Administrative Structure and Functions of Drug Regulatory Authorities in India. 2015;
14. parliament of india rajya sabha 59 th (presented to the rajya sabha on 8 may, 2012) th (laid on the table of the lok sabha on 8 may, 2012) th (presented to the rajya sabha on 8 may, 2012) rajya sabha secretariat new delhi rajya sabha secretariat new delhi. [cited 2022 May 23]; Available from: http://rajyasabha.nic.in
15. Huremović D. Brief History of Pandemics (Pandemics Throughout History). Psychiatry of Pandemics [Internet]. 2019 [cited 2022 Jun 23];7. Available from: /pmc/articles/PMC7123574/
16. Parliament Of India Rajya Sabha 59 Th (Presented To The Rajya Sabha On 8 May, 2012) Th (Laid On The Table Of The Lok Sabha On 8 May, 2012) Th (Presented To The Rajya Sabha On 8 May, 2012) Rajya Sabha Secretariat New Delhi Rajya Sabha Secretariat New Delhi. [cited 2022 Jun 23]; Available from: http://rajyasabha.nic.in
17. Shetty Y, Brahma S, Manjesh P. Compassionate drug use: Current status in India. Perspect Clin Res [Internet]. 2020 Jan 1 [cited 2022 Jun 23];11(1):3. Available from: /pmc/articles/PMC7034137/
18. Prashant Reddy vs Ministry Of Health & Family ... on 26 May, 2020 [Internet]. [cited 2022 Jun 23]. Available from: https://indiankanoon.org/doc/115080764/
19. home [Internet]. [cited 2022 Jun 23]. Available from: https://cdsco.gov.in/opencms/opencms/en/Home/
20. National Pharmaceutical Pricing Authority | Department of Pharmaceuticals [Internet]. [cited 2022 Jun 23]. Available from: https://pharmaceuticals.gov.in/national-pharmaceutical-pricing-authority.

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RESEARCH ARTICLE

Optimization of Vitamins and Amino Acids on the Amylase Enzyme Production by *Bacillus* Spp. in Solid State Fermentation

N.Kiruthiga[1] and N.Pandeeswari[2]*

[1]II M.Sc, (Agri. Microbiology), Department of Agricultural Microbiology, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India
[2]Assistant Professor, Department of Agricultural Microbiology, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India

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***Address for Correspondence**
N.Pandeeswari
Assistant Professor,
Department of Agricultural Microbiology, Faculty of Agriculture,
Annamalai University, Annamalai nagar,
Chidambaram, Tamil Nadu, India
E-Email: kiruthiga0110@gmail.com

ABSTRACT

Amylase is one of the most frequently used enzymes in industry. These enzymes break down starch molecules into polymers comprised of glucose units. The food, fermentation, and pharmaceutical industries, among others, can all benefit from the employment of amylases in industrial processes. Although there are many different sources of amylases, including plants, animals, and bacteria, microbial enzymes frequently meet industrial needs. The bacterium was removed from the cassava trash. By using staining techniques, motility tests, plating under specific conditions, and biochemical testing, the bacteria that had been isolated were identified as *Bacillus amyloliquefaciens, Bacillus licheniformis,* and *Bacillus subtilis*. Following incubation, the blue colour surrounding the microbial colonies in the starch agar medium disappeared, showing that these Bacillus spp. were producing amylase. Waste from the cassava plant served as the substrate for the synthesis of amylase. Solid state fermentation was carried out to manufacture amylase using these *Bacillus* spp. The purpose of this study was to determine the best vitamin and amino acid source for the maximum enzyme production by *Bacillus* spp. In a sequential order, various process parameters were optimized for maximum amylase production. Amylase activity was determined by methods such as decrease in starch-iodine color intensity and Plate assay.

Keywords: Amylase, Amino acid, *Bacillus* pp, Cassava waste, Vitamin.

INTRODUCTION

The most crucial items made by plants, animals, and microorganisms for human use are enzymes. These are the bio-catalysts, which are crucial for all phases of metabolism and biochemical processes. On an industrial scale, some processes have made use of certain enzymes as organic catalysts. Microbial enzymes are among the several types of enzymes and are recognised to be excellent enzymes derived from various microorganisms, especially for usage in commercial enterprises. Enzymes like amylases are replacing many chemical processes, which helps to develop environmentally friendly products and outputs. Amylases are enzymes that break down starch (Souza and Magalhaes., 2010). The Japanese scientist Jokichi Takamine initially created these enzymes in 1894 in Peoria, Illinois (USA) from a source of fungi, and they were utilised as a medicinal aid for the treatment of digestive diseases. According to Azzopardi *et al*., (2016), there are three primary forms of amylases: beta-amylase, found in plants and microorganisms, and gamma-amylase, found in both animals and plants. Alpha-amylase is found in animals, people, microbes, and plants. A characteristic secretory enzyme among them is α-Amylase (1,4-a-D-glucan glucanohydrolase, EC 3.2.1.1). Amylases are starch hydrolyzing enzymes that produce a variety of compounds, such as dextrins and progressively smaller polymers made of glucose units. Amylases are crucial for the starch, detergent, beverage, and textile industries. Commercial production of amylases from microorganisms accounts for 25 to 33 percent of the global enzyme industry. For biotechnological applications in the food, fermentation, textile, and paper sectors, amylolytic enzymes that digest starch are essential. The amylases can be obtained from a wide range of sources, including bacteria, plants, and animals.

In the starch processing sector, microbial amylases have nearly completely replaced chemical hydrolysis of starch because they meet industrial needs, are widely available on the market, and are effective (Sen *et al*., 2014). The ability of microbes to produce amylases in large quantities at low costs is their greatest advantage. In order to create enzymes with the required characteristics, microbes can also be easily controlled. Currently, the global market for enzymes is estimated to be around US $ 2.7 billion and is growing at a pace of 4% per year (Abd-Elhalem*et al*., 2015). Today, *Bacillus* bacterial species are used to create the majority of amylase enzymes. Enzyme production can be greatly increased by media engineering, the addition of various nutrients, and the optimization of ideal growth conditions, all of which promote the growth of microorganisms and the manufacture of better enzyme. In this study, various vitamin and amino acid sources were evaluated for their ability to produce a significant amount of amylase enzyme. Recently, solid state fermentation (SSF) has received a lot of interest and is thought to be a useful substitute for the production and utilisation of enzymes (Govarthanan*et al*., 2015). Organic wastes are used as a substrate in the solid state fermentation process, which lowers production costs and the amount of capital needed to create the amylase enzyme. By using organic wastes as substrates, it also increases awareness of the importance of waste management and the sharp increase in waste materials.

The root vegetable cassava, also known as *Manihot esculenta*, is native to Brazil and Paraguay but before the advent of the Colombus, it spread throughout the tropical areas of South and Central America. It ranks as the sixth-largest food crop in the world right now and one of the most crucial in tropical regions. Cassava is frequently called to as "bread of the tropics," "meal of the poor," or "poverty fighter" in tropical Africa because to its drought resistance and ability to flourish in practically any type of soil, even marginal soils. It is not a well-known or well-liked food crop in Western countries. Cassava is a 3 to 5 metre tall perennial herbaceous plant native to the tropics. Long petioles hold the palmate, deeply indented leaves, which have three to seven lobes, to a short stem. Small greenish-yellow blooms grow panicles, which develop into seed capsules and mature into seed capsules that ripen into seed capsules that rupture to release seeds. The plant's roots generate sizable sweet potato-like starchy tubers. Cassava is one of the most adaptable plants in terms of habitat suitability. It can live in both humid tropical climes and dry circumstances and can adapt to grow in nutrient-poor soil where other plants cannot. This low-maintenance plant defends itself against a range of predators using its poisonous latex, which freely pours through the leaves. The ideal environments for it to grow are tropical ones. Waste management had become a major issue across the globe in both developed and underdeveloped countries. Developing countries currently face a challenge that industrialised countries are only partially capable of fixing due to ineffective and dishonest waste management practises. For instance, it has long

been common practise in Nigeria to release solid waste into the environment unsorted. Mountains of solid trash are frequently piled up in open fields, incomplete buildings, and abandoned land as a result. Since agricultural waste, in particular garbage from cassava crops, makes up a larger fraction of the total wastes produced each year, the Salem District of Tamil Nadu suffers a disposal problem. As a potential industrial development for the utilisation of cassava waste, the bioconversion of cassava waste into proteins, biomolecules, organic acids, and other food-related substances has been researched. As a result, the study's main objectives were to identify and characterise amylolytic bacteria from cassava waste dumps as well as to partially characterise the synthesis of enzymes and their properties in connection to the influence of various vitamins and amino acid sources. The study was carried out at a temperature 50°C and at pH 6.0. The best carbon and nitrogen source for the maximum enzyme production was found to be Maltose and Beef extract from the previous studies. The incubation period and age of inoculum was found to be 3 days and 2 days for optimum production of amylase enzyme.

MATERIALS AND METHODS

Isolation of *Bacillus* spp.

The solid cassava waste (Cassava baggase) was used to isolate amylolytic bacteria. The bacteria will be isolated on nutrient agar plates using the serial dilution method. In this situation, the appropriate bacteria will be inoculated on NA media using 0.1 ml of the sample's (10^5) dilution. For 24 hours, the infected medium will be incubated at 37°C. Utilizing colony forming units per millilitre, discrete colonies that developed on the plates will be counted (cfu ml^{-1}). To create the pure culture, the isolates will be subcultured once more on slants. For the slants, it will be kept at 4 degrees.

Screening and identification of amylase producer

During storage at 4°C, isolated bacterial colonies will be further examined for their amylolytic capability by being inoculated on a starch agar plate. Plates with inoculations will undergo three days of incubation at 37°C. Gram's iodine solution (1 g of iodine crystals and 2.0 g of potassium iodide will be dissolved in 100 ml of distilled water, stored at room temperature), which can be used to detect amylolytic bacteria, will be extensively poured over the plates after three days of incubation (Pokhrel*et al*., 2013).

Characterization of bacterial isolates

Iodine and starch combine to create a dark blue mixture that completely covers the agar. The positive colonies show a distinct zone of hydrolysis around the colonies when flooded with gram's iodine solution, whereas the negative colonies do not, as observed by a blue-black colouring on starch agar. (Parmar *et al*., 2012, Benkiar*et al*., 2013, Kaur *et al*., 2012).

Optimization of culture conditions

The effect of culture conditions the present study will be carried out at different vitamin sources (Biotin, Riboflavin, Thiamine, Pyridoxine and Ascorbic acid) and different sources of amino acids (L-Lysine, Tyrosine, Cysteine, Glycine and Alanine) for the aim of producing high quantity of amylase enzyme.

Solid state fermentation

Twenty grams of starch and five grammes of substrate will be combined with a bacterial amylase production containing (g/l) KH_2PO_4, NH_4NO_3, KCl, $MgSO_4.7H_2O$, and $FeSO_4.7H_2O$. Distilled water will then be added to this mixture to get the necessary moisture level (Cassava Solid Waste). The flask contents will be well mixed before being autoclaved at 121°C for 20 minutes. Solid state fermentation will be performed using 2 ml of each suspension of *Bacillus amyloliquefaciens, Bacillus licheniformis,* and *Bacillus subtilis* for a total of 72 hours at 30°C with a starting moisture content for the substrate of 64%. (Castro *et al*., 2010).

Enzyme extraction

The cultures were then given 22 ml of 0.1 M phosphate buffer (pH-6.5), and they were shaken on a rotary shaker for 30 minutes at 19°C and 140 rpm. This mixture was filtered through a cheesecloth filter and centrifuged at 8000 rpm and 4°C for 15 minutes. Whattman Number-1 filter paper was used to separate the centrifuged supernatant filtrate, which was then used for more research.

Estimation of amylase activity

The rate of maltose release from starch, which was measured by its capacity to decrease 3,5 dinitrosalicylic acid, served as a proxy for the amylase activity (DNSA). The amount of amylase that can release 1 mg of maltose per minute at 25°C was found to be equal to one unit of amylase activity. In phosphate buffer, the substrate 1 percent cassava baggase was gelatinized. One millilitre of the substrate and one millilitre of the enzyme solution made up the reaction mixture. After being incubated for 5 minutes at 25°C, it was stopped by adding 1 ml of the DNSA colour reagent. In a water bath, the combination was heated for five minutes at 100°C. After cooling, it was diluted with ten millilitres of distilled water. The optical density was measured at 540 nm after the reaction mixture had stood at room temperature for 15 minutes. Amylase activity was measured in units as follows:

$$\text{Enzyme activity (Units/ml)} = \frac{\mu\text{g of maltose released}}{\text{Volume of enzyme taken(1 ml)} \times \text{Time of incubation}}$$

Assay of enzyme activity

Decrease in starch-iodine color intensity

Starch reacts with iodine to create a compound that is deep blue in colour. The starch then gradually began to hydrolyze, changing from white to red and brown. Numerous studies have suggested numerous techniques for the quantitative measurement of amylase in order to support this characteristic. This approach measures the decline in the iodine colour response to determine the amylase's dextrinizing activity.

Plate assay

The plate test made use of starch-modified agar plates. The agar plates were made by combining 1.5:2 agar and starch. A well with a diameter of roughly 10 mm was aseptically carved out with a cork borer after the agar had set. The culture filtrate was poured into the well, which was then incubated at 37°C overnight. The well's hydrolytic zone could be seen, and the agar had been covered with a coating of 1% iodine solution. Sterilized water was added to the separate well to maintain the viability of the negative control.

RESULT AND DISCUSSION

Singh *et al*., (2015) confirmed the production of amylase enzyme from *Bacillus* isolates using iodine on starch agar medium and the creation of a clear zone around bacterial growth. The amylase enzyme was produced by *Bacillus amyloliquefaciens, Bacillus licheniformis,* and *Bacillus subtilis* using cassava waste from the Sago processing firm in Salem. The bacterial strain used in this study that made amylase more efficiently was initially described as being gram-positive, one micron long, spore-forming, and having core spores that were frequently smaller than the cell. Using Gram staining, a motility test, a selective medium, and biochemical tests, the *Bacillus* spp. were extracted and identified (*Bacillus amyloliquefaciens, Bacillus licheniformis,* and *Bacillus subtilis*). We have previously reported the optimum pH, temperature, carbon source, nitrogen source, incubation period and age of inoculum for alpha amylase production by *Bacillus amyloliquefaciens, Bacillus subtilis, Bacillus licheniformis*. To further characterize the enzyme production, in this study we determine the best vitamin and amino acid source. Effects of different vitamin sources (Biotin, Riboflavin, Thiamine, Pyridoxine and Ascorbic acid) on amylase enzyme production by *Bacillus amyloliquefaciens*, *Bacillus licheniformis*, *Bacillus subtilis* are described in Figure-1. As shown in Figure-1, among the various vitamins, thiamine stimulated the growth and enzyme activity of the bacterial isolate followed by pyridoxine , riboflavin, biotin and ascorbic acid. Similar observation was made by Ali *et al*., (2010) by *Bacillus* sp on amylase

production. In this present study, the enzyme production was found maximum at a vitamin source Thiamine (565 Uml^{-1}) recorded by *B. licheniformis* and found minimum at vitamin source Ascorbic acid (314 Uml^{-1}) recorded by *B. subtilis* (Figure 1). After determining the best vitamin source for maximum amylase enzyme production, different sources of amino acids, L-Lysine, Tyrosine, Cysteine, Glycine and Alanine were tested for cell growth and amylase production. Among the amino acids tested, cysteine was found to be the best amino acid for amylase enzyme production followed by glycine, tyrosine, L-lysine and alanine (Figure-2). The result was found similar to the observation made by Varalakshmi *et al*., (2012) by *Pseudomonas* sp. 2 on amylase enzyme production. Effects of different amino acids on amylase production by *Bacillus amyloliquefaciens*, *Bacillus licheniformis*, *Bacillus subtilis* are described in Figure-2. In this present study, the enzyme production was found maximum at an amino acid source cysteine (663 Uml^{-1}) recorded by *B. licheniformis* and found minimum at alanine (450 Uml^{-1}) recorded by *B. subtilis* **(**Figure 2).

CONCLUSION

The natural bacterial flora of the cassava baggase, which was procured from Salem's agricultural enterprise, was discovered in the present investigation to produce amylase. 5 of the 15 bacterial isolates evaluated for the production of extracellular amylase in starch agar medium were found to be positive as evidenced by the presence of a zone of hydrolysis on starch agar plates. In order to make amylase, the bacteria *Bacillus amyloliquefaciens, Bacillus licheniformis* and *Bacillus subtilis* were used, with cassava waste serving as the substrate during solid state fermentation. The amylase activity of each of the 5 bacterial isolates determined during the preliminary screening was quantitatively evaluated. It is possible to further characterise the chosen bacterial isolate that showed a significant quantity of amylase activity for a number of advantageous commercial applications. The selected bacterial isolate that showed considerable amylase activity can be further tested for different vitamin and amino acid sources for the growth of bacterial species and for maximum enzyme production.

REFERENCES

1. Abd-Elhalem, B.T., El-Sawy, M., Gamal, R.F., and Abou-Taleb, K.A. (2015). Production of amylases from *Bacillus amyloliquefaciens* under submerged fermentation using some agro-industrial by-products. *Ann. Agr. Sci*, 60(2), 193–202.
2. Ali, S,S., Nazeer, M and Riaz, T. 2010. Effect of salt stress and Vitamin B1 on amylase production potential in locally isolated bacteria. *Punjab University Journal of Zoology*, 25(1-2): 67-80.
3. Azzopardi, E., Lloyd, C., Teixeira, S.R., Conlan, R.S and Whitaker, I.S. (2016). Clinical applications of amylase: Novel perspectives. *Surgery,*160 (1), 26-37.
4. Benkiar, A., Nadia, Z.J., Badis, A., Rebzani, F., Soraya, B.T., Rekik, H., Naili, B., Ferradji, F.Z., Bejar, S., and Jaouadi, B. (2013). Biochemical and molecular characterization of a thermo and detergent-stable alkaline serine keratinolytic protease from *Bacillus circulans* strain DZ100 for detergent formulations and feather-biodegradation process. *Int. Biodeterior. Biodegrad,* 83, 129–138.
5. Castro, A.M., Carvalho, D.F., Freire, D.M.G., and Castilho, L.R. (2010). Economic analysis of the production of amylases and other hydrolases by *Aspergillus awamori i*n solid state fermentation of babassucake. *Enzyme Res,* 1, 9.
6. Govarthanan, M., Selvankumar, T., Selvam, K., Sudhakar, C., Vincent, A., and Kamala-Kannan, S. (2015). Response surface methodology based optimization of keratinase production from alkali-treated feather waste and horn waste using *Bacillus* sp. *MG-MASC-BT. J. Ind. Eng. Chem,* 27, 25–30.
7. Kaur, A., Kaur, M., Samyal, M.L., and Ahmed, Z. (2012). Isolation, characterization and identification of bacterial strain producing amylase. *J. Microbiol. Biotech. Res,* 2(4), 573-579.
8. Parmar, D and Pandya, A. (2012). Characterization of Amylase Producing Bacterial Isolates. *Bull. Environ. Pharmacol. Life Sci,* 1, 42-47.
9. Pokhrel, B., Wanjare, P., Singh, S. (2013). Isolation, screening and characterization of promising-amylase producing bacteria from sewage enriched soil. *Int. J. Adv. Biotechnol. Res*, 4(2), 286-290.

10. Sen, S.K., Dora, T.K., Bandyopadhyay, B., Mohapatra, P.K.D., and Raut, S. (2014). Thermostable alpha-amylase enzyme production from hot spring isolates *Alcaligenes faecalis* SSB17 – Statistical optimization.*Biocatal. Agric. Biotechnol.*
11. Singh, V., Sharma, R., and Sharma, P. (2015). Isolation, screening and optimization of amylase producing *Bacillus* sp. from soil. *Asian Pac J Health Sci,* 2(3), 94-103.
12. Souza, P.M.D and Magalhaes, P.D.O. (2010). Application of microbial α-amylase in
13. industry-a review. *Brazilian Journal of Microbiology*, 41(4), 850–861.
14. Varalakshmi, K.N., Pallavi, N.U., Arpita, B., Bhuvaneshwari, S., Mrudula, P and Priyanka, G. 2012. Production and partial purification of α-amylase from *Pseudomonas* sp. 2 under solid-state fermentation. *Turkish Journal of Biochemistry*, 37(1): 21-28.

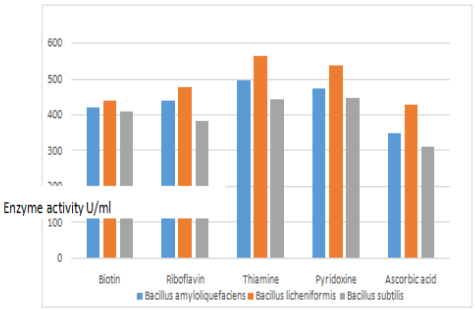

Fig 1: Effect of vitamins on the amylase production

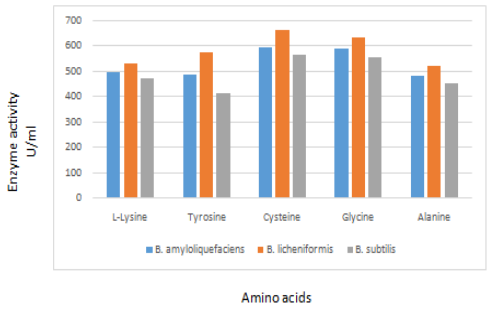

Fig 2 : Effect of amino acids on the amylase production

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RESEARCH ARTICLE

Impact of Integration of IoT and Cloud Computing and their Role in Smart Cities: Case Study of Ahmedabad

Archana Sharma and Prateek Jain*

Assistant Professor, Amity University Noida, Uttar Pradesh, India.

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***Address for Correspondence**
Prateek Jain*,
Assistant Professor,
Amity University Noida,
Uttar Pradesh, India.
Email:prateekjain2010@gmail.com

ABSTRACT

IoT contains more information about the world than we have ever accessed before, and this interconnectedness is generating vast amounts and varieties of data from numerous devices, objects, people and systems at a supreme volume and velocity. Within the ecosystem of interconnected and interactive systems all devices must be integrated, work together, and communicate seamlessly with all connected systems and infrastructure. The data produced from these multiple interactions must be secure, analysed, integrated and actionable. For smart cities that have information gathered from people, things, houses, and other sources, applications for the IoT on the cloud may be helpful. This information is processed and assessed to control and observe many systems, including those for managing waste disposal, transportation networks, electric utilities, resource management, proficiency, digital libraries, healthcare facilities, and other opportunities. A cloud service provider provides third parties with the ability to incorporate IoT data within electronic devices running on the IoT using public cloud services that can update the IoT environment. The authors examined cloud based IoT apps and their functions in smart cities in this research with the case study of Ahmedabad.

Keywords: IoT, Cloud Computing, Smart City, Sensor, Cloud of Things, ICT, API

INTRODUCTION

As the cloud computing, which is the next evolution in internet-based computing, Now, it's possible to leverage information technology capabilities as a service. The Internet of Things has the potential to boost throughput, performance, and effectiveness when intelligent devices depart the cloud infrastructure environment.. Urban areas that systematically work to achieve environmental sustainability, urban system authority, better public health, knowledge development, and network-driven advancement are mentioned to as smart cities [1-3]. Smart cities also

see for themselves where records in addition to communication technologies are headed. The next phase of internet-based computing, cloud computing, enables the transmission of information and communication technology (ICT) resources across a network. Increased productivity, performance, and payload are some benefits of cloud infrastructure for IoT. The manner that cloud computing has made it possible to create, distribute, and sell electronic packaging. As a result, IoT and cloud are now very near to emerging internet technologies that are compatible with IoT systems. IoT is an organization of web-enabled devices that practice sensors to gather data from their surroundings, process it, and transmit it over the network. The Internet of Things (IoT) has established the prior few decades and is now present in practically all practical applications [4, 5]. These days, there are billions of linked, different gadgets that produce a lot of diverse data (big data). These various items, which can be connected, actuated, or monitored, are represented in Fig. 1. They include sensors, actuators, household appliances, cellphones, smart devices, autos, and a variety of other objects. Using heterogeneous access networks, these devices are not only connected to one another but also to the internet [6–8].

These numerous networked devices seek to create a sophisticated and sustainable society and economy. The biggest issue with these IoT devices is their limited ability to process and store real-time data due to their IoT sensors' poor computing and storage capabilities. The dependability, efficiency, high performance, scalability, and ubiquitous accessibility of these IoT devices are necessary for them to deliver the anticipated benefits. IoT platforms typically make use of cloud computing advantages to store, process, and present a sizable amount of acquired data [9–12].
The IoT is mostly focused on problems that develop in a dynamic, shared environment. IoT is a large category made up of a variety of adaptive and unconventional devices with constrained storage, power supply, and performance capabilities. These limitations, which include complicated problems like compatibility, efficiency, full functionality, and availability, provide a barrier and a hindrance to the development of IoT systems. The most intriguing approach that could be used in conjunction with IoT to get over these constraints is cloud computing. Shared resources (network, storage, computers, and software) are offered via the cloud and are distinguished by their accessibility, affordability, and aesthetic appeal.To collect, transfer, analyze, process, and store data, this platform may make use of cloud resources and services. To gather, transmit, search for, analyze, and store data produced by complicated scenarios, it may also leverage cloud resources and services. The cloud based IoT platform to create apps is shown in Figure 2.Every project in the private and public sectors, including industrial systems, emergency deliveries, public transportation, public safety, city lighting, and other urban applications, has embraced the Internet of Things. Cities are connecting more as the IoT grows, allowing them to improve the dependability and responsiveness of emergency services as well as the effectiveness of infrastructure construction. In the approaching years, researchers are ready to examine cutting-edge ideas for smart cities that utilize IoT solutions. The articles that were published between 2012 and 2021 are shown in Table 1.

Cloud Is the Key for Internet-Based Computing

The next stage in the development of Internet-based computing is cloud computing, which enables the delivery of ICT services as a carrier. Cloud computing allows for the connection of various essential resources, including computer capabilities, infrastructure (such as servers and storage), systems, and business processes [4]. With the advent of cloud computing, it has become simpler to create adaptable business models that let companies utilize resources as their operations expand. The development of flexible business models that allow businesses to use resources as their operations grow has gotten easier with the introduction of cloud computing. Contrary to businesses that offer conventional web-based services (like web hosting), cloud computing enables instant access to cloud delivery without a drawn-out provisioning process. In cloud computing, each provisioning and withdrawal of resources can happen again. Applications and resource records can communicate in the cloud thanks to APIs (application programming interfaces), which also let consumers access cloud services. Payment options include invoicing and rating providers, which offer the assistance needed to use the rating aid and to make payments in advance. Monitoring and assessing performance: In addition to the integrated physical computing system and its methodologies, cloud computing infrastructure offers a carrier management environment for monitoring and assessing performance.

Security

The cloud based architecture provides secure operations to safeguard sensitive data. The two primary commercial factors influencing the uptake of cloud computing and related services are as follows: (1) A commercial organization. Cloud computing offers two benefits: (1) cost savings; and (2) flexible, timely, and necessary access to computer resources as needed to achieve corporate objectives. By transforming capital expenditures (CapEx) into operating costs, cloud computing promises to save costs. This is because cloud computing favors pre-existing management and enables more flexible scheduling and resource allocation.

How Does the Cloud Allow IoT Applications?

IoT applications that use the cloud are expanding and interacting with one another online. The introduction of cloud based IoT applications as well as service hosting and deployment are made possible by the cloud. In addition, cloud computing provides a suitable Internet platform for the archiving and processing of data from smart devices, including connected cars, smart grids, smart cities, Wi-Fi, sensors, and actuator networks. It is possible to set up network setups rapidly and efficiently. But software is used for back-end activities, enabling rolling back, location monitoring, content labeling, and performance monitoring [11–15]. Cloud computing also strengthens the reliability of IoT systems. By combining the cloud and IoT, developers may create backups of hardware and software that run in the cloud, making them more error-tolerant. They can also be utilized to track data offline. To support their IoT response, developers can also build up virtual servers, run programs, and launch a database.

Integration of Cloud and IoT

The capability of cloud computing to revolutionize service delivery patterns over the whole present IT (Information Technology) industry has attracted the interest of both academia and industry throughout the world. It offers criteria for service provisions with low initial outlay, anticipated performance, high availability, exceptional fault-tolerance capabilities, infinite scalability, and more [39].According to [40], the services are alienated into three tiers: Computing resources like processing power or storage are made available through infrastructure as a service, or IaaS. Platform as a Service (PaaS) allows software developers to design their applications in line with the specifications of a certain platform starved of having to burden about the supporting hardware infrastructure. Since SaaS (Software as a Service) refers to the actual software programmes that are downloaded and used, it is the cloud computing layer that is most visible to operators. In addition to the fundamental layers, there are additional levels that are introduced and discussed in the literature, including DaaS (Data as a Service), NaaS (Network as a Service), IPMaaS (Identity and Policy Management as a Service), and others. Everything as a Service, or XaaS, is a theory that encourages "pay as you go" and lets customers use service providers' products while only being charged for the resources they really use. The authors introduce it in [41]. Within the framework of the Internet of Things, this strategy offered feedback to the so-called ultimate cloud consumers, developers, and service providers. The authors of [42] give a comprehensive analysis of one fascinating scenario that makes CoT possible. They concentrate on the internet of things (IoT) infrastructure's four conceptually layered Sensing according to a Service model.

Owners of Sensors as well as Sensors is a collection of sensors that explains how their owner controls them and makes decisions regarding whether or not to publish their data within the cloud. Before publishing sensors online, sensor publishers look for available sensors, get in touch with the owners, and get their permission. Extended service providers interact with a variety of sensor publishers to select sensors based on customer demands. Sensor data users who need to register to access the information. Numerous advantages and benefits are promised by the Sensing as a Service approach. To name a few, there are several advantages, including the capacity to reuse and share sensor data (if someone has already deployed the sensors, others can access them by paying a fee to the sensor owner), lower data acquisition costs because the data are shared, and gather previously inaccessible data (thanks to the business model, companies are encouraged to "sell" them sensor data).

IoT Cloud Architecture

Several components are implanted to create an intelligent network of interconnected items, including sensor technology, gateways, RFID, and other smart technologies [43]. Consider the figure 3 shows a basic IoT cloud infrastructure. IoT sensors and devices that people wear provide the raw data for the perception layer. Data is accessed via internet gateways at the network layer. The edge computing layer is used for data pre-processing and cleansing. On the cloud platform, further data analytics and prediction tasks were carried out utilising a variety of machine learning algorithms. The major objective of the IoT is to improve and simplify human life, either by assisting people in making better decisions or by assisting them in living with less stress, less monotonous labour, and less human contact through IoT computer technology, according to the IoT's advocate [44].

IoT Cloud Platforms

According to the IoT's proponent [45], the main goal of the IoT is to enhance and simplify human existence, either by supporting people in making better decisions or by assisting them in doing so while also reducing stress, tedious work, and human contact.

Importance of Cloud-Based IoT Applications for Smart Cities

Cities have shifted to IoT and connection technology for a variety of reasons. IoT systems enable sensors to collect data to manage appliance consumption, potentially saving a significant amount of money. When determining whether to go offline or online, cost is a crucial consideration because deploying and maintaining IoT apps is easier. Efficiency is utmost crucial variable [46]. In addition, costs are declining, and communications' power output and endurance enable new scenarios that were before impractical. Service providers essentially physically visit the website to analyze and construct communications infrastructure for the most demanding solutions. Wireless communication provides monitoring and management of IoT transmission through a variety of analytics. To enable firmware updates, apply security fixes to all completed plans, and receive automatic problem notifications, administrators can do this. As it should be, especially in operational situations, diminished support is frequently to blame when smart road illumination and tracking equipment are maintained.

Smart City can be built using the six steps below.

Phase 1: A complete smart city platform

A complete smart city platform is available at the beginning level. Create a simple implementation design for smart cities to begin with. You can improve newly released services while maintaining overall performance and create the foundation for further development [5]. The basis of the smart city solution is comprised of the several needs listed below. Things in a Network with Intelligence Smart cities combine IoT and smart devices with sensors and actuators. Data collection and transmission to a dependable cloud control platform are the goals of the sensors. Actuators make it possible for equipment to work by adjusting the brightness of lights or managing the flow of water through a leaky pipe. using secure communication gateways Each Internet of Things (IoT) device has two main parts: hardware and software. Applications play a big role in how data moves between objects. Gateways should be used to control the data. Data collection and compression are made simpler by these gates, which examine data before sending it to the cloud. The cloud is a component of the city's smart solution for ensuring secure data transmission across neighbourhood gates. understanding of the pool The pool of data was initially developed as a tool for record-keeping. The case for smart cities is supported by data sources. Information is obtained from the pool and given to the location upon request. A sizable record shop large databases are part of vast record warehouses. In contrast to statistics pools, it has incredibly well-organized data. The real data is removed, changed, and sent into a sizable data warehouse after it has been found.

Phase 2: Data Evaluation and observation

At the entry level, a whole smart city platform is accessible. Start by developing a straightforward implementation strategy for smart cities. While preserving overall performance and laying the groundwork for future growth, you can enhance recently released services [5]. The several needs stated below make up the foundation of the smart city

solution. Things with Intelligence in a Network IoT, smart devices, sensors, and actuators are all combined in smart cities. The sensors' objectives are to gather data and transmit it to a reliable cloud control platform. By controlling the flow of water via a leaking pipe or regulating the brightness of lights, actuators enable equipment to function. making use of secure communication gateways Hardware and software make up the two fundamental components of every Internet of Things (IoT) device. Applications have a major impact on how data is transferred between objects. The data should be controlled via gateways. These gates, which inspect data before transmitting it to the cloud, simplify data collecting and compression. The city's smart approach for assuring secure data flow across neighbourhood gates includes the cloud. knowledge of the pool The database was initially created as a tool for keeping records. Data sources help make the case for smart cities. The pool provides information that is requested by the location. a large record store Huge record warehouses include large databases. It has data that is exceptionally well arranged, unlike statistics pools. Once the genuine data is discovered, it is altered, erased, and sent into a big data warehouse.

Phase 3: Data analysis

The extent of data formed by communities, transportation systems, and digitization is enormous and growing swiftly. The production process is greatly sped up by new IoT technology like devices and cloud services. Our understanding of urban environments and the efficiency of urban movement can be considerably improved through analysis, modeling, and information extraction from this data. A large amount of historical sensory data is scanned by ML algorithms in order to evaluate development and provide precise models. Actuators on Internet of Things devices receive orders from their control package models. Unlike standard traffic modes, which are designed to display a notice for a set period of time, traffic scenarios allow intelligent visitors to change the entry timings. In order to distinguish between different types of visitors to a location, govern signal timing, enable acceleration of the average car speed, and avoid traffic based on out-of-date sensor systems, ML algorithms were constructed.

Phase 4: A deft control

Control systems ensure that smart city technology is highly automated by sending commands to their actuators. They give instructions to the right staff on how to handle a specific issue. Typically, machine learning is integrated into rule-based management systems (ML). Falsely based system standards are manually expressed whereas models created using ML techniques are used in ML-controlled applications. These trends are discovered using statistical tests that are reevaluated, approved, and updated on a regular basis.

Phase 5: Automated traffic control

In addition to the possibility of autonomous control, smart city initiatives should always include a customer-driven option (for example, in an emergency condition). User programmes are used to run it. Users of user programmes can connect to the municipal management platform to discover IoT devices, manage them, and receive alerts and warnings. For instance, a smart traffic control system uses GPS information from smart phones to detect traffic jams. Consequently, a notification instructing local cars to take alternative routes is automatically sent out. A tourist management system is used in the field of smart cities to monitor crowding in real-time and implement visitor ideas to lower the number of site visitors in congested locations. To lessen environmental harm caused by visitors, the smart city also includes a visitor management response and a smart air tracking system. Using a computer application, a visitor center's staff can receive notifications concerning busy regions. To lessen and reroute traffic congestion, the robot investigators are given instructions on how to manage the notifications.

Phase 6: Combining many options

Integrating IoT-based various solutions demands for growing functionality more so than the variety of senses, and this should be done.

Smart City Applications

A collection of companies that handle topics like traffic, wastewater management, emergency services, tourism, etc. are referred to as "smart cities." Innovative new city jobs are anticipated to grow more popular and technology-focused based on the demands of certain use cases. Figure 3 depicts some smart city applications.

Systems for Lighting in Smart Cities

Utmost common IoT application for smart cities is lighting, and several governments already rely on IoT to reduce costs and consumption. A ruggedized Digi wr44r router class is part of the system and offers connectivity and authentication for moving a smart pole with several device nodes. These are just a few possible uses for smart lighting. Natural perceptions, security cameras, electronic billboards, electric vehicle charging stations, and wireless technology access. Street light control and maintenance are made feasible and cost-effective by the usage of IoT in smart homes. By adding sensors to streetlights and connecting them to a cloud management service, the lighting may be synchronized [47]. To improve the lighting schedule, smart lighting systems track the movement of light, people, and vehicles. They then combine this information with historical and contextual data (such as specific functions, public distribution systems, the time of year, etc.) and perform analysis. The lights around a road are activated when a pedestrian crosses it.

Transportation

Transportation infrastructure is a part of smart city applications that is expanding quickly. In terms of cost savings, security, route management, and enhanced passenger experience, smart cities and the transportation sector stand to gain greatly. Many communities have seen a drop in shipping as a result of the advent of buses, trains, and wireless passenger connectivity in recent years, but many are currently witnessing additional developments.

Water Management

Smart city programs such as wastewater management, water tracking, and environmental restoration all make use of water management software [48]. In places like state-owned corporations and adjacent towns, IoT packages are becoming more prevalent. It promotes productivity, enhances visibility of remote tanks and water management plans, and lowers the cost of monitoring and supporting their facilities. It also improves access to ageing infrastructure. The gateway connects to a network of services that help with a variety of problems, including water levels and tank pressure. Digi far-flung is a remote-control tool that can connect IoT systems and devices into modules and sensors as well as test the individual parts of an IoT distribution tool. U.S. Water was created in response to a request from Digi to set up a regional, remote tracking and management solution using their cutting-edge technology. U.S. Water provides water treatment services to commercial customers across the United States and Canada.

Smart Tourism

Finding methods to enhance site traffic is one of the hardest problems that big cities face.. One of the fussiest cities in the world is Los Angeles, which has developed an effective shipping plan to handle tourists. Real-time updates on traffic crashes are provided by sensors buried in the pavement to an essential traffic control platform, which analyzes the information and modifies the site visitor lights to match the flow of traffic. None of these techniques require human participation; instead, To forecast where traffic will travel, they examine previous data.. To accomplish this, local governments mandated IoT development and required user feedback on smart sites. Smart traffic solutions use sensors to assist drivers in determining the range, location, and speed of their vehicles in addition to GPS data collected by cell phones [49]. To reduce traffic congestion, intelligent visitor lights that are linked to a cloud control platform simultaneously enable time measurement and light management built on the status of the visitors. Intelligent traffic management systems may also predict where people will cross and take precautions to prevent power shortages by looking at historical data. They used tourist replies, for instance, to help manage traffic flow because due to highest tourist visitors in the world. Street surface sensors and closed-circuit televisions on a strong

guest management platform deliver simulated actual information on traffic movement. Through applications, the platform analyzes data and notifies users about traffic jams and misuse of road signs.

Smart Parking

One may also utilize intelligent parking systems that can identify when a car has left a parking space. Ground sensors notify the vehicle about the available parking space through an android app to the user. The availability of parking spaces is determined by smart parking which is also helpful in providing a real time parking map using the GPS. Drivers can use a map on their phone instead of their own memory to get the parking space and get their vehicle parked.

Smart Remote

The solutions provided by IoT in Smart Cities will help the citizens to get application administration customer services. People may use smart remote for operating various devices like television, AC [51]. For instance, a homeowner might turn off the geyser installed in his home by using a smart IoT based remote. Furthermore, the Utility companies can also notify local bodies of emergencies and send out experts to handle concerns.

Waste Administration

Waste management systems take care of any environmental problems brought on by inefficient waste chains in addition to reducing operational expenses and enhancing waste chain efficiency. These responses consist of a waste can stage sensor and a phone notification for the truck driver's management platform when they cross the border. The message assists individuals to avoid empty drains by having them take the advised action. These techniques may serve as a guide for many open garbage collection operators. Garbage tracing, the adoption of methodologies, and performance monitoring are promoted through IoT-powered city-based reactions that help enhance waste collection schedules [52]. A sensor in each waste field counts the amount of trash that is present. The trash management system finds sensor data, examines it, and notifies the mobile app on the vehicle. The container is fully empty after the truck driver has poured out all its contents. Tracking the essential elements necessary for a healthy environment is made simple by the IoT smart city solutions offered by the neighborhood. For example, a large city might use a cloud platform to assemble a sensory group from all around the water system and identify the biggest portion of waste.

Social Security

Real-time analytics, tracking, and replacements for social security are provided via IoT and smart cities. PSS's can predict the severity of crime by a data combination from the city's gadgets and CCTV recordings with readings and feeds from social media. These social security apps would help the police deter or punish criminal activity. Utilizing networked gadgets in the smart city is the solution. For instance, after a crime, the information from the device is transmitted to a cloud platform, the data is analysed, and the perpetrator is located. Between the handgun and the mobile device that reported hearing a gunshot, the platform measures the distance and the amount of time that has passed. Next, the cloud program can notify law enforcement through a android application [53].

Air Control Platform

The various tools of IoT can be helpful in the real-time pollution forecasts and detection. Cities can pinpoint the major point of occurrence of their emissions problems and thereby they can contemplate strategic approaches to diminish air pollution. Regulations adhere to the law and can be applied, for instance, to seize control of nearby aircraft for tourists. It is crucial to monitor the volume of greenhouse gasses in the atmosphere [54].

Advantages of IoT and Cloud computing Integration

IoT and cloud system integration has a number of benefits. Here are a few perks that are listed.

Analysis

Aggregated sensor networks are collected using the cloud computing prototype, together with substantial amounts of unstructured sensor data. This integration makes this knowledge available for research.

Scalability

If an organization requires more resources, it can add any new cloud merchant services without incurring additional costs. This is referred to as a cloud of sensors' scalability.

Visualization

The sensor's cloud infrastructure provides a platform for innovation to gather and access sensor data from diverse sources.The Collaboration-Sensor cloud enables several categories of shops to exchange sensor data, thus connecting several physical sensor networks. The allocation of data storage and unneeded processing facilities, as well as the provision of an application that can manage a large amount of data, are all ways to improve data processing and storage. Sensor clouds with dynamic service processing can access their data at any time and from any location.

Flexibility

It enables the user to extend the capabilities of the earlier computer technique. It enables us to communicate and store sensor data in a setting that allows for a variety of uses. Rapid response time is made possible by the combination of wireless sensor networks (WSN) with cloud computing. As a result, it is regarded as a real-time application.

Automation

Cloud computing for sensors heavily relies on automation. Additionally, it lengthens the transmission time for necessary adjustments.

Smart City: Case study of Ahmedabad

Ahmedabad was one of the first 20 smart cities to be chosen by the Indian government in 2016 as part of the SMARTNET project, which the government had previously announced. The goal of the program is to encourage the growth of smart cities through centralized financial support. The government intends to create smart cities as part of this project, and the outcomes should become apparent by 2022. To combat the growing traffic and congestion on the roadways, Ahmedabad intends to use renewable energy sources and an intelligent traffic system [55]. A projected metro proposal is under way, and Ahmedabad has a dedicated bus lane known as BRTS (Bus Rapid Transit System). The government wants to make a universal card that may be used to pay for parking, BRTS, and other services. Our smart gadgets would also have an app that would allow us to check our balance and trips. Additionally, the app would have a real-time parking feature that would display the closest parking spaces nearby. The anticipated parking space would result in less traffic and more room on the roads [56].

Ahmedabad experiences traffic issues, same as Porto. Due to the narrow roads and heavy traffic, Ahmedabad's older neighborhoods are particularly dangerous due to this issue. A simple sensor on the trash dumpsters in this kind of situation could assist in reducing traffic. When the garbage can is full, the sensor will indicate it, and the garbage trucks will plan their routes accordingly. This reduces emissions and aids in traffic management. Like Porto, the Wi-Fi network from the buses may gather data from across the city to create a wireless network throughout the city.
The Ahmedabad municipal government has recently started using an intelligent transit management system to enable real-time data collection and give passengers and traffic controllers feedback. To help commuters, determine the precise time that a bus would arrive at the station, it would display real-time traffic in various locations. Along with this Automatic Fare collection has also been targeted on Bus stations to ease operations [56].

The potential to profit from tourism exists in Ahmedabad. It is the first World Heritage Site in India. Being a responsible city entails looking out for both its visitors and its residents. Tourists want to visit cities that are safe and convenient to navigate through, therefore a smart city enhances tourism because of this. Windmills and solar panels have been built at various offices and open fields, respectively, to encourage clean and green energy. To promote a healthy lifestyle and lower pollution levels, specialized bike rental stations have been set up across the city. By reason of the narrow roads and dense traffic, creating dedicated bike tracks like in European nations is still difficult. To communicate with the public and inform them of the most recent government plans and initiatives, the administration has developed an Ahmedabad website [57]. Ahmedabad is a historic city that predate 500 years. The majority of cities on the earth are the remains of earlier cultures.

This results in the lack of a major plan or development strategy for the city. Lack of a principle plan impedes growth and development because there isn't a clear city architecture. This is one of the biggest issues in cities that have supported civilization for a very long time[58]. Ahmedabad, a walled city with a 600-year history, is renowned for its twisting lanes and age-old buildings. Government must watch out to prevent innovation from obstructing or destroying historical sites. In cities, it is still very challenging to combine outdated architecture with contemporary technology**.** The smart city's digital security component presents yet another significant difficulty. The government must protect the information it gathers from its citizens from fraud and abuse while also ensuring its integrity and authenticity. A citizen's right to privacy must also be respected by the government, which is why it is forbidden to track someone's whereabouts without that person's consent [58]. Cybercrimes have been caused by the lack of clear and established regulations that precisely outline clear limits on data tracking and protect user privacy. Smart cities are essentially large networks of interconnected IoT devices, which makes them vulnerable to cyber security threats and unauthorized tracking of user data. It's important to create the concept of smart cities in a way that safeguards user privacy. This fine line between users and service providers requires mutual understanding.

CONCLUSION

With the aid of cloud-based enabling technologies, accurate information might be accessed, analyzed, and managed to help professionals, companies, and individuals make better decisions and raise the standard of living for people. With the help of connected cars and smart houses, people engage in smart city settings using their mobile devices. It's possible to cut costs and boost efficiency when gadgets and information are linked to a city's physical infrastructure. Cities may improve resource distribution, speed up rubbish collection, lower accident rates, and eliminate pollutants with the help of the Internet of Things. In this paper we have examined and discussed cloud based IoT applications and their functions in smart cities. Additionally, we have discussed the applications for smart cities, and the convergence of IoT and the cloud. Further, we have showcased the case study of Ahmedabad which must deal with issues including pollution, waste management, congestion, and transportation. With the aid of smart cities, they have tried to resolve the said issues. In addition, technology must be shared so that it is available to everyone worldwide since, at the end of the day, we are all part of humanity. Using sustainable technology and renewable energy, we can address the new issues that the growing population and climate change provide. Less traffic and pollution are possible in smart cities, which would boost citizens' general well-being.

REFERENCES

1. Khan, Z.; Pervez, Z.; Ghafoor, A. Towards Cloud Based Smart Cities Data Security and Privacy Management. In Proceedings of the 2014 IEEE/ACM 7th International Conference on Utility and Cloud Computing, IEEE, London, UK, 8-11 December. 2014; pp. 806–811.
2. Khan, Z.; Kiani, S.L. A Cloud-Based Architecture for Citizen Services in Smart Cities. In Proceedings of the 2012 IEEE Fifth International Conference on Utility and Cloud Computing, IEEE, Chicago, IL, USA, 5-8 November. 2012; pp. 315–320.

3. Suciu, G.; Vulpe, A.; Halunga, S.; Fratu, O.; Todoran, G.; Suciu, V. Smart cities built on resilient cloud computing and secure IoT. In Proceedings of the 2013 19th International Conference on Control Systems and Computer Science, IEEE, Bucharest, Romania 29-31 May 2013; pp. 513–518.
4. Alshammari, M.O., Almulhem, A.A., and Zaman, N.: „Internet of Things (IoT): Charity Automation", International Journal of Advanced Computer Science and Applications (IJACSA), 2017, 8, (2)
5. Farahani, B., Firouzi, F., and Chakrabarty, K.: „Healthcare IoT": "Intelligent Internet of Things" (Springer, 2020), pp. 515-545.
6. Alamri, M., Jhanjhi, N., and Humayun, M.: „Blockchain for Internet of Things (IoT) Research Issues Challenges & Future Directions: A Review", Int. J. Comput. Sci. Netw. Secur, 2019, 19, pp. 244-258
7. Khan, A., Jhanjhi, N., Humayun, M., and Ahmad, M.: „The Role of IoT in Digital Governance": „Employing Recent Technologies for Improved Digital Governance" (IGI Global, 2020), pp. 128-150
8. Suciu, G., Suciu, V., Martian, A., Craciunescu, R., Vulpe, A., Marcu, I., Halunga, S., and Fratu, O.: „Big data, internet of things and cloud convergence–an architecture for secure e-health applications", Journal of medical systems, 2015, 39, (11), pp. 141
9. Bhatt, C., Dey, N., and Ashour, A.S.: „Internet of things and big data technologies for next generation healthcare", 2017
10. Diro, Abebe, Haftu Reda, Naveen Chilamkurti, Abdun Mahmood, N. Z. Jhanjhi, and Yunyoung Nam. "Lightweight Authenticated-Encryption Scheme for Internet of Things Based on Publish-Subscribe Communication." IEEE Access (2020).
11. Firouzi, F., Rahmani, A.M., Mankodiya, K., Badaroglu, M., Merrett, G.V., Wong, P., and Farahani, B.: „Internet-of-Things and big data for smarter healthcare: From device to architecture, applications and analytics", in Editor (Ed.)^(Eds.): „Book Internet-of-Things and big data for smarter healthcare: From device to architecture, applications and analytics" (Elsevier, 2018, edn.),
12. Almusaylim, Z.A., Alhumam, A., and Jhanjhi, N.: „Proposing a Secure RPL based Internet of Things Routing Protocol: A Review", Ad Hoc Networks, 2020, pp. 102096
13. González-Zamar, M.-D.; Abad-Segura, E.; Vázquez-Cano, E.; López-Meneses, E. IoT Technology Applications-Based Smart Cities: Research Analysis. Electron. 2020, 9, 1246.
14. Saravanan, K.; Julie, E.G.; Robinson, Y.H. Smart cities & IoT: Evolution of applications, architectures &technolo-gies, present scenarios & future dream. In Internet of Things and Big Data Analytics for Smart Generation; Springer: Cham, Switzerland 2019, pp. 135–151
15. Shamsir, S.; Mahbub, I.; Islam, S.K.; Rahman, A. Applications of sensing technology for smart cities. 2017 IEEE 60th Int. Midwest Symp. Circuits Syst. 2017, 1150–1153.
16. Saha, H.N.; Auddy, S.; Chatterjee, A.; Pal, S.; Sarkar, S.; Singh, R.; Singh, A.K.; Sharan, P.; Banerjee, S.; Sarkar, R.; et al. IoT solutions for smart cities. In Proceedings of the 2017 8th Annual Industrial Automation and Electromechanical Engineering Conference (IEMECON), Institute of Electrical and Electronics Engineers (IEEE), Bangkok, Thailand, August 16–18, 2017; pp. 74–80.
17. Song, H.; Srinivasan, R.; Sookoor, T.; Jeschke, S. Smart Cities: Foundations, Principles, and Applications; John Wiley & Sons, New York, USA, 2017.
18. Sookhak, M.; Tang, H.; He, Y.; Yu, F.R. Security and Privacy of Smart Cities: A Survey, Research Issues and Challenges. IEEE Commun. Surv. Tutor. 2019, 21, 1718–1743.
19. Park, E.; Del Pobil, A.P.; Kwon, S.J. The Role of Internet of Things (IoT) in Smart Cities: Technology Roadmap-oriented Approaches. Sustain. 2018, 10, 1388.
20. Mehmood, Y.; Ahmad, F.; Yaqoob, I.; Adnane, A.; Imran, M.; Guizani, S. Internet-of-things-based smart cities: Recent advances and challenges. IEEE Commun. Mag. 2017, 55, 16–24.
21. Visvizi, A.; Lytras, M.D. Sustainable Smart Cities and Smart Villages Research: Rethinking Security, Safety, Well-being, and Happiness. Sustain. 2019, 12, 215.
22. Talari, S.; Shafie-Khah, M.; Siano, P.; Loia, V.; Tommasetti, A.; Catalão, J.P. A review of smart cities based on the IoT concept.
Energies 2017, 10, 421.
23. Delsing, J. Smart City Solution Engineering. Smart Cities 2021, 4, 643–661.

24. Lanza, J.; Sánchez, L.; Gutiérrez, V.; Galache, J.A.; Santana, J.R.; Sotres, P.; Muñoz, L. Smart city services over a future Internet platform based on IoT and cloud: The smart parking case. Energies 2016, 9, 719.
25. Syed, A.; Sierra-Sosa, D.; Kumar, A.; Elmaghraby, A. IoT in Smart Cities: A Survey of Technologies, Practices and Challenges.
Smart Cities 2021, 4, 429–475.
26. Almalki, F.A.; Alsamhi, S.H.; Sahal, R.; Hassan, J.; Hawbani, A.; Rajput, N.S.; Saif, A.; Morgan, J.; Breslin, J. Green IoT for Eco- Friendly and Sustainable Smart Cities: Future Directions and Opportunities. Mob. Networks Appl. 2021, 1–25, 1–25.
27. Lea, R.; Blackstock, M. City Hub: A Cloud-Based IoT Platform for Smart Cities. In Proceedings of the 2014 IEEE 6th International Conference on Cloud Computing Technology and Science, Institute of Electrical and Electronics Engineers (IEEE), NW Washington, D.C. United States, December 15–18, 2014; pp. 799–804.
28. Sikder, A.K.; Acar, A.; Aksu, H.; Uluagac, A.S.; Akkaya, K.; Conti, M. IoT-enabled smart lighting systems for smart cities. In Proceedings of the 2018 IEEE 8th Annual Computing and Communication Workshop and Conference (CCWC), IEEE, Las Vegas, NV, USA, January 8–10, 2018; pp. 639–645.
29. Ding, H.; Li, X.; Cai, Y.; Lorenzo, B.; Fang, Y. Intelligent data transportation in smart cities: A spectrum-aware approach.
IEEE/ACM Trans. Netw. 2018, 26, 2598–2611.
30. Ramos, H.M.; McNabola, A.; López-Jiménez, P.A.; Pérez-Sánchez, M. Smart Water Management towards Future Water Sustainable Networks. Water 2020, 12, 58.
31. Chung, N.; Lee, H.; Ham, J.; Koo, C. Smart Tourism Cities' Competitiveness Index: A Conceptual Model. In Information and Communication Technologies in Tourism 2021; Springer Science and Business Media LLC: 2021, virtual conference, Berlin, Germany, January 19–22, 2021; pp. 433–438.
32. Biyik, C.; Allam, Z.; Pieri, G.; Moroni, D.; O'fraifer, M.; O'connell, E.; Khalid, M. Smart Parking Systems: Reviewing the Literature, Architecture and Ways Forward. Smart Cities 2021, 4, 623–642.
33. Miyasawa, A.; Akira, S.; Fujimoto, Y.; Hayashi, Y. Spatial demand forecasting based on smart meter data for improving local energy self-sufficiency in smart cities. IET Smart Cities 2021,3, 107–120.
34. Khalifeh, A.; Darabkh, K.A.; Khasawneh, A.M.; Alqaisieh, I.; Salameh, M.; AlAbdala, A.; Rajendiran, K. Wire-less Sensor Networks for Smart Cities: Network Design, Implementation and Performance Evaluation. Electronics 2021, 10, 218.
35. McCurdy, A.; Peoples, C.; Moore, A.; Zoualfaghari, M. Waste Management in Smart Cities: A Survey on Public Perception and the Implications for Service Level Agreements. EAI Endorsed Trans. Smart Cities 2021, Article in press.
36. Chatterjee, U.; Bhunia, G.S.; Mahata, D.; Singh, U. Smart Cities and Their Role in Enhancing Quality of Life. Quality of Life: An Interdisciplinary Perspective. 2021, 127–143.
37. Múnera, D.; Aguirre, J.; Gómez, N.G. IoT-based air quality monitoring systems for smart cities: A systematic mapping study.Int. J. Electr. Comput. Eng. (2088–8708) 2021, 11, 3470–3482.
38. P Kasznar, A.P.; WA Hammad, A.; Najjar, M.; Linhares Qualharini, E.; Figueiredo, K.; Soares, C.A.P.; N Haddad, A. Multiple Dimensions of Smart Cities' Infrastructure: A Review. Buildings 2021, 11, 73.
39. ZHOU, MINQI AND ZHANG, RONG AND ZENG, DADAN AND QIAN, WEINING. Services in the Cloud Computing era: A survey. In *Proceedings of Universal Communication Symposium* (2010).
40. PATIDAR, SHYAM AND RANE, DHEERAJ AND JAIN, PRITESH. A Survey Paper on Cloud Computing. In *Proceedings of Second International Conference on Advanced Computing & Communication Technolo- gies*(2012).
41. BANERJEE, PRITH AND ET AL. Everything as a Service: Powering the New Information Economy. *Computer 44*, 3 (2011).
42. PERERA, CHARITH AND ZASLAVSKY, ARKADY B. AND CHRISTEN, PETER AND GEORGAKOPOULOS, DIMITRIOS. Sensing as a service model for smart cities supported by internet of things. Trans. Emerging Telecommunications Technologies 25, 1 (2014)
43. Chen S, Xu HuihanzhiLiu D , Hu B and Wang H 2014 *IEEE INTERNET OF THINGS JOURNAL*, VOL. **1**, NO. 4, AST 2014

44. Lea, R.; Blackstock, M. City Hub: A Cloud-Based IoT Platform for Smart Cities. In Proceedings of the 2014 IEEE 6th International Conference on Cloud Computing Technology and Science, Institute of Electrical and Electronics Engineers (IEEE), NW Washington, D.C. United States, December 15–18, 2014; pp. 799–804.
45. Ramos, H.M.; McNabola, A.; López-Jiménez, P.A.; Pérez-Sánchez, M. Smart Water Management towards Future Water Sustainable Networks. *Water* 2020, *12*, 58.
46. Chung, N.; Lee, H.; Ham, J.; Koo, C. Smart Tourism Cities' Competitiveness Index: A Conceptual Model. In *Information and Communication Technologies in Tourism 2021*; Springer Science and Business Media LLC: 2021, virtual conference, Berlin, Germany, January 19–22, 2021; pp. 433–438.
47. Biyik, C.; Allam, Z.; Pieri, G.; Moroni, D.; O'fraifer, M.; O'connell, E.; Khalid, M. Smart Parking Systems: Reviewing the Literature, Architecture and Ways Forward. *Smart Cities* 2021, *4*, 623–642.
48. Miyasawa, A.; Akira, S.; Fujimoto, Y.; Hayashi, Y. Spatial demand forecasting based on smart meter data for improving local energy self-sufficiency in smart cities. *IET Smart Cities* 2021,*3*, 107–120.
49. Khalifeh, A.; Darabkh, K.A.; Khasawneh, A.M.; Alqaisieh, I.; Salameh, M.; AlAbdala, A.; Rajendiran, K. Wire-less Sensor Networks for Smart Cities: Network Design, Implementation and Performance Evaluation. *Electronics* 2021, *10*, 218.
50. McCurdy, A.; Peoples, C.; Moore, A.; Zoualfaghari, M. Waste Management in Smart Cities: A Survey on Public Perception and the Implications for Service Level Agreements. *EAI Endorsed Trans. Smart Cities* 2021, Article in press.
51. Múnera, D.; Aguirre, J.; Gómez, N.G. IoT-based air quality monitoring systems for smart cities: A systematic mapping study. *Int. J. Electr. Comput. Eng. (2088–8708)* 2021, *11,* 3470–3482.
52. P Kasznar, A.P.; WA Hammad, A.; Najjar, M.; LinharesQualharini, E.; Figueiredo, K.; Soares, C.A.P.; N Haddad, A. Multiple Dimensions of Smart Cities' Infrastructure: A Review. *Buildings* 2021, *11*, 73.
53. Severino, A.; Curto, S.; Barberi, S.; Arena, F.; Pau, G. Autonomous Vehicles: An Analysis Both on Their Distinc-tiveness and the Potential Impact on Urban Transport Systems. *Appl. Sci.* 2021, *11*, 3604.
54. Alam, T.; Khan, M.A.; Gharaibeh, N.K.; Gharaibeh, M.K. Big Data for Smart Cities: A Case Study of NEOM City, Saudi Arabia. In *TRANSBALTICA XI: Transportation Science and Technology*; Springer Science and Business Media LLC: 2021; pp. 215–230.
55. Alam, T. Cloud Computing and its role in the Information Technology. *IAIC Trans. Sustain. Digit. Innov.* 2020, *1*, 108–115.
56. Song, H.; Bai, J.; Yi, Y.; Wu, J.; Liu, L. Artificial Intelligence Enabled Internet of Things: Network Architecture and Spectrum Access. *IEEE Comput. Intell. Mag.* 2020, *15*, 44–51.
57. https://www.iifl.com/blogs/major-challenges-upcoming-smart-cities-india.
58. Elmaghraby, Adel &Losavio, Michael. (2014). "Cyber Security Challenges in Smart Cities: Safety, security and privacy". Journal of Advanced Research. 5. 10.1016/j.jare.2014.02.006.

Table 1: Review of Literature

Authors	Ref.	Year	Title
Khan *et al.*	[1]	2014	Towards cloud - based smart cities, data security, and privacy management
Khan *et al.*	[2]	2012	A cloud-based architecture for citizen services in smart cities
Suciu	[3]	2013	Smart cities built on resilient cloud computing and secure IoT
RoyandSarddar	[4]	2016	The Role of Cloud of Things in Smart Cities
Silva *et al.*	[5]	2018	Towards sustainable smart cities: Are view of trends, architectures, components, and open challenges in smart cities
Chai *et al.*	[6]	2021	Role of BIC(Big Data, IoT, and Cloud)for Smart Cities
Rubí *et al.*	[7]	2021	An IoT -based plat form for environment data sharing in smart cities
Kaur *et al.*	[8]	2016	Building smart cities applications using IoT and cloud-based architectures
Saleem *et al.*	[9]	2020	Building smart cities applications based on IoT technologies: A review

Dlodlo *et al.*	[10]	2016	Internet of things technologies in smart cities
Hyman *et al.*	[11]	2019	Secure controls for smart cities; applications in intelligent transportation systems and smart buildings
Curry *et al.*	[12]	2016	Smart cities–enabling services and applications
González-Zamar *et al.*	[13]	2020	IoT technology applications-based smart cities: Research analysis
Saravanan *et al.*	[14]	2019	Smart cities & IoT: evolution of applications, architectures& technologies, present scenarios & future dream
Shamsir *et al.*	[15]	2017	Applications of sensing technology for smart cities
Saha *et al.*	[16]	2017	IoT solutions for smart cities
Song *et al.*	[17]	2017	Smart cities: foundations, principles, and applications
Sookhak *et al.*	[18]	2018	Security and privacy of smart cities: a survey, research issues, and challenges
Park *et al.*	[19]	2018	The role of IoT in smart cities: Technology roadmap-oriented approaches
Mehmood *et al.*	[20]	2017	Internet-of-things-based smart cities: Recent advances and challenges
Visvizi *et al.*	[21]	2020	Sustainable smart cities and smart villages research: Rethinking security,safety,well-being, and happiness
Talari *et al.*	[22]	2017	A review of smart cities based on the IoT concept
Delsing *et al.*	[23]	2021	Smart City Solution Engineering
Lanza *et al.*	[24]	2016	Smart city services over a future Internet plat form based on IoT and cloud: The smart parking case
Syed *et al.*	[25]	2021	IoT in Smart Cities :A Survey of Technologies, Practices, and Challenges
Almalki et al.	[26]	2021	Green IoT for Eco-Friendly and Sustainable Smart Cities: Future Directions and Opportunities
Lea *et al.*	[27]	2014	City hub: A cloud based IoT plat form for smart cities
Sikder *et al.*	[28]	2018	IoT-enabled smart lighting systems for smart cities
Ding *et al.*	[29]	2018	Intelligent data transportation in smart cities: A spectrum-aware approach
Ramos *et al.*	[30]	2020	Smart water management towards future water sustainable networks
Chung *et al.*	[31]	2021	Smart Tourism Cities' Competitiveness Index: A Conceptual Model
Biyik *et al.*	[32]	2021	Smart Parking Systems: Reviewing the Literature, Architecture and Ways Forward
Miyasawa *et al.*	[33]	2021	Spatial demand forecasting based on smart meter data for improving local energy self-sufficiency in smart cities
Khalifeh *et al.*	[34]	2021	Wireless Sensor Networks for Smart Cities: Network Design, Implementation and Performance Evaluation
McCurdy *et al.*	[35]	2021	Waste Management in Smart Cities: A Survey on Public Perception and the Implications for Service Level Agreements
Chatterjee *et al.*	[36]	2021	Smart Cities and Their Quality of Life: An Interdisciplinary Perspective
Múnera *et al.*	[37]	2021	IoT- based air quality monitoring systems for smart cities: A systematic mapping study
PKasznar *et al.*	[38]	2021	Multiple Dimensions of Smart Cities' Infrastructure: A Review

Table 2. IoT Cloud Plat forms and Their Features

S. No.	IoT Platforms	Features
1	Sales force IoT Cloud	IoT Cloud manages massive amounts of data from devices including computers, sensors, websites, clients, and partners who are connected to the cloud.
2	AWS IoT Core &Analytics	utilizing a changeability of commercially available tools and sensors with assistance from protocols like HTTP, MQTT, and Network Sockets, for instance. Data preprocessing is carried out by AWS IoT Analytics, including cleaning and filtering of sensor data.
3	Oracle IoT	Asset Management Cloud provides real-time data on asset health and usability as well as alerts and failure predictions. Oracle Stream Analytics is used to perform computational operations on a steady stream of enormous volume of data.
4	Particle IoT	Asset Management Cloud provides real-time data on asset health and usability as well as alerts and failure predictions. Oracle Stream Analytics is used to perform computational operations on a steady stream of enormous volume of data.
5	Predix	Is a platform for the production, implementation and maintenance of industrial machinery applications. This platform safely connects devices, gathers data, examines the data, and gives users feedback.
6	SQL Stream	For users of Kafka, Kinesis, and other streams, SQLstream offers straightforward integrati on and analysis, and it does real-time data analysis. It offers continuous real-time ML.
7	UBidot	Tools for data gathering, analysis, and visualisation are available through the Ubidots IoT platform. This framework supports a platform that enables REST APIs that are Microsoft Azure-compatible
8	sAzure Stream analytics	offers real-time device data analytics and real-time analytical intelligence as a Microsoft product. Data from sensors and other devices is processed by analytics from Azure and displayed. Power Business Intelligence is used to help.
9	Watson IoT Platform	This IoT framework is a creation of Ayla Network. Analytics and business intelligence are integrated with it. The key strength of the platform is cognitive computing, which gives its users deep insights into their data.

Fig. 1. Internet of Things.

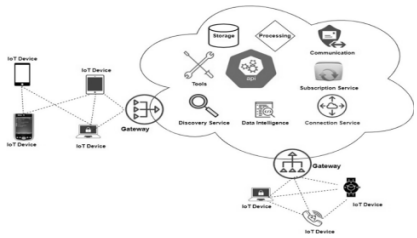

Fig. 2. Cloud-based IoT System.

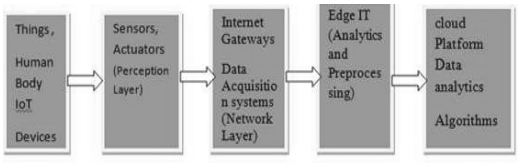

Fig. 3. Basic IoT Cloud Structure

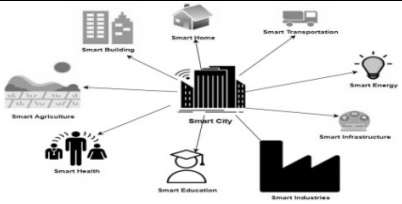

Fig.4. Smart cities applications.

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REVIEW ARTICLE

Nanoparticles Synthesis from Marine Bacteria and Its Applications - An Overview

J.Tharani[1], R.Ishwarya[1], M.Geetha[1] and D.Sangeetha[2]*

[1]Research Scholar, Department of Microbiology, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India
[2]Assistant Professor, Department of Microbiology, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India

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***Address for Correspondence**
D.Sangeetha
Assistant Professor,
Department of Microbiology,
Annamalai University, Annamalai Nagar,
Chidambaram, Tamil Nadu, India
Email: sangeethadau@gmail.com

ABSTRACT

Nanotechnology refers to the creation and utilization of materials whose constituents exist at the nanoscale; and, by convention, be up to 100 nm in size. The increased interest in nanomedicine for a wide range of biological functions demands the search for raw materials to create nanomaterials. Marine derived materials , either whole extract or pure components are used in synthesis of nanoparticles due to their ease of availability, low cost production and low toxicity towards eukaryotic cells. These marine-derived nanomaterials have been employed to kill infectious diseases caused by microorganisms. Due to their exceptional properties including antibacterial activity, high resistance to oxidation and high thermal conductivity, nanoparticles have attracted considerable attention in recent years. Nanoparticles can be synthesized chemically or biologically. Metallic nanoparticles that have immense applications in industries are of different types, namely, Gold, Silver, Alloy, magnetic etc. The current review is focused on the overview of Nanoparticles and its biological applications.

Keywords: Nanoparticles, Marine Bacteria, Applications, Synthesis.

INTRODUCTION

Many bacteria, fungi and plants have shown the ability to synthesise metallic nanoparticles and all have their own advantages and disadvantages. In recent years the topic of nanoparticles has received particular interest in a wide range of fields. The term “nano” comes from the Greek word “nanos” meaning dwarf and denotes a measurement on the scale of one-billionth of a metre in size. Synthesis of nanoparticles is a most importance to expand their

biomedical applications. One of the options to achieve this goal is to use microorganisms to synthesize nanoparticles. Nanoparticles particles having one or more dimensions of the order of 100 nm or less have attracted great attention due to their unusual and fascinating properties, and applications advantageous over their bulk counterparts[1,2]. Despite that the latter methods are able to produce large quantities of nanoparticles with a defined size and shape in a relatively short time, they are complicated, outdated, costly, and inefficient and produce hazardous toxic wastes that are harmful, not only to the environment but also to human health. Heavy metals ions are toxic to most bacteria, highly resistant strains are available that absorb them and reduce them to the metallic state. This results in the formation of nanoparticles that can be harvested to provide a source of these valuable metals [3,4]. The "biogenic" approach is further supported by the fact that the majority of the bacteria inhabit ambient conditions of varying temperature, pH, and pressure. The particles generated by these processes have higher catalytic reactivity, greater specific surface area, and an improved contact between the enzyme and metal salt in question due to the bacterial carrier matrix[5,6]. Nanoscience is rapidly making an impact in all domains of human life due to its wide applications[7].Biological methods are the key solution to this problem. The potential ability of microorganisms to synthesize nanoparticles has led microbiologist around the world to screen array of microorganisms for this capability and develop a simple and cost effective method to synthesize nanoparticles of constant size, shape and monodispersity[8].Microorganisms often produce inorganic materials of nano-size either extracellularly or intracellularly. Microbial systems are able to detoxify heavy metals by virtue of their ability to reduce the metal ions or convert the soluble toxic ions into insoluble non-toxic metal nanoparticles. A great deal of study has been carried out on synthesis of nanoparticles by prokaryotic bacteria since they are the easiest organisms to handle and can be manipulated most easily. A new branch of nanotechnology existing, which is bio-nanotechnology that integrates principles of biology with physical and chemical procedures to generate nano-sized particles with specific functions[9,10,11] .Bacteria are able to form nanoparticles both intracellularly via bioaccumulation and extracellularly using its enzymes. Nanoparticle synthesis was optimized and characterized to determine its size shape and conformity[12].There are important links between the way nanoparticles are synthesised and their potential uses. These features can be exploited for next generation biosensors, electronics, catalysts and antimicrobials. Metallic nanoparticles are one important and widely studied group of materials, showing great diversity and many different uses.

Chemical and Physical Method of Nano Synthesis

Metallic nanoparticles can be synthesised in many different ways. In order to study the biological methods of synthesising nanoparticles (NPs), a clear understanding of the current chemical and physical methods is needed to allow comparisons to be made and a basis for improvement to become evident. There is an abundant volume of research on the synthesis of metallic nanoparticles available in the literature. The chemical method used to produce silver nanoparticles (AgNPs) of cube and tetrahedron shape by Wiley et al, it was achieved by heating $AgNO_3$ and Ethylene glycol to 148°C. The resulting nanoparticles were single crystals and had a size range of 30-80 nm in diameter and the reaction time was only 15 minutes. However the temperature needed for the reaction to occur was relatively high therefore requiring a significant amount of energy if commercial volumes were to be made. A method using polyamide fabrics developed by Montazer et al. successfully formed AgNPs. The NPs were observed under SEM and were found to be between 20 and 150 nm across with an average size of 90 nm. A small number of aggregates was seen which were attributed to the boiling point temperature during the reaction. The polyamide fabric itself was used as the reducing agent for the Ag+ and was found to stabilise the synthesised NPs. This important result meant that the use of extra stabilising agents was redundant. The fabric was subsequently tested for its antibacterial activity which showed high bactericidal effects even after 30 washing cycles. Stable platinum nanoparticles (PtNPs) have been produced using a physical method, which does not require the reducing agents that cause contamination in the nanoparticles produced. Laser ablation that creates nanoparticles by heating up bulk material using a laser beam by Mafuné et al. He and his group performed this method in both SDS and pure water and found in both cases that stable PtNPs were produced. Subsequently the size distribution of the PtNPs was measured under TEM and was found to be 1-7 nm, which proved to be too small for isolating them using centrifugation.

Preparation of Nanopartciles

Nanomaterials can be fabricated through two main methods"top-down" and "bottom-up"[13].The top-down approach basically works with the material in its bulk form and the size reduction to the nanoscale is then achieved by specialized ablations, e.g., lithography, thermal decomposition, laser ablation, mechanical milling, etching, and sputtering [14]. The "bottom-up" approach is more preferable for the preparation of nanoparticles, where involving a homogeneous system wherein catalysts (e.g., reducing agent and enzymes) synthesize nanostructures that are controlled by catalyst properties, reaction media, and conditions (e.g., solvents, stabilizers, and temperature). For instance, chemical reduction method is the most common synthetic pathway for metal nanoparticles synthesis [15]. In the case of silver nanoparticles, the chemical reduction method is carried out based on the reduction of aqueous silver nitrate in an appropriate operating medium using chemical reductants such as sodium citrate or branched polyethylenimine. In this way, negatively charged silver nanoparticles can be obtained from the process using sodium citrate acting as reductant, while positively charged silver nanoparticles can be synthesized from the reaction with branched polyethylenimine as reductant. Thus, the physiochemical properties, surface, and morphological characteristics of nanoparticles can possibly be controlled depending on the subsequent application through variation in precursor concentrations and reaction conditions[16].

Biosynthesis of Nanoparticles by Micro Organisms

Research has focused heavily on prokaryotes as a means of synthesising metallic nanoparticles. Due to their abundance in the environment and their ability to adapt to extreme conditions, bacteria are a good choice for study. They are also fast growing, inexpensive to cultivate and easy to manipulate. Growth conditions such as temperature, oxygenation and incubation time can be easily controlled .Biological entities and inorganic materials have been in constant touch with each other ever since inception of life on the earth. Due to this regular interaction, life could sustain on this planet with a well-organized deposit of minerals. Studies have found that many microorganisms can produce inorganic nanoparticles through either intracellular or extracellular routes. This section describes the production of various nanoparticles via biological methods following the categories of metallic nanoparticles including gold, silver, alloy and other metal nanoparticles, oxide nanoparticles consisting of magnetic and nonmagnetic oxide nanoparticles, sulfide nanoparticles, and other miscellaneous nanoparticles[17]. Some typical metal nanoparticles produced by microorganisms are summarized in Table. Nanoparticles can be synthesized chemically or biologically. Many adverse effects have been associated with chemical synthesis methods due to the presence of some toxic chemical absorbed on the surface. Eco friendly alternatives to Chemical and physical methods are Biological ways of nanoparticles synthesis using microorganisms, enzymes, fungus, and plants or plant extracts. The development of these eco friendly methods for the synthesis of nanoparticles is evolving into an important branch of nanotechnology which have many applications.

Gold Nanoparticles:

Morphologically, round, triangular or hexagonal AuNPs has been bio-diminishment of wrote about cell surface in *E. coli* DH5α with morphologies. Besides, this synthesis has been connected with plasma layer related NADPH-subordinate proteins and carotenoids in Rhodobacter capsulatus intervened biosynthesis of AuNPs. Gold nanoparticles (AuNPs) are used in immunochemical studies for identification of protein interactions. They are used as lab tracer in DNA fingerprinting to detect presence of DNA in a sample. They are also used for detection of aminoglycoside antibiotics like streptomycin, gentamycin and neomycin. Gold nanorods are being used to detect cancer stem cells, beneficial for cancer diagnosis and for identification of different classes of bacteria. *Bacillus subtilis* 168 encouraged gold (Au+3) particles to Au0 nanoparticles with octahedral morphology has been accounted for onto their cell dividers [18]. Another bacterium, *Geobacter ferrireducens* diminished Au particles in periplasmic space to deliver AuNPs. *Shewanella* algae likewise decreased Au+3 particles to natural AuNPs at 25°C in periplasmic space and on its cell surface. Under various pH conditions distinctive sizes of NPs were watched [19]. A *cyanobacterium*, *Plectonema boryanum* UTEX485, synthesized AuNPs at 25 to 200°C with the assistance of some external layer proteins, lipopolysaccharides and phospholipids. This blend has been connected with indicated detoxification systems in microscopic organisms [20]. Gold and its compounds have long been used as medicinal agents throughout the history of civilization with its earliest record dating back to 5000 years ago in Egypt [21-25].

Silver Nanoparticles

Silver is a gleaming, very ductile, and malleable element but slightly harder than gold, with a symbol of Ag and atomic number of 47. It is one of the basic elements that make up our planet. In nature, it exists as a native element, as an alloy combining with other metals (e.g., gold) and as minerals (e.g., chlorargyrite and argentite). Chemically, silver possess four different oxidation states, i.e., Ag^0 , Ag^{1+}, Ag^{2+}, and Ag^{3+} [26]. However, it is a chemically inactive element, but it can be reacted with nitric acid or hot concentrated sulfuric acid, forming soluble silver salts. It also possesses an excellent conductivity of heat and electricity, yet its applications in electrical industry have greatly been limited due to its greater cost [10]. As for metallic silver form, it is insoluble in water, but its metallic salts such as silver nitrate, $AgNO_3$, and silver chloride, AgCl, are water-soluble. Klaus and coworkers have shown that the bacterium *Pseudomonas stutzeri* AG259, isolated from a silver mine, when placed in a concentrated aqueous solution of silver nitrate, played a major role in the reduction of the Ag+ ions and the formation of silver nanoparticles (AgNPs) of well-defined size and distinct topography within the periplasmic space of the bacteria [27]. AgNPs were synthesized in the form of a film or produced in solution or accumulated on the surface of its cell when fungi, *Verticillium, Fusarium oxysporum*, or *Aspergillus flavus*, were employed [28-31].Some other silver nanoparticles produced by microorganisms are listed.

Alloy Nanoparticles

Bimetallic alloy nanoparticles were prepared following the above mentioned procedure, using different ratios of silver and copper ion concentrations and ascorbic acid as a reducing agent. The solutions were kept under microwave for 90 s. The change in the colour of the solution indicated the formation of alloy nanoparticles with different composition. The alloys rich in silver were somewhat orange while those rich in copper were red. Alloy nanoparticles are of great interest due to their applications in catalysis, electronics, as optical materials, and coatings [31,32]. Reported the synthesis of bimetallic Au-Ag alloy by *F. oxysporum* and argued that the secreted cofactor NADH plays an important role in determining the composition of Au-Ag alloy nanoparticles [32]. studied Au-Ag alloy nanoparticles biosynthesized by yeast cells. Fluorescence microscopic and transmission electron microscopic characterizations indicated that the Au-Ag alloy nanoparticles were mainly synthesized *via* an extracellular approach and generally existed in the form of irregular polygonal nanoparticles. Electrochemical investigations revealed that the vanillin sensor based on Au-Ag alloy nanoparticles modified glassy carbon electrode was able to enhance the electrochemical response of vanillin for at least five times. Sawle et al., demonstrated the synthesis of core-shell Au-Ag alloy nanoparticles from fungal strains *Fusarium semitectum* and showed that the nanoparticle suspensions are quite stable for many weeks [33].

Palladium Nanoparticles

Pd is a member of the Platinum Group Metals (PGM) which is a collection of highly catalytically active metals and are currently being primarily used as catalysts for dehalogenation and hydrogenation reactions [34].$PdCl_2$ and BLs (molar ratio 1 : 2, resp.) were separately dissolved in freshly prepared solvent (absolute ethanol and Milli-Q water in 1 : 1.5) using 1 MLH magnetic stirrer. Then, the BLs solutions were added drop wise to metal compound solution with continuous stirring at room temperature. After 10 h, the mixture turned from light red brown to greenish color and after 16 h, precipitates were formed. The precipitates were filtered off, washed several times with chilled water/ethanol in 1 : 1 ratio, and kept overnight in vacuum oven at room temperature for absolute dryness [35]. Recently it has been shown that zero valent palladium (Pd0) nanoparticles can be synthesised using bacteria found at Alpine sites heavily contaminated with heavy metals. Of all the variety of heavy metal resistant bacteria that they have found in that environment, they found that *Pseudomonas* cells were involved in producing catalytically active nanoparticles which were successfully used in reductive dehalogenation of tri and tetra-chlorinated dioxin congeners [34]. Macaskie et al. suggested that *Escherichia coli*, can also synthesise Pd0 nanoparticles with the help of hydrogenases found in the bacterium [36]. In both studies mentioned, the Pd nanoparticles were found to be formed on the cell envelope of the bacteria which makes them attractive as they are easily accessible.

Selenium Nanoparticles

Selenium has photo-optical and semiconducting properties that have applications in photocopiers and microelectronic circuit devices. Few selenite- and selenate-respiring bacteria such as *Sulfurospirillum barnesii*, *B. selenitireducens* and *Selenihalanaerobacter shriftii* synthesized extracellularly stable uniform nanospheres of diameter ~300 nm of elemental selenium Se0 with monoclinic crystalline structures. These bacteria have also produced small amounts of Se0 intracellularly [37]. Microbial synthesis of elemental selenium (Se0) nanospheres resulted in unique, complex, compacted nanostructured arrangements of Se atoms. These arrangements were resulted due to the dissimilatory reductions that were subtly different in different microbes.

Titanium Nanoparticles

Titanium nanoparticles of spherical aggregates of 40–60 nm were produced extracellularly using the culture filtrate of *Lactobacillus sp*. at room temperature [38]. These titanium nanoparticles were lighter in weight and high resistance to corrosion and have enormous applications in automobiles, missiles, airplanes, submarines, cathode ray tubes and in desalting plants and has promising future role in cancer chemotherapy and gene delivery.

Magnetic Nanoparticles

Magnetic nanoparticles are recently developed new materials, due to their unique micro configuration and properties like super paramagnetic and high coercive force, and their prospect for broad applications in biological separation and biomedicine fields. Magnetic nanoparticles like Fe_3O_4 (magnetite) and Fe_2O_3 (maghemite) are known to be biocompatible. They have been actively investigated for targeted cancer treatment (magnetic hyperthermia), stem cell sorting and manipulation, guided drug delivery, gene therapy, DNA analysis, and magnetic resonance imaging (MRI) [39]. *Magnetotactic* bacteria synthesize intracellular magnetic particles comprising iron oxide, iron sulfides, or both [40, 41]. In order to distinguish these particles from artificially synthesized magnetic particles (AMPs), they are referred to as bacterial magnetic particles (BacMPs) [41]. BacMPs, which are aligned in chains within the bacterium, are postulated to function as biological compass needles that enable the bacterium to migrate along oxygen gradients in aquatic environments, under the influence of the Earth's geomagnetic field [42]. BacMPs can easily disperse in aqueous solutions because they are enveloped by organic membranes that mainly consist of phospholipids and proteins. Furthermore, an individual BacMP contains a single magnetic domain or magnetite that yields superior magnetic properties [43]. Since the first report of *magnetotactic* bacteria in 1975, various morphological types including *cocci*, *spirilla, vibrios,* ovoid bacteria, rod-shaped bacteria, and multicellular bacteria possessing unique characteristics have been identified and observed to inhabit various aquatic environments [43,44]. *Magnetotactic cocci*, for example, have shown high diversity and distribution and have been frequently identified at the surface of aquatic sediments. The discovery of this bacterial type, including the only cultured *Magnetotactic coccus* strain MC-1, suggested that they are microaerophilic. Magnetic nanoparticles like Fe_3O_4 (magnetite) and Fe_2O_3 (maghemite) are known to be biocompatible.

Other Nanoparticles

In biological systems, a large variety of organisms form organic/inorganic composites with ordered structures by the use of biopolymers such as protein and microbe cells. In addition to nanoparticles mentioned above, $PbCO_3$, $CdCO_3$, $SrCO_3$, PHB, $Zn_3(PO_4)_2$, and CdSe nanoparticles were reported to be synthesized by microbes [45]. $SrCO_3$ crystals were obtained when challenging fungi were incubated with aqueous Sr^{2+} ions [46]. The authors believed that secretion of proteins during growth of the fungus Fusarium oxysporum is responsible for modulating the morphology of strontianite crystals and directing their hierarchical assembly into higher order superstructures. Zinc phosphate nanopowders were synthesized with yeasts as biotemplates [47]. Yan et al. demonstrated the synthesis of $Zn_3(PO_4)_2$ powders with butterfly-like microstructure with a size range of 10–80 nm in width and 80–200 nm in length [48]. Kumar et al. showed that highly luminescent CdSe quantum dots can be synthesized by *F. oxysporum* at room temperature [49].

Application of Nanoparticles

Nanomedicine has tremendous prospects for the improvement of the diagnosis and treatment of human diseases. Use of microbes in biosynthesis of nanoparticles is an environmentally acceptable procedure. Nanotechnology has potential to revolutionize a wide array of tools in biotechnology so that they are more personalized, portable, cheaper, safer, and easier to administer. Nanomedicine is a burgeoning field of research with tremendous prospects for the improvement of the diagnosis and treatment of human diseases [50]. Dispersed nanoparticles are usually employed in nanobiomedicine as fluorescent biological labels [51,52], drug and gene delivery agents [53,54], and in applications such as biodetection of pathogens [55], tissue engineering [56,57], tumor destruction via heating (hyperthermia) [58], MRI contrast enhancement [59], and phagokinetic studies [60]. While the field of biosynthesized nanoparticles is relatively new, researchers have already started exploring their use in applications such as targeted drug delivery, cancer treatment, gene therapy and DNA analysis, antibacterial agents, biosensors, enhancing reaction rates, separation science, and MRI. Here, we provide some examples to illustrate these applications.

Multidisciplinary in nature

Nanotechnology is a multidisciplinary field in nature regarding investigations and applications [61]. In most recent couple of decades, inquiries about in building, physical science, natural chemistry and microscopy have prompted magnificent additions of worry in portrayal minor particles and their promising ramifications in various territories of material science. Enhance the analytic and treatment Nanotechnology is a multidisciplinary field in nature regarding investigations and applications .

Nano-medicine

Delivering the drugs precisely and safely to their target sites at the right time to have a controlled release and achieve the maximum therapeutic effect is a key issue in the design and development of novel drug delivery systems. Targeted nanocarriers must navigate through blood-tissue barriers to reach target cells. They must enter target cells to contact cytoplasmic targets via specific endocytotic and transcytotic transport mechanisms across cellular barriers [62]. Although this they can likewise be utilized for discovery of pathogens, tissue designing [63], tumor destruction, contrast change in (MRI) and phagokinetic examinations. Because of their small size, nanoparticle drug carriers can bypass the blood-brain barrier and the tight epithelial junctions of the skin that normally impede delivery of drugs to the desired target site. Secondly, as a result of their high surface area to volume ratio, nanocarriers show improved pharmacokinetics and biodistribution of therapeutic agents and thus minimize toxicity by their preferential accumulation at the target site [64]. Magnetic nanoparticles like Fe_3O_4 (magnetite) and Fe_2O_3 (maghemite) are known to be biocompatible. They have been actively investigated for targeted cancer treatment (magnetic hyperthermia), stem cell sorting and manipulation, guided drug delivery, gene therapy and DNA analysis, and MRI [65]. *Magnetotactic* bacteria (MTB) MC-1 with *magnetosomes* was also used as drug delivery agent. Felfoul et al. applied *magnetotaxis* to change the direction of each MTB embedded with combination of nanoparticles magnetite and the flagella to steer in small-diameter blood vessels [66]. However, in order to guide these MTBs towards a target, it is essential to be able to image these living bacteria in *vivo* using an existing medical imaging modality. It was shown that the *magnetosomes* embedded in each MTB can be used to track the displacement of these bacteria using an MRI system, since these *magnetosomes* disturb the local magnetic field affecting T1 and T2 relaxation times during MRI. In addition to a high surface-to-volume ratio, AuNPs have unique size- and shape-dependent optical and electronic properties. The surfaces of AuNPs can also be readily modified with ligands containing functional groups such as thiols, phosphines, and amines, which exhibit affinity for gold surfaces [25]. Gold nanoparticles have emerged as a promising scaffold for drug and gene delivery that provide a useful complement to more traditional delivery vehicles. Silver nanoparticles have been widely used as a novel therapeutic agent extending its use as antibacterial, antifungal, antiviral and antiinflammatory agent. Kalishwaralal et al., found silver nanoparticles, produced by *Bacillus licheniformis*, have the potential of anti-angiogenic [64].

Incomprehensible as antimicrobial

Amongst various metal NPs, AgNPs have been widely considered surgical gloves and covers, antibacterial injury dressings, bed lines and so forth [67]. They likewise have various applications in the fields of gadgets, catalysis and

indicative therapeutics. The nanoparticles were also evaluated for their increased antimicrobial activities with various antibiotics against Gram-positive and Gram-negative bacteria. The antibacterial activities of ampicillin, kanamycin, erythromycin, and chloramphenicol were increased in the presence of AgNPs against test strains.

Biosensors

Similarly, gold nanoparticles (AuNPs) are additionally used for various purposes e.g. as labels for biosensors, for cure of hyperthermia and being equipped for conveying vast estimated biomolecules, give non-dangerous intends to quality and medication liberation to the objective locales [68]. Platinum nanoparticles (PtNPs) are utilized for the treatment of various illnesses, for example, growth and oxidative anxiety issue; in addition, they are utilized as a part of gadgets for planning of a novel memory component.

Reaction rate Enhancement Agent

Magnetic nanoparticles have been used to improve the microbiological reaction rates. In fact, magnetic nanoparticles were utilized not only for their catalytic function but also for their good ability to disperse. Shan et al. made use of the coated microbial cells of *Pseudomonas delafieldii* with magnetic Fe_3O_4 nanoparticles to fulfill desulfurization of dibenzothiophene [69]. The application of an external magnetic field ensured that the cells were well diffused in the solution even without mixing and enhanced the possibility to collect cells for reuse. The results showed that the desulfurization efficiencies of *P. delafieldii* were not reduced and the cells could be reused several times.

Clinical operations

Titanium nanoparticles have been applied in the pharmaceutical industry as drug delivery vehicles and in excipient formulations [68,72-75]. Used in cellular therapy, such as cell labeling and targeting and as a tool for cell biology research to separate and purify cell populations. Also used in: tissue repair; drug delivery; magnetic resonance imaging; hyperthermia; magnetofection [68,77]. Used for covering urinary catheters, surgical instruments and bone prostheses. Additionally, silver has been used in water and air filtration to eliminate microorganisms. AgNPs have been added to soft tissue Conditioners for prosthetic devices [78-80]. Palladium nanostructures have also emerged as self-therapeutics. A few examples recently demonstrated their anti-microbial and cytotoxic pharmacological activities. For example, Adams et al. reported the size dependent high anti-microbial activity of Pd NPs. The particles showed higher growth inhibition against *S. aureus*, compared to *E. Coli*, highlighting Pd NPs as useful anti-microbial agents especially for gram positive bacteria [70]. Balbin et al. reported that Pd NPs supported on *mesoporous silica*-based materials displayed relatively high cytotoxic activity against four tested human cancer cell lines [71].

CONCLUSION

The use of microrganisms including bacteria, yeast, fungi and actinomycetes can be classified intracellular and extracellular synthesis according to the location where nanoparticles are formed. Nanomedicine is a burgeoning field of research with tremendous prospects for the the improvement of the diagnosis and treatment of human diseases. Research is currently carried out manipulating microorganisms at the genomic and proteomic levels. Nanoparticles are having a great deal of utilizations in different fields like antimicrobials, additives, paints, biosensors and makeup With the recent progress and the ongoing efforts in improving particle synthesis efficiency and exploring their biomedical applications, it is hopeful that the implementation of these approaches on a large scale and their commercial applications in medicine and health care will take place in the coming years.

REFERENCES

1. M. C. Daniel and D. Astruc, “Gold nanoparticles: assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology,” Chemical Reviews, vol. 104, no. 1, pp. 293–346, 2004.

2. H. Kato, "*In vitro* assays: tracking nanoparticles inside cells," Nature Nanotechnology, vol. 6, no. 3, pp. 139–140, 2011.
3. Gordon RB, Bertram M, Graedel TE. Metal stocks and sustainability. Proc Natl Acad Sci U S A 2006;103:0509498103 [pii]:1209–1214
4. Pantidos N, Horsfall LE. Biological synthesis of metallic nanoparticles by bacteria, fungi and plants. J Nanomed Nanotechnol 2014;05.
5. R. Bhattacharya and P. Mukherjee, "Biological properties of "naked" metal nanoparticles," Advanced Drug Delivery Reviews, vol. 60, no. 11, pp. 1289–1306, 2008.
6. K. Simkiss and K. M. Wilbur, Biomineralization, Academic, New York, NY, USA, 1989.
7. Filipponi, L. and Sutherland, D. (2013), "Nanotechnologies: Principles, Applications, Implications and Hands-on Activities", A compendium for educators, Luksemburg.
8. Babu, M.M.G., Sridhar, J. and Gunasekaran, P. (2011), "Global transcriptome analysis of Bacillus cereus ATCC 14579 in response to silver nitrate stress", J. Nanobiotech., 9(1), 1-12.
9. Kathiresan K, Manivannan S, Nabeel MA, Dhivya B (2009) Studies on silver nanoparticles synthesized by a marine fungus, Penicillium fellutanum isolated from coastal mangrove sediment. Colloids Surf B 71(1):133–137. doi:10.1016/j.colsurfb.2009.01.016.
10. Wang MY, Shen T, Wang M, Zhang D, Chen J (2013) One-pot green synthesis of Ag nanoparticles-decorated reduced graphene oxide for efficient nonenzymatic H2O2 biosensor. Mater Lett 107:311–314. doi:10.1016/j. matlet.2013.06.031.
11. Roduner E (2006) Size matters: why nanomaterials are different. Chem Soc Rev 35(7):583–592. doi:10.1039/B502142C.
12. Kapil Punjabi, Shraddha Mehta, Snehal Yedurkar , Rajesh Jain , Sandeepan Mukherjee , Avinash Kale and Sunita Deshpande "Extracellular synthesis of silver nanoparticle by Pseudomonas hibiscicola – Mechanistic approach" Advances in Nano Research, Vol. 6 No. 1 (2018) 81-92 DOI: https://doi.org/10.12989/anr.2018.6.1.081
13. Forough Mehrdad, Farhadi K (2010) Biological and green synthesis of silver nanoparticles. Turkish J Eng Env Sci 34:281–287. doi:10.3906/muh-1005-30.
14. Abou El-Nour KMM, Aa Eftaiha, Al-Warthan A, Ammar RAA (2010) Synthesis and applications of silver nanoparticles. Arab J Chem 3(3):135–140. doi:10.1016/j.arabjc.2010.04.008.
15. Pal Sovan Lal, Utpal Jana PK, Manna GP, Mohanta Manavalan R (2011) Nanoparticle: an overview of preparation and characterization. J Appl Pharma Sci 1(6):228–234
16. Mason Cynthis, Vivekanadhan Singaravelu, Misra Manjusri, Mohanty AK (2012) Switchgrass (Panicum virgatum) extract mediated green synthesis of silver nanoparticles. World J Nano Sci Eng 2:47–52.
17. Xiangqian Li,1, 2 Huizhong Xu,3 Zhe-Sheng Chen,2 and Guofang Chen4 " Biosynthesis of Nanoparticles by Microorganisms and Their Applications" . Hindawi Publishing Corporation Journal of Nanomaterials Volume 2011, Article ID 270974, 16 pages doi:10.1155/2011/270974.
18. Bazylinski DA, Frankel RB, Jannasch HW. Nature 1988;334:518.
19. Konishi Y, Ohno K, Saitoh N, Nomura T, Nagamine S, Hishida H, et al. J Biotechnol 2007;128:648.
20. Lengke M, Southam G. Geochim Cosmochim Acta 2006;70:3646.
21. P. Mukherjee, R. Bhattacharya, C. R. Patra et al., Nanogold in Cancer Therapy and Diagnosis, vol. 7, chapter 3, Wiley-VCH, Weinheim, Germany, 2007.
22. S. Mahdihassan, "Alchemy, Chinese versus Greek, an etymological approach: a rejoinder," American Journal of Chinese Medicine, vol. 16, no. 1-2, pp. 83–86, 1988.
23. G. J. Higby, "Gold in medicine: a review of its use in the West before 1900," Gold bulletin, vol. 15, no. 4, pp. 130–140, 1982.
24. C. R. Patra, R. Bhattacharya, D. Mukhopadhyay, and P. Mukherjee, "Fabrication of gold nanoparticles for targeted therapy in pancreatic cancer," Advanced Drug Delivery Reviews, vol. 62, no. 3, pp. 346–361, 2010.
25. D. A. Giljohann, D. S. Seferos, W. L. Daniel, M. D. Massich, P. C. Patel, and C. A. Mirkin, "Gold nanoparticles for biology and medicine," Angewandte Chemie International Edition, vol. 49, no. 19, pp. 3280–3294, 2010.
26. Riedel Sebastian, Kaupp Martin (2009) The highest oxidation states of the transition metal elemtns. Coord Chem Rev 253(5–6):606–624. doi:10.1016/j. ccr.2008.07.014.

27. T. Klaus, R. Joerger, E. Olsson, and C. -G. Granqvist, "Silverbased crystalline nanoparticles, microbially fabricated," Proceedings of the National Academy of Sciences of the United States of America, vol. 96, no. 24, pp. 13611–13614, 1999.
28. N. Jain, A. Bhargava, S. Majumdar, J. C. Tarafdar, and J. Panwar, "Extracellular biosynthesis and characterization of silver nanoparticles using Aspergillus flavus NJP08: a mechanism perspective," Nanoscale, vol. 3, no. 2, pp. 635– 641, 2011.
29. N. Vigneshwaran, N. M. Ashtaputre, P. V. Varadarajan, R. P. Nachane, K. M. Paralikar, and R. H. Balasubramanya, "Biological synthesis of silver nanoparticles using the fungus Aspergillus flavus," Materials Letters, vol. 61, no. 6, pp. 1413– 1418, 2007.
30. K. C. Bhainsa and S. F. D'Souza, "Extracellular biosynthesis of silver nanoparticles using the fungus Aspergillus fumigatus," Colloids and Surfaces B, vol. 47, no. 2, pp. 160–164, 2006.
31. S. Senapati, D. Mandal, A. Ahmad et al., "Fungus mediated synthesis of silver nanoparticles: a novel biological approach," Indian Journal of Physics A, vol. 78A, no. 1, pp. 101–105, 2004.
32. D. Zheng, C. Hu, T. Gan, X. Dang, and S. Hu, "Preparation and application of a novel vanillin sensor based on biosynthesis of Au-Ag alloy nanoparticles," Sensors and Actuators B: Chemical, vol. 148, no. 1, pp. 247.
33. B. D. Sawle, B. Salimath, R. Deshpande, M. D. Bedre, B. K. Prabhakar, and A. Venkataraman, "Biosynthesis and stabilization of Au and Au-Ag alloy nanoparticles by fungus, Fusarium semitectum," Science and Technology of Advanced Materials, vol. 9, no. 3, Article ID 035012, pp. 1–6, 2008.
34. Schlüter M, Hentzel T, Suarez C, Koch M, Lorenz WG, et al. (2014) Synthesis of novel palladium(0) nanocatalysts by microorganisms from heavy-metalinfluenced high-alpine sites for dehalogenation of polychlorinated dioxins. Chemosphere 117C: 462-470
35. Nithin kumar sharma et al.,2016 "From Synthesis to Biological Impact of Pd (II) Complexes: Synthesis, Characterization, and Antimicrobial and Scavenging Activity", Hindawi Publishing Corporation Biochemistry Research International Volume 2016, Article ID 4359375, 8 pages http://dx.doi.org/10.1155/2016/4359375.
36. Lloyd JR, Yong P, Macaskie LE (1998) Enzymatic recovery of elemental palladium by using sulfate-reducing bacteria Appl Environ Microbiol 64: 4607- 4609
37. Oremland RS, Herbel MJ, Blum JS, Langley S, Beveridge TJ, Ajayan PM, et al. Appl Environ Microbiol 2004;70:52
38. Prasad K, Jha AK, Kulkarni AR. Nanoscale Res Lett 2007;2:248.
39. T. X. Fan, S. K. Chow, and D. Zhang, "Biomorphic mineralization: from biology to materials," Progress in Materials Science, vol. 54, no. 5, pp. 542–659, 2009.
40. D. A. Bazylinski, R. B. Frankel, B. R. Heywood et al., "Controlled biomineralization of magnetite (Fe3O4) and greigite (Fe3S4) in a magnetotactic bacterium," Applied and Environmental Microbiology, vol. 61, no. 9, pp. 3232–3239, 1995.
41. A. Arakaki, H. Nakazawa, M. Nemoto, T. Mori, and T. Matsunaga, "Formation of magnetite by bacteria and its application," Journal of the Royal Society Interface, vol. 5, no. 26, pp. 977–999, 2008.
42. R. Blakemore, "Magnetotactic bacteria," Science, vol. 190, no. 4212, pp. 377–379, 1975.
43. R. H. Thornhill, J. G. Burgess, and T. Matsunaga, "PCR for direct detection of indigenous uncultured magnetic cocci in sediment and phylogenetic analysis of amplified 16S ribosomal DNA," Applied and Environmental Microbiology, vol. 61, no. 2, pp. 495–500, 1995.
44. S. Spring and K. H. Schleifer, "Diversity of magnetotactic bacteria," Systematic and Applied Microbiology, vol. 18, no. 2, pp. 147–153, 1995
45. A. Sanyal, D. Rautaray, V. Bansal, A. Ahmad, and M. Sastry, "Heavy-metal remediation by a fungus as a means of production of lead and cadmium carbonate crystals," Langmuir, vol. 21, no. 16, pp. 7220–7224, 2005.
46. D. Rautaray, A. Sanyal, S. D. Adyanthaya, A. Ahmad, and M. Sastry, "Biological synthesis of strontium carbonate crystals using the fungus Fusarium oxysporum," Langmuir, vol. 20, no. 16, pp. 6827–6833, 2004.
47. S. R. K. Pandian, V. Deepak, K. Kalishwaralal, J. Muniyandi, N. Rameshkumar, and S. Gurunathan, "Synthesis of PHB nanoparticles from optimized medium utilizing dairy industrial waste using Brevibacterium casei SRKP2: a green chemistry approach," Colloids and Surfaces B, vol. 74, no. 1, pp. 266–273, 2009.
48. S. Yan, W. He, C. Sun et al., "The biomimetic synthesis of zinc phosphate nanoparticles," Dyes and Pigments, vol. 80, no. 2, pp. 254–258, 2009.

49. S. A. Kumar, A. A. Ansary, A. Abroad, and M. I. Khan, "Extracellular biosynthesis of CdSe quantum dots by the fungus, Fusarium oxysporum," Journal of Biomedical Nanotechnology, vol. 3, no. 2, pp. 190–194, 2007.
50. B. Fadeel and A. E. Garcia-Bennett, "Better safe than sorry: understanding the toxicological properties of inorganic nanoparticles manufactured for biomedical applications," Advanced Drug Delivery Reviews, vol. 62, no. 3, pp. 362–374, 2010.
51. W. C. W. Chan and S. Nie, "Quantum dot bioconjugates for ultrasensitive nonisotopic detection," Science, vol. 281, no. 5385, pp. 2016–2018, 1998.
52. F. Tian, A. Prina-Mello, G. Estrada et al., "A novel assay for the quantification of internalized nanoparticles in macrophages," Nanotoxicology, vol. 2, no. 4, pp. 232–242, 2008.
53. D. Cui, F. Tian, S. R. Coyer et al., "Effects of antisense-mycconjugated single-walled carbon nanotubes on HL-60 cells," Journal of Nanoscience and Nanotechnology, vol. 7, no. 4-5, pp. 1639–1646, 2007.
54. D. Pantarotto, C. D. Partidos, J. Hoebeke et al., "Immunization with peptide-functionalized carbon nanotubes enhances virus-specific neutralizing antibody responses," Chemistry and Biology, vol. 10, no. 10, pp. 961–966, 2003.
55. R. L. Edelstein, C. R. Tamanaha, P. E. Sheehan et al., "The BARC biosensor applied to the detection of biological warfare agents," Biosensors and Bioelectronics, vol. 14, no. 10- 11, pp. 805–813, 2000.
56. A. De la Isla, W. Brostow, B. Bujard et al., "Nanohybrid scratch resistant coatings for teeth and bone viscoelasticity manifested in tribology," Materials Research Innovations, vol. 7, no. 2, pp. 110–114, 2003.
57. J. Ma, H. Wong, L. B. Kong, and K. W. Peng, "Biomimetic processing of nanocrystallite bioactive apatite coating on titanium," Nanotechnology, vol. 14, no. 6, pp. 619–623, 2003.
58. M. Shinkai, M. Yanase, M. Suzuki et al., "Intracellular hyperthermia for cancer using magnetite cationic liposomes," Journal of Magnetism and Magnetic Materials, vol. 194, no. 1, pp. 176–184, 1999.
59. R. Weissleder, G. Elizondo, J. Wittenberg, C. A. Rabito, H. H. Bengele, and L. Josephson, "Ultrasmall superparamagnetic iron oxide: characterization of a new class of contrast agents for MR imaging," Radiology, vol. 175, no. 2, pp. 489–493, 1990.
60. W. J. Parak, R. Boudreau, M. Le Gros et al., "Cell motility and metastatic potential studies based on quantum dot imaging of phagokinetic tracks," Advanced Materials, vol. 14, no. 12, pp. 882–885, 2002.
61. Panigrahi S, Kundu S, Ghosh SK, et al. Selective one-pot synthesis of copper nanorods under surfactantless condition. Polyhydron. 2006;25(5):1263-9.
62. B. Fadeel and A. E. Garcia-Bennett, "Better safe than sorry: understanding the toxicological properties of inorganic nanoparticles manufactured for biomedical applications," Advanced Drug Delivery Reviews, vol. 62, no. 3, pp. 362–374, 2010.
63. Baesman SM, Bullen TD, Dewald J, et al. Formation of tellurium nanocrystals during anaerobic growth of bacteria that use Te oxyanions as respiratory electron acceptors. Appl Environ Microbiol. 2007;73(7):2135-43.
64. R. Vaidyanathan, K. Kalishwaralal, S. Gopalram, and S. Gurunathan, "Nanosilver—the burgeoning therapeutic molecule and its green synthesis," Biotechnology Advances, vol. 27, no. 6, pp. 924–937, 2009.
65. Fan T.X., Chow S.K. and Zhang D., Biomorphic mineralization: from biology to materials, Progress in Materials Science, 54(5), 542–659, (2009).
66. O. Felfoul, M. Mohammadi, and S. Martel, "Magnetic resonance imaging of Fe3O4 nanoparticles embedded in living magnetotactic bacteria for potential use as carriers for in vivo applications," in Proceedings of the 29th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBS '07), pp. 1463–1466, 2007.
67. Bellucci S. Nanoparticles and nanodevices in biological applications. Lecture Notes in Nanoscale Sci Technol. 2009;4:1-198
68. Acosta TLS, Lopez MLM, Nunez ARE, et al. Biocompatible metal-oxide nanoparticles: Nanotechnology improvement of conventional prosthetic acrylic resins. J Nanomater. 2011:941561
69. G. Shan, J. Xing, H. Zhang, and H. Liu, "Biodesulfurization of dibenzothiophene by microbial cells coated with magnetite nanoparticles," Applied and Environmental Microbiology, vol. 71, no. 8, pp. 4497–4502, 2005.
70. C. P. Adams, K. A. Walker, S. O. Obare and K. M. Docherty, PLoS One, 2014, 9, e85981.
71. A. Balbin, F. Gaballo, J. Ceballos-Torres, S. Prashar, M. Fajardo, G. N. Kaluderovic and S. Gomez-Ruiz, RSC Adv., 2014, 4, 54775–54787
72. Salata O. Applications of nanoparticles in biology and medicine. J Nanobiot. 2004;2(1):3.

73. Contado C, Pagnoni A. TiO2 in commercial sunscreen lotion: Flowfield-flow fractionation and ICP-AES together for size analysis. Anal Chem. 2008;80(19):7594-608.
74. Guo Y, Zhou Y, Jia D, et al. Fabrication and in vitro characterizationof magnetic hydroxycarbonate apatite coatings with hierarchicallyporous structures. Acta Biomater. 2008;4(4):923-931.
75. Sikong L, Kooptarnond K, Niyomwas S, et al. Photoactivityand hydrophilic property of SiO2 and SnO2 co-doped TiO2 nanocompositethin films. Songklanakarin Journal of Science & Technology. 2010;32(4):413-8.
76. Arbab AS, Bashaw LA, Miller BR, et al. Characterization of biophysical and metabolic properties of cells labeled with superparamagnetic ironoxide nanoparticles and transfection agent for cellular MR imaging. Radiology. 2003;229(3):838-46.
77. Sanpo N, Wen C, Berndt CC, et al. Antibacterial properties of spinelferrite nanoparticles. In: Mendez A, editor. Microbial pathogens andstrategies for combating them: Science, technology and education. Spain: Formatex Research Centre. 2013;239-50.
78. Limbach LK, Wick P, Manser P, et al. Exposure of engineered nanoparticles to human lung epithelial cells: Influence of chemical composition and catalytic activity on oxidativestress. Environ Sci Technol. 2007;41(11):4158- 63.
79. Nino MN, Martínez CGA, Aragon PA. Characterization of silver nanoparticles synthesized on titanium dioxide fine particles. Nanotech. 2003;19(6):065711.
80. Ahn SJ, Lee SJ, Kook JK. Experimental antimicrobial orthodontic adhesives using nanofillers and silver nanoparticles. Dent Mater. 2009;25(2):206-13.
81. Gandhi H and Khan S. Biological Synthesis of Silver Nanoparticles and Its Antibacterial Activity. J Nanomed Nanotechnol. 2016;7:366.
82. Kumar D et al. Nanoindentation and VSM of Silver Nanowires Synthesized by Microwave-Polyol Process. J Nanomater Mol Nanotechnol. 2016;5:1.
83. Ashok CH et al. CuO/TiO2 Metal Oxide Nanocomposite Synthesis via Room Temperature Ionic Liquid. J Nanomater Mol Nanotechnol. 2016;5:1
84. Beveridge TJ, Murray RGE. J Bacteriol 1980;141:876.
85. Zonaro, E., Piacenza, E., Presentato, A., Monti, F., Dell'Anna, R., Lampis, S., et al. (2017). Ochrobactrum sp. MPV1 from a dump of roasted pyrites can be exploited as bacterial catalyst for the biogenesis of selenium and tellurium nanoparticles. Microb. Cell Factories 16:215. doi: 10.1186/s12934-017-0826-2
86. Subramaniyan, S. A., Sheet, S., Vinothkannan, M., Yoo, D. J., Lee, Y. S., Belal, S. A., et al. (2018). One-pot facile synthesis of Pt nanoparticles using cultural filtrate of microgravity simulated grown P. chrysogenum and their activity on bacteria and cancer cells. J. Nanosci. Nanotechnol. 18, 3110–3125. doi: 10.1166/jnn.2018. 14661
87. De Windt D, Aelterman P, Verstraete W. Environ Microbiol 2005;7:314.
88. Lang C, Schuler D. J Phys Condens Matter 2006;18:S2815–28
89. Bharde A, Wani A, Shouche Y, Joy PA, Prasad BLV, Sastry M. J Am Chem Soc 2005;127:9326.
90. Senapati S, Ahmad A, Khan MI, Sastry M, Kumar R. Small 2005;1:517

Table 1: Biosynthesis of Nanoparticles by Micro Organisms

S.No	Micro Organism	Nanoparticle	Location	Size	Reference
a	*Bacillus subtilis* 168	Au	Octahedral	5.25nm	[84]
b	*Escherichia coli*	Au	Spherical	15-57	[81-83]
c	*Lactobacillus spp.*	Au-Ag	Hexagonal	9-25	
d	*Fusarium oxysporum*	Au/Ag Alloy	ND	8-14	[90]
e	*S. oneidensis* MR–1	Pd	Not available	ND	[87]
f	*M.gryphiswaldense*	Magnetite	/Cubo-octahedral elongated hexagonal prismatic	35-120nm	[88]
g	*Actinobacter sp.*	Magnetite	Quasi-spherical	10-40nm	[89]
h	*Ochrobactrum sp* MPV	Te	Spherical, rod		[85]
i	*P. aeruginosa* SNT1	Se	Spherical /contour	ND	[87]
j	*Penicillium chrysogenum*	Pt	Not available	5-40	[86]

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RESEARCH ARTICLE

BA-ANFIS: Biometric Authentication using Adaptive Neuro-Fuzzy Inference System

N. P. Shiju[1]* and D. Kannan[2]

[1]Ph.D. Research Scholar, Department of Computer Science, Pollachi College of Arts and Science, Pollachi, Coimbatore Tamil Nadu, India.
[2]Professor, Department of Computer Science, Pollachi College of Arts and Science, Pollachi, Coimbatore, Tamil Nadu, India.

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***Address for Correspondence**
N. P. Shiju,
Ph.D. Research Scholar,
Department of Computer Science,
Pollachi College of Arts and Science,
Pollachi, Coimbatore**,** Tamil Nadu, India.

ABSTRACT

Biometrics, such as fingerprints, face, irises, palm prints, veins, finger knuckle-prints (FKPs), ears, voice, signature, and so on, is the most secure and extensively used technique for detecting personal identification and validating public security and privacy protection requirements. Hand-based biometrics has gained more attention from academics and engineers in recent decades than other biometrics owing to its adaptability and user acceptance. This article presents a framework for biometric authentication based on an adaptable neuro-fuzzy inference system (BA-ANFIS). Fingerprint image enhancement is a critical step in obtaining minutiae from input fingerprint pictures. MEA's performance is dependent on the quality of fingerprint pictures. We suggested a novel image improvement approach that combines Wedge Fuzzy Integrated Clip Limited Adaptive Histogram Equalization (WFI-CLAHE) with the minutiae extraction algorithm (MEA), which extracts minutiae from fingerprint photos, to improve the Image. In addition, the statistical characteristic of fingerprints is extracted. The Morph approach segmentation obtains the best features using PCA and SPCA algorithms. Finally, the ANFIS algorithm has been used for fingerprint authentication. The experimental findings are compared to current methodologies and classification performance measures.

Keywords: ANFIS, Biometric, PCA, WFI-CLAHE, MPC,

INTRODUCTION

In today's electronic environment, authentication is a critical responsibility. Traditional authentication techniques rely on tokens, making passwords insecure and dangerous. These methods have many disadvantages, including the possibility of a token being lost, stolen, or shared and large passwords being difficult to remember. Authentication methods based on a human's biometric features may overcome the limitations of existing systems. Human biometric features are divided into two categories: behavioral and physiological traits. Behavioral features are associated with human conduct, such as stride, voice, signature, etc. Physiological features, however, are connected to the form or shape of portions of the human body, such as a fingerprint, face, iris, etc. Such biometric features seen in the human body may be collected using proper sensors. The fingerprint is one of the most prominent biometric qualities used to verify a person since it is simple to make, fast to process, and widely accepted. Recently, biometric characteristics have emerged as essential authentication system components. This is because biometric characteristics are based on physiological or behavioral characteristics that are unique to each person. Biometric aids are regarded as essential components of highly secure identification and verification systems as solutions to security breaches, transaction fraud, and other issues in various applications that directly impact ordinary people's lives. This is particularly true in delicate situations, such as financial transactions, restricted access zones, the handling of personal data, and privacy. Real-time biometric authentication systems are also available, especially for applications involving scattered computer resources and where application logins, data protection, remote access, transaction security, etc., correlate with individual personality features. The primary benefit received is security, which strengthens reliability and trust.

In most cases, biometric identification is the most reliable transaction method. The identification of individuals based on their physical characteristics or characteristics is known as biometric authentication. In human-computer interaction (HCI) systems, biometrics is used as an identity and access control approach. The recognition of fingerprints is a well-developed biometric technique used in criminal investigations. The entire Image, finger ridges, or prominent portions derived from the ridges are utilized to build significant representations of the finger (minutiae). These properties form a fingerprint orientation field, ultimately giving the distinguishing elements for person authentication. Fingerprint identification is a popular method because of its user-friendliness, low cost of fingerprint sensors, non-intrusive scanning, and fairly adequate performance. Commercial automated fingerprint recognition systems (FRS) have seen substantial performance advancements in recent years. Biometric systems are divided into static and dynamic biometric systems. Human static traits, such as fingerprints, iris, and faces, remain constant. While human dynamic qualities such as voice, ECG, keyboard and touch dynamics, and so on, change with time. The key performance assessment criteria for biometrics identification systems are efficiency, accuracy, robustness, security, and privacy [3]. Impersonation and spoofing assaults against biometrics systems are common, lowering the recognition system's performance. Therefore, robust and trustworthy person identification systems need secure, non-replicable, and spoof-resistant systems [4] [5]. This paper proposes an ANFIS-based multimodal biometric person authentication system that uses the user's face, palm vein, and fingerprints.

The main contributions of this research work are
- Preprocessing using the WFI-CLAHE method
- Segmentation using Morph Algorithm
- Feature Extraction using Principle Component analysis
- Classification and Prediction using ANFIS Method.

The following is how the paper is structured. Section II discusses prior research on multimodal biometric authentication systems. Section III describes the suggested technique in full. Included in Section IV are the experimental results and discussion. Section V concludes the essay by describing the open framework for developing the proposed system.

BACKGROUND STUDY

Abazi, B. *et al.* [2] Mobile devices are evolving, and the capacity to analyze data pushes the mobile device business to unprecedented success. They provide geolocation, online browsing, and mobile-only apps. These mobile device services have increased the demand for essential resources, such as 546. Ali, S. *et al*. [3] Traditional authentication methods, such as PIN and password, have significant disadvantages compared to biometric-based human authentication systems. The security of the biometric data, saved in the database as a user template after registration and cannot be altered, is a problem with these systems since it is permanently associated with the human body and cannot be altered. Biometric user template security systems safeguard biometric data. Because fingerprints are one of the most extensively used biometric identifiers, there is a need for a method to preserve fingerprint template data. This article offers a comprehensive technique for protecting the fingerprint template. It creates a three-dimensional, non-invertible fingerprint template based on the minutiae position and single points in the fingerprint picture—the suggested method deals with intra-class alterations such as rotation and translation. Babamir, F. S., *et al*. [7] Our digest-based biometric authentication strategy for client-server systems communicating across an untrusted public network has been enhanced. Unprotected public networks necessitate that client-server systems prioritize attack resistance and cost. However, it lacked anonymity and mutual authentication. When using an offline dictionary and MITM methods, our present work can withstand these assaults and is resistant to password guessing.

It also has features like anonymity and password-changing security. Cherifi, F. *et al*. [9] We suggested an effective continuous and real-time authentication mechanism for mobile devices based on human prehensile motions acquired by inertial signal sensors. We developed a dataset of prehensile motions and used a Hidden Markov Model-Universal Background Model to identify users (HMM-UBM). The suggested technique collects real-time data on prehensile movement, generates feature vectors, trains the HMM-UBM model, and validates user IDs using gravity signals. A prototype of the suggested method was developed for performance assessment, and its effectiveness was tested in terms of Equal Error Rate (EER) and authentication latency. The suggested method significantly increases Precision and latency. Walmart, V. R., & Brindha, M. [11] A biometric-based privacy-preserving authentication system has been developed that saves the authentication data table on an untrusted server. The system protocol employs three hashing algorithms: Secure Hash Algorithm-256, Chaotic map, and Paillier cryptosystem. The suggested technique protects the confidentiality of the authentication data table. The suggested system incorporates a one-of-a-kind picture symmetric cipher. An innovative biometric image cryptosystem may be applied to any biometric image, such as an iris or fingerprint, for use in various biometric identity systems. In a new biometric picture cryptosystem, the biometrics input dictates the control values of the keystreams needed during confusion-diffusion rounds.

Jamdar, C., & Boke, A. [14] System for verifying face biometric identity. Face to multimodal biometrics will be studied and evaluated using various face images. The person verification strategy enhanced recognition accuracy compared to unimodal and privies systems. Mason, J. *et al*. [17] Physical measures may be used to identify humans. These measures are referred to as biometrics. Integrating biometric data with the real world creates biometric applications and devices in society. We provide a biometric solution for the healthcare industry. Our biometric technology takes an innovative method of identifying new and registered patients inside healthcare information systems. Srinivasan, P. [25] An ATMEGA controller was used to build a biometric sensor-based automotive ignition system. The technology reads the user's fingerprint before activating the ignition system. The car will start and be ready to drive if the driver is permitted. Yang, W. *et al*. [29] The proposed approach is used to characterize the MP fingerprint minutia feature descriptor. S MP has been evaluated for recognizing fingerprints from four public databases. The suggested technique generates projection matrices from local feature slots while modifying the user-specific key M.

Problem Specification

Because the CLAHE method is not automated and requires two input parameters, we presented the WFI-CLAHE Method. Because the present biometric authentication system has low Accuracy, we improved biometric authentication accuracy.

MATERIALS AND METHODS

We presented a BA-ANFIS architecture for effective biometric authentication in this part. We utilized biometric pictures as a benchmark dataset in this system. The WFI-CLAHE Method is used to preprocess the images. The Morph technique was used to segment the image. PCA algorithms are used to choose the best features. Finally, the categorization was completed using ANFIS algorithms.

PREPROCESSING

Image preprocessing refers to operations performed on images at the most fundamental level of abstraction to enhance the image data by reducing undesired distortions or enhancing key image properties in preparation for further processing. It does not affect the quantity of image data. Its approaches exploit the significant redundancy in photos. In photographs, neighboring pixels from the same object have the same or equivalent brightness value. If a warped pixel can be isolated, it may be rectified by averaging the values of its neighboring pixels.

Noise Removal

It is difficult to distinguish road segments in noisy pictures accurately. This is true if the Image displays two-way traffic with very close lanes. Furthermore, the Image may include a variety of non-interesting components. This section describes the suggested noise removal-based technique for denoising and reconstructing traffic video frame pictures from the traffic video dataset. Noise reduction may be performed using IRA and iterative weight modifications.

$(a, w) = arg.\min||b - B||\left(\frac{2}{b}\right) + \beta||a - w$ ------- (1)

The first term deals with sparsity, while the second term iteratively decreases noise and acts as the regularisation parameter. The optimization problem is divided into two sub problems by Equation (1). (1) estimating image patches based on measurements; (2) repeatedly denoising image patches by adjusting their weights;

$a^{(s+1)} = arg.\min||b - B||\frac{2}{2} + \beta$ ------ (2)

$w^{(s+1)} = arg.\min||b - B||\frac{2}{2} + \beta$ --------- (3)

In the above equations, are s represents the current iteration. The term B minimizes the reconstruction error. β represent the feature maps. An iterative gradient technique can be employed to estimate $a^{(s+1)}W^{(s+1)}$ represents the error by refining weights. The proposed architecture has shown in figure 1, which consists of preprocessing, segmentation, feature extraction, and classification details.

Histogram Equalization using WFI function

Contract stretching, histogram equalization, soft masking, and spatial filtering are all required. Micro calcifications vary widely in size and form, as does the contrast between ROIs and surrounding tissues. This method reduces image noise, accentuates image edges, and shows digital images. Before picture production, other procedures for medical image processing of coherent echo data may be applied. This study utilizes a clip-value-based approach for Image preprocessing (WFI-CLAHE). Image enhancement may increase contrast in various applications because of its simplicity, convenience of usage, and effectiveness. Examining medical imaging and radar data are two examples. While flattening the probability distribution, histogram equalization broadens the dynamic range of grey levels. To increase the contrast of the image. Histogram equalization improved photos by employing the WFI to map the grey levels of the source image. Sub-Picture Histogram Equalization, a unique image contrast enhancement approach based on lighting, is introduced in this paper.

Grayscale pictures with low light are very effective in retaining entropy and regulating the pace of improvement. The Sub-Image Histogram Equalization approach enables controlled improvement in addition to optimizing

entropy. The proposed procedure should result in better low-light photos. The purpose of the transformation function is to produce an image with a uniform histogram as the output.
Let $M = \{M(i,j)\}$ identifier for a picture comprised of L distinct grey levels

$M = \{M_0, M_1, M_2 \dots \dots . M_{L-1}\}$ the density function p for a given image M[M_k].

Information theory predicts that the message source's entropy will reach its maximum value.

SEGMENTATION

Convert the Image to binary format, then do the morphological operation ion, and thin the Image.
Binary pictures (pixels that can only be one of two colors, often black and white) may include flaws. Sound and texture may be adjusted when a simple threshold produces binary zones—morphological operations in image processing attempt to overcome these flaws by considering the Image's shape and structure.

FEATURE EXTRACTION

Local and global features are used to extract the features. PCA and LDA algorithms are used to extract local characteristics. NPCA, SpPCA, and LBA approaches are used to extract global features.

Principal Component Analysis

Step 1: The covariance matrix is calculated.
Step 2: The covariance matrix's Eigenvectors and Eigen values.
Step 3: Transform the Image using its Eigenvectors.

Local Discriminant Analysis

Step 1. Project data into a vector space
Step 2: Magnifies the inter-class variance as well as degrades the intra-class variance
The Global features are extracted with the following features.

Modular Principal Component Analysis

Step 1: Divide the Image into smaller regions
Step 2: For Each sub-regions, find the eigenvector and covariance matrix, transform the Image with eigenvectors of the sub-region

Subpattern Principal Component Analysis

Step 1: Convert the image into a vector
Step 2: Partitioned into equally sized sub patterns with non - overlapping
Step 3: Convert to submatrix from the subpattern vector
Step 4: Apply PCA to each sub-matrix and combine the features.

Local Binary Pattern

Step 1: Dividing the image into a matrix(3x3)
Step 2: Set a pixel value as the center pixel and collect the pixels around it
Step 3: If the neighborhood value is larger than or equal to the value of the central pixel, set the threshold to 1; otherwise, set the threshold to 0.
Step 4: Collect the binary values according to clockwise or counterclockwise direction, and then convert the binary code to decimal.
Step 5: Replace the center pixel value with the resultant decimal and repeat for all other pixel values in the picture.

MODULAR PRINCIPAL COMPONENT ANALYSIS

Because of differences in expression, lighting, and posture, most automated face recognition algorithms assess faces as a single unit [14-17]. This overlooks the fundamental difference between expression-invariant and expression-sensitive facial characteristics. MPCA is one of these upgrades above PCA. Using this MPCA approach, the whole face image is subdivided into smaller regions, and feature extraction is conducted for each area. As in PCA, calculate the mean sub-region, covariance matrix, eigenvectors, and weight set for each sub-region of the training set. The weights will be more representative of the face's regional data. Under normal conditions, the weights of the face photographs will be comparable to the weights of a single face image in the same place. The specified face belongs to the class of faces with the smallest Euclidean distance. This technique improves Accuracy by using unaffected regions of the face since some modifications to face photos do not influence the whole facial information. As a consequence, it is anticipated that using the following MPCA technique would result in greater recognition rates. Furthermore, unlike PCA, it selects a small number of core components to find the precise matching facial picture.

This procedure subdivides each picture in the training set into smaller N areas. Consequently, the size of each sub-image is r c/N. These sub-images may be mathematically expressed as

$$I_{ij}(x,y) = I_i\left(\frac{r}{\sqrt{y}}(j-1) + x, \frac{c}{\sqrt{y}}(j-1) + y\right)\forall_{i,j} \qquad (1)$$

where I range from 1 to X, where X represents the total number of pictures in the training set, j ranges from 1 to Y, where Y represents the number of sub-images, and m and n range from 1 to r/ Y and c/ Y, respectively. The next step is calculating the mean picture of all the training sub-images.

$$\varphi = \frac{1}{X.Y}\sum_{i-1}^{X}\sum_{j-1}^{Y} I_{ij} \qquad (2)$$

Subtracting the image matrix with its mean,

$$Y_{ij} = I_{ij} - \varphi \qquad (3)$$

The covariance matrix is calculated as follows using these sub-images,

$$C = \frac{1}{X.Y}\sum_{i-1}^{X}\sum_{j-1}^{Y} Y_{ij}.Y_{ij}^{T} \qquad (4)$$

From the covariance matrix, Eigen values and Eigenvectors are computed. The covariance matrix yields a symmetric result. Multiplying the eigenvectors and producing the main components yields the picture weights,

$$PC = \left(I_{ij} - \varphi\right).\ v\ \ \forall_{ij} \qquad (5)$$

Next, the test image is classified, and the test sub-image weights are obtained using eigenvectors as stated in the following Equation:

$$\Omega A = PC^{T}.\left(T_{test\,j} - \phi\right); A = 1,2 \ldots\ldots\ldots M, \forall j \qquad (6)$$

The Euclidean distance between two weight vectors (feature vectors) thus serves as a measure of similarity. Finding the minimum Euclidean distance k between a test point and a training point is the mathematical definition of recognition.

$$\varepsilon_k = \sqrt{||\Omega_a - \Omega_k||^2}; k = 1, \ldots\ldots M \qquad (7)$$

To extract significant information from the sample images of two databases, we have to choose an optimal number of PCs. We can achieve this by calculating variance

$$variance = \frac{\gamma_i}{\sum_{i=1}^{M}\gamma_i} \times 100 \qquad (8)$$

SPCA

The SpPCA is divided into two stages. In the third step, a whole original pattern represented by a vector is divided into a collection of non overlapping subpatterns of equal size. All subpatterns with identical original feature components are extracted from the training set to create training subpattern sets. Then, PCA is performed on each of these subpattern sets. We are given a specific set of instructions.

patterns $M = \{M_1, M_2, \dots, M_y\}$ with each column vector $M_i (i = 1,2, \dots, N)$

Possessing m measurements A whole original design is divided into K non overlapping d-dimensional subpatterns and molded into a d-by-K matrix in the first step.

$M = (M_{i1}, M_{i2}, \dots, M_{ik}), with\ M_{ij} = (m_{i(d+1),\dots,m_i(jd)})^T$ being the jth subpattern of M_i and i=1,2,…,y and j=1,2,…, K. and then according to the second step, we construct PCA for the jth subpattern set

$SP_j = \{M_{ij}, i = 1,2, \dots, N\}, j = 1,2, \dots, K\ to\ seek\ its\ pro-jection\ vectors\ \emptyset_j = (\varphi_{j1}, \varphi_{j2} \dots, \varphi_{jl})$

We are minimizing the reconstructed (local) error (RCE) or maximizing the total spatial dispersion. Similar to the Eigenface method [2], the jth total subpattern scatters (sub-scatter) matrix is initially calculated.

$$s_j as\ s_j = (\frac{1}{N}) \sum_{i=1}^{N} (M_{ij} - M_j)(M_{ij} - M_j)^T, where\ M_j = \left(\frac{1}{N}\right) \sum_{i=1}^{N} m_{ij}, j = 1,2, \dots, K$$

Are subpatterns denotative? Here, it is simple to show that all total sub-scatter matrices are positive semi-de3nite matrices with d d scales. Then, find the third set of projection sub-vectors independently using the following eigenvalue–eigenvector system while adhering to the constraints:

$\emptyset_j^T \emptyset_j = I_j, j = 1,2, \dots, k,$
$S_j \emptyset_j = \emptyset_j A_j,$

Where Ij is an identity matrix, _j is a diagonal matrix made up of Sj's 3rd l largest nonnegative eigenvalues in descending order, and their 3rd l local eigenvectors make up j. After obtaining all projected subvectors from partitioned subpattern sets, we may extract subfeatures Yj from each subpattern of a given full pattern $Z = (Z_1, Z_2, \dots, Z_k)$ where Zj is a subpattern with the dimension matching each partition (2):

$Y_j = \emptyset_j^T Z_j j = 1,2, \dots, K.$

Then combine them into the following global characteristic:

$N = (N_1^T, N_2^T, \dots, M_K^T)^T = (Z_1^T, \emptyset_1\ Z_2^T, \emptyset_2, \dots, Z_K^T, \emptyset_K)^T.$

It is not difficult to conclude that when K=1 and d=x, SPCA simplifies to PCA. In addition, the dimension of N remains the same as the size of the projection vector discovered using PCA on the whole pattern. We can now classify patterns using the closest neighbor (NN) method [3] based on the synthesized global characteristics.
It is made as follows: for any unknown whole pattern m, if $i = \arg min\{||\emptyset^T(m_I - m_i)||^2, then\ m \in class\ i.$
The decision may be modified in proportion to the relevant decision functions.

$fPCA(m) = [x - (m_I + m_i)/2]^T \emptyset^T \emptyset [m_I - m_i],$ where m_I and m_i are the ith and Ith whole training patterns
PCA results in a projection matrix represented by. Similarly, for spPCA, analogous decision functions are constructed by

$fSpPCA(m) = \sum_{k=1}^{k}[\, x^{(k)} - \frac{m_i^k + m_i^k}{2]^T \emptyset_k^T} \emptyset_k \left[m_I^k - m_i^{(k)}\right]$,

where K and k are de3ned as before, and m(k); m(k)

I and x(k) l corresponds to the kth subpatterns of the m, mi, and my patterns. The PCA decision function is linear. However, the SpPCA decision function combines K piece-wise linear functions in d-dimensional space that is the outcome of distinct k. SpPCA has the potential to enhance PCA's performance and attractiveness dramatically. When K is set to 1, the decision functions of both models are the same, emphasizing that PCA is a unique instance of SpPCA.

CLASSIFICATION

A neuro-fuzzy technique, a blend of neural networks and fuzzy logic, has been developed to overcome individual flaws and give more attractive qualities. The ultimate purpose of using such a system is to eliminate imprecise information in a picture, such as a pixel greyness ambiguity, geometrical image segmentation, and ambiguous scene interpretation. This takes advantage of the systems' learning and descriptive capabilities, yielding excellent interpretability and accuracy outcomes.

Workflow of ANFIS-based authentication system

Figure 2 displays the workflow for the proposed authentication system. Initially, it constructs a user profile model based on behaviorally-representative characteristics. There are two sorts of activities: foreground and background. The user cannot control background operations, such as receiving SMS and phone calls. On the other hand, the foreground activities are those that the user initiates, such as making a phone call or connecting to the Internet. These qualities are used to evaluate foreground and background behavior:

Features : $(h_1, h_2, h_3, \dots, h_n), (a_1, a_2, a_3, \dots, a_m)$ – (8)

Adaptive real-time foreground and background anomaly scores are generated via a time-window-based profiling method to quantify the irregularity of the present user's activity. Due to the dynamic nature of user behavior, a generalized ranking system (see section III C) is implemented to maintain a list of the top n most recent or relevant occurrences for each feature (excluding the Screen On/Off feature). Section III C explains that the strange score is calculated using the values gathered by the ranking algorithm within each feature category. Using the scoring

functions described below, the strange score for each attribute is computed:

Functions :$(T_{h1}, T_{h2}, \dots, T_{hn}), (T_{a1}, T_{a2}, \dots, T_{am})$ – (9)

Because the foreground anomaly score PT_{fore} is generated gradually based on all the events that occur during a period; it may be expressed as:

$PT_{fore} = \sum_{k=1}^{n} \sum_{l=0}^{num_k} T_{hk}(event_{kl})$ ---------- (10)

Where $event_{kl}$represents the l_{th} occurrence of a foreground feature event $h_k$is the total number of foreground features, and $num_k$is the number of events that have happened for a particular foreground feature h_k. The model's computation of the background anomaly score PTback may be expressed as:

$$PTback = \sum_{k=1}^{m} (T_{ak}(a_k) - \rho \times o_k) \quad - \qquad (11)$$

Where is the damping factor that accommodates for lengthy periods of inactivity and permits a gradual decrease in the anomaly score, given that o_kIs the inactivity duration, and m is the total number of background features?

Calculating the following foreground and background references is the responsibility of the reference calculation module.

$$\begin{cases} rehfore_k = h_{ref}\left(rehfore_{k-1}, PT_{fore_k}\right) \\ rehfore_k = h_{ref}\left(rehfore_{k-1}, PT_{back_k}\right) \end{cases} - \quad (12)$$

The foreground and background anomaly scores and their respective references are then used as input parameters of the trained ANFIS model to estimate the real-time threat level. Therefore, the risk level of the present user, as determined by the decision module, may be stated as:

$$threat\ level\ =\ ANFIS(p1, p2, \ldots, pk) - \quad (13)$$

where in our case, we have four inputs:
$p1\ =\ PTfore, p2\ =\ PTback, p3\ =\ (PTfore - rehfore), p4\ =\ (PTback - rehback)$.

The computed *threat level* is then used to make final authentication decisions.

Algorithm for BA-ANFIS
Step 1: Get the Finger Print image from the dataset
Step 2: Enhance the Image by WFI – CLAHE
Step 3: Apply a Morphological image on the fingerprint to get a thin image of the fingerprint
Step 4: Extract the local features by Principal Component Analysis and Local discriminant Analysis
Step 5: Extract global features by Mpca, SpPCA, and LBP.
Step 6: Fusion of the features
Step 7: Authentication detected by ANFIS Classification
Step 8: Performance Analysis

A neuro-fuzzy approach, a combination of neural networks and fuzzy logic, has been created to overcome individual defects and provide more desired traits. The ultimate goal of using such a system is to remove ambiguous information from a photograph, such as a pixel greyness ambiguity, geometrical image segmentation, and ambiguous scene interpretation. This uses the systems' learning and descriptive capabilities, yielding highly interpretable and accurate results.

RESULTS AND DISCUSSION

The proposed BA-ANFIS framework has been implemented by using MATLAB 2018Ra. The experimental results are shown in figure 2 to figure 7.
Figure 2 depicts the input biometric image. And the WFI-CLAHE Method has been applied, as illustrated in Figure 3. Figure 4 depicts the binary image result. Finally, Figure 5 shows the ANFIS categorized thinned image. Figure 6 depicts categorization metrics such as Accuracy, sensitivity, specificity, and Precision. Figure 7 depicts the Performance assessment chart. The Precision is 95.7143 percent.

CONCLUSION

This article explains the BA-ANFIS Framework for finger vein and finger image-based biometric authentication. Using the WFI-CLAHE approach, the Image was preprocessed. The Morph method was used for segmentation. Utilizing PCA, LDA, and LBP, the features are extracted. The categorization was accomplished using the ANFIS algorithm with a 95.7413 percent success rate. Experiments demonstrate that the attempt to use the intrinsic relationship of texture on both the upper and lower sides of the finger is effective and promising for multimodal biometrics, with higher recognition accuracy and fewer authentications than other multimodal and unimodal

identification methods. There are ongoing efforts to enrich our database and optimize finger rotation performance. ANFIS classifiers are used to classify the feature correctly.

REFERENCES

1. Alpar, O. (2021). Biometric keystroke barcoding: A next-gen authentication framework. Expert Systems with Applications, 177, 114980. doi:10.1016/j.eswa.2021.114980
2. Abazi, B., Qehaja, B., & Hajrizi, E. (2019). Application of biometric models of authentication in mobile equipment. IFAC-PapersOnLine, 52(25), 543–546. doi:10.1016/j.ifacol.2019.12.602
3. Ali, S. S., Baghel, V. S., Ganapathi, I. I., & Prakash, S. (2020). Robust biometric authentication system with a secure user template. Image and Vision Computing, 104004. doi:10.1016/j.imavis.2020.104004
4. Buriro, A., Crispo, B., & Conti, M. (2019). AnswerAuth: A bimodal behavioral biometric-based user authentication scheme for smartphones. Journal of Information Security and Applications, 44, 89–103. doi:10.1016/j.jisa.2018.11.008
5. Brostoff, G. (2019). How AI and biometrics are driving next-generation authentication. Biometric Technology Today, 2019(6), 7–9. doi:10.1016/s0969-4765(19)30086-4
6. Bera, B., Das, A. K., Balzano, W., & Medaglia, C. M. (2020). On the design of biometric-based user authentication protocol in a smart city environment. Pattern Recognition Letters, 138, 439–446. doi:10.1016/j.patrec.2020.08.017
7. Babamir, F. S., & Kirci, M. (2019). Dynamic digest-based authentication for client-server systems using biometric verification. Future Generation Computer Systems. doi:10.1016/j.future.2019.05.025
8. Bruno, A., Cattaneo, G., Ferraro Petrillo, U., & Capasso, P. (2021). PNU spoofing: a menace for biometrics authentication systems? Pattern Recognition Letters. doi:10.1016/j.patrec.2021.07.008
9. Cherifi, F., Omar, M., & Amroun, K. (2021). An efficient biometric-based continuous authentication scheme with HMM prehensile movements modeling. Journal of Information Security and Applications, 57, 102739. doi:10.1016/j.jisa.2020.102739
10. De Oliveira Nunes, I., Eldefrawy, K., & Lepoint, T. (2019). SNUSE: A secure computation approach for large-scale user re-enrollment in biometric authentication systems. Future Generation Computer Systems, 98, 259–273. doi:10.1016/j.future.2019.03.051
11. Falmari, V. R., & Brindha, M. (2021). Privacy preserving biometric authentication using Chaos on remote untrusted server. Measurement, 177, 109257. doi:10.1016/j.measurement.2021.109257
12. Hasimah Ali, Wahyudi, & Salami, M. J. E. (2009). Keystroke pressure based typing biometrics authentication system by combining ANN and ANFIS-based classifiers. 2009 5th International Colloquium on Signal Processing & Its Applications. doi:10.1109/cspa.2009.5069216
13. Hasimah Ali, Wahyudi, & Salami, M. J. E. (2009). Keystroke pressure based typing biometrics authentication system by combining ANN and ANFIS-based classifiers. 2009 5th International Colloquium on Signal Processing & Its Applications. doi:10.1109/cspa.2009.5069216
14. Jamdar, C., & Boke, A. (2017). Multimodal biometric identification system using fusion level of matching score level in single modal to multimodal biometric system. 2017 International Conference on Energy, Communication, Data Analytics and Soft Computing (ICECDS). doi:10.1109/icecds.2017.8389858
15. Kaur, H., & Khanna, P. (2020). Privacy preserving remote multi-server biometric authentication using cancelable biometrics and secret sharing. Future Generation Computer Systems, 102, 30–41. doi:10.1016/j.future.2019.07.023
16. Meng, X. (2008). Study on the Model of E-Commerce Identity Authentication Based on Multi-biometric Features Identification. 2008 ISECS International Colloquium on Computing, Communication, Control, and Management. doi:10.1109/cccm.2008.263
17. Mason, J., Dave, R., Chatterjee, P., Graham-Allen, I., Esterline, A., & Roy, K. (2020). An Investigation of Biometric Authentication in the Healthcare Environment. Array, 100042. doi:10.1016/j.array.2020.100042
18. Menaga, S., Paruvathavardhini, J., Pragaspathy, S., Dhanapal, R., & Jebakumar Immanuel, D. (2021). An efficient biometric based authenticated geographic opportunistic routing for IoT applications using secure wireless sensor network. Materials Today: Proceedings. doi:10.1016/j.matpr.2021.01.241

19. Mahfouz, A., Mahmoud, T. M., & Eldin, A. S. (2017). A survey on behavioral biometric authentication on smartphones. Journal of Information Security and Applications, 37, 28–37. doi:10.1016/j.jisa.2017.10.002
20. Oduah, U. I., Kevin, I. F., Oluwole, D. O., & Izunobi, J. U. (2021). Towards a high-precision contactless fingerprint scanner for biometric authentication. Array, 11, 100083. doi:10.1016/j.array.2021.100083
21. Pallikonda Rajasekaran, M., Suresh, M., & Dhanasekaran, U. (2014). Multimodal biometric recognition using sclera and fingerprint based on ANFIS. 2014 International Conference on Recent Trends in Information Technology. doi:10.1109/icrtit.2014.6996159
22. Rakesh Kumar, M., & Parthasarathy, V. (2020). A secure fuzzy extractor based biometric key authentication scheme for body sensor network in Internet of Medical Things. Computer Communications. doi:10.1016/j.comcom.2020.01.077
23. Qi, M., Chen, J., & Chen, Y. (2018). A secure biometrics-based authentication key exchange protocol for multi-server TMIS using ECC. Computer Methods and Programs in Biomedicine, 164, 101–109. doi:10.1016/j.cmpb.2018.07.008
24. Rajan, Y., & Patil, J. (2016). Satyaapan: Novel technique for identification using biometric data. 2016 International Conference on ICT in Business Industry & Government (ICTBIG). doi:10.1109/ictbig.2016.7892697
25. Srinivasan, P., Anthoniraj, S., Anguraj, K., Kumarganesh, S., & Thiyaneswaran, B. (2021). Development of keyless biometric authenticated vehicles ignition system. Materials Today: Proceedings. doi:10.1016/j.matpr.2021.03.632
26. S., A., & AnilKumar, K. S. (2021). Secure mobile internet voting system using biometric authentication and wavelet based AES. Journal of Information Security and Applications, 61, 102908. doi:10.1016/j.jisa.2021.102908
27. Tian, Y., Li, Y., Sengupta, B., Li, N., & Su, C. (2020). Leakage-resilient biometric-based remote user authentication with fuzzy extractors. Theoretical Computer Science. doi:10.1016/j.tcs.2020.02.001
28. Xing, S., Xue, H., & Yan, X. (2008). Research on Model of Biometric Identification Emergency Response in Electronic Commerce. 2008 International Conference on Management of e-Commerce and e-Government. doi:10.1109/icmecg.2008.28
29. Yang, W., Wang, S., Shahzad, M., & Zhou, W. (2021). A cancelable biometric authentication system based on feature-adaptive random projection. Journal of Information Security and Applications, 58, 102704. doi:10.1016/j.jisa.2020.102704
30. Zhang, Q. (2018). Deep Learning of Electrocardiography Dynamics for Biometric Human Identification in era of IoT. 2018 9th IEEE Annual Ubiquitous Computing, Electronics & Mobile Communication Conference (UEMCON). doi:10.1109/uemcon.2018.8796676

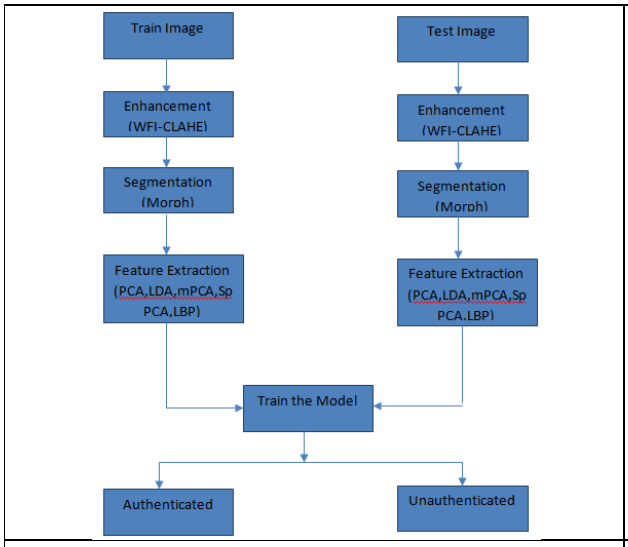

Figure 1: BA-ANFIS Framework architecture

Figure 2: Biometric Input Image

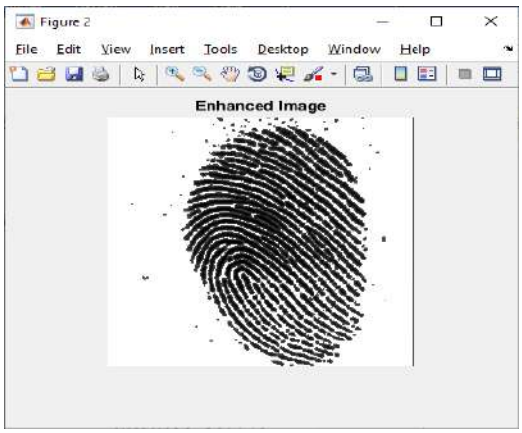

Figure 3: Enhanced Image

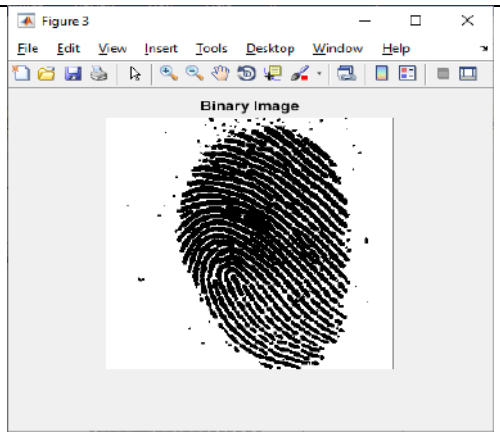

Figure 4: Binary Image

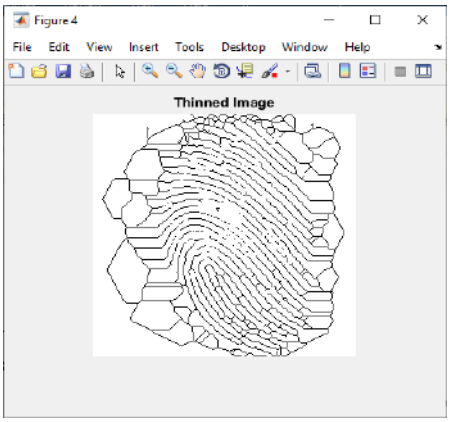

Figure 5: Thinned Image

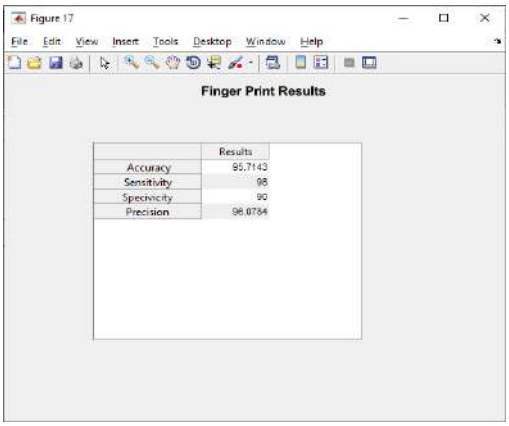

Figure 6: ANFIS Classification metrics results.

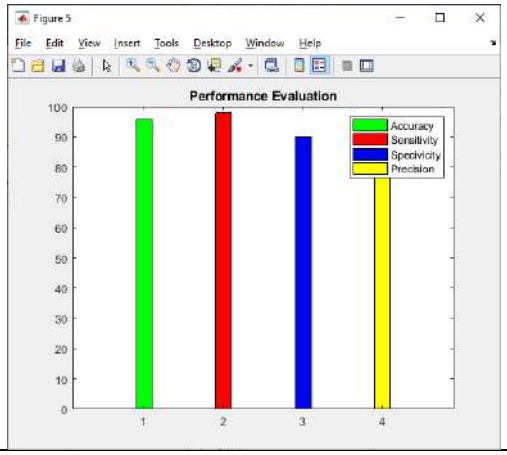

Figure 7: Performance evaluation with classification metrics.

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RESEARCH ARTICLE

Perceptions of the Pradhan Mantri Jandhan Yojana among Bengaluru City Slum Dwellers

Divya.U[1], Noor Firdoos Jahan[2], Sunil M Rashinkar[3]* and D.Jogish[4]

[1]Assistant Professor, Department of Management Studies, Dayananda Sagar Academy of Technology and Management, Bengaluru, Karnataka, India.
[2]Professor, RV Institute of Management, Bengaluru, Karnataka, India.
[3]Assistant Professor, School of Management, Presidency University, Bengaluru, Karnataka, India.
[4]Professor, Department of Management Studies, Sai Vidya Institute of Technology, Bengaluru, Karnataka, India.

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***Address for Correspondence**
Sunil M Rashinkar,
Assistant Professor,
School of Management,
Presidency University,
Bengaluru, Karnataka, India.
Email: getrashinkar@gmail.com

ABSTRACT

In order to achieve long-term economic development, inclusive growth is required. To promote inclusive growth in the country, every government must prioritise financial inclusion. Financial inclusion measures are not new in India. The RBI and the Government of India have undertaken several initiatives, including nationalisation of banks, expansion of banks and their branches, Bank Mitra, the Swabhimaan campaign, and so on. Despite several financial inclusion initiatives, poverty and exclusion continue to dominate the Indian economy six decades after independence. However, the Government of India and the Reserve Bank of India have not given up on their attempts to achieve total financial inclusion in India. The Pradhan Mantri Jan Dhan Yojana is one of the main efforts launched by the Modi government to promote full financial inclusion (PMJDY). The plan was introduced with the goal of providing everyone access to financial services, beginning with a basic banking account, an overdraft capacity, and a Rupay debit card with built-in accident insurance. The study focused primarily on the perceptions of urban slum residents in Bengaluru city regarding the PMJDY initiative. The study's key results were that slum dwellers believed that the KYC standards for creating Jan Dhan accounts are easy, that bank staff/Bank Mitras are extremely cooperative, and that direct benefit payments to these accounts are helpful. Overall, slum residents in Bengaluru city are hesitant to operate the Jan Dhan account on their own.

Keywords: Pradhan Manti Jandhan Yojana (PMJDY), Perception, Financial literacy, Slum dwellers

INTRODUCTION

India is the world's biggest democracy, the fifth-largest in nominal GDP, and the third-largest in PPP (purchasing power parity). Despite all evidence of progress and development in India, a huge portion of the population remains impoverished. According to the Oxford Poverty and Human Development Initiative and the United Nations Development Program's multidimensional poverty index (MPI), the poverty rate in India is about 55%. (2010). To promote inclusive growth in a country, every government must prioritise financial inclusion. It is the integration of financially excluded groups of society into the economy's formal financial structure. Financial inclusion is not a new endeavour in India. The Reserve Bank of India and the Government of India have launched several initiatives, including bank nationalisation, the development of co-operative and regional rural banks, the Bank Mitra model, the Swabhimaan Campaign, and so on. Despite these different financial inclusion policies, poverty and exclusion continue to dominate the Indian economy six decades after independence. However, the Government of India and the Reserve Bank of India did not quit their attempts to attain total financial inclusion (Chowhan, S. S., & Pande, J. C. 2014). The Pradhan Mantri Jan Dhan Yojana is an important effort launched by the Modi government to promote full financial inclusion (PMJDY). The plan was introduced with the goal of providing universal access to financial services through the provision of a basic banking account, overdraft capacity, Rupay debit card, and built-in life and accident insurance coverage. The plan was included into the Guinness Book of Records for opening the most number of accounts in the shortest amount of time. R. Khuntia (2014).

Six pillars of Jan Dhan Yojana

- **Sub Service Area (SSA) approach**

The RBI is mapping about 6,00,000 villages to provide at least one fixed point banking outlet, i.e. branch banking (brick and mortar model) or branchless banking (business correspondence model), serving to 1,000 to 1,500 families.

- **Basic bank account with overdraft facility and Rupay debit card**

The primary goal of PMJDY is to equip financially excluded poor individuals with a basic saving bank account with an integrated Rupay debit card and an overdraft capability of up to Rs 10000.

- **Financial Literacy**

The RBI has clearly recognised the importance of financial literacy in the success of financial inclusion initiatives. As a result, it insisted on banks, particularly in rural regions, having specialised financial literacy centres to educate the public on financial matters.

- **Credit guarantee fund**

The credit guarantee fund was intended to protect banks from overdraft facility defaults.

- **Microinsurance**

One of the major goals of the PMJDY initiative was to provide access to insurance products. It was thought that issuing microinsurance was more significant.

- **Swavalamban pension scheme**

Access to unorganised sector pension schemes like as Swavalamban was another pillar of PMJDY.V. Gupta (2015).

LITERATURE REVIEW

Shettar, R. M. (2016) investigated Jan Dhan difficulties and barriers for effective plan implementation. The main challenges identified in this study include duplication of Jan Dhan accounts as more than one account was opened by the same person with different branches of banks due to the relaxation of KYC norms; the government did not

make any budgetary provision for providing security against OD default; LIC's need to fix nominal premiums for insurance coverage; and providing overdraft facility was left to the discretion of banks, so they may not offer this facility due to its unavailability. Other obstacles include providing basic infrastructural facilities for Bank Mitras such as laptops, micro ATMs, internet access, and so on, and private banks may levy hidden fees for Jan Dhan accounts, which may impede financial inclusion. Irrinki and Burlakanti (2017) studied the awareness and impression of the Pradhan Mantri Jan Dhan Yojana among 125 Thallarevu Mandal residents. According to the study's major findings, about 97.6 percent of respondents were aware of the initiative. Many respondents stated that the Jan Dhan account was not their first bank account and that they had another bank account before creating the Jan Dhan account. The majority of respondents said they created a Jan Dhan account to save money. The majority of them have been handed an Aadhaar card or ration card as evidence of address in order to register an account. 41 percent of respondents thought they received all of the Jan Dhan benefits on schedule. They can classify elements into five categories in factor analysis, such as wholesomeness, awareness, leverage eyewash, and other aspects.

Vaishali Khandelwal (2017) conducted a study of 71 respondents in Udaipur to assess the knowledge and advantages of the Pradhan Mantri Jan Dhan initiative. He conducted a descriptive study of demographic data and used the chi-square test to see if there was a link between demographic characteristics and awareness. He discovered that the strategy was well-known among the responders. They were aware that the plan provided life and accidental insurance coverage. The survey found that the government's attempt to raise public knowledge of the Jan Dhan scheme was a success. In terms of their impressions of the plan, they believed the government had made a very excellent step for the benefit of everybody. The system was beneficial in encouraging individuals to save and protected them from being exploited by money lenders. They also saw the strategy as beneficial to poverty alleviation and overall economic growth. The chi-square test found a link between respondents' age and awareness.

Dasgupta A. and Anklesaria E. (2015) investigated the difficulties in implementing the Pradhan Mantri Jan Dhan Yojana scheme. The key obstacles of this system are insufficient infrastructure in rural locations, inactivity of bank accounts, financial illiteracy among the underprivileged sector of the population, and the problem of account duplication, among other things. Satpathy, I *et al*. (2015) examined financial inclusion in Bhuvaneswar and discovered that respondents with bank accounts were aware of the Jan Dhan initiative, whereas respondents without bank accounts were unaware of Jan Dhan accounts.Approximately 78 percent of male respondents and 95 percent of financially excluded respondents were unaware of the Pradhan Mantri Jan Dhan initiative. They proposed that the government and banks launch an intensive campaign to raise awareness of the plan among the financially excluded strata of society.

Objectives of the study

- To study the slum dwellers' perception towards Pradhan Mantri Jan Dhan Yojana accounts.
- To evaluate the association between the Pradhan Mantri Jandhan account's perception and demographic variables of respondents.

METHODOLOGY

The descriptive research approach was used in this study. The study's scale is essentially an interview schedule. The PMJDY perception scale was heavily influenced by research investigations done by Irrinki and Burlakanti (2017) and Khandelwal (2017). (2017). A five-point Likert scale ranging from 5-Strongly agree to 1-Strongly disagree was used to determine the respondents' degree of statement agreement with the Pradhan Mantri Jandhan Yojana. Jandhan account users in Bengaluru city slums were chosen as the sampling unit for the study. The study included a sample size of 300 respondents. For the study, non-probability sampling approaches such as purposive and judicious sampling were used. The Karnataka Slum Development Board statistics were used as the sampling frame for the sample selection. The slum board divides Bengaluru's slums into two categories: declared slums and undeclared slums.The study took into account the designated slums. The declared slums were separated into five groups based

on Bengaluru geographical regions such as Bengaluru South, North, East, West, and Central in order to obtain a sample with similar traits and attributes of the people. Two slums were chosen from each of these regions depending on the number of households in the slum. Each slum's number of respondents was chosen in proportion to the overall number of households. Each of these selected houses is polled with one Respondent. Primary data was acquired from 500 slum inhabitants, and 459 of them were able to supply us with complete information in the interview schedule, while 41 interview schedules were discarded owing to erroneous and missing replies. 91.8 percent of people responded. The face-to-face interview approach was used for data collection, which resulted in a high response rate. The slum responders were really helpful in supplying information. For data analysis, R- Programming was used in the study. For data analysis, statistical procedures such as mean, standard deviation, t-test, and ANOVA were used.

ANALYSIS AND RESULTS

According to the above data, the Demographic information of Jan Dhan account holders show that 45.3 percent were 'Female' and 54.7 percent were 'Male.' 60 percent of Jan Dhan account holders were in the age category of '36-45 years,' 22.3 percent were in the age group of 'less than 35 years,' 11.7 percent were in the age group of '46-55 years,' and 6percent were in the age group of 'more than 56 years.' In terms of education, 70.6 percent of respondents studied up to 'High School or lower,' 18 percent of Jan Dhan account holders had 'No Formal Education,' and 11.4 percent studied up to 'PUC or above.' Concerning employment, 55.3 percent of respondents had 'PFTE,' 25 percent had 'PPTE,' 11.7 percent were 'Self-employed,' and 8 percent were 'Unemployed.' In terms of occupation, 88.7 percent of Jan Dhan account holders were performing 'Jobs,' 6% were doing 'Other' jobs, and just 5.3 percent owned their own 'Business.' 59.3 percent of respondents had a monthly salary between '5,000 and 10,000,' 35 percent had a monthly income between '10,000 and 20,000,' and 5.7 percent had a monthly income of 'less than 5,000.' Concerning family size, 84 percent of them belong to the 'Up to 5' family size, while 16 percent belong to the 'More than 5' family size. **H_{01}**- There is no statistically significant difference in the mean values of 'PMJDY perception' between Male and Female.

Interpretation

An independent sample t-test was performed between males (M=3.46, SD=0.75) and females (M=3.37, SD=0.93) to see if there was a significant difference in mean Perception towards Pradhan Mantri Jan Dhan Yojana. At the 5% level of significance, the mean difference of 0.09 is not statistically significant. (t (458) = -0.88, p=.038, > 0.05). As a result, the Null hypothesis, "There is no significant difference in mean values of 'PMJDY perception' among Male and Female respondents," is accepted. **H_{02}**- There is no significant difference in mean values of 'PMJDY Perception' between families with less than five children and those with more than five children.

Interpretation

An independent sample t test was used to assess if there is a significant difference in mean values of Perception towards Pradhan Mantri Jan Dhan Yojana between families with more than five members (M=3.44, SD=1.17) and families with less than five members (M=3.42, SD=0.75). At the 5% level of significance, the mean difference of 0.02 is not statistically significant (t (458) = 0.08, p=.0.94 > 0.05). As a result, the Null hypothesis is accepted: "There is no significant difference in mean values of 'PMJDY Perception' for families with less than five and more than five people."H_{03}- There is no statistically significant difference between educational groups in mean values of 'PMJDY Perception.'

Interpretation

A one-way ANOVA test was used to see if 'PMJDY Perception' differed across groups with varying levels of education. Respondents were divided into three groups: those with no education (n=54), those with a high school diploma or less (n=212), and those with a postsecondary diploma or above (n=34). The mean values of PMJDY Perception increased from 'PUC and above' (M=3.11, SD=0.56) to 'No education' (M=3.45, SD=0.79) to 'High school and below' (M=3.49, SD=1.05) in that order, but the differences in mean values between these education groups were

not statistically significant at the 5% level of significance [F (2, 298) = 2.21, p =0.11 > 0.05]. As a result, the null hypothesis, "There is no significant difference in mean values of 'PMJDY Perception' between Educational groups," is accepted. H_{04}- There is no statistically significant variation in the mean values of 'PMJDY Perception' across age groups.

Interpretation

A one-way Anova test was used to assess whether 'PMJDY Perception' differed by age group. Respondents were divided into four groups: those under the age of 35 (n=67), those between the ages of 36 and 45 (n=180), those between the ages of 46 and 55 (n=35), and those above the age of 56 (n=18). The 'PMJDY Perception' rose in that sequence from 'Less than 35 years' (M=3.32, SD=0.68) to 'Between 36 and 45 years' (M=3.36, SD=0.82) to 'Greater than 56 years' (M=3.41, SD=0.57) to 'Between 46 and 55 years' (M=3.75, SD=1.08). At the 5% level of significance, the mean differences between these age groups were statistically significant [F (2, 298) =3.08, p=0.03 0.05]. As a result, the Null hypothesis is rejected. After rejecting the null hypothesis based on test findings, the alternative hypothesis "There is a substantial difference in mean values of 'PMJDY Perception' between Age groups" is accepted. H_{05}- There is no statistically significant difference in the mean values of 'PMJDY Perception' across income groups.

Interpretation

A one-way ANOVA test was used to assess if 'PMJDY Perception' differed by income group. Respondents were divided into three groups: those earning between Rs10000 and Rs20000 (n=105), those earning between Rs5000 and Rs10000 (n=178), and those earning less than Rs5000 (n=17). In that sequence, the mean PMJDY Perception went from 'less than Rs 5000' (M=3.31, SD=0.69) to 'between Rs5000 and Rs10000' (M=3.41, SD=0.87) to 'between Rs 10000 and Rs 20000' (M=3.52, SD=0.84). The mean income differences were not statistically significant [F (3, 457) =0.89, (p) 0.41 > 0.05]. As a result, the null hypothesis, "There is no significant difference in mean values of 'PMJDY Perception' between Income groups," is accepted. H_{06}- There is no significant difference among mean values of 'PMJDY Perception' across Occupational groups

Interpretation

A one-way ANOVA test was used to see if 'PMJDY Perception' differed across profession respondents. PMJDY respondents were divided into three categories: "Business" (n=16), "Job" (n=266), and "Others" (n=18). In that sequence, the mean PMJDY perception climbed from 'Business' (M=3.30, SD=0.52) to 'Job' (M=3.42, SD=0.87) to 'Others' (M=3.62, SD=0.46). At the 5% level of significance, the mean differences between these occupational categories were not statistically significant [F (2, 297) = 3.53, (p) 0.51> 0.05]. As a result, the Null hypothesis, "There is no significant variation in the mean values of PMJDY Perception between occupational categories," is accepted.

DISCUSSION

In terms of their perceptions of the Pradhan Mantri Jan Dhan Yojana, slum dwellers believe that the KYC standards for creating Jan Dhan accounts are easier, that free life and accidental insurance coverage provides social security, and that the Jan Dhan plan is helpful to them. They also stated that bank employees/Bank Mitras are quite helpful, and that direct benefit payments to these accounts are beneficial. They both agreed that the zero balance option is really useful. Overall, slum residents in Bengaluru city are hesitant to operate the Jan Dhan account on their own. The hypothesis testing found that there was little variation in PMJDY perception between male and female respondents, family sizes, education groups, profession groups, and income groups. However, there was a statistically significant variation in PMJDY perception among respondents of various ages. The middle-aged group of 36-45 years old is more conscious of PMJDY. When compared to other age groups, the view of PMJDY is good in the 46-55 age group.

CONCLUSION

PMJDY is a significant step forward in India's financial inclusion. The policy was carefully thought out, with features such as KYC relaxation, zero balance accounts, Rupay debit card, OD facility, inbuilt life and accidental coverage, access to government pension and insurance plans, usage of the Bank Mitra model, direct benefit transfer, and so on.Though the PMJDY scheme was well planned, there have been some flaws in its implementation, such as the OD facility and free insurance coverage not being provided to all account holders, banks not appointing many Bank Mitras because the government did not budget for financial assistance to implement these benefits, the relaxation of KYC led to vast duplication of bank accounts, and accounts were also opened by people who already had bank accounts (Shettar, R. M. 2016). People's use of Jan Dhan accounts has been relatively restricted due to widespread financial illiteracy, and they have been hesitant to handle the accounts. They have not taken out a loan or invested in any of the government's insurance or pension plans. The initiative proved effective in transferring gas subsidies and other benefits directly to recipients' accounts. To summarise, the Pradhan Mantri Jan Dhan Yojana is a good move made by the government towards financial inclusion, and it is a significant milestone in India's financial inclusion, however the plan is not well executed. The plan is less successful today due to a variety of practical issues.

REFERENCES

1. Chowhan, S. S., & Pande, J. C. (2014). Pradhan Mantri Jan Dhan Yojana: A giant leap towards financial inclusion. International Journal of Research in Management & Business Studies, 1(4), 19-22.
2. Dasgupta, A., & Anklesaria, E. (2015) "Pradhan Mantri Jan Dhan Yojana.", KPMG report.
3. Gupta, S. K. (2015). Performance Appraisal of the Pradhan Mantri Jan Dhan Yojana. IOSR Journal of Business and Management (IOSR-JBM), 17(9), 35-39.
4. Gupta, V. (2015). Pradhan Mantri Jan-Dhan Yojana: An Effort towards Financial Inclusion. IUP Journal of Bank Management, 14(2), 49.
5. Irrinki., & Burlakanti., (2017). Perception on Pradhan Mantri Jan Dhan Yojana: A study with reference to Thallarevu mandal. International Journal of Research, Granthaalayah, Vol.5, Issue 6, ISSN- 2350-0530(O), ISSN- 2394-3629(P).
6. Khuntia, R. (2014). Pradhan Mantri Jan Dhan Yojana (PMJDY): A new drive towards financial inclusion in India. ZENITH International Journal of Business Economics & Management Research, 4(11), 10-20.
7. Nanda, K., & Kaur, M. (2017). Bank-led Financial Inclusion and Human Development: Evidence from India. South Asian Journal of Management, 24(1).
8. Satpathy, I., Patnaik, B. C. M., & Das, P. K. (2015). Banishing Financial Untouchability of the Poor through PMJDY–A Tryst with New Destiny. *Mediterranean Journal of Social Sciences*, *6*(2), 184-184.
9. Shettar, R. M. (2016). Pradhan Mantri Jan DhanYojana: Issues and Challenges. IOSR Journal of Business and Management (IOSR-JBM) Volume, 18, 17-24.
10. Shettar, R. M. (2016). Pradhan Mantri Jan DhanYojana: Issues and Challenges. IOSR Journal of Business and Management (IOSR-JBM) Volume, 18, 17-24.
11. Vaishali Khandelwal. (2017). A Study on Awareness and Benefits of Pradhan Mantry Jan Dhan Yojana. Imperial Journal of Interdisciplinary Research (IJIR), Vol 3, Issue 3, ISSN 2454-1362, http://www.onlinejournals.in.

Table 1: Earlier Financial inclusion campaign v/s Pradhan Mantri Jan Dhan Yojana approach

Earlier Financial Inclusion Approach	PMJDY approach
a. Swabhimaan campaign covered villages with a population of more than 2000 and thus limited geographical coverage has happened.	a. In PMJDY the focus is shifted to households and Self Service Area (SSA) approach for complete coverage of the whole nation.
b. Earlier approaches concentrated more towards rural areas for financial inclusion	b. In PMJDY equal focus is provided for both urban and rural areas for financial inclusion.
c. In Earlier schemes, Bank-Mitra used to visit villages on fixed days.	c. Under PMJDY fixed point Bank-Mitra's is appointed for each Self Service Areas consisting of

	1000-1500 households or 3 to 4 villages.
d. Earlier financial inclusion schemes concentrated on just opening bank accounts which led to large dormant accounts.	d. To avoid dormancy in bank accounts the PMJDY accounts are integrated with Direct Benefit Transfer, OD facility, and accessibility to insurance and pension products.
e. The interoperability of bank accounts was not there in earlier schemes.	e. To encourage the interoperability of accounts, the Rupay debit card is issued to account holders.
f. Mobile banking was not used	f. Mobile banking is introduced.
g. Very cumbersome KYC formalities were adopted in earlier schemes of financial inclusion which made opening bank accounts difficult.	g. Simplified KYC and e-KYC norms are introduced under PMJDY schemes as per RBI guidelines to make the account opening an easy task.
h. No guidelines were there for fixing remuneration for Bank Mitra	h. The minimum remuneration of Rs 5000 is fixed for Bank Mitra.
i. A recent survey from RBI revealed that 47% of the Bank Mitra's appointed under earlier schemes were untraceable.	i. Viability and sustainability of bank Mitra's were considered as very crucial.
j. Monitoring of financial inclusion schemes was left to banks	j. Monitoring mechanism at different levels such as central, State and district was introduced.
k. Not much focus was provided for financial literacy under earlier schemes.	k. Financial literacy cells were established in rural branches by banks.
l. State and district did not take an active involvement in earlier financial inclusion schemes.	l. State and district level monitoring committees were set up.
m. Providing credit was not encouraged	m. Overdraft, facility was provided after satisfactory operation of accounts for 6 months.
n. No grievance redressal system.	n. Grievance redressal system was introduced

Source: www.pmjdy.gov.in

Table 2: Demographic information of respondents

Variables understudy	Categories	Jan Dhan account holders in slums	
		Frequency	Percentage
Gender	Female	136	45.3 %
	Male	164	54.7 %
	Total	**300**	**100.0 %**
Age group (in years)	Less than 35	67	22.3 %
	36–45	180	60.0 %
	46–55	35	11.7 %
	Greater than 56	18	6.0 %
	Total	**300**	**100.0 %**
Educational level	No Formal Education	54	18.0 %
	High school or below	212	70.7 %
	PUC or Above	34	11.3 %
	Total	**300**	**100.0 %**
Employment	Permanent full time employment	166	55.3 %
	Permanent part time employment	75	25.0 %
	Self-employed	35	11.7 %
	Unemployed	24	8.0 %
	Total	**300**	**100.0 %**

Occupation	Business	16	5.3 %
	Job	266	88.7 %
	Others	18	6.0 %
	Total	**300**	**100.0 %**
Income (in thousands [K])	10K-20K	105	35.0 %
	5K-10K	178	59.3 %
	Less than 5K	17	**5.7 %**
	Total	**300**	**100.0 %**
Family size	More than 5	48	16.0 %
	up to 5	252	84.0 %
	Total	**300**	**100.0 %**

Table 3: Showing t-test result on PMJDY Perception among Male and Female Respondents

		Female (n=136)	Male (n=164)	t_value	P_value	Remarks
PMJDY Perception	Mean	3.37	3.46	-0.88	0.38	Non sig
	SD	0.93	0.75			

Tablet 4: Showing t-test result on PMJDY Perception between Family Size

		Greater than five (n=48)	Less than five (n=252)	t_value	P_value	Remarks
PMJDY Perception	Mean	3.44	3.42	0.08	0.94	non sig
	SD	1.17	0.75			

Table 5: One-way ANOVA test on PMJDY Perception among Educational groups

		No Education	High School & Below	PUC & above	F-value	P-value	Remarks
PMJDY Perception	Mean	3.45	3.49	3.11	2.21	0.11	Non-sig
	SD	0.79	1.05	0.56			

Table 6: Showing One way Anova test on PMJDY Perception among age groups

		Less than 35 yrs	36yrs–45yrs	46yrs–55yrs	Greater than 56yrs	F-value	P-value	Remarks
PMJDY Perception	Mean	3.32	3.36	3.75	3.41	3.08	0.03	5%_sig.
	SD	0.68	0.82	1.08	0.57			

Table 7: Showing One way Anova on mean of PMJDY Perception between Income groups

		Rs 10000-20000	Rs 5000-10000	Less than Rs 5000	F-value	P-value	Remarks
PMJDY Perception	Mean	3.52	3.41	3.31	0.89	0.41	Non-sig
	SD	0.84	0.87	0.69			

Table 8: Showing One way Anova test result on PMJDY Perception between occupations

		Business	Job	Others	F-value	P-value	Remarks
PMJDY Perception	Mean	3.3	3.42	3.62	0.68	0.51	Non-sig
	SD	0.52	0.87	0.46			

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REVIEW ARTICLE

A Review of Herbal Flora of Chhattisgarh and It's Utilization by the Tribal's for Medicinal Purposes

Pushpa P. Gupta[1] , Amit Roy[2], Shashikant Chandrakar[3], Akhilesh Kumar[4], Shiv Kumar Bhardwaj[4], Bibekananda Meher[1] and Poonam Sahu[4]*

[1]Associate Professor, Department of Pharmacology, Columbia Institute of Pharmacy, Raipur, Chhattisgarh, India.
[2]Professor and Principal, Columbia Institute of Pharmacy, Raipur, Chhattisgarh, India
[3]Associate Professor, Department of Pharmaceutics, Columbia Institute of Pharmacy, Raipur, Chhattisgarh, India.
[4]Assistant Professor, Department of Pharmacology, Columbia Institute of Pharmacy, Raipur, Chhattisgarh, India.

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***Address for Correspondence**
Poonam Sahu,
Assistant Professor,
Department of Pharmacology,
Columbia Institute of Pharmacy,
Raipur, Chhattisgarh, India.
Email: poonamsahu152@gmail.com

ABSTRACT

In this work there is mention of how the indigenous tribes of Chhattisgarh utilize the medicinal values of plants to treat various ailments. Chhattisgarh is known as herbal state, Chhattisgarh is located in the Chhattisgarh Plain, which forms the upper Mahanadi River basin. The basin proper lies at an elevation that ranges from about 800 to 950 feet (250 to 300 meters) above sea level. It is a structural plain with topographic variations resulting from extensive denudation. More than 40% of Chhattisgarh is forest area where we find a huge diversity of plants and animals. Similarly almost 40% of population comes from tribal region. This state with such huge forest land is house to a large number of aromatic and medicinal plants. The indigenous people to large extent use the produce from the forest to sustain their livelihood as well as to maintain their primary health care. This review is a short description of some very important medicinal plants along with their traditional uses.

Keywords: Chhattisgarh, Health Care, Tribal, Indigenous Medicine, Traditional uses.

INTRODUCTION

Plants play an important role as novel sources of varieties of pharmaceutical products [1]. Whole plants or their parts as powders extract and also in fresh form have been used since ages for medicinal purposes [2]. Medicinal plants play a most important role in the health care system. The indigenous people in every part of world with their experience of ages have collected the forest produce for their livelihood as well as medicinal needs [3]. The Chhattisgarh state houses about 40% of tribal population and its land are covered with forest having a remarkable biological diversity. It is therefore a need to document and preserve this knowledge and helping to save and sustain this remarkable biodiversity [4]. The common tribal communities in Chhattisgarh are Gond, Baiga, Korwa, Oraon, Kamar, Halba, and Binjhwar. These tribes have their own set of knowledge that is restricted to their specific location [5]. The Gonds are the largest tribal group in terms of population and are mainly concentrated in the southern part of the state. The distribution of the Gond is wide from Bastar, Bilaspur, Durg, and Raigarh in Chhattisgarh. More than 20 % of Gonds in Chhattisgarh live in the Bastar region only.

There are 3 major sub-tribes of Gond in Dorla, Bastar – Maria, Muria. The Halba Tribals are usually dispersed all over Chhattisgarh. The Gond tribe and Halba use many medicinal plant species for the treatment of common ailments. Traditional health practices and Indigenous health care practices can provide valuable clues to medical scientists in discovering medicine for modern diseases [6]**.** Baigas the second largest grouping with their unique life style and culture. They are inhabitants of kabirdham, Bilaspur and Surguja districts. They utilize the forest produce for production of herbal medicinal products and traditional handicrafts. The baigas are an agricultural community and at the same time they harness produce from forest to sell them in local markets [7]. In different districts of Chhattisgarh there are several tribes namely korwa, Raj-gonds, Nagvanshi, Oraon, Kanwal, Kamar etc. They inhabit in sometimes in deep forest and also in plains and mountains of Chhattisgarh. They are all engaged in collecting produce from forest and selling them in local market. Some of them are civilized and use modern medical facilities like Binjhawar tribe [8, 9, 10, 11, 12].

Objective of the Study

The main objective of this study is to review and document the medicinal properties of some popularly used herbs. These are extensively used by the tribal population of Chhattisgarh. This review shall help many researchers to carry out their work in future.

Medicinal Plants and its uses by Tribal of Chhattisgarh

Herbal preparations are natural, safe, and produce desired pharmacological effects. In Chhattisgarh state, there are many tribal communities that live and have a great deal of knowledge about the uses of medicinal plants [13]. In this state majority of tribal communities still depend on forest produce for their daily needs and health care [14]. In the table below a brief summary is given about the herbs, their local name, family, their plant part used and their main medicinal properties. Table 1

CONCLUSION

Medicinal plants have been found to provide a variety of substances that provides livelihood and health benefits to the inhabitants of that specific area where they grow. They are presently considered as a vital contributor to food industries, agricultural products and Pharmaceuticals. A number of researche is being carried out throughout the world to harness their chemotherapeutic potential for the treatment of array of diseases.

In Chhattisgarh the tribal's comprise of more than 40% of population and they have amassed a huge knowledge about the medicinal and other benefits of the plants that grows in their surrounding forest. The medicinal men among the tribals who are as baigas and guniyas are the healers who prescribed the medicinal plants in their community as formulations, extracts or in any other form. The knowledge that they have, has been obtained by experience of several generations only by word of mouth that is registered in their memory.

The above observations necessitates that this ethno medical knowledge should be documented so that it can pave way for popularizing and promoting the traditional knowledge which may be lost if are not written and published. In the present short review we see that Chhattisgarh is richly bestowed with a variety of medicinal herbs having medicinal values. Finally it is to be concluded that only a small number of species have been listed here however there are a considerably greater number. In this review it has also been tried to record the ancient knowledge so that the cultural tradition is brought in front of the scientific community. This will help in conservation of medicinal plants, if possible to preserve them through cultivation and scientifically authenticating their medicinal values so that they can be brought in open market for benefit of a much larger population.

REFERENCES

1. Soni A, Mathur S, and Motvani D. A Review on Some Threatened Medicinal Plant Species in Chhattisgarh: Therapeutic Potential and Conservation Concerns. International Journal of Advances in Science, Engineering and Technology. 2018;6(4):84-88.
2. Patel DK. Medicinal plants in G.G.V Campus, Bilaspur, Chhattisgarh in central India, International journal of medicinal Aromatic plants 2012;2(2):293-300.
3. Singh RS, Shahi SK. Diversity of medicinal plants of Ratanpur region of Bilaspur district (Chhattisgarh). J Med Plants. 2017;5:276-81.
4. Tiwari AK. Indigenous knowledge for treating skin disease in some selected districts of Chhattisgarh (India). International Journal of Recent Scientific Research. 2015; 6(2):2654-7.
5. Mitra M. Ethnomedicine and Health Management Practices among the Hill Korwa and Birhor of Chhattisgarh: An Anthropological Appraisal. In: Conservation of Medicinal Plants. Eds. R.N. Pati & D.N. Tewari. A.P.H. Publishing Corporation, New Delhi. 2009;302-338.
6. Painkra VK, Jhariya MK, and Raj A. Assessment of knowledge of medicinal plants and their use in tribal region of Jashpur district of Chhattisgarh, India. Journal of Applied and Natural Science. 2015;7(1):434-42.
7. Soni PK, Varoda A, Mitra M. Ethnomedicinal practices among Gond & Halba tribes of Chhattisgarh, India. International Journal of Science and Research. 2013;5(9):955-8.
8. Babu R, Panda AN. Socio-Economic status of the Baiga tribe of Chhattisgarh in India. International Journal of Multidisciplinary Research and Development. 2016;10(3):182-6.
9. Ekka A. Some rare plants used by Hill-Korwa in their healthcare from Chhattisgarh. Int J Life Sci Biotechnology Pharma Res. 2013;2:198-203.
10. Chakma T, Rao PV, Meshram PK, Singh SB. Health and nutrition profile of tribals of Madhya Pradesh and Chhattisgarh. In Proceeding of National Symposium on Tribal Health 2006.
11. Shukla R, Chakravarty M. Study of traditional medicinal practices among the Raj-Gonds of Korba district of Chhattisgarh. Studies on Ethno-Medicine. 2010;4(3):211-6.
12. Shukla R, Chakravarty M, Goutam MP. Ethno medicinal practices among the Binjhwar tribe of Chhattisgarh, India. Global Journal of Research on Medicinal Plants & Indigenous Medicine. 2013;2(7):525.
13. Kurre RK. Wide variety of medicinal plants and their uses in Jashpur District of Chhattisgarh. J Environ Sci Toxicol Food Technol. 2015;1(1):01-5.
14. Beck NR, Samal P. Traditional medicinal plants used by the tribes and rural people of Bilaspur district, Chhattisgarh (India). Research Journal of Pharmacy and Technology. 2012; 5(10):5.
15. Yadav DK, Jhariya MK, Kumar A, and Sinha R. Documentation and ethnobotanical importance of medicinal plants found in Sarguja district. Journal of Plant Development Sciences. 2015;7(5):439-46.
16. Arjariya A and Chaurasia K. Some medicinal plants among the tribes of Chhatarpur district (MP) India. Ecoprint: An International Journal of Ecology. 2009;16:43-50
17. Raj A, Toppo P. Assessment of floral diversity in Dhamtari district of Chhattisgarh. Journal of Plant Development Sciences. 2014; 6(4):631-5.
18. Sharma GN, Dubey SK, Sharma P, and Sati N. Medicinal values of bael (Aegle marmelos) (L.) Corr.: A review. Int J Curr Pharm Rev Res. 2011;2(1):12-22.

19. Sundarkar P, Govindwar R, Nyamati SB, Alladwar N, Thombre V, Soni A, and Raj A. Use of Aloe vera in dentistry. Journal of Indian Academy of Oral Medicine and Radiology. 2011;23(5):389.
20. Gupta DK, Gupta G. Diversity of Ethno Medicinal Plant in Dist. Balod (CG). IOSR Journal of Pharmacy and Biological Sciences. 2017;12(3):80-9.
21. Jain N, Sharma V, Ramawat KG. Therapeutic potentials of medicinal plants traditionally used during postpartum period and their molecular targets. Journal of Ecobiotechnology. 2011;3(10):30-9.
22. Biswas D, and Jadhav MA. Diversity of Medicinal Plants In Kalinga University, New Raipur (CG). European Journal of Molecular & Clinical Medicine; 7(3):2020.
23. Thakur S, Tiwari KL, Jadhav SK. Approaches for Conservation of an Ethno medicinal Plant: Asparagus racemosus Willd. On Line Journal of Biological Sciences. 2015;15(3):126.
24. Shukla R, and Chakravarty M. Study of traditional medicinal practices among the Raj-Gonds of Korba district of Chhattisgarh. Studies on Ethno-Medicine. 2010;4(3):211-6.
25. Ahmad S, Maqbool A, Srivastava A, and Gogol S. Biological detail and therapeutic effect of azadirachta indica (neem tree) products-a review. Evidence Based Med. Healthcare. 2019;6(22):1607-12.
26. Medicinal herbs of Chhattisgarh, India having less known traditional uses. 80. Gandhraj (Gardenia jasminoides, family Rubiaceae). Paramparik.blogspot.com. 2022 [cited 6 April 2022]. Available from: https://paramparik.blogspot.com/2014/06/medicinal-herbs-of-chhattisgarh-india 2753.html.
27. Naik SK, Behera S, Rath SK, Patra JK. A comprehensive scientific overview of Blepharispermum subsessile DC (Asteraceae), a conservation concern medicinal plant with promising pharmaceutical potential. Indian Journal of Traditional Knowledge (IJTK). 2019;19(1):208-17.
28. Vinodia Shilpa, Dixit A K, Das B, Sahu B, Harish Dhiwar, Darakhsa K. Sheikh, Mery A. Kerketta. Traditional phytotherapy among the Gond tribes of janjgir-champa, Chhattisgarh (India). Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences. 2019;5(1):765-72.
29. Lal S, and Sahu MS. Ethno botanical observations from Sitanadi Wild life sanctuary Dhamtari, Chattisgarh, India. International Journal of Pharmacy & Life Sciences. 2016;7(9).
30. Sahu PK, Masih V, Gupta S, Sen DL, and Tiwari A. Ethnomedicinal plants used in the healthcare systems of tribes of Dantewada, Chhattisgarh India. American Journal of Plant Sciences. 2014.
31. Bothara SB, Singh S. Pharmacognostical studies of seeds on some plants belonging Chhattisgarh. Pharmacognosy Journal. 2012 Mar 1;4(28):24-30.
32. Dubey A, Bhardwaj S, Pandey AK, Khalkho AD, Jain S, and Rai A. A new species of acremoniula g. Arnaud ex cif. On Butea Monosperma (LAM.) Taub.–an important medicinal plant from forest flora of jashpur (cg). Journal of Experimental Biology and Agricultural Sciences. 2019;7(6):600-5.
33. Ahirwar RK. Diversity of ethnomedicinal plants in Boridand forest of district Korea, Chhattisgarh, India. American Journal of Plant Sciences. 2015;6(02):413.
34. Sharma V, Rajani GP. Evaluation of Caesalpinia pulcherrima Linn. for anti-inflammatory and antiulcer activities. Indian Journal of Pharmacology. 2011;43(2):168.
35. Oudhia PP. Common rice weeds used for first aid by Chhattisgarh farmers. Agric Sci Dig. 2001;21(4):273-4.
36. Husain N, Chauhan D, Trak TH. Some Ethno-Medicinally Important Plants from Chhattisgarh, India, with Caution Stigma in Reference to their Abortifacient Activity. Int J Sci Res. 2017;6(7):2046-50.
37. Virmani R, Virmani T, Singh C, Sorout G, Gupta J. Hidden potential of natural herb Carissa carandas (Karonda). Res Pharm Health Sci. 2017;3:294-302.
38. Lahare RP, Yadav HS, Dashhare A, Bisen YK. An Updated Review on Phytochemical and Pharmacological Properties of Catharanthus rosea. Saudi Journal of Medical and Pharmaceutical Sciences. 2020;6(12):759-66.
39. Sharma AK. Medicinal and aromatic plants. Aavishkar Pub. Jaipur (Rajasthan); 2007:62–79.
40. Harwansh RK, Pareta SK, Patra KC, Rahman MA. Preliminary phytochemical screening and anthelmintic activity of Chloroxylon swietenia root extract. International Journal of Phytomedicine. 2010;2(3).
41. Nadkarni KM. [Indian materia medica]; Dr. KM Nadkarni's Indian materia medica: with Ayurvedic, Unani-Tibbi, Siddha, allopathic, homeopathic, naturopathic & home remedies, appendices & indexes. 1. Popular Prakashan; 1996.

42. Anuj SK, Srivastava P, Tiwari BN. Plant (Cissus quadrangularis) with various ethno pharmacological action: A review. Journal of Pharmacy Research. 2011;4(6):1887-90.
43. Patel S, Tiwari S, Pisalkar PS, Mishra NK, Naik RK, Khokhar D. Indigenous processing of Tikhur (Curcuma angustifolia Roxb.) for the extraction of starch in Baster, Chhattisgarh. 2015; 6(3):213-220.
44. Chakraborty A, Kosariya SS, Chakravarty M. A Review of Ethnomedicinal Practices for Gynaecological Disorders among the Various Tribes of Chhattisgarh. Journal of Environmental Science, Toxicology and Food Technology. 2015;1(2):55-62.
45. Tiwari PR, Soni IN, Patel SU. Study of vegetation in Pt. Ravishankar Shukla Uni-versity campus, Raipur Chhattisgarh with special reference to the statistics department. Indian J Sci Res. 2014;4:121-6.
46. Shukla R, Chakravarti M, Goutam MP. Ethno medicinal practices among the Binjhwar tribe of Chhattisgarh, India. Global Journal of Research on Medicinal Plants & Indigenous Medicine. 2013;2(7):525.
47. Kurre RK. Wide variety of medicinal plants and their uses in Jashpur District of Chhattisgarh. Journal of Environmental Science, Toxicology and Food Technology. 2015;1(1):01-5.
48. Patel PK, Janghel V, Chandel SS, Sahu J. Madhuca indica (Mahua)-pharmaceutical, nutraceutical and economical importance for tribal people of Chhattisgarh state. International Journal of Pharmaceutical and Phytopharmacological Research. 2019;9(3):16-28.
49. Madharia P, Jahan A, Verma P. Ethnomedicinal plants and their conservation in Chhattisgarh State: Review and Perspectives. Journal of Environmental Science, Toxicology and Food Technology. 2015;1(4):46-50.
50. Singh VK, Dwivedi P, Chaudhary BR, Singh R. Immunomodulatory effect of Gymnema sylvestre (R. Br.) Leaf Extract: an in vitro study in rat model. PloS one. 2015 ;10(10):0139631.
51. Kumari, I., H. Kaurav, and G. Chaudhary. "Hedychium Spicatum Buch-Ham. (Kuchri), A Treasure House of Essential Oils". International Journal Of Current Pharmaceutical Research,2021;13(4):25-31
52. Chakrabortty S, Choudhary R. Hemidesmus indicus (anantmool): rare herb of Chhattisgarh. Indian J. Sci. Res. 2014;4(1):89-93.
53. Rahangdale CP, Patley RK, Yadav KC. Phytodiversity of ethnomedicinal plants in Sacred Groves and its traditional uses in Kabirdham district of Chhattisgarh. Indian Forester. 2014;140(1):86-92.
54. Bargali SS, Shrivastava SK. Exploration of valuable medicinal vegetal wealth from the tribal belt of Bastar district in Chhattisgarh. The Botanica. 2002;52:75-82.
55. Sharma AK, Bajpai S, Shrivastava S, Kanungo VK. Inventorying medicinal plants in urban homegardens of Raipur, Chhattisgarh. Int J Herb Med. 2014;2(1):43-50.
56. Bisht VK, Negi JS, Bhandari AK, Sundriyal RC. Traditional use of medicinal plants in district Chamoli, Uttarakhand, India. Journal of Medicinal Plants Research. 2013;7(15):918-29.
57. Sharma SD, Sahu K, Chandrol GK, Jain PK, Sharma V. Ethnobotanical survey of five villages of Durg District of Chhattisgarh, (India). Int J Adv Res Biol Sci. 2016;3(10):104-10.
58. Ekka A, Ekka NS. Madhuca longifolia var. latifolia: An Important Medicinal Plant used by tribes of North–East part of Chhattisgarh. , Online International Interdisciplinary Research Journal, Vol-IV, 2014.
59. Kumar S. Study of forest based indigenous technology at Bastar region of Chhattisgarh. International journal of research and analytical reviews. 2016;3(3):304-11.
60. Singh RA, Singhal KC, and Khan NU. Antifilarial activity of Mallotus philippinensis Lam. on Setaria cervie (Nematoda: Filarioidea) in-vitro. Indian Journal of Physiology and Pharmacology.1997;41(4):397–403.
61. Kumar, V.P., Chauhan, N.S., Padh, H. and Rajani, M., 2006. Search for antibacterial and antifungal agents from selected Indian medicinal plants. Journal of Ethnopharmacology. 2006;107(2):182–188.
62. Gangwar M, Goel RK, and Nath G. Mallotus philippinensis Muell. Arg (Euphorbiaceae): ethnopharmacology and phytochemistry review. BioMed research international. 2014;1-13
63. Kenwat R, Prasad P, Satapathy T, Roy A. Martynia annua: an overview. Pharmaceutical and Biosciences Journal. 2013:07-10.
64. Prabhas L, Agrawal M, Tamrakar V. Prologue phytochemical analysis of traditional medicinal plants used by tribes in Gariyaband district (Chhattisgarh)-India. Int J Innov Res Sci Eng. 2016;2(11):123-30.
65. Mohan SM, Pandey B, Rao SG. Phytochemical analysis and uses of Mimosa pudica Linn. Chhattisgarh. IOSR J Environ Sci Toxicol Food Technol. 2015;1(3).

66. Sonewane K, Chouhan SS, Rajan M, Chauhan NS, Rout OP, Kumar A, Baghel GS, and Gupta PK. Pharmacological, ethnomedicinal, and evidence-based comparative review of Moringa oleifera Lam.(Shigru) and its potential role in the management of malnutrition in tribal regions of India, especially Chhattisgarh 2022.
67. Bagachi A, Semwal A, and Bharadwaj A. Traditional uses, phytochemistry and pharmacology of Morus alba Linn.: A review. Journal of Medicinal Plants Research. 2013;7(9):461-9.
68. Arjariya A, and Chaurasia K. Some medicinal plants among the tribes of Chhatarpur district (MP) India. Ecoprint: An International Journal of Ecology. 2009;16:43-50.
69. Toppo RK. Study of traditional knowledge of medicinal plants and their use by tribal people of sitapur and bagicha block in Chhattisgarh, india. International Journal of Engineering Applied Sciences and Technology.2020;4(10):145-149.
70. Pandey AK. An Ethnobotanical Study of Medicinal Plants in Atal Nagar (New Raipur) of Chhattisgarh, India. International Research Journal of Plant Science. 2021;12(1):1-8.
71. Ramteke V, Kurrey VK, Panigrahi TK, Yadav P. Horse gram (Kulthi): pulse of rural peoples in Chhattisgarh. Innovative Farming. 2016;1(4):205-8.
72. Chamoli RT, Minj S, Singh V. Phytochemical chemical characters of Nyctanthes arbor-tristis Linn.: A promising medicinal plant. Journal of Medicinal Plants. 2019;7(6):141-3.
73. Prakash PA, and Gupta N. Therapeutic uses of Ocimum sanctum Linn (Tulsi) with a note on eugenol and its pharmacological actions: a short review. Indian journal of physiology and pharmacology. 2005;49(2):125.
74. Minu V, Harsh V, Ravikant T, Paridhi J, Noopur S. Medicinal plants of Chhattisgarh with anti-snake venom property. Int J Curr Pharm Rev Res. 2012;3(2):1-0.
75. Sandey H, Sharma A. Study on ethnomedicinal plants of Achanakmar-Amarkantak Tiger reserve of Chhattisgarh. J. Sci. Let. 2016;1(3):216-22.
76. Sapiens K, Nagal P, Prakash P. Ethno-medicinal Plants of Sihawa, Chhattisgarh Used in Herbal and Folk Remedies in Indian System of Medicine. Int. J. Phar. & Biomedi. Rese. 2021;8(3):6-12.
77. Nair LD, Sar SK, Arora A, and Mahapatra D. A Comparative study on analysis of zinc concentration conducted on few medicinal plants of Chhattisgarh. International Journal of Current Research in Chemistry and Pharmaceutical Sciences. 2014;1(5):40-3.
78. Mehrotra NN, Ojha SK, Tandon S. Drug development for cardiovascular diseases from ayurvedic plants. Feature. 2007;1:89.
79. Jena J, and Gupta AK. Ricinus communis Linn: a phytopharmacological review. International Journal of Pharmacy and Pharmaceutical Sciences. 2012;4(4):25-9.
80. Verma r. and Shrivastava k. Phytochemical analysis and anti-bacterial assessment of root extract of asparagus racemosus (shatavari) found in ambikapur, surguja (Chhattisgarh). Indian journal of applied research. 2018;8(6):26-28.
81. Maravi MK, Ahirvar BP, Chaudhry S. Ethno medicinal plants use by two sympatric tribes of Central India. Int J Adv Sci Technol. 2017;3(1):37-48.
82. Devi, C.A., Swamy, G.S.K., Pandey, B.B., Naik, N., Sampath, P.M., Suresha, G.J. and Chavan, M.L., 2018. Studies on success of different age rootstocks of softwood grafting in jamun (Syzygium cuminii Skeels). International Journal of Current Microbiology and Applied Sciences. 2018;7(2):3158-65.
83. Jangdey MS, Gupta A, Kaur CD, Saraf S. Assessment of Utilization, Value addition and characterization of Tamarind: A Natural Gum of Chhattisgarh. International Journal of Pharmaceutical Research & Allied Sciences. 2016, 5(2):323-334.
84. Pandey AK, Mandal AK. Sustainable Harvesting of Terminalia arjuna (Roxb.) Wight & Arnot (Arjuna) and Litsea glutinosa (Lour.) Robinson (Maida) Bark in Central India. Journal of Sustainable Forestry. 2012;31(3):294-309.
85. Chandra U. Study on the medicinal plants found in sakri block of takhatpur, Chhattisgarh. World Journal of Pharmaceutical Research. 2019:8(6);1603-1611.
86. Sinha MK. Ethnobotany in relation to health and livelihood security in district Bastar of Chhattisgarh state.2011
87. Chauhan D, Shrivastava A.K. and Patra S, International Journal of Current Microbiology and Applied Sciences 2014;3(4): 611-622.

88. Pandey D, Khandel P, Verma P. Exploration of the unique blend of traditional knowledge and medicinal plants from Bastar, Chhattisgarh. India. J Chem Biol Res. 2018;35(2):517-26.
89. Krutika J, Tavhare S, Panara K, Kumar PA, Karra N, Studies of Ashwagandha (*Withania somnifera* Dunal). International Journal of Pharmaceutical & Biological Archives 2016;7(1):1-11.
90. Ekka A, Tirkey A, Kujur N. Cluster and principal component analysis (PCA) in ashwagandha (*Withania somnifera* (L.) Dunal) for root traits. International Journal of Communication Systems. 2021;9(1):3012-6.

Table 1: Detail About Herbal Floras of Chhattisgarh And It's Utilization by the Tribal's for Medicinal Purposes.

S. No	Botanical Name	Local name	Family	Plant parts	Uses	Ref.
1	***Achyranthes aspera Linn.***	Chirchitti	Amaranthaceae	Whole plant	Twigs for tooth pain, roots tied to women for easy delivery, leaves in scorpion sting and in skin eruptions. The leaves and camphor are burned together and used in cataract and paste of root is applied in snake bite.	[15]
2	***Adina cord folia Roxb.***	Haldu	Lythraceae	Bark	Used for skin diseases.	[16]
3	***Aegle mormelos***	Bel	Rutaceae	Bark Leaves Fruit	Liver toxicity, diabetes, , microbial and fungal infection, stomach, inflammation, and pain, sun stroke, eye problems, head pain, ulcer, jaundice, dysentery and in diarrhea.	[17]
4	***Aloe vera***	Ghritkumari	Asphodelaceae	Whole plant	Gel of this plant to treat ulcers and lesions of oral cavity. The fleshy part of the plant is good for use as facial cream and to treat skin burn. The plant is also used as antibacterial, and antiviral which accelerates wound healing. The resin and gums are purgative and used in constipation.	[19]
5	***Andrographis paniculata***	Bhui-neem/ kalmegh	Acanthaceae	Leaves	Diabetes, choleretic bitter tonic, blood purifiers, and malarial fever, and anthelmintic.	[20]
6	***Anogeissuss latifolia***	Dhawra, Bakli	Combretaceae	Gum	Wound healing, repair damaged tissue, cure back pain, potential antioxidant and hepatoprotective.	[21]
7	***Argemone maxicana***	Pilikateri	Papaveraceae	Whole plant both fresh and dried	Skin disease, jaundice, seed oil anthelmintic, wound healing, , and as a purgative	[22]
8	***Asparagus racemosus***	Shatavari	Asparagaceae	Root	Increasing the secretion of milk in lactating women, controlling blood sugar levels (with	[23]

					Azardichta indica)	
9	***Azadiricta indica***	Neem	Meliaceae	Whole plant	Fever, tuberculosis, skin diseases chickenpox, and malaria	[24],[25]
10	***Bixa orellana Linn.***	Sinduri	Bixaceae	Leaves, Fruit	Leaves of the plant are bitter, cooling, and appetizer. Fruit is, carminative, purgative, anthelmintic, vulnerary, alexiteric, healers wounds, ulcers, tumours, stone in bladder, useful in diseases of abdomen, enlargement of spleen, and in bronchitis etc.	[26]
11	***Blepharis perum Wight (DC)***	Rasnajadi	Sterculiaceae	Leaves	Use for the treatment of arthritis and related joint pain, Inflammation of the intestines, diarrhoea, rhinitis, common cold, scabies, skin diseases, wound, various ophthalmic problems, and gynaecological disorders.	[27]
12	***Blumea lacera***	Kukurmutta	Asteraceae	Leaves	Leaf extract of this plant are applied on cuts and wounds to stop bleeding, and also used to cure ring worms.	[28]
13	***Bombax ceiba***	Semal/ silk cotton tree	Bombacacea	Root	Used for surgical dressing in the case of wounds and to increase sexual vigor.	[29]
14	***Borassus flabellifer Linn***	Tadi	Arecaceae	Leaves, Sap	Liver and spleen diseases, in inflammatory affections and leprosy, and in dysentery.	[30]
15	***Buchnania lanzan (Spreng) Roxb.***	Char	Anacardiaceae	Root, Stem, Fruit	Fruit of the plant is sour, sweet, fattening, binding cooling, laxative, aphrodisiac, cures fevers, biliousness, thirst, ulcers, and blood diseases. Juice of the leaves is purgative, digestive, and has aphrodisiac properties. Seeds are used as expectorant, tonic to body and brain, stomachic. It is also used as antioxidant, and antiulcer.	[31]
16	***Butea monosperma***	Palash	Papilionaceae	Root, Stem, Leaves, Flower, and Seed.	Antihepatotoxic, antigout, antidiabetic, antileprotic, anticonvulsant, antimicrobial, antistress, antioxidant, antidiarrheal, and anti-inflammatory.	[32]
17	***Caesalpinia bonduc (L) Roxb.***	Gotarun	Caesalpiniaceae	Stem	Treat asthma or other cough complaints.	[33]

18	***Caesalpinia pulcherniama Linn.***	Guletura	Leguminoceae	Leaves, Flower, Bark, and Seed.	Anti-inflammatory and antiulcer activities.	[34]
19	***Caesulia axioaris Roxb.***	Belonda	Compositae	Whole plant	For minor injuries, cuts, and wounds.	[35]
20	***Cajanus cajan***	Rahad	Fabaceae	Stem, Root, Leaves, and Seed.	Leaves are used for healing wounds, effective against tongue sores, gum inflammation, spongy gums also used to cure lungs, cough, bronchitis, stop blood flow, and also used for malaria. Leaf paste is useful for jaundice, diabetes, measles and piles. Dried root powder is good for purifying blood. Leaves and seeds are applied as poultice over the breast to induce lactation.	[36]
21	***Carissa spinarum Linn.***	Karunda	Apocynaceae	Fruit, Root	Used for the treatment of cancerous wounds and to destroy the maggots. Unripe fruit is good appetizer, cooling astringent, anthelmintic, acidic, stomachic, leaf decoctions of this plant are used in the commitment of remittent fever.	[37]
22	***Catharanthus***	Sadabahar	Apocynaceae	Leaves	Antioxidants, anti-inflammatory, antithrombotic, anti-allergic, vasodilatory effects, antifungal, antibacterial and antiviral activity.	[38]
23	***Chlorophytum borivilianum***	Safed musli	Asparagaceae	Root	Joint pain, diarrhea, diabetes, blood purifier, tonic for lactating mothers.	[39]
24	***Choroxylon swietenia D.C.***	Bhira	Meliaceae	Bark	Antifertility, antifeedant, larvicidal, mosquito repellent, astringent antimicrobial, antioxidant, anti-inflammatory, hepatoprotective activity, and anthelmintic agent.	[40]
25	***Cissus quadrangular***	Hadjod	Vitaceae	Stem	Anthelmintic, digestive, dyspeptic, tonic, analgesic in eye and ear diseases, fracture and in complaints of the back and spine, and also used in asthma, and treatment of irregular menstruation.	[41],[42]

26	***Curcuma angustifolia***	Tikhur	Zingiberaceae	Rhizomes	Used as a herbal drink especially during summer season, works as cooling agent for the stomach, source of nutrition, curing worms and stomach aches.	[43]
27	***Cynodon dactylon***	Dub ghas	Gramineae	Grass	Treatment of Bleeding during Pregnancy and threatened abortion.	[44]
28	***Datura stramonium Linn.***	Datura	Solanaceae	Seed, Stem	Arthritis, fever, ulcer, skin diseases, cough, and asthma.	[45]
29	***Diospyros melanoxylon Roxb.***	Tendu	Ebenaceae	Bark	Treatment of Painful menses / Blood discharge.	[46]
30	***Dolichos biflorus***	Kulthi	Fabaceae	Root	Body ache, Stone	[47]
31	***Ficus benghalensis***	Bargad	Moraceae	Fruit, Latex, Bark, and Steam	Use in the treatment of diabetes, skin troubles like eczema and ringworm and also use in pain, weakness, fracture, toothache problems, heel cracks.	[48]
32	***Ficus religiosa***	Peepal	Moraceae	Whole Plant	Treatment of diabetes, asthma, hypertension, vomiting, gastrointestinal pain, infection, neurological and sexual disorders.	[49]
33	***Gloriosa superba Linn.***	Kalihari	Liliaceae	Rhizome	Treatment of various diseases like indigestion vitiated Kapha, fever, obstructed labor, arthritis, skin diseases and cardio-myopathy.	[50]
34	***Gymnema sylvestre Roxb Br.***	Gumar	Asclepidaceae	Leaves	Treat diabetes, Asthma, Obesity, and Immunomodulator.	[51]
35	***Hedychium spicatum***	Kachri	Zingiberaceae	Tuber	Anti-inflammatory, antipyretic, anti-microbial, hepatoprotective, anti-diabetic, antihelminthic, and in sexual enforcement.	[52]
36	***Hemidesmus indicus Linn.***	Anant mool	Apocynaceae	Root	Helps in improves quality and quantity of sperms. Curing dyspepsia, dysentery, indigestion, cough, bronchitis, uterine hemorrhage, leucorrhoea, dysuria, blood diseases, diuretic, diaphoretic, depurative, mmunosuppressant, refrigerant, antisyphilitic, antileucorrhoeic, galactogenic, aphrodisiac, anti-	[53]

					diarrhoeal, and antirheumatic. The drug is also useful in fever, skin diseases, thirst, vomiting, anemia, debility and poisoning.	
37	***Hibiscus lampas* Cav.**	Ban Kapas	Malvaceae	Root	Used as digestive	[54]
38	***Hydrocotyle asiatica***	Brahmi booti	Apiaceae	Whole plant	The plant is used against rheumatism, cures skin diseases and leprosy, and also used as nerve tonic, and blood purifier.	[55]
39	***Jatropha curcas L.***	Ratanjot	Euphorbiaceae	Leaves, Seed oil	As purgative and for skin diseases.	[56]
40	***Lantana camaral***	Bantulsa	Verbenaceae	Root, Leabes, Oil	Used in skin disease, and insect bites	[57]
41	***Leptidenea reticulate* L.**	Jivanti	Asclepidaceae	Whole plant	Ear infection	[58]
42	***Madhuca indica***	Mahua	Sapotaceae	Fruit	Useful for treating skin diseases, headache, rheumatism, chronic constipation, and piles. Sometimes used as an emetic, antimicrobial, hepatoprotective, bone joining potentials and wound healing.	[59]
43	***Mallotus philipinensis***	Rohini	Euphorbiaceae	Leaves Fruit, Seed	Anti-inflammatory, antifilarial, antimicrobial, antibacterial, immune-regulatory activity and also used as carminative, purgative, alexiteric, in bronchitis, abdominal diseases, spleen enlargement, and as an antiparasitic.	[60],[61],[62]
44	***Martynia annua L.***	Baghnakha	Martyniaceae	Leaves, Fruit.	Anthelmintic, antibacterial, analgesic, antipyretic, antifertility, antinociceptive, antioxidant, anticonvulsant antidiabetic, CNS depressant, and wound healing activity.	[63]
45	***Mentha piperita***	Piperment	Lamiaceae	Leaves	Aromatherapy, fatigue, bronchitis, flu, ulcer, halitosis, lice herbal tea, sinus, analgesic, astringent. anti-oxidant, and skin tonic,	[64]
46	***Mimosa pudica L.***	Chuimui	Mimosaceae	Root , Leaves, Seed	Used for the disease related to blood and bile, bilious fever, piles, jaundice, ulcer, leprosy, and smallpox.	[65]
47	***Moringa oleifera***	Munga	Moringaceae	Leaves, Fruit,	Control obesity, diabetes, asthma, cardiac, liver, and	[66]

				Root	gastrointestinal, infective, and disorders, of brain such as alzheimer's disease and depression.	
48	***Morus alba***	Sahtut	Moraceae	Leaves, Fruit, Bark, Stems.	Used to treat fever, sore throats, inflamed eyes, headaches, vertigo, as expectorants, gargle and mouthwash, prevent premature graying of the hair and to treat dizziness, blurred vision, insomnia and ringing in the ears, as a antibacterial, diaphoretic, purgative, and hypoglycaemic , antispasmodic, antirheumatic, diuretic, and hypotensive.	[67]
49	***Mucuna pruriens***	Kewanch	Fabaceae	Seed, Roots	Used as aphrodisiacs and in male sterility, in dysentery, uterine trouble, kill stomach worms in children, and also use for cough and cold.	[68], [69]
50	***Murraya koenigii***	Meetha neem	Rutaceae	Leaves	Used in diarrhoea, dysentery, useful for preventing hair loss, microbial growth and stomach ache, pain of kidney, and as antidiabetic.	[70]
51	***Mycrotyloma uniflorum***	Kulthi	Fabaceae	Seeds	Used for bronchitis, asthma, heart disease, urinary discharges and treatment of kidney stones.	[71]
52	***Nyctanthes arbortristis***	Harsingar	Oleaceae	Leaves, Flower, Fruit	Anti-oxidant, anti-inflammatory, Anti-leishmanis, anti-fungal, anti-viral, anti-pyretic, anti-malarial, and antihistaminic.	[72]
53	***Ocimum sanctum***	Tulsi	Lamiaceae	Whole plant	Used for the treatment of bronchial asthma, bronchitis, arthritis, painful eye diseases, dysentery, diarrhea, skin diseases, chronic fever, malaria, and insect bite. The plant has also been suggested to have antidiabetic, anticancer, antifertility, antifungal, and antimicrobial, cardioprotective, hepatoprotective, antiemetic, analgesic, antispasmodic, diaphoretic, and adaptogenic actions.	[73]
54	***Pergularia daemia (Forsk.) Chiov.***	Utran	Asclepiadaceae	Leaves	Treatment of snakebite.	[74]

55	***Piper betle Linn.***	Pan	Piperaceae	Leaves, Root	Decoction of leaves used for healing wounds, Roots used to produce sterility in women.	[75]
56	***Plumbago zeylanica***	Chitrak	Plumbaginaceae	Root, Stem	Used in fever and skin disease treatment and give relief from flatulence.	[76]
57	***Pongamia pinnata***	Karanj	Fabaceae	Seed, Bark, Leave.	Fresh bark used for piles, used for skin disease and arthritis, wounds, ulcers, and diarrhea, Seed Cakes used manure, roots and leaves used as fish poison.	[77]
58	***Rauvolfia serpentina (Linn.) Benth. ex Kurz***	Sarpgandha	Apocynaceae	Leaves	Anti-hypertensive, tranquiliser, anti-atherosclerotic, anti-obesity,	[78]
59	***Ricinus communis Linn.***	Jada	Euphorbiaceae	Seed, Root	Used as an anti-oxidant, laxative, purgative, and antihistaminic, antimicrobial, fungicide antinociceptive, immunomodulatory, antiasthmatic, anti-inflammatory, hepatoprotective, lipolytic, antiulcer, antidiabetic, antifertility, central nervous system stimulant, wound healing, fertilizer, larvicidal and insecticidal.	[79]
60	***Ruta graveolens L.***	Sadab	Rutaceae	Whole plant.	Ear ache, rheumatism of the joints, and when the plant juice is mixed with the juice of Allium cepa, the preparation is taken orally to stop vomiting.	[80]
61	***Schleichera oleosa***	Kosum	Sapindaceae	Bark	Stomachache, and bloody stools.	[81]
62	***Semecarpus anacradium***	Bhelva	Anacardiaceae	Fruit, Seeds	Headache, diarrhoea, paralysis, worms, leg ache, wound, and piles.	[81]
63	***Shorea robusta***	Sarai	Dipterocarpaceae	Bark	Delivery convalescence, diarrhoea, and weakness,	[81]
64	***Syzygium cumini***	Jamun	Myrtaceae	Fruits, Seeds, Leaves	Control diabetes, dysentery, diarrhoea, edema, ringworm, fever.	[82]
65	***Tamaridus indica***	Imli	Leguminaceae	Root, Fruit, Leaf	Antidiabetic, antivenomic, antimicrobial, antioxidant, hepatoprotective, antimalarial, laxative, antiasthmatic, and anti-hyperlipidemic activity.	[83]
66	***Terminalia arjuna***	Arjun	Combretaceae	Stem, Bark	Treatment of heart failure, coronary artery disease,	[84]

					biliousness, sores, as an antidote to poison, and viral diseases, and for hypercholesterolemia. It has also been found to have antioxidant, antibacterial, and antimutagenic properties, wound healing, and hypotensive activity.	
67	***Terminalia chebula***	Harra	Combretaceae	Fruit,	Control blood pressure, remove cough, in dentifrices, immunomodulator, cardiac and nervine tonic, laxative and to treat indigestion, used on scabies and bleeding gum.	[85], [86]
68	***Terminaliabellirica***	Bahera	Combretaceae	Fruit, Bark	Fever, cough, cold, purgation antidiarrheal therapy and digestive.	[87]
69	***Tinosporacordifolia***	Guduchi	Menispermaceae	Stem, Barks, Climber	Malaria, fever, cold, liver disorder.	[88]
70	***Withaniasomnifera***	Ashwagandha	Solanaceae	Root	Used as an anti-inflammatory, neurosis, for swellings, tumors, rheumatism, and as a sedative and hypnotic, in anxiety asthma, colds, chills, and also used to increase the tone of uterus after miscarriage or birth.	[89], [90]

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RESEARCH ARTICLE

Isolation and Characterization of *Pseudomonas viridiflava* from *Capsicum annuum* Seeds

Neetu Soni[1]*, Laxmi Meena[2] and Ashwani Kumar Verma[2]

[1]Research Scholar, Department of Botany, Raj Rishi Government College, Alwar, Rajasthan, India.
[2]Assistant Professor, Department of Botany, Raj Rishi Government College, Alwar, Rajasthan, India.

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***Address for Correspondence**
Neetu Soni,
Research Scholar,
Department of Botany,
Raj Rishi Government College,
Alwar, Rajasthan, India.
Email: sonineetu378@gmail.com

ABSTRACT

The present study has investigated presence of *Pseudomonas viridiflava* in seeds of *Capsicum annum* L. by use of its biochemical traits. Total twenty five bacterial colonies were obtained from the diseased seeds of capsicum and identified as pseudomonas spp. through numerous biochemical tests. Further these were confirmed as *Pseudomonas viridiflava* on the basis of LOPAT test, carbohydrate utilization, HR (hypersensitive reaction) and pathogenicity test. The discoloured and symptom showing seeds were plated on nutrient agar medium and total 42 bacterial colonies were selected on the basis of colony morphology and response on King's B medium. From these isolates, totally 25 colonies were sorted for further identification test. They were Gram negative, rod shaped, and utilized glucose, mannitol, inositol but unable to consume sucrose and starch in medium. Their host and pathogenicity test confirmed the pathovar as *Pseudomonas viridiflava*.

Keywords: Bacterial leaf spot, *Capsicum annum*, *Pseudomonas viridiflava*, biochemical test.

INTRODUCTION

The role of fresh fruits and vegetables in nutrition and healthy diet is well recognized and in recent years, many countries have undertaken various initiatives to encourage consumers to eat more of these products. The health aspect together with increasing consumer demands for variety and availability, and the changing structure of global trade has led to an increase in trade of fruits and vegetables (Abd-Alla *et al.* 2011). Chilli (*Capsicum annum* L.) is most important spice. Chilli is grown for its fruit because its dried fruit have a good pungency. Chilli belong to the genus capsicum, family *Solanaceae*. Capsicum mainly cultivated in tropical region because it necessary a long and hot

summer climate for its growth. It is originated in Mexico, Southern Peru and Bolivia (Villalon, 1981). The world's hottest chilli species is "Naga jolokia". India is largest producer, consumer and exporter of chilli in the world. Agriculture products can be exposed to microbial contamination through a variety of sources. Since fruits and vegetables are produced in a natural environment, they are vulnerable to contamination by human pathogens. The majority of diseases associated with fresh fruits and vegetables are primarily those transmitted by the faecal-oral route, and therefore, are a results of contamination at some point in the process. (De Rover 1998). Besides being used as Spice, medically, fungicide, it is attacked by many pathogenic bacteria which annually results in heavy yield losses (Aktar and Shamsi, 2014; Politi *et al.,* 2012; Zhang *et al.,* 2013; Salehi *et al.,* 2018; Mir *et al.* 2019). Bacterial disease is leading cause of postharvest losses of potatoes (Ceponis *et al.* 1984), tomatoes (Ceponis *et al*. 1986), peppers (Ceponis *et al*. 1987), lettuce (Ceponis *et al*. 1985). It is causes by a group of plant pathogens that includes pectolytic, *Pseudomonas fluorescence* and *P. viridiflava* (Lund 1983). The pathovars of *Pseudomonas viridiflava* have a wide host ranges they are known for infecting mostly economically important crop species, thus it is considered as the most common pathogen of plants (Lamichhane *et al.* 2015). Additionally, regular occurrences of diseases caused *by P. viridiflava* pathovars remain as a global threat to production of different crops (Xin *et al.* 2018; O'Malley and Anderson 2021.) such as diseases of potato, eggplant, tomato and lettuce (Shane and Baumer, 1984) have been a matter of concern.

One of the latest examples of such distressing effects of this pathogen is bacterial leaf spot (BLS) disease caused by pathovar *capsici sp.* in *capsicum annum* grown worldwide including India. BLS is a devastating disease of capsicum and is triggered by bacterium *Pseudomonas viridiflava capsici.* It is a seed-borne disease as it transmits from seeds to seedling and plants. It was first detected in the United States in the year 1972 and till then it has been reported from many countries of the world and causes heavy destruction of yield by infecting juvenile plants of capsicum (Styer *et al.,* 1980). The displaying characteristic symptoms of this disease start with water- soaked, dark brown- to- black, necrotic on pepper (*Capsicum annum* L.) leaves, irregular circles of chlorotic soft tissue. In some severe stage of disease plants show apical growth resulting in death of the infected plants. It shows variability in symptom development pattern also as sometimes little; moist, dark-green spots are emerged on lower side of leaves which is later covers upper layer also with brownish drawn-out de-coloration (Hellmers, 1955). As mentioned above, the Capsicum has immense importance as a source of Spices compounds, details on its microbial pathogens and their early identification process is a necessity to decide approaches of crop protection so that monetary losses can be prevented, particularly for small-scale producers.

MATERIALS AND METHODS

Field survey and sample Collection

For collecting samples of *Capsicum annum* were performed in the year 2019-2020 and total 110 samples of seeds were collected from fields of different villages of Alwar districts, of Rajasthan state. In initial investigation, the symptomatic seeds were distinguish on the basis of paleness of cotyledons, wrinkles or any other spot.

Screening of the pathogen

Out of the 110 samples, 65 samples showed discoloration and spots on seeds which later yielded in isolation of 42 bacterial colonies. On application of specific identification methods, 25 colonies were identified as *Pseudomonas virdiflava capsici*. Which were further confirmed by host and pathogenicity assays. Before doing biochemical tests, symptomatic seeds were washed with sterilized distilled water, and surface disinfested by washing in sodium hypochlorite (10%) for 1 min, a Second washing in ethyl alcohol (70%) for 1 min, followed by triple washing in sterile distilled water. (Cotter *et al*. 1985). Now the symptomatic tissue was investigated under a light microscope (40X) to check for bacterial streaming. The second test was electron microscopy screening for the presence of virus particles (Golding *et al*.2016). third an all seed tissue was plated on potato dextrose agar (PDA: Sogma Aldrich Ltd.) to check for the presence of fungi and lastly, the seeds sample was plated on agar medium to grow potentially present plant pathogenic bacteria. The different bacterial colony grown under the seeds were picked and transferred on semi

selective King's B (KB) medium for further identification process. The isolates which showed *Pseudomonas viridiflava capsici* type colony appearances were exposed to many biochemical tests.

Nutritional and biochemical analysis

In isolates 25 bacterial colony were selected for their reaction towards Gram's- staining, KOH solubility, and catalase activity, LOPAT test (Levan production, Oxidase activity, Potato soft rot, and hypersensitive response) for early identification of pseudomonas spp. (Klement *et al.,* 1990; Peix *et al.,* 2018; Patyka *et al.,* 2019). In addition to these initial tests, strains were eveluted for their ability to hydrolyase gelatin and esculin and to utilize betaine, L-lactate and homoserine as the sole carbon source according to Schaad *et al.* (2001). The isolates were tested for development of fluorescence also by streaking on KB (King's medium B) and observed under UV light (Mohan and Schaad, 1987). Likewise, leaves, stems and seeds showing symptoms of *Pseudomonas viridiflava* were disinfected and their small parts were transferred on NA and tested for nutritional and biochemical features as stated above.

Pathogens and Host reaction

The isolates showing characteristic test of *Pseudomonas viridiflava* were assessed for pathogenicity test on *Capsicum annum* and other hosts such as Potato (*Solanum tuberosum*), Tomato (*Solanum lycopersicum* L.), eggplant (*Solanum melongena)*) (Robinson *et al*. 2004). The leaves of host plants were punctured and the bacterial suspension maintained at 1×107 CFU/mL was injected in the middle vein while the control was inoculated with sterile tap water only (Rhodehamel and Durbin 1985; Peix *et al.,* 2018). The inoculated leaves were then placed in at 27±2°C and looked every day for development of any necrotic or pathogenic symptoms (Schaad and Kendrick, 1975; Saettler *et al.,* 1989).

RESULTS AND DISCUSSION

The *P. viridiflava* capsici hosts members of family *Solanaceae* and incites apical chlorosis and bacterial leaf spots (Gulya *et al.,* 1982; Hellmers, 1955; Rhodehamel and Durbin, 1985; Rhodehamel and Durbin, 1989; Shane and Baumer, 1984). Formerly also, it has been isolated from various plants and from weeds showing apical chlorosis. On the contrary, it is assessed for antagonistic activity for controlling Canada thistle in soybean and woolly leaf bursage in cotton (Budde and Ullrich *et al.* 2000; Sheikh *et al..,* 2001; Gronwald *et al.,* 2002). In this study, overall 65 seed samples displayed atypical colour and spots on seed coat which further brought out 42 types of bacterial colonies. From these isolates, 25 colonies were developed on NA medium and characterized as *Pseudomonas viridiflava* on the basis of their response for nutritional and biochemical analytic tests as shown in table no. 1. The colonies developed on NA were whitish, mucoid, raised, smooth and glistering (Fig.2). All the isolates were Gram's negative and positive for KOH and catalase test and rod shaped. The isolates produced a yellow – green to blue fluorescence on King's B (KB) medium, confirming that the isolates are fluorescent pseudomonads (Mohan and Schaad, 1987). This pigment has been used as characteristic of many *P. viridiflava* strains (Lamichhane and Varvaro, 2013; Tymon and Inglis, 2017; Fuenzalida-Valdivia *et al.* 2022).

These fluorescent pseudomonads were looked for their group on the basis of LOPAT test and they were -+--+ and confirmed their position in LOPAT group IB (Young and Fletcher 1997). Suzuki *et al*. (2003) also used LOPAT and other test to differentiate *P. viridifalva pisi* from infected pea plants. The most studied *P. viridiflava* bacterial models had same pattern in some other study viz. *Pseudomonas* pathovar from tomato (Whalen *et al*. 1991) and cauliflower (Weibe *et al.* 1993) showed same response for KB medium and LOPAT test. The results of morphology of colony and LOPAT directed that all the purified colonies are strains of *Pseudomonas viridiflava capsici.* The results of different phenotypic (biochemical) tests lead to the confirmation of pathovar. Further positive results for D-Galctose, Gluconate, Glucose, inositol, mannitol, sorbitol utilization and negative results for starch hydrolysis and Glycerol and sucrose specified that all isolates are *Pseudomonas virdiflava* which was further confirmed by host and HR test. In previous studies too, similar responses of biochemical and hypersensitivity reactions of Pseudomonas virdiflava have been observed in capsicum and other plants (Lydon *et al.* 2011; Song *et al.* 2015). In earlier studies also similar pattern has been observed in various members of family *Solanaceae* (Rhodehamel and Durbin 1985; Lydon *et al*. 2011;

Suzuki *et al*. 2003; Zhang *et al.,* 2002). Even there are surprising reports of symptom development by this pathogen in plants of *Brassicaceae, Asteraceae* and *Cucurbitaceae* family (Gulya *et al.* 1982). There are reports that host range of pathovars of Pseudomonas is expanding frequently like in Ageratum, Cirsium, Cordyline, Anemone, Argyranthemum Fuchsia, Lavandula, Arabidopsis thaliana, Geranium, (Zavala *et al*. 2022). This is the reason the *P. viridifalva* has attracted scientists due to its diverse host range and toxin production (Xin *et al*. 2018). In some key studies, the selective characteristics of pathogen of Capsicum were established on the basis of specific biochemical features (Jeevan *et al*. 2021). For *B. altitudinis*, *P. aeruginosa, B. aryabhattai*, *B. wiedmannii* and many more, biochemical characterization method was applied successfully (Margarita *et al*. 2017; Shah *et al*. 2022). In experiments exploring antimicrobial properties of *Pseudomonas virdiflava* it was identified and distinguished on the basis of nutritional requirements Grădilă *et al.* 2022). For other pathovars of *P. virdiflava* biochemical and pathogenicity tests have made its early identification easier as commended in various studies (Chaturvedi *et al.* 2018; Jangir *et al.* 2018; Chaturvedi *et al.* 2015). Though there are developments of enormous advanced techniques for identification of bacterial pathogens from plants, the LOPAT, biochemical and pathogenicity tests are still important as colony type and difference in carbon source utilization plays a significant role in deciding pathovar of a species (Marques *et al*. 2016; Tymon and Inglis, 2017; Gomila *et al.* 2017; Chaturvedi *et al.* 2018; Saint-Vincent *et al*. 2020). Thus, the present study has established identification of *Pseudomonas virdiflava capsici* on by exhausting its biochemical traits.

CONCLUSION

We have performed an inclusive phenotypic and biochemical analysis of 25 strains of *Pseudomonas viridiflava capsic*i isolated from the seeds of *Capsicum annum L*. The strains showed ability to utilize D-Galctose, Gluconate, Glucose, inositol, mannitol and sorbitol as sole carbon source in medium. The significance of LOPAT and other biochemical tests has been established in many molecular detection experiments also and in our study we successfully demonstrated that these methods are vital to decide pathovar of *Pseudomonas viridiflava*. We have provided an extensive detail of these test methods and results which may be proven as a great source of reference in further

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REFERENCES

1. Hellmers E. 1955. Bacterial leaf spot of African capsicum (caused by *Pseudomonas* sp. n. Acta Agric. Scand. 5:185-200.
2. Klement, Z. 1963. Rapid detection and the pathogenicity of pathogenicity of phytopathogenic Pseudomonas. Nature. 199:299-300.
3. Shane WW, Baumer JS. 1984. Apical chlorosis and leaf spot of Jerusalem artichoke incited by *Pseudomonas* Plant Dis. 68:257-260. Styer DJ, Durbin RD. 1982. Isolation of *Pseudomonas* from sunflower in Wisconsin. Plant Dis. 66:601
4. Rhodehamel NH, Durban RD. 1985. Host range of strains of *Pseudomonas virdiflava* Plant Dis. 69: 589-591.
5. Ceponis MJ, Cappellini RA, Lightner GW (1985) Disorders in crisphead lettuce shipments to the New York market, 1972–1984. Plant Dis 69:1016–1020
6. Ceponis MJ, Cappellini PA, Lightner GW (1986) Disorders in tomato shipments to the New York market, 1972–1984. Plant Dis 70:261–265
7. Ceponis MJ, Cappellini RA, Lightner GW (1987) Disorders in fresh pepper shipments to the New York market, 1972–1984. Plant Dis 71:380–382

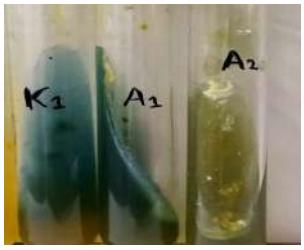
8. Cotter JL, Fader RC, Lilley C, Herndon DN (1985) Chemical parameters, antimicrobial activities, and tissue toxicity of 0.1 and 0.5% sodium hypochlorite solutions. Am Soc Microbial 28:118–122
9. Mohan SK, Schaad NW. 1987. An improved agar plating assay for detecting *Pseudomonas* and P. s. pv.*phaseolicola* in contaminated bean seed. Phytopathology. 77:1390–1395.
10. Rhodehamel NH, Durbin RD. 1989. Two new hosts of *Pseudomonas virdiflava* Plant Dis. 73:368.
11. Whalen MC, Innes RW, Bent A, Staskawicz BJ. 1991. Identification of *Pseudomonas syringae* pathogens of Arabidopsis and a bacterial locus determining avirulence on both Arabidopsis and soybean. Plant Cell. 3: 49–59.
12. Wiebe WL. 1993. Characterization of *Pseudomonas virdiflava* and Comparison with P. s. tomato. Plant Dis. 77: 414.
13. Abd-Alla MH (1994) Phosphatases and the utilization of organic phosphorus by Rhizobium leguminosarum biovar viceae phosphatases. Lett Appl Microbial 18:294–296
14. Rover De (1998) Microbiological safety evaluations and recommendations on fresh produce. Food Control 6:321–347
15. Agrios GN. Plant Pathology. 5th Ed. The Netherlands: Elsevier Academic Press; 2005
16. Lund BM. Bacterial spoilage. In: Demis C (Ed.). Postharvest pathology of fruits and vegetables. London: Academic press; 1983: 219– 5
17. Abd-Alla MH, Bashandy SR, Schnell S, Ratering S (2011) Isolation and characterization of Serratia rubidaea from dark brown spots of tomato fruits. Phytoparasitica 39(2):175–183
18. Young JM and Fletcher MJ. (Eds) 1997. International Collection of Microorganisms from Plants: Catalogue Accession 1-12989 (3rd Edn), Manaaki Whenua Landcare Research, Auckland, pp 271 Gulya TJ, Urs R, Banttari EE. 1982.
19. Budde IP, Ullrich M. S. 2000. Interactions of *Pseudomonas pv.glycinea* with host and nonhost plants in relation to temperature and phytotoxin synthesis. Mol. Plant-Microbe Interact. 13:951-961.
20. Bruhn CM (1995) Consumer attitudes and market response to irradiated food. J Food 58:175–181
21. Sheikh T, Wheeler TA, Dotray PA, Zak JC. 2001. Biological Control of Woollyleaf Bursage (*Ambrosia grayi*) with *Pseudomonas* and Weed Technol. 15(2):375–381. doi:10.1614/0890-037x(2001)015[0375:bcowba]2.0.co
22. Gronwald JW, Plaisance KL, Ide DA, Wyse DL. 2002. Assessment of *Pseudomonas* as a biocontrol agent for Canada thistle. Weed Sci. 50:397-404.
23. Suzuki A, Togawa M, Ohta K, Takikawa Y. 2003. Occurrence of white top of pea caused by a new strain of *Pseudomonas virdiflava* pv.*pisi*. Plant Dis. 87:1404-1410.
24. Krieg NR., Ludwig W., Whitman WB., Hedlund BP., Paster BJ., Staley JT., Ward N., Brown D. Bergey's manual of systematic bacteriology, 2nd edn, vol. 4, New York: Springer– Verlag; 2010
25. Lydon J, Kong H, Murphy C, Zhang W. 2011. The Biology and Biological Activity of *Pseudomonas* spp. Pest Technol. 5(1):48-55.
26. Politi FA, Figueria CM, Aruio AM, Sampieri BR, Mathias MI, Szabo MP, Bachara GH, Santos LCD, Vilegas W, Pietro RC. 2012. Acaricidal activity of ethanolic extract from aerial parts of Asteraceae against larvae and engorged adult females of *Rhipicephalus sanguineus* (Latreille, 1806). Parasit Vectors. 17 (5):295.
27. Zhang JX, Lin BR, Shen HF, Pu XM, Wang ZW, Zeng DQ, Huang N. 2013. First Report of Bacterial Soft Rot on *Capsicum annum* Caused by *Dickeya dieffenbachiae* in USA Plant Dis. 97(2): 282-282
28. Lamichhane JR, Varvaro LA. 2013. New medium for the detection of fluorescent pigment production by pseudomonads. Plant Pathol. 62: 624–632.
29. .Aktar M, Shamsi S. 2014. Report on alternaria blight of *Tagetes erecta* and Ta*getes patula* caused by *Alternaria alternata* (Fr.) Keissler. J. Asiat. Soc. Bangladesh, Sci. 40(1): 133–140.
30. Chaturvedi S, Kulshrestha S, & Agrawal KP. 2015. Characterization of *Pseudomonas savastoni* pv *phaseolicola* in seed lots of mung bean (*Vigna radiata* L. Wilczek) collected from Rajasthan, India. Int. Res. J. Biological Sci. 4(10):57-61.
31. Lamichhane JR, Messean A, Morris CE. 2015. Insights into epidemiology and control of diseases of annual plants caused by the *Pseudomonas* species complex. J. Gen. Plant Pathol. 81(5): 331–350. doi:10.1007/s10327-015-0605-z
32. Marques E, Borges, RCF, Uesugi, CH. 2016. Identification and pathogenicity of *Pseudomonas cichorii* associated with a bacterial blight of gerbera in the Federal District. Horticulture Brasileira. 34:244-248. DOI http://dx.doi.org/10.1590/S0102-053620160000200015

33. Margarita G, Antonio B, Magdalena M, Elena GV, Jorge L. 2017. Clarification of Taxonomic Status within the *Pseudomonas* Species Group Based on a Phylogenomic Analysis. Front Microbiol. 8:2422 URL=https://www.frontiersin.org/article/10.3389/fmicb.2017.02422 DOI=10.3389/fmicb.2017.02422 ISSN=1664-302X
34. Tymon LS, Inglis DA. 2017. Identification and pathogenicity of *pseudomonas* genomospecies 1 phylogroup 2B causing leaf spots and fruit warts on cucurbits in Western Washington, U.S. J. Plant Pathol. 99(3):713-722.
35. Chaturvedi S, Agrawal K, Kulshrestha S, Narayan A. 2018. Molecular Identification of *Pseudomonas syringae (savastoni)* pv.*phaseolicola* in Mung Bean (*Vigna radiata* L. Wilczek) Seeds Grown in Rajasthan State, India. Int. J. Curr. Microbiol. App. Sci. 7(11):1859-1866.
36. Jangir R, Sankhla IS, Agrawal K. 2018. Characterization, incidence, transmission and biological control of *Ralstonia solanacearum* associated with soybean [*Glycine max* (L.) Merrill] in Rajasthan, India. Res. Crop. 19(3):472–479.
37. Peix A, Ramírez-Bahena MH, Velazquez E. 2018. The current status on the taxonomy of Pseudomonas revisited: An update. Infect. Genet. Evol. 57: 106–116.
38. Salehi B, Valussi M, Morais-Braga M, Carneiro J, Leal A, Coutinho H, Vitalini S, Kręgiel D, Antolak H, Sharifi-Rad M, Silva N, Yousaf Z, Martorell M, Iriti M, Carradori S, & Sharifi-Rad J. 2018. *T*agetes spp. Essential Oils and Other Extracts: Chemical Characterization and Biological Activity. Molecules (Basel, Switzerland). *23*(11):2847.
39. Patyka V, Pasichnyk L, Butsenko L, Petrychenko V, Zubachev S, Dankevych L, Gnatiuk Y, Huliaiva H, Tokovenko I, Kalinichenko A. 2019. Express Diagnostics of Phytopathogenic Bacteria and Phytoplasmas in Agrophytocenosis. (Eds) Suszanowich D, Patyka V and Wyd-wo I. Drukarnia Swietego Krzyza, Opole, Poland. ISBN 978-83-7342-684-9.
40. Mir RA, Ahanger MA, Agarwal RM. 2019 from Mandap to Medicine and from Ornamentation to Remediation. Am. J. Plant Sci. 10(2) 309-33
41. Saint-Vincent PM, Ridout M, Engle NL, Lawrence TJ, Yeary ML, Tschaplinski TJ, Newcombe G, Pelletier DA. 2020. Isolation, Characterization, and Pathogenicity of Two *Pseudomonas* Pathovars from *Populus trichocarpa* Seeds. Microorganisms.
42. Jeevan U, Kurian PS, Sreelatha U, Mathew D, Narayanankutty C. 2021. Morphological, symptomatological and molecular characterization of Enterobacter cloacae causing bacterial wilt and Indian Phytopathol. 75: 279-285. doi:10.1007/s42360-02100414310.1007/s42360-021-00414.
43. Riaz M, Ahmad R, Rahman NU, Khan Z, Dou D, Sechel G, Manea R. 2020. Traditional uses, Phyto-chemistry and pharmacological activities. J. Ethnopharmacol. 255: 112718. doi:10.1016/j.jep.2020.112718
44. O'Malley MR, Anderson JC. 2021. Regulation of the *Pseudomonas* Type III Secretion System by Host Environment Signals. *Microorganisms.* 9(6):1227.
45. Shah D, Khan MS, Aziz S, Ali H, Pecoraro L. 2022. Molecular and Biochemical Characterization, Antimicrobial Activity, Stress Tolerance, and Plant Growth- Promoting Effect of Endophytic Bacteria Isolated from Wheat Varieties. Microorganisms. 10: 21. https://doi.org/10.3390/microorganisms10010021
46. Molecular and Genomic Characterization of the *Pseudomonas virdiflava* Phylogroup 4: An Emerging Pathogen of *Arabidopsis thaliana* and *Nicotiana benthamiana*. Microorganisms. 10: 707.
47. Fuenzalida-Valdivia I, Gangas MV ,Zavala D, Herrera-Vasquez A, Roux F, Meneses C, Blanco-Herrera F. 2022. Draft Genome Sequence of *Pseudomonas* RAYR-BL, a Strain Isolated from Natural Accessions of Arabidopsis thaliana Plants. Microbiol. Resource. Announc. 11: e01001-21.
48. Höfte M., DeVos P. Plant pathogenic Pseudomonas species. In: plant-associated bacteria. Dordrecht. The Netherlands: Springer; 2007:507–33.

Table no. 1: Biochemical and physiological characterization of isolates strains of *Pseudomonas viridiflava capsici* isolated from seeds of *Capsicum annum L.*

S. NO.	Test	Response	S.no	Test	Response
1	Gram reaction	Negative	16	Mannitol	Positive
2	5%NaCl tolerance	Negative	17	Utilization from Glucose	Positive
3	7%NaCl tolerance	Negative	18	Sucrose	Negative
4	Catalase	Positive	19	Sorbitol	Positive
5	Mucoid growth	Positive	20	Erythritol	Positive
6	Growth at 37	Positive	21	Maltose	Positive
7	Growth at 40	Positive	22	Ribose	Positive
8	Hydrolysis of gelatin	Positive	23	Dextrose	Negative
9	Hydrolysis of aesculin	Positive	24	Triacetin	Negative
10	Hydrolysis of starch	Negative	25	L-Valine*	Negative
11	Hydrolysis of Fructose	Negative	26	*myo*-Inositol	Positive
12	Esculin	Negative	27	Gelatin liquefaction	Negative
13	Citrate utilization	Negative	28	Tobacco Hypersensitivity test	Negative
14	Oxidase	Positive	29	Indole test	Negative
15	Amylase	Negative			

A- Catalase hydrolyse Positive



B- Citrate hydrolyse Negative

C- Potato soft rot Negative

D- Indole Test Negative

E-Hypersensitivity test Negative

F- Oxidase positive

Fig.1. Results of LOPAT explain that the response of pathogen *Pseudomonas viridiflava capsici* was -+--+ that includes it in LOPAT 1B group

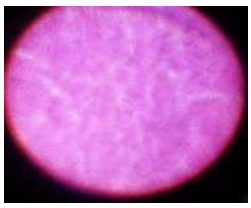
			
A: Rod shaped colonies in Gram's Negative	**B: White, glistening and mucoid colonies of *Pseudomonas virdiflava capsici* on NA**	**C: Bubbles indicating KOH solubility test**	**D: Whitish glistening colony on NA**

Fig. 2: Results of colony morphology, Gram's staining and KOH solubility test

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RESEARCH ARTICLE

Knowledge on Disaster among Residents in Mador Village, Kattumannarkoil

Prabhakaran.A[1]* and V.Selvanayaki[2]

[1]Research Scholar / Tutor, Department of Community Health Nursing, V.M.A. College of Nursing, Vinayaka Mission's Research Foundation – Deemed to be University, Salem, Tamil Nadu, India.
[2]Professor, Department of Obstetrics and Gynecological Nursing, V.M.A. College of Nursing, Vinayaka Mission's Research Foundation – Deemed to be university, Salem, Tamil Nadu, India.

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***Address for Correspondence**
Prabhakaran. A,
Research Scholar / Tutor,
Department of Community Health Nursing,
V.M.A. College of Nursing,
Vinayaka Mission's Research Foundation – Deemed to be University,
Salem, Tamil Nadu, India.
Email: prabhakaranyellow5@gmail.com

ABSTRACT

A descriptive study was conducted to assess the knowledge on Disaster among residents in Mador village. Data was collected from 50 residents. The simple random sampling technique was adopted and information was gathered. Highest percentage (80%) of the residents was in the age group of 26-33 years. Highest percentages (60%) of the residents were male. Majority (89%) of resident's religion were Hindu. Highest percentages (62%) of resident's educational status were high school education. Highest percentages (52%) of resident's occupation were daily wagers. Majority (80%) of residents was married. Highest percentage (48%) of resident's monthly income was 10,000 – 15,000 rupees. Most of the (60%) residents were nuclear family. Majority (90%) of residents have previous experience of disaster. Further Area wise distribution of mean, SD and mean percentage of knowledge of residents regarding disaster preparedness reveals that the mean score of disaster (2.6 ± 1.32) which is 55.24%, the mean score of cyclone (3.58 ± 1.61) which is 51.19%, the mean score of flood (3.15 ± 1.13) which is 46.03% and the mean score of drought (2.3 ± 0.94) which is 37.56% which reveals average knowledge about disaster preparedness.. The overall level of knowledge on disaster shows that highest percentage (46%) of residents had average knowledge. Lowest percentage (8%) of residents had very poor knowledge. (34%) of residents had poor knowledge and (12%) of residents had good knowledge regarding disaster. The present study assessed the knowledge of residents about disaster. The resident's knowledge level was found to be average further educational interventional programme need to be created to improve resident's level of knowledge.

Keywords: Disaster, Residents (People), Knowledge.

INTRODUCTION

Disaster is a sudden, calamitous event bringing great damage, loss, and destruction and devastation to life and property. The damage caused by disasters is immeasurable and varies with the geographical location, climate and the type of earth surface/degree of vulnerability. Emergencies and disaster not only affect health and well being of people, frequently large number of people are displaced, killed or injured or subjected to greater risk of epidemics. Considerable economic harm is also common. Disaster cause great harm to the existing infrastructure and threaten the future of sustainable development. Disasters are not confined to a particular part of the world, they can occur anywhere and at any time. Major emergencies and disaster have occurred throughout the history, as the world's population grows and resources become more limited, community are increasingly becoming vulnerable to the hazards that cause disaster .Disasters have, in recent years become an undeniably grim feature in our lives. Barely had the world begun the process of recovery after the devastation of the tsunami, which in its wake overran all geographical, social and economic demarcations, then the hurricane Katrina brought the world's most powerful nation to its feet. In the interim there was the Mumbai deluge, the terrorist strikes at London and innumerable local disasters, which had an equally devastating impact. Natural disasters are often described as the wrath of God. In fact, they are the wrath of nature increasingly, the wrath of nature that has been tampered with. Manmade disasters are sometimes the misuse of science and sometimes accidents. Asia tops the list of causalities due to natural disasters. It is almost impossible to prevent the occurrence of natural disasters and their damage. However, it is possible to reduce the impact of disasters by adopting suitable disaster mitigation strategies and disaster preparedness.

Statement of the problem
A Study to Assess the Knowledge on Disaster among residents in Mador village, Kattumannarkoil

Objectives
To assess the level of knowledge about Disaster among residents.

METHODOLOGY

Research approach
A cross sectional survey approach was used to assess the knowledge on Disaster among residents

Research design
A descriptive research design was adopted to assess the Knowledge on Disaster among residents

Setting of the study
The study was conducted in Mador village, Kattumannarkoil

Population and Sample
The population under study was residents (people) in the age group of 18 – 49 years and who were presented during the period of data collection was the sample of the study.

Sample size
The sample comprised of 50 people living in Mador village, Kattumannarkoil

Sampling technique
Simple random sampling was adopted to select the samples for this study.

Description of the tool
Closed ended questionnaire was used to assess the knowledge on Disaster among residents.

The tool consists of two sections.

- Part A – Demographic data
- Part B – Knowledge questionnaire

Part – A: Demographic data

It consist of demographic characteristics such as age, sex, religion, education, occupation, income, marital status, type of family and previous disaster experience

Part - B: Knowledge questionnaire

Structured questionnaire consist of 37 multiple choice questions, related to information about disaster, cyclone, flood, drought. Each right answers carrying one mark. Each wrong answers carrying zero mark.

RESULT AND DISCUSSIONS

A descriptive cross sectional study was conducted to assess the knowledge on Disaster among residents in Mador village, Kattumannarkoil. The data collected from 50 residents by using simple random sampling technique.

Area wise distribution of mean, SD and mean percentage of knowledge of residents regarding disaster reveals that the mean score of disaster (2.6 ± 1.32) which is 55.24%, the mean score of cyclone (3.58 ± 1.61) which is 51.19%, the mean score of flood (3.15 ± 1.13) which is 46.03% and the mean score of drought (2.3 ± 0.94) which is 37.56% which reveals average knowledge about disaster preparedness. (Table No.1).

Assessment of level of knowledge of the residents about disaster preparedness

Percentage wise distribution of residents according to their overall level of knowledge about disaster shows that highest percentage (46%) of residents had average knowledge. Lowest percentage (8%) of residents had very poor knowledge. (34%) of residents had poor knowledge and (12%) of residents had good knowledge regarding disaster. (Fig. No.1)

CONCLUSION

The present study assessed the knowledge of residents about disaster. The resident's knowledge level was found to be average further educational interventional programme need to be created to improve resident's level of knowledge.

REFERENCES

1. All ender, J.A, and Spradley. B.W., (2000). "COMMUNITY HEALTH NURSING - CONCEPTS AND PRACTICE"., 5th editions., Philadelphia., Lippincott, Pp: 1224-1231.
2. B.T.Basavanthappa.,(2015)., "COMMUNITY HEALTH NURSING"., 2nd edition., Jaypee brother's Medical publishers (P) Ltd., New Delhi., Pp:953-977.
3. Basavanthappa BT, (2001), "NURSING RESEARCH- PRINCIPLES AND METHODS", 6th edition, Jaypee brothers, New Delhi, Pp: 115-176.
4. Das. B.C and Malini. S., (2005)., "HOSPITAL DISASTER MANAGEMENT PLAN, TEXT BOOK OF COMMUNITY MEDICINE-RECENT ADVANCES". 1st edition., Delhi., Ahuja Publications., Pp: 3146-3158.
5. Dave. P.K. and et.al. (2000)., "EMERGENCY MEDICAL SERVICES AND DISASTER MANAGEMENT"., 1st edition., New Delhi., Jayvee., Pp:1454-1468.
6. "FIRST AID AND EMERGENCY CARE". (2004). 8th edition. NRBrothers Publishers., Indore., Pp: 214-255.
7. Gebbie K.M, and Qunesh.K, (2000), "DISASTER - A BASIC OVERVIEW, PREPARING STUDENTS FOR DISASTER PREPAREDNESS", 1st edition. New Jersey, Prentice Hall, Pp:17
8. Park's., (2021). "TEXTBOOK OF PREVENTIVE AND SOCIAL MEDICINE". 24th edition., M/s. Banarsidas Bharat publishers., Jabalpur., India.,Pp: 700-707.

9. Sreevani.R., (2005). "ROLE OF A NURSE IN DEALING PSYCHOSOCIAL PROBLEMS OF DISASTER VICTIMS"., Nightingale nursing time., Volume: 1(3)., Pp: 4.
10. Waff, TV., (2005)., "DISASTER MANAGEMENT HEALTH FOR THE MILLIONS"., Feb., Pp:8-9.

Table.1. Area wise distribution of mean, SD and mean percentage of knowledge about disaster preparedness among residents **n=50**

Variables	Total score	Mean	Standard deviation	Mean percentage
Disaster	5	2.6	1.32	55.24
Cyclone	7	3.58	1.61	51.19
Flood	7	3.15	1.13	46.03
Drought	7	2.3	0.94	37.56

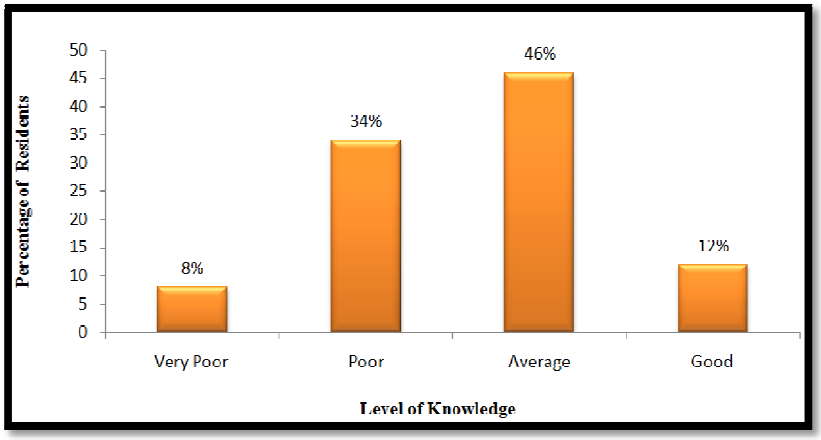

Fig. 1. Bar diagram showing overall percentage of residents according to their level of knowledge about disaster.

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RESEARCH ARTICLE

Assessment of Heavy Metal Contaminations in the Lake Water Resources of Coimbatore City

P. R. Soumya[1], S. Brindha[1] and K.S. Tamil Selvi[2]*

[1]Research Scholar, Department of Botany, PSGR Krishnammal College for Women, Peelamedu, Coimbatore, Tamil Nadu, India.
[2]Assistant Professor, Department of Botany, PSGR Krishnammal College for Women, Peelamedu, Coimbatore, Tamil Nadu, India.

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***Address for Correspondence**
K.S. Tamil Selvi,
Assistant Professor,
Department of Botany,
PSGR Krishnammal College for Women,
Peelamedu, Coimbatore, Tamil Nadu, India.
Email: tamilselviks@psgrkcw.ac.in

ABSTRACT

Pollution of aquatic ecosystem with toxic heavy metals is a major socio-environmental issue. Heavy metals are common pollutants in water resources due to anthropogenic activity. The present research work was focused to evaluate the concentration of heavy metals such as chromium, cobalt, copper, nickel, and zinc in Singanallur, Sulur and Ukkadam lakes, Coimbatore, Tamil Nadu. Water samples were collected in the months of January, May, July and November and analyzed for heavy metals by using AAS. The results revealed that, chromium, cobalt, copper, nickel were present in all the water samples collected during the period of study except zinc. The average concentration of the heavy metals was in the order Cr<Cu<Zn<Co<Ni and they were within the maximum permissible limit recommended by WHO for drinking water. The present investigation revealed that, the water in the three lakes was moderately contaminated with heavy metal pollution.

Keywords: Water samples, Heavy metal Contamination, Drinking water

INTRODUCTION

Lakes are "important natural resources for freshwater, replenishing of ground water, habitats for wide variety of flora and fauna, key factor for climatic changes and considered as one of the most functional ecosystems". Industrialization and urbanization is a major threat to water environment and adversely affect the biological diversity. Expeditive growth of human population, rapid development of industrial, agricultural based sectors have resulted in discharging of toxic wastes and municipal waste materials into the wetland. Lake water quality has deteriorated due to eutrophication and heavy metal pollutions and has been evident in the lakes surrounding the cities. Water pollution, especially with heavy metal contamination represent a biggest environmental issue in this modern world (Zan *et al.,* 2011, Hou *et al.,* 2013, Crispin and Shivakumar, 2020). Heavy metals are persistent, non biodegradable contaminant that create significant effect on natural ecosystem. Discharge of effluent from urban and agricultural run off etc. are the major sources of pollutants. At low concentration heavy metals pose, toxic effect on human, animal and aquatic organisms. previous research works reported that, the heavy metals may enter into the human body mainly through air, water, soil and food (Lu *et al.,* 2004, Hassan *et al.,* 2007, Chatterjee *et al.,* 2011, Chiodi *et al.,* 2011, Hemambika *et al.,* 2011).

Heavy metals like copper, zinc and nickel are known as "essential trace metals" which are vital for normal metabolic activity of human body. Excessive intake of these heavy metals may damage human health. Chromium, copper, nickel and zinc released from mining, smelting and electroplating industries can cause large amount of pollutions. High level exposure of chromium cause liver and kidney damage, skin ulceration and also affects the central nervous system. Continuous exposure to Copper may cause kidney damages. Nickel and Copper are considered as tumor promoting factors on human beings. Close contact with nickel powder may cause nasopharyngeal carcinoma. Exposure to higher concentration of zinc may cause anemia and cholesterol problems in human beings (Rajkumar *et al.*, 2011, Gautam *et al.*, 2014). The heavy metal toxicity to the environment and to human beings is a serious challenge. Therefore, regular monitoring of water quality is mandatory for development of successful healthy society. Hence the research work was conducted to analyse seasonal variation of heavy metals (Co, Cr, Cu, Ni and Zn) in different water bodies in Coimbatore city.

MATERIALS AND METHODS

Study area and sample collection

Singanallur, Sulur and Ukkadam lakes in Coimbatore city were selected for the study. Water samples were collected during the months of January, May, July and November using sterilized containers. pH of the samples were checked using pH meter. The samples were acidified with appropriate amount of nitric acid (HNO_3) to a pH <2 to avoid precipitation. The samples were stored in refrigerator.

Digestion of water sample

50ml water samples were digested with 2.5ml of single acid (HNO_3) on a hot plate and filtered through What man filter paper No:42 and used for analysis of Cr, Co, Cu, Ni and Zn using Atomic Absorption Spectrometer.

RESULTS

Water samples were collected from Singanallur, Sulur and Ukkadam lakes during the months of January, May, June and November and evaluate the concentration of Cr, Co, Cu, Ni and Zn. The concentration of heavy metals of water samples collected from Singanallur, Sulur and Ukkadam lakes were depicted in the Table 1. Concentration of Cr in water samples collected from Singanallur, Sulur and Ukkadam lakes ranged between 0.001±0.0012mg/L and 0.005±0.0007mg/L. Maximum (0.005±0.0007mg/L) concentration of Cr was found in Sulur lake during the month of May. Minimum (0.001±0.0054mg/L) concentration of Cr was found in Singanallur lake during the months of January and July, Sulur lake during the month of January and Ukkadam lake during the month of November. The

concentration of Cr was noticed within the maximum permissible limit (0.05mg/L) of WHO for drinking water. Concentration of Co was observed in water samples, collected from Singanallur, Sulur and Ukkadam lakes to be in the range of 0.009±0.01039mg/L - 0.022mg/L±0.0083mg/L, 0.012±0.0065mg/L - 0.039±0.00577mg/L and 0.007±0.0079mg/L - 0.025±0.00618mg/L respectively during the period of study. Concentration of Co was higher (0.039±0.00577mg/L) in Sulur lake in the month of January, compare to the other two lakes. Concentration Co was not exceeded the maximum permissible limit prescribed by WHO (0.05mg/L).The concentration of Cu in Singanallur, Sulur and Ukkadam lakes ranged from 0.004±0.00081mg/L - 0.005±0.00115mg/L, 0.002±0.00421mg/L - 0.006±0.00306mg/L and 0.003±0.00110mg/L - 0.006±0.00069mg/L respectively. Among the three lakes, Sulur (0.006±0.00306mg/L) and Ukkadam (0.006±0.00069mg/L) lake registered higher concentration of Cu in the months of May and November. Concentration of Cu was within the maximum permissible limit of WHO (2.0mg/L) for drinking water.

Concentration of Ni varied from 0.011±0.00710mg/L - 0.037±0.01703mg/L, 0.008±0.00999mg/L - 0.028±0.00878mg/L and 0.008±0.00491mg/L -0.024±0.00433mg/L in Singanallur, Sulur and Ukkadam lake respectively. However, maximum (0.037±0.01703mg/L) concentration of Ni was noticed in Singanallur lake in the month of July and minimum concentration of Ni was recorded in Sulur lake in the month of July (0.008±0.00999mg/L) and Ukkadam lake in the month of November (0.008±0.00491mg/L). Concentration of Ni was within the maximum permissible limit of WHO (0.07mg/L) for drinking water. The highest (0.029±0.00041mg/L) and lowest (0.001±0.00543mg/L) concentration of Zn was observed in Singanallur lake during the months of May and January respectively. Concentration of Zn in lake water samples were varied from 0.013±0.00855mg/L - 0.020±0.00676mg/L, 0.001±0.00543mg/L - 0.014±0.00156mg/L and 0.004±0.00346mg/L - 0.029±0.00041mg/L in Singanallur, Sulur and Ukkadam lakes respectively during the period of study. Concentration of Zn was within the permissible limit of WHO (3.0mg/L) for drinking water.

Average Concentration of heavy metals

The Average concentration of Cr was found to be higher (0.0028mg/L) in L2, whereas lower (0.0015mg/L) in L1. The average concentration of Co showed higher (0.0215mg/L) in L2 and lower (0.0150mg/L) in L3. The average concentration of Cu showed maximum (0.0048mg/L) inL1and minimum (0.0040mg/L)in L3. The higher concentration of Ni (0.0217mg/L) and Zn (0.0175mg/L) were found L1 and lower concentration of Ni (0.0177mg/L) and Zn (0.0063mg/L) were found in L3. Among the five heavy metals, Ni recorded as maximum concentration (0.0217mg/L) in L1 and Cr recorded as minimum (0.0015mg/L) concentration in L1. The average concentration of Cr, Co, Cu, Ni and Zn in water samples were analyzed, they were in the order Cr<Cu<Zn<Co<Ni (Table-2).

DISCUSSION

The study was conducted to analyse the concentrations of heavy metals (Cr, Co, Cu, Ni and Zn) in lake water samples. Heavy metals are toxic pollutants have significantly contributed to contaminate air, soil and water and directly or indirectly enter into food chain. The major sources of heavy metals are municipal waste, power industry, transport, waste dumping sites and fertilizers etc (Szyczewski *et al.,* 2009; Ahmad *et al.,* 2010). The results showed that, all the heavy metals were within the maximum permissible limit recommended by WHO for drinking water. Accumulation of Cr in the aquatic system is due to presence of sewages, septic tank wastes and plastic wastes etc. Previous results reported by Mohanraj *et al* (2000) showed that, concentration of Cr in Singanallur lake and Ukkadam lake was found to be 52µg/L and 29.8µg/L respectively. According to Batvari and Surendran (2015), the water samples of Chemberambakkam lake showed concentration of chromium to be 0.019 - 0.035 mg/L. Manikandan *et al* (2016) reported that the concentrations of Cr in Ukkadam and Singanallur lakes were found to be 0.0310±0.000mg/L and 0.0475±0.000mg/L respectively, during the month of June to September. During the months of October - November, concentrations of Cr in Ukkadam and Singanallur lakes were found to be 0.0595±0.000mg/L and 0.0633±0.000mg/L respectively. Similar result was reported by Crispin and Sivakumar (2020), which showed that, concentration of Cr in Singanallur, Sulur and Ukkadam lake ranged as 0.01mg/L to 0.02 mg/L. Municipal waste,

laundry chemicals, paint and pigments are the primary sources of Cr in surface water (Dixit and Tiwari, 2008). In the present study, concentrations of Co was within the maximum permissible limit recommended by WHO (0.05mg/L). Batvari and Surendran (2015) reported that, concentration of Co in water samples of Chemberambakkam lake was ranged as 0.015 - 0.099mg/L. According to Palanisamy *et al* (2021), concentration of Co in Singanallur and Ukkadam lakes as 0.22±0.004µg/L and 0.21±0.003µg/L respectively, whereas, concentration of Co in other lakes of Coimbatore was reported as 0.15±0.007µg/L (Kurichi lake), 0.11±0.009µg/L (Perur lake), 0.20±0.003µg/L (Selvachinthamani lake) and 0.52±0.16µg/L (Valankulam lake).

Mohanraj *et al* (2000) noted that the concentration of Cu in Singanallur and Ukkadam lakes were 44µg/L and 18µg/L respectively. According to Sridevi *et al* (2003), low concentrations of Cu in water may due to the bioaccumulation by macrophytes. The earlier results reported by Manikandan *et al* (2016) showed that, the concentration of Cu varied from 0.1810±0.005mg/L - 0.3501±0.006mg/L and 0.0254±000mg/L - 0.3240±0.005mg/L in Ukkadam and Singanallur lakes respectively during the period of study. Crispin and Sivakumar (2020) recorded the concentration of Cu in Singanallur, Sulur and Ukkadam lake ranged from 0.01mg/L to 0.08mg/L. According to Brahma and Misra (2014), the concentration of Cu in water samples of Rupahi Beel was lower than the permissible limit, prescribed by WHO. The similar result was reported by Kar *et al* (2008) in surface water, collected from river Ganga in West Bengal. Batvari and Surendran (2015) reported the concentration of Cu (0.015- 0.02mg/L) in water samples of Chemberambakkam lake. Increase in nickel concentration in lakes may due to many reasons. Septic tank and metal manufacturing units are the common factors induce the Ni contamination. Continuous discharge of industrial effluents and municipal waste water without treatment in water systems are primar concern. Mohanraj *et al* (2000) have noted the concentration of Ni was 23µg/L and 4.6µg/L in Singanallur and Ukkadam lakes respectively. Manikandan *et al* (2016) reported that, the concentration of Ni in Ukkadam lake during the months of June - August (0.0283±0000mg/L) and March - May (0.1177±0.005mg/L), whereas, concentration of Ni in Singallure lake during the months of June - August (0.0429±3.333mg/L) and March - May (0.0944±0.000mg/L).

Puri and Yenkie (2011) reported the seasonal effect on maximum concentration of Ni in water samples of Futala (0.052mgL^{-1}), Ambazari (0.029mgL^{-1}) and Gorewada (0.052mgL^{-1}) lakes during the months of June - August and July – September. It is due to evaporation of water from lakes during summer and subsequent dilution due to precipitation and run off from catchment area during rainy season (Puri *et al.,* 2014). The significant difference in Ni concentrations in lakes due to the presence of an anthropogenic activity. According to Brahma and Misra (2014), the concentration of Ni in water of Rupahi Beel was lower than the permissible limit, prescribed by WHO. The similar result was reported by Kar *et al* (2008) in surface water, collected from river Ganga in West Bengal. According to Batvari and Surendran (2015), the concentration of Ni in water samples of Chemberambakkam lake was recorded as 0.030-0.084mg/L. According to Damodharan (2013), run of agricultural and industrial waste effluents is the major source of zinc into water bodies. Mohanraj *et al* (2000) reported that the concentration of Zn in Singanallur and Ukkadam lakes were 95µg/L and 34µg/L respectively. According to Brahma and Misra (2014), the concentration of Zn in water samples of Rupahi Beel was lower than the permissible limit, prescribed by WHO. This result support for our result. The similar result was reported by Kar *et al* (2008) in surface water collected from river Ganga in West Bengal. The water samples collected from Chemberambakkam lake showed concentration of Zn (0.018 - 0.026mg/L) was within the maximum permissible limit prescribed by WHO (Batvari and Surendran, 2015). The heavy metal concentrations in all studied lakes showed distinct temporal and spatial variations. The deviation of level of existence of heavy metal in lakes were found divergent from each other due to variation of solubility of existing forms of metal in water, as well as availability of heavy metals in the environment (Puri *et al.,* 2014).

CONCLUSION

Rapid urbanization and industrialization with anthropogenic activity, extremely affects the different kinds of aquatic ecosystem in the country. Water reservoirs with heavy metal pollutions or contamination affects the human population. Thus, the present investigation was conducted to study the concentration of heavy metal content in

Singanallur lake, Sulur lake and Ukkadam lake during the month of January, May, July and November. The results showed that, concentration of Cr Co, Cu, Ni and Zn were within the maximum permissible limit of WHO. Thus, the results revealed that, the water in the three lakes were moderately contaminated with heavy metal pollution.

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REFERENCES

1. Zan F, Huo S, Xi B, Li Q, Liao H, Zhang J. Phosphorous Distribution in the sediments of a shallow eutrophic lake, lake Chaohu, China. J. Environ. Earth. Sci 2011; 62: 1643-1653. https://doi.org/10.1007/s12665-010-0649-5
2. Hou D, He J, Lub C, Renb L, Fand Q, Wang J, Xie Z. Ecotoxi. Enviro. Safety 2013; 93: 135 - 144.
3. Crispin CA, Sivakumar R. Urbanization and Industrialization Impact on Surface Water in Coimbatore-Sulur Subwatershed. International Journal of Recent Technology and Engineering 2020; 8(6): 5097:5101.
4. Lu X, Kruatrachue M, Pokethitiyook P, Homyok K. Removal of cadmium and zinc by water hyacinth, Eichhornia crassipes. Science Asia 2004; 30(93): 103. https://dx.doi.org/10.2306/scienceasia15131874.2004.30.03
5. Hasan SH, Talat M, Rai S. Sorption of cadmium and zinc from aqueous solutions by water hyacinth (Eichchornia crassipes). Bioresource technology 2007; 98(4): 918-928. https://doi.org/10.1016/j.biortech.2006.02.042
6. Chatterjee S, Chetia M, Singh L, Chattopadhyay B, Datta S, Mukhopadhyay SK. A study on the phytoaccumulation of waste elements in wetland plants of a Ramsar site in India. Environmental monitoring and assessment 2011; 178(1): 361-371. https://doi.org/10.1007/s10661-010-1695x
7. Chiodi LB, Escalante A, Haeften GV, Moreno V, Gerpe M. Assessment of Heavy Metal Accumulation in Two Aquatic Macrophytes: a Field Study. J. Braz.Soc. Ecotoxicol 2011: 6 (1): 57-64.
8. Hemambika B, Rani MJ Kannan VR. Biosorption of heavy metals by immobilized and dead fungal cells: A comparative assessment. Journal of Ecology and the Natural Environment 2011; 3(5): 168-175. https://doi.org/10.5897/JENE.9000022.
9. Rajkumar M, Ae N, Prasad MNV, Freitas H. Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. Trends in biotechnology 2010; 28(3): 142-149. https://doi.org/10.1016/j.tibtech.2009.12.002.
10. Gautam RK, Sharma SK, Mahiya S, Chattopadhyaya MC. Contamination of heavy metals in aquatic media: transport, toxicity and technologies for remediation in *Heavy Metals In Water: Presence, Removal and Safety*, 2014; p.p -1-24. 10.1039/9781782620174-00001
11. WHO. World Health organization.Geneva, 2011.
12. Szyczewski P, Siepak J, Niedzielski P, Sobczyński T. Research on heavy metals in Poland. Pol. J. Environ. Study 2009; 18(5): 755.
13. Ahmad MK, Islam S, Rahman MS, Haque MR and Islam MM. Heavy metals in water, sediment and some fishes of Buriganga River, Bangladesh. Int J Environ Res 2010; 4: 321-332. http://dx.doi.org/10.22059/IJER.2010.24
14. Mohanraj R, Sathishkumar M, Azeez PA, Sivakumar R. Pollution status of wetlands in urban Coimbatore, Tamilnadu, India. Bulletin of environmental contamination and toxicology 2000; 64(5): 638-643. https://doi.org/10.1007/s001280000051.
15. Batvari BPD, Surendran A. Assessment of heavy metal contamination in Chemberambakkam lake water, Chennai, Tamil Nadu, India. Journal of Chemical and Pharmaceutical Research 2015; 7(3): 865-869.
16. Manikandan R, Ezhili, N, Muthulakshmiandal N, Favas PJC, Venkatachalam P. Assessment of physicochemical characteristics and the level of nutrient contents as well as heavy metal ions in waters of three lakes at Coimbatore, Tamil Nadu, India. J. Mater. Environ. Sci 2016; 7(7): 2259-2266.
17. Dixit S, Tiwari S. Impact Assessment of Heavy Metal Pollution of Shahpura Lake, Bhopal, India. Int. J. Environs. Res 2008; 2(1) : 37- 42.

18. Palanisamy S, Eswaran R, Muniasamy M. Intensity of Pollution and Heavy Metals Toxicity on Surface Water Bodies of Coimbatore, Tamilnadu, India - A Case Study. Applied Ecology and Environmental Sciences 2021; 9(2): 247-259. https://doi.org/10.12691/aees-9-2-17
19. Sridevi B, Sharief SD, Dawood N, Noorjahan CM, Prabakar K. Bioabsorption of nickel and zinc by water hyacinth- Eichhornia sp. Ecology Environment and Conservation 2003; 9: 361-365.
20. Brahma NK, Misra AK. Study of heavy metals content in water, water hyacinth and soil of Rupahi Beel, Nagaon, Assam (India). Archives of Applied Science Research 2014; 6(5): 7-11.
21. Kar D, Sur P, Mandai SK, Saha T, Kole RK. Assessment of heavy metal pollution in surface water. International Journal of Environmental Science and Technology 2008; 5(1): 119-124.https://doi.org/10.1007/BF03326004
22. Puri PJ, Yenkie MKN, Choudhary RG, Meshram SU, Rana DB, Awale LS. Impact Assessment of Heavy Metal Pollution in Various Lakes, Nagpur, India. Asian Academic Research Journal of Multidisciplinary 2014; 1(22): 385-397
23. Damodharan U. Bioaccumulation of heavy metals in contaminated river water-Uppanar, Cuddalore, South East coast of India.Perspectives in Water Pollution 2013; 23-34.

Table-1.Concentration of heavy metals in lakes

S.No	Water Samples	Season	Cr (mg/L)	Co (mg/L)	Cu (mg/L)	Ni (mg/L)	Zn(mg/L)
1.	L1	M1	0.001± 0.0013	0.017± 0.01305	0.005± 0.00064	0.016± 0.00629	0.013± 0.00855
2.	L2		0.001± 0.0012	0.039± 0.00577	0.004± 0.00104	0.021± 0.00589	0.001± 0.00543
3.	L3		0.002± 0.0021	0.025± 0.00618	0.003± 0.00110	0.019± 0.00346	0.029± 0.00041
4.	L1	M2	0.002± 0.0023	0.022 ± 0.0083	0.004± 0.00081	0.011±0.00710	0.020±0.00676
5.	L2		0.005±0.0007	0.012± 0.0065	0.006± 0.00306	0.020±0.00548	0.004±0.04677
6.	L3		0.003±0.0015	0.007± 0.0079	0.004± 0.00121	0.020±0.00346	-
7.	L1	M3	0.001±0.0054	0.013± 0.0029	0.005± 0.00398	0.037±0.01703	0.019±0.00964
8.	L2		0.003±0.0060	0.018± 0047	0.005± 0.00225	0.008±0.00999	0.014±0.00156
9.	L3		0.002±0.0009	0.010± 0.0048	0.003± 0.00110	0.024±0.00433	0.009±0.00497
10.	L1	M4	0.002±0.0016	0.009 ± 0.01039	0.005± 0.00115	0.023±0.01490	0.018±0.00104
11.	L2		0.002±0.0011	0.017 ± 0.00375	0.002±0.00421	0.028±0.00878	-
12.	L3		0.001±0.0007	0.018 ± 0.00918	0.006± 0.00069	0.008±0.00491	0.004±0.00346

Datas are Mean (n=3)

L1- Singanallur lake, L2 - Sulur Lake, L3 - Ukkadam Lake

M1 - January, M2 - May, M3 – July, M4 – November

Table 2: Average Concentration of heavy metals

S. no	Heavy metals (mg/L)	Water samples		
		L1	L2	L3
1	Cr	0.0015	0.0028	0.0020
2	Co	0.0153	0.0215	0.0150
3	Cu	0.0048	0.0043	0.0040
4	Ni	0.0217	0.0192	0.0177
5	Zn	0.0175	0.0063	0.0140

L1- Singanallur Lake, L2- Sulur Lake, L3 - Ukkadam Lake

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RESEARCH ARTICLE

A Deep Learning Approach for Detecting Pneumonia using Convolutional Neural Network Model on X-Ray Images

Devavarapu Sreenivasarao[1]*, S. Anu H Nair[2], T.Venkat Narayana Rao[3] and K.P. Sanal Kumar[4]

[1]Research Scholar, Department of CSE, Annamalai University, Chidambaram, Tamil Nadu, India and Assistant Professor, Department of CSE, Sreenidhi Institute of Science and Technology, Hyderabad, Telangana, India.
[2]Assistant Professor, Department of CSE, Annamalai University, Chidambaram (Deputed to WPT Chennai), Tamil Nadu, India.
[3]Professor and HoD of CSE (IoT), Sreenidhi Institute of Science and Technology, Hyderabad, Telangana, India.
[4]Assistant Professor, Department of Computer Science, RVGA College, Chengalpattu, Tamil Nadu, India.

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***Address for Correspondence**
Devavarapu Sreenivasarao,
Research Scholar, Department of CSE,
Annamalai University, Chidambaram,
Tamil Nadu, India.
Email: sreenivasaraod@sreenidhi.edu.in

ABSTRACT

Pneumonia is one of the most terminal diseases in the world. It is caused due to a viral or bacterial attack on the lungs or due to fungi formation in the lungs. Several types of research and surveys are evident that if it is not treated at the right time it may lead to death. Pneumonia becomes deadly when infants and old people are affected. It may also cause sudden death due to cardiac arrest. So, it is necessary that it is treated at an early stage. Generally, pneumonia is detected by analysing the chest x-ray images by a lung specialist. This study helps to detect pneumonia using deep learning techniques without consulting a specialist at an early stage. Nowadays, neural networks are playing a prominent role in building machine learning models. As neural networks can think like a human and make decisions. This approach is going to be widely used in the upcoming Artificial Intelligence projects. Convolutional Neural networks are one of the widely used image classification techniques. In this study Convolutional neural network models are employed to identify pneumonia. The Kaggle chest x-ray images dataset is used for this study. The experiment involves building of convolutional neural network model through several layers using ReLU and SoftMax as activation functions. Numerous data augmentation procedures are

employed to develop the accuracy and efficiency of the model. The results obtained are quantified to ascertain the extent of precision in detecting pneumonia**.**

Keywords: Pneumonia, Convolutional Neural Network, RelU, epoch and Softmax.

INTRODUCTION

Pneumonia is a ailment that causes in lungs. Sometimes the air sacs in the lungs attain some aliment and infections that causes cough and difficulty in breathing. It can be caused by some bacteria or fungi. It mainly infects children, older people and can be cured. Increase in the symptoms may lead to severe health conditions. It can be detected by X-ray, CT-SCAN, and analysing the medical diagnosis. As the computer technology is growing rapidly from generation to generation, many new methods have evolved to resolve various problems. Diagnosis of pneumonia is challenging task that entails experience, talent and professional knowledge. By the growth of the computer vision technology the detection of the disease had made easier by using the image processing techniques which implements Machine Learning algorithms to detect the disease. This type of detection process not only decreases time effort but also improves accuracy for the detection [1] [3]. It employs machine learning algorithms for detection of disease and the algorithms used are convolutional neural networks (CNN) and Transfer Learning [3]. In CNN the layers that are present describe the functions that involve processing of the network. Deep learning is also used for the detection as a leading machine-learning tool and the CNN comes under this preview.

The deep neural networks can automatically learn from the given dataset and can build a model after training through dataset. It can interpret the data images taken for further testing. In recent years the image processing involved in the medical field has significantly improved and it builds a model at a faster rate and with a higher precision. This image processing technique has begun to yield positive results through computer technology. Using deep learning algorithms to detect the pneumonia by analysing the X-rays can improve the processing of X-ray images by the radiologists and can decrease the error rate [2][4]. The deep learning also uses the Compressed Sensing (CS) to reconstruct the signal to find the solutions for various systems. By CS process the data acquiring takes place faster and the diagnosis accuracy increases. Recently, the CS is widely used in the deep learning medical imaging which improves the accuracy of diagnosis rapidly. Deep learning approaches outperformed machine learning methods in numerous computer vision and medical imaging tasks, detection, classification and segmentation. This study presents a solution for the Pneumonia Detection for pneumonia regions. The proposed approach uses deep convolution neural networks (CNNs), augmentations and multi-task learning [2]. The algorithm automatically traces the lung opacities on chest radiographs and demonstrates the detection of the disease.

Proposed Architecture

The proposed architecture of the model states the steps that are involved in the model evaluation as shown in figure 1.In this architecture we have various steps in a way the model is evaluated and each step undergoes different operations. In each step, we can use any algorithm or technique to get maximum accuracy.
The steps involved in proposed method are

Selection of Dataset

In this step the dataset is chosen and the dataset chosen are the images of X-rays.

Transfer image into the system

The image is uploaded into the system by using the web framework.

Extracting Features in Background

After transferring the image to the system, then data augmentation techniques are applied and identify hidden patterns and extract unknown features from the image. This step ensures that the model is trained accurately. Feature extractions indulge in reducing the number of resources required to depict a large variety of data. All the required features are extracted from the dataset [5] [7].

Classifying Images

In this step the classification of images is done. The training of the dataset operation takes place to recognize them using labeled images [3]. Pneumonia detection: After the classification the model is build by the training dataset and the results are validated. In this stage the image is validated [6] and the output is generated as a binary output i.e. as pneumonia being detected if the person is infected with pneumonia or normal if he is not infected with the pneumonia disease.

METHODS USED

Different methods are used in proposed architecture. They are

Softmax Function

Softmax function takes an N-dimensional vector of arbitrary values and produces another N-dimensional vector with real values which are in the range (0,1) that add up to 1. It transforms the N-dimensional vector into a probability distribution that is used for predictions and represented as:

$$S_i = \frac{e^{a_i}}{\sum_{j=1}^{N} e^{a_j}}$$

here, S_i is the softmax output for i^{th} value in our input vector of size N. Si is continually positive i.e $S_i>0$ because of the exponents. As the numerator seems in the denominator summed up with some other positive numbers i.e. $S_i< 1$. Hence, this property permits us to derive a probability distribution for the classes in the classification. Normally, the Softmax is used along with the cross-entropy loss function in a neural network based classifier [10]. We first revisit the formulation of original softmax classifier. Its formulation can be written as

$$p(y = k|x) = \frac{exp(o_k(x, w))}{\sum_{t=1}^{c} exp(o_t(x, w))}$$

where $o_t(x, w)$ is the output logic for class t. As mentioned above, softmax is intrinsically a linear classifier.

Rel u (Rectified linear Unit)

ReL U signifies rectified linear unit, and is a kind of activation function. Mathematically, it can defined as y = max(0, x). ReLU is a commonly used activation function in neural networks, especially in CNNs. If you are not sure which activation function is to be used in your network, ReLU is typically a best choice. The following are the variants that are present in Relu.

Leaky Relu

Leaky ReLU has a little slope for negative values, in its place of zero altogether. For example, leaky ReLU may have y = 0.01x when x < 0.

Parametric Relu

Parametric ReLU (PReLU) is of type leaky ReLU i.e. instead of having a predetermined slope like 0.01, it makes it assumes a parameter for the neural network to figure out itself as y = ax when x < 0.

Implementation

Before starting any classification project selection of dataset must be the first step. We can select the dataset which is already available such as kaggle.com or we can prepare our own dataset as this dataset is test on professional grounds. Preparing our own dataset takes lot of time and one should collect the data such that it satisfies all the edge cases, so we should be very careful while selecting our data. After selecting the dataset, we must should build test cases. Then separate the dataset into testing data and training data. While building testing and training data we should be careful such that all the possible edge cases and constraints are present in both the datasets. The training of the model should precise be so that it would promise accurate results when encountered real world problems [8][9].

The kaggle dataset which contains various chest x-ray images. The dataset is split into 3 parts which contains Training Data, Testing Data and Evaluation Data. The count of images is specified in the Table.1. The dataset contains both normal and pneumonia effected person's chest X-ray images. The figure 2 and figure 3 shows how a normal and Pneumonia images look like.

Data pre-processing

After selecting the dataset we need to pre process the dataset. Data pre-processing [6] is a technique which is used to transform the data such that the machine learning model can understand the data to encode and parse the data for any errors or outliners or missing values for eliminated. In this study it is obvious to transform the data such that it is acceptable by neural networks. Because, the dataset contains variables like normal, pneumonia, which are written in string format, so it is mandatory to convert them into binary vectors. Later it requires mapping of the categorical values to integer values.

Data Augmentation

Data Augmentation [7] is the most powerful and useful technique used in neural networks. Neural networks deals with lot of complex and millions of training data. So, it is necessary that we should generalize the data to avoid over fitting. It contains various methods like flipping the images, rotating the images, shifting parts of images, cropping the images, zooming in or zooming out images, changing the brightness, changing the contrast, converting into grayscale etc. These methods not only help in regularization but also helps in improving performance of the model. In this study we are going to resize the data and rotate the data and zoom it by 10%. We can use predefined data augmentation techniques which are present in python's keras module which contains methods like Image Data Generator. After completing the above steps our dataset is ready for training the system. Now apply convolutional neural networks to build the model. A neural network is a computing model which works similar to our brain and nervous system. It contains sets of algorithms which are used to identify patterns and interpret data [8]. It contains input layer, hidden layers and output layer. It contains several nodes which are interconnected with each other. It uses activation functions based on which the neurons are fired. There are various types of neural networks like convolutional neural networks, recurrent neural networks and Artificial Neural Networks. The use of convolutional neural networks is vital because these are specially designed to identify patterns in images and classing them as they can also handle large amount of data.

Convolutional Neural Networks

Convolution Neural Networks (CNN) [3] are currently one of the most efficient deep learning models which are used for classifying images. These models helps in learning unvarying features hierarchically. They first identity lower level patterns or features and then they combine them to identify complicated patterns or features. These features are identified from various layers of the network. Each layer of the network contains some specific number of neurons. These are represented in the form of three dimensions length, depth and width [10]. To realize convolutional neural network structure, we shall observe it as two discrete parts. In input, images are presented as a matrix of pixels. It has 2 dimensional grayscale image. The color is symbolized by a third dimension, i.e. depth 3 to represent the fundamental colors (Red, Green, Blue). The first part of the convolutional neural network is the convolutive part. It is used to extract features of the images [3]. This study include rectified linear activation function (ReLU) [4]. It is used in CNN other than various activation functions like sigmoid, hyperbolic tangent because it has various advantages. It helps in reducing the exponential growth of computation while operating CNN. It eliminates saturation problem.

ReLU [4] overcomes the gradient descent problem and allows the models to learn faster and perform better. This study uses SoftMax [5] activation function as a switch. The SoftMax [5] function is employed as an activation function in the output layer of neural network models [7] that forecast a multinomial probability distribution. SoftMax units obviously represent a probability distribution on a discrete variable with k probable values, so that they would be used as a switch. The model for a convolutional neural network comprise of convolution layers, pooling layers and full connection layers. The convolution layer and pooling layer are superimposed interchangeably. After passing through the complete connection layers, a SoftMax layer [5] is coupled to map the probability of every category to the output of the network. The input layer takes all the training data. After that input layer is connected to various convolutional pooling layers which are then connected to dense layers. As it is already mentioned that it is going to use ReLU as activation function [3], employed in each layer. Finally, the last layer is connected to output layer. It contains 3 nodes and use of SoftMax [5] as activation function. The SoftMax function is used in output layer to produce the output which contains the probality[9] of the classes. This study uses Cross Entropy Loss function and Adam optimizer to minimize using the loss value in neural networks. Now testing phase is initiated to test the model using any predefined fitting functions like fit, fit generator [10] etc.

The Proposed Algorithm

1. Loading test images.
2. Reshaping the image to any standard format like 200X200
3. Data augmentation to increase the data and reduce over fitting.
3.1. Rotation range 10 degree
3.2. Zoom range 0.1
3.3. Width shift range 0.1
3.4. Height shift range 0.1
4. Convolutional Neural Network construction
4.1. Input layer
4.2. Convolutional 2d layers
4.3. Max pool 2d layers
4.4. Dense layers
4.5. Output layers
5. Compile the model using optimizers like Adam optimizer.
6. Fit the model and choose required number of epochs.

RESULTS AND DISCUSSION

The accuracy of the model is depicted in the form of figures given below. The figures represent the test results after training and testing process for the model. Figure 4 represents the confusion matrix of the model. Confusion matrix is one of the most used statistical classification tool. It is used to visualize the performance of the machine learning model. It is used to compare the predicted and the normal results. It uses a m*m square matrix where m is the number of class labels. It represents the true positives, true negatives, false positives and false negatives. From the Figure 4, it is clearly visible that our model has almost identified all the bacterial images accurately. As we are now concentrated on identifying only pneumonia we can ignore the cases of identifying the virus images as bacterial images because both represent that pneumonia is present. So, both the classes are considered same. Based on this it can be ascertained say this model can identify pneumonia accurately. The accuracy scores and loss values are represented in the figure 5 and figure 6. The accuracy score is used to evaluate the classification models. A line graph is plotted that compares these scores. The loss value determines how well we trained our model. From the accuracy and loss value graphs we can clearing say that the performance of the model keeps increasing, though both the accuracy scores and loss values look altering in these thirty epochs. We can also observe that model does not suffer from over fitting [11].

Training and Validation Accuracy

The table.2 shows training and validation precision of the developed model with reference to the value of epochs. It is evident from table.2 that as the training effort increases the accuracy component also improves.

CONCLUSION

Convolutional neural networks is a good technique in image classification. We can expect many more image classification and object detection projects using this algorithm. The model that is proposed can detect pneumonia accurately using x ray images. This model can be further modified by adding new activation functions and new data augmentation techniques so that the accuracy and efficiency of the model improves. In the future as there will be automation of all the things, one can expect that medical field can also be automated like robots acting as doctors for detecting diseases. So, this model provides a solution for detecting pneumonia which can be automated at a affordable price and speedy results. This study can acts as a base for such technology. With the slight modification of the algorithm we can detect many diseases like brain tumour detection, cancer detection and identify different patterns and detect an object which can be used in self driving cars etc. It would be interesting to see how people are going to use CNN and change the world with their intelligence in the near future. For a training model with more than 5 samples this system promises 94% of accuracy.

REFERENCES

1. Gaurav Labhane, Rutuja Pansare, Saumil Maheshwari "Detection of Pediatric Pneumonia from Chest X-Ray Images using CNN and Transfer Learning" 3rd International Conference on Emerging Technologies in Computer Engineering: Machine Learning and Internet of Things (ICETCE-2020), 07-08 February 2020, (IEEE Conference Record # 48199).
2. Saad Albawi, Tareq Abed Mohammed, Saad Al-Zawi "Understanding of a convolutional neural network." 2017 International Conference on Engineering and Technology(ICET).
3. Nadia Jmour,Sehia Zayen,Afef Abdelkrim "Convolutional neural networks for image classification" 2018 International Conference on Advanced Systems and Electric Technologies(IC_ASET).
4. Hidenori Ide, Takio Kurita "Improvement of learning for CNN with ReLU activation by sparse regularization" 2017 International Joint Conference on Neural Networks (IJCNN).
5. Meiqi Wang,Siyuan Lu,Danyang Zhu,Jun Lin,Zhongfeng Wang, "A High-Speed and Low-Complexity Architecture for Softmax Function in Deep Learning" 2018 IEEE Asia Pacific Conference on Circuits and Systems (APCCAS).
6. Raphael Benedict G. Luta,Renann G. Baldovino,Nilo T. Bugtai "Image preprocessing using quick color averaging approach for color machine vision (CMV) systems" 2017IEEE 9th International Conference on Humanoid, Nanotechnology, Information Technology, Communication and Control, Environment and Management (HNICEM).
7. Agnieszka Mikołajczyk,Michał Grochowski "Data augmentation for improving deep learning in image classification problem" 2018 International Interdisciplinary PhD Workshop (IIPhDW).
8. Rajpurkar, Pranav, et al. "Chexnet: Radiologist-level pneumonia detection on chest x-rays with deep learning." arXiv preprint arXiv:1711.05225 (2017).
9. Stephen, Okeke, et al. "An Efficient Deep Learning Approach to Pneumonia Classification in Healthcare." Journal of healthcare engineering 2019 (2019).
10. Kermany, D. K., and M. Goldbaum. "Labeled optical coherence tomography (OCT) and Chest X-Ray images for classification." Mendeley Data 2 (2018).
11. T.Venkat Narayana Rao, Dr. A. Govardhan , Syed Jahangir Badashah ", An improved Lossless Embedding and Extraction-A Data Hiding Mechanism " , International journal of computer science & Information Technology (IJCSIT), April 2010.

Table.1: Dataset Classification

	Pneumonia	**Normal**
Training	2992	2972
Testing	854	849
Validation	8	6
Total	3854	3827

Table.2: Training and Validation

Epochs	**Training Accuracy**	**Validation Accuracy**
1	0.5962	0.6189
2	0.6191	0.6245
3	0.6197	0.6476
4	0.6437	0.6551
5	0.6475	0.6701
6	0.6716	0.6838
7	0.6781	0.6979
8	0.7071	0.7106
9	0.7109	0.7165
10	0.7148	0.7383
11	0.7339	0.7622
12	0.7448	0.7732
13	0.7727	0.7821
14	0.7818	0.7938
15	0.7649	0.8039
16	0.8029	0.8150
17	0.7947	0.8212
18	0.8116	0.8397
19	0.8205	0.8365
20	0.8276	0.8401

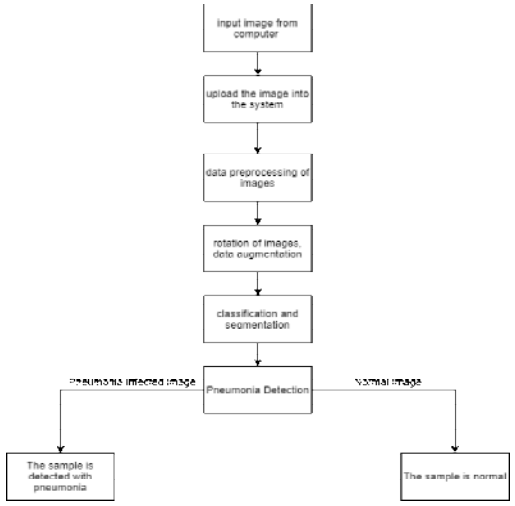

Figure 1: The Proposed Architecture

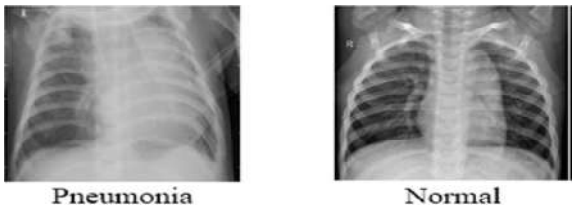

Figure 2: Pneumonia chest X-ray

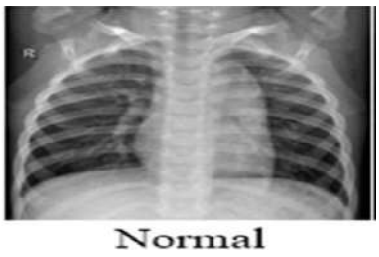

Figure 3: Normal Chest X-ray

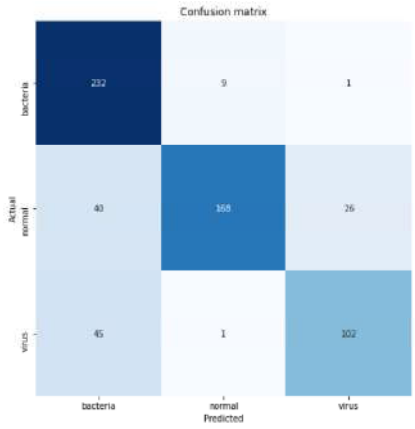

Figure 4: Confusion Matrix

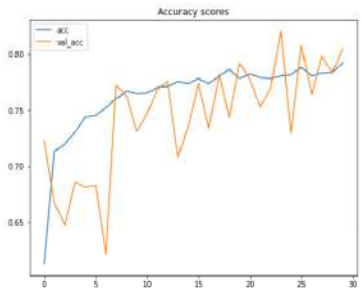

Figure 5: Accuracy Scores

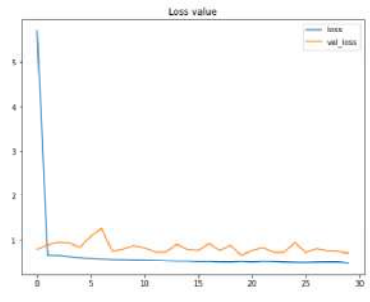

Figure 6: Loss Value Decrease

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RESEARCH ARTICLE

Neural Network System based on Real time Object Detection and Recognition for Video Surveillance Systems

Raja Reddy Duvvuru[1]*, K. Anitha Reddy[2], P. Chandana Priya[3], K.Vimala Kumar[4] and B. Sudharshan Reddy[5]

[1]Associate Professor, EEE Department, Malla Reddy Engineering College, Secundrabad, Telangana, India.
[2]Assistant Professor, EEE Department, Malla Reddy Engineering College, Secundrabad, Telangana, India.
[3]Assistant Professor, Department of CSE, Malla Reddy University, Secundrabad, Telangana, India.
[4]Assistant Professor, Department of EEE, JNTUKUCEN, Narasaraopet, Guntur, India.
[5]Professor, CIVIL Department, Malla Reddy Engineering College, Secundrabad, Telangana, India.

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***Address for Correspondence**
Raja Reddy Duvvuru,
Associate Professor,
EEE Department,
Malla Reddy Engineering College,
Secundrabad, Telangana, India
Email: rajajntuacep@gmail.com

ABSTRACT

In this work, one of the crucial issues that many of the previous works are concentrating on is the recognition and tracking of the item. Due to the increasing demand for video surveillance system applications including traffic control, medical image processing, and satellite image processing, object recognition and tracking are particularly well-liked. This method is also among the most potent ones used in applications based on artificial intelligence, machine learning, and computer vision. Understanding the type of images, attributes, locations of each image in space, and tracking the movements of each object while it is moving are the main goals of these fundamental object recognition based systems. Since human identification has received so much attention in the research that has already been done, many object detection apps focus primarily on it. This section outlines a novel method for object recognition that uses the CNN methodology to recognise both live and non-living objects. The major goal of this section is to provide a framework for classifying items into living and non-living categories. Once they have been discovered, they can then be categorised using the support vector machine technique, which is useful for identifying theft utilising surveillance systems.

Keywords: CNN, Image processing, SVM.

INTRODUCTION

In modern Object recognition is one of the main challenges facing most computer vision work. Increasing demands on surveillance, security, traffic management and medical imaging are particularly popular in object detection and tracking [1]. It is also a product of strong algorithms in machine learning, computer vision and hardware advances that enable a couple of minutes of highly data-intensive calculations. The ultimate aim of the vision-based detection is to understand the type of objects in the picture, their characteristics, and their location in the space and to move or track the object. In the area of image processing, numerous research projects have been undertaken and successfully carried out. Automatic surveillance systems based on real-time videos of public spaces are becoming necessary due to growing concerns about public safety and security. These surveillance systems must be installed in busy and critical locations such as markets, malls, renowned eateries, train stations, etc [2-4]. They are also most in demand for traffic control and examination, activity recognition and tracking, fault detection in industrial applications, and semantic video indexing, without restricting their application to security beneath public places. The approach utilised is to initially detect the target of interest in individual frames in order to do the high level objectives of categorization or tracking a target from video stream [5-7].

Background subtraction is a surveillance technique that is employed in various works. By separating the background and foreground pixels in the frame being processed, this approach extracts the foreground object, or the target that is moving. Many researchers have taken full use of this method's advantages, particularly its performance when a stationary video camera is present and its lighting invariance. A crucial factor to take into account is creating a background model of the video frame that was collected. For feature extraction for detection of objects, textures such as Local Pattern Binary (LBP) [8-10] of the image have been considered. Pixel neighbourhood operations are used to compute LBP characteristics. Histogram of oriented gradients is a common feature descriptor for object detection. HOG features are shape descriptors that represent an object in specific directions in terms of intensity gradients. In [11], the researchers took advantage of HOG [12] functions, citing their invariance properties in regard to transformations such as rotation, deformities and conditions of illumination.

System Design for the proposed method

The system design phase gives the proposed research project an abstract representation that outlines the entire workflow of the study and how each module must be executed and integrated by the effective application development.

Design diagram- High level

This design diagram describes the representation of all the modules in the proposed technique and provides solution for the services offered by the system to produce the high quality design for the research work. In a multi-project model, such an outline is important to ensure that each supporting element design is consistent with the neighboring designs and the large picture. All the services of the proposed system, the platform used and the process of implementation should be described in the brief manner and any important change needed to be done and integrated to be specified in this stage. Furthermore, all major commercial, legal, environmental, safety and safety matters should be considered briefly [13-15].

Design Diagram-High level

The bubble graphs used in this diagram can be classified as dfds. One of the simplest graphical representations, as seen in figures 1 and 2, is the data flow diagram [16].

Case Diagram

Case diagram is shown in the figure below. The functionality for the communication will be documented and carried out in accordance with how 3 explains the interaction between the application framework and the end user.

Characters who participate in the process are referred to as on-screen characters, while those who perform outside the parameters are referred to as performing artists. The primary goal of this design diagram is to explain how each module communicates with the others in a way that aids in the execution of the work [17-19].

Design Diagram-Activity

The activity diagram describes in the figure .4, presents the important activities carried out in the research work. In this diagram the circles represents the start of the activity and the end of an activity and the rectangle boxes defines the modules of each proposed research work [20-21].The purpose of activity diagram in the proposed framework is to make sure the workflow of the application development is implemented according to the desired requirements provided by the end users. The developer will refer these design diagrams for the implementation of the work.

Process of identification of objects and the classification technique

Step 1: Input the video that contains both human and non-living moving objects.

Step 2: Pre-processing the input video: The input video to be pre-processed is two different steps:

- Divide the input video into frames and store individually.
- Once frames are generated apply the morphological operations over the input video.

Image Pre-Processing and Annotation

Image Pre-Processing involves processing or cleaning of images. This step focuses on removal of noise and distortion, sharpening, intensity normalization, etc. The VOC dataset is refined with only person images and annotated according to the format of YOLO model. A text file is created for each image in the same directory with the same name that contains object number and object coordinates on this image, for each object in new line. The object numbers an integer number of object from zero to total number of classes – 1, and object coordinates are float values relative to width and height of image, it can be equal from (0.0 to 1.0]. The ID card images are only pre-processed [22-25].

Training the YOLO model and testing

After pre-processing and annotation, the person dataset is divided into training and testing datasets. We train the YOLO model using training dataset until we get a better mean Average Precision (map). After the training, it is tested with testing dataset. YOLO is a full convolution network consisting built using darknet-53. It detects objects at three different strides (8, 16 and 32) which help to detect smaller objects. The provided input image will be divided into S*S grid and each of the cell will be made of the coordinated of (x, y, w, h) and the confidence of the object. The representation of the coordinates x, y defines the position of the boundary box which is relatively grid in nature. The coordinates with w, h is represented as the width and height of the detected boundary box. The probability of each grid is predicted for C categories. The confidence is determined by the probability model that includes the target and the prediction detection box [26]. The object is defined as Pr which stands for the target object that falling under the cell. If the confidence is presents then it is defined using:

$$C\ (Object) = Pr\ (Object)*IOU\ (Prod, Truth) \quad (1)$$

The cell doesn't contain any kind of the object and the confidence values is defined as C (object)] =0. The IOU is defined as the overlapping rate for which the bound of candidate and the truth value of the ground can be defined as the ration of union and the intersection of the grounds [11-13].Then the classification of the objects is achieved by classifying them into their respective categories. Here, multi class SVM classification is used by supervised learning for the output of the object class. The quality of CNN classification is determined over video data set checking: The category output is the class to which an object is identified. In a single frame, a vector containing all classes detected is generated as an output for multiple objects of different lasses.

Extraction of features for the identification of Non-Living objects

This section presents the detailed study of how the extraction of features has been implemented and the mathematical model related to the techniques used for feature extraction technique used will be described [27].

Corner Detector using Shi-Tomasi Technique

The Harris Corner Detector is an experimental model that detects the corner features from the video frames that provides higher texture features with minimal propositional changes. The harries technique is mainly used to detect the corners of the frames and it is carried out as follows:

The intensity of the pixel proposition is defined in I(x, y) for the position (x, y) of each window frame of the input video, and if the window moves by small margin the shift (e, v), will be marked with I(x+u, y+v). Since the main objective is to locate regions or windows with small displacements in the image, the intensity is expressed mathematically.

$$E(u,v) = \sum_{x,y} w(x,y)[I(x+u, y+v) - I(x,y)]^2 \quad \text{..... (1)}$$

The weight function is defined with 'W' and the high intensity variation in the window frames may lead to the result of E (u, v).

The Taylor's series and simplification technique of Equation 1, provides the results in,

$$M = \sum w(x,y) \begin{bmatrix} I_x^2 & I_x I_y \\ I_x I_y & I_y^2 \end{bmatrix} \quad \text{.... (2)}$$

The Eigen values of matrix is defined as M is used to find out suitable corners sing the score value,

$$S = |M| - k(trace(M))^2 \quad \text{..... (3)}$$

If λ_1, λ_2 Are Eigen values of M, then |M| = $\lambda_1 \lambda_2$ andtrace (M) = $\lambda_1 + \lambda_2$ Each corners of the frame is represented using S, to predict the high score value. The smaller change in the calculation is calculated using the Shi-Tomasi technique, that leads to the determining the most suitable corner defined in 'n', rather than identifying the each and every corner of the feature [28].

Shi-Tomasi score for corner detection follows-

$$S = min(\lambda_1, \lambda_2). \quad \text{..... (4)}$$

If the score, S exceeds a threshold it is considered as a corner.

Determining the optimal flow using Lucas-Kanade technique

The optical flow of the detected fame has to be determined, that track the movement of the objects in frame when the object is moving or during the rotation of the camera. The optical flow of any kind of entity from one frame to another frame in a video is to be determined. The proposed algorithm Lucas-Kanade [29-31] is mainly based on the type of optical flow theory, where all the video frames remain in the similar kind of intensity and the pixels in the frames have similar kind of movements and the pixels rate between successive each frames smaller in nature. A pixel rate [32] with the intensity rate defined as I(x,y,t) in a frame at time interval defined as t after the movement with a small displacement defined as (d_x, d_y) in the consecutive frames with a difference of time d_t Is expresses as:

$$I(x,y,t) = I(x + d_x, y + d_y, t + d_t) \quad \text{..(5)}$$

The flow of an optimal equation for the movement of objects in an image is given as-

$$\frac{\partial_f}{\partial_x}\frac{d_x}{d_t} + \frac{\partial_f}{\partial_y}\frac{d_y}{d_t} + f_t = 0 \quad \text{..... (6)}$$

The optical flow motion of the vectors using Lucas-Kanade method is defined by solving Equation (6):

$$\begin{bmatrix} u \\ v \end{bmatrix} = \Sigma_i \begin{bmatrix} f_{x_i}^2 & f_{x_i} f_{y_i} \\ f_{x_i} & f_{y_i}^2 \end{bmatrix}^{-1} \Sigma_i \begin{bmatrix} -f_{x_i} f_{t_i} \\ -f_{y_i} f_{t_i} \end{bmatrix} \quad \text{..... (7)}$$

The representation of the displacement defined I (u, v) represents the of the object between consecutive frames.

RESULTS AND DISCUSSIONS

The framework can be integrated within the Mat lab tool kit that makes it possible to use its toolboxes for the computer vision and machine learning to easily integrate mathematical computations for the identification and classification of artifacts. After extraction, the video processing is carried out smoothly on each frame. The toolbox for image processing includes several filtering and refining functions required to process an identified image before processing. A maximum of 15 videos containing standard products of various classes are collected. The dataset has been divided into three groups–5 video training datasets and 8 video test data sets required for vector support machine technique. Figure 6 below shows the histogram of frames extracted from video 1 in which different objects are recognized in the same way after extracting characteristics, as in Figure 7 and Figure 8 various objects detected from video 6 and video 7 respectively. The final results are drawn using the proposed CNN framework where the input is given to the framework that contains both human and non-living objects in the video dataset. The input video will be processed and detects the background and fore-ground of the objects. Initially pre-processing is applied to remove noise in the video and then morphological operations are applied to analyze the color pixel of the detected frames.

CONCLUSION

This work presented a new approach for object recognition using Vector Machine based classification in Video Surveillance Systems and Lucas-Kanade technique. In this article, the artifacts are correctly identified and their position from an unknown location is calculated. First, object recognition using Shi-Tomasi and Lucas-Kanade techniques will be stored, and the context subtraction will be applied when an object from extracted frames of the input video is recognized. Then the classification of the objects in their individual categories is accomplished by supervised learning with the help vector machine classification. The precision of the technique being proposed is analyzed by the total number of frames detected by object compared to the total number of frames. In this chart four input videos from different sources of various sizes and backgrounds were taken, and for each video we should achieve 92 percent accuracy. Where frames vary from 500 to 1500 for each video, the exactness of the identification of the objects is 80 to 95% for each video.

REFERENCES

1. C S Pillai, AnandaBabu J, “Object Recognition using Lucas-Kanade Technique and Support Vector Machine Based Classification in Video Surveillance Systems”, International Journal of Engineering and Advanced Technology(IJEAT), Vol. 9 Issue -1,October-2019, ISSN:2249-8958.
2. Elliott, D.: Intelligent video solution: A definition, Security, pp. 46–48, 2010.
3. Avidan, S.: Ensemble tracking, IEEE Trans. Pattern Anal. Mach. Intell., vol. 29, no. 2, pp. 261– 271, Feb. 2007.
4. Kushwaha, A., Sharma, C., Khare, M., Srivastava, R., Khare, A.: Automatic multiple human detection and tracking for visual surveillance system. 2012 International Conference on Informatics, Electronics Vision (ICIEV), pp. 326-331.
5. B. D. Lucas, T. Kanade, "An iterative image registration technique with an application to stereo vision," in IJCAI, 1981.

6. M. Everingham, L. Van Gool, C. K. Williams, J. Winn, A. Zisserman, "The Pascal Visual Object Classes (VOC) Challenge", IJCV, 2010, pp. 30.
7. Avidan, S.: Ensemble tracking, IEEE Trans. Pattern Anal. Mach. Intell., vol. 29, no. 2, pp. 261– 271, Feb. 2007.
8. Khan, Z., Gu, I.: Joint feature correspondences and appearance similarity for robust visual object tracking, IEEE Trans. Inf. Forensics Security, vol. 5, no. 3, pp. 591–606, Sep. 2010.
9. Dalal, N., Triggs, B.: Histograms of oriented gradients for human detection, in Proc. CVPR, 2005, pp. 886–893.
10. Wang, L.: Abnormal walking gait analysis using silhouette-masked flow histograms, in Proc. ICPR, 2006, vol. 3, pp. 473–476.
11. Wang, S., Lee, H.: A cascade framework for a real-time statistical plate recognition system, IEEE Trans. Inf. Forensics Security, vol. 2, no. 2, pp. 267–282, Jun. 2007.
12. Yu, X., Chinomi, K., Koshimizu, T., Nitta, N., Ito, Y., Babaguchi, N.: Privacy protecting visual processing for secure video surveillance, in Proc. ICIP, 2008, pp. 1672–1675.
13. Park, U., Jain, A.: Face matching and retrieval using soft biometrics, IEEE Trans. Inf. Forensics Security, vol. 5, no. 3, pp. 406–415, Sep. 2010.
14. Cong, Y., Yuan, J., Luo, J.: Towards scalable summarization of consumer videos via sparse dictionary selection, IEEE Trans.Multimedia, vol. 14, no. 1, pp. 66–75, Feb. 2012.
15. Cong, Y., Gong, H., Zhu, S., Tang, Y.: Flow mosaicking: Real-time pedestrian counting without scene-specific learning, in Proc. CVPR, 2009, pp. 1093–1100.
16. k. Cheng, Q. Liu, R. Tahir, L. K. Eric and L. He, "Multi-Camera Logical Topology Inference via Conditional Probability Graph Convolution Network," *2021 IEEE International Conference on Multimedia and Expo (ICME)*, 2021, pp. 1-6, doi: 10.1109/ICME51207.2021.9428335.
17. L. H. Jadhav and B. F. Momin, "Detection and identification of unattended/removed objects in video surveillance," *2016 IEEE International Conference on Recent Trends in Electronics, Information & Communication Technology (RTEICT)*, 2016, pp. 1770-1773, doi: 10.1109/RTEICT.2016.7808138.
18. R. Alimuin, A. Guiron and E. Dadios, "Surveillance systems integration for real time object identification using weighted bounding single neural network," *2017IEEE 9th International Conference on Humanoid, Nanotechnology, Information Technology, Communication and Control, Environment and Management (HNICEM)*, 2017, pp. 1-6, doi: 10.1109/HNICEM.2017.8269461.
19. G. Pavithra, J. J. Jose and T. A. Chandrappa, "Real-time color classification of objects from video streams," *2017 2nd IEEE International Conference on Recent Trends in Electronics, Information & Communication Technology (RTEICT)*, 2017, pp. 1683-1686, doi: 10.1109/RTEICT.2017.8256886.
20. K. S. Kumar, S. Prasad, P. K. Saroj and R. C. Tripathi, "Multiple Cameras Using Real Time Object Tracking for Surveillance and Security System," *2010 3rd International Conference on Emerging Trends in Engineering and Technology*, 2010, pp. 213-218, doi: 10.1109/ICETET.2010.30.
21. B. Hdioud, A. Ezzahout, Y. Hadi and R. O. Haj Thami, "A real-time people tracking system based on trajectory estimation using single field of camera view," *2013 International Conference on Computer Applications Technology (ICCAT)*, 2013, pp. 1-4, doi: 10.1109/ICCAT.2013.6522038.
22. S. Sen, A. K. Das and S. P. Chowdhury, "Saving Electrical Power in a Surveillance Environment," *2009 Seventh International Conference on Advances in Pattern Recognition*, 2009, pp. 274-277, doi: 10.1109/ICAPR.2009.38.
23. V. Mathew, T. Toby, A. Chacko and A. Udhayakumar, "Person re-identification through face detection from videos using Deep Learning," *2019 IEEE International Conference on Advanced Networks and Telecommunications Systems (ANTS)*, 2019, pp. 1-5, doi: 10.1109/ANTS47819.2019.9117938.
24. J. Tang, R. Ding and X. Tian, "Real-Time Target Detection Algorithm Based on Background Modeling," *2013 Fourth International Conference on Digital Manufacturing & Automation*, 2013, pp. 970-974, doi: 10.1109/ICDMA.2013.227.
25. S. Alfasly, B. Liu, Y. Hu, Y. Wang and C. -T. Li, "Auto-Zooming CNN-Based Framework for Real-Time Pedestrian Detection in Outdoor Surveillance Videos," in *IEEE Access*, vol. 7, pp. 105816-105826, 2019, doi: 10.1109/ACCESS.2019.2931915.

26. L. Qu, J. Wang, S. Xin, M. Qin and J. Dong, "A System for Detecting Sea Oil Leak Based on Video Surveillance," *2011 Third Pacific-Asia Conference on Circuits, Communications and System (PACCS)*, 2011, pp. 1-3, doi: 10.1109/PACCS.2011.5990183.
27. A. Singh, T. Anand, S. Sharma and P. Singh, "IoT Based Weapons Detection System for Surveillance and Security Using YOLOV4," *2021 6th International Conference on Communication and Electronics Systems (ICCES)*, 2021, pp. 488-493, doi: 10.1109/ICCES51350.2021.9489224.
28. X. Song, L. Wang, H. Wang and Y. Zhang, "Detection and identification in the intelligent traffic video monitoring system for pedestrians and vehicles," *The 7th International Conference on Networked Computing and Advanced Information Management*, 2011, pp. 181-185.
29. B. Ashwini, B. Deepashree, B. N. Yuvaraju and P. S. Venugopala, "Identification of vehicles in traffic video," *2016 International Conference on Signal Processing, Communication, Power and Embedded System (SCOPES)*, 2016, pp. 588-593, doi: 10.1109/SCOPES.2016.7955507.
30. C. -Y. Wang, P. -Y. Chen, M. -C. Chen, J. -W. Hsieh and H. -Y. M. Liao, "Real-Time Video-Based Person Re-Identification Surveillance with Light-Weight Deep Convolutional Networks," *2019 16th IEEE International Conference on Advanced Video and Signal Based Surveillance (AVSS)*, 2019, pp. 1-8, doi: 10.1109/AVSS.2019.8909855.
31. Z. Zhen-Jie and W. Qi, "Research on Detection and Tracking of Moving Vehicles in Complex Environment Based on Real-Time Surveillance Video," *2020 3rd International Conference on Intelligent Robotic and Control Engineering (IRCE)*, 2020, pp. 42-46, doi: 10.1109/IRCE50905.2020.9199246.
32. Y. Chen, B. Wu, H. Huang and C. Fan, "A Real-Time Vision System for Nighttime Vehicle Detection and Traffic Surveillance," in *IEEE Transactions on Industrial Electronics*, vol. 58, no. 5, pp. 2030-2044, May 2011, doi: 10.1109/TIE.2010.2055771.

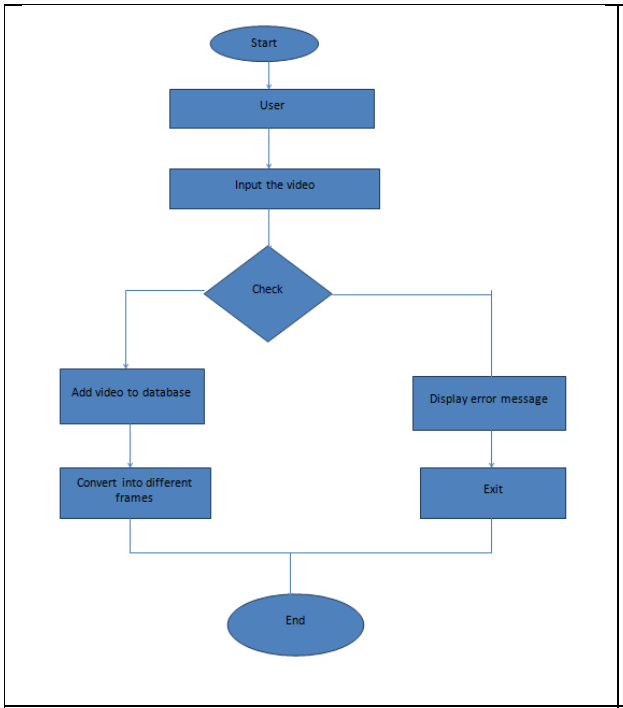

Fig. 1: Data Flow Diagram for Add Database

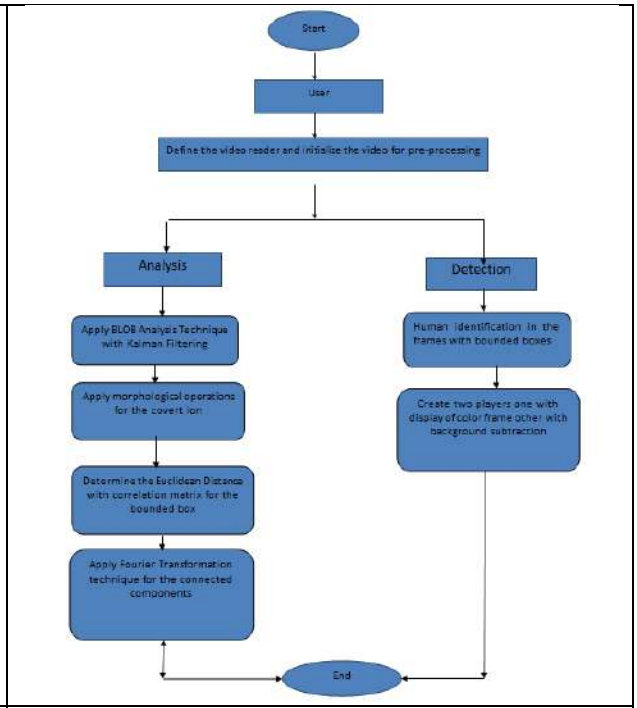

Fig. 2: Data Flow Diagram for Proposed Technique

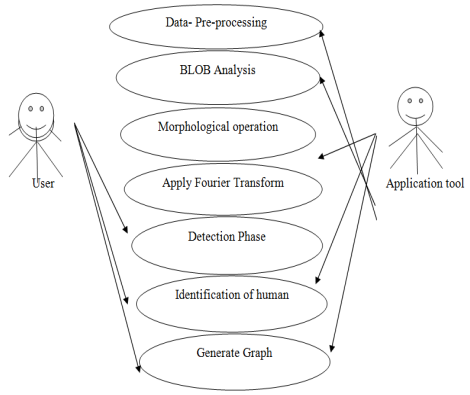

Figure 3: The Use case diagram for Proposed Technique

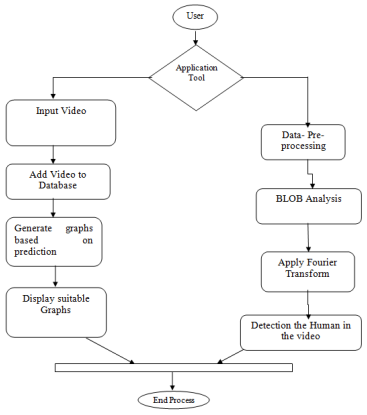

Figure 4: The activity diagram for Proposed Technique

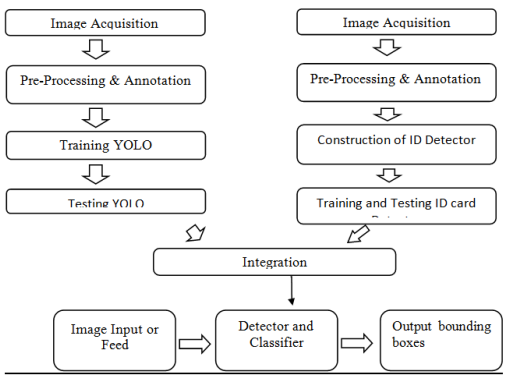

Figure 5: System Architecture of proposed Framework

Figure 6: Frame extraction for the detected objects in the input video 1

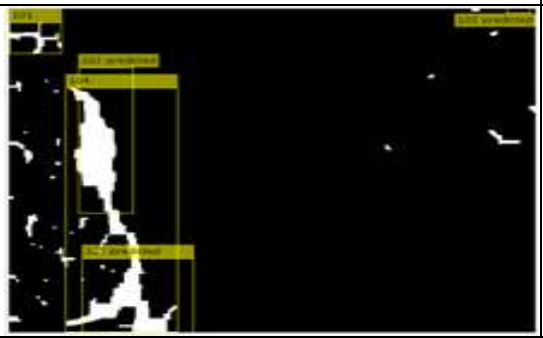

Figure 7: Frame extraction for the detected objects in the input video2

Figure 8: Frame extraction for the detected objects in the input video3

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RESEARCH ARTICLE

An Improved Face Recognition Model for Image-based Attendance System

Prabavathy Balasundaram*[1] , Lekshmi Kalinathan[1] and Anu Lekshmi[2]

[1]Associate Professor, Department of Computer Science and Engineering, Sri Sivasubramania Nadar College of Engineering, Chennai, Tamil Nadu, India
[2]UG Student, Department of CSE ,VIT University, Chennai, Tamil Nadu, India

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***Address for Correspondence**
Prabavathy Balasundaram
Associate Professor,
Department of Computer Science and Engineering,
Sri Sivasubramania Nadar College of Engineering,
Chennai, Tamil Nadu, India
Email: prabavathyb@ssn.edu.in

ABSTRACT

In educational institutions, conventionally the attendance of the students is recorded manually. It is required for every student to satisfy the minimum attendance to appear for his/her examinations. This necessitates the attendance process to be error-free. However, the conventional system is prone to proxy-attendance and human errors. Hence, it mandates the necessity of an automatic image-based attendance system utilizing face recognition techniques. Many of the existing face recognition models utilized video footage of the entire classroom session, which in turn demands high-end cameras. Further, many have utilized similarity approaches to detect the face. However, the similarity measures fail to identify the pattern when there is change in facial lighting conditions, visual alterations, and poses. Thus, the idea of this paper is to propose and build an improved face recognition model that utilizes pre-trained models, namely, MTCNN and Face Net with a suitable classification model for classroom images.

Keywords: Face Recognition Techniques, MTCNN, FaceNet, Classifiers, Similarity Measures

INTRODUCTION

Recording and maintaining attendance logs of students is integral to most schools and universities. It is a very crucial factor that closely correlates with academic performance. Hence, several institutes enforce a minimum attendance criterion as an eligibility factor for students to appear in exams. Due to the consequent emphasis on the significance of attendance, students often point out discrepancies in logs made through conventional methods like roll-call or sign-in-sheet and claim to have attended sessions in which they have been marked absent. These time-inefficient, labor-intensive methods, which are susceptible to human error, make the authenticity of these claims hardly

determinable. Hence, there arises the need for a more sophisticated attendance system that can rule out the possibility of such issues. Token-based methods using Radio Frequency Identification (RFID) tags, Wi-Fi, or Bluetooth connections do eliminate some of these disadvantages. However, in addition to high investment costs, this solution also has an inherent limitation in handling bogus attendance. Considering similar disadvantages in other biometric methods such as iris scanning, fingerprint scanning, and voiceprint recognition, it can be concluded that face recognition is the best method for attendance registration in a classroom. From the literature, it was realized that most of the papers adopted similarity measures for comparing faces where classifiers could have been used to achieve enhanced performance. Secondly, many of the existing methods have utilized high-resolution video inputs for which dedicated cameras have to be mounted in every classroom. Finally, most of the models were trained and tested on small custom datasets. Those who have worked with the benchmark dataset did not consider the balancing of the data. This increases the chances of the models producing false results on images subjected to conditions of poor illumination or noise.

Hence, the objective of this research work is to propose a cost-effective Improved Face Recognition Model (I-FRM) that overcomes the limitations of existing image-based attendance systems in terms of accuracy, feasibility, and cost, while considering classrooms to be unconstrained environments. Through a dedicated mobile application that requires capturing 2-3 images of a classroom at different intervals (to record late attendance), a file can be downloaded in CSV format which will contain the attendance details of every student registered with the classroom's database. Thus, the need for a dedicated camera to be mounted in every classroom can be disregarded. Figure 1 shows the image-based attendance system that utilizes the proposed I-FRM model in a real classroom. At first, the students register themselves with 10 images of each in the system. This dataset is utilized to build the model. To this model, three group images of a classroom at different intervals are fed. From these group images, the individual faces are extracted. Furthermore, for every face, features will be extracted, and their corresponding face embeddings will be found. Subsequently, the I-FRM model is utilized to detect the name of every face. Further, these names were utilized to mark the attendance in the CSV file. The rest of the paper is organized as follows. Section 2 discusses the research work related to image-based attendance systems. Section 3 discusses in detail the proposed improved face recognition model, I-FRM. Section 4 provides detailed performance analysis to support the proposed model, and Section 5 concludes the paper with the significant results of this work.

Literature survey

In few-shot learning, faces are preprocessed prior to training the model using Multi-task Cascaded Neural Network (MTCNN). One shot learning is used by a Siamese network based on the ResNet34 model architecture. A HAAR cascade is used to detect and extract faces. The extracted faces are then passed through an embedding creation module. Face embedding of incoming images taken by a smartphone camera is compared with the embedding of all the students to mark the attendance. The model has achieved an accuracy of 97% on the LFW dataset and around 85% on classroom images [1]. In another work, a Modified Local Binary Pattern Histogram (MLBPH) algorithm is used to divide the face image into blocks, calculate a histogram for each block, and combine Local Binary Pattern Histograms (LBPH) into a single histogram. The histograms of input images are compared with the histograms of images in the database using the classifier. The experiments on classroom images reported a precision of 97% [2]. In the proposed SeetaFace face recognition model, Faster R-CNN outputs coordinates of all the students in the classroom after detecting them from the frames of the input video. It does the same along with face detection, key point positioning, and face recognition. The paper compared the fastness of Faster R-CNN and SeetaFace face detection by increasing the change of face pose. The identity of students is determined using the algorithm forward or backward, considering that the seats of students are fixed during a lecture [3]. In this proposed face recognition model, Haar Cascade is used for face detection and ANN back propagation is used for recognition. The facial recognition processor compares the data from the database with the face testing image, which satisfies a minimum threshold condition set by the system. The model achieved an accuracy of 95% on classroom image frames [4]. Face detection was done using the Viola-Jones algorithm, and face recognition was carried out with simple classifiers like logistic regression, linear discriminate analysis, and k-nearest neighbor. The system achieved an accuracy of 97.29% with linear discriminate analysis [5]. A single-sample face recognition was proposed for which a two-phase method

combining data augmentation and CNN transfer learning was studied. Faces are detected using MTCNN. In this paper, MobileNetV2, ResNet50V2, DenseNet121, InceptionV3, and VGG16 CNNs were evaluated, and it was proved that DenseNet121 was the best model for the single sample per person face recognition problem. The model showed an average accuracy of 99.6% on classroom images [6].For detection, MTCNN is used, DSST for tracking, and Inception-ResNet-V1 for extracting facial features from surveillance video. The cosine distance between these features and face features in the multimodal face feature reference gallery is calculated to find the closest face feature in tracklets to the reference face feature [7]. Face detection is done by making use of HAAR-like features, Integral Image, AdaBoost Algorithm, and Cascade Classifiers. Face images are extracted from video frames using the Viola-Jones algorithm followed by recognition using AlexNet CNN trained on the face database of students. It showed accuracies of 98.44% on a dataset with frontal faces under proper lighting and angles and 90% on images subjected to lighting, obscure features, and faces at different angles relative to the camera [8].

Face recognition was implemented using the SIFT (Space invariant feature transformation) algorithm, which is based on an approach where a small set of significant features are used to describe the variation between images of faces [9]. Captured images are enhanced and fed into a face detection module based on Viola and Jones's algorithm, followed by face recognition where the faces are verified one by one using the EigenFace method [10].Faster RCNN is used to detect the boundary boxes of all the faces in each video frame. These boxes are given to the face tracking module to fix the face on a standard frame. These face tracks are given to CNN to find features. Features of the reference photo and features of the photos in the entire video are compared using a cosine similarity distance measure [11]. Faces are detected using the Tiny Face Detector, which creates three copies of an image and feeds them into a shared CNN model to predict the potential face location on the image [12]. Using non-maximum suppression (NMS), the face location data is merged and ranked to find the best face location in the image. Detected faces are recognised using dlib, which internally computes similarity using Euclidean distance [12]. Faster R-CNN is used for detection, FaceNet for creating embeddings, and SVC for face classification. A two-tier authentication method is used to process faces. The primary tier improves the accuracy of face detection and face classification, while the second tier is based on a constant monitoring approach that enables the calculation of the time an individual has spent in the classroom to grant attendance only to those that were present for longer than the specified threshold. The model showed an accuracy of 93.33% on classroom images [13]. Faces are detected, cropped, and normalized from every frame. The facial recognition process uses the output of facial detection, which is the normalized face images. The ANN extracts the features of the face images and compares them with the face features stored in the database. The one with the highest similarity will be chosen as the student who owns the face image that was processed. The system achieved an accuracy of 87% on classroom surveillance videos [14].

Haar features are used to detect faces. Face positioning is altered to resolve the issue of projecting faces [15]. A CNN will be trained to create 128 vectors for each face. The detected faces are matched with the encodings from the training set. The Euclidean distance is calculated for each of the faces in the database. An accuracy of 96.15% was achieved on experiments with 25 images per student. Face recognition Discrete Wavelet Transforms (DWT) and Discrete Cosine Transform (DCT) are combined to extract the features of a student's face, which after facial objects are classified by applying Radial Basis Function (RBF). The system achieved a success rate of 82% [16]. Face recognition is implemented using the Gabor wavelet (Gabor filter). It identifies faces quickly in large numbers. With direct direction, the best result of 97% was achieved. For detection, the HOG feature with cascade regression is used [18]. The K-NN predicts registered faces by obtaining the current frame by searching for the k most similar features using Euclidean distance and the highest number of the same possible labels are found. A suitable distance threshold is configured to exclude the unregistered person from the prediction. It marks the attendance of an individual whenever the person's face has trained within five seconds. It achieved an accuracy of 92.5% in an experiment with images of 40 participants [17].

MATERIALS AND METHODS

This section describes in detail the MTCNN technique and the proposed method to create a Face Recognition System.

Proposed Improved Face Recognition System

In the proposed I-FRM Model shown in Figure 2, first the input face images are preprocessed. Subsequently, these preprocessed images are sent to MTCNN, which consists of cascaded neural network models, namely, P-Net, R-Net, and O-Net. From these preprocessed images, top 'n' bounding boxes are generated by the P-Net. These boxes are further refined by the R-Net. From these finer boxes, the O-Net extracts key feature locations such as the positions of eyes, nose, and mouth. These key features are sent to FaceNet to create face embeddings. Furthermore, multiple classifiers have been used to find the best suitable model for face recognition.

Preprocessing

The Labeled Faces in the Wild (LFW) dataset, downloaded from the Kaggle website, has been considered for building the model. This dataset contains images of 5,749 different individuals. It exhibits natural variability in the pose, lighting, focus, resolution, facial expression, age, gender, race, accessories, make-up, occlusions, background, and photographic quality. While 1,680 people have two or more images, the remaining 4,069 people have just a single image. Hence, the dataset is imbalanced. When the data is imbalanced, the standard classifier algorithms are inherently biased toward major classes. Hence, the dataset must be preprocessed to maintain the balance of the dataset.

Face Detection

During face detection, faces in the image are detected. There are various face detection methods, namely, HAAR, HOG, and MTCNN. The Haar cascade face detection method has a simple architecture that works in nearly real-time on the CPU. In addition, it can detect images at different scales [1-2,19]. However, the major drawbacks are that it gives false results and it does not work on non-frontal images. DLib's HOG face detection is the fastest method on CPU, which can work on frontal and slightly on non-frontal images. However, it is incapable of detecting small images and handling occlusions. This method often excludes some parts of the chin and forehead during the detection phase.The MTCNN face detection method works for faces having different facial lighting conditions, visual alterations, and poses [6-7]. It performs significantly better since it outputs arbitrary rectangular bounding boxes rather than square boxes as done by Haar. Hence, MTCNN has been chosen to detect the face. Algorithm 1 describes the procedure for the extraction of key facial landmarks by MTCNN. Every image will be passed into the 3 networks, namely, P-Net, R-Net, and O-Net of MTCNN [20]. In P-Net, for every image, a set of images is generated with different scales. A kernel of size 12x12 pixels is superimposed on every scaled image and an 'n' number of bounding boxes are generated randomly over that image. Confidence scores for these bounding boxes with respect to the kernel are calculated based on the following equation (1).

$$L_i^{\text{det}} = -\left(y_i^{\text{det}} \log\left(p_i \right) + \left(1 - y_i^{\text{det}}\right)\left(1 - \log\left(p^i \right)\right)\right) \quad \text{....(1)}$$

where the probability of face sample x_i is represented by p_i which is predicted by the MTCNN. y_i^{det}stands for ground truth [21]. Top n bounding boxes are selected as surviving bounding boxes based on their confidence scores. These surviving boxes of all the input images are scaled to the size of original image. These boxes are given as an input to the R-Net. The process followed in P-Net has been repeated to generate finer surviving bounding boxes based on the following equation (2).

$$L_i^{box} = \| y_i^{\wedge box} - y_i^{box} \|_2^2 \quad \text{....(2)}$$

where$y_i^{\wedge box}$ is the regressed target, y_i^{box} is the ground-truth including width, top-left coordinates, and height.

Again the boxes generated from R-Net are given as an input to O-Net. This generates key facial landmarks for every bounding box based on the following equation (3).

$$L_i^{landmark} = \| y_i^{\wedge landmark} - y_i^{landmark} \|_2^2 \quad \text{....(3)}$$

where $y_i^{\wedge landmark}$ represents the network's regressed feature coordinate and $y_i^{landmark}$ represents the ground truth.

Generation of Face Emebddings

During this stage, biological components of faces are extracted. These biological components are the features of a face that differ from person to person. There are various methods that extract various combinations of features, commonly known as nodal points. No two people can have all the nodal points like each other, except for identical twins. Face recognition APIs are a popular way to detect human faces with feature points. Another way to detect facial features is to utilize a deep neural network. However, though deep neural networks extract more meaningful features when compared to machine learning, they require a huge amount of data. Hence, instead of building the deep network from scratch, the FaceNet model can be used to extract salient features from input faces. It is a pre-trained model that has been trained on a large dataset to remember how to encode facial images. Furthermore, face recognition APIs use block-level processing rather than pixel-level processing. Hence, the FaceNet model is used to generate the embeddings for the obtained facial landmarks corresponding to every image.

Feature classification

Various classification methods are used to differentiate the face embeddings among different classes to identify the best suitable model.

RESULTS AND DISCUSSION

This section describes in detail the datasets used for experimentation and discusses the results of the proposed methods.

Dataset Description

The LFW dataset consists of 13,233 images of 5,749 people. Of these, 1,680 people have two or more images, while many people have only one image. To overcome this challenge of an imbalanced classification dataset where the distribution of images in all the classes is not the same, a selective sampling strategy has been used to select <=k samples for each class. Thus, the Balanced LFW (B-LFW) dataset consists of 1,430 images for 143 people with 10 samples each. This B-LFW dataset is utilized for building the proposed I-FRM model. The LFW dataset is a benchmark dataset that reflects the nature of classroom images, including natural variability in the pose, lighting, focus, resolution, facial expression, age, gender, race, accessories, make-up, occlusions, background, and photographic quality.5 Celebrity Faces is a toy dataset that is used for experimenting with computer vision techniques. It has a training directory consisting of 14 to 25 photos for each of the following celebrities:

- Ben Afflek
- Elton John
- Jerry Seinfeld
- Madonna
- Mindy Kaling

Performance metric used

The proposed system is tested with the metrics, namely, accuracy, precision, recall, F1-score and ROC-AUC.

- Accuracy refers to the proportion of true results among the total number of cases examined. This metric is useful for classifying problems that are well balanced.

- Precision refers to the proportion of predicted positives that are truly positive. Recall refers to the proportion of true positives to actual positives.
- The F1-score metric is helpful in reducing false negatives. Hence, it is helpful to compare the performance of classifiers.
- The ROC-AUC metric is useful in determining the ability of the classifier to distinguish different classes.

Implementation

The proposed I-FRM system has 4 processes, including preprocessing, face detection, feature extraction, and feature classification. MTCNN is a pre-defined model written in Python to detect nodal points of the faces in terms of bounding boxes. The B-LFW dataset is fed into MTCNN to detect the bounding boxes around faces. Further, these bounding boxes are sent to a pre-defined model, FaceNet, to generate the embeddings. These embeddings are sent to various classifiers to perform the classification of labels. The entire system has been built using a computing machine with the configuration of Intel Core i5-8265U, 256GB SSD, 8GB DDR4 RAM, 2400 MHz, and NVIDIA 4 GeForce MX150.

Performance of I-FRM model with and without FaceNet Embedding

Objective

To study the performance of the I-FRM model with and without FaceNet embedding. Experiments were conducted to test the proposed I-FRM with the consideration of combinations of MTCNN and FaceNet pre-trained models. MTCNN generates the key facial landmarks of the faces represented as bounding boxes as shown in Figure 3. In one experiment, the features of the bounding boxes were only considered to build a model. In the other, the output of MTCNN is sent to the FaceNet model to generate embeddings, and this was used to build the model. Models have been trained with both B-LFW and 5 Celebrity Faces datasets. For both the models, all the performance metrics were measured and plotted as graphs, as shown in Figures 4 and 5. It is inferred from all the graphs that the models trained with MTCNN+FaceNet outperform when compared to those of models that were trained with MTCNN alone, irrespective of the datasets. The reason behind the performance of the models is that models using MTCNN utilise all the features of the bounding box, whereas the model with MTCNN+FaceNet considers the significant features in the bounding box represented as embeddings.

Performance of I-FRM model with similarity and classification approaches

Objective

To study the performance of the I-FRM model using similarity and classification approaches. Experiments were conducted to test the proposed I-FRM by constructing the models using a few of the classification and similarity approaches. Various classifiers, namely, Support Vector Machine, Logistic Regression, Decision Tree, Random Forest, and Gaussian Naïve Bayes, have been used to build the model to classify the labels. Various similarity measures such as KNN, Cosine similarity, Jaccard distance, Euclidian distance, and Manhattan distances were utilised to find the closest distance between test samples among training samples to classify the labels. The accuracies of the models were compared across the different approaches and plotted as shown in Figure 6 and 7. It is inferred from the graphs that classification approaches have outperformed similarity approaches. The rationale behind, the result is that the similarity measure used for classification leads to poor results even when half of the features of test and training samples match.

Performance of I-FRM model using classifiers

Objective

To study the performance of the I-FRM model using various classifiers. To study the performance of the proposed I-FRM model, it is implemented with both B-LFW and 5-celebrity-faces datasets. The Balanced LFW (B-LFW) dataset consists of 1430 images for 143 people with 10 samples each. The 5-celebrity-faces dataset consists of 92 images in total. Each celebrity consists of around 20 images. Experiments were conducted with 5 fold cross-validation of training data. In each fold, a random set of images was considered. Various classifiers, namely, Support Vector

Machine, Logistic Regression, Decision Tree, Random Forest, and Gaussian Naïve Bayes were used. Table 1 and Table 2 show the corresponding results.

Performance of I-FRM model using AUC-ROC

Objective

To study the performance of the I-FRM model with the AUC-ROC metric. The AUC–ROC (Area Under Receiver Operating Characteristics) curve is a salient performance metric for multi-class classification. The AUC curve denotes the degree of separability. That is, it clearly shows how much the model is capable of distinguishing between classes. The higher the AUC, the better the model is to predict the classes. The models built for different datasets were measured to plot the AUC curves. It is inferred from Figure 8 that the SVM classifier performs better when compared to the other classifiers when compared to the datasets used. In summary, the proposed Improved Face Recognition Model (I-FRM) was implemented. A benchmark dataset was analyzed for class imbalance, and the random sampling method was used to balance the dataset. This balanced LFW and 5 Celebrity Faces datasets were used to conduct experiments. In the I-FRM model, MTCNN detects the face and the FaceNet model generates its embedding. Various experiments were conducted to investigate the proposed models built with MTCNN alone and with MTCNN+FaceNet and found that MTCNN+FaceNet outperforms the others. Furthermore, experiments were conducted to investigate the models built to perform classification tasks based on similarity measures and ML classifiers and found that the classifiers outperform the similarity approaches. In addition, experiments were conducted to investigate various classifiers and found that SVM outperforms the other classifiers with an accuracy of 97.5%.

REFERENCES

1. Bhat, A., Rustagi, S., Purwaha, S. R., &Singhal, S. (2020). Deep-learning based group-photo Attendance System using One Shot Learning. In: Proceedings of International Conference on Electronics and Sustainable Communication Systems (ICESC), 546-551.
2. Kumar, A. , Sharma, M., Gautam, S. P., Kumar, R. & Raj, S. (2020). Attendance Management System using Facial Recognition. In: Proceedings of International Conference on Decision Aid Sciences and Application (DASA), 228-232.
3. Ping, C., Da-Peng, H., &Zu-Ying, L. (2020). Automatic Attendance Face Recognition for Real Classroom Environments. In: Proceedings of the 2nd International Conference on Big Data and Internet of Things, 65-70.
4. Prangchumpol, D. (2020). Face Recognition for Attendance System Using Neural Networks Technique. In: Proceedings of the 3rd International Symposium on Computer Science and Intelligent Control, 1-5.
5. Sunaryono, D., Siswantoro, J. &Anggoro, R. (2021). An android based course attendance system using face recognition. Journal of King Saud University-Computer and Information Sciences, 33, 304-312.
6. Filippidou, F. P. &Papakostas, F. A. (2020). Single Sample Face Recognition Using Convolutional Neural Networks for Automated Attendance Systems. In: Proceedings of 4th International Conference on Intelligent Computing in Data Sciences (ICDS), 1-6.
7. Jin, K., Xie, X., Wang, F., Gao, X., & Shi, G. (2018). Real-Time Face Attendance Marking System in Non-cooperative Environments. In: Proceedings of 2nd International Conference on Video and Image Processing, 29-34.
8. Ali, M., Zahoor. H. U., Ali, A. & Qureshi, M. A.(2020). Smart Multiple Attendance System through Single Image. In: Proceedings of IEEE 23rd International Multitopic Conference (INMIC), 1-5.
9. Dhope, M.G., &Bhombe, D. L. (2021). Face Recognition Based Attendance System using SIFT Algorithm. International Research Journal of Engineering and Technology, 4, 1916--1918.
10. Wagh, P., Thakare, R., Chaudhari, J. &Patil, S. (2015). Attendance system based on face recognition using eigen face and PCA algorithms. In: Proceedings of International Conference on Green Computing and Internet of Things (ICGCIoT), 303-308.

11. Cheng, Q., Rong, B., Sun, B., & He, J. (2019). Class Attendance Checking System based on Deep Learning and Global Optimization. In: Proceedings of the 8th International Conference on Computing and Pattern Recognition, 331-336
12. Al-Fahsi, R. D., Pardosi, A. P., Winanta, K. A., Kirana, T., Suryani, O. F., &Ardiyanto, I. (2019). Laboratory Attendance Dashboard Website Based on Face Recognition System. In: International Electronics Symposium (IES), 19-23.
13. Mehta, R., Satam, S., Ansari, M. &Samantaray, S. (2019). Real-Time Image Processing: Face Recognition based Automated Attendance System in-built with "Two-Tier Authentication" Method. In: Proceedings of International Conference on Data Science and Engineering (ICDSE), 1-6.
14. Castillo, R. V., Catanaoan, M. J., Obliopas, J. F. &Linsangan, N. B. (2018). Class attendance generation through multiple facial detection and recognition using artificial neural network. In: Proceedings of International Conference on Machine Learning and Machine Intelligence, 38-42.
15. Kumar, S., Kathpalia, D., Singh, D., & Vats, M. (2020). Convolutional Neural Network based Automated Attendance System by using Facial Recognition Domain. In: Proceedings of 4th International Conference on Intelligent Computing and Control Systems (ICICCS), 654-659.
16. Lukas, S., Mitra, A. R., Desanti, R. I., &Krisnadi, D. (2016). Student attendance system in classroom using face recognition technique. In: Proceedings of International Conference on Information and Communication Technology Convergence (ICTC), 1032-1035.
17. Widyarto, S., Nugraha, A. K., Sumali, N., Wulan, R. &Yulianti, S. (2017). Face Identification Attendance System using Gabor Wavelet Method. Selangor Science & Technology Review, 1, 55-62.
18. Tee, T. X., &Khoo, H. K. (2020). Facial Recognition using Enhanced Facial Features k-Nearest Neighbor (k-NN) for Attendance System. In: Proceedings of 2nd International Conference on Information Technology and Computer Communications, 14-18.
19. Budi, S., Karnalim, O., Handoyo, E. D., Santoso, S., Toba, H., Nguyen, H., Malhotra, V. (2018). IBAtS-Image based attendance system: A low cost solution to record student attendance in a classroom. In: Proceedings of IEEE International Symposium on Multimedia (ISM), 259-266.
20. Zhang, K., Zhang, Z., Li, Z. &Qiao, Y. (2016). Joint face detection and alignment using multitask cascaded convolutional networks. IEEE Signal Processing Letters, 23(10),1499–1503.
21. Ma, M., & Wang, J. (2018). Multi-view face detection and landmark localization based on MTCNN, In: Proceedings of Chinese Automation Congress (CAC), 4200–4205.

Algorithm 1. MTCNN Algorithm

Algorithm 1 Working Principle of MTCNN Algorithm

Input:
Images from TrainSet
Output:
bounding box, 5 facial landmarks, and confidence score for each image

for *every image i in TrainSet* **do**
  Create a set S_i of varying scaled sizes
  // Working of P-Net
  Set S_i, as the input for P-Net
  for *every image j in set* S_i **do**
    Superimpose centrally, the kernel of size 12 x 12
    Generate n, random bounding boxes
    Calculate the distance of every bounding box with respect to the kernel
    Assign confidence score to every bounding box based on its corresponding distance
    Select top m, bounding boxes based on the confidence scores
    // Choose the surviving bounding boxes using Non-Maximum Suppression method
    for *every bounding box b, from top m bounding boxes* **do**
      Calculate overlapping area for b, over the kernel
    end
    Select k bounding boxes based on maximum overlapping areas as surviving bounding boxes
    Convert the coordinates of surviving bounding box into coordinates of image i
    Reshape the surviving bounding boxes into square shapes
  end
  Resize the surviving bounding boxes into 24 x 24 pixels to form a set $bb_{i-PNetout}$
  // Working of R-Net
  Set $bb_{i-PNetout}$ as the input for R-Net
  Repeat the steps 3-6 for the input to generate surviving bounding boxes
  Resize the surviving bounding boxes into 48 x 48 pixels to form a set $bb_{i-RNetout}$
  // Working of O-Net
  Set $bb_{i-RNetout}$ as the input for O-Net
  for *every I in* $bb_{i-RNetout}$ **do**
    Extract key points
  end
  Output a bounding box along with its key points and confidence score
end

Table 1. Classifiers' performance for proposed I-FRM Model with the B-LFW-celebrity dataset

Classifier/ Performance Metric	Logistic Regression	SVM	Decision Tree	Random Forest	Gaussian Naïve Bayes	Best Score
Accuracy	0.97303	0.975025	0.411602	0.914075	0.908095	SVM
Precision	0.968531	0.968531	0.365711	0.906061	0.929494	SVM
Recall	0.974126	0.974825	0.425874	0.916783	0.918182	SVM
F1-Score	0.966620	0.967786	0.370203	0.899720	0.910758	SVM

Table 2. Classifiers' performance for proposed I-FRM Model with the 5-celebrity dataset

Classifier/ Performance Metric	Logistic Regression	SVM	Decision Tree	Random Forest	Gaussian Naïve Bayes	Best Score
Accuracy	1.0	1.0	0.869591	1.0	0.988889	SVM
Precision	1.0	1.0	0.848667	1.0	0.986667	SVM
Recall	1.0	1.0	0.85	1.0	0.99	SVM
F1-Score	1.0	1.0	0.838698	1.0	0.986286	SVM

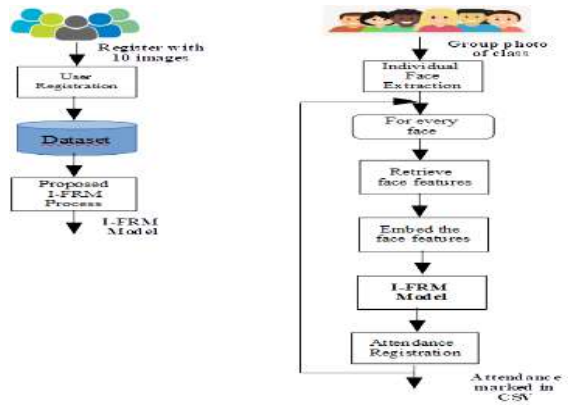

Figure 1. I-FRM Model in Image based Attendance System

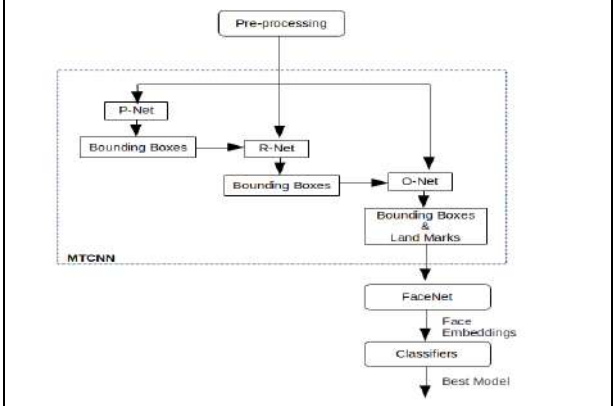

Figure 2. Proposed Improved Face Recognition Model

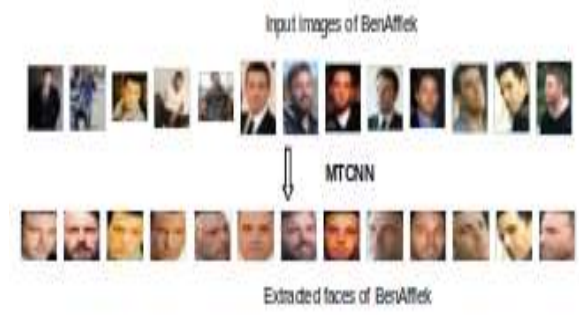

Figure 3. Output of MTCNN

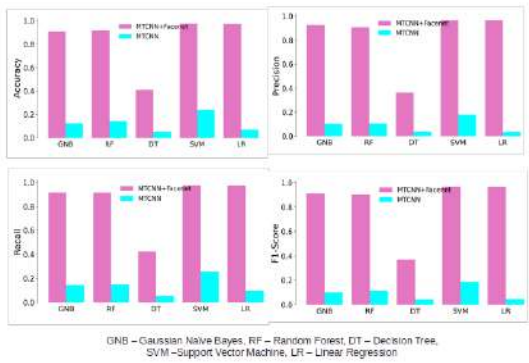

Figure 4. Performance of I-FRM Model with and without FaceNet for B-LFW dataset

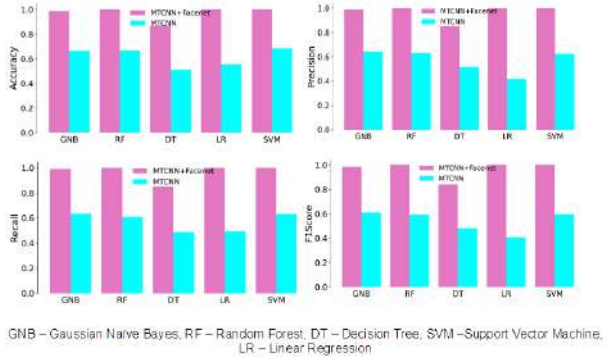

Figure 5. Performance of I-FRM Model with and without FaceNet for 5-celebrity dataset

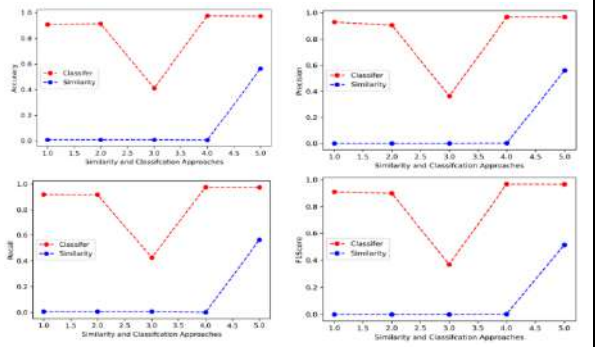

Figure 6. Performance of I-FRM Model with similarity and classification approaches for B-LFW dataset

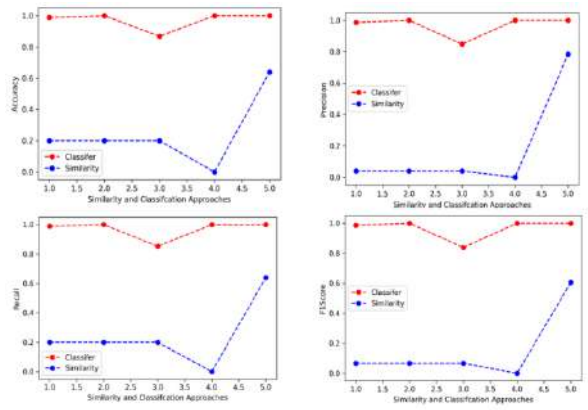

Figure 7. Performance of I-FRM Model with similarity and classification approaches for 5-celebrity dataset

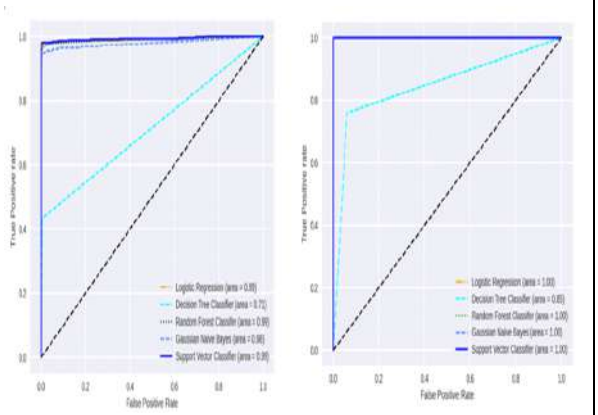

Figure 8. Performance of I-FRM Model uses Classifiers with AUC-ROC curve for B-LFW and 5-celebrities dataset

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REVIEW ARTICLE

Depression – A Review

Simran Gupta[1]* and Neeraj Kumar[2]

[1]Master of Pharmacology (Pharmacology), Department of Pharmacology, School of Pharmaceutical Sciences, Sri Guru Ram Rai University, Patel Nagar, Dehradun, Uttarakhand, India.
[2]Assistant Professor, Department of Pharmacology, School of Pharmaceutical Sciences, Sri Guru Ram Rai University, Patel Nagar, Dehradun, Uttarakhand, India.

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***Address for Correspondence**
Simran Gupta
Master of Pharmacology,
Department of Pharmacology,
School of Pharmaceutical Sciences,
Sri Guru Ram Rai University, Patel Nagar,
Dehradun, Uttarakhand, India.
Email: guptasimran7088@gmail.com

ABSTRACT

Researchers in India have long paid close attention to the disease of depression. Numerous research from India have been published in the last 50–60 years that focus on various aspects of this illness that is frequently present. Epidemiology, numerous psycho-social risk factors, symptomatology, diagnosis, the effects of depression, therapies and treatment-related difficulties were some of the various areas examined. There are many different neurochemical hypotheses, and there are several synthetic antidepressants on the market today, but not everyone who suffers from this condition responds to them effectively. Further limiting its therapeutic utility are the adverse effects and medication interactions. In contrast, herbal remedies are widely utilized around the world because to their broad application, therapeutic efficacy, and few side effects. This has expedited scientific study into the antidepressant action of herbal remedies.

Keywords: Depression, neurotransmitters, stress, treatment, anti depressants and herbs

INTRODUCTION

One of the most prevalent neuropsychiatric disorders is depression, which has a lifetime prevalence of close to 17% [1]. According to WHO predictions, depression will overtake heart disease as the second most common disease in the world by the end of the next ten years. One out of every five women now suffer from depression. Not just adults, but 2% of schoolchildren and 5% of teenagers also experience depression, most of whom go unrecognised. The most

frequent cause for people to visit a psychiatrist is depression, despite the fact that the general public believes that all psychiatric issues are depression [2]. One of the most important risk factors for ischemic heart disease is depression [3]. Considerable mood depression and functional impairment are the chief clinical symptoms of severe depression. The symptoms of several anxiety disorders, such as panic-agoraphobia syndrome, severe phobias, social anxiety disorder, posttraumatic stress disorder, and obsessive-compulsive disorder, coincide with those of depressive illnesses [4]. Depression can range from a very mild condition that approaches on normality to a severe psychotic depression accompanied by hallucinations and delusions. Depression is characterized as disorders of mood rather than disruptions of thought or cognition [5]. In terms of prevalence, misery, dysfunction, morbidity, and economic cost, depression is an illness of substantial public health significance. Women experience depression more frequently than males. According to the Global Burden of Disease report, uni - polar depressive episodes had a point prevalence of 1.9% for men and 3.2% for women, and a one-year prevalence of 5.8% for males and 9.5% for women. If demographic and epidemiological trends continue, depression is predicted to account for 5.7% of all illness burden by 2020 and rank as the second biggest cause of years lived with a disability adjusted for age (DALYs),In numerous research, the prevalence of depression in community samples has been calculated, and the prevalence rates have ranged from 1.7 to 74 per thousand people [6].

The monoamine theory, proposed in 1965, mania can occur from an excess of the monoaminergic (nor-adrenaline and/or 5-hydroxytryptamine) transmission in the central nervous system, whereas depression is thought to be caused by a functional deficit of these transmissions. The notion was founded on the observation that pharmaceuticals like reserpine can induce depression and that established antidepressant medications, like as tricyclic antidepressants and mono-amine oxidase inhibitors, can promote monoaminergic transmission [7]. The mono-amine theory in its simplest form is not convincingly supported by biochemical tests on depressed patients. In depression, plasma cortisol typically responds abnormally slowly to exogenous steroids (dexamethasone suppression test), which may indicate faulty mono-amine transmission in the hypothalamus. Recent research reveals that depression may be linked to decreased neurogenesis and neurodegeneration in the hippocampus. Although the mono-amine hypothesis in its simplest form is insufficient to explain depression, the most effective therapeutic strategy continues to be pharmaceutical modulation of mono-amine transmission [8].

TYPES OF DEPRESSION

As with numerous other disorders, depression manifests itself in a variety of ways.

A. Major depression is characterised by a variety of symptoms that make it difficult to work, sleep, eat, and take pleasure in once-pleasurable activities. These incapacitating depressive episodes can happen just once, twice, or multiple times in a lifetime.

B. Dysthymia, A less severe kind of depression is characterized by long-lasting, persistent symptoms that do not incapacitate you but prevent you from operating at "full steam" or from feeling happy. Major depressive episodes are occasionally experienced by people with dysthymia.

C. Manic-depressive or bipolar is not nearly as common as other depressive disorders. Cycles of mania or exhilaration and despair are involved. The mood swings can be abrupt and dramatic at times, but most of the time they are gradual. Any or all of the other symptoms of a depressive illness may be present when one is in the depression cycle. Any or all of the symptoms described under "mania" may be present during the manic cycle. Mania frequently has an embarrassing and problematic impact on thinking, judgement, and social behavior [9,10].

SYMPTOMS OF DEPRESSION

Not every sign of manic or depressive illness manifests itself in every person. A few or several symptoms may be experienced by certain people. Additionally, each person's symptom severity may be different [11-13].

i. Prolonged sadness, anxiety, or emptiness.
ii. A feeling of impotence or pessimism
iii. Guilt, worthlessness, and hopelessness.
iv. Loss of enjoyment or interest in past-time interests and pursuits, especially sex.

v. Insomnia, morning awakenings, or excessive snoozing.
vi. Appetite, weight loss, or gain from eating too much.
vii. Lowered energy, tiredness, and sluggishness,
viii. Suicidal ideas, attempts, or thoughts of death.
ix. Anxiety and agitation.
x. Problems with memory, concentration, or decision-making.
xi. Persistent physical problems like headaches, stomach issues, and chronic pain that do not improve with treatment.

CAUSES OF DISEASE – ENVIRONMENTAL FACTORS AND GENE COMPONENT

Genetic Causes of Depression

Serotonin transporter (SLC6A4), serotonin 2A receptor (5HTR2A), tyrosine hydroxylase (TH), the limiting enzyme for dopamine synthesis, tryptophan hydroxylase 1 (TPH1), and catechol-o-methyltransferase (COMT) functional polymorphism have received the majority of attention in published genetic association studies of mood disorders (dopamine catabolism)[14]. Although it has long been recognized that depressive disorders can run in families, it was unclear until relatively recently whether a person's sensitivity to these conditions was inherited or if something else, such as their environment, was the real cause. Depressive diseases can, to some extent, be inherited, according to researchers who study depression[15]. Bipolar disorder is heavily influenced by genetics. A parent with a history of clinical depression is present in about 50% of people with bipolar disorder. Whenever a parent has bipolar disorder, there is a 25% risk that their child will also experience clinical depression of some kind. The likelihood that a kid would likewise develop bipolar illness is between 50 and 75 percent higher if both parents have the condition. In comparison to people without such siblings, those with bipolar disorder may have an 8–18 fold increased risk of developing bipolar disorder and a 2–10 fold increased risk of major depressive illness [16].

Environmental Causes of Depression

Stress, traumatic experiences, and childhood challenges are examples of environmental factors that contribute to depression. These are occurrences that occur in our daily lives and can happen to anyone. They are regarded as independent variables.

Stress

Stressful conditions, how a person responds to stress in their body and mind, and the emergence of clinical depression all seem to be interconnected in a very complicated way. A stressful situation and the emergence of depression are causally related. Both negative and positive stress is possible. Divorce, losing a job, losing a relationship, and losing a loved one are all examples of negative stress. Moving to a new city, getting ready for a new career, and organizing a wedding are a few examples of constructive stress. Environmental events can cause both positive and negative stress, which can occur before depression [17].

Traumatic Events

The loss of a loved one, a serious sickness, the dissolution of a marriage, or a substantial financial loss are examples of traumatic occurrences in people's life. Events of this nature frequently result in emotional distress since they undermine a person's feeling of stability and control in their lives [18].

Synthetic Chemicals:-

We consume synthetic chemicals every day from many sources. Preservatives, chemicals, and hormones are present in a large number of our foods, along with sprayed pesticides, air pollution, and water pollution. According to studies, pollution of the air and water alone can lead to cancer and other ailments. More research is being done on the relationship between synthetic pollutants and depressive and major depressive episodes[19].

PHARMACOLOGICAL MANAGEMENT OF MAJOR DEPRESSION

To increase mono-amine levels at the synaptic cleft, conventional antidepressants either (i) block presynaptic mono-amine transporter proteins, which remove released transmitters from the extracellular space; (ii) inhibit the enzyme mono-amine oxidase, which breaks down catecholamine neurotransmitters; or (iii) interact with pre- or postsynaptic receptors that control mono-amine transmitter release and or neuronal firing rate [21]. It has been suggested that depression may be caused by a lack of nor-adrenaline, 5-HT, and dopamine at their receptor sites in the brain, while antidepressant medications raise extracellular mono-amine concentrations. Although the effects of antidepressants on mono-amines can be observed right away after delivery, it usually takes a few weeks of ongoing treatment for therapeutic responses to manifest. This concept is known as the mono-amine depression hypothesis. It's possible that information processing issues in the brain network, rather than chemical imbalance, are what causes depression because of the therapeutic delay of antidepressants [22]. In actuality, BDNF is a critical predictor of antidepressant efficacy since traditional antidepressants work by boosting BDNF in the fore-brain areas, especially in the hippocampus. The brain's neuroplasticity is induced by BDNF action, which lessens the symptoms of depression [23].

TREATMENT

Both medicine and psychotherapy are effective treatments for mild depression. For moderate to severe depression, a strategy combining medication and psychotherapy may be necessary.

Drug Treatment:-

When taking the initial antidepressant, 50 to 65 % recover. No antidepressant has a higher level of effectiveness or faster time to action than any other. The ability to match a patient's symptoms to a side effect profile, the presence of medical and mental health co-morbidity, and previous responses can all influence a decision. Antidepressant-treated patients should be continuously monitored for potential worsening of depression or suicidality, particularly at the start of medication or when the dose is changed [24]. Antidepressants are thought to work therapeutically through altering neurotransmitters and neurotransmission. According to the Mono-amine Hypothesis, which is a scientific theory, mono-amines like dopamine, serotonin, and nor-epinephrine are not active enough in the brain to produce depression. The effectiveness of tricyclic antidepressants and mono-amine oxidase inhibitors (MAOIs) in the treatment of depression was unintentionally discovered in the 1950s [25]. By blocking the enzyme mono-amine oxidase, mono-amine oxidase inhibitors (MAOIs) prevent the degradation of the monoamine neurotransmitters serotonin, nor-epinephrine, and dopamine. This results in higher levels of these neurotransmitters in the brain and greater neurotransmission [26]. Serotonin, nor-epinephrine, and, to a much lesser extent, dopamine are just a few of the neurotransmitters that are prevented from re-uptake by tricyclic antidepressants (TCAs). The most used antidepressants now are selective serotonin re-uptake inhibitors (SSRIs), which stop serotonin from being reabsorbed (thereby increasing the level of active serotonin in synapses of the brain). Other cutting-edge antidepressants have differing effects on nerve cell receptors or nor-epinephrine re-uptake [27-28]. Although SSRIs, MAOIs, and TCAs raise serotonin levels, other medications stop serotonin from binding to 5-HT2A receptors, arguing that it is oversimplified to refer to serotonin as the "happy hormone." In fact, it's typical for patients to feel worse during the first few weeks of treatment since the prior antidepressants accumulate in the circulation and the serotonin level rises. One theory for this is that 5-HT2A receptors evolved as a saturation signal, warning an animal to cease exploring for food, a partner, etc., and to start looking for predators (people who use 5-HT2A antagonists frequently gain weight). A hypothalamic-pituitary-adrenal (HPA) axis that is hyperactive and similar to the neuroendocrine (cortisol) response to stress is one idea for the origin of depression. Antidepressants help to normalize HPA axis function, which is affected by these HPA axis abnormalities, which contribute to the onset of depression symptoms [29].

Frequent Initial Visits

Early on in treatment, patients must have frequent visits to monitor their progress, suicidal ideation, side effects, and psychological support networks.

Continuation Therapy

The risk of a major depressive episode relapsing is reduced by continuation therapy (9–12 months after the acute symptoms disappear). Based on their history of relapse and other clinical criteria, certain patients should be given the option of long-term maintenance or lifetime medication therapy[30].

Education / Support

Education and assistance for patients are crucial. Social stigma and patient resistance to receiving a diagnosis of depression are still issues.

SIDE EFFECTS OF LONG TERM THERAPY

When depression episodes are in their acute phase and untreated symptoms are at their worst, antidepressants are essential for treating them. But after repeated use, a process he calls oppositional tolerance kicks in to help the brain adjust to the drug's effects. Like every system in the body, the brain makes an effort to resume its normal balance of neurotransmitter creation, release, and re-uptake whenever its regular operation is disrupted. According to the theory, if a drug unnaturally raises serotonin or nor-epinephrine levels in the brain, the neurobiology of the system will respond by decreasing its own production of the neurotransmitter. Long-term antidepressant use will eventually cause the brain to develop a mechanism to counteract its effects. It's possible that taking antidepressants is what's creating the issue[31]. Here is evidence that, in patients who no longer react to antidepressants, discontinuing the medication can cause symptoms to return as the brain adjusts once more, this time to the drug withdrawal. However, discontinuing the drug doesn't have any impact on some people. They still experience recurrent depression. These patients run the risk of developing a chronic condition that returns if antidepressant therapy is resumed in response. Tardive dysphoria is this [32].

ALTERNATIVE FORMS OF THERAPY FOR DEPRESSION

There is no proof that any complementary medicine or at-home remedy works to treat moderate to severe depression. However, home treatments may help some persons with minor depression by promoting calm. Depression symptoms can be alleviated by relaxation. It can also aid in coping with some of the reasons of depression, including physical pain, anxiety, role changes, and even sorrow.

Acupuncture

A traditional Chinese medicine is acupuncture. Acupuncturists place needles into spots on meridians with the goal of redressing the imbalance and restoring health on the basis of the theory that two types of "energies" travel through "meridians" throughout the body and that an imbalance of these energies creates sickness. Western acupuncturists disagree with these Taoist views and believe that acupuncture has neurophysiology's effects that contribute to its purported health benefits. Studies demonstrating that needling can raise endorphin levels thus give the putative mechanism for acupuncture's potential role in treating depression. Normally, doctors or (more frequently) non-medically trained therapists do acupuncture in specialist clinics (NMQTs). There may be a need for a series of 6 to 12 treatments, with each session typically lasting 20 minutes [33-34].

Herbal medicine

The use of plants, plant parts, or plant extracts in the treatment of disease is known as medical herbalism (also known as phytotherapy in Europe). It has a long history in all medicinal cultures, and a lot of the pharmaceuticals we use today have their origins in plants. There are a wide variety of compounds found in each plant, making it often challenging to pinpoint which compounds and how much of each are responsible for a given pharmacological activity. Thus, the mechanism of action can be complicated, but it could be understood or investigated using standard pharmacological techniques. Although the general public typically believes that plant-based medications have no side effects, this belief can be seriously deceptive[35].

Homeopathy

According to the "like heals like" principle, which is the foundation of homeopathy, a remedy (often but not always made from plants) that generates specific symptoms in a healthy person can be used as a treatment for patients who exhibit such symptoms. Homeopaths also contend that "potentiating" a remedy involves dilutions in small amounts followed by vigorous shaking. This process increases rather than decreases the medicine's potency. They believe that even very small concentrations of the original treatment will have significant clinical effects. 62 Both physicians and NMQTs practice homeopathy. Typically, a first consultation takes longer than an hour [36].

Aromatherapy

Aromatherapists (often NMQTs) combine soft massage methods with plant-based essential oils. After trans-dermal resorption, these oils are expected to have particular pharmacological effects. One session would take approximately 30 minutes, and a course of 6 to 12 sessions would typically be advised. Despite the fact that aromatherapy is recommended for treating depression and is regarded as useful by certain patients [37].

Exercise

There are many different types of physical activity, such as recreational and professional activities, one-off workouts, and regular exercise. They might react differently physiologically. It is useful to make the distinction between power (primarily anaerobic) exercise and regular endurance (mainly aerobic) exercise for the topic that follows. Exercise can be done at home alone or with supervision (such as a physiotherapist) for the treatment of depression. In actuality, a mixed strategy works best [37].

Dance and movement therapy

A dance therapist (often an NMQT) encourages patients to express themselves through movement in order to involve them and improve well-being. Group sessions might be arranged for treatments to include more social interaction. Sessions usually run between 30 and 40 minutes, and frequent (monthly, for example) repetitions are usually advised.

Music therapy

The active or passive application of music to enhance health and well-being is known as music therapy. Patients engage in musical activity or listen to carefully selected music under the guidance of a music therapist while receiving treatment (usually an NMQT). Depending on the patient's personality and condition, the music will vary.

Massage therapy

The practice of massage treatment comes in a variety of shapes and traditions. In this article's context, massage often involves a gentle physical stroking method across the body (usually the back). One of the many intricate physiological and psychological impacts of this is the relaxation of the muscles and the psyche. 72 A typical prescription would consist of a series of about 6 twice-weekly sessions, with each session lasting 20 to 30 minutes and being provided by an NMQT.

PLANTS WITH ANTIDEPRESSANT ACTIVITY [38].

Plant-based medications are becoming more popular and are being researched for a variety of diseases, including neurological conditions like depression. Many plants have been investigated for their potential as antidepressants.

CONCLUSION

A major public health issue, depression is a significant medical illness. Understanding the symptoms, potential causes, and treatments of the condition is crucial for enhancing the well-being of those who are affected, even if the onset of depression is probably the result of a number of variables. In order to decide whether and how long to continue treatment, it is also necessary to research the progression of depressive illnesses that are now experienced

worldwide. Studies should assess the cost-effective treatment modalities that are readily applicable in the primary care environment for the efficient treatment of depression. The natural compounds that have been taken into consideration show promise as potential antidepressant treatments. Treatment for a variety of neurological illnesses like anxiety and depression might benefit greatly from the use of these medications in the creation of a multi-component herbal formulation.

REFERENCES

1. Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. Arch Gen Psychiatry 62:593– 602, 2005
2. Robins L., Regier D., Psychiatric Disorders in America, New York: Free Press (2004)
3. Murray CJL, Lopez AD, eds. The global burden of disease. Boston: Harvard University Press, 1996
4. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. Washington, DC: APA Press; 2000
5. Rang HP, Dale MM, Ritter JM, Flower RJ. Pharmacology. London: Churchill Livingstone Elsevier; 2008
6. Lopez AD, Mathers CD, Ezzati M, Jamison DT, Murray CJ. Global Burden of Disease and Risk Factors. Washington: The World Bank; 2006
7. Baker GB, Dewhurst WG. Biochemical theories of affective disorders. In: Dewhurst WG, Baker GB, eds. Pharmacotherapy of affective disorders. Beckenham: Croom Helm; 1985:239-45
8. Baldessarini RJ. Fifty years of biomedical psychiatry and psychopharmacology in America. In: American psychiatry after World War II: (1944–1994). Menninger R, Nemiah, J, eds. Washington DC: American Psychiatric Press; 2000:371–412
9. Katon W. The epidemiology of depression in medical care, Int J Psychiatry Med, 17, 93-112 (2006)
10. Judd L.L., The clinical course of unipolar major depressive disorders Arch Gen Psychiatry, 54, 989-991 (2008)
11. Consensus Development Panel, NIMH/NIH Consensus Development Conference statement Mood disorders: pharmacologic prevention of recurrences, Am J Psychiatry,142, 469-476 (2003)
12. Pennix B.W., Guralnik J.M., Ferrucci L., Simonsick E.M., Deeg D.J., Wallace R.B., Depressive symptoms and physical decline in community-dwelling older persons JAMA, 276, 1720-1726 (2007)
13. Wells K.B., Stewart A., Hays R.D. et al., The functioning and well-being of depressed patients Results from the Medical Outcomes Study, JAMA, 262, 914-919 (2002)
14. Howland R., General health, health care utilization, and medical comorbidity in dysthymia, Int J Psychiatry Med 23, 211-238 (2005)
15. Williams J.W. Jr, Kerber C.A., Mulrow C.D., Medina A. and Aguilar C., Depressive disorders in primary care: prevalence, functional disability and identification, J Gen Intern Med., 10, 7-12 (2008)
16. Wagner R., Burns B.J., Yarnall K., Sigmon A., Walker R. and Gaynes B.N., Minor depression in family practice: functional morbidity, comorbidity, service utilization and outcomes, Psychol Med., 30(6), 1377-1390 (2000)
17. Kessler R.C., Nelson C.B., McGongale K.A., Liu J., Swartz M. and Blazer DG., Comorbidity of DSMIIIR major depressive disorder in the general population, Results from the US National Comorbidity Survey, Br J Psychiatry, 168, 17-30 (2006)
18. Regier D.A., Narrow W.E., Rae D.S., Manderscheid R.W., Locke B.Z. and Goodwin F.K., The de facto US mental and addictive disorders service system, Epidemiologic catchment area prospective 1-year prevalence rates of disorders and services, Arch Gen Psychiatry, 50, 85-94 (2005)
19. Gerber P.D., Barrett J., Barrett J., Manheimer E., Whiting R. and Smith R., Recognition of depression by internists in primary care, a comparison of internist and gold standard psychiatric assessments, J Gen Intern Med,. 4, 7-13 (2003)
20. Rajput MS, Sinha S, Mathur V, Agrawal P. Herbal antidepressants. IJPFR. 2011;1(1):159-69
21. Nemeroff, C.B.; Owens, M.J. Treatment of mood disorders. Nat. Neurosci. 2002, 5, 1068–1070
22. Castrén, E. Is mood chemistry? Nat. Rev. Neurosci. 2005, 6, 241–246

23. Björkholm, C.; Monteggia, L.M. BDNF—A key transducer of antidepressant effects. Neuropharmacology 2016, 102, 72–79
24. Feightner J.W., Early detection of depression. In, Canadian Task Force on the Periodic Health Examination, Canadian Guide to Clinical Preventive Health Care, Ottawa, Health Canada, 450-454 (2004)
25. Banerjee S., Shamash K., MacDonald A.J. and Mann A.H., The use of the SelfCARE (D) as a screening tool for depression in the clients of local authority home care services—a preliminary study, Int J Geriatr Psychiatry. 13, 695-699 (2006)
26. Beekman A.T., Deeg D.J., Van Limbeek J., Braam A.W., De Vries M.Z. and Van Tilburg W., Criterion validity of the Center for Epidemiologic Studies Depression scale (CES-D), results from a community-based sample of older subjects in The Netherlands, Psychol Med., 27, 231-235 (2011)
27. Broadhead W.E., Leon A.C. and Weissman M.M., et al., Development and validation of the SDDS-PC screen for multiple mental disorders in primary care, Arch Fam Med., 4, 211-219 (2003)
28. Burnam M.A., Wells K.B., Leake B. and Landsverk J., Development of a brief screening instrument for detecting depressive disorders, Med Care, 26, 775-789 (2011)
29. Hoyl MT, Alessi CA, Harker JO, et al., Development and testing of a five-item version of the Geriatric Depression Scale, J Am Geriatr Soc., 47,873-878 (2008)
30. Leon A.C., Olfson M. and Weissman M.M., et al., Brief screens for mental disorders in primary care, J Gen Intern Med., 11, 426-430 (2012)
31. Leung K.K., Lue B.H., Lee M.B. and Tang L.Y., Screening of depression in patients with chronic medical diseases in a primary care setting, Fam Pract., 15, 67-75 (2009)
32. Lustman P.J., Clouse R.E., Griffith L.S., Carney R.M. and Freedland K.E., Screening for depression in diabetes using the Beck Depression Inventory, Psychosom Med., 59, 24 (2011)
33. Filshie J, White A, eds. Medical Acupuncture: A Western Scientific Approach. Edinburgh, Scotland: Churchill Livingstone Inc; 1998
34. Han JS, Terenius L. Neurochemical basis for acupuncture analgesia. Annu Rev Pharmacol Toxicol. 1982;22:193-220
35. Ernst E. Harmless herbs? a review of the recent literature. Am J Med. 1998;104:170-178.
36. Ernst E, Hahn EG, eds. Homoeopathy: A Critical Appraisal. Oxford, England: Butterworth-Heinemann; 1998.
37. Mojay G. Oils for depression. Int J Aromatherapy. 1994;6:18-23. Hughes JR. Psychological effects of habitual aerobic exercise: a critical review. Prev Med. 1984; 13:66-78
38. Rajput MS, Sinha S, Mathur V, Agrawal P. Herbal antidepressants. IJPFR. 2011;1(1):159-69.

Table 1: Herbs With Antidepressant Properties

Plant	Family	Model	Mechanism
Agapanthus Campanulatus	Agapanthus	FST, TST	By monoaminergic system
Allium cepa	Liliaceae	FST	Acting hypothalamic-pituitary-adrenal axis
Banxia houpu	Lamiaceae	FST, TST	Increasing 5-HT and dopamine levels
Curcuma longa (turmeric)	Zingiberaceae	FST, OFT, MAO assay	Through MAO A inhibition
Crocus sativus L. (Saffron)	Iridaceae	FST	Uptake inhibition of dopamine and norepinephrine
Glycyrrhiza glabra (Liquorice)	Leguminosae	FST, TST	Increase in brain nor-epinephrine and dopamine by glycirrhizin
Hypericum perforatum	Hypericaceae	Kinetic Analyses synaptosomes of mouse brain	Inhibition of serotonin uptake by elevating [Na+]
Melissa officinalis	Lamiaceae	FST	Enhancement of norepinephrine neurotransmission

Nardostachys jatamansi	Valerianaceae	FST, TST, MAO assay	MAO inhibition
Ocotea duckei	Lauraceae	FST	Periphernal neuromuscular blockade
Paeonia lactiflora	Paeoniaceae	FST, TST, OFT	Central monoaminergic neurotransmitter system
Rhazya stricta	Apocynaceae	MAO assay	MAO inhibitory activity
Salvia divinorum	Lamiaceae	FST, TST	FST, TST Mediated through opioid and endocannabinoid systems
Securidaca longepedunculata	Polygalaceae	FST	By adrenergic mechanisms
Tabebuia avellanedae	Bignoniaceae	FST, TST	Dependent on the monoaminergic system
Tagetes lucida	Asteraceae	FST, MSBT	Involvement of serotonergic brain systems
Vitis vinifera	Vitaceae	FST, TST	Increase of 5-HT levels
Xysmalobium undulatum	Asclepiadaceae	FST, TST	By monoaminergic system
Zizyphus xylopyrus	Rhamnaceae	FST, TST	Regulation of α2-adrenergic receptor

FST- forced swim test, TST- tail suspension test, MSBT- male sexual behavioral test, OFT- open field test.

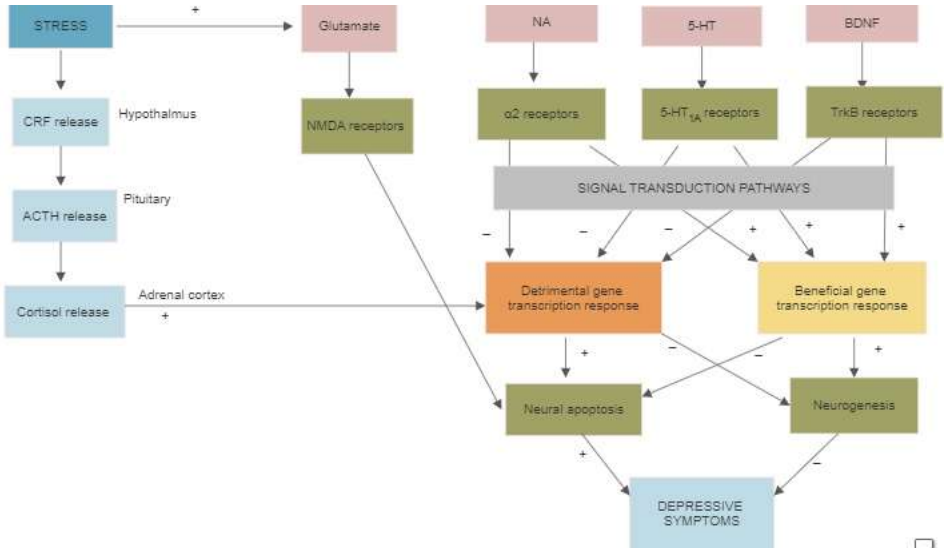

Figure 1: Mechanisms Believed To Be Involved in the Pathophysiology of Depression [20]

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RESEARCH ARTICLE

An Economics Analysis on Mango Export Potentiality in India

K. Buvaneswari[1]* and G. Ramanathan[2]

[1]Ph.D., Research Scholar, Department of Agricultural Economics, Annamalai University, Chidambaram, Tamil Nadu, India
[2]Professor and Head, Department of Agricultural Economics, Annamalai University, Chidambaram, Tamil Nadu, India

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***Address for Correspondence**
K. Buvaneswari
Ph.D., Research Scholar,
Department of Agricultural Economics,
Annamalai University, Chidambaram,
Tamil Nadu, India.
Email: buvaneswarieco@gmail.com

ABSTRACT

The present study is an attempt to analyse growth, instability and direction of trade of mango exports from India. Compound Annual Growth Rate, Cuddy-Della Valle Index and Markov chain analysis are the tools used for analysing data from 2012 to 2021. The growth rate of 1987-2022 export in terms of quantity is negative (-11.34% per annum) and the growth rate of exports in terms 2012-2022 of value is positive (13.43% per annum). The instability index is low (109.28%) for exports in quantity terms and is medium (130.68%) for exports in value terms. It was evident from the transitional probability matrices developed through Markov chain analysis that UAE was the most loyal buyer for Indian mango. The attention should be focused on the market requirement and specifications of Bahrain, Nepal, Bangladesh, Saudi Arabia and the UK were the most stable buyers of Indian mango. The study suggested the need to diversify India mango market.

Keywords: Growth Rate, Instability Index, Mango Exports, Markov Chain Analysis

INTRODUCTION

In the present scenario, there was a great deal of interest among policymakers and trade analysts in the role of horticultural products as a principal means of agricultural diversification and foreign exchange earnings in developing countries. The area under horticulture increased by 2.6 percent per annum and annual production increased by 4.8 percent over the last decade. The production of horticultural crops was estimated to be 311.71 million tonnes in 2018-19 from an area of 25.43 million ha [1]. Fruits, an important component of the horticulture sector, are valued as a rich source of minerals and vitamins, providing more energy per unit weight and higher

returns to the growers [2]. The total production of fruits increased from 50.9 million tonnes to 97.35 million tonnes from 2004-05 to 2018-19. The export of horticultural crops plays a major role in India's export earnings and employment generation. The export of horticultural produce earns not only foreign exchange but also provides much- needed competitiveness in production, productivity and quality as compared to its competitors in the global market. There has been an impressive growth in the export of horticultural products from India, especially fruits and during 2019-20, the total export of fruits from India was 0.51 million tonnes, whereas it imports 6.61 million tonnes [3].Mango is the main fruit of Asian countries, though it is produced all over the world. The global estimated production of mango was 55.86 lakh tonnes on an area of 5.59 lakh hectares. It is quite a matter of pride that 25.63 lakh tones of mango were produced on an area of 2.58 lakh ha in India. India is the leading nation in mango production, contributing nearly 45.89 per cent of global production, followed by China (9.25 per cent) and Pakistan (6.06 per cent). The prominent mango-growing states are Andhra Pradesh, Uttar Pradesh, Karnataka, Bihar, Gujarat and Tamil Nadu. As such Uttar Pradesh ranks first in mango production with a share of 23.47 per cent and the highest productivity. India is one of prominent exporters of fresh mangoes to the world. The country exported 49,658.68 MT of fresh mangoes to the world for the worth of 400.21 crores (456.11 million) during 2019-20 [3]. The major export destinations of Indian mango are United Arab Emirates, UK, USA, Oman and Qatar. Despite a significant mango production base, this weak position of India in its major export markets can be attributed to its differences in varietal preferences and seasonal disparities. Therefore, against this backdrop, the present study will provide us with the present status and export performance of Indian mango in the world market by focusing on related aspects.

METHODOLOGY

Nature and Sources of Data

The study was conducted to analyse the export competitiveness of mango using secondary data. The time-series data covering 1987-1988 to 2021-2022 on export quantity, export value, unit values were obtained from FAO and APEDA websites. The tabular analysis was used to know the trends in export and export competitiveness. Inferences were drawn using averages and percentages

Compound Annual Growth Rate

The exponential function of the following form was used to estimate the compound annual growth rate [4].

$Y=ab^t$ …… (1)

By taking logarithm on both sides, it may be written as

Log y = log a + t × log b… (2)

Y = A + B × t

Where

Y = log y

A= log a

B= log b

Y= area (ha) / production (tons) and productivity (Kg / ha)

t= time elements, where1,2 and n represent different years.

A= intercept

B= regression co- efficient

Compound growth rate

= (Antilog of B- 1) × 100

t = r/ SE (r)

Where,

r = Compound Growth Rate

SE= Standard Error

Instability Index

The extent of variability in the quantity and value of exports examined by using Cuddy-Della Valle Index [5]. In time series data with long-term trends, the simple coefficient of variation overestimates the degree of instability, whereas the Cuddy-Della Valle index corrects the coefficient of variation. The Cuddy-Della Valle Index's calculation formula is

$$CDV\ (\%) = C.V \times \sqrt{1 - \overline{R^2}}$$

Where,
CVD = Cuddy-Della Valle Index
C.V = Coefficient of variation (in percent)
$\overline{R^2}$= Adjusted Coefficient of determination

Markov chain analysis

In the Markov chain analysis, a transitional probability matrix 'P' is developed, whose elements, P_{ij}indicate the probability of exports switching from country 'i' to country 'j' over time. The diagonal component Pij, where i=j, measures the likelihood that a country will maintain its market share or to put it another way, the commitment of an importing nation to a specific nation's products. In the context of current application, structural change was treated as a random process with eight importing countries for mango [6]. The assumption was that the average export of mango exports from a country amongst importing countries in any period depends only on the export in the previous period and this dependence is same for all the periods. This was algebraically expressed as

$$E_{jt} = \sum_{i=1}^{n} (E_{it-1})P_{ij} + e_{jt}$$

Where,
E_{jt}= Exports from India to the j^{th} during the year t
E_{it-1} = Exports to thei^{th}country during the year t-1
P_{ij} = The Probability that exports will shift from i^{th} country to j^{th} country
e_{jt} = The error term which is statistically independent of E_{it-1} and
n = The number of importing countries
The transition probabilities P_{ij}, which can be arranged in a (c × r) matrix, have the following properties:

$$\sum_{i=1}^{n} P_{ij} = 1 \text{ and } 0 < P_{ij} < 1$$

Thus, the expected share of each importing country during period 't' is obtained by multiplying the exports to these countries in the previous period (t-1) with the transition probability matrix. The probability matrix is estimated for exports from India and also exports are predicted for the period from 2012 to 2022. As a result, the transitional probability matrix (T) was calculated using a linear programming (LP) framework utilising a technique known as mean absolute deviation minimization (MAD)

Min OP* + Ie
Subject to,
XP* + V = Y
GP* = 1
P* > 0
Where,
P* - vector of the probabilities P_{ij} to be estimated
O - a vector of zeros
I- an appropriately dimensional vectors of areas

e - the vector of absolute errors (|U|)
Y- the vector of exports to each country
X- a block diagonal matrix of lagged values of Y
V- the vector of errors
G -is a grouping matrix that adds the row components of P that are ordered in P* to one.

RESULTS AND DISCUSSION

Growth and Instability Analysis of Mango Exports

Mango is an important horticultural crop, and India is one of the major mango producers globally, growing more than half of the global supply. Despite a tremendous spurt in the domestic production base of selected fruits, only a tiny part of mango was exported, nearly five percent of its production [7]. A detailed analysis of the export competitiveness of the Indian mango was carried out to know its performance in international markets. The annual compound growth rates of mango. During the WTO period (1987-2002) and Post WTO (2002-2012) period also the total mango export registered a negative growth rate of 4.67 percent, 8.16 percent which can increased to 13.43 percent in recent period (Table. 1). Interestingly, during recent WTO period (2012-2022), most of the countries registered negative growth rate but exemplary growth rate for Bangladesh. In addition, the instability index also increased for eight countries by exemplary higher overall instability (161.16 percent). The instability index is low (109.28) for exports in quantity terms and is medium (130.68) for exports. (Table. 2)

Market Composition of Indian Fresh Mango Export

Premium mango markets of the European Countries and developed countries have enacted and enforced plant health controls and certification system. Mango export to premium markets of the world by India is subjected to several measures to guarantee safe exports. Consumer preferences, protection of brand image, strict food regulations in the developed countries during 1990s have forced many countries to raise their food safety standards [8]. Super markets in the developed countries have responded to the changing regulatory and demand for all the products [9] Major importing countries of Indian mango are gulf countries like Bahrain, Qatar, UAE, Saudi Arabia, Kuwait; European Countries like United Kingdom, Netherland, USA, Singapore and Bangladesh. (Table. 4) In 2012 – 2013 total export of fresh mango on 55,584.97 MT but 2021-2022 total export of fresh mango it will be decreased in 26,376.94 MT (Table 4). UAE retained (45.27) per cent of its original share followed by Nepal (17.65%), UK (12.49%) and others (12.21%). During the period, India was losing its stronghold in new markets for mango export which was a bad sign for mango export in international market. It can be inferred that India has consolidated its position of mango export to its already existing markets.

Export share of fresh mango (Quantity) from India

The direction of fresh mangoes export from India during the period 2012-2022 has been changing over time. The dynamics in the direction of mango export and changing pattern in the trade, by shift in export share from one country to another country were analysed using Markov chain model. The trend in sustaining existing markets, the gains and losses in the export share of fresh mangos by importing countries were obtained from the transition probability matrix values. The diagonal elements represent the probability of retention of existing quantity of trade in fresh mango the future. For instance, the probability of retention of existing quantity of trade by UAE was estimated as 67.86 per cent. The major importing countries taken for analysis of trade in fresh mango exports during the period 2012-2022 were UAE, UK, QATAR, Kuwait, Nepal, Bahrain, Saudi Arabia and Bangladesh along with the remaining countries grouped under “others”. It is clearly evident that India’s export fresh mango UAE was retained to the tune of 67.86 per cent. The remaining 0.4 percent UK, 1.27 percent Nepal, 6.78 percent Bangladesh and 18.29 percent others country (Table.3). India retained 10.51 percent of its previous export share to UK. India could retain only 10.51 percent of its previous share to Kuwait, India’s previous export to Nepal 41.66 percent, 53.01 percent export share to Bahrain, 0.45 percent of its export shares to other countries. However, India retained 100 percent of its

previous export share to Saudi Arabia. India could not retain its previous export share to Qatar. The share of Qatar's market was lost to Kuwait (17.53%), Nepal (0.25%), Saudi Arabia (0.98%) and Bangladesh (69.97%).

CONCLUSION

India is the largest mango producing nation globally and their export can contribute significantly to the country's economic growth. Despite a vast production base, the export share of India is not very impressive, which is the cause of great concern. The model Markov Chain Analysis was applied to the export data and the transitional probability matrix provided a great deal of information on where to sell Indian mango to get the highest benefits. For mango, UAE was the loyal buyer with 67.86 per cent of the retention capacity of its previous year. Therefore, efforts are also needed to improve the efficiency of production and quality of mango to stabilize the markets and make the product acceptable and price competitive in other importing countries. Although India has a vast production base yet, the yield estimates were not that promising compared to the world, therefore, horticulturists also need to focus on this issue. The study suggests the need to diversify the India mango market. Steps should be taken by government exploit existing markets and explore new markets in India.

REFERENCES

1. Aarathi, L. R., Kumar, S., Negi, D., & Singh, D. R. (2012). Prevailing Standards and Dimensions Governing Sanitary and Phyto-Sanitary Compliance in Indian Black Pepper Supply Chain. Agricultural Economics Research Review, 25(1). https://EconPapers.repec.org/RePEc:ags:aerrae:126044
2. Anonymous. (2022). Horticultural statistics at a glance 2018 https://agricoop.nic.in/sites/default/files/Horticulture%20Statistics%20at%20a%20Glance-2018.pdf
3. Cuddy, J. D. A., & Valle, P. A. D. (February,1978). Measuring the instability of time series data. Oxford Bulletin of Economics and Statistics, 40(1), 79–85.
4. Dolan, C., & Humphrey, J. (2000). Governance and Trade in Fresh Vegetables: The Impact of UK Supermarkets on the African Horticulture Industry. The Journal of Development Studies, 37(2), 147–176.
5. India Export Statistics. (2022). APEDA. https://agriexchange.apeda.gov.in/indexp/genReport_combined.aspx
6. Jacob, A., & Job, E. (2015). Pepper production and export from India: Growth and instability analysis. International Journal of Current Research, 7(09),20388-20391
7. Mohit, Rajesh, & Aditi. (2016). Quantification of changing structure of indian mango exports using markov chain analysis. Produits Pharmaceutiques, 8(60), 3381–3384.
8. Shilpa, Sharma, & Ajit. (2021). Trends in production and export of major fruits in India. Indian Journal of Economics and Development, 17(2), 462–467.
9. Shilpa, Sharma, R., & Sharma, A. (2019). Trends, Growth and Variability of Apple in Himachal Pradesh: 1973-2016. Agricultural Science Digest - A Research Journal, 39(04). doi: 10.18805/ag.d-5003

Table: 1 Compound Growth Rates of Export in Fresh Mangoes from India During 1987 to 2022.

S.No	Country	1987-88 to 2001-2002	2002-2003 to 2011-2012	2012-2013 to 2021-2022	over all 1987 to 2022
1	UAE	-1.64	6.06	-10.38	2.66
2	UK	6.57	11.75	8.75	4.25
3	Qatar	-2.19	20.18	10.38	3.76
4	Kuwait	1.62	7.25	-8.39	-1.49
5	Nepal	34.01	-91.71	2.70	32.37
6	Bahrain	-0.17	-97.69	-3.88	-1.39
7	Saudi Arabia	0.64	-101.43	-17.70	-5.59

8	Bangladesh	83.18	4.00	-51.31	-1.40
9	Others	24.27	-7.43	4.57	5.19
10	Total	-4.67	-8.16	13.43	-11.34

Table: 2 Instability of Export in Fresh Mango from India During 1987 to 2022.

S.NO	Country	1987-88 to 2001-2002	2002-2003 to 2011-2012	2012-2013 to 2021-2022	over all 1982 to 2022
1	UAE	16.54	19.89	16.76	**35.13**
2	UK	31.03	22.71	39.11	41.86
3	Qatar	53.89	72.6	30.83	81.46
4	Kuwait	37.17	41.38	84.07	72.04
5	Nepal	134.37	55.65	68	59.63
6	Bahrain	48.07	33.16	39.77	0.11
7	Saudi Arabia	43.35	36.18	39.01	47.28
8	Bangladesh	56.26	33.5	75.37	114.73
9	Others	36.61	35.97	46.27	49.98
10	Total	109.28	161.16	130.68	102.41

Table 3. Transition probability matrix of fresh mango export during period from 2012 to 2021

Country	UA E	UK	Qatar	Kuwait	Nepal	Bahrain	Saudi Arabia	Bangladesh	Others
UAE	**0.67869**	0.00457	0.00000	0.00000	0.12705	0.00000	0.00000	0.00678	0.18291
UK	0.00000	**0.10513**	0.07940	0.00000	0.07053	0.64602	0.09892	0.00000	0.00000
Qatar	0.00000	0.00000	**0.00000**	0.17532	0.02599	0.00000	0.09892	0.69978	0.00000
Kuwait	0.00000	0.40035	0.00000	**0.10513**	0.01558	0.00000	0.05931	0.41962	0.00000
Nepal	0.00000	0.35451	0.22883	0.00000	**0.41665**	0.00000	0.00000	0.00000	0.00000
Bahrain	0.00000	0.17872	0.11932	0.00000	0.04412	**0.53015**	0.12769	0.00000	0.00000
Saudi Arabia	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	**1.00000**	0.00000	0.00000
Bangladesh	0.00000	0.00000	0.02723	0.27515	0.04959	0.00000	0.11788	**0.53015**	0.00000
Others	0.00000	0.00556	0.25566	0.00455	0.62375	0.03552	0.00257	0.02641	**0.04598**

Table 4. Fresh Mango Export from India to its Major Trading Partners (2012-2022)

	2012-13	2013-14	2014-15	2015-16	2016-17	2017-18	2018-19	2019-20	2020-21	2021-22
UAE	37598.64 (67.64)	23046.65 (55.83)	29231.9 (67.98)	19974.29 (54.31)	28483.16 (53.99)	23542.53 (47.87)	16398.18 (35.26)	16567.22 (33.36)	12756.4 (60.65)	11939.6 (45.27)
U K	3304.48 (5.94)	3381.08 (8.19)	329.8 (0.77)	1496.28 (4.07)	3030.79 (5.74)	3728.45 (7.58)	4014.06 (8.63)	4356.26 (8.77)	2471.69 (11.75)	3293.49 (12.49)
Qatar	1522.89 (2.74)	770.08 (1.87)	998.1 (2.32)	1016.25 (2.76)	2254.19 (4.27)	2321.89 (4.72)	2877.58 (6.19)	2744.95 (5.53)	1762.11 (8.38)	1964.3 (7.45)
Kuwait	828.16 (1.49)	4601.44 (11.15)	787.28 (1.83)	748.35 (2.03)	1100.19 (2.09)	1300.31 (2.64)	1057.03 (2.27)	1170.85 (2.36)	515.96 (2.45)	638.83 (2.42)
Nepal	2237.62 (4.03)	1106.44 (2.68)	3574.93 (8.31)	8704.85 (23.67)	9415.38 (17.85)	7878.09 (16.02)	6975.07 (15.00)	11975.04 (24.12)	384.89 (1.83)	4656.15 (17.65)
Bahrain	497.49 (0.90)	634.54 (1.54)	658.71 (1.53)	747.79 (2.03)	1086 (2.06)	1288.28 (2.62)	765.08 (1.64)	700.33 (1.41)	382.63 (1.82)	335.55 (1.27)
Saudi Arabia	1665.43 (3.00)	1721.91 (4.17)	2171.49 (5.05)	1399.91 (3.81)	2371.99 (4.50)	2670.5 (5.43)	1638.53 (3.52)	1517.39 (3.06)	166.41 (0.79)	329.09 (1.25)
Bangladesh	4650.21 (8.37)	2899.85 (7.02)	2475.33 (5.76)	46.3 (0.13)	1158.2 (2.20)	168 (0.34)	4813.62 (10.35)	3038.11 (6.12)	0 (0.00)	0 (0.00)

Others	3280.05 (5.90)	3117.94 (7.55)	2770.57 (6.44)	2645.17 (7.19)	3860.99 (7.32)	6281.95 (12.77)	7970.88 (17.14)	7587.71 (15.28)	2593.44 (12.33)	3219.93 (12.21)
Total	55,584.97 (100.00)	41,279.93 (100.00)	42,998.11 (100.00)	36,779.19 (100.00)	52,760.89 (100.00)	49,180.00 (100.00)	46,510.03 (100.00)	49,657.86 (100.00)	21,033.53 (100.00)	26,376.94 (100.00)

Fig. 1 Compound Growth Rates of Export in Fresh Mangoes from India During 1987 to 2022.

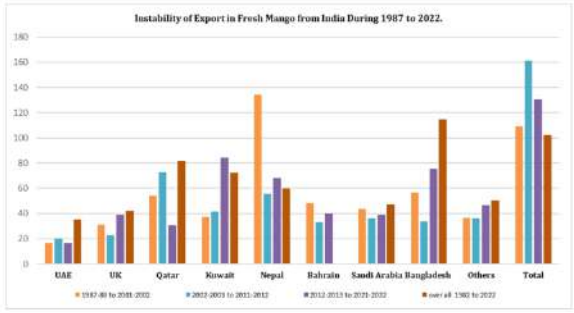

Fig. 2: Instability of Export in Fresh Mango from India During 1987 to 2022.

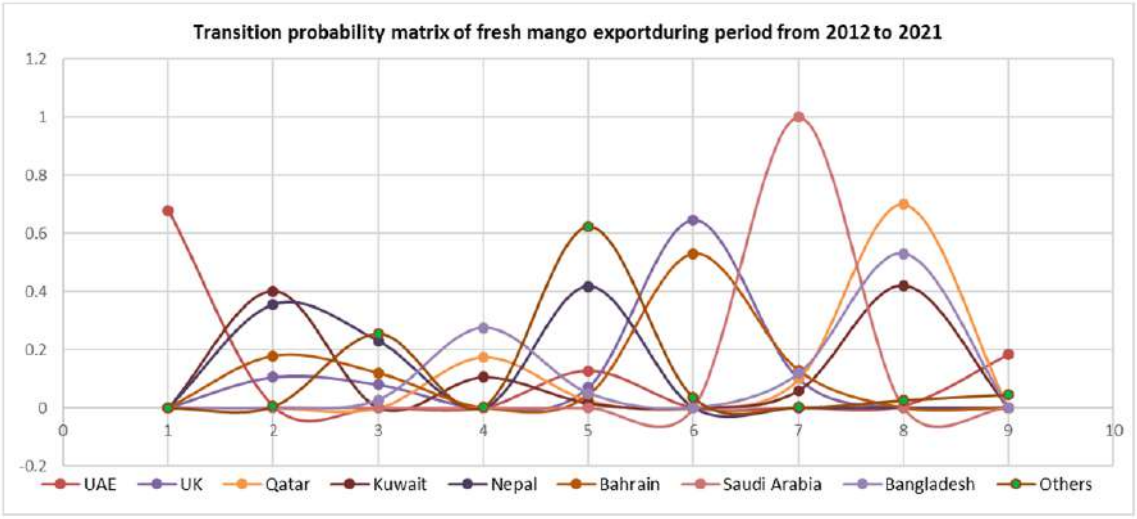

Fig. 3: Transition probability matrix of fresh mango export during period from 2012 to 2021

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RESEARCH ARTICLE

Effect of an Indigenously Developed Synbiotic Complex on Indoxyl Sulphate in Patients with Chronic Kidney Disease

Divya. J.S[1], Hemamalini. A.J[2]*, Soundararajan. P[3] and Priya Iyyer[4]

[1]Ph.D Research Scholar, Sri Ramachandra Institute of Higher Education Research, Chennai, Tamil Nadu, India.
[2]Professor and Head, Department of Clinical Nutrition, Sri Ramachandra Institute of Higher Education Research, Chennai, Tamil Nadu, India.
[3]Professor and Head, Department of Nephrology, Saveetha University, Chennai, Tamil Nadu, India.
[4]Associate Professor and Head, Department of Biotechnology, Women's Christian College Chennai, Tamil Nadu, India.

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***Address for Correspondence**
Hemamalini. A.J
Professor and Head,
Department of Clinical Nutrition,
Sri Ramachandra Institute of Higher Education Research,
Chennai, Tamil Nadu, India.
Email: hemamalini.aj@sriramachandra.edu.in

ABSTRACT

Patients with chronic kidney disease (CKD) show an increase in bowel aerobic bacteria that produce uremic toxins and decreased anaerobic bacteria such as bifid bacteria and lactobacillus which are reported to be helpful in reducing uremic toxins. However, to date, quality intervention trials investigating the novel treatment of evaluating effect of probiotic therapy in CKD are limited. The aim of this study is to assess the effectiveness of synbiotics as a potential treatment against the synthesis of uremic toxins, specifically, indoxyl sulphate (IS). An indigenous synbiotic complex, containing prebiotic and probiotics was prepared, standardized and a daily dose of 100 ml of synbiotic complex containing ($20*10^9$) CFU was supplemented for twelve weeks to the randomly assigned 40 participants in the experimental group, after obtaining their informed consent. In all patients, Glomerular Filtration Rate, Creatinine, indoxyl sulphate and blood uric acid were evaluated. Stats: Mann Whitney U test was used to test the median values between two groups and Wilcoxon Signed rank test was used to compare the median values within control groups and experimental groups. All statistical tests were performed using SPSS 23.0 and any statistical tests with p – value less than 0.05 was considered as statistically significant**.** There was a significant reduction in serum IS level in the supplemented group ($p=0.000 < 0.05$)with respect to the baseline measurement. The synbiotic complex also significantly reduced the creatinine levels which also reflected in the significant improvement in the GFR($p<0.001$)**.** This indigenous synbiotic

complex has significantly influenced the reduction in levels of BUN and Creatinine. The levels of uremic toxin indoxyl sulphate was also significantly reduced among the study participants in the experimental group while there is no significant changes in the baseline uremic toxins and other renal parameters among the control group. Hence, this indigenously developed synbiotic complex, can be considered as an effective adjuvant treatment strategy for bringing down the uremic toxin indoxyl sulphate.

Keywords: Uremic toxins. Probiotics. Prebiotics, Synbiotics, Chronic kidney disease, Indoxyl sulphate, Blood urea

INTRODUCTION

Currently, many studies prove that certain food items have some valuable effect on specific functions associated with human health and have some significant nutritional value. Such type of foods is currently commonly consumed and preferred by physicians to preventing or treating diseases [1] . Thus, the concept of "functional food" has arisen, and it is defined as a product, modified food or nutritional ingredient that can exert beneficial health effects other than its traditional nutritional value [2]. Probiotics, prebiotics and synbiotics have obtained a relevant role in the field of functional foods. It is proposed that very nearly 66% of people with uremia have anomalies in the gastrointestinal mucosa and disequilibrium in the GI ecosystem [3]. Most of these progressions occur at the ileum level and in the colon, where the combined genetic material of the microorganisms assumes a significant part. The uremic syndrome is provoked by a progressive number of compounds that are normally excreted by kidneys in healthy individuals. At least 90 compounds, often called uremic toxins, like indoxyl sulphate (IXS) and p-cresylsulphate (PCS), have been found increased in subjects with ESRD creating great harm to biological systems [5][6]. In addition, in a study of Schepers *et al*., it has been shown that PCS stimulates the basic leukocyte activity with pro-inflammatory effects, inhibiting the activated leukocyte function and inducing endothelial disorder [7]. A condition that could induce cardiovascular events. PCS, a phenol 108 Da MW, is a terminal product of protein catabolism, produced by intestinal bacteria that metabolize tyrosine and phenylalanine [8,9]. IXS causes an endothelial disorder of the uremia, promoting the proliferation of smooth muscle cells through the activation of growth factors derived from platelets and inducing a significant production of free radicals by endothelial cells [10,11]. The aim of this study is to Develop the Probiotic Enhanced Prebiotic supplementation, A Synbiotic complex in reducing the levels of Indoxyl Sulphate, one of the uremic toxins .

OBJECTIVES

- To develop and standardize a probiotic enhanced prebiotic supplement- a symbiotic complex
- To supplement the Synbiotic complex and evaluate its effect on the biochemical parameters- such as creatinine, blood urea nitrogen, indoxyl sulphate test.

Hypothesis

- There is no significant effect of supplementation of the Synbiotic complex on the creatinine, blood urea nitrogen, indoxyl sulphate test

MATERIALS AND METHODS

The study comprised of two phases

Phase: I Development and Standardisation of the Synbiotic complex.

Phase: II Supplementation of Synbiotic complex to the study participants

Phase I: Development and standardization of the Synbiotic Complex comprised of 4steps

STEP: I *Synbiotics* is defined as a mixture of probiotics and prebiotics that beneficially affects the host by improving the survival and activity of beneficial microorganisms in the gut." (Ranganathan et al., 2010).

Development of Synbiotic Complex was carried out in four steps:

SELECTION OF PREBIOTIC

Uremic illness is considered to be due largely to the accumulation of organic waste products that are normally cleared by the kidney. Uremic retention solutes are generated in part in the gastrointestinal tract (GIT), with the gut microbiota and the ensuring micro-biometabolome playing a significant role in the proliferation of uremic retention solutes (T Niwa etal.,2011). Moreover, such a uremic toxin is produced to a greater degree by the intestinal bacterial flora in uremic patients because of abnormally high levels of urea and creatinine, which diffuse into the gut and may affect the composition of microflora. The intake of lactic acid, such as lactobacillus species, and streptococcus thermophilus effectively restores the disturbed microflora to a normal one it ferments carbohydrates to produce acetic acid and lactic acid that inhibit the intestinal putrefaction. This raises the possibility that lactobacillus to uremic patientsmay reduce the level of indoxyl sulphate by normalization of the microflora. It was found that the intake of prebiotic inulin, enriched with oligofructose, could significantly reduce serum concentrations of IXS. Cereals were found to be effective in reducing uremic toxins among the patients with chronic kidney disease (Ishikawa Ramos et al.,2019). Prebiotic are largely reported to be present in different types of cereals (Lamsal and Faubion,2009). In order to prepare the Synbiotic complex four cereals were chosen as prebiotic base, based on its prebiotic content (Ranganathan etal., 2013, M. Rossi etal., G.R. Gibson, 2000). The Following cereals were chosen for the study:Finger Millet or Eleusine coracana (Ragi) (Anisseri and Gudipati, 2012), Pearl millet or Pennisetum glaucum(Bajra) (Lamsal B.P, 2009). Avena sativa(Oats)and Hordeum vulgare (Barley) (Codex alimentarius 2010).

Selection of probiotic Culture:

Based on the extensive survey of published research probiotic microorganisms such as *Lactobacillus acidophillus, Lactobacillus casei, Streptococcus thermophilus Lactobacillus bulgaricus* were found to be effective in reducing uremic toxins among the patients in chronic kidney Disease (Gibson etal., GR, Roberfroid MB (1995). Therefore, curd and yogurt which contains *Lactobacillus Species* were chosen as the probiotic culture medium.

STEP -III Preparation of the Synbiotic complex

The selected prebiotic cereals were completely washed and sun dried for period of 24hrs. Dried samples were grounded into a fine powder using standard techniques. The powders were stored at room temperature in individual sterile containers for further use in the experiment. The finely grounded Cereal powders namely Finger Millet or *Eleusine coracana*(Ragi) Pearl millet or *Pennisetum glaucum*(Bajra), *Avena sativa* (Oats), *Hordeum vulgare* (Barley)were individually prepared into a thicker consistency, by using 10g of the cereal powder boiled in 150ml of water at 100^0 C for 15minutes. Each Mixture was then allowed to cool at room temperature for a period of 20-25mins.To this prebiotic base 25ml each of curd and yogurt was added and subjected to fermentation for period of 12hrs overnight under room temperature. This fermented product was carried under Aseptic conditions to the NABL Accredited lab for evaluating the microbial potentials.

Evaluation of CFU of the culture medium

Sterilization of Autoclave was carried out using standard techniques.

MRS Agar

Lactobacilli MRS Agar was used for the cultivation of *Lactobacilli*. *Lactobacilli* MRS Agar is based on the formulations of deMan, Rogosa and Sharpe (MRS). This medium supports luxuriant growth of lactobacilli from oral, faecal, dairy, and other sources. Enzymatic Digest of Animal Tissue, Beef Extract, and Yeast Extract are the carbon, nitrogen, and vitamin sources used to satisfy general growth requirements in *Lactobacilli* MRS Agar. Dextrose is the fermentable carbohydrate. Sodium Acetate is an inhibitory agent. Sodium Acetate and Ammonium Citrate act as selective agents as well as energy sources. Potassium Phosphate is the buffering agent. Magnesium Sulphate and Manganese

Sulphate provide cations used in metabolism. Polysorbate 80 is a surfactant, facilitating uptake of nutrients by lactobacilli. Agar is the solidifying agent.

Serial dilution

One mL from the sample was serially diluted up to 10^{-12} dilutions. Then 0.1 mL from 10^{-2}to10^{-12}dilution was used for pour plate method of enumeration of bacterial colonies. Nutrient agar and MRS Agar were used. Then the plates were incubated at 37°C to 48°C for 37 hours. Microscopic evaluation of numbers of colony forming units was carried out using standard techniques. The prebiotic mixture containing Barley with curd and yogurt was found to be having optimal CFU that is $60*10^9$ with *lactobacillus*, *Streptococcus* species. The colony forming units and shelf life of the developed symbiotic complex was ascertained using repeated estimations for two consecutive batches, using standard procedure. This Synbiotic complex was selected to be the product of supplementation and was further subjected to organoleptic evaluation before supplementation. Organoleptic evaluation of the Synbiotic complex was carried out using 9- point Hedonic Scale among 30 panellist comprising Nutritionist, non-nutritionist and patients with CKD stages 3&4. Organoleptic evaluation obtained high accessibility Scores from the panellist and hence the product was Considered as suitable for supplementation. Nutrient analysis of the finalised product was carried out in NABL accredited lab.

Supplementation Phase

After Screening 125 Patients with CKD visiting OPD of a Multi-speciality care centre based on inclusion and exclusion criteria, 80Patients were chosen to be the study Participants based on the inclusion and exclusion criteria and were assigned randomly to experiment and control group, with 40 in each group, after obtaining their informed consent. During the course of the study period, there were five dropouts from the study group due to personnel issues. The study participants lost for follow up were replaced with new set of CKD chosen based on inclusion Criteria. The sample size for the study was estimated using the study, "Effect of Probiotics on Human Blood Urea levels in patients with chronic renal failure, Paola Vanessa Miranda Alatriste *et al.,* 2011. The sample size was calculated by Assuming equal sample size (k = 1) for both cases and controls, with an additional mean difference of 0.5 units, level of significance of 5% and the power of 80%. The required minimum sample size for each group was calculated to be n= 40. Thus, for first group, n A = k * n B = 1 * 40 = 40, The study participants were assigned randomly to the study group and control group, through Computer-generated randomization of participants to either the experimental or the control group. Informed consent was obtained from the study participant before the initiation of supplementation, and IEC approval obtained. The supplementation of the Synbiotic complex developed using Barley, Curd and yogurt was provided to study participants with CKD stage 3 and 4, selected based on inclusion and exclusion criteria. The product to be supplemented was handed over to the study participants in five individual sterile containers with 100ml each given for a period of five days for consumption. It was distributed to the study participants in sterile containers packed using thermocol box maintained at 4^0 C and instructed to be refrigerated at 20^0 C immediately after removing from the thermocol box and consumed half an hour before breakfast after thawing to room temperature, everyday. The product of supplementation was given once in five days. The adherence to the consumption of supplementation was ensured on day-to-day basis through telephone calls and review at OPD once in five days. The nutritional status of the study participants was assessed at baseline and at end of the supplementation period using the validated SGA questionnaire. Anthropometry parameters such as Height and Weight of the study participants were measured using standard techniques, at baseline and at the end of the supplementation period. The Impact of the developed Synbiotic complex on the renal profiles such as creatinine and Blood Urea Nitrogen and the uremic toxin Indoxyl Sulphate compound was analysed at baseline and at the end of the supplementation period of 12 weeks.

Statistical Analysis

The variables used in this study is a combination of quantitative and qualitative variables. Descriptive statistics was performed for continuous variables and frequency distribution is used for qualitative variables. Mann Whitney U test was used to test the median values between two groups and Wilcoxon Signed rank test was used to compare the median values within control groups and experimental groups. Chi square test for independence was used to assess

the association between two qualitative variables. All statistical tests were performed using SPSS 23.0 and any statistical tests with p – value less than 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

This figure represents the percentage of patients diagnosed with stage 3 and 4 Chronic Kidney Disease. In the experimental group 15% and in the control group 13% of the study participants were diagnosed with CKD 4 and 85% and 87% were diagnosed to be in stage 3 of CKD in both the groups respectively. There was a significant reduction in the number of severely malnourished between the study group and control group. Following supplementation in the experiment group there was significant improvement in the status of malnutrition though the difference observed was not statistically significant among the mild to moderate malnourished subjects' status of malnourished improved. The table- 3 presents Anthropometric data of the study participants in both the groups It can be observed, that there is no significant weight difference within the experimental and the control group. Creatinine is cleared by the kidneys with minimal tubular reabsorption. Creatinine is together with urea, the most widely known "uremic toxin", and is usually assessed whenever a reduction in kidney function is suspected. This is mainly because creatinine evaluation is cheap, widely accessible and relatively well reflects the renal function. It also forms the basis for estimation of estimated glomerular filtration rate (eGFR) and thus is a major component of all principal eGFR equations. Creatinine accumulates in the blood when GFR decreases in the setting of renal dysfunction. As a result, serum creatinine levels are commonly used as a surrogate for GFR and renal function. Vaz Perez et al., (2009). Glomerular filtration rate is considered the best overall measurement of kidney function and correlates well with disturbance in renal function. Small-scale studies using creatinine-based GFR estimates suggest that probiotic supplementation may be helpful in delaying disease progression in those with CKD.

The table above depicts the Urea, Creatinine and GFR values of the study participants. It is evident from the table that there is a significant reduction in creatinine and a significant improvement in the GFR rate, among the study participants in the experimental group after supplementation with the Synbiotic complex. No such significant improvement/reduction in the relevant renal parameters were observed in the control group The supplementation with Synbiotic complex for the period of 12 weeks to the experimental group has brought down indoxyl sulphate significantly ($p<0.001$) .As regards indoxyl test (IXS) solute is metabolized by the liver from the indol, which is produced by the intestinal flora as a metabolite of tryptophan. IXS causes an endothelial disorder of the uremia, promoting the proliferation of smooth muscle cells through the activation of growth factors derived from platelets and inducing a significant production of free radicals by endothelial cells. IXS appears to have a clinically important role in aortic stiffness and vascular calcification of IXS. It come from bacterial fermentation of the proteins in the large intestine: the colonic microbiota degrades tryptophan to indole. Therefore, in renal failure conditions, the altered intestinal bacterial metabolism changes serum concentrations of IXS, it was found that the intake of prebiotic inulin, enriched with oligofructose, could significantly reduce serum concentrations of IXS (Megan Rossi 2014). Probiotics have been defined as living organisms in food and dietary supplements that, upon ingestion, can improve the health of the host beyond their inherent basic nutritional content Lulis etal., 2013. In comparison, prebiotic are nondigestible food ingredients that stimulate the growth and/or activity of bacteria in the GIT. There is a deficit of relevant clinical trials in the scientific literature. This clinical study employed a lactobacillus species and it reported significant reduction in uremic toxins. Ranganathan etal.,2010have also reported efficacy, following symbiotic supplements among chronic kidney disease patients in reducing uremic toxins.

CONCLUSION

Treatment with the synbiotic complex resulted in significant reduction of serum Indoxyl sulphate and Creatininein patients with advanced CKD. Given the demonstrated association of this gut–derived uremic toxin with cardiovascular disease and CKD progression, future studies are needed to explore the effect of long-term administration of synbiotics with a higher amount of prebiotic component on clinical outcomes in CKD. More

research into cost-effective treatments, such as pre- and probiotics, is critical given the rising prevalence of CKD, high mortality and morbidity rates, and high treatment costs.

REFERENCES

1. De las Cagigas Reig AL and Anesto JB. Prebióticos y probióticos, una relación beneficiosa. Revista Cubana Aliment Nutr,2002; 16: 63-8.
2. Chow J. Probiotics and prebiotics: A brief overview. J Ren Nutr.2002; 12 (2): 76-86.
3. Kang JY. The gastrointes tinal tract in uremia. Dig Dis Sci,1993; 38 (2): 257-68.
4. Cohen G, Glorieux G, Thornalley P, Schepers E and Meert N. European Uremic Toxin Work Group (EUTox): Review on uraemic toxins III: recommendations forhandling uraemic retention solutes in vitro—towards a standardized approach for research on uraemia. Nephrol Dial Transplant,2007; 22: 3381–3390.
5. Vanholder R, Schepers E, Pletinck A, Nagler EV and Glorieux G. The uremic toxicity of indoxyl sulfate and p-cresyl sulfate: a systematic review. J Am Soc Nephrol, 2014; 25: 1897–1907.
6. Schepers E, Glorieux G and Vanholder R. The gut: The forgotten organ in uremia. Blood Purif , 2010; 29: 130–136.
7. Barreto FC, Barreto DV, Liabeuf S, Meert N and Glorieux G. Serum indoxyl sulfate is associated with vascular disease and mortality in chronic kidney disease patients. Clin J Am Soc Nephrol, 2009; 4: 1551–1558.
8. Ruben Poesen, Liesbeth Viaene, Kristin Verbeke, Patrick Augustijns and Bert Bammens. Cardiovascular disease relates to intestinal uptake of p-cresol in patients with chronic kidney disease. BMC Nephrology, 2014.
9. Wu IW, Hsu KH, Lee CC, Sun CY and HsuHJ. p- Cresyl sulphate and indoxyl sulphate predict progression of chronic kidney disease. Nephrol Dial Transplant, 2015; 26: 938–947.
10. Fishberg AM. Hypertension and Nephritis, ed 5, Philadelphia, Lea and Febiger, 1954.
11. De Preter V, Vanhoutte T, Huys G, Swings J, Rutgeerts P and Verbeke K. Effect of lactulose and Saccharomyces boulardii administration on the colonic urea-nitrogen metabolism and the bifidobacteria concentration in healthy human subjects. Aliment Pharm Ther, 2006; 23: 963-974.
12. Viaene L, Lutgarde T, Yu J, Yanping L and Yumei G. Heritability and Clinical Determinants of Serum Indoxyl Sulfate and p-Cresyl Sulfate, Candidate Biomarkers of the Human Microbiome Enterotype. PLoS One, 2014; 9(5): e79682.
13. D'Apolito M, Du X, Zong H, Catucci A and Maiuri L. Urea-induced ROS generation causes insulin resistance in mice with chronic renal failure. J Clin Invest, 2010; 120: 203–213.
14. Wu IW, Hsu KH, Hsu HJ, Lee CC and Sun CY. Serumfree p-cresyl sulfate levels predict cardiovascular and all-cause mortality in elderly hemodialysis patients—a prospective cohort study. Nephrol Dial Transplant, 2012; 27: 1169–1175.
15. Wu IW, Hsu KH, Lee CC, Sun CY and HsuHJ. p- Cresyl sulphate and indoxyl sulphate predict progression of chronic kidney disease. Nephrol Dial Transplant, 2015; 26: 938–947.
16. Torre A and Vargas F. Effects of short-term administration of a lactobacillus casei drink (yakult) on the intestinal flora, intestinal permeability, endotoxins, cytokines, ammonium and nitric oxide in patients with chronic renal failure. National Autonomous University of Mexico, México D.F, 2004.
17. Rossi M, Johnson DW, Morrison M, Pascoe EM, Coombes JDS, Forbes JM, Szeto C-C, McWhinney BC, Ungerer JPJ and Campbell KL. Synbiotics easing renal failure by improving gut microbiology (SYNERGY): A randomized trial. Clin J Am Soc Nephrol, 2016; 11: 223–231.
18. Vaziri ND, Liu SM, Lau WL, Khazaeli M, Nazertehrani S, Farzaneh SH, Kieffer DA, Adams SH and Martin RJ. High amylose resistant starch diet ameliorates oxidative stress, inflammation, and progression of chronic kidney disease. PLoS One, 2014; 9:e114881, 2014.

Table 1: Nutritional status of the study participants assessed using SGA tool

Study Groups	SGA [n=80]		
	Mild	Moderate	Severe
Experiment (n=40)	33	5	1
Control (n=40)	32	4	4

Table 2: Anthropometry Data of the Study Participants

Parameter	**Study Groups**		
	Experiment n=40	Control n=40	p-Value
Height (Cms)	164.0±10.14	160.33±9.65	NS
Weight (Kg)	65.12±10.68	62.66±10.71	NS

Parameter	**Study Groups**		
	Experiment n=40	Control n=40	p-Value
Height (Cms)	164.0±10.14	160.33±9.65	NS
Weight (Kg)	66.22±10.17	62.53±10.81	NS

Table-3: Comparison of mean biochemical parameters of the subjects experimental and control groups

Parameter	**Experiment n=40**			**Control n=40**		
	Initial	**Final**	**p- Value**	**Initial**	**Final**	**p-Value**
Urea(mg/dl)	43.34±17.64	41.0±14.22	NS	45.02±12.41	44.03±10.67	NS
Cr(mg/dl)	2.69±1.18	1.95±1.03	p<0.001*	2.20±0.94	2.18±0.81	NS
GFR (mL/min/1.73m^2)	32.88±13.5	47.91±19.45	P<0.001*	39.81±15.36	38.82±14.98	NS

Table 4: Indoxyl Sulphate levels Of The Experiment and The Control Group

PARAMETER	INDOXYL SULPHATE(IXS/mg/l)		p-VALUE
	Initial	Final	
Experiment (n=40)	1.29±0.40	0.60±0.31	p<0.001*
Control (n=40)	1.37±0.49	1.49±0.47	NS

Fig. 1: The following figure represents the patients with CKD among the study participants

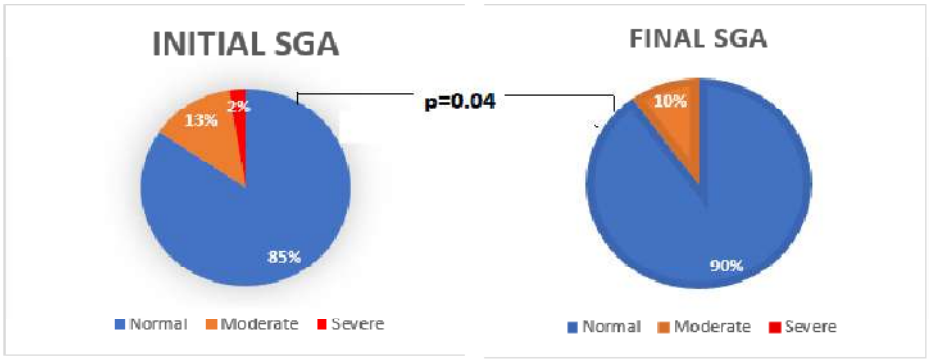

Fig. 2: Experimental Group: Nutritional data of the study participant at the end of the study period p=0.04* (p <0.05 Significant)

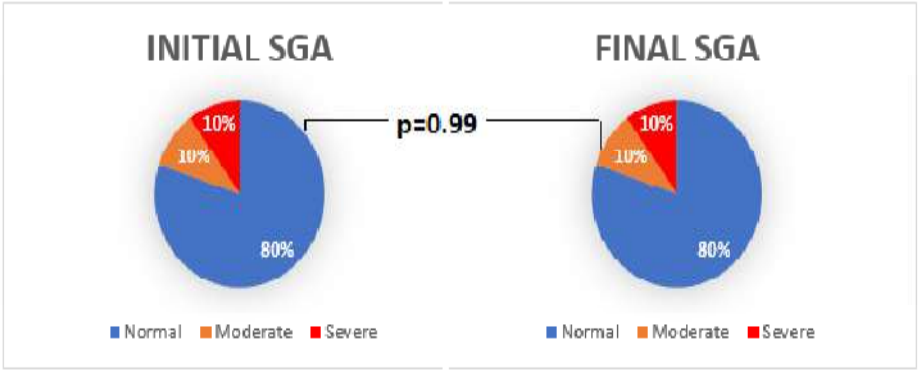

Fig. 3: Control Group: Nutritional data of the study participant at the end of the study period p=0.99 (p>0.05 NS)

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RESEARCH ARTICLE

Consumer (Rural Farmers) Awareness towards Consumer Rights in FMCG

Anandan Aritheertham[1], Bimal Thapa[2], Jeevanantham.N[3], Jenniffer Ravichandran[1] and M. Sampath Nagi[4]*

[1]Assistant Professor, Government Law College, Coimbatore, Tamil Nadu, India.
[2]Assistant Professor, Department of Management, Sikkim Alpine University, Namchi, Sikkim, India.
[3]Assistant Professor, Department of Business Administration, Government Arts and Science College, Valparai, Tamil Nadu, India.
[4]Assistant Professor, Department of Management, Pondicherry University, Port Blair Campus, Andaman and Nicobar Island, India.

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***Address for Correspondence**
M. Sampath Nagi
Assistant Professor,
Department of Management,
Pondicherry University, Port Blair Campus,
Andaman and Nicobar Island, India.
Email: sampathnagi@gmail.com

ABSTRACT

Fast Moving goods became a basic necessity in human life. In this paper, an effort has been made to seek out the determination of consumer awareness about their rights in FMCG. On the opposite hand, consumers should be aware not only of the commercial aspects of sale and get of products but also of the health and security aspect. Therefore, Consumer exploitation should be bought down with proper awareness to the consumers on their rights and the way them may act with things, if they feel they have been cheated. The research study transpires in Tamil Nadu state. The study refers to rural farmers residing at Tamil Nadu State (Sampath & Sathish, 2021). Since the population of study is not clearly available, the researcher considered the population to be infinite would be infinite. The primary data were collected from 100 respondents using a structured questionnaire. The collected primary data have been analysed using the SPSS software (Statistical Packages for Social Sciences). The Frequency Distribution, Measures of Central Tendency, Measures of Dispersion and Bi variate correlation were used to analyse the data.This study concludes that majority of the respondents showed low level of awareness about right to choose, heard healthy environment and safety. Respondents who are aware about the consumer rights in seek redressal but they never lodge complaint against exploitation. Similarly, it proved that no consumers are willing to file case in the consumer court due to complicated procedure of filing complaint and due to wastage of time and money and it is suggested to conduct consumer

education and consumer awareness programs, public campaigns among rural and uneducated people and government should take necessary actions to minimize the procedure of filing case, speed up the redressal programs and provide various support to the consumers for their redressal.

Keywords: Consumer protection act, Consumer rights and FMCG

INTRODUCTION

Consumer awareness is that the understanding and knowledge that a buyer should have of his rights as a customer. The awareness is extremely important for the customer since it permits him to induce the foremost from what he buys. Consumer awareness is making the consumer awake to their rights. Consumer awareness could be a marketing term. It implies that consumers note or are aware of products or services, its characteristics and also the other marketing P's (place to shop for, price, and promotion). Consumer protection has earned a very important place within the political, economic and social agendas of the many nations. In India, the government has taken many steps including legislative, to safeguard consumers. Education could be a lifelong process of continually acquiring relevant information, knowledge and skills. Consumer education is a very important a part of this process and could be a basic consumer right that has got to be introduced at the school level. Consumers by definition include all citizens who are, by and huge the most important group, who are affected by almost all government, public or private decisions. The most important step in consumer education is awareness of consumer rights. However, consumer education is incomplete without the responsibilities and duties of consumers, and this influences individual behaviour to a good extent. Consumer Awareness is an act of constructing sure the customer or consumer is aware of the data about products, goods, services, and consumers rights. Consumer awareness is vital so buyer can take the proper decision and make the proper choice.

Consumer Protection Act 2019

In 2019, the consumer Protection Act was gone by the govt of India. The consumer Protection Act is known as COPRA. The most objective of this act is to make a decision the complaints of the consumers immediately and to create proceedings easy. A three-tier judiciary system has been established under COPRA at district, state and national level to resolve the disputes of consumers. The forum at district level hears the cases related to the claims up to Rs. 50 lakhs. The claims from Upto Rs. 2 Crore are heard within the state level forum. The forum at national level hears the cases with the claims of above Rs. 2 Crore. The Consumer Protection Act 2019 provides certain rights to consumers to protect themselves from unfair trade practices resorted by the seller. Every consumer must have awareness about their rights, at the identical time consumer should have awareness about responsibilities. A consumer must take certain precautions during purchase of the products. Consumer rights and consumer responsibilities both are knotted together. To protect the consumer rights, different mechanisms are established at different levels. Consumer Protection Act could be a weapon within the hands of consumers to fight against exploitation by traders, manufacturers and sellers on one hand and providers of services on the opposite. The Act provides effective, people oriented, broad based enacted with an objective to produce better protection of the interests of the consumers and to form provision for the establishment of Consumer Councils and other authorities for the settlement of consumer disputes. Unlike other laws, which are basically punitive or preventive in nature the provisions of the Act are compensatory. It's a matter of great satisfaction that we are able to legitimately boast that we now have in our country a statute, which provides simpler protection to the consumers than any corresponding legislation operative in countries, which are considered to be much more advanced and industrialized.

Consumer

Any person who buys goods and services for personal consumption and not for commercial purpose or for resale is consumer. Every consumer consumes different commodities and services from our birth to death. All commercial activity revolves around the consumer. Consumers are said to be the king of the fashionable market. They buy and consume numerous and form of products each day. They are the pillars of any economic development and are the

foundation for economic building and responsible for the transformation of resources into productive things Nevertheless, in reality, the consumer is cheated in numerous ways by middlemen. Exploitations is also within the variety of adulteration, under-weight of products, selling goods of inferior quality (Sampath et. al, 2022) and duplicated goods, charging higher prices, misleading advertisement within the media etc. the consumer isn't sure of getting quality goods manufactured and preserved in hygienic condition and at competitive prices. There's a requirement that customers be protected. Consumers are protected against fraudulent trade practices and, promote their general welfare, and establish standards of conduct for business and industry. It also emphasizes the rights of the consumers.

Fast Moving Consumer Goods (FMCG)

The Indian FMCG sector is that the fourth largest sector of the Indian economy. The FMCG industry is over 115 years old. Consumable items (other than groceries / pulses) that one must frequent regular intervals. These are items which are used daily, so have a fast rate of consumption, and a high return. FMCG can broadly be categorized into three segments which are home items as soaps, detergents, household accessories, etc, secondly attention items as shampoos, toothpaste, shaving products, etc and eventually. Thirdly Food and Beverages as snacks, processed foods, tea, coffee, edible oils, soft drinks etc.

History of FMCG in India

In India, companies like ITC, HLL, Colgate, Cadbury and Nestle are a dominant force within the FMCG sector well supported by relatively less competition and high entry barriers. These companies were, therefore, able to charge a premium for their products. During this context, the margins were also on the upper side. With the gradual opening from the economy over the last decade, FMCG companies are forced to fight for a market share. FMCGs can be divided into several different categories including Processed foods **(**Cheese products, cereals, and boxed pasta), Prepared meals (ready-to-eat meals), Beverages (bottled water, energy drinks & juices), Baked goods (cookies, croissants & bagels), Fresh, frozen foods, and dry goods (fruits, vegetables, frozen peas, carrots, raisins & nuts), Medicines (aspirin, pain relievers & other medication that can be purchased without a prescription), Cleaning Products (baking soda, oven cleaner, and window &glass cleaner), Cosmetics and Toiletries (hair care products, concealers, toothpaste & soap) and Office Supplies (pens, pencils & markers).

Forms of Consumer Exploitation

Consumer exploitation may be a variety of exploitation during which the consumers are exploited or cheated by the businessmen in terms of poor quality of product, underweight, under-measurement, duplicate articles and etc. A number of the common ways by which the consumers are exploited by the businessmen are underweight and under-measurement, sub-standard quality, high prices, duplicate articles, adulteration & impurity, lack of safety devices, artificial scarcity, false and incomplete information, unsatisfactory after sale services and rough behaviour. In order to safeguard consumer interest, six consumer rights were initially envisioned by consumer rights activists of namely: Right to Safety, Right to information, Right to Choice, Right to Heard, Right to Redress and Right to Consumer Education. In time, two more important rights were added viz.: Right to Basic Needs and the Right to a Healthy and Sustained environment. These two rights are very closely linked with the realities of developing countries were environment plays a really vital role as a resource and support structure for the people.

Right to Safety - Consumer right is defined because the right to be protected against marketing of products and services which are hazardous to life and property in areas like healthcare, food processing and pharmaceuticals, Automobiles, Travel, Domestic Appliances, Housing and etc.

Right to Choose - The right to be assured access to a variety of products at competitive prices, without any pressure to impose a sale, i.e., freedom of choice. Consumer Protection Act, 2019 defines this right as "the right to be assured, wherever possible, to own access to a variety of products and services at competitive prices".

Right to Seek Redressal - The right to "seek redressal against unfair trade practices or restrictive trade practices or unscrupulous exploitation of consumers" is defined because the right to redressal within the Consumer Protection Act 2019. Consumer courts like District Consumer Disputes Redressal Forums at the district level, State Consumer Disputes Redressal Commissions and National Consumer Disputes Redressal Commissions are established through the consumer Protection Act.

Right to Consumer Education - This right simply ensures that the consumers in India have access to informational programs and materials that might enable them to create better purchasing decisions.

Right to be Heard - This right is meant to empower Indian consumers to fearlessly voice their complaints and concerns against products and firms to make sure their issues are handled efficiently and expeditiously.

Right to be Informed - This consumer right is defined because the „the right to be informed about the standard, quantity, potency, purity, standard and price of products or services, because the case could also be so on protect the consumer against unfair trade practices.

Right to Basic Needs - Right to basic needs ensures basic goods and services which guarantee survival. It includes adequate food, clothing, shelter, health care, education and sanitation to guide a decent life.

Right to Healthy Environment - The right to physical environment that may enhance the standard of life. It includes protection against environmental dangers over which the individual has no control. It acknowledges the necessity to guard and improve the environment for present and future generations.

Duties and Responsibilities of Consumers

- The consumer contains a certain responsibility to hold as an aware consumer can bring changes within the society and would help other consumers to fight the unfair practice or be aware of it.
- They should be aware of their rights under the consumer Protection Act and may practice the identical just in case of need.
- They should be awake to the merchandise they're buying. Should act as a cautious consumer while purchasing the merchandise.
- If just in case a product is found of anything false or not satisfactory a complaint should be filed.
- The consumer should ask for a Cash Memo while making a purchase. A customer should check for the quality marks that are introduced for the authenticity of the standard of the merchandise like ISI or Hallmark etc.

LITERATURE REVIEW

Tyagi et.al (2019), studied about the "consumer buying behavior towards selected FMCG products". The motive of the paper is to identify the factors affecting consumer buying behaviour towards FMCG products and finally effecting their decision-making process. The study reveals that consumer behaviour is largely affected by place, product, price, promotion, physiological and psychological factors. However, effect of these factors also differs from product to product. Finally, to conclude it is said that almost every FMCG company has been riding the waves of growth in the last 20 years and it won't be any different in the future. The winners however will innovate more complex but significantly insightful models and use technology to create flexible supply chain, innovative products and communication ideas and satisfy even more consumer requirements. Together with this, the government has to create an enabling environment and tackle number of urban issues for the industry to truly reach its potential. Jamuna (2014), "Consumers' Awareness and Attitudes Towards Consumer Protection Act 1986" analysed 280 respondents of Virudhunagar district to understand their level of awareness and attitudes towards consumer rights and responsibilities. The researcher included six socio-economic variables i. e. age, gender, education, occupation, legal status and monthly income out of which age and gender were significant to consumer problems. The educated

consumers can take rational decisions while buying products, so a necessity of proper consumer education should be imposed by the government at the college and college level. The government should take strict action against unscrupulous traders and also proper training and guidance in terms of consumer legislation should be provided to the rural consumers (Sampath & Sathish, 2021). Mohan and Suganthi (2013), "Rural Consumers Awareness about Consumer Rights" conducted analyses within the Udumalpet Taluk of Tirupur district of Tamil Nadu to know the level of awareness of rural consumers about the buyer rights. The study was based on both primary and secondary sources where 500 consumers of Udumalpet were selected to know the association between demographic factors of rural consumers and also the level of awareness. For the analysis, Chi-square test was used. The study reveals that the agricultural consumers above 40 years old were highly aware of the consumer rights. Based on the gender, the result reveals that 10% male were highly aware while the females were less aware as compared to males. Level of awareness supported legal status reveals that married people were less aware as compared to unmarried people while on the basis of occupation, people who were employed were highly aware. The awareness should be done through various modes like digital media, electronic media, medium within the style of articles, pamphlets, booklets, etc. The panchayats should even be involved to teach rural consumers. The study reveals that the demographic factors don't influence the level of awareness in regard to consumer rights. Sundaram and Balramalingam (2012), "Women Awareness on Consumer Rights – A Study with reference to Vellore city", revealed the awareness of girls about consumer rights in Vellore city that a sample of 450 women were interviewed. The researcher found that 6.

95% of the respondents weren't knowledgeable, 56% were less knowledgeable, 27.1% had medium knowledge while 10. 15% were highly intimate the consumer rights. They found that the academic qualification, family income and monthly income have a positive correlation and significant association with awareness of consumer rights while legal status and occupation showed a negative correlation but significant association in terms of consumer rights awareness. They suggested that those respondents whose level of information was low because of lack of income, the government should create awareness and training camps through which they get knowledge about consumer rights. They also suggested that the government and media should collaborate to teach consumers at mass level. Chandra (2011), has done research on "Consumer perception and awareness about consumer rights and consumer Protection act - A study in district Raipur". The study was conducted among the 400 consumers of Raipur district from urban, semi-urban and rural areas (Sampath & Sathish, 2021). The study revealed that there have been no VCOs in Raipur district to spread consumerism, even district forum was facing plenty of problems like delay within the settlement of cases because of vacant post for extended period, involvement of lawyers, inefficient working system, lack of staff, in competency of members, etc. He suggested that the government should encourage members of the forums for settlement of cases within time norms, lesser involvement of lawyers, proper education to the consumers through seminars, conferences, short films in regional languages, spread knowledge about the quality marks and labels, and encourage VCOs to market and protect the consumer rights. The study was limited to know the level of awareness and therefore the issues faced by the consumers. It also identified problems faced by the district consumer forum but evaluated performance supported the responses of the district forum authorities only.

Objective

The researcher has formulated the following objective exclusively for the farmers residing in the rural parts of Tamil Nadu and they are

- To understand the socio-economic factors of the farmers residing in the rural parts of Tamil Nadu on consumer protection awareness.
- To determine level of awareness and utilisation of the consumer right by the farmers residing in the rural parts of Tamil Nadu.

METHODOLOGY OF THE STUDY

Area of the Study

Area of the study refers to rural farmers residing at Tamil Nadu State (Sampath & sathish, 2021). Since the population of study is not clearly available, the researcher considered the population to be infinite would be infinite

Sources of Data

The study involves primary data and secondary data. For the purpose of the study, the primary data were collected from 100 respondents using a structured questionnaire. The questionnaire comprises of eight acts like "Right to Safety", "Right to information", "Right to Choose", "Right to Heard", "Right to Redress", "Right to Consumer Education", "Right to a Healthy" and "Sustained environment". The "awareness" and "utilisation" of the above-mentioned rights were asked to the respondents and responses were filled by the researcher.

Sampling Design

For the purpose of the study, 100 respondents were selected using convenience sampling method. Convenience Sampling is a non-probability sampling technique where subjects are selected because of their convenient accessibility proximity to the researcher. This sample is used because it allows the researcher to obtain basic data and trends regarding his study without the complications of using a randomized sample.

Data Analysis

The collected primary data have been analysed using the SPSS software (Statistical Packages for Social Sciences). The reliability and validity of the questionnaire were also tested. The Cronbach alpha value was found to be above 0.8 and proved that the questionnaire is good fit for the current research. The Frequency Distribution, Measures of Central Tendency, Measures of Dispersion and Bi variate correlation were used to analyse the data.

RESULTS AND DISCUSSION

Frequency Distribution

The Frequency Distribution has been used to analyse the "Socio-Economic Factors of the Farmers". The profiles measured under the "Socio-Economic Factors of the Farmers" are "Gender", "Age", "Educational Qualifications", "Income" and "Marital Status". The results of the analysed are displayed in the Table.1. below.

Gender

The majority of the respondents are males with 65 per cent and 35 per cent of the respondents are females.

Age

The table clearly shows that majority of the respondents belong to the age category of 38 - 48 Years, 15 per cent belong to 28 - 38 Years, 10 per cent belong to 48 - 58 Years and the remaining 4.6 per cent 18 Years - 28 Years.

Educational Qualifications

The table it is much clear that 31 per cent of the respondents are postgraduates, 28 per cent are uneducated, 16 per cent have diploma or higher secondary education, 14 per cent are under graduate and remaining 11 per cent are professionals.

Income

From the table it is much clear that 36 per cent of the respondents belong to the income group of Rs. 10000 to Rs. 15000, 28 per cent belong to income group of Rs.20001 to Rs. 25000, 22 per cent belong to income group of Rs.15001 to

Rs. 20000, 14 per cent belong to income group of Rs. 25001 to Rs. 30000and remaining 6 per cent earn above Rs. 30000.

Marital Status

The majority of the respondents are married with 68 per cent and 32 per cent of the respondents are unmarried.

Descriptive Analysis

The Descriptive Analysis (Measures of Central Tendency & Measures of Dispersion) has been used to analyse the **"**Consumer Protection Right Act (COPRA 2019)". The Variables (Acts) measured under the "Consumer Protection Right Act (COPRA 2019)" are "Right to Safety", "Right to information", "Right to Choose", "Right to Heard", "Right to Redress", "Right to Consumer Education", "Right to a Healthy" and "Sustained environment"**.** The results of the analysed are displayed in the Table.2. and Figure.1. below. The respondents (rural farmers) are having a neutral feel with the variable "Right to Seek Redressal" with a mean value 2.13. Similarly, the respondents are having neutral feel with the variables "Right to Basic Needs" with a mean value 2.07. In the same way the respondents neutral feel with the variable "Right to Consumer Education" with a mean value 2.01. Likewise, the respondents disagree with the variable "Right to safety" with a mean value, 1.91. In the same way the respondents disagree with the variables "Right to healthy environment" with a mean value 1.66. Correspondingly the respondents disagree with the variables "Right to be heard" with a mean value 1.06. And finally, the respondents disagree with the variables "Right to Choose" with a mean value 1.02.

Bivariate Correlation

The relationship between the variables is analysed using bivariate correlation. The relationship between the variables like (right to information, right to choose, right to safety, right to be heard, right to redressal and right to consumer education), (right to information, right to choose, right to safety, right to be heard, right to redressal and right to consumer education) and responsiveness (right to information, right to choose, right to safety, right to be heard, right to redressal and right to consumer education). The correlation analysis is as follows;

Positive Correlation

- The independent variable Awareness Right to Safety has positive correlation with the dependent variable Awareness like Right to Choose (0.379), Right to be Heard (0.443), Right to Seek Redressal (0.163), Right to Consumer Education (0.136), and Right to Basic Needs (0.112).
- The independent variable Awareness Right to Information has positive correlation with the dependent variable Awareness like Right to be Heard (0.180), Right to Seek Redressal (0.381), Right to Basic Needs (0.214) and Right to healthy Environment (0.308).
- The independent variable Awareness Right to choose has positive correlation with the dependent variable Awareness like Right to be Heard (0.473), Right to Seek Redressal (0.151), Right to Consumer Education (0.263) and Right to Healthy Environment (0.095).
- The independent variable Awareness Right to Heard has positive correlation with the dependent variable Awareness like Right to Seek Redressal (0.241), Right to Consumer Education (0.198), Right to Basic Needs (0.109) and Right to healthy Environment (0.146).
- The independent variable Awareness Right to Seek Redressal has positive correlation with the dependent variable Awareness like Right to Basic Needs (0.307), and Right to Healthy Environment (0.585).
- The independent variable Awareness Right to Basic Needs has positive correlation with the dependent variable Awareness Right to Healthy Environment (0.607).

No Correlation

- The independent variable Awareness Right to safety has no correlation with the dependent variable Awareness Right to Information and Right to Healthy Environment.
- The independent variable Awareness Right to Information has no correlation with the dependent variable Awareness Right to choose and Right to Consumer Education.

- The independent variable Awareness Right to Choose has no correlation with the dependent variable Awareness Right to Basic Needs.
- The independent variable Awareness Right to Seek Redressal has no correlation with the dependent variable Awareness Right to Consumer Education.
- The independent variable Awareness Right to Consumer Education. has no correlation with the dependent variable Awareness Right to Basic Needs and Right to Healthy Environment.

CONCLUSION

This study concludes that majority of the respondents showed low level of awareness about right to choose, heard healthy environment and safety. Respondents who are aware about the consumer rights in seek redressal but they never lodge complaint against exploitation. Similarly, it proved that no consumers are willing to file case in the consumer court due to complicated procedure of filing complaint and due to wastage of time and money and it is suggested to conduct consumer education and consumer awareness programs, public campaigns among rural and uneducated people and government should take necessary actions to minimize the procedure of filing case, speed up the redressal programs and provide various support to the consumers for their redressal. The Consumers Forum which has been set up under the Consumer Protection Act, give the necessary guidance and legal advice to the consumers in approaching the court regarding the consumer problems. Government machineries and Consumer Voluntary Organizations should take special attention in creating consumer awareness relating to unfair trade practices, consumer acts proper awareness program should be conducted in all levels of the society. The subjects of consumer protection, consumer rights and consumer responsibilities should be introduced from primary school onwards.

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Conflict of Interest

The authors certify that, they have had no affliction with or involvement in any organisation or entity with any financial interest, or non-financial interest in subject matter, or material discussed in the manuscript.

Authors' Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

REFERENCES

1. Dr. A. K. Chandra (2011), "Consumer Perception and Awareness about Consumer Rights and Consumer Protection Act: A Study in District Raipur". The International Journals Research Journal of Social Science & Management, 1, 59-69.
2. Dr. N. Sundaram and C. Balaramalingam (2012), "Women Awareness on Consumer Rights – A Study with Reference to Vellore City". International Journal of Research in Commerce, Economics and Management, 2 (12), 51-52.
3. Dr. S. Mohan, V. Suganthi, (2013), "Rural Consumers Awareness about Consumer Rights", International Journal of Innovative Research &Development, 2 (1), 136-141.

4. S. Jamuna (2014), Consumers' Awareness and Attitudes Towards Consumer Protection Act 1986 – A Study with Reference to Consumers in Virudhunagar District – Indian Journal of Applied Research, 4 (4), 7-10.
5. Dr. Arabinda Debnath and Mrs. Manashi Mazumdar, (2015), "An evaluative study on consumer rights in the context of business" ISSN: 2349-6959 Volume-I, Issue-IV.
6. Dr. M. Sampath Nagi and Mr. A. Anandan, (2020), "Consumer Protection Act and the Patients' Rights", Aut Aut Research Journal, Vol. 11, No. 4, Pp. 86 – 95.
7. Dr. M. Sampath Nagi, Vijayakanthan. S and Arasuraja. G, (2022), *"Service Quality towards Retail Stores Across Coimbatore District",* Indian Journal of Natural Sciences, Vol. 12, No. 70, Pp. 38370 – 38374.
8. Dr. M. Sampath Nagi, M. Kannan and P. Ramasubramaniam, "*Challenging Scenario Faced by Exporters of Garment Industry",* Indian Journal of Natural Sciences, Vol. 12, No. 70, Pp. 38728 – 38734.
9. M. Sampath Nagi and K. Prakash (2021)*, "Client Satisfaction Towards Transportation and Logistics Services Providers, Tamil Nadu",* Global Journal for Research Analysis, Vol. 10, No. 07, Pp. 01 – 03.
10. M. Sampath Nagi and D. Sathish Kumar, (2021), *"Perception of the Farmers towards the Quality of Service Provided by Co-Operative Banks",* Indian Journal of Natural Sciences, Vol. 12, No. 66, Pp. 30725 – 30732.
11. Tyagi, Vibhuti & Tyagi, Ajay & Pandey, Vivek. (2019). *"A Case Study on Consumer Buying Behavior towards Selected FMCG Products"*. 10.13140/RG.2.2.16421.96485.

Table.1. Socio-Economic Factors of the Farmers

Profile	Measuring Labels	Frequency	Percent
Gender	Male	65	65.0
	Female	35	35.0
Age	18 Years - 28 Years	5	5.0
	28 Years - 38 Years	15	15.0
	38 Years - 48 Years	70	70.0
	48 Years - 58 Years	10	10.0
Educational Qualifications	Professional	11	11.0
	Post Graduation	31	31.0
	Under Graduation	14	14.0
	HSC / Diploma	16	16.0
	Uneducated	28	28.0
Income	Rs. 10000 to Rs. 15000	36	36.0
	Rs.15001 to Rs. 20000	22	22.0
	Rs.20001 to Rs. 25000	28	28.0
	Rs. 25001 to Rs. 30000	14	14.0
	Above Rs. 30000	6	6.0
Marital Status	Married	68	68.0
	Unmarried	32	32.0
*** Source - Primary Data**			
Number of Respondents (N) - 100			

Table 2: Awareness towards Consumer Protection Right Act (COPRA, 2019) towards FMCG

Measuring Questions	Mean	Sd	Measuring Questions	Mean	Sd
1. Right to Safety	1.91	.286	2. Right to Informed	1.06	.313
3. Right to Choose	1.02	.141	4. Right to be Heard	1.06	.270
5. Right to Seek Redressal	2.13	.525	6. Right to Consumer Education	2.01	.860
7. Right to Basic Needs	2.07	.821	8. Right to Healthy Environment	1.66	.670
*** Source - Primary Data**					

Table 3: Correlation between the Variables of Awareness towards Consumer Protection Right Act (COPRA, 2019) towards FMCG

H_0: There is no significant correlation between the variables of awareness towards consumer protection right act (COPRA, 2019) towards FMCG.

Variable		RS	RI	RC	RH	RSR	RCE	RBN	RHE
RS	PC	1							
	Sig.								
RI	PC	.057	1						
	Sig.	.111							
RC	PC	.379**	.027	1					
	Sig.	.000	.448						
RH	PC	.443**	.180**	.473**	1				
	Sig.	.000	.000	.000					
RSR	PC	.163**	.381**	.151**	.241**	1			
	Sig.	.000	.000	.000	.000				
RCE	PC	.136**	.069	.263**	.198**	.069	1		
	Sig.	.000	.051	.000	.000	.051			
RBN	PC	.112**	.214**	.033	.109**	.307**	-.031	1	
	Sig.	.002	.000	.356	.002	.000	.388		
RHE	PC	.047	.308**	.095**	.146**	.585**	.063	.607**	1
	Sig.	.191	.000	.008	.000	.000	.076	.000	

****. Correlation is significant at the 0.01 level (2-tailed).**	**PC - Pearson Correlation**
***. Correlation is significant at the 0.05 level (2-tailed).**	**N – Number of Respondents**
RS - Right to Safety	**RSR - Right to Seek Redressal**
RI - Right to Informed	**RCE - Right to Consumer Education**
RC - Right to Choose	**RBN - Right to Basic Needs**
RH - Right to be Heard	**RHE - Right to Healthy Environment**

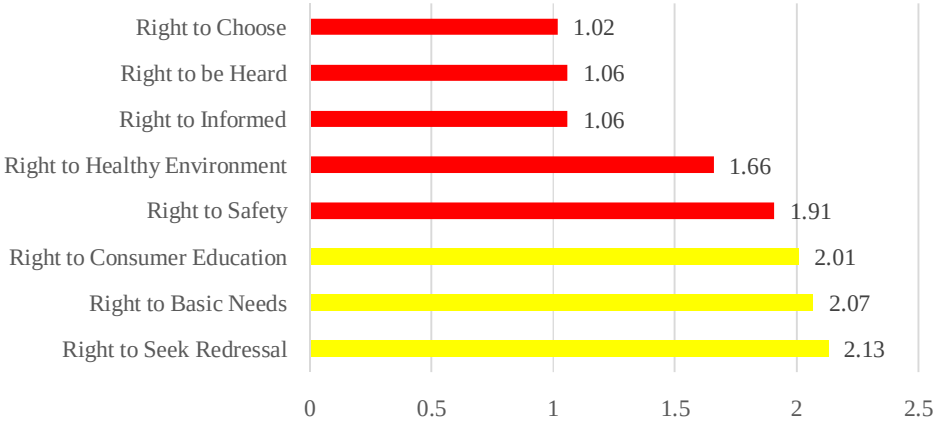

Fig. 1: **Awareness towards Consumer Protection Right Act (COPRA, 2019) towards FMCG**

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RESEARCH ARTICLE

Extracellular Synthesis of Silver Nanoparticles Mediated by *Streptomyces thermocarboxydus* Strain PRO 33 and its Antimicrobial Activity

K.M. Prashanth[1]* and R. Onkarappa[2]

[1]Research Scholar, Department of Microbiology, Sahyadri Science College, Shivamogga, Karnataka, India
[2]Professor, Department of Microbiology, Sahyadri Science College, Shivamogga, Karnataka, India

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***Address for Correspondence**
K.M. Prashanth
Research Scholar,
Department of Microbiology,
Sahyadri Science College,
Shivamogga, Karnataka, India
Email: prashanth201821@gmail.com

ABSTRACT

In the present study, we demonstrate the systematic and thorough synthesis of silver nanoparticles (Ag-NPs) using *Streptomyces thermocarboxydus* strain PRO 33. The produced Ag-NPs were characterized by UV–Vis, FTIR, Scanning Electron Microscopy (SEM)-Energy Dispersive X-Ray (EDX), X-ray powder diffraction (XRD), Dynamic light scattering (DLS), and zeta potential studies. The agar well diffusion technique was used to test antibacterial activity against *R. equi* MTCC 3551, *S. typhimurium* NCIM 2501, *E. faecalis* NCIM 5253, *V. cholerae* MTCC 2906, *S. aureus* NCIM 2073, *S. flexneri* NCIM 5265, *E. coli* NCIM 2138, *B. cereus* NCIM 2217, *C. albicans* NCIM 3628, *C. neoformans.* Based on the findings, it was clear that Ag-NPs produced by *Streptomyces thermocarboxydus* PRO 33 have the potential to be used as antimicrobial agents.

Keywords: *Streptomyces thermocarboxydus***,** Extracellular Synthesis, Ag-NPs, Antimicrobial activity.

INTRODUCTION

Nanotechnology is an exciting area of research dealing with the production, design, and manipulation of nanostructures ranging in size from 1 to 100 nm. Metal nanoparticles are composed of 20 to 15,000 atoms, resulting in their significantly reduced size[6] [34]. Chemical and biological features of nanoparticles have strong empathy for target molecules, especially proteins, structural stability in granularity, particle aggregation dependent on the type of surface modification, increased photoemission, and enhanced surface catalytic activity [26]. Nanoparticles have recently discovered a wide range of uses in microelectronics, diagnostics, catalysis, antimicrobials, biomolecular detection, and therapies [44]. Nanoparticles can be synthesized utilizing a variety of chemical and physical processes,

including electrochemical methods, irradiation-assisted methods, microwave-assisted methods, and the sol-gel approach [33][8].Chemical processes are complex and involve side effects, such as the production of poisonous substances that are hazardous to both the environment and human health [26]. The physical methods employ the production of nanoparticles with definite shapes and sizes. Production of a high amount of waste by-products, high energy utilization, and time consumption are the major drawbacks of this process. When compared to chemical and physical methods of nanoparticle production, biological synthesis is both environmentally benign and cost-effective [40].Without the use of any harsh, harmful, or expensive chemicals, biological approaches for the synthesis of various nanoparticles are achieved. Microbes and plants are used as sources for nanoparticle manufacturing in biological approaches. The discovery of environmentally acceptable ways for synthesizing nanoparticles with the requisite morphology, size, and dispersity has been a major focus for researchers [24] [27]. Nanoparticles synthesized by biological means have benefits over those synthesized by physical or chemical means [31]. This biologically synthesized nanoparticle has been shown to suppress pathogens [25]. Many microorganisms, including bacteria, actinomycetes, fungi, and viruses, have been examined for their ability to produce numerous nanoparticles, notably silver (Ag), gold (Au), zinc (Zn), palladium (Pd), magnesium (Mg), copper (Cu), iron (Fe), lead (Pb), and titanium (Ti)[12] [13] [14].

Actinomycetes are a species of microbe that resembles fungi morphologically and bacteria physiologically. They were once considered to be transitional forms of bacteria and fungi [42]. Actinomycetes are Gram-positive bacteria that develop substrate and aerial mycelium, produce spores and have a high G+C concentration in their DNA [16] [21] [36] [43]. These are mostly saprophytic, aiding in the breakdown of biopolymers like lignocelluloses, hemicelluloses, keratin, pectin, and chitin. Actinomycetes are the primary source of therapeutically significant metabolites utilized as anti-infective and anticancer medicines [48]. Actinomycetes have been used in the biological synthesis of nanoparticles, and as a result, Actinomycetes have been termed bio-nano factories for the synthesis of nanoparticles [2] [14] [28]. The nanoparticles synthesized using actinomycetes show remarkable stability and polydispersity [23]. Actinomycetes possess several metal resistance mechanisms, working on the principle of chemical detoxification and proton anti-transporters-based energy-dependent ion efflux from the cell. Following this concept, actinomycetes detoxify metal ions into insoluble non-toxic metallic nanoparticles by extracellular precipitation, biomineralization, or intracellular bioaccumulation [17].

The *Streptomyces* genus is the largest member of Actinomycetes, with 900 species identified. It is estimated that >100,000 secondary metabolites are produced by the *Streptomyces* genus which includes antibiotics, enzymes, antitumor agents, pharmacological agents, and immunosuppressant's [10] [40]**.** Many researchers have shown that *Streptomyces* sp. can synthesize nanoparticles that have antimicrobial properties [46][1]**.** In many previous reports, various members of Actinomycetes such as *Thermomonospora* sp. [2]. *Streptomyces viridogens* [4]. *Streptomyces albogriseolus* [37], *Nocardia* sp [22], S**.** *hydroscopicus* [41], *Streptomyces* sp. NH21 strain [46] *Streptomyces* sp. PRO 15 [32]has been employed in the synthesis of many nanoparticles. The environment consists of many multidrug resistance pathogens which cause serious health problems by causing different diseases with high fatality in humans [7]**.** To address this issue, there is a desire for effective, inexpensive, and safe antimicrobials derived from microorganisms found in nature [11].Because of their conductivity, stability, catalytic, and antibacterial capabilities, silver nanoparticles (Ag-NPs) have garnered a lot of interest in recent years [30]. The biosynthesized Ag-NPs with characteristic properties are used in the fields of catalysis, microelectronics, optics, electrical batteries, antimicrobials, cell imaging, bio-labeling, and biosensors [18]. The current research focuses on the extracellular production of silver nanoparticles utilizing the *Streptomyces thermocarboxydus* PRO 33 strain isolated from coffee plantation soil in Kodagu, Karnataka, India. UV–Vis spectroscopy, Fourier-transform infrared spectroscopy (FTIR), DLS and Zeta Potential, XRD, SEM, and Energy Dispersive X-Ray Analysis (EDX) were used to analyze the synthesized silver nanoparticles. The agar well diffusion method was used to investigate the antimicrobial properties of the synthesized nanoparticles.

MATERIALS AND METHODS

Synthesis of Ag-NPs from *Streptomyces thermocarboxydus* strain PRO 33

Streptomyces thermocarboxydus strain PRO 33 was isolated from the soil of a coffee plantation in Kodagu, Karnataka. The partial 16S rRNA gene sequence was deposited in GenBank (Accession No: OM232052). *Streptomyces thermocarboxydus* PRO 33 was cultured in starch casein nitrate broth and incubated on a rotary shaker for 7 days at 30°C (120 rpm). The broth was then centrifuged for 20 minutes at 8000 rpm to separate the cells. The synthesis of Ag-NPs was carried out by using equal volume (1:1 ratio) of cell-free extract and 1mM silver nitrate (AgNO3). The pH was adjusted to 8.0. The prepared solution was incubated in the rotary shaker (120 rpm) at 30 °C for 4 days in the dark [46][3].

Characterization of synthesized Ag-NPs

UV-Visible Spectroscopy analysis

The change in color of the solution revealed the formation of Ag-NPs. Later, using a UV–Vis. spectrophotometer, a physicochemical investigation was done to confirm the synthesis of Ag-NPs by measuring the UV-visible spectrum at 380 to 700 nm. (ELICO SL-159Elico Ltd., India) [20].

FTIR analysis

The functional groups in biogenic Ag-NPs were investigated using FTIR measurements (PerkinElmer FTIR C94012, PerkinElmer USA). A small amount of dried Ag-NPs powder was examined for FTIR readings. The infrared spectra were recorded at a resolution of 4 cm^{-1} and in the wavelength range of 4,000 to 400 cm^{-1}. The IR shifts in the maxima of the peaks in various locations of the spectra were studied and compared to earlier studies [19].

SEM and EDX analysis

The morphology and elemental content of dried Ag-NPs powder were examined using a scanning electron microscope (Hitachi, S-3400N, Japan) in conjunction with EDX analysis. A carbon-coated plate was used to hold the powdered nanoparticle. The plate was then gold-coated using sputter-coating equipment to improve the conductivity and precision of the image [50].

Dynamic light scattering (DLS) and Zeta potential analysis

A Malvern Zetasizer Ver. 7.12 was used to determine particle size and zeta potential. DLS with a laser of wave length 633 nm and a fixed scattering angle of 173° was used to determine the hydrodynamic diameter. The particle size was calculated under the assumption that the particle was spherical. At a constant temperature of 25°C, each sample was measured three times [28].

X-ray diffraction analysis

The dried powders of AgNPs were employed for X-ray diffraction analysis X-ray Diffractometer X'Pert, Malvern Panalytical United Kingdom. The diffraction patterns were captured between 20° and 80° (2Θ), using a current of 30 mA, CuK radiation, and a voltage of 40 kV. The crystal structures were calculated by comparing the obtained values of inter-planar spacing and diffraction pattern matching intensities to the standard theoretical values in the JCPDS database [35].

Antimicrobial property of Synthesized Ag-NPs

The agar diffusion technique was used to investigate the antibacterial activity of Ag-NPs against the following test microorganisms: *R.equi* MTCC 3551, *S.typhimurium* NCIM 2501, *E.faecalis* NCIM 5253, *V. cholerae* MTCC 2906, *S.aureus* NCIM 2073, *S. flexneri* NCIM 5265, *E. coli* NCIM 2138, *B.cereus* NCIM 2217, *C.albicans* NCIM 3628, *C.neoformans* NCIM 3541 Using sterile cotton swabs, bacterial cultures were inoculated on Müller Hinton agar (MHA) and Yeast cultures on Sabouraud Dextrose agar (SDA). Wells were cut out in each plate with a sterile gel borer, and 100 µL of biosynthesized Ag-NPs were employed as a test sample. Streptomycin sulfate and Nystatin

were employed as conventional antibiotics against the clinical isolates, and 1mM silver nitrate solution was used as a control. The inoculated plates were incubated for 24 hours at 37°C [5].

RESULTS AND DISCUSSION

Synthesis of Ag-NPs from *Streptomyces thermocarboxydus* PRO 33

The current work focused on the extracellular synthesis of Ag-NPs by *Streptomyces thermocarboxydus* strain PRO 33(Fig 1)isolated from coffee plantation soil in Kodagu, Karnataka, India (lat: 12°30'41.8 N long: 75°49'19.8 E). Nanoparticles can be made from different types of metals, including silver, gold, zinc, and platinum, and have a wide range of biological applications. Biosynthesized Ag-NPs are less hazardous to nature and can limit the growth of several pathogenic microbes, as well as powerful antiviral and anticancer effects. Adding 1mMol/l AgNO3 to the cell-free supernatant at pH 8.0 resulted in the extracellular production of Ag-NPs from *Streptomyces thermocarboxydus* strain PRO 33. After 4 days of incubation in the dark, the color changed from creamy white to brown, indicating the synthesis of Ag-NPs (Fig 2). Manivasagan *et al.,* 2013, Vijayabharathi *et al.,* 2018, and Fouda *et al* 2019 also reported similar findings. To our best knowledge, this is the first report on the synthesis of extracellular silver nanoparticles utilizing *Streptomyces thermocarboxydus.*

Characterization of synthesized Ag-NPs

UV-Visible Spectroscopy analysis

Figure 3 depicts the UV-visible spectrum of Ag NPs generated using *Streptomyces thermocarboxydus strain* PRO 33 cell-free extract. Due to the inter-band shift and plasmon movement of the NPS, the UV-visible spectrum exhibits a strong surface plasmon resonance band around 440 nm, as well as a sharp peak, which are known to be related to the size and shape of Ag NPs [45]. When the size of the NPs varies, the surface plasmon resonance band often shifts to higher wavelength regions [29]. This finding is consistent with the qualitative examination of Ag-NPs. Silva-Vinhote *et al*., 2017, and Madakka *et al*., 2018 reported similar findings.

FTIR analysis

The FTIR spectra of *Streptomyces thermocarboxydus* strain PRO 33 supernatant and biosynthesized Ag-NPs were depicted in Fig. 6 respectively. Several vibrational frequencies were observed in the range of 400 to 4000 cm−1. The FTIR spectrum of Ag-NPs synthesized from PRO 33 supernatant revealed four peaks, positioned at 3342.76,1637.82, 649.35, and 608.87cm−1.The first strong and broad absorption peak at 3342.76 cm−1 arose due to the presence of an O-H stretching assigned for alcohol, while the spectral peak at1637.82 cm−1 was assigned to the C=C stretching of alkene. Finally, the 2-weak peaks positioned at 649.35 cm−1 and 608.87cm−1 were assigned to (CA-Cl) or (CA-Br) stretch halo compounds. The O-H stretching of alcohol and the C=C stretching of alkenes may be implicated in the stabilization of Ag-NPs [7] [26] [46]. Proteins in biomass filtrate play an important role in the stabilization and capping of Ag-NPs, as evidenced by the various groups represented in FTIR spectra [50]. FTIR analysis is used to describe materials and, in particular, to identify inorganic mixtures. Furthermore, FTIR provides information on the molecular and structural forms of organic and inorganic materials [8].

SEM and EDX analysis

SEM was used to examine the topology of the produced Ag-NPs, as shown in Figure 5a. The images show that the strain PRO 33-mediated Ag-NPs are asymmetrical. For elemental analysis of biosynthesized Ag-NPs, the EDX spectrum (Fig. 5b) was recorded. The optical absorption peak was seen at around 3 keV, demonstrating the presence of metallic silver as a result of SPR stimulation. The oxygen (33.10%) and carbon (18.71%) peaks in the EDX spectra could be attributable to leftover materials surrounding the NPs or to the SEM grid employed for sample preparation [9]. Some peaks were identified across the EDX spectra, including N (11.06%), Na (7.65%), Si (0.20%), P (1.95%), S (0.83%), Cl (2.32%), K (1.89%) and Ag (22.28%). This research verifies metabolites in cell-free filtrate's capacity to decrease and stabilize silver nanoparticles. Because of their SPR, the optical absorption peaks of silver nanoparticles

occurred at roughly 3 keV [8]. EDX profiles revealed that the Ag-NP peak was located in about 3 keV areas, confirming the development of crystalline Ag-NPs.

DLS and Zeta potential analysis

Figure 6a depicts the particle size distribution measurement for Ag-NPs, which shows an average particle size of 43.82 nm. DLS measurements yield the hydrodynamic diameter of a sphere (i.e., particle diameter with hydration shell) with the same volume as the particle and the additional solvent or stabilizer traveling with the particle [51]. The zeta potential of Ag-NPs was -31.5 mV. (Figure 8b). The zeta potential value indicated that the surface of Ag-NPs is negatively charged.

X-ray diffraction analyses

Figure 7 depicts the typical XRD pattern of the produced Ag-NPs. Peaks at 32.2°,38.2°, 44.53°, and 64.59° correspond to silver planes (102), (111), (200), and (220), respectively. This XRD spectrum confirms the crystalline structure of silver nanoparticles. The available literature (JCPDS, File No. 4-0783) supports the silver nanoparticles by indexing the acquired XRD peaks to a face-centered cubic structure of silver. The additional tiny peak at 46.2° suggests the presence of bioorganic compounds/proteins happening at the surface of the Ag-NPs during production [39]. These bioorganic substances' reflecting peaks were weaker than those associated with the crystalline structure of Ag-NPs. The reflection planes indicative of a face-centered cubic (FCC)shape of metallic silver are visible in the powder XRD pattern matching Ag NPs. There were no other peaks found, indicating the excellent purity of the produced Ag NPs [15] [38].

Antimicrobial property of Synthesized Ag-NPs

The biosynthesized Ag-NPs exhibited potent antibacterial activity against a wide range of pathogenic bacteria and fungi (Fig. 8). The antimicrobial activity of biosynthesized Ag-NPs against pathogenic microbes was tested in triplicate and is expressed as mean ±SD. The highest activity was observed against *E. faecalis*, *R. equi,* and *S. aureus*, with inhibition zones of 22.6±0.5 mm, 21±1mm,and 21.3±0.5 mm, respectively, at a volume of 100 mL Ag-NPs suspension; the lowest activity was observed against *E. coli*, with inhibition zones of 17.3±0.5mm. At the same time, Ag-NPs inhibited *B.cereus* and *S.typhimurium* by 20±0.57mm, *V.cholerae*by 19.6±0.5mm and *S.flexinerae* by 19.33±0.5mm. Similarly, the maximum antifungal activity was observed against *C.albicans*, with an inhibition zone of 15.3±0.5 mm, while the lowest activity was reported against *C. neoformans*, with an inhibition zone of 12.5±0.5 mm**.** These results are supported by reports of Sheik *et al.,* 2019 and Salem *et al.,* 2022.Many scientific reports have explained the possible modes of action for Ag-NPs' antimicrobial mechanism; interestingly, as illustrated in the possible mechanism, contact with silver species causes bacterial cellular damage, the formation of reactive oxygen species, enzyme inactivation, ribosome disassembly, protein degradation, interruption of the electron transport chain, and apoptosis in pathogenic microbes [31].

CONCLUSION

The current study describes a straightforward approach for synthesizing Ag-NPs using *Streptomyces thermocarboxydus* strain PRO 33 cell-free extract. The first indication of Ag-NPs being formed was a change in the color of the reaction mixture, which was confirmed by an absorption maximum at 440 nm in UV–Vis. analysis. IR bands in the FTIR spectrum were used to identify the biomolecules involved in the reduction, stabilization, and capping of Ag-NPs. Scanning electron microscopy was used to establish the morphological aspects of the bio-reduced Ag-NPs. The chemical composition of the produced silver nanoparticles was revealed by EDX examination to be 22.28 percent Ag, as well as several other components. The crystalline nature of the nanoparticles was confirmed by X-ray diffraction analysis. The average grain size was evaluated by dynamic light scattering, and the zeta potential of the synthesized silver nanoparticles was found to be -31.5 mV. The biosynthesized Ag-NPs showed substantial antibacterial efficacy against bacterial and fungal infections. The findings could pave the way for the development of a new strategy to combat antimicrobial resistance.

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REFERENCES

1. Abd-Elnaby, H. M., Abo-Elala, G. M., Abdel-Raouf, U. M., & Hamed, M. M. (2016). Antibacterial and anticancer activity of extracellular synthesized silver nanoparticles from marine *Streptomyces rochei* MHM13. The Egyptian Journal of Aquatic Research, 42(3), 301–312. https://doi.org/10.1016/j.ejar.2016.05.004
2. Ahmad, A., Mukherjee, P., Senapati, S., Mandal, D., Khan, M. I., Kumar, R., & Sastry, M. (2003). Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. Colloids and Surfaces B: Biointerfaces, 28(4), 313–318. https://doi.org/10.1016/S0927-7765(02)00174-1
3. Al-Dhabi, N., Mohammed Ghilan, A.-K., &Arasu, M. (2018). Characterization of Silver Nanomaterials Derived from Marine *Streptomyces* sp. Al-Dhabi-87 and Its In Vitro Application against Multidrug Resistant and Extended-Spectrum Beta-Lactamase Clinical Pathogens. Nanomaterials, 8(5), 279. https://doi.org/10.3390/nano8050279
4. Balagurunathan, R., Radhakrishnan, M., Rajendran, R. B., & Velmurugan, D. (2011). Biosynthesis of gold nanoparticles by actinomycete *Streptomyces viridogens* strain HM10. Indian j. Biochem. Biophys., 48, 6.
5. Bizuye, A., Gedamu, L., Bii, C., Gatebe, E., &Maina, N. (2020). Molecular-Based Identification of Actinomycetes Species That Synthesize Antibacterial Silver Nanoparticles. International Journal of Microbiology, 2020, 1–17. https://doi.org/10.1155/2020/8816111
6. Chen, X., &Schluesener, H. J. (2008). Nanosilver: A nanoproduct in medical application. Toxicology Letters, 176(1), 1–12. https://doi.org/10.1016/j.toxlet.2007.10.004
7. Dhanaraj, S., Thirunavukkarasu, S., Allen John, H., Pandian, S., Salmen, S. H., Chinnathambi, A., &Alharbi, S. A. (2020). Novel marine Nocardiopsis dassonvillei-DS013 mediated silver nanoparticles characterization and its bactericidal potential against clinical isolates. Saudi Journal of Biological Sciences, 27(3), 991–995. https://doi.org/10.1016/j.sjbs.2020.01.003
8. Fouda, A., Hassan, S. E.-D., Abdo, A. M., & El-Gamal, M. S. (2020). Antimicrobial, Antioxidant and Larvicidal Activities of Spherical Silver Nanoparticles Synthesized by Endophytic *Streptomyces* spp. Biological Trace Element Research, 195(2), 707–724. https://doi.org/10.1007/s12011-019-01883-4
9. Ganesh Babu, M. M., Sridhar, J., & Gunasekaran, P. (2011). Global transcriptome analysis of Bacillus cereus ATCC 14579 in response to silver nitrate stress. Journal of Nanobiotechnology, 9(1), 49. https://doi.org/10.1186/1477-3155-9-49
10. Gautham. S. A., Sunita C. Mesta, & R. Onkarappa. (2016). Characterization and antimicrobial spectrum of a potent Streptomyces sp. Gos2 isolated from western ghats of karnataka, india. International Journal of Pharmacy and Pharmaceutical Sciences, 8(9), 310. https://doi.org/10.22159/ijpps.2016.v8i9.12547
11. Gebreyohannes, G., Moges, F., Sahile, S., & Raja, N. (2013). Isolation and characterization of potential antibiotic producing actinomycetes from water and sediments of Lake Tana, Ethiopia. Asian Pacific Journal of Tropical Biomedicine, 3(6), 426–435. https://doi.org/10.1016/S2221-1691(13)60092-1
12. Ghorbani, H. R., Safekordi, A. A., Attar, H., &Sorkhabadi, S. M. R. (2011). Biological and Non-biological Methods for Silver Nanoparticles Synthesis. Chem. Biochem. Eng. Q., 10.
13. Gokulakrishnan, R., Ravikumar, S., & Raj, J. A. (2012). In vitro antibacterial potential of metal oxide nanoparticles against antibiotic resistant bacterial pathogens. Asian Pacific Journal of Tropical Disease, 2(5), 411–413.https://doi.org/10.1016/S2222-1808(12)60089-9
14. Golinska, P., Wypij, M., Ingle, A. P., Gupta, I., Dahm, H., & Rai, M. (2014). Biogenic synthesis of metal nanoparticles from actinomycetes: Biomedical applications and cytotoxicity. Applied Microbiology and Biotechnology, 98(19), 8083–8097. https://doi.org/10.1007/s00253-014-5953-7
15. Jose M., Martin Britto Dhas S.A., Arul Doss Daisy., & Jerome Das S. (2016). Synthesis and characterization of nano

spheres decorated silver bromide nanorods using a twostep chemical reduction route. Optik 127, 8019–8023.http://dx.doi.org/10.1016/j.ijleo.2016.06.015

16. Kekuda, P., Onkarappa, R., &Jayanna, N. (2014). Characterization and Antibacterial Activity of a Glycoside Antibiotic from *Streptomyces variabilis* PO-178. Science, Technology and Arts Research Journal, 3(4), 116. https://doi.org/10.4314/star.v3i4.17
17. Kumari, S., Tehri, N., Gahlaut, A., &Hooda, V. (2020). Actinomycetes mediated synthesis, characterization, and applications of metallic nanoparticles. Inorganic and Nano-Metal Chemistry, 1–10. https://doi.org/10.1080/24701556.2020.1835978
18. Ma, L., Liu, J., Su, W., Zeng, X., Liu, X., Li, W., Deng, J., & Tang, J. (2018). Extracellular Biosynthesis, Characterization and Cytotoxic Effect of Silver Nanoparticles by *Streptomyces coelicoflavus* KS-3. Journal of Nanoscience and Nanotechnology, 18(12), 8133–8141. https://doi.org/10.1166/jnn.2018.16388
19. Mabrouk, M., Elkhooly, T. A., & Amer, S. K. (2021). Actinomycete strain type determines the monodispersity and antibacterial properties of biogenically synthesized silver nanoparticles. Journal of Genetic Engineering and Biotechnology, 19(1), 57. https://doi.org/10.1186/s43141-021-00153-y
20. Madakka, M., Jayaraju, N., & Rajesh, N. (2018). Mycosynthesis of silver nanoparticles and their characterization. MethodsX, 5, 20–29. https://doi.org/10.1016/j.mex.2017.12.003
21. Manasa M, Pallavi S, Onkarappa R, &PrashithKekuda T.R. (2014). Antibacterial activity of bioactive *streptomyces* species MPPO-02 against clinical isolates of burn, dental caries and urinary tract infections. Journal of Biological & Scientific Opinion, 2(1), 57–61. https://doi.org/10.7897/2321-6328.02113
22. Manivasagan, P., Venkatesan, J., Senthilkumar, K., Sivakumar, K., & Kim, S.-K. (2013). Biosynthesis, Antimicrobial and Cytotoxic Effect of Silver Nanoparticles Using a Novel Nocardiopsis sp. MBRC-1. BioMed Research International, 2013, 1–9. https://doi.org/10.1155/2013/287638
23. Manivasagan, P., Venkatesan, J., Sivakumar, K., & Kim, S.-K. (2014). Actinobacteria mediated synthesis of nanoparticles and their biological properties: A review. Critical Reviews in Microbiology, 1–13. https://doi.org/10.3109/1040841X.2014.917069
24. Menon, S., Agarwal, H., Kumar, S. R., & Kumar, S. V. (2017). Green synthesis of silver nanoparticles using medicinal plant *acalyphaindica* leaf extracts and its application as an antioxidant and antimicrobial agent against food borne pathogens. International Journal of Applied Pharmaceutics, 9(5), 42. https://doi.org/10.22159/ijap.2017v9i5.19464
25. Nasrollahi, A., Pourshamsian, K., &Mansourkiaee, P. (2011). Antifungal activity of silver nanoparticles on some of Fungi. International Journal of Nano Dimension.1(3): 233-239. https://doi.org10.7508/ijnd.2010.03.007
26. Nayaka, S., Chakraborty, B., Bhat, M. P., Nagaraja, S. K., Airodagi, D., Swamy, P. S., Rudrappa, M., Hiremath, H., Basavarajappa, D. S., &Kanakannanavar, B. (2020). Biosynthesis, characterization, and in vitro assessment on cytotoxicity of actinomycete-synthesized silver nanoparticles on Allium cepa root tip cells. Beni-Suef University Journal of Basic and Applied Sciences, 9(1), 51. https://doi.org/10.1186/s43088-020-00074-8
27. Nayantara, & Kaur, P. (2018). Biosynthesis of nanoparticles using eco-friendly factories and their role in plant pathogenicity: A review. Biotechnology Research and Innovation, 2(1), 63–73. https://doi.org/10.1016/j.biori.2018.09.003
28. Nélly Mara Silva-Vinhote, Nelson Eduardo Durán Caballero, Taciana de Amorim Silva,PatrickVerasQuelemes, Alyne Rodrigues de Araújo, Ana Carolina Mazarin de Moraes, Ana Lygia dos Santos Câmara, João Paulo Figueiró Longo, Ricardo BentesAzevedo,Durcilene Alves da Silva, José Roberto de Souza de Almeida Leite& Maria Francisca Simas Teixeira (2017). Extracellular biogenic synthesis of silver nanoparticles by Actinomycetes from amazonic biome and its antimicrobial efficiency. African Journal of Biotechnology, 16(43), 2072–2082. https://doi.org/10.5897/AJB2017.16148
29. Nguyen, C. K., Tran, N. Q., Nguyen, T. P., & Nguyen, D. H. (2017). Biocompatible nanomaterials based on dendrimers, hydrogels and hydrogel nanocomposites for use in biomedicine. Advances in Natural Sciences: Nanoscience and Nanotechnology, 8(1), 015001. https://doi.org/10.1088/2043-6254/8/1/015001
30. Otari, S. V., Patil, R. M., Nadaf, N. H., Ghosh, S. J., &Pawar, S. H. (2012). Green biosynthesis of silver nanoparticles from an actinobacteria *Rhodococcus* sp. Materials Letters, 72, 92–94. https://doi.org/10.1016/j.matlet.2011.12.109

31. Pallavi S.S., Rudayni, H. A., Bepari, A., Niazi, S. K., & Nayaka, S. (2022). Green synthesis of Silver nanoparticles using *Streptomyces hirsutus* strain SNPGA-8 and their characterization, antimicrobial activity, and anticancer activity against human lung carcinoma cell line A549. Saudi Journal of Biological Sciences, 29(1), 228–238. https://doi.org/10.1016/j.sjbs.2021.08.084
32. Prashanth, K. M. &Onkarappa R. (2021). Germicidal Properties of Biosynthesised Gold Nanoparticles from Streptomyces sp. Bioscience Biotechnology Research Communications, 14(3), 1118–1123. https://doi.org/10.21786/bbrc/14.3.32
33. Qingmin Z., Yan L., Dongsheng X., &Zhennan G. (2001).Preparation of silver nanowire arrays in anodic aluminum oxide templates. Journal of Materials Science Letters, 20, 925– 927.
34. Rai, M., Yadav, A., &Gade, A. (2009). Silver nanoparticles as a new generation of antimicrobials. Biotechnology Advances, 27(1), 76–83. https://doi.org/10.1016/j.biotechadv.2008.09.002
35. Rajivgandhi, G., Maruthupandy, M., Muneeswaran, T., Anand, M., Quero, F., Manoharan, N., & Li, W.-J. (2019). Biosynthesized silver nanoparticles for inhibition of antibacterial resistance and biofilm formation of methicillin-resistant coagulase negative Staphylococci. Bioorganic Chemistry, 89, 103008. https://doi.org/10.1016/j.bioorg.2019.103008
36. Salimath, M. B., &Onkarappa, R. (n.d.). Streptomyces MSR-V21, A Broad-Spectrum Antibiotic Producing Actinomycetes Isolated from Shettyhalli Reserve Forest, Western Ghats of Karnataka, India. 6.
37. Samundeeswari, A., Dhas, S. P., Nirmala, J., John, S. P., Mukherjee, A., & Chandrasekaran, N. (2012). Biosynthesis of silver nanoparticles using *actinobacterium* S *treptomyces albogriseolus* and its antibacterial activity: Biosynthesis of nanoparticles using actinobacteria. Biotechnology and Applied Biochemistry, 59(6), 503–507. https://doi.org/10.1002/bab.1054
38. Shameli, K., Mansor Bin Ahmad, M., Mohsen, Z., Yunis, W. Z., Ibrahim, N. A., &Rustaiyan, A. (2011). Synthesis of silver nanoparticles in montmorillonite and their antibacterial behaviour. International Journal of Nanomedicine, 581. https://doi.org/10.2147/IJN.S17112
39. Shankar, S. S., Rai, A., Ankamwar, B., Singh, A., Ahmad, A., & Sastry, M. (2004). Biological synthesis of triangular gold nanoprisms. Nature Materials, 3(7), 482–488. https://doi.org/10.1038/nmat1152
40. Sheik, G. B., Abdel Raheim, A. I. A., Alzeyadi, Z. A., &AlGhonaim, M. I. (2020). Extracellular Synthesis, Characterization and Antibacterial Activity of Silver Nanoparticles by a Potent Isolate *Streptomyces* sp. DW102. Asian Journal of Biological and Life Sciences, 8(3), 89–96. https://doi.org/10.5530/ajbls.2019.8.15
41. Shivaji, S. W., Arvind, M. D., & Zygmunt, S. (2014). Biosynthesis, optimization, purification and characterization of gold nanoparticles. African Journal of Microbiology Research, 8(2), 138–146. https://doi.org/10.5897/AJMR10.143
42. Shobha, K. S., &Onkarappa, R. (2014). Actinomycetes of Western Ghats Karnataka, India. 8(5), 172-178.
43. Shobha, K. S., Gautham, S. A., &Onkarappa, R. (2012). In vitro antioxidant activity of antibacterial metabolites from Streptomyces species KSRO 04. International research journal of pharmacy, 4(12), 24–29. https://doi.org/10.7897/2230-8407.041206
44. Siddiqi, K. S., Husen, A., & Rao, R. A. K. (2018). A review on biosynthesis of silver nanoparticles and their biocidal properties. Journal of Nanobiotechnology, 16(1), 14. https://doi.org/10.1186/s12951-018-0334-5
45. Siddiqui, M. R., Khan, M., Khan, Adil, Tahir, Tremel, W., Khathlan, &Warthan. (2013). Green synthesis of silver nanoparticles mediated by *Pulicaria glutinosa* extract. International Journal of Nanomedicine, 1507. https://doi.org/10.2147/IJN.S43309
46. Składanowski M., Wypij M., Laskowski D., Golinska P., Dahm H., & Rai M. (2017) Silver and gold nanoparticles synthesized from *Streptomyces* sp. isolated from acid forest soil with special reference to its antibacterial activity against pathogens. Journal of Cluster Science, 28(1), 59–79.
47. Sowani, H., Mohite, P., Munot, H., Shouche, Y., Bapat, T., Kumar, A. R., Kulkarni, M., &Zinjarde, S. (2016). Green synthesis of gold and silver nanoparticles by an *actinomycete Gordoniaamicalis* HS-11: Mechanistic aspects and biological application. Process Biochemistry, 51(3), 374–383. https://doi.org/10.1016/j.procbio.2015.12.013
48. Sukanya, M. K., Saju, K. A., Praseetha, P. K., & Sakthivel, G. (2013). Therapeutic Potential of Biologically Reduced Silver Nanoparticles from Actinomycete Cultures. Journal of Nanoscience, 2013, 1–8. https://doi.org/10.1155/2013/940719

49. Sunita C. Mesta, R. Onkarappa, Chittara S, Dinesh J, & Chaitra K. (2010). Screening of antimicrobial and antioxidant activities of *endophytic* actinomycetes isolated from *Rhizophora mucronata* and *Sonneratia caseolaris*. 8, 8.
50. Vijayabharathi, R., Sathya, A., & Gopalakrishnan, S. (2018). Extracellular biosynthesis of silver nanoparticles using *Streptomyces griseoplanus* SAI-25 and its antifungal activity against *Macrophomina phaseolina* , the charcoal rot pathogen of sorghum. Biocatalysis and Agricultural Biotechnology, 14, 166–171. https://doi.org/10.1016/j.bcab.2018.03.006
51. Vujačić, A., Vodnik, V., Sovilj, S. P., Dramićanin, M., Bibić, N., Milonjić, S., &Vasić, V. (2013). Adsorption and fluorescence quenching of 5,5′-disulfopropyl-3,3′-dichlorothiacyanine dye on gold nanoparticles. New Journal of Chemistry, 37(3), 743. https://doi.org/10.1039/c2nj40865a

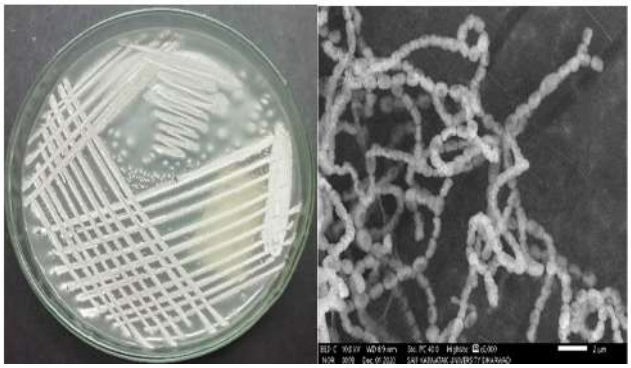

Fig 1: *Streptomyces thermocarboxydus* strain PRO 33 isolates on Starch Casein Nitrate and Electron microscopic image

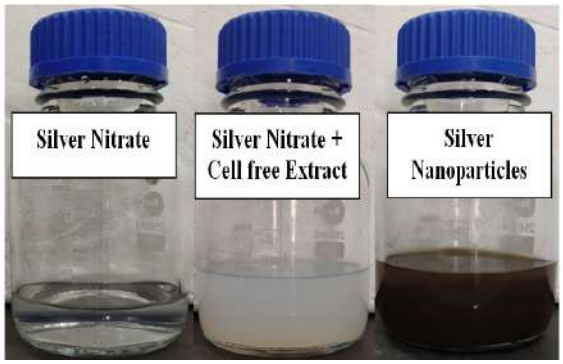

Fig 2: Synthesis of Ag-NPs

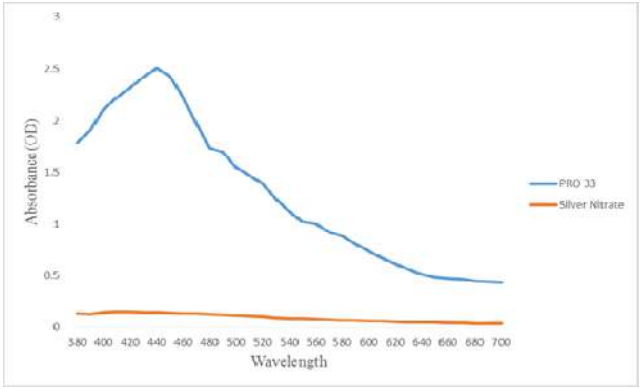

Fig 3: UV-Vis. absorption spectrum AgNPs synthesized

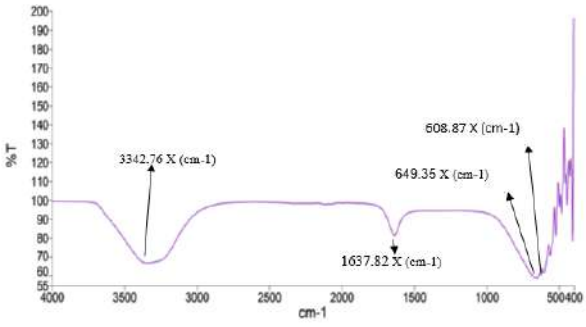

Fig 4: FTIR spectra of Ag-NPs Synthesized from *Streptomyces thermocarboxydus* PRO 33

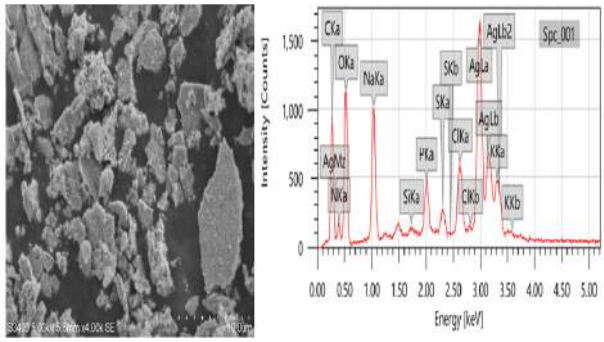

Fig 5: (a)SEM image of synthesized Ag-NPs (b) EDX spectrum of Synthesized Ag-NPs

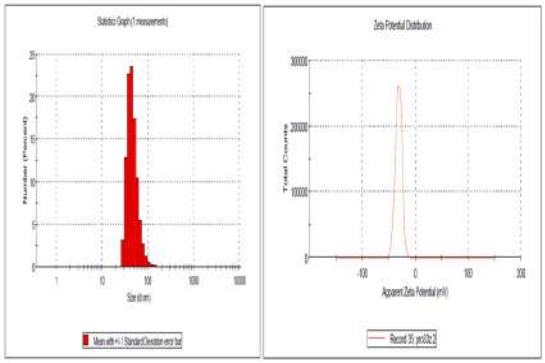

Fig 6: (a)DLS measurement of synthesized Ag-NPs (b) Zeta potential of synthesized Ag-NPs

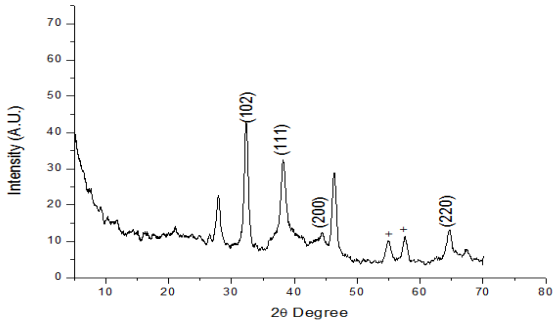

Fig 7: XRD pattern of synthesized Ag-NPs

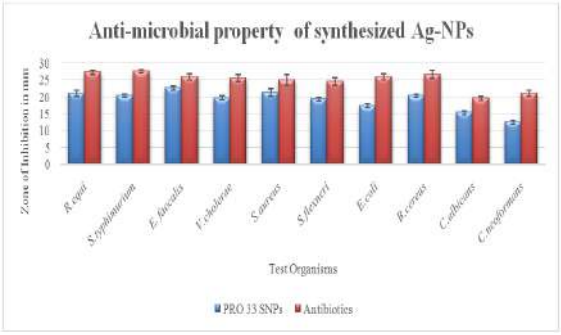

Fig 8: Anti-microbial property of synthesized Ag-NPs

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RESEARCH ARTICLE

Prevalence of Overweight and Obesity among Adolescent Girls in Selected Colleges, Puducherry

Hemavathi. P[1]* and Malathi. S

[1]Ph.D Research Scholar, Vinayaka Mission's Annapoorana College of Nursing, Vinayaka Mission's Research Foundation (Deemed to be University), Salem, Tamil Nadu, India.
[2]Professor, Department of Community Health Nursing, Vinayaka Mission's Annapoorana College of Nursing, Vinayaka Mission's Research Foundation (Deemed to be University), Salem, Tamil Nadu, India

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***Address for Correspondence**
Hemavathi. P
Ph.D Research Scholar,
Vinayaka Missions Annapoorana College of Nursing,
Vinayaka Mission's Research Foundation (Deemed to be University),
Salem, Tamil Nadu, India.
Email: breezehema@gmail.com

ABSTRACT

Adolescent period Overweight and obesity are becoming public health problem among adolescent girls globally. Adolescent period is more crucial period where the remarkable physiological and hormonal changes occur in the body. Observational cross-sectional study was conducted to measure the overweight and obesity among 364 adolescent girls from selected colleges, Puducherry. Results shows that 45.9% of them were 18 years, 57.1% attained menarche at 12-13 years, 50.3% of them were from Arts background, 89.8% of them were nonvegetarian, 94.5% of them were Hindus, 41.4% had monthly income of Rs 5000-10000, 61.3% of them belong to joint family, 61.8% of them from urban. Health history depicts that 9.3% had person health problems, 38.5% and 12.6% of them family history of DM and PCOS respectively. Anthropometric measurements shows that Mean weight was 53.87 $\pm$11.9, Height 2.01 $\pm$0.05 and BMI 21.8 $\pm$4.6, BMI shows that 5.5%,16.7% 29.4% and 48.4% of them were obese, overweight, underweight and normal respectively. Statistically significant association found between BMI and all the sociodemographic variable at $p<0.01$ and also between BMI and personal medical and family health history of the adolescent girls at $p<0.05$.Study concludes that prevalence of overweight and obesity was 16.7% and 5.5% respectively among adolescent girls.

Keywords: Adolescent girls, overweight, obesity, Body Mass Index

INTRODUCTION

BACKGROUND

Overweight and obesity are becoming public health problem among adolescent girls globally. Adolescent period is more crucial period where the remarkable physiological and hormonal changes occur in the body. Report shows that obesity is the predisposing cause for negative reproductive health where it is associated with Polycystic Ovarian Disease and Infertility [1]. Obesity is the one of the leading factors for cardio vascular diseases, hypertension, diabetes mellitus and several other diseases [2].

NEED FOR THE STUDY

Transition from School to College brings many lifestyle changes in adolescent girls including changes in dietary habits, physical inactivity and excessive screen time. They start enjoying canteen food items where mostly beverages, baked, fried and fast-food items are available. They tend to skip meals and fascinated to unhealthy dietary habits. Physical activity and outdoor games also not as good like in the school. All these factors put the adolescent girls at increased risk of obesity worldwide. Cross-sectional Study on Prevalence and contributing factors for adolescent obesity in present era was conducted in Uthrakand, India. Results shows that 6.8% and 17.1% of the adolescents were obese and overweight respectively, 53.8% had normal BMI and 22.3% were underweight. BMI was good among athletic adolescents and high among adolescents who had more than 2 h of screen time were more obese[3]. Cross-sectional study was conducted to measure the prevalence of overweight and obesity among 2,465 students in the age group of 10 to 18 year in five schools and two polytechnic colleges, Puducherry. Results found that overweight and obesity was 9.7% and 4.3% respectively among study participants and no difference found in the prevalence of obesity between males and females[4]. Hence the investigator is interested to measure the prevalence of overweight and obesity among adolescent girls in the age of 17 to 19 years attending Colleges.

OBJECTIVES

1. To measure the prevalence of overweight and obesity among Adolescent girls
2. To associate BMI with selected sociodemographic variables of the adolescent girls
3. To associate BMI with personal medical and family health history of the Adolescent girls.

METHODOLOGY

Research Approach: Quantitative approach
Research design: observational cross-sectional design
Setting of the study: Bharathidasan Government College for women (BGCW), and Tagore Govt. Arts and Science College (TAC), Puducherry.

Variables
Demographic variables: Age, discipline, age at menarche, dietary pattern, religion,monthly family income, type of family and residence
Study variables: Overweight and Obesity

Population of the study: Adolescent girls studying in colleges, Puducherry.

Sample: Adolescent girls studying in selected Government Arts and Science College, Puducherry.

Sample size: the sample size comprised of 364 adolescent girls with an expected proportion of 0.35, precision of 5%, confidence level of 95%[5].

Sampling technique: simple random sampling technique by using lottery method eight courses were selected from colleges. Study participants were screened by census method.

Sampling criteria

Inclusion criteria

- Adolescent girls aged 17-19 years
- Willing to participate in the study

Exclusion criteria

- Not willing and not available during data collection

Instruments and tools

Tool consists of three sections

- **Section A:** Demographic data consist of Age, discipline, age at menarche, religion, dietary pattern, monthly family income and residence
- **Section B:** Personal Health and Family health History of Diabetes Mellitus (DM) and Poly cystic Ovary Syndrome (PCOS)
- **Section C:** Anthropometric variable includes Height, weight and BMI (weight in kg/ height in m^2) measured using Omron weight machine and height scale.

Data Collection Method

Data was collected from November 2021 to December 2021. Total Under Graduate Courses available in the selected arts and science colleges were listed and eight courses from were randomly selected by using lottery method. Study participants were screened by census method. Informed consent was obtaining from the study participants who meets the inclusion criteria. structured interview questionnaire was used to collect data on demographic variables, personal health and family health history of DM and PCOS. Anthropometric variables were measured by Omron Weight machine and height scale and BMI was calculated according WHO scale. Researcher spent 10 – 15 minutes for each study participants.

Data Analysis plan

Data were entered in excel sheet and analyzed with SPSS version 27. The Data was analyzed in an orderly manner by using descriptive and inferential statistics.

Ethical Considerations

- Permission obtained from Institutional Ethical committee, VMACON, Salem
- Directorate of technical and higher education, Puducherry.
- Principal of Selected Arts and Science College, Puducherry.

A written informed consent and assent was obtained from the study participants needfully before data collection.

FINDINGS AND INTERPRETATION

Section A: Distribution of Adolescent girls according to their socio demographic variable

Percentage-wise distribution of adolescent girls according to sociodemographic data shows that 45.9% of them were 18 years, 57.1% attained menarche at 12-13 years, 50.3% of them were from Arts background, 89.8% of them were nonvegetarian, 94.5% of them were Hindus, 41.4% had monthly income of Rs 5000-10000, 61.3% of them belong to joint family, 61.8% of them from urban. (Table no.:2).

Section B: Distribution of Adolescent girls according to their Personal health history

Percentage-wise distribution of adolescent girls according to Health history depicts that 9.3% had person medical history where as majority (90.7%) had no personal medical history. (Table no.:3). Percentage-wise distribution of adolescent girls according to person health problem shows that highest (35.3%) had Wheezing &Asthma, 20.6%,

17.6% and 14.7% had PCOD. Irregular menstruation and gastric ulcer respectively and only 11.8% had thyroid problem. (Table no.:4). Percentage-wise distribution of adolescent girls according to family history of Diabetes Mellitus shows that highest (61.5%) had no history of DM where as 38.5% had family history of DM (Table no.:5). Percentage-wise distribution of adolescent girls according to family history of Polycystic Ovary Syndrome shows that majority (87.4%) had no history of PCOS whereas 12.6% had family history of PCOS (Table no.:6). Mean and SD of anthropometric variable of the adolescent girls shows that Mean weight was 53.87± 11.9, Mean Height 2.01 ± 0.05 and Mean BMI 21.8 ± 4.6(Table no.:7). Percentage-wise distribution of adolescent girls according to BMI shows that highest (48.4%)had normal body weight, 16.7% of them were overweight,29.4% of them were underweight where as only 5.5% of them were obese.(Table no.:8). Table 8 shows that there was statistically significant association found between BMI and all the sociodemographic variable at $p<0.01$. Table 9 shows that there was statistically significant association found between BMI and personal medical and family health history of the adolescent girls at $p<0.05$.

DISCUSSION

Demographic data shows that 45.9% of them were 18 years, 57.1% attained menarche at 12-13 years, 50.3% of them were from Arts background, 89.8% of them were nonvegetarian, 94.5% of them were Hindus, 41.4% had monthly income of Rs 5000-10000, 61.3% of them belong to joint family, 61.8% of them from urban. Health history depicts that 9.3% had person health problems, 38.5% and 12.6% of them family health history of DM and PCOS respectively. Anthropometric variable shows that Mean weight was 53.87 ±11.9, Mean Height 2.01 ±0.05 and Mean BMI 21.8 ±4.6. More or less similar results found by AL-Mahrouqi Z (2021) in his study titled, Prevalence of Obesity among Omani Adolescent Girls and reported Mean weight 57.8 16.0 and Mean BMI was 23.4± 6.3[6].BMI classification shows that 5.5%,16.7% 29.4% and 48.4% of them were obese, overweight, underweight and normal body weight respectively. Study results were supported by M Shashidhar Kotian (2010) who conducted study on Prevalence and Determinants of Overweight and Obesity Among 900 adolescents in the age group of 12 to 15 years School going adolescent children in South Karnataka, Ind shows that prevalence of overweight and obesity among adolescents girls was 10.5% and 4.3% respectively. Statistically significant association found between BMI and all the sociodemographic variable at $p<0.01$ and also between BMI and personal medical and family health history of the adolescent girls at $p<0.05$.

IMPLICATIONS

Community Health Nurse plays major role in the health promotion of adolescent girls. Study findings help the nursing students to provide health education on healthy lifestyle to the adolescents to maintain ideal body weight in the Schools, Colleges and Community. The future researcher may focus on the health risk associated with obesity among adolescent girls.

CONCLUSION

Study concludes that prevalence of overweight and obesity was 16.7% and 5.5% respectively among adolescent girls.Lifestyle habits of current generation adolescent girls increases the risk of obesity. Awareness on healthy lifestyle is prime important to halt the rate of obesity and related health problem.

Source of support: Nil
Conflict of Interest: Nil

REFERENCES

1. Norman RJ, Dewailly D, Legro RS, Hickey TE. Polycystic ovary syndrome. Lancet. 2017 Aug 25;370(9588):685-97. doi: 10.1016/S0140-6736(07)61345-2. PMID: 17720020.

2. Abdelaal M, le Roux CW, Docherty NG. Morbidity and mortality associated with obesity Ann Transl Med. 2017;5:161
3. Seema S, Rohilla KK, Kalyani VC, Babbar P. Prevalence and contributing factors for adolescent obesity in present era: Cross-sectional Study. J Family Med Prim Care. 2021 May;10(5):1890-1894. doi: 10.4103/jfmpc.jfmpc_1524_20. Epub 2021 May 31. PMID: 34195121; PMCID: PMC8208200.
4. Prasad R V, Bazroy J, Singh Z. Prevalence of overweight and obesity among adolescent students in Pondicherry, South India. Int J NutrPharmacolNeurol Dis 2016;6:72-5
5. Mangla, A. , Dhamija, N. , Gupta, U. and Dhall, M. (2019) Lifestyle Trends and Obesity among College Going Girls of Delhi. *Health*, 11, 201-210. doi: 10.4236/health.2019.112018.
6. AL-Mahrouqi Z. The prevalence of obesity among omani adolescent girls. International Journal of Contemporary Medical Research 2021;8(3):C10-C13.
7. Kotian MS, S GK, Kotian SS. Prevalence and determinants of overweight and obesity among adolescent school children of South karnataka, India. Indian J Community Med. 2010 Jan;35(1):176-8. doi: 10.4103/0970-0218.62587. PMID: 20606948; PMCID: PMC2888353.
8. Novosad, Shannon & Khan, Supriya& Wolfe, Bruce n & Khan, Akram. (2013). Role of Obesity in Asthma Control, the Obesity-Asthma Phenotype. Journal of allergy. 2013. 538642. 10.1155/2013/538642.

Table 1: BMI was scored based on WHO classification[8].

Sl.No	BMI	Nutritional status
1	Below 18.5	Underweight
2	18.5–24.9	Normal weight
3	25.0–29.9	Overweight
4	30.0–34.9	Obesity class I
5	35.0–39.9	Obesity class II
6	Above 40	Obesity class III

Table 2: Frequency and percentage distribution of Adolescent girls according to socio demographic variables

n= 364

Sl.No	Socio demographic variables	Frequency (f)	Percentage (%)
1.	Age in year		
	17	62	17.0
	18	167	45.9
	19	135	37.1
2.	Age at Menarche		
	10-11 years	16	4.4
	12-13 years	208	57.1
	14-15 years	132	36.3
	Above 15 years	8	2.2
3.	Discipline		
	Arts	183	50.3
	Science	181	49.7
4.	Dietary Pattern		
	Vegetarian	37	10.2
	Non-vegetarian	327	89.8
5.	Religion		
	Hindu	344	94.5

	Christian	9	2.5
	Muslim	11	3.0
6.	Monthly family Income in Rupees		
	<5000	63	17.3
	5000-10000	151	41.4
	10001-15000	73	20.1
	>15000	77	21.2
7.	Type of family		
	Nuclear	131	36.0
	Joint	223	61.3
	Extended	10	2.7
8.	Place of residence		
	Urban	225	61.8
	Rural	139	38.2

Table 3: Frequency and percentage distribution of Adolescent girls according to Personal medical history

n= 364

Sl.no	Personal health history	Frequency (f)	Percentage (%)
1	Yes	32	9.3
2	No	332	90.7

Table 4: Frequency and percentage distribution of Adolescent girls according to Personal health problem

n= 32

Sl.no	Personal health problem	Frequency (f)	Percentage (%)
1	PCOD	8	20.6
2	Irregular menstruation	6	17.6
3	Thyroid	4	11.8
4	Wheezing &Asthma	12	35.3
5	Gastric ulcer	2	14.7

Table 5: Frequency and percentage distribution of Adolescent girls according to family history of Diabetes Mellitus

n=364

Sl.no	Family history of DM	Frequency (f)	Percentage (%)
1	Yes	140	38.5
2	No	224	61.5

Table 6: Frequency and percentage distribution of Adolescent girls according to family history of Polycystic Ovary Syndrome

n==364

Sl.no	Family history of PCOS	Frequency (f)	Percentage (%)
1	Yes	42	12.6
2	No	322	87.4

Table 7: Mean and SD of weight, Height and BMI n==364

Sl. No	Anthropometric variable	Mean	SD
1	Weight in Kg	53.87	11.9
2	Height in Meter	2.01	0.05
3	BMI	21.8	4.6

Table 8: Frequency and percentage distribution of Adolescent girls according to BMI

n=364

Sl. No	BMI classification	Frequency (f)	Percentage (%)
1	Underweight	107	29.4
2	Normal	176	48.4
3	overweight	61	16.7
4	obesity	20	5.5

Table 9: Association of BMI with selected sociodemographic variables of the adolescent girls

n=364

S. No.	Socio Demographic Variables	Body Mass Index		
		df	χ^2	p value
1	Age in years	6	79.389[a]	.000
2	Age at menarche	9	252.297[a]	.000
3	Discipline	3	12.588[a]	.006
4	Dietary pattern	3	41.394[a]	.000
5	Religion	6	173.559[a]	.000
6	Monthly family income	9	308.247[a]	.000
7	Type of family	6	180.965[a]	.000
8	Residence	3	190.823[a]	.000

p<0.01 highly significant

Table 10: Association of BMI with personal medical and family health history of the adolescent girls

n=364

S. No.	Medical History	Body Mass Index		
		df	χ^2	p value
1	Personal health history	3	511.764[a]	.000
2	Family history of PCOS	3	10.800[a]	.013
3	Family history of DM	3	69.828[a]	.000

p<0.05 significant

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RESEARCH ARTICLE

A Study on Entrepreneurial Orientation among Business School Students in Kollam District Kerala

Mohan Alex Medayil[1]* and R. S. Balakumar[2]

[1]Research Scholar, Department of Management, Christhuraj College (Affiliated to Bharathidasan University), Panjapur, Edamalaipatti Pudur, Tiruchirappalli, Tamil Nadu, India.
[2]Research Supervisor and Assistant Professor, Department of Management, Christhuraj College (Affiliated to Bharathidasan University), Panjapur, Edamalaipatti Pudur, Tiruchirappalli, Tamil Nadu, India.

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***Address for Correspondence**
Mohan Alex Medayil
Research Scholar,
Department of Management,
Christhuraj College (Affiliated to Bharathidasan University),
Panjapur, Edamalaipatti Pudur, Tiruchirappalli,
Tamil Nadu, India.

ABSTRACT

The role of entrepreneurship in work creation and joblessness decrease is grounded by earlier studies. Consequently, one of the approaches to address the joblessness issue among young graduates in Kollam district Kerala is to investigate how pioneering direction could be embraced into the more extensive setting of the country's schooling framework. Exact research tracked down that earlier investigations on business venture instruction in territory of Kerala, India will in general zero in on auditing instructive arrangements, issue and difficulties. The idea of the entrepreneurial orientation determinants past pioneering training stays ambiguous. Subsequently, this investigation propels the conversation in business training by auditing determinants for innovative direction from business school students in Kollam district Kerala, India. Throughout the long term it has been discussed that are Entrepreneurs are born or made so it is imperative to recognize and consider the different variables that support PG students, particularly the MBA gradutates to go into business. It is basically an excursion which makes a character of a person, as somebody supporting self and society on the loose. Thus, it merits understanding those reasons/factors that make somebody not the same as others and have such goal to get into business venture while others are not propelled to dive into it. This exact survey is comprehend the explores done in understanding the inspiring elements to turn into a business visionary, particularly among the board understudies, as they are the best fit to become start a business or take their current privately-run company ahead.

Keywords: : Entrepreneurship, Entrepreneurial, Orientation, Intention, Business School, Kerala

INTRODUCTION

A well known Nike motto urges individuals to "do what needs to be done!" For individuals and associations that have fostered a pioneering direction, "get it done!" is a lifestyle. While frequently connected with beginning new pursuits, a pioneering direction can be entirely significant to set up associations as well. (Astrini, N. J., et al 2020) Below we describe each of the five uniqueness associated with an entrepreneurial orientation: autonomy, proactiveness, competitive aggressiveness, innovativeness, and risk-taking. A focal part of business and methodology is enterprising orientation (EO). It reflects administrative vision and educates the authoritative endeavors needed to deliver advancements that make an incentive for clients and organizations that serve them.(Singh, S. K., & Gaur, S. S. 2018) Entrepreneurship and innovation discuss how we succeed in business. Since Peter Drucker himself mentioned that there is a close relationship between entrepreneurship and innovation, there was a lot of knowledge of on these two. In fact, many academic researchers are publishing studies that identify entrepreneurs and determine how their characteristics relate to the performance of companies. Entrepreneurship is made up of individuals with personality and entrepreneurship, so the overall performance of the company is competitive. Entrepreneur Orientation (EO) knows a range of traits that exist at levels of entrepreneurial entrepreneurs. In academic research, this EO theme is relevant to corporate management practices.(Lewrick, M., et al 2010). Education for entrepreneurship and innovation: "Management capabilities for sustainable growth and achievement". WJESMD. Some researchers conducted a survey of students in the business program indicating that entrepreneurs are studying in the business program because they are starting their own business.

Many factors affect them to start a business, and one of them is their entrepreneurial orientation (EO). Some studies use EO as an important tool to define the characteristics of a business that seeks and defines opportunities that exist in the external environment. The EO measurement itself uses 5 components, autonomy, innovation, aggressiveness, risk-taking and competitive aggression. According to researcher stated, it is important to understand and measure entrepreneurial orientation at the individual level to "Entrepreneur Entrepreneurship Entrepreneur Enlargement." Factors that contribute to success and vice versa. During the 1730s, Richard Cantillon utilized the French time-frame business person, or really "funeral director," to counsel the individuals who embrace independent work while additionally tolerating a questionable return. In resulting years, advertisers have furthermore been known as pioneers of most recent musings (Thomas Edison), people who find and sell new combinations of variables of assembling (receipt Gates' packaging of Microsoft's product), and the individuals who misuse entrepreneurial plans to extend little associations (Mark Zuckerberg at facebook). The ordinary variables of those originations of advertisers are that they experiment and that a few people could create something out of open doors that others can't. Entrepreneurial Orientation (EO) is a key idea when leaders are creating strategies inside the expectations of experimenting and misusing conceivable outcomes that various organizations can't abuse. EO alludes to the strategies, practices, and decision making assortments of gatherings that demonstration innovatively (Lumpkin and Dess, 1996). Any partnership's degree of EO can be perceived via analyzing the manner in which it piles up comparative with five measurements: (1) autonomy, (2) competitive aggressiveness, (3) innovativeness, (4) proactiveness, (5) and risk taking. these measurements are likewise pertinent to individuals.

Problem Statement

The biggest problem facing our economic system is the unemployment rate. India has a higher unemployment rate than all other countries, and currently has the largest unemployment of its graduates. In general, the lack of views and understanding of young people on unemployment leads to a major problem in unpopularity and unemployment in a large sector that provides information to some workers, even if not a twist of fate that there are many business colleges in the Kollam district, Kerala State. There is extremely little expertise on it. It is important to understand the factors that expect a for-profit entrepreneurial view of the reality that entrepreneurial behavior is the result of the formation of positive attitudes and motivation. They are more solid and more consistent and are not afraid of difficult situations that can be directly committed and re-committed (Ault, J.K. & Spicer, A.2020). One of the important discoveries of the screen record of global entrepreneurship in 2015 is how the views differ from 1 person

to further promote the appearance of entrepreneurship as a work. . (Kumari, M.2021) The expertise of this attitude may be important not only to motivate domestic entrepreneurship, but also to support reporting orders.

Objectives

The objectives of the research are as follows:

- To understand the demographic profile of business school students and Institutional Support of the business school students to start their own business.
- To study and analysis of entrepreneurial orientation and business guidance from business schools students
- To study the relationship between entrepreneurial orientation and entrepreneurial opportunities.

LITERATURE REVIEW

(Bernoster, I., et al 2020) even though the literature on affect (i.e., the volume to which an individual subjectively experiences emotions and emotions) is burgeoning inside the field of entrepreneurship, have an effect on has no longer received enough interest with recognize to an essential antecedent to entrepreneurial achievement—entrepreneurial orientation. in this paper, we check out the role of both tremendous and negative have an effect on in entrepreneurial orientation (i.e., the strategic posture of a firm/individual with appreciate to proactiveness, innovativeness, and risk taking) and entrepreneurial achievement. The consequences of our evaluation, primarily based on two samples (337 Dutch sole proprietors and 254 French small commercial enterprise owners), display that positive affect is undoubtedly related to entrepreneurial orientation, while poor have an effect on is negatively associated with entrepreneurial orientation for sole proprietors. With appreciate to entrepreneurial success, results are blended. the existing study contributes to the information of the role of affect in entrepreneurial orientation. It also contributes to the literature on entrepreneurial fulfillment, the last goal in the field of entrepreneurship.(Musara, M., & Nieuwenhuizen, C. 2020) The informal economy plays a vital role in solving the social currency problems faced by many countries in the world. The informal entrepreneurial movement accounts for 10-20% of GDP in advanced economies and 60% in growing economies. In South Africa, informal areas account for 15% to 17% of general employment and approximately 5.2% of the country's GDP. But little or no attention has been paid to how informal entrepreneurial spirit shapes one's entrepreneurial orientation and the emergence of entrepreneurial leadership, and vice versa. Considering that a large number of successful marketers in Africa now start to do business in the informal area, people are not interested in the concept of male or female business orientation, and the rise of business management in the informal sector is worrying. This newsletter provides a multi-level assessment of the emergence of business management in the informal area of South Africa.

We use inspiring ideas of debt and social identity from poverty to wealth to increase the inclusive framework that emerges in informal neighborhood business management. For the sake of illustration, a brief case study of successful business leaders emerging from the leisure area is provided. This document aims to provide valuable information on the following aspects, one of which is the under-researched but fast-growing business environment, the entrepreneurial spirit in informal areas, and how this environment affects the direction of individual business and the rise of business management. This provides new insights into previously hidden areas and further studies how to advance business development research and practice. (Al Mamun, A., et al 2017) The purpose of this paper is to report on the study performed so that you can increase a valid degree for entrepreneurial orientation, particularly inside the context of low-earnings families in Malaysia. most previous studies examined the constructs of threat-taking and innovativeness because the additives of entrepreneurial orientation; however, a scarce variety of researchers targeted on other big attributes of entrepreneurial orientation, such as proactiveness or autonomy. therefore, this have a look at has tested Creativity and Innovativeness, threat Taking, Proactiveness, and Autonomy, also offering an device to measure Entrepreneurial Orientation. The look at adopted a cross-sectional design, at the same time as quantitative data was collected from 800 households throughout 4 districts in Kelantan, Malaysia, the use of structured interviews. based totally at the reliability and validity checking out, the take a look at finalized the instrument to 17 gadgets yielding 4 elements, i.e., Creativity & Innovativeness (4 objects), risk Taking (3 items), Proactiveness (five objects), and Autonomy (five items). The findings of the reflective hierarchical version display

that Autonomy is the very best contributor to entrepreneurial orientation most of the low-income families in Kelantan, observed through Proactiveness, Creativity, & Innovativeness, and chance-Taking. destiny researchers may want to similarly enlarge the evolved degree by way of go-analyzing the device supplied in this look at across extraordinary income-level organizations in the course of developing and developed countries.(Martens, C. D. P., et al 2018) Entrepreneurial orientation (EO) is a strategic posture of an business enterprise, and it's miles related to primary regulations and practices for the development of entrepreneurial movements searching out developing aggressive benefits. This have a look at develops and exams a model of the relationship among entrepreneurial orientation and mission fulfillment in Brazilian context. As quantitative research, a survey was used to collect records. A sample of one hundred valid solutions from assignment practitioners changed into treated through the structural equation modeling technique. As studies implications, the main result factors out the positive association between the entrepreneurial orientation and the undertaking success, contributing to the development of this research subject and supporting to decrease the space within the literature that addresses the relationship between assignment success and EO. In practical terms, expertise that innovativeness, threat taking, proactiveness, autonomy and aggressive aggressiveness (the size of the EO) can contribute to assignment success and also can indirectly impact on organizational performance, should assist corporations get aggressive advantage while developing correlate elements. in the end, the consequences recommend that practices of mission control may be aligned to the company's entrepreneurial orientation to permit firms to achieve higher effects of their tasks and generate a aggressive benefit. On other hand, given the percentage of the effect of EO on assignment success (23 %) identified on this take a look at, it is crucial that undertaking management specialists increase their horizon to understand other factors that have an effect on assignment fulfillment.

RESEARCH METHODOLOGY

Researchers apply technical research design so that the study population is a student of a business school studying at a university in the Kollam District, Kerala region of India. It belongs to Technical Education in India. A list of colleges and universities that belong to kerala University and offer MBA programs in the column area approved by AICTE was obtained from UGC's website. The sample designs used in the study are systematic random sampling with a total sample size of 450 using scientific formulas, with 45 respondents from each of the 10 institutions offering Technical Education India related MBA programmes. A pilot study using 30 samples tested in advance for improvement of the questionnaire. The reliability and validity of the content tested in the survey. Basic data, questionnaires collected using structured questionnaires were self-managed. The study statistical tools used in the analysis are frequency analysis, Anova, and multiple regression analysis. Statistical Package for Social Sciences-SPSS version 16 was used for analysis.

DATA ANALYSIS AND DISCUSSION

Demographic analysis

The demographic profile of the respondents collected based on gender, age group, location, parent's occupation, parent's educational qualification, entrepreneurial orientation questions, institutional support questions their close associates' entrepreneurs and their interest in the entrepreneurial activity in their business schools. The above table 1 to 8 inferred that the dominance of male students. There are male students (66%) and the female students (34%) out of all the students. The most of the students falls between the age group of 18-22 and 23-27.The most of business student's parents are working in Public sector (31.78%) and followed by self-employed parents (28.22%). Location of the business school students Urban (63.77%) followed by Semi-urban (20.22%). The most of the parents are Post Graduate Degree qualified (61.55%) and followed by UG (26.22%), The most of the parents annual income 3L to 5L (48.67%) and followed by Below 3L (24.89%) and maximum business school students are willing to take new business as job(63.77%) and Business school students' Institutional Support mentioned majority is entrepreneurship club (31.78%) and followed by startup club (28.22%).

Chi-Square Test

Table 9 Chi-Square Tests- Institutional support of the business school students versus Starting a business gives opportunity to be economically Independent.

H_0: There is no significant relationship between Institutional support of the business school students versus Starting a business gives opportunity to be economically Independent

H_1: There is a significant relationship between Institutional supports of the business school students versus Starting a business gives opportunity to be economically Independent

From the above table 9 In Pearson Chi-square value, were a low significance value of .000 (typically below 0.05) is observed. Hence, the Null hypothesis is rejected and alternative hypothesis is accepted and inferred that there exist a relationship between two variables namely Institutional supports of the business school students versus Starting a business gives opportunity to be economically Independent.

Table 10 Chi-Square Tests - Institutional supports of the business school students versus students have a strong tendency for high-risk tasks

H_0: There is no significant relationship between Institutional supports of the business school students versus students have a strong tendency for high-risk tasks

H_1: There is a significant relationship between Institutional supports of the business school students versus students have a strong tendency for high-risk tasks

From the above table 10 In Pearson Chi-square value, were a low significance value of .000(typically below 0.05) is observed. Hence, the Null hypothesis is rejected and alternative hypothesis is accepted and inferred that there exist a relationship between two variables namely business school students versus students have a strong tendency for high-risk tasks.

Table 11 Chi-Square Tests - Institutional supports of the business school students versus More entrepreneurship related programme on business school campus could help their to start own businesses

H_0: There is no significant relationship between Institutional supports of the business school students versus More entrepreneurship related programme on business school campus could help their to start own businesses

H_1: There is a significant relationship between Institutional supports of the business school students versus More entrepreneurship related programme on business school campus could help their to start own businesses.

From the above table 11 In Pearson Chi-square value, were a low significance value of .000(typically below 0.05) is observed. Hence, the Null hypothesis is rejected and alternative hypothesis is accepted and inferred that there exist a relationship between two variables namely Institutional supports of the business school students versus More entrepreneurship related programme on business school campus could help their to start own businesses.

ANOVA and Multiple Comparisons Post-Hoc Bonferroni Test

From the above table 13, it is inferred that in one-way ANOVA, Significance indicates the significance level of the F-test. Small significance value (<. 05) indicates group, the difference between variables namely Starting a business gives opportunity to be economically Independent. (0.013),Students have a strong tendency for high-risk tasks (0.001) and More entrepreneurship related programme on business school campus could help their to start own businesses(0.012)

Regression analysis

The above table 14 displays are R, R2, adjusted R2 and standard error R (. 461) denotes the multiple correlation coefficient .i.e. it is the correlation between the observed and predicted values of the dependent variable. R^2 (.198) is the proposition of variation in the dependent variable explained by the regression model. Sample R2 tends to optimistically estimates how well the model fits the population. Adjusted R^2 (.171) attempts to correct R^2 to more closely reflect the goodness of fit of the model in the population. The above table 15summarizes the results of the analysis of variance. Sum of squares, degrees of freedom, mean square are displayed for two sources of variations, regression and residual. The above output for regression displays information about the variations accounted for by the model. The output for a total (368.693) is the sum of information for regression (67.012) and residual (296.682). A

model with the large regression sum of squares in comparison with residual sum of squares indicates that the model accounts for the most of the variation in the dependent variable. F statistics (93.590) are the regression mean square dividend residual mean squared. Regression degree of freedom is the numerated degree of freedom and the residual degree of freedom is the denominator degree of freedom for the 'F' statistics. The total number degree of freedom is the number of cases minus 1. If the significance of 'F' statistics is small (0.05), then the independent variable does a good work in explaining the variation in the dependent variable. From the above table 16 , Significance value .000 and beta value .471 and overall Business school guidance and Government support is absolutely necessary to start and run a business is the significant variables.

CONCLUSION

The performance and entrepreneurial orientation exercise amongst enterprise school college students are slight. In standard, entrepreneurial orientation undoubtedly impacts ventures performance, and mainly, proactiveness, risk-taking, and independent dimensions undoubtedly decide commercial enterprise overall performance. the extent of affect is growing as upcoming enterprise are worried with the commercial enterprise activity for the reason that entrepreneurial orientation is better and contributed more to the mission's overall performance in this industry. We want to measure it because we remember this statistics to be crucial to identify the success of individuals beginning their personal organizations as commercial enterprise owners or entrepreneurs with entrepreneurial competencies and expertise given at business schools and universities.

REFERENCES

1. Ault, J. K., & Spicer, A. (2020). State fragility as a multi-dimensional construct for international entrepreneurship research and practice. Asia Pacific Journal of Management, 37(4), 981–1011.
2. Al Mamun, A., Kumar, N., Ibrahim, M. D., & Bin Yusoff, M. N. H. (2017). Validating the measurement of entrepreneurial orientation. Economics and Sociology, 10(4), 51–66.
3. Astrini, N. J., Rakhmawati, T., Sumaedi, S., Bakti, I. G. M. Y., Yarmen, M., & Damayanti, S. (2020). Innovativeness, Proactiveness, and Risk-taking: Corporate Entrepreneurship of Indonesian SMEs. In IOP Conference Series: Materials Science and Engineering (Vol. 722).
4. Bernoster, I., Mukerjee, J., & Thurik, R. (2020). The role of affect in entrepreneurial orientation. Small Business Economics, 54(1), 235–256.
5. Kumari, M. (2021). Role of Entrepreneurship in Indian Economy. SSRN Electronic Journal. orientation perspective. Entrepreneurship: Theory and Practice, 33(1), 47–69.
6. Lewrick, M., Omar, M., Raeside, R., & Sailer, K. (2010). Education for entrepreneurship and innovation:"Management capabilities for sustainable growth and success". World Journal of Entrepreneurship, Management and Sustainable Development.
7. Musara, M., & Nieuwenhuizen, C. (2020). Informal sector entrepreneurship, individual entrepreneurial orientation and the emergence of entrepreneurial leadership. Africa Journal of Management, 194–213.
8. Martens, C. D. P., Machado, F. J., Martens, M. L., Silva, F. Q. P. de O. e., & Freitas, H. M. R. de. (2018). Linking entrepreneurial orientation to project success. International Journal of Project Management, 36(2), 255–266.
9. Singh, S. K., & Gaur, S. S. (2018). Entrepreneurship and innovation management in emerging economies. Management Decision.
10.

Table 1. Business School Students Age - Frequency

Age	Frequency	Percentage
18 to 22	241	53.56
23 to 27	150	33.33
Above 27	59	13.11
Total	450	100.0

Table 2. Business school students – Gender Frequency

Gender	Frequency	Percentage
Male	297	66.00
Female	153	34.00
Total	450	100.0

Table 3. Business school students – Location frequency

Location	Frequency	Percentage
Urban	287	63.77
Rural	72	16.0
Semi-Urban	91	20.22
Total	450	100.0

Table 4. Business school students' Parent's Occupation

Parent's Occupation	Frequency	Percentage
Public sector	143	31.78
Private sector	96	21.33
Self-employed	127	28.22
Retired	33	7.33
Others	51	11.33
Total	450	100.0

Table 5. Parent's Education Qualification of Business school students

Parent's Qualification	Frequency	Percentage
SSLC	8	1.77
HSC	35	7.77
UG	118	26.22
PG	277	61.55
PHD	12	2.67
Total	450	100.0

Table 6. Annual income of parents

Parent's Annual Income	Frequency	Percentage
Below 3L	112	24.89
3L-5L	219	48.67
6L-8L	64	14.22
9L-10L	20	4.44
Above 10L	35	7.77
Total	450	100.0

Table 7. Business school students' Institutional Support

Institutional Support	Frequency	Percentage
Entrepreneurship Club	143	31.78
Business Club	80	17.77
Startup Club	127	28.22
Entrepreneurship Development Cell (EDC)	84	18.67
Other	16	3.56
Total	450	100.0

Table 8. Are you willing to take new business as a Job?

Business School Students opinion	Frequency	Percentage
Yes	287	63.77
No	163	36.22
Total	450	100.0

Table 9. Chi-Square Tests- Institutional support of the business school students versus Starting a business gives opportunity to be economically Independent.

	Value	df	A symp. Sig. (2-sided)
Pearson Chi-Square	39.752(a)	12	.000
Likelihood Ratio	40.540	12	.000
Linear-by-Linear Association	.083	1	.773
N of Valid Cases	450		

A 4 cells (20.0%) have expected count less than 5. The minimum expected count is 2.56.

Table 10. Chi-Square Tests - Institutional supports of the business school students versus students have a strong tendency for high-risk tasks

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	54.457(a)	16	.000
Likelihood Ratio	71.251	16	.000
Linear-by-Linear Association	14.496	1	.000
N of Valid Cases	450		

A 7 cells (28.0%) have expected count less than 5. The minimum expected count is .06.

Table 11. Chi-Square Tests - Institutional supports of the business school students versus More entrepreneurship related programme on business school campus could help their to start own businesses

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	47.904(a)	12	.000
Likelihood Ratio	53.285	12	.000
Linear-by-Linear Association	8.018	1	.005
N of Valid Cases	450		

A 4 cells (20.0%) have expected count less than 5. The minimum expected count is 3.87.

Table 12: ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Starting a business gives opportunity to be economically Independent.	Between Groups	13.399	4	3.350	3.194	.013
	Within Groups	420.111	445	1.064		
	Total	433.510	449			
students have a strong tendency for high-risk tasks	Between Groups	16.696	4	4.174	5.121	.001
	Within Groups	322.494	445	.816		
	Total	339.190	449			
More entrepreneurship related programme on business school campus could help their to start own businesses	Between Groups	15.535	4	3.884	3.257	.012
	Within Groups	468.465	445	1.186		
	Total	484.000	449			

Table 13 : Multiple Comparisons

Bonferroni

Dependent Variable	(I) 6. Institutional Support	(J) 6. Institutional Support	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Starting a business gives opportunity to be economically Independent.	Entrepreneurship Club	Business Club	-.049	.141	1.000	-.45	.35
		Startup club	-.244	.129	.590	-.61	.12
		EDC	.472	.208	.238	-.12	1.06
		Others	-.175	.224	1.000	-.81	.46
	Business Club	Entrepreneurship Club	.049	.141	1.000	-.35	.45
		Startup Club	-.195	.145	1.000	-.60	.21
		EDC	.521	.218	.176	-.10	1.14
		Others	-.126	.234	1.000	-.79	.53
	Startup Club	Entrepreneurship Club	.244	.129	.590	-.12	.61
		Business Club	.195	.145	1.000	-.21	.60
		EDC Others	.715(*)	.210	.007	.12	1.31
		Others	.069	.227	1.000	-.57	.71
	EDC	Entrepreneurship Club	-.472	.208	.238	-1.06	.12
		Business Club	-.521	.218	.176	-1.14	.10
		Startup Club Others	-.715(*)	.210	.007	-1.31	-.12
		Entrepreneurship Club	-.647	.279	.211	-1.44	.14
	Others	Business Club	.175	.224	1.000	-.46	.81
		Startup Club	.126	.234	1.000	-.53	.79
		EDC	-.069	.227	1.000	-.71	.57
			.647	.279	.211	-.14	1.44
students have a strong tendency for high-risk tasks	Entrepreneurship Club	Business Club	-.186	.124	1.000	-.54	.16
		Startup club	-.292	.113	.099	-.61	.03
		EDC	-.749(*)	.182	.000	-1.26	-.24
		Others	-.416	.197	.349	-.97	.14
	Business Club	Entrepreneurship Club	.186	.124	1.000	-.16	.54
		Startup Club	-.106	.127	1.000	-.46	.25
		EDC	-.563(*)	.191	.034	-1.10	-.02
		Others	-.230	.205	1.000	-.81	.35
	Startup Club	Entrepreneurship Club	.292	.113	.099	-.03	.61
		Business Club	.106	.127	1.000	-.25	.46
		EDC Others	-.457	.184	.135	-.98	.06

		Others	-.124	.199	1.000	-.68	.44
	EDC	Entrepreneurship Club	.749(*)	.182	.000	.24	1.26
		Business Club	.563(*)	.191	.034	.02	1.10
		Startup Club	.457	.184	.135	-.06	.98
		Others					
		Entrepreneurship Club	.333	.245	1.000	-.36	1.02
	Others	Business Club	.416	.197	.349	-.14	.97
		Startup Club	.230	.205	1.000	-.35	.81
		EDC	.124	.199	1.000	-.44	.68
			-.333	.245	1.000	-1.02	.36
More entrepreneurship related programme on business school campus could help their to start own businesses	Entrepreneurship Club	Business Club	-.364	.149	.153	-.79	.06
		Startup club	-.233	.136	.874	-.62	.15
		EDC	-.660(*)	.220	.028	-1.28	-.04
		Others	-.447	.237	.598	-1.12	.22
	Business Club	Entrepreneurship Club	.364	.149	.153	-.06	.79
		Startup Club	.131	.153	1.000	-.30	.56
		EDC	-.297	.231	1.000	-.95	.35
		Others	-.083	.247	1.000	-.78	.61
	Startup Club	Entrepreneurship Club	.233	.136	.874	-.15	.62
		Business Club	-.131	.153	1.000	-.56	.30
		EDC	-.428	.222	.550	-1.05	.20
		Others					
		Others	-.214	.239	1.000	-.89	.46
	EDC	Entrepreneurship Club	.660(*)	.220	.028	.04	1.28
		Business Club	.297	.231	1.000	-.35	.95
		Startup Club	.428	.222	.550	-.20	1.05
		Others					
		Entrepreneurship Club	.213	.295	1.000	-.62	1.05
	Others	Business Club	.447	.237	.598	-.22	1.12
		Startup Club	.083	.247	1.000	-.61	.78
		EDC	.214	.239	1.000	-.46	.89
			-.213	.295	1.000	-1.05	.62

* The mean difference is significant at the .05 level.

Table 14 : Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.461(a)	.198	.171	.810

a Predictors: (Constant), Institutional support will help them to start their own business

Table 15: ANOVA (b)

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	72.011	1	67.012	93.590	.000(a)
	Residual	296.682	396	.725		
	Total	368.693	397			

a Predictors: (Constant), Institutional support will help them to start their own business

b Dependent Variable: [Overall, Business school guidance and Government support is absolutely necessary to start and run a business]

Table 16: Coefficients (a)

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	2.299	.168		13.671	.000
	Institutional support will help them to start their own business	.394	.041	.471	9.617	.000

a Dependent Variable: [Overall, Business school guidance and Government support is absolutely necessary to start and run a business]

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RESEARCH ARTICLE

Effect of Flat footwear on Functional Balance among Young Females

Prerna Sharma[1]* and Daksha Davey[2]

[1]Research Officer at Hindustan latex Family Planning Promotion Trust, Noida, Uttar Pradesh, India.
[2]Physiotherapist (Self – Employed) at Noida, Uttar Pradesh, India.

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***Address for Correspondence**
Prerna Sharma
Research Officer at Hindustan latex Family Planning Promotion Trust,
Noida, Uttar Pradesh, India

ABSTRACT

Balance is an essential part of life for all people. All movements must be performed in static or dynamic environments to maintain a high quality of life, thus individuals must be able to attain physical homeostasis and maintain stability throughout both standing and ambulatory activities. Flat-soled shoes are linked to aberrant lower-leg kinematics. Excessive use of flats has been related to discomfort, with an increase in heel pain. At heel contact, the flat footwear delivers a lower peak vertical force. Flat shoes cause the tibialis anterior (TA) muscle to contract in response to the ankle angle during the swing phase of walking. As a result, the study's goal is to see how flat footwear affects functional balance in young females. This study included 60 females between the ages of 18 and 28, who had been wearing flats for the previous two years. The Multi-factorial fall risk screening Questionnaire was used to screen all of the participants followed by Four Square Step Test (FSST) and the Functional Reach Test were used to determine functional balance (FRT). This study found the positive statistical correlation between FSST score and duration of wearing flats (r = 0.74). A negative correlation was also observed between FRTS score and duration of wearing flats (r = –0.78) and though the results are negative but statistically significant which represents tendency of negative correlation.

Keywords: Functional balance, Multi factorial fall risk screening questionnaire, FSST and FRT

INTRODUCTION

Adulthood is a typical stage of human physical and mental development. Adults are frequently exposed to images and ideas of how they should appear, behave, and act [1]. Human health is defined as their level of functional or metabolic efficiency. It describes a person's overall physical and emotional well-being and frequently refers to their lack of illness, trauma, or suffering [2]. Since maintaining balance is a crucial living need for all humans, balance control has been thoroughly investigated in a number of studies. It is crucial for people to be able to attain body equilibrium and maintain stability during peaceful standing and ambulatory activities as all motions, whether static or dynamic, are required to maintain a high quality of life [3, 4]. One of the most important factors in achieving

postural balance is the anatomy of foot. The contribution of the foot mechanism towards the stability of postural standing has attracted attention from various investigators. The toe is in charge of providing a stable surface area that stays in contact with the ground and serves to relay pertinent sensory proprioception information to the central nervous system, whereas it has been demonstrated that the normal arch in the foot plays an important role in shock absorption and propulsion phases [5].Standing balance control relies substantively on proprioception (i.e., our sense of body position and movement). Ankle joint proprioception appears to be of particular importance, as it provides the most salient information regarding standing body sway [6]. According to general consensus, the human body will attempt to orient the centre of mass (COM), which is equivalent to the total body mass at which the average of the mass distribution for each body segment may be assumed to be concentrated, against disturbance. The somatosensory, vestibular, and visual systems are thought to be the main physiological inputs of balance control [7].

A healthy person will instinctively try to return to COM inside the base of support by unconsciously controlling the position of the body. According to Winter et, al, a person's foot activity directly affects where the COP is located. According to him, an increase in evertor activity will cause the COP to shift medially, whereas an increase in invertor activity will cause the COM to shift laterally and move in a lateral manner, causing the COM to shift medially [6]. It goes without saying that carrying out daily tasks effectively depends on balance control systems functioning properly. Humans lack these balance control systems by nature due to a relatively high centre of mass and a bipedal stance. To maintain postural control, the body's centres of mass must be continuously maintained over an ever-changing base of support [8].Three major sensory system works together to provide the central nervous system with the information needed to counteract balance perturbations. The visual, vestibular and somatosensory system all help detect changes in the environment that could lead to fall. The visual system uses the eyes to provide feedback about changing environmental conditions and the body’s position in the environment. The vestibular system relies on input from the inner ear to sense linear and angular acceleration as well as to maintain a steady gaze and an upright vertical stance [9]. One of the most sensitive parts of the human body is the skin on the bottom of the foot, which is crucial in detecting balancing information [10].

Since shoes alter the interface between mechanoreceptors and the external environment, they must be taken into account when looking at factors that influence postural control [8]. Shoes that don't have the natural shape and function of the foot will eventually change the foot's morphology and biomechanical behaviour [12]. While the fundamental function of footwear is to protect the foot and aid in propulsion, footwear influences postural stability and the resultant risk of slips, trips, and falls through altering somatosensory feedback to the foot and ankle and influencing frictional conditions at the footwear/floor interface. Footwear and features of footwear is responsible to affect the balance and gait in young people [11]. Flat footwear is associated with abnormal kinematics in the lower leg. The excessive wearing of flats has been linked to discomfort; it leads to an increase in heel pain. The flat footwear produces a decreased peak vertical force at heel contact [13]. Peak vertical force at heel contact is reduced by flat shoes [13]. During the swing phase, the flats on the foot would activate the toe flexor muscles (FHL, FDL and TP) and as FHL, FDL, and TP also cross the ankle joint they would imply a plantar flexion moment at the ankle, which would reduce dorsiflexion and increase activity of the TA. Decreased dorsiflexion during the swing phase of gait, the activity of the toe flexors to grip the flats may also affects the windlass mechanism of foot as the first MTP joint extends which results in the plantar fascia tightens. Flats also affect the gait dynamics of foot [14, 15]. Therefore the study aims to find out the effect of flat footwear on functional balance in young females.

MATERIALS AND METHODS

This study was included 60 females between the ages of 18 and 28, who had been wearing flats for the minimum of two years. The entire study was described to the subjects, and they gave their informed consent for the participation in the study. The Multi-factorial fall risk screening Questionnaire was used to screen all of the respondents, and only those who met the inclusion criteria were included in the study. The heel height and flatness of each subject's foot wear were measured along with the demographic details of the participants. The subjects' functional balance (FRT)

was assessed using the Four Square Step Test (FSST) and the Functional Reach Test. During the FSST, participants had to step over four 90-cm-long walking sticks that were arranged in a cross on the ground in a predetermined order. The subjects starting position is in square 1, with square 2 to their left. Then, the participant's starts by stepping forward, to the right, backward, and to the left into each quadrant in the clockwise direction, followed by the reverse sequence in the counterclockwise direction (i.e, the sequence 2, 3, 4, 1, 4, 3, 2, 1). Both feet must make contact in each quadrant. The participant was instructed to complete the sequence as fast as possible without touching the sticks. The time taken to complete the sequence was recorded. The trial was considered a failure and repeated when the sequence was not completed correctly, the subject lost her balance, or a foot touched a cane. Trials were only repeated to a maximum of twice when the participant fails during the task. Each participant performed 1 practice trial and 2 trials for the score. Best score was noted as a score. Further, Functional Reach Test was done by utilizing a straightforward clinical tool consisting of a leveled "yardstick" fastened to the wall at correct acromion height. The participant was instructed to stretch their arm forward and make a fist. The location of the third metacarpal's end along the yardstick was then noted. The position of the third metacarpal was once more noted as the subjects were then instructed to reach as far forward as they could without losing their balance. Mean difference between positions 1 and 2 over three trials were noted as a result.

RESULT

Continuous data (weight, height, BMI, duration of wearing flats, FSST score and FRT score) were summarized as Mean ± SD (standard deviation). The primary outcome measures of the study were functional balance. A two-tailed (α = 2) $p<0.05$ was considered statistically significant. Analyses were performed on SPSS software. The baseline demographic characteristics age, weight, height and BMI is summarized in table 1 and also depicted in figure 1.Four square step test and functional reach test score were analyzed with the duration of wearing flats and BMI for the Pearson's correlation coefficient, and the values of each parameter are presented in **Table 2 which** reveals that there is an overall positive correlation between FSST score and duration of wearing flats (r = 0.74) which is statistically significant. A negative correlation was observed between FRTS score and duration of wearing flats (r = –0.78) and though the results are negative but statistically significant which represents tendency of negative correlation. No correlation has been found between BMI and functional balance.

DISCUSSION

Flat footwear is associated with abnormal kinematics in the lower leg. The excessive wearing of flats has been linked to discomfort; it leads to an increase in heel pain. The flat footwear produces a decreased peak vertical force at heel contact. The tibialis anterior (TA) muscle contracts in flat shoes in proportion to the ankle angle during the swing phase of locomotion [13]. The four square step test and the functional reach test were used in the current study to evaluate functional balance. The FSST is a brand-new clinical examination that involves stepping and direction changes to assess dynamic standing balance. Healthy active adults aged younger than 30 years can complete the FSST in less than six second and forward reach test is a dynamic balance test, it measures of postural control with balance [16]. As age are the most significant and sensitive factor for this test, we were used normal measurement in inches according to the age group. Biomechanical effect of footwear on human movement influences postural responses to footwear. A study conducted in 2000 revealed that the type of footwear affects the measurement obtained with the FRT [14].Therapeutic footwear and insoles offers an external modifiable additional factor with the potential to influence balance. Footwear characteristics, including heel collar height, sole hardness, and trad has been found to influence quantities measure of balance. Joanne S. Paton in 2013 found that footwear choices influenced balance confidence in day to day life activities [17]. Study by Perry SD in 2017 concluded that the influence of different midsole material and even the presence of it impair the dynamic balance system in healthy young females [15]. Footwear serves as a support area for the body and if the support area is unstable or inadequate then the static

and dynamic stability of a person is compromised. Kristen in 1998 has also revealed that functional balance performance was superior for subjects those wearing shoes as compared to slipper or barefoot condition [12].

CONCLUSION

In summary, this is the first study to evaluate the effect of flat footwear on functional balance in young females and the concluding observation shows that there is a significant effect of flat footwear on functional balance in young females. However BMI has not been found associated with the flat footwear on functional balance. The results of this study can be helpful for young adult to choose their footwear wisely and to prevent the alteration of foot anatomy and maintaining a good functional balance. Furthermore, this study suggests the need for research on association of specific type of foot wears with functional balance among young as well as elderly women.

REFERENCES

1. Tanner JL, Arnett JJ. The emergence of emerging adulthood: The new life stage between adolescence and young adulthood. In Routledge handbook of youth and young adulthood 2016 Oct 14 (pp. 50-56). Routledge.
2. Miles L. Physical activity and health. Nutrition bulletin. 2007 Dec;32(4):314-63.
3. Julia Greve; Angelica Alonso; Ana Carolina P.G. Bordini; Gilberto Luis Camano. Correlation between body mass index and postural balance. vol.62 no.6 São Paulo 2007.
4. Ku PX, Abu Osman NA, Yusof A, Wan Abas WA. The effect on human balance of standing with toe-extension. PLoS One. 2012 Jul 25;7(7):e41539.
5. Han J, Anson J, Waddington G, Adams R, Liu Y. The role of ankle proprioception for balance control in relation to sports performance and injury. BioMed research international. 2015 Oct 25;2015.
6. Winter DA. Human balance and posture control during standing and walking. Gait & posture. 1995 Dec 1;3(4):193-214.
7. Zabjek KF, Coillard C, Rivard CH, Prince F. Estimation of the centre of mass for the study of postural control in idiopathic scoliosis patients: a comparison of two techniques. European Spine Journal. 2008 Mar;17(3):355-60.
8. Massion J. Postural control system. Current opinion in neurobiology. 1994 Dec 1;4(6):877-87.
9. Gaerlan MG. The role of visual, vestibular, and somatosensory systems in postural balance (Doctoral dissertation, University of Nevada, Las Vegas).
10. Horgan NF, Crehan F, Bartlett E, Keogan F, O'Grady AM, Moore AR, Donegan CF, Curran M. The effects of usual footwear on balance amongst elderly women attending a day hospital. Age and ageing. 2009 Jan 1;38(1):62-7.
11. Arulsingh W, Pai GS. Does foot wear influence the occurrence of foot defects, deformities and diseases in long and middle distance runners?-An analytic cross sectional study. European Journal of Sports and Exercise Science. 2014;3(3):42-60.
12. Brooks KC, Jonkman SL, Schmitz MT. The influence of footwear on functional balance in a population of institutionalized elderly women (Doctoral dissertation, Grand Valley State University).
13. Shroyer JF. Flip-flops: Fashionable but functionally flawed. Lower Extremity Review Magazine. 2010.
14. Arnadottir SA, Mercer VS. Effects of footwear on measurements of balance and gait in women between the ages of 65 and 93 years. Physical Therapy. 2000 Jan 1;80(1):17-27.
15. Perry SD, Radtke A, Goodwin CR. Influence of footwear midsole material hardness on dynamic balance control during unexpected gait termination. Gait & posture. 2007 Jan 1;25(1):94-8.
16. Branthwaite H, Chockalingam N, Greenhalgh A. The effect of shoe toe box shape and volume on forefoot interdigital and plantar pressures in healthy females. Journal of foot and ankle research. 2013 Dec;6(1):1-9.
17. Paton JS, Roberts A, Bruce GK, Marsden J. Does footwear affect balance?: the views and experiences of people with diabetes and neuropathy who have fallen. Journal of the American Podiatric Medical Association. 2013 Nov 1;103(6):508-15.

Table 1. Demographic measurements of participants

Variables	Mean ± SD
Age	20.9±1.6
Height	158±5
Weight	52.4±5
BMI	21±1
Duration	3.9±2.2
FSST Score	7.4±1.5
FRT Score	11.3±2

Table 2. Pearson's correlation coefficient between parameters and p-values

Parameter	Pearson correlation (r)	P Value
FSST score and duration of wearing flats	0.74	<0.00001
FRTS score and duration of wearing flats	-0.78	<0.00001
BMI and FSST score	-0.06	0.648
BMI and FRTS score	-0.03	0.820

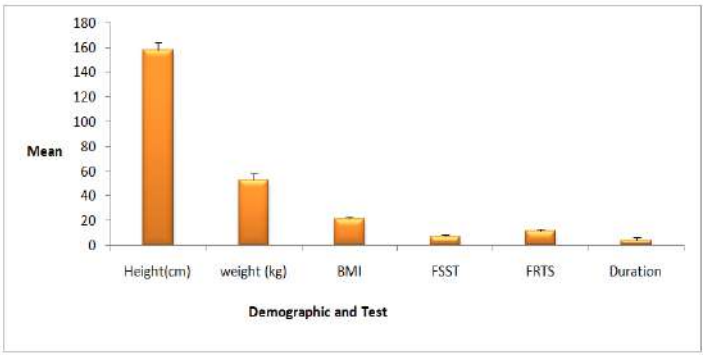

Figure 1. Mean and standard deviation of demographics of participants

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RESEARCH ARTICLE

Green Synthesis of Silver Nanoparticles from *Chaetomorpha antennina* (Bory) Kützing and its Antibacterial Activity

Rakkimuthu R[1]*, Nishana A[2], Sathishkumar P[3] Anandakumar A.M[3], Sowmiya D[3] and Poonkodi K[4]

[1]Assistant Professor and Head, PG and Research Department of Botany, Nallamuthu Gounder Mahalingam College, Pollachi, Tamil Nadu, India
[2]PG Student, PG and Research Department of Botany, Nallamuthu Gounder Mahalingam College, Pollachi, Tamil Nadu, India.
[3]Assistant Professor, PG and Research Department of Botany, Nallamuthu Gounder Mahalingam College, Pollachi, Tamil Nadu, India
[4]Assistant Professor and Head, PG Department of Chemistry, Nallamuthu Gounder Mahalingam College, Pollachi, Tamil Nadu, India.

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***Address for Correspondence**
Rakkimuthu R
Assistant Professor and Head,
PG and Research Department of Botany,
Nallamuthu Gounder Mahalingam College,
Pollachi, Tamil Nadu, India
Email: biorakki@gmail.com

ABSTRACT

A simple method for the green synthesis of silver nanoparticles (AgNPs) from Green algae *Chaetomorpha antennina* present in the coastal region of Kerela. The formation of silver nanoparticles was characterized by UV–vis and FTIR. The UV absorption spectra at 430 nm revealed the characteristic spectra of the silver nanoparticles. The Fourier Transform Infrared (FTIR) spectra indicated the presence of polyphenols or protein, alkenes, amide II and amide III of aromatic rings. Synthesised silvernano particles were tested for antibacterial activity against *Escherichia coli*, *Klebsiella pneumoniae, Streptococcus aureus* and *Pseudomonas aeruginosa*. At 100µl concentration, the largest zone of inhibition (18mm) was found in *E. coli* and *P. aeruginosa* while the least zone of inhibition (16mm) was observed in *K. pneumoniae* and *S. aureus.* This type of research could also serve as a model for the future development of nanomedicines or focused algal drug delivery.

Keywords: *Chaetomorpha antennina*, Green algae, Ultraviolet visible spectroscopy, FTIR, Antibactrial activity

INTRODUCTION

Nanotechnology is emerging as a rapidly growing field with its application in science and technology for the purpose of manufacturing new materials at the nano scale level [1]. Silver nanoparticles (AgNPs) are non-toxic to humans and are most effective at low concentrations against bacteria, viruses, and other eukaryotic microorganisms. AgNPs have potential applications in the biomedical field and has several advantages over physical and chemical methods due to its cost effectiveness, compatibility for medical and pharmaceutical applications as well as large scale commercial production [2]. Bio-extracts from a varied set of microorganisms, ranging from bacteria to fungi and algae, are used in the green production of nanoparticles as a reducing and sometimes capping agent [3]. AgNPs have been successfully synthesized using several plant extracts [4]. India is blessed with a longest coastline. So it gives an opportunity to investigate the vast bio resources especially seaweeds. In India 1,553 species of seaweeds belonging to 271 genera are recorded [5]. Seaweeds are natural and renewable living resources found in the marine ecosystem, and they are used for food, feed, and medicine. More than 60 elements, macro and micronutrients, proteins, carbohydrates, vitamins, and aminoacids can be found in seaweeds [6]. Some of the seaweeds are used for nanoparticles synthesis and their various medical applications especially antibacterial activity [7,8] and water filters, bio sensors, in controlling plant pathogens and antifungal activity [9].With current antibiotic therapy in treating infectious diseases, antimicrobial drug resistance is the most serious problem all over the world [10]. Recently, a lot of attention has been paid to seaweeds in terms of isolating and creating novel antimicrobial compounds. Many unique bioactive chemicals have been identified from marine organisms over the past four decades [11]. *Chaetomorpha antennina* is a marine green alga, which belongs to the family Cladophoraceae. Literature survey showed that there is no data available regarding silver nanoparticles synthesis in *Chaetomorpha antennina* in kerela costal area. The current study aimed to evaluate the antibacterial activity of green synthesised Silver nano particles synthesized from the green seed weed.

MATERIALS AND METHODS

Collection of plant material

The green alga *C. antennina* was collected from Chavakkad coast, Thrissur, kerala and the algae was identified by PG and Research Department of Botany, NGM College.

Preparation of Seaweed extracts

C. antennina fresh seaweed was shade dried and powdered. 25gm of powder were mixed with 500 ml of distilled water and boiled for 30-40 minutes. Filter the content with Whatman no.1 filter paper and stored it on room temperature for synthesis of SNPs.

Silver Nanoparticle synthesis

Ammonium solution (2.5ml) was added to 5ml of 1mM $AgNO_3$ solution, followed by addition of algal extract 1-10 ml and the final volume was adjusted to 50 ml by adding the appropriate amount of de-ionized water in Erlenmeyer flask. The Erlenmeyer flask was incubated at 37^0C under agitation (200rpm) for 24-72 hrs [12].

Characterization of silver nanoparticles

UV-VIS Spectra analysis

The silver nanoparticles were confirmed by measuring the wave length of reaction mixture in the UV-vis spectrum of the Labman spectrophotometer at a resolution from 300-700 nm.

FTIR analysis of nanoparticles

Perkin-Elmer spectrometer FTIR spectrum in range of 4000-400 cm^{-1} at a resolution of 4 cm^{-1} is used for detection of various functional bonds in nanoparticles being synthesized. The samples were mixed with KBr and thin sample disc

was prepared by pressing with the disc preparing machine and placed in the Fourier transform infrared spectroscopy [FTIR] for the analysis of the nanoparticles.

Antibacterial activity of synthesized silver nanoparticles

The AgNPs synthesized from *C. antennina* was tested for their antibacterial activity by well diffusion method [13] against human pathogenic organisms such as *E. coli*, *Klebsiella pneumonia, Streptococcus aureus* and *Pseudomonas aeruginosa*. Clinically isolated these microorganisms were obtained from Department of Microbiology, PSG institute of Medical Sciences & Research, Peelamedu, Coimbatore.The pure culture of all these stains was subcultured on nutrient broth at 35ºC on rotary shaker at 200 rpm. The petriplates were sterilized using autoclave at 121^{0}C for 15-20 minutes. Freshly prepared nutrient agar medium was poured into sterilized petriplates and allowed to cool. Each strain was spread uniformly on the individual plate using sterile cotton swabs. Wells of size 6mm was made on nutrient agar plates using gel puncture. Using micropipette 50µl and 100µl of the sample of nanoparticles solution was poured into wells on all plates and negative control ampicillin 25µl (1mg/L) and Positive control distilled water was added to all plates. After incubation at 35ºC for 18 hours, the different levels of zone of inhibition were measured.

RESULTS AND DISCUSSION

Synthesis of silver nanoparticles

The aqueous extract of *Chaetomorpha antennina* (10 ml) was added to the 90 ml aqueous solution of Silver Nitrate in 250 ml conical flask and kept in room temperature for 72 hours. The colour of solution turned from yellow to brown indicates the formation of silver Nanoparticles (Figure-1). The solution colour changed from pale yellow to brown colour due to the excitation of surface plasmon vibrations. Similar results were reported in *Sargassum muticuml*[14]; *Caulerpa racemosa* [15]; *P. boergesenii*[16]. Due to the excitation of surface plasmon vibration, the brown seaweed *P. boergesenii* showed colour change from brownish to pale yellow colour [16]. In the present study, biosynthesis of silver nanoparticles was evidenced by the colour change of the reaction mixture (algae extract and silver nitrate). It confirmed the presence of AgNPs in the *Chaetomorpha antennina* with colour change.

UV-VIS spectra analysis

The formation of metal nanoparticles was confirmed by one of the important technique UV-Vis spectrum. In the present study synthesized silver nanoparticles of *Chaetomorpha antennina* was treated with UV-Visible spectrophotometer for confirming the presence of silver nanoparticles. An absorbance peak at 440 nm was recorded and it indicated the synthesis AgNPs (Figure-2). The formation of AgNPs in the present study was confirmed through comparison with Rajeshkumar *et al*. [17]. They reported that the brown algae *Padina tetrastromatica* silver nanoparticles exhibited a single absorbance band at 440 nm at 15 minutes and steadily increased in intensity at 24 hrs without any shift in the peak. The formation of AgNPs in the present study was also confirmed through comparison with Roy and Suparna report [18]. They also reported the surface plasmon resonance band corresponding to formation of AgNPs was occurred at 435.5 nm for *Chaetomorpha antennina.* There is some inappropriateness when compared with the observations of Vishnu kiran and Murugesan [15] in which the bands corresponding to the surface plasmon resonance of AgNPs arised at 420 nm for red algae *Halymenia porphyroides*.

FTIR analysis of the leaf extract and nanoparticles

An important biomolecule responsible for the reduction of silver ions to silver nanoparticles was identified using FT—IR analysis. Figure-3 shows the FT—IR spectrum of *Chaetomorpha antennina* assisted silver nanoparticles. The band at 3423.65 cm^{-1} represents O—H stretching groups in polyphenols (or) protein enzymes (or) polysaccharides. Our result was agreed with Rajeshkumar *et al*. [17]. The band at 1633.71 cm^{-1} corresponds to C=C stretching groups of conjugated alkenes. The peak at 1384.89 cm^{-1} corresponding to amide II and amide III of aromatic rings either may by poly phenols. The band at 2061.90 - 2081.19cm-1. Corresponds to N=C=S stretching groups of isothiocyanate. The band at 501.49 - 545.85 cm^{-1} corresponds to C- Cl / C- Br stretching groups of halogens. The similar result was

observed in *Padina boergesenii*, the band at 1383.88 cm^{-1} and 1627.92 cm^{-1} assigned to the C-H and C=C stretch alkanes group respectively. The band at 2207.58 cm^{-1} corresponding to the S-H bend Mercaptans group. The band seen at 3448.72 cm^{-1} corresponding to the O-H stretching carboxylic acids group [16]. FTIR spectrum of the SNPS of *Sargassum wightii* showed peaks at 3411, 2925, 1584, 1425 and 1033 cm−1. The peaks at 3411 cm−1 (H–bonded hydroxyl groups), 2925 cm−1 (–OH stretching), 1584 and 1425 cm−1 (asymmetrical and symmetrical vibration of carboxylate ions) and 1033 cm−1 (C–O stretching of alcoholic groups) [19]. In present study showed some appropriateness and inappropriateness groups of molecules were observed.

Antibacterial activity of plant extracts *Chaetomorpha antennina*

The silver nanoparticles were synthesized from *C. antennina* and tested for their antibacterial activity by well diffusion method against pathogenic organism like *E. coli*, *Klebsiella pneumoniae*, *Streptococcus aureus* and *Pseudomonas aeruginosa*. The result was compared with the standard broad spectrum antibiotic Ampicillin (10 mg/ml), which was used as positive control and the distilled water served as negative control. The zone of inhibition was measured all the tested organisms and tabulated. (Table-1) The maximum zone of inhibition was observed in *E. coli* (18 mm) and *P. aeroginosa* (18mm) and minimum zone of inhibition was observed *K. pneumonia* (16) and *S. aureus* (16) at 100µl concentration. The maximum zone of inhibition was observed in *E. coli* (17mm) and minimum zone of inhibition was observed in *Streptococcus aureus* (14 mm) at 50µl concentrations. Figure 4 showed the antibacterial activities of different concentration of synthesized silver nanoparticles were lower than of positive control ampicillin except *Pseudomonas aeruginosa*. No zone of inhibition was observed in negative control (distilled water). Roy and Anantharaman [18] reported that the biosynthesized SNPs from *Chaetomorpha antennina* was used for antibacterial study against six pathogenic bacteria. The zone of inhibition showed in *E. coli*, *P. aeroginosa, K. pneumonia and P. mirabilis* were 11.6, 8.1, 4.4 and 2 mm respectively. In this study zone of inhibition showed more significant in *E. coli* (17mm), *P. aeroginosa* (15), *K. pneumonia* (15) at low concentration. Shanmugam *et al*. [20] reported that synthesized SNPS of *Sargassum whitii* showed the zone of inhibition in *E. coli* (2mm), *P. aeroginosa* (5mm) and *S. aureus* (12mm). In our present study antibacterial activity at 50 µl concentration showed more remarkable activity than SNPS of *Sargasssum whitii.* Synthesized SNPS of fresh water green alga *Pithophora oedogonia* showed the maximum zone of inhibition against *P. aeroginosa* (17.2mm) and followed by *E. coli* (16.8mm) [21]. Similar result was obtained in SNPS of *C. antennina*. The above result concluded that the algal mediated AgNPs shows a wide range of biological activity against microorganisms which can be applied in the medical field in future.

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REFERENCES

1. Albrecht, MA, Evans, CW and Raston, CL. Green chemistry and the health implications of nanoparticles. *Green chemistry,* 2006, 8, 417-432.
2. Mallikarjuna K, Narasimh, G, Dillip, GR, Praveen, B, Shreedhar, B, Sree Lakshmi, C, Reddy, BVS and Deva Prasad Raju, B. Green Synthesis of Silver Nanoparticles using *Ocimum* leaf extract and their characterization. *Dig J. of Nan & Bios*, 2011, 6 (1), 181 – 186.
3. Sudha, SS, Rajamanickam, K, Rengaramanujam, J. Microalgae mediated synthesis of silver nanoparticles and their antibacterial activity against pathogenic bacteria. *Ind J Expt* Biol, 2013, 9, 393-399.
4. Li, S, Shen , Y, Xie, A, Yu, X, Qiu, L, Zhang, L. Green synthesis of silver nanoparticles using *Capsicum annuum* L. extract. *Green Chem*, 2007, 9(8), 852.
5. Oza, RM and Zaidi, SH. A Revised Checklist of Indian Marine Algae. National Marine Data Centre on Algae and Marine Chemicals, CSMCRI, Bhavnagar, India, 2001.

6. Jensen, Present and future needs for algae and algal products. *Hydrobiologia*, 1993, 260 (1), 15–23.
7. Shanmugam, N, Rajkamal, P, Cholan, S, Kannadasan, N, Sathishkumar, K, Viruthagiri,G and Sundaramanickam, A. Biosynthesis of silver nanoparticles from the marine seaweed *Sargassum wightii* and their antibacterial activity against some human pathogens. *Appl. Nanosci*. 2014, 4, 881–888.
8. Venkatpurwar, V and Pokharkar, V. Green synthesis of silver nanoparticles using marine polysaccharide: study of *in vitro* antibacterial activity. *Mater. Lett*, 2011, 65, 999-1002.
9. Kumar, P, SenthamilSelvi, S and Govindaraju, M. Seaweed-mediated biosynthesis of silver nanoparticles using *Gracilaria corticata* for its antifungal activity against *Candida* sp. *Appl Nanosci*, 2013, 3, 495–500.
10. Manikandan, S, Ganesapandian, S, Singh,M and Kumarguru, AK. Emerging of Multi drug Resistance Human Pathogens form Urinary Tract Infections. *Curr Res Bacteriol*, 2011, 4(1), 9-15.
11. Faulkner, DJ. Marine natural products.*Nat. Prod. Rep*, 2002, 19, 1-48.
12. Kasthuri, J, Veerapandian, S and Rajendiran, N. Biological synthesis of silver and gold nanoparticles using apiin as reducing agent. Colloids and Surfaces B: Biointerfaces, 2009, 68, 55–60.
13. Azoro, C. Antibacterial activity of crude extract of *Azadiracta indica* on *Salmonella typhi*. *World J of Bioechnol*, 2002, 3, 347 – 357.
14. Susan Azizi, Farideh Namvar, Mahnaz Mahdavi, Mansor Bin Ahmad and Rosfarizan Mohamad. Biosynthesis of Silver Nanoparticles Using Brown Marine Macroalga, *Sargassum muticum* aqueous extracts, *Materials,* 2013, 6, 5942-5950.
15. Vishnu Kiran, M and Murugesan, S. Bio-synthesis of silver nano particles from marine alga *Halymenia poryphyroides* and its antibacterial efficacy, *Int. J. Curr. Microbiol. App. Sci,* 2014, 3(4), 96-103.
16. Senthilkumar, P, Bhuvaneshwari, Janani Prakash, Lakshmi Priya and Ranjith Santhosh Kumar. Green synthesis and characterization of silver nanoparticles from aqueous extract brown seaweed of *Padinaboergesenii* and its antifungal activity, *World Journal of Pharmacy and Pharmaceutical Sciences,* 2015, 4(10), 1858-1870.
17. Rajeshkumar, Kannan, C and Annadurai, G. Synthesis and characterization of antimicrobial silver nanoparticles using Marine Brown seaweed *Padina tetrastromatica*, *Drug Invention Today,* 2012, 4(10), 511-513.
18. Suparna Roy and Anantharaman, P.. Biosynthesis of silver nanoparticles by *Chaetomorpha antennina* (Bory de Saint-Vincent) Kutzing with its antibacterial activity and ecological implication*, J Nanomed Nanotechnology,* 2017, 8, 467.
19. Govindaraju. K, Kiruthiga, V, Ganesh Kumar, V and Singaravelu, G. Extracellular synthesis of silver nanoparticles by a marine alga, *Sargassum grvilli* and their antibacterial effects, *J. Nanosci. Nanomed,* 2009, 9, 9.
20. Shanmugam, N, Rajkamal, P, Cholan, S, Kannadasan, N, Sathishkumar, K, Viruthagiri,G and Sundaramanickam, A. Biosynthesis of silver nanoparticles from the marine seaweed *Sargassum wightii* and their antibacterial activity against some human pathogens. *Appl. Nanosci*, 2014, 4, 881–888.
21. Sankar Narayan Sinha, Dipak Paul, Nilu Halder, Dipta Sengupta and Samir Kumar Patra.. Green synthesis of silver nanoparticles using fresh water green alga *Pithophoraoedogonia* (Mont.) Wittrock and evaluation of their antibacterial activity, *Appl. Nanosci***,** 2014, 5, 703-709.

Table 1: Antibacterial activity of silver nanoparticles from *Chaetomorpha antennina*

S.No	ORGANISM	ZONE OF INHIBITION(mm)			
		Synthesized silver nanoparticles		Ampicillin 25 µl (10 mg/ml)	Distilled water (50µl)
		50 µl	100µl		
1	*Escherichia coli*,	17	18	20	0
2	*Klebsiella pneumoniae*	15	16	20	0
3	*Streptococcus aureus*	14	16	19	0
4	*Pseudomonas aeruginosa*	15	18	18	0

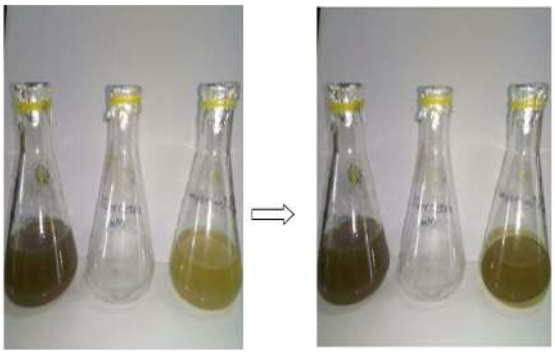

Fig. 1: 1 mM $AgNo_3$ with seaweed extract solution turned from yellow to bright yellow and turned to brown

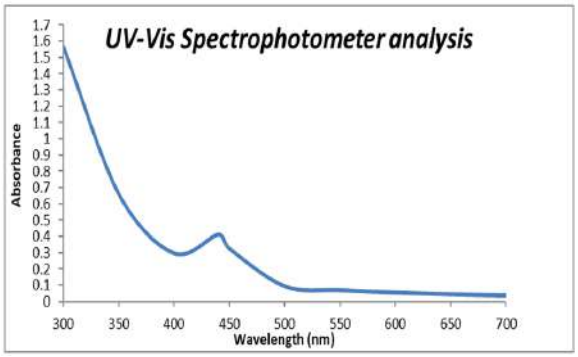

Fig. 2: UV-Vis spectra of SNPs synthesized from the extract of *Chaetomorpha antennina*

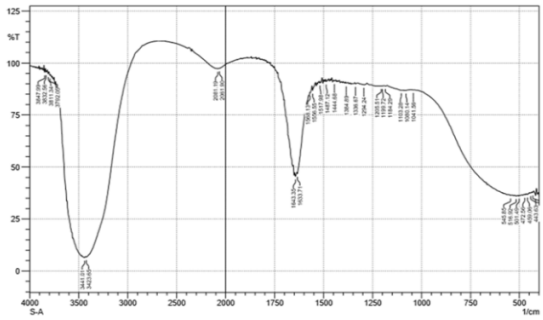

Fig. 3: FT-IR spectra of SNPs synthesized from the seaweed extract of *Chaetomorpha antennina*

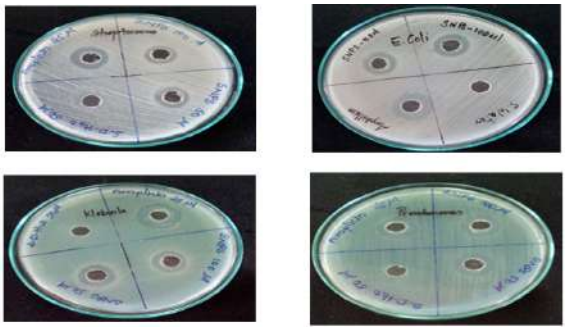

Fig. 4: Antibacterial activity of synthesized silver nanoparticles from *Chaetomorpha antennina* seaweed extract

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RESEARCH ARTICLE

Common Reservoirs of Pathogens and Its Types Causing Nosocomial Infection

M. Geetha[1], R. Ishwarya[1], J. Tharani[1] and D. Sangeetha[2]*

[1]Ph.D Research Scholar, Department of Microbiology, Faculty of Science, Annamalai University, Annamalai Nagar, Tamil Nadu, India.
[2]Assistant Professor, Department of Microbiology, Faculty of Science, Annamalai University, Annamalai Nagar, Tamil Nadu, India.

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***Address for Correspondence**
D. Sangeetha
Assistant Professor,
Department of Microbiology,
Faculty of Science,
Annamalai University, Annamalai Nagar,
Tamil Nadu, India.
Email: sangeethadau@gmail.com

ABSTRACT

Nosocomial infections are the serious concern in the medical field due to the emergence of multidrug resistant pathogens. Hospital environment plays an important role in the transmission of deadly infections through patients, health care workers and the inanimate materials are the major sources of causing Healthcare Associated Infections (HAIs). Among these various sources from contaminated environmental surface can easily transmit the nosocomial pathogens. To prevent the disease, we can utilize some antimicrobial agents like broad spectrum antibiotics, hygiene guidelines and disinfection strategy. And apart from the antibiotics there is a necessity for novel drug discovery to defence against nosocomial pathogens. To control the spreading of resistant pathogens in the hospital environment, everyone must knowledge about its sources and route of transmission. Therefore, this review describes the common sources, types of nosocomial pathogens and infections.

Keywords: Nosocomial infection, Disease transmission, Fomites, Resistant pathogens

INTRODUCTION

Nosocomial infections are the hospital acquired infections which contracted to the inpatient of the hospital. These infections were reported to be 7% and 10% in developed and developing countries, respectively. WHO reported that

the 15% of the patients undergone medical care were affected with these infections. Bacteria, Fungi and Viruses are the nosocomial pathogens which are transferred by the reservoirs such as patient immediate environment, contaminated hands of healthcare personnel and the diseased patients [1]. It is recognized as any bacterial infection which is acquired by 48 to 72 hours of after hospital admission [2]. Nosocomial infections are mainly caused by the specific set of pathogens known as ESKAPE pathogens. These pathogens are highly infectious with their multi drug resistance mechanisms. There is critical need of Novel invention of drug is important against these potential pathogens [3]. High economic burden and mortality rate was caused by the ESKAPE pathogens [4]. Nosocomial pathogens survive for a long duration on the surface and continuously transmits infection through the health workers. Nosocomial pathogens are predominantly transferred by the contaminated hands of health care personal so effective disinfectant of hospital surface equipment's and hand hygiene may reduce the outbreaks of infectious pathogen. Totally 20 to 40 % of the nosocomial infections are transmitted by the contaminated hands of healthcare workers who are acquired pathogens directly from the patients or from the contaminated hospital environment surface [5]. Nosocomial infections or the hospital acquired infections are causing serious threat in the emergence of multidrug resistant pathogens. Health care workers and the inanimate objects in the hospital environment are the major reservoir of the nosocomial infections [6]. Hence, the present review centralised on the basic sources, types of nosocomial pathogens and infections.

Sources of Nosocomial Infection

Fomites

Fomites are the patient care items or an inanimate object which are contaminated with the nosocomial pathogens. Outbreaks due to the fomites can be controlled by the regular cleaning and disinfection of the surfaces [7]. Fomites associated pathogens are mostly MDROs which transmits nosocomial infection through direct contact with the contaminated objects, indirectly with the hands of healthcare workers and also by the other sources like air, water and food. [8], [9]. In the hospital environment infected patients are the major source of pathogens, they released infectious agent from their environment and can be viable in the surface, water and air. Patient's environment plays an important role in the outbreaks of nosocomial infection it includes all the equipment, medical devices, bed, bathroom, phone and personal belongings [10]. Contaminated thermometer can transmit the infection of ESBL producing *K. pneumoniae* in a neonatal unit. [11]. Hospital environment and the objects in the hospitals are the important reservoir of the transmission of multidrug resistant pathogens. A review was conducted in Ethiopia revealed 70% of the fomites associated contamination by the pathogens. Among that ampicillin resistant *K. pneuminia* was most prevalence followed by the *citrobactersp [12]*. (Table 1: showed the common types of fomites and surface in the hospital environment).

Resistant pathogens on Fomites

Antibiogram of the gram positive and gram negative bacteria isolated from the inanimate objects of the hospitals in Ethiopia showed emergence of 100% resistance to the antibiotics. [13]. Out of 232 samples analysed by the Bhatta *et al*., *Staphylococcus aureus* was the dominant pathogen. It was the multidrug resistant and produced biofilm to survive on objects firmly. This study insisted to maintain the sterilized environment and hand hygiene of the healthcare workers. [14].Critically ill adult patients and neonatal intensive care units were seriously affected with the fomites related outbreaks. They were mainly infected with multidrug resistant organisms causing deadly infections and was occurred by the improper hygienid disinfection practices [15]. Due to the biofilm formation it would not eliminate by using surface disinfectant and can survive for a prolonged duration on dry surface area [16]. Bacterial and fungal strains were isolated from the inanimate objects of the operation theatres by swab and settle plate methods. All the pathogens are showed moderate resistance to antibiotics. Fomites are the major source of Nosocomial infection mainly through the hand contact during surgery. So preventive measures should be followed in the operation theatres [17] [18].Outbreaks due to nosocomial pathogens such as carbapenem resistant *enterobacteriaciae* and coagulase negative *Staphylococci* were occurred by the direct contaminations of fomites. Following the regular cleaning practice can control the fomite related outbreaks [19].

Survival time of pathogens on Fomites

Nosocomial pathogens are predominantly transferred by the inanimate surfaces of the hospital environment and the pathogens were survived for hours to weeks under laboratory condition. Highly potential nosocomial pathogens are persisted on the fomites for a prolonged duration. So proper preventive measures should be taken to reduce the spread of infection. [20] [21]. Nosocomial infection causing pathogens are persisted on the fomites for longer duration. Gram positive and gram negative bacteria such as *Enterococcus, Staphylococcus aureus, E. coli, Klebsiella* spp., *Pesudomonas aeruginosa, shigella* spp. Persist for a month on the dry surfaces of the hospital environment. Fungal and yeast pathogens are survived for 4 to 5 months on the surface and some viruses can be live for few days to week. So continuous decontamination with the effective disinfectant only reduce the outbreaks of the nosocomial infection. Survival time on fomites refer Certain factors increase the viability of the nosocomial pathogens on the surface of dried fomites such as load of pathogen, Relative humidity and low temperature. These three factors determine the persistence of nosocomial pathogens [22]. Medial charts of the general wards and the special units could transmit the specific pathogens such as *Staphylococcus aureus (17.8%),* Methicillin-resistant *Staphylococcus aureus (9.3%), Streptococcus viridians (9.4%), Escherichia coli (11.2%), Klebsiella pneumonia (7.5%), and Acinetobacter baumannii* (7.5%). So proper hand hygiene is essential before and after using of medical charts [23]. Load of pathogens are more around the patients in the hospital environment. The equipment's and the healthcare workers are considered as a dominant reservoir of the Nosocomial infections. They spread infections by direct contact with the patients or indirect contact with the fomites [24] [25].One of the study included that the hospital sterile rooms also loaded with the 40% of Multidrug resistant organisms and among that VRE were the common pathogen which were in 19% of the total isolates were recovered [26]. Stethoscope has been carried the group of microorganisms among that majority of that are non-pathogenic in nature. Pathogens such as *Pseudomonas aeruginosa, Staphylococcus aureus, Clostridium difficile and Vancomycin resistant enterococci* are frequently isolated from the contaminated stethoscope. From this review they concluded that the there is no evidence of causing HCAI by the contaminated stethoscope. But Stethoscope carries pathogens which is mainly acquired from the skin of patients [27]. Hospital environment are the major source of nosocomial infection, and is mainly influenced by certain characteristics such as load of microorganisms in the air, rate of activity, supporting material for the growth of pathogens and temperature. Among that the temperature, quantity of pathogens and route of entry into the host are the main factors which determines the nosocomial infection. Gram positive bacteria usually adheres on the dry surface whereas gram negative bacteria viable on the moist surface [28].

Health care workers

Poor hand hygiene practice of healthcare workers will result in the spread of ICU associated nosocomial infections. These infections are transferred by direct contact with the patients or indirectly by the objects in the hospital environment. These kind of cross contaminated infections cause serious concern in the immunocompromised patients when infected with the multidrug resistant pathogens [29]. The infections associated with the healthcare personal who are direct contact with the patients and the medical devices were screened for the study. Samples were collected from the PICU and NICU neonatal and paediatric ICU of the hospital in Dehradun. Totally 260 samples were taken from the inanimate objects, Nasal and hand swabs. Antibiotic susceptibility pattern was noted for all the isolated pathogens with the effective antibiotics. From this study we observed that the HAIs are mainly occurred by the health care workers so proper sterilization of the equipment and following the hand hygiene will reduce the infection rate [30]. Cross contamination of the nosocomial pathogen through the hands of healthcare personal who were acquired pathogens directly from the patients or from the hospital environmental fomites. They concluded that the environmental contamination placed the major role to transfer Healthcare Associated Infections (HAIs). They also suggested that the hand hygiene and decontamination of the hospital area will reduce the risk of HAIs [31]. Qualitative and quantitative analysis of nosocomial infection were done by the [32]. Coagulase negative *Staphylococcus (*CNS), Methicillin Resistant *Staphylococcus aureus* (MRSA) and *Klebsiella pneumonia* were the most abundant nosocomial pathogen. From this research they concluded that the nosocomial infection mainly transferred by the direct contact with the contaminated equipment and other environmental objects. So all the health personals should follow the standard bio cleaning procedures which will reduce the rate of mortality of the infection.

Air and waterborne contamination

Outbreaks of invasive pulmonary *Aspergillosis* of *Aspergillus* spp were reviewed by the Vonberg and Gastmeier and from their study they concluded that the half of the infection solely acquired from the construction and renovation works of hospital surface. It induces the burst of spores settled on the surface. Immunocompromised patients were developed *Aspergillosis* even for a less dose of Aspergillus (1cfu/m3). Kanamori and colleagues also reviewed 28 construction related outbreaks of HAIs from 1976 to 2014 and found *Aspergillus Sp*. Was the dominant pathogen which caused infection in the rate of 60% mortality. Water in the Hospital environment plays an important reservoir of pathogens such as gram negative bacteria, mycobacteria, protozoa and fungi. Water system in the hospitals are fully established throughout by the pipelines. This favours the growth of biofilm forming bacteria with exopolysaccharide, proteins and nucleic acid. This protects the pathogens from the disinfection and other treatments. Water borne outbreaks mainly occur in certain population such as neonates, immunocompromised patients, bed ridden patients, Surgical patients and patients undergone transplantation [33] . Most of the water related infections were caused by pathogens which are associated with water. Based on that water pathogens are Water based (Legionellosis), water borne (Fecal contamination by *E. coligastroenteritis*) and water related [34]. Patients are acquired water borne infections by direct and indirect contact of contaminated water system, ingestion and inhalation of water sources [35].

Types of Nosocomial Infection

Figure 2: Major types of Nosocomial infections

Central line-associated bloodstream infections

Central Venous Catheters (CVCs) are the essential tool in the medical field for assisting a treatment processes. But the unavoidable harmful effects of CVC are the blood stream associated infections. CVCs are of different types based on the requirement of the patients. It is widely applied in the ICU and sometimes non ICU and outpatient settings also used CVCs. [36] [37].Gram negative bacteria such as *Staphylococcus aureus* and coagulase negative *Staphylococci* and the *Candida sps* are the major cause of CLABSI. Blood culture of infected pathogen are for the diagnosis of the infection and the same pathogen not to be isolated from another site of infection [38]. CLABSIs are majorly caused by the intra vascular access procedures. New methodologies should be used for the early detection and better treatment of IVD related nosocomial infections [39]. Vascular access is considered an effective treatment method for diagnosing and delivering fluids, blood products and medications [40]. CLABSIs can be detected by the Machine Learning Algorithm (MLA) and recorded in the Electronic Health Record (EHR). This data will be very useful for the early detection of infections and can assist to reduce the healthcare cost [41]. Catheter related infections are highly infective but can be managed by following the hand hygiene practice [42] analysed the effective skin antisepsis. They suggested that the Chlorhexidine alcohol was the best antiseptic which can be used to reduce the catheter related infections. Transoesophageal echography and ophthalmological examination are the treatment for the CLBSI patients infected with *S. aureus, Enterococci* and *Candida sp*. Sequential antibiotic therapy should be recommended for the chronic infected patients [43].

Catheter-Associated Urinary Tract Infection (CAUTI)

Indwelling devices favour the formation of biofilm and leads to the development of the bacteriuria. The duration of the cauterization determines the rate of infections. It is estimated that the 20% of the catheter associated infections noted in the acute care facilities and the 50% in the long term care facilities. Limitation in the usage of catheter and the improve the quality of the catheters by avoiding the biofilm formation can reduce the mortality rate of the CAUTI [44]. Nosocomial urinary tract infections are accomplished by the contaminated catheters which directly inoculated microbial pathogens and increases the chances of acquiring biofilm formations on the mucosal cells of the bladder and causes irritation [45].One of the study concluded that nosocomial catheter associated infections are mainly caused by the *Candida* species. It is considered as the second leading pathogen which causes Catheter associated candiduria in the hospitalised patients [46]. According to the CDC guidelines, duration of the catheter insertion should be less to all the patients especially to immunocompromised patients, women and aged persons. And they also avoid the use of routine antimicrobials [47]. Most of the catheter associated nosocomial infections are

asymptomatic but mild fever, peripheral leucocytosis are the common symptoms of this infection to predict for diagnosis [48]. The study conducted in the prevalence of nosocomial urinary tract infections of the hospitalized ICU patients. They revealed that the *Candida albicans* and the non albican species are the predominant drug resistant pathogen of catheter associated urinary tract infections followed by *E. coli*, *Klebsiella* spp, *Pseudomonas and Acetobacter* spp. All the pathogens are MDR so proper schedule should be followed in the antibiotic dosage [49].

Surgical Site Infections (SSI)

Surgical site infections (SSI) are the wound infection which mainly acquired by the usage of invasive procedures and 2 to 5% of the patients were affected worldwide [50] [51]. Elderly people, Patients who stayed prolonged duration in the hospital and also patients with certain wounds are susceptible for the SSI [52]. *E. coli, S. aureus and P. aeruginosa* were the most common pathogen which cause SSI in the hospital environment [53]. From their study of 49 different nosocomial infections surgical site wound infection showed high rate of incidence. They insisted the preventive measures to take against the multidrug resistant nosocomial pathogen which spread more infection. Coryne form bacteria are the emerging nosocomial pathogen with multi drug resistant potential and caused SSI. One of the study revealed that the patients infected with the *Corynebacteria* showed fever and post-operative wound infections leads to the prolonged stay in the hospital. [54]. Surgical site infections were occurred by the various operative procedures such as coronary bypass surgery, abdominal surgery, Cardiac and breast surgery [55]. Surgical site infections occurred after HTO (High tibial osteotomy) were analysed and reported. According to them male sex, smoking and longer anaesthesia, use of artificial bone are major risk factors of SSI [56].

Ventilator-Associated Pneumonia (VAP)

Nosocomial infection occurred in the ICU were analysed by the [57]. They found that pneumonia was the frequent infections among the other nosocomial infections. And the length of hospital stay in the ICU influences the incidence of nosocomial infections. Incidence of Pneumonia along with UTI and BSI were reported frequently in ICU by the gram negative bacilli over 20 years of screening study [58].Gram negative bacilli were associated with all the four infections and among that UTI was predominantly caused by them. Pneumonia caused by the *Acinetobacter sp*. was increased in ICU from 1986 to 2003. VAP is one of the nosocomial infection which are usually occurred in the patients using mechanical ventilator of the intensive care unit [59]. Men were seriously affected by the VAP than women. It was estimated that 79% of the men were reported to get VAP [60]. VAP mainly caused by the prolonged mechanical ventilation and this also increases the duration of the ventilation as well as the ICU stay in the hospital. In the first day of ventilation 5% of the people can get the VAP and at the 30 days exposure of the mechanical ventilation 65% of the people acquiring VAP [61]. VAP is the second commonly occurred nosocomial infection which caused infections more in the paediatric intensive care unit (PICU) patients [62]. According to the one study report VAP increases the hospital stay in the paediatric intensive care units and also cause financial burden. It can be diagnosed accurately by the Broncho alveolar lavage of the infected patients. But standard techniques should be needed to diagnose VAP in PICU. Bacteria are the major cause of VAP, which is acquired by the healthcare workers or the environmental objects. Common pathogens include *Pseudomonas* species and other highly resistant Gram-negative bacilli, *Staphylococci*, *Enterobacteriaceae*, *Streptococci* and *Haemophilus* species. Antibiotic resistant pathogen as *Pseudomonas* and *Acinetobacter* species and methicillin resistant strains of *Staphylococcus aureus* [63].

Nosocomial Pathogens

Bacteria

Staphylococcus aureus

Staphylococcus aureus are the major nosocomial pathogen and showed resistance to chloramphenicol, erythromycin, cephalexin and tetracycline. Emergence of MDR pathogens creates serious concern in the patients and the healthcare communities [64]. Methicillin Resistant *Staphylococcus aureus* (MRSA) can be frequently isolated from the environmental surface. Air borne *Staphylococcus aureus* was contaminated 73% of the room surface and 65% of the patients were infected in the contaminated room. Health care workers plays an important role in transferring the MRSA directly from the patient and indirectly from the contaminated surfaces. Around 42% of the infection transmitted from the HCWs gloves indirectly [65]. Mobile communication devices are the major reservoir of the

pathogenic bacteria such as *Staphylococcus aureus* nearly 9 to 25% of the MCDs are contaminated with the pathogens. This can be reduced with following the hand hygiene, disinfection and avoiding the use of MCDs in the highly risk areas of the hospitals [66]. Resistance in *Staphylococcus* were studied by the *et al*., They screened the blood stream s. aureus isolates and coagulase negative isolates from the hospitals in US. They observed that the emergence of methicillin resistant was increased in US centres and preventive measures must be needed to control the [67]. Pathogens involved in the ICU acquired infections and the mortality of the infections were studied by the Vincent *et al.,* A case reports of 1417 ICUs were studied. Among the various isolates Methicillin Resistant *Staphylococcus aureus* were obtained in maximum percentage [68].

Clostridium difficile

One study found that *Clostridium difficile* was the dominant pathogen which caused hospital acquired infections such as pneumonia and gastro intestinal tract infections in US hospitals [69]. *C. difficile* infections are mainly transmitted by the spores and is carried by the animate and inanimate objects. It was estimated that the infants are more susceptible for the *C. difficile* infection than the adults. In addition, hospitalized patients are highly susceptible *difficile*-associated diarrhoea (CDAD) were considered as the nosocomial infection and is transferred by the spores of C. difficile mainly after the antimicrobial therapy [70] reviewed the guidelines to control the spread of C. difficile associated CDAD. The risk of developing CDI is 8 to 10 times higher to this disease [71]. *Clostridium difficile* infection (CDI) was mainly transferred in the hospital environment. According to the cox regression analysis there is the increased mortality associated with the hospital acquired CDI [72]. *Clostridium* during antimicrobial treatment and 4 weeks thereafter, and three times in the next 2 months [73]. According to the European Society of Clinical Microbiology and Infection (ESCMID), patients infected with the *C. difficile* were divided into various groups. Guidelines for the treatment procedures also advised and the antibiotics recommended for the infection are metronidazole, vancomycin and fidaxomicin. For severe cases faecal transplantation are recommended [74].

Multidrug resistant gram negative bacteria

ESKAPE pathogens possessed different mechanism for resistance against antibiotics such as inactivation by enzymes, modification of target, altered cell permeability, biofilm production and efflux pumps. Due to the multidrug resistance of ESKAPE pathogens exerts a problem in disease diagnosis and treatment. It also caused a high economic burden, mortality and morbidity [75]. Because of the resistance mechanism of the pathogens treatment with antibiotics are not effective so novel drug should be formulated instead of antibiotics. Gram negative bacteria are the major source of HAIs, out of 11,437 isolates screened 19 gram negative strains were identified. They found that the GNBs were high average resistance to the antibiotics. Resistance were showed for the last line drugs such as carbapenem antibiotics so this survey study conveyed the alarming stage of the antibiotics resistance on the GNBs [76]. Virulence of the nosocomial pathogens can be analysed by the Antibiotic sensitivity assay and biofilm determination. Antibiotic determinant genes of the nosocomial pathogens are transferred by the horizontal gene transfer mechanism. Whole genome sequencing is the ideal procedure to confirm the antibiotic resistant genes of the pathogens [77]. Enteric bacteria are the common nosocomial pathogens which are isolated from the patients and the environment. This bacterium showed high antibiotic resistance. So antibiotics prescription should be in the précised manner to avoid the emergence of the multi drug resistant pathogens [78].

Viruses

Nosocomial infections are commonly caused by the viruses. Due to the lack of the consistent monitoring system incidence could not be identified but it is estimated that 5% of the nosocomial infection noted when routine surveillance has been performed [79]. Hospitalized patients and the paediatrics are suffered more by the nosocomial viral respiratory infections. It increases the morbidity and mortality of hospitalized patients. It was estimated that 10-fold increase in the paediatric cases in US hospitals [80]. Viruses can be transferred by the contaminated hand, nasal and faecal oral route. Hepatitis B and C are the predominant virus which cause fatal diseases than the other viruses include, HIV, rotavirus, Influenza and Herpes simplex virus [81] [82]. Among the different types of respiratory viruses parainfluenza virus type 3 (PIV3) showed highest rate of nosocomial infection. Infection rate are differed based on the treatment procedure and the patient's immune health [83].

Fungi

Candida species are the common opportunistic pathogen which caused hospital acquired infections in the immunocompromised patients. *C. albicans, C. parapsilosis, C. glabrata are the* important fungal pathogens associated with nosocomial infections. (Magill 2018). Recently Nosocomial meningitis caused by Co infections of *Candida utilis* and *Stenotrophomonas maltophilia* after the neurosurgery were identified and treated successfully [84].

CONCLUSION

From the present study we can conclude that the nosocomial infections are mainly transmitted by the inanimate objects in the hospital environment. Health care workers plays a major role in the spread of nosocomial infections. Emergence of MDR pathogens in the hospital environment causes a serious issue in the medical field. Improper and excessive use of antibiotics causes the multidrug resistant in the pathogens. In order to implement the control of nosocomial infection all the health care personal should aware of the sources of nosocomial infections. By using disinfectants of the hospital environment and on the medical equipment's can reduce the cross contamination of the nosocomial infections. Therefore, novel strategies should be implemented to control the infection and to treat the antibiotic resistant nosocomial pathogens.

REFERENCES

1. Khan, H. A., Baig, F. K., & Mehboob, R. (2017). Nosocomial infections: Epidemiology, prevention, control and surveillance. *Asian Pacific Journal of Tropical Biomedicine*, *7*(5), 478-482.
2. Jenkins, D. R. (2017). Nosocomial infections and infection control. *Medicine*, *45*(10), 629-633.
3. Ma, Y. X., Wang, C. Y., Li, Y. Y., Li, J., Wan, Q. Q., Chen, J. H., &Niu, L. N. (2020). Considerations and caveats in combating ESKAPE pathogens against nosocomial infections. *Advanced Science*, *7*(1), 1901872.
4. Founou, R. C., Founou, L. L., &Essack, S. Y. (2017). Clinical and economic impact of antibiotic resistance in developing countries: a systematic review and meta-analysis. *PloS one*, *12*(12), e0189621.
5. Weber, D. J., Rutala, W. A., Miller, M. B., Huslage, K., &Sickbert-Bennett, E. (2010). Role of hospital surfaces in the transmission of emerging health care-associated pathogens: norovirus, Clostridium difficile, and Acinetobacter species. *American journal of infection control*, *38*(5), S25-S33.
6. Kim, Y. A., Lee, H., & Lee, K. (2015). Contamination of the hospital environmental by pathogenic bacteria and infection control. *Korean Journal of Nosocomial Infection Control*, *20*(1), 1-6
7. Rutala, W. A., & Weber, D. J. (2016). Disinfection and sterilization in health care facilities: an overview and current issues. *Infectious Disease Clinics*, *30*(3), 609-637.
8. Centers for Disease Control and Prevention (CDC 2012). Principles of epidemiology in public health practice, 3rd ed: an introduction to applied epidemiology and biostatistics. Washington, DC: Public Health Foundation, 2012.
9. Otter JA, Yezli S, French GL. The role played by contaminated surfaces in the transmission of nosocomial pathogens. Infect Control HospEpidemiol 2011; 32:687–99. 8
10. Provincial Infectious Diseases Advisory Committee. (2012). *Best Practices for Environmental Cleaning for Prevention and Control of Infections in All Health Care Settings.* Public Health Ontario.
11. Macrae, M.B., Shannon, K.P., Rayner, D.M., Kaiser, A.M., Hoffman, P.N., French, G.L. (2001). A simultaneous outbreak on a neonatal unit of two strains of multiply antibiotic resistant Klebsiellapneumoniae controllable only by ward closure. J Hosp Infect 2001; 49:183–92.
12. Kiros, T., Damtie, S., Eyayu, T., Tiruneh, T., Hailemichael, W., &Workineh, L. (2021). Bacterial Pathogens and Their Antimicrobial Resistance Patterns of Inanimate Surfaces and Equipment in Ethiopia: A Systematic Review and Meta-analysis. *BioMed research international*, *202*
13. Birru, M., Mengistu, M., Siraj, M., Aklilu, A., Boru, K., Woldemariam, M., &Manilal, A. (2021). Magnitude, Diversity, and Antibiograms of Bacteria Isolated from Patient-Care Equipment and Inanimate Objects of

Selected Wards in Arba Minch General Hospital, Southern Ethiopia. *Research and Reports in Tropical Medicine*, *12*, 39.

14. Bhatta, D. R., Hamal, D., Shrestha, R., HosuruSubramanya, S., Baral, N., Singh, R. K., &Gokhale, S. (2018). Bacterial contamination of frequently touched objects in a tertiary care hospital of Pokhara, Nepal: how safe are our hands?. *Antimicrobial Resistance & Infection Control*, *7*(1), 1-6.
15. Kanamori, H., Rutala, W. A., & Weber, D. J. (2017). The role of patient care items as a fomite in healthcare-associated outbreaks and infection prevention. *Clinical Infectious Diseases*, *65*(8), 1412-1419.
16. Russotto, V., Cortegiani, A., Fasciana, T., Iozzo, P., Raineri, S. M., Gregoretti, C., &Giarratano, A. (2017). What healthcare workers should know about environmental bacterial contamination in the intensive care unit. *BioMed Research International*, *2017*.
17. Nwankwo, E. (2012). Isolation of pathogenic bacteria from fomites in the operating rooms of a specialist hospital in Kano, North-western Nigeria. Pan African Medical Journal, 12(1).
18. Matinyi, S., Enoch, M., Akia, D., Byaruhanga, V., Masereka, E., Ekeu, I., &Atuheire, C. (2018). Contamination of microbial pathogens and their antimicrobial pattern in operating theatres of peri-urban eastern Uganda: a cross-sectional study. BMC infectious diseases, 18(1), 1-9.26
19. Schabrun, S., &Chipchase, L. (2006). Healthcare equipment as a source of nosocomial infection: a systematic review. *Journal of Hospital Infection*, *63*(3), 239-245.
20. Wißmann, J. E., Kirchhoff, L., Brüggemann, Y., Todt, D., Steinmann, J., & Steinmann, E. (2021). Persistence of pathogens on inanimate surfaces: a narrative review. *Microorganisms*, *9*(2), 343.
21. Suleyman, G., Alangaden, G., &Bardossy, A. C. (2018). The role of environmental contamination in the transmission of nosocomial pathogens and healthcare-associated infections. *Current infectious disease reports*, *20*(6), 1-11.
22. Kramer, A., Schwebke, I., &Kampf, G. (2006). How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. *BMC infectious diseases*, *6*(1), 1-8.
23. Chen, K. H., Chen, L. R., & Wang, Y. K. (2014). Contamination of medical charts: an important source of potential infection in hospitals. *PloS one*, *9*(2), e78512.
24. Adams, C. E., Smith, J., Watson, V., Robertson, C., & Dancer, S. J. (2017). Examining the association between surface bioburden and frequently touched sites in intensive care. *Journal of Hospital Infection*, *95*(1), 76-80.
25. Hayden, M. K., Blom, D. W., Lyle, E. A., Moore, C. G., & Weinstein, R. A. (2008). Risk of hand or glove contamination after contact with patients colonized with vancomycin-resistant enterococcus or the colonized patients' environment. *Infection Control & Hospital Epidemiology*, *29*(2), 149-154.
26. Shams, A. M., Rose, L. J., Edwards, J. R., Cali, S., Harris, A. D., Jacob, J. T., & Noble-Wang, J. A. (2016). Assessment of the overall and multidrug-resistant organism bioburden on environmental surfaces in healthcare facilities. *infection control & hospital epidemiology*, *37*(12), 1426-1432.
27. O'flaherty, N., & Fenelon, L. (2015). The stethoscope and healthcare-associated infection: a snake in the grass or innocent bystander? *Journal of Hospital Infection*, *91*(1), 1-7.
28. Chinn, R. Y., &Sehulster, L. (2003). Guidelines for environmental infection control in health-care facilities; recommendations of CDC and Healthcare Infection Control Practices Advisory Committee (HICPAC).
29. Russotto, V., Cortegiani, A., Raineri, S. M., &Giarratano, A. (2015). Bacterial contamination of inanimate surfaces and equipment in the intensive care unit. *Journal of intensive care*, *3*(1), 1-8.
30. Mir, M. A., Ashraf, M. W., Tripathi, V., & Mir, B. A. (2021). Isolation, characterization and prevention of various microbial strains in NIC unit and PIC unit. *Scientific Reports*, *11*(1), 1-12.
31. Suleyman, G., Alangaden, G., &Bardossy, A. C. (2018). The role of environmental contamination in the transmission of nosocomial pathogens and healthcare-associated infections. Current infectious disease reports, 20(6), 1-11.
32. Ahmed, E. H., Hassan, H. A. M., El-Sherbiny, N. M., &Soliman, A. (2019). Bacteriological monitoring of inanimate surfaces and equipment in some referral hospitals in Assiut City, Egypt. *International journal of Microbiology*, *2019*.
33. Vonberg, R., &Gastmeier, P. (2006). Nosocomial aspergillosis in outbreak settings. *Journal of Hospital Infection*, *63*(3), 246-254.

34. Ashbolt, N.J., (2015). Environmental (saprozoic) pathogens of engineered water systems: understanding their ecology for risk assessment and management. Pathogens 2015;4(2):390–405
35. Kanamori, H., Weber, D.J., Rutala, W.A. (2016). Healthcare outbreaks associated with a water reservoir and infection prevention strategies. Clin Infect Dis. 2016;62(11):1423–35
36. Mermel, L. A., Allon, M., Bouza, E., Craven, D. E., Flynn, P., O'Grady, N. P., & Warren, D. K. (2009). Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infection: 2009 Update by the Infectious Diseases Society of America. *Clinical infectious diseases*, *49*(1), 1-45.
37. Kallen, A. J., Patel, P. R., & O'Grady, N. P. (2010). Preventing catheter-related bloodstream infections outside the intensive care unit: expanding prevention to new settings. *Clinical Infectious Diseases*, *51*(3), 335-341.
38. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. *Am J Infect Control*. 2008;**36**(5):309–332.
39. Crnich, C. J., & Maki, D. G. (2001). The role of intravascular devices in sepsis. *Current infectious disease reports*, *3*(6), 496-506.
40. Timsit, J. F., Rupp, M., Bouza, E., Chopra, V., Kärpänen, T., Laupland, K., &Zingg, W. (2018). A state of the art review on optimal practices to prevent, recognize, and manage complications associated with intravascular devices in the critically ill. *Intensive care medicine*, *44*(6), 742-759.
41. Rahmani, K., Garikipati, A., Barnes, G., Hoffman, J., Calvert, J., Mao, Q., & Das, R. (2022). Early prediction of central line associated bloodstream infection using machine learning. *American Journal of Infection Control*, *50*(4), 440-445.
42. Mimoz, O., Lucet, J. C., Kerforne, T., Pascal, J., Souweine, B., Goudet, V., & CLEAN Trial Investigators. (2015). Skin antisepsis with chlorhexidine–alcohol versus povidone iodine–alcohol, with and without skin scrubbing, for prevention of intravascular-catheter-related infection (CLEAN): an open-label, multicentre, randomised, controlled, two-by-two factorial trial. *The Lancet*, *386*(10008), 2069-2077.
43. Rupp, M. E., &Karnatak, R. (2018). Intravascular catheter–related bloodstream infections. *Infectious Disease Clinics*, *32*(4), 765-787.
44. Nicolle, L. E. (2014). Catheter associated urinary tract infections. *Antimicrobial resistance and infection control*, *3*(1), 1-8.
45. Vergidis, P., & Patel, R. (2012). Novel approaches to the diagnosis, prevention, and treatment of medical device-associated infections. *Infectious disease clinics*, *26*(1), 173-186.
46. Padawer, D., Pastukh, N., Nitzan, O., Labay, K., Aharon, I., Brodsky, D., &Peretz, A. (2015). Catheter-associated candiduria: Risk factors, medical interventions, and antifungal susceptibility. *American journal of infection control*, *43*(7), e19-e22.
47. Gould, C.V., Umscheid, C.A., Agarwal, R.K., Kuntz, G., Pegues, D.A., (2010). Guideline for prevention of catheter-associated urinary tract infections 2009. *Infect Control HospEpidemiol*. 2010 Apr. 31(4):319-26.
48. Tambyah, P. A., & Maki, D. G. (2000). Catheter-associated urinary tract infection is rarely symptomatic: a prospective study of 1497 catheterized patients. *Archives of internal medicine*, *160*(5), 678-682.
49. Bizuayehu, H., Bitew, A., Abdeta, A., &Ebrahim, S. (2022). Catheter-associated urinary tract infections in adult intensive care units at a selected tertiary hospital, Addis Ababa, Ethiopia. *Plos one*, *17*(3), e0265102.
50. Horan, T. C., Gaynes, R. P., Martone, W. J., Jarvis, W. R. &Emori, T. G. (1992). CDC definitions of nosocomial surgical site infections, 1992: a modification of CDC definitions of surgical wound infections. Infect Control HospEpidemiol 13, 606–608 (1992
51. Smyth, E. T. M., McIlvenny, G., Enstone, J. E., Emmerson, A. M., Humphreys, H., Fitzpatrick, F., & Hospital Infection Society Prevalence Survey Steering Group. (2008). Four country healthcare associated infection prevalence survey 2006: overview of the results. *Journal of Hospital Infection*, *69*(3), 230-248.
52. Fan, Y., Wei, Z., Wang, W., Tan, L., Jiang, H., Tian, L., &Nie, S. (2014). The incidence and distribution of surgical site infection in mainland China: a meta-analysis of 84 prospective observational studies. *Scientific reports*, *4*(1), 1-8.
53. Wen, X., Ren, N. & Wu, A. Distibution of pathogens and antimicrobial resistance: an analysis of China healthcare-associated infection cross-sectional survey in 2010 (in Chinese). Chin J Infect Control 11, 1–6 (2012

54. Rizvi, M., Rizvi, M. W., Sultan, A., Khan, F., Shukla, I., & Malik, A. (2013). Emergence of coryneform bacteria as pathogens in nosocomial surgical site infections in a tertiary care hospital of North India. *Journal of infection and public health*, *6*(4), 283-288.
55. Singh, S., Chakravarthy, M., Rosenthal, V. D., Myatra, S. N., Dwivedy, A., Bagasrawala, I., & Chacko, F. (2015). Surgical site infection rates in six cities of India: findings of the International Nosocomial Infection Control Consortium (INICC). *International health*, *7*(5), 354-359.
56. Kawata, M., Jo, T., Taketomi, S., Inui, H., Yamagami, R., Matsui, H., .& Tanaka, S. (2021). Type of bone graft and primary diagnosis were associated with nosocomial surgical site infection after high tibial osteotomy: Analysis of a national database. *Knee Surgery, Sports Traumatology, Arthroscopy*, *29*(2), 429-436.
57. Dasgupta, S., Das, S., Chawan, N. S., &Hazra, A. (2015). Nosocomial infections in the intensive care unit: Incidence, risk factors, outcome and associated pathogens in a public tertiary teaching hospital of Eastern India. *Indian journal of critical care medicine: peer-reviewed, official publication of Indian Society of Critical Care Medicine*, *19*(1), 14.
58. Weinstein, R. A., Gaynes, R., Edwards, J. R., & National Nosocomial Infections Surveillance System. (2005). Overview of nosocomial infections caused by gram-negative bacilli. *Clinical infectious diseases*, *41*(6), 848-854.
59. Vincent, J. L., Bihari, D. J., Suter, P. M., Bruining, H. A., White, J., Nicolas-Chanoin, M. H., & Hemmer, M. (1995). The prevalence of nosocomial infection in intensive care units in Europe: results of the European Prevalence of Infection in Intensive Care (EPIC) Study. *Jama*, *274*(8), 639-644.
60. Sharpe, J. P., Magnotti, L. J., Weinberg, J. A., Brocker, J. A., Schroeppel, T. J., Zarzaur, B. L., et al. (2014). Gender disparity in ventilator-associated pneumonia following trauma: identifying risk factors for mortality. *J. Trauma Acute Care Surg.* 77, 161–165.
61. Abdelrazik, O. A., and Salah Abdelazim, M. (2017). Ventilator-associated pneumonia in adult intensive care unit prevalence and complications. *Egypt. J. Crit. Care Med.* 5, 61–63
62. Chang and Schibler, A. (2016). "Ventilator Associated Pneumonia in Children," *Paediatric Respiratory Reviews*, vol. 20, pp. 10–16, 2016.
63. Park, D. R. (2005). The microbiology of ventilator-associated pneumonia. *Respiratory care*, *50*(6), 742-765.
64. Tolera, M., Abate, D., Dheresa, M., &Marami, D. (2018). Bacterial nosocomial infections and antimicrobial susceptibility pattern among patients admitted at HiwotFana Specialized University Hospital, Eastern Ethiopia. *Advances in medicine*, *2018*.
65. Boyce, J.M., Potter-Bynoe, G., Chenevert. C., King, T. (1997). Environmental contamination due to methicillin-resistant Staphylococcus aureus: possible infection control implications. Infect Control HospEpidemiol. 1997;18(9):622–7
66. Brady, R. R., Verran, J., Damani, N. N., & Gibb, A. P. (2009). Review of mobile communication devices as potential reservoirs of nosocomial pathogens. *Journal of Hospital Infection*, *71*(4), 295-300.
67. Diekema, D. J., Pfaller, M. A., Schmitz, F. J., Smayevsky, J., Bell, J., Jones, R. N., & SENTRY Participants Group. (2001). Survey of infections due to Staphylococcus species: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, and the Western Pacific region for the SENTRY Antimicrobial Surveillance Program, 1997–1999. *Clinical Infectious Diseases*, *32*.
68. Vincent, J. L., Rello, J., Marshall, J., Silva, E., Anzueto, A., Martin, C. D., & Epic II Group of Investigators. (2009). International study of the prevalence and outcomes of infection in intensive care units. *Jama*, *302*(21), 2323-2329.
69. Magill, S. S., Edwards, J. R., Bamberg, W., Beldavs, Z. G., Dumyati, G., Kainer, M. A., &Fridkin, S. K. (2014). Multistate point-prevalence survey of health care–associated infections. *New England Journal of Medicine*, *370*(13), 1198-1208.
70. Vonberg, R. P., Kuijper, E. J., Wilcox, M. H., Barbut, F., Tüll, P., Gastmeier, P., &Wiuff, C. (2008). Infection control measures to limit the spread of Clostridium difficile. *Clinical Microbiology and Infection*, *14*, 2-20.
71. Leffler, D.A., Lamont, J.T. (2015) Clostridium difficile infection. N Engl J Med 373:287–288
72. Oake, N., Taljaard, M., van Walraven, C., Wilson, K., Roth, V., & Forster, A. J. (2010). The effect of hospital-acquired Clostridium difficile infection on in-hospital mortality. *Archives of internal medicine*, *170*(20), 1804-1810.
73. Hensgens, M.P., Goorhuis, A., Dekkers, O.M., Kuijper, E.J., (2012). Time interval of increased risk for Clostridium difficile infection after exposure to antibiotics. J AntimicrobChemother 67:742–748

74. Debast, S.B., Bauer, M.P., Kuijper, E.J. Escmid (2014) European Society of Clinical Microbiology and Infectious Diseases: update of the treatment guidance document for Clostridium difficile infection. ClinMicrobiol I.
75. Santajit, S., &Indrawattana, N. (2016). Mecanismos de resistencia a losantimicrobianosenpatógenos ESKAPE. *Biomed Res Int*, *2016*, 2475067.
76. Jain, N., Jansone, I., Obidenova, T., Simanis, R., Meisters, J., Straupmane, D., &Reinis, A. (2021). Antimicrobial Resistance in Nosocomial Isolates of Gram-Negative Bacteria: Public Health Implications in the Latvian Context. Antibiotics, 10(7), 791.
77. Korotetskiy, I. S., Jumagaziyeva, A. B., Reva, O. N., Kuznetsova, T. V., Shvidko, S. V., Iskakbayeva, Z. A., &Myrzabayeva, A. H. (2019). Isolation and characterization isolates of nosocomial infections. (5), 199-209.
78. Duedu, K. O., Offei, G., Codjoe, F. S., &Donkor, E. S. (2017). Multidrug resistant enteric bacterial pathogens in a psychiatric hospital in Ghana: implications for control of nosocomial infections. *International Journal of Microbiology*, *2017*.
79. Valenti, W. M., Hall, C. B., Douglas, R. G., Menegus, M. A., &Pincus, P. H. (1980). Nosocomial viral infections: I. Epidemiology and significance. *Infection Control & Hospital Epidemiology*, *1*(1), 33-37.
80. Chow, E. J., &Mermel, L. A. (2017). Hospital-acquired respiratory viral infections: incidence, morbidity, and mortality in pediatric and adult patients. In *Open forum infectious diseases* (Vol. 4, No. 1). Oxford University Press.
81. Ducel, J.F., Nicolle, L. (2002). Prevention of hospital-acquired infections. 2002, WHO.
82. Emily, R.M., Sydnor, T.M.P. (2011). Hospital epidemiology and infection control in acute-care settings. ClinMicrobiol Rev 2011; 24(1): 141-73.
83. Martin, D., Hönemann, M., &Liebert, U. G. (2021). Dynamics of nosocomial parainfluenza virus type 3 and influenza virus infections at a large German University Hospital between 2012 and 2019. *Diagnostic Microbiology and Infectious Disease*, *99*(3), 115244.
84. Al Musawa, M., Asdaq, S. M. B., Alattas, M., Qutub, M., Bamogaddam, R. F., Yamani, A., &Aldabbagh, Y. (2021). Candida utilis and Stenotrophomonasmaltophilia causing nosocomial meningitis following a neurosurgical procedure: A rare co-infection. *Journal of Infection and Public Health*, *14*(11), 1715-1719.

Table 1: List of common Fomites and Environmental surface

Fomites	Environmental surface
Operation lamp Suction pump Forceps Floor Wall Scissors Trolley Boyel's machine Bed rails Surgical lamp Ventilator	Dry inanimate Surface Cotton Fabric Ceramic Floor Moist Surface Synthetic fiber

Table 2: Common Pathogens, Host factors and Reservoirs of the Nosocomial infection

Nosocomial Pathogen	Host factors	Reservoir
Bacteria	Age	Fomites
Fungi	Immunity	Humans
Virus	Nutrition	Animate objects
Parasites	Therapy	
	Radiation	
	Surgery	
	Chronic illness	

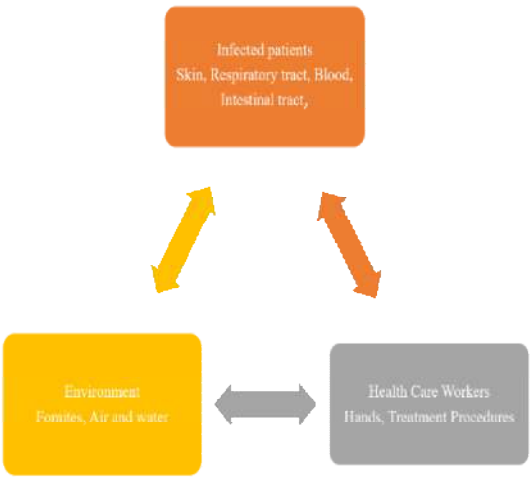

Figure 1. Transmission of Nosocomial infection

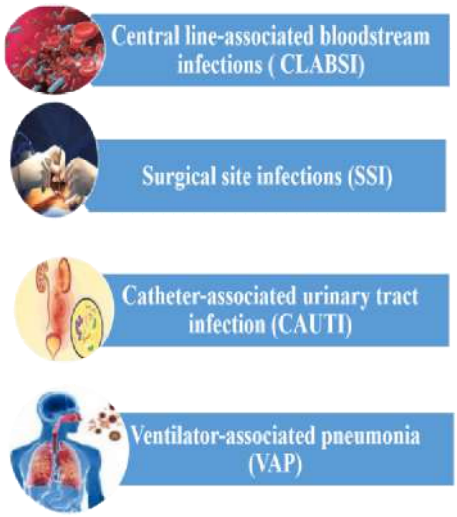

Figure 2. Major types of Nosocomial infections

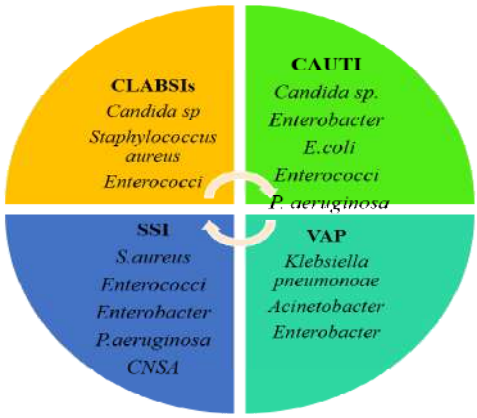

Figure 3. Pathogens associated with Nosocomial Infections

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RESEARCH ARTICLE

An Analysis on the Market Intermediaries and Various Marketing Practices Adopted by Small Tea Growers with Special Reference to Assam Tea Industry

Bhabajyoti Saikia [1] * and Homeswar Goswami [2]

[1]Assistant Professor, Department of Management, Assam down town University, Guwahati, Assam, India.
[2]Professor Emeritus, Department of Management, Assam down town University, Guwahati, Assam, India.

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***Address for Correspondence**
Bhabajyoti Saikia,
Assistant Professor,
Department of Management,
Assam down town University,
Guwahati, Assam, India.
Email: saikia.bhabajyoti@gmail.com

ABSTRACT

Small Tea Growers in India especially Assam has seen tremendous growth related to numbers, area as well as tea supply. This particular sector can provide enormous job opportunities along with livelihood for the rural community in Assam. It has further improved the standard of living of those communities. This has emerged as a recent phenomena and not much of in depth research has been found in the marketing practices adopted by them. This paper tries to cover the various roles played by the market intermediaries and also the different marketing channels which are preferred by the small tea growers of the Assam region. This study has been carried out in tea districts of Upper Assam and the reason behind choosing this region is that highest numbers of STGs are found to be in this region and also the production of tea is also very high compared to other parts of Assam. Many focus group discussions have been carried out and also an interview schedule was also used. Additionally, one survey has also been carried out amongst the STGs of that region and used as a tool for collection of data such that an appropriate conclusion can be made. Research shows, the various market intermediaries involved are small tea growers, self help groups, processors and agents. It has been found that the STGs do come across various challenges in marketing their produce which are mainly green leaf and price fluctuations and buying behavior of processor plays an important role. Further, after research it has been found that the STGs do not follow a specific channel for marketing their produce. The outcome of this study is of great relevance and there is much scope for further research in this area.

Keywords: Assam, BLF, Channels, Marketing, STGs, SHGs, Tea Estate.

INTRODUCTION

Tea is considered as one of the most preferred drink in the World after water. It is quite refreshing and thirst-quenching beverage. It can be found that during the last few decades, tea is becoming like a commodity in India. After China, India is the second largest tea producer in the World. It greatly contributes to India`s GDP growth and also in the foreign exchange earnings. Various forms of tea in India need to start with green leaf from the tea plant which is called as *Camellia sinensis* where the Indian version of that tea plant is termed as *Camellia sinensis* Assamica [2]. The tea leaves for use needs to be hand-picked from the tea bush for ensuring of plucking of the right buds and leaves [1]. Indian tea is found to be one of the finest in the World and owes strong geographical indications, large investments in tea processing units, regular innovations, increased product mix and also various strategic market expansions. The tea sector of India is very important for the rural community of Assam and it serves as a major livelihood opportunities. Research says that there are sixteen tea growing states in India where Assam, West Bengal, Tamil Nadu and Kerala contributes about 95% of the country`s tea production in total. Assam alone produces more than 50% of the total production of India [3, 5]. However, the tea industry is going through various changes in the form of structures ad significant changes can be noticed during the last few decades and one amongst them is the increasing number of Small Tea Growers (STG). Assam as a state has experienced exponential growth with the increase in the formation of STGs and sources from All Assam Small Tea Growers Association (AASTGA) there are around 3,44,222 registered Small Tea Growers during the year 2019-2020 and they cultivates in around 1,60,948 hectares of the Assam region [2]. According to the Tea Board of India, small tea cultivation includes plantations of up to 10.12 hectares (25 acres). Growers took full advantage of the favorable weather, suitable land, and other infrastructural facilities that were readily available in Assam, which resulted in the growth of STGs [7].

The rising trend of small holding tea growers is primarily due to tea estates' failure to meet yield targets as well as a consistent decline in tea quality [1]. People were interested in utilizing their land in tea plantations because it provides a consistent income for a longer period of time with a relatively small investment [5]. The potential exists for local youngsters to experience employment satisfaction through active participation in this industry [9]. The STGs do, however, have a number of difficulties in promoting their products. It is challenging for the marketing system to transport their goods effectively and rapidly since they grow their tea in widely separated geographic areas and in relatively small quantities. Additionally, the quality of the tea cannot be preserved longer after being picked from the garden due to chemical changes [4]. As a result, STGs have very few options and are compelled to sell to whichever customer is nearby and willing to pay quickly. Small tea growers (STGs) confront a variety of issues, including inadequate infrastructure, a lack of knowledge about tea, a low price for tea leaves, and exploitation by tea brokers [2, 6]. Given the significance of STGs, it is imperative that researchers, policymakers, and the general public comprehend the numerous problems and difficulties they encounter. This paper attempted to comprehend the functions of numerous market intermediaries, the various marketing channels for fresh leaf, and the most desired marketing channel for STGs.

METHODOLOGY

This study's objective is to determine the role of various market intermediaries in the marketing of green tea leaf by Small Tea Growers (STGs) in the region, as well as the most preferred channel for marketing of leaf by small tea growers. The research is more exploratory in nature. Primary and secondary data have been gathered. Secondary data on the number of various market intermediaries in the study area was obtained from the Tea Board of India. A survey of STGs in the study area was used to collect primary data on the role of each intermediary and the most preferred channel for marketing. The respondents have been given a well-structured interview schedule. The five Assam districts - Tinsukia, Dibrugarh, Sibsagar, Jorhat, and Golaghat, which house more than 65% of the STGs in the area, are used as the sample locations. The Self Help Groups (SHGs) established by STGs were used to contact the sample respondents. Before distributing the schedule, the context and goals of the study are discussed, and a focus

group discussion is held. 32 SHGs were included in the sample, which was conveniently selected from five districts according to the area covered by STG holdings where each of the SHGs had an average of 100 STGs.

RESULTS AND DISCUSSIONS

The data and information gathered through the brainstorming method was compiled and discussed under two headings: i) the role of market intermediaries and ii) the various channels used by STGs to market fresh green leaves.

Market Intermediaries' Role in Green Leaf Marketing

Producers (STGs), Self-help groups (SHGs), commission agents, and factories are among the various factors involved in the marketing of green tea leaf. The following is a brief description of their role and scenario in the study area:

Small Tea Growers STGS (Producers)

Producers are the people who actually grow green leaf tea on various types of land. They are divided into minor tea growers, proprietary tea gardeners, and corporate or estate tea growers based on the size of their land holdings. They are the most important players in the value chain of tea. Small Tea Growers (STGs) are individuals or groups with plantation areas up to 10.12 hectares, according to the Tea Board of India. According to a survey conducted by the Assam State Government and the Tea Board of India, upper Assam districts such as Tinsukia, Dibrugarh, Sivsagar, Golaghat, and Jorhat have the highest concentration of STGs. Assam's STGs are now a major force in the industry, accounting for 42.17% of the state's total tea production as in 2017-18. According to the study carried out, the majority of 46% of STGs have landholdings that are between 6 and 10 bighas in size on average. Tea production is the only source of income for the majority of STGs. The majority of STGs use manual plucking, and in order to keep labor costs down, most STGs do not adhere to the traditional plucking cycle. None of them perform the plucking with the ideal 6–7 rounds. 50% or majority of the people do eight to nine days' worth of plucking rounds. The STGs also differ in their post-harvest procedures, such as temporary storage in gardens, loading, shipping, unloading, etc.

Self Help Groups

STGs have formed several groups in tea-growing areas, with the majority of them registering as Self Help Groups (SHGs) and participating in various activities. The group is made up of like-minded and homogeneous STGs from a specific region/village. SHGs' main functions include supplying inputs for tea cultivation, training on cultivation, plucking and post-harvesting activities, and so on. Aside from that, SHGs in some places act as major value chain intermediaries, collecting green leaf from their fellow members and supplying it directly to factories. This significantly reduces the involvement of other intermediaries who would normally exploit the STGs. For better price realization, SHGs are encouraged to supply green leaf directly to the factory. So far, 332 small tea grower SHGs have been formed. Furthermore, in order to encourage small tea growers to establish their own tea manufacturing factories and obtain a higher price for their produce, the Tea Marketing Control Order, 2003 was amended, and mini tea processing factories were exempted from obtaining any registration/no objection certificate. Data on the proportion of STGs in the study area who participate in collective activities has been collected, and the total area of STGs tea falls under the collectives. The details are shown in the table below (table.1). Even though the STGs formed a number of SHGs, there are very few of them actively engaged in the procurement of green leaf throughout the entire study area. The STGs cluster is strong in the Assam state districts of Golaghat, Dibrugarh, and Jorhat, where the majority of them are located.

Commission Agents (CA)

Green leaf agents are another name for commission agents. They primarily assist purchased leaf factories in obtaining green leaf tea from small tea growers. They also act as an agent for supplying manures or extending credit to small tea growers from purchased leaf factories. They charge a fixed commission per kg of leaf from growers rather than factories. They assist growers in transporting their produce and locating markets where the produce can be sold at a higher price. The farmers are relieved of the responsibility of carrying leaves from the garden to the factories because the "agents" collect leaves from their doorsteps. Additionally, the grower is guaranteed a quick

market for his produce. Agents are significant participants in this value chain. Thus, in terms of any loss resulting from a delay in shipping or the withering of the leaves, the agent is a "risk absorber" and the producer is a "risk averter", minimizing the impact on the grower. In this way, the crop is indirectly insured. The agency may offer the grower an advance. They can meet their financial needs as a result. The agent is also a trusted member of the locality, occasionally even a STG himself. Since the creation of the STGs, the agent-grower business connection has persisted as a workable model, thriving on goodwill and mutual trust.

Processors (estate factories / bought leaf factories (BLFS)

Processors are important players in the tea value chain. They are either estate factories or BLFs owned by corporate gardens. According to Tea Board data from 2019, Assam has approximately 747 estate factories and 329 BLFs. In addition to their own garden leaf, corporate gardens with factories purchase from STGs to meet their gap in fresh leaves. BLFs are the processors who buy green leaf tea from small tea growers within a 20-kilometer radius. The factory must process at least 25 lakh kgs of green leaf per year in order to be established. Tea leaves are obtained from small tea growers, self-help groups, or proprietary tea gardens. Factory purchasing practices primarily include the mechanism for determining the percentage of fine and coarse leaf, the acceptance level, pricing mechanisms, the time for green leaf to arrive at the factory, and so on. According to the study, there is no standard practice across different factories. Most BLFs do not set strict quality leaf standards, such as fine and coarse leaf percentages, loading and unloading methods, transportation specifications, and, most importantly, timing. BLF performs a random quality check by sorting the leaves and sets the price. However, growers complain that sorting is not uniform.

Marketing channels and practices preferred by STGS for marketing green leaf

The research conducted in the study area revealed some issues concerning the marketing of green tea leaf. Selling to commission agents is more convenient for the majority of STGs. Agents collects green leaves from STGS and delivers them to BLFs. It can be seen that the agents have relatively greater bargaining power, and they play a minor role in price fixing. The small tea growers are concerned that the quality of their produce is far superior to that of competitors and also from BLF's personal estates. Unfortunately, both get mixed up in the process, which is morally unacceptable. Additionally, collecting agents compete with one another, and typically a grower doesn't form a bond with a particular agent. They divide up the produce and sell it to several brokers. Fresh leaf prices vary from season to season and location to location. BLFs lower the price during peak season and justify it by saying that there is "no place in the factory to keep leaf." In times of leaf shortage, farmers haggle for a higher price. In the study area, there is no standard practice or specific pattern followed by SHGs/STGs in marketing their produce. As previously stated, no SHG markets their entire collected leaf through a single channel. The table below shows the various channels through which leaf is sold, as well as the percentage of SHGs that use each of the channels (table.2.). Green leaf is typically sold to the factory through a commission agent (First Channel).

From STGs, they get a commission per kilogram. BLFs typically enter into agreements for the volume of supply of green leaves with middlemen like leaf agents or producers. Due to their lack of organization and unionization, STGs have limited bargaining power with factories. The price of green leaf finally decreases as a result of a lack of market information, inadequate storage facilities, transportation issues, etc. The majority of producers have agreements with these commission brokers because they use advance credit for various purposes, forcing them to sell their produce to them at the stipulated price. Some STGs are selling their produce straight to the BLF in order to avoid these problems (Second Channel). The majority of STGs who live within 3 to 4 kilometers of the factory favor this channel. In the case of third channel, small tea growers established a self-help group that typically has thirty members. They transport their produce to the BLF after gathering it. The commission charge is a significant savings, and because pooling and transportation are done collectively, transportation costs are reduced. They save money on commission and get a good price for their produce. Due to a lack of transportation facilities, SHG were unable to transport their produce directly to the BLF in some cases. In such cases, commission agents with their own transportation arrangements are involved in marketing their produce to BLF (Fourth Channel). In the study area, it can be observed that the improved marketing channel (Fifth Channel) is on the rise. The Self Help Group establishes its own mini-factory to process green leaf tea. They receive a fair price for their produce through this channel. These channels

encourage farmers to supply higher-quality leaf while also increasing price realization. BLF also has the potential to obtain high-quality products that are competitively priced through dealers, industries, and auction houses. There are relatively little STGs marketing through fourth, fifth and sixth channels. Many corporate factories are concerned that the quality delivered by STGs won't be uniform.

CONCLUSION

This study reveals a few new insights into STGs' preferred marketing strategies and distribution channels for fresh tea leaf. The STG's power and contribution to the supply of tea are increasing on yearly basis. The market intermediates, including agents, processors, SHGs, and STGs, play a crucial role. STGs do not have a preferred standard channel. Many channels have been attempted to be understood by the study. Future research might be done to determine the price spread in each channel and the proportion of STGs. Similar research can also be done in other regions of the nation, such as Tripura and Arunachal Pradesh, where there are several STGs. The possibility of alternative strategies for enhancing the market competitiveness of the most vulnerable and significant stakeholder, the small tea grower, may also be investigated through future studies.

REFERENCES

1. Kakati, N., (2011), "Problems of Small Tea Growers in Assam with Reference to Lakhimpur District", Indian Journal of Applied Research, Volume-1.
2. Das, K., (2014), "The Tea Smallholdings of Assam: Sector for Competitive and Inclusiveness", DVS publishers, Guwahati.
3. Goowalla, H., (2015), "A Study on the Problem and Prospect of Small Tea Growers in Assam with Special Reference to Jorhat District", International Journal of Scientific Engineering and Applied Science, Volume-1.
4. Jeshurun, S. B., & Jenefa, L. (2019), "An Empirical study on Satisfaction towards Selected Herbal Medicines", History Research Journal, 5(6), 30 - 36.
5. Baruah, P., (2008), "The Tea Industry of Assam: Origin and Development", EBH Publisher House.
6. Bhowmik, S. K., (1991), "Small Growers to Prop Up Large Plantations", Economic and Poltical Wekkly, Volume-26.
7. Das, K., (2012), "The Small Holding In Assam: Is There A Way Out", Economic and Political Weekly, 47(11): 23-25.
8. Deb, B. J., Sengupta K. & Roy D. B., (2008), "Globalization and North –East India", Concept Publishing Company, New Delhi.
9. Jeshurun, S. B., Nirmala, D. R., & Sivakumar S. (2020), "Effectiveness of Sales Endorsement Techniques – An Emperical Review", Our Heritage, 68(1), 10739 - 10743.

Table 1: Status of STGs Collectives in NER during 2018

Sl. No.	Name of Sector	Stated Strength of STG	STG Member Strength within SHG	Total Area of Tea	Area of Tea under SHG
1	Dibrugarh – Tinsukia (70 SHGs)	46180	3878 (8.40)	50396	4023.91 (7.98)
2	Jorhat-Sivasagar-Golaghat-Karbi Anglong (90 SHGs)	37469	4047 (10.80)	41985.31	3632.96
3	Silchar-Barak valley (5 SHGs)	236	155 (65.68)	459.36	414.41 (90.21)
4	Guwahati-	1349	929	1835.04	1285

	Goalpara-Bongaigaon-Baksa-Dhubri-Kokrajhar-Chirang (13 SHGs)				(70.02)
5	North Bank including Nagaon (45 SHGs)	16708	1815 (10.86)	20478.67	2353.7 (11.49)
6	**Assam (223 SHGs)**	**103694**	**11464 (11.05)**	**115154.4**	**12109.98 (10.52)**

Source: Tea Board of India.

Table 2: Various marketing channels preferred for marketing of green leaf by STGs

Channel Option	Channel Name	Percentage of STGs
First Channel	Conventional Market Channel (STG – Commission Agents – Factory)	65
Second Channel	Direct Market Channel (STG – BLF)	5
Third Channel	Establishment of SHGs (STG – SHG – Factory)	20
Fourth Channel	SHG marketing through agents (STG – SHG – Commission Agents – Factory)	6
Fifth Channel	SHG owned BLF	1
Sixth Channel	Procurement of Green Leaf Tea by Estate Factory (STG – Estate Factory)	2

Source: Approximate estimation based on survey.

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RESEARCH ARTICLE

CAGR Analysis on Area, Production and Productivity of King of Fruits (Mango) – Evidence from Krishnagiri District of Tamil Nadu

K.Buvaneswari[1]* and G. Ramanathan[2]

[1]Ph.D., Research Scholar, Department of Agricultural Economics, Annamalai University, Chidambaram, Tamil Nadu, India.
[2]Professor and Head, Department of Agricultural Economics, Annamalai University, Chidambaram, Tamil Nadu, India.

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***Address for Correspondence**
K.Buvaneswari,
Ph.D., Research Scholar,
Department of Agricultural Economics,
Annamalai University,
Chidambaram, Tamil Nadu, India.
Email: buvaneswarieco@gmail.com

ABSTRACT

A study was undertaken using the secondary data drawn from both State and Central Government sources regarding the area, production and productivity of mango. The study revealed that the reports of the area, production and productivity of data are collected from various statistical sources. The data were analyzed CAGR (Compound Annual Growth Rate) using MS Excel. According to CAGR the area (0.73 ha), production (3.95 MT) and productivity (3.15 MT), was in the increasing state during 2003 to 2021 in India. In Tamil Nadu, CAGR is Positive for area (1.23 ha), which shows a declining trend in production (-0.018 MT) and productivity (-1.23 MT), positive for area indicates an increase in mango cultivation in Tamil Nadu. In the Krishnagiri district, there has been no significant change for 2003 to 2020. Decreasing in the area (-0.48 ha), production (-1.99), productivity (-1.51 MT) in Krishnagiri District. Cuddy-Della Valle index provides best estimates and instability was found to be more in production (30.32) and productivity (31.47) of mango is Krishnagiri district and Area was high in India (6.53). Coppock Index values are high in area Tamil Nadu (39.56), Krishnagiri has high in production (49.57) and productivity (49.68). High coppock index values are represents greater instability.

Keywords: Mango, CAGR, Coefficient of variation, CDVI, Coppock Index.

INTRODUCTION

Mango is a very delicious and widely liked fruit by peoples all over the world. It is called the "King of Fruits". Mango is one of the most consumed fresh fruits in the world. It dominates the Indian fruit basket; it comes first among the top-rated delicious fruits and mango farmers are used to getting considerable income. Variable geological and geographical conditions in India are favorable for growing an extensive array of horticultural crops throughout the country. It is a historical fruit because it was cultivated in South Asia for thousands of years in the ancient days of its cultivation. It is available in sweet and sour tastes, used in different ways of consumption. Mango occupies a unique and vital place among the fruit crops of India. It is now playing a crucial role in the country's national economy. In India, 45% of all tropical fruits produced are mangoes (NHB 2019). With 24.7 million tons, India accounts for almost half of the world's production, followed by Indonesia, China, Mexico and the Philippines. The aggregated production of ten countries is responsible for roughly 80% of the world's mango production (Paulmurugan 2021). India is the home of about 1,000 varieties of mango as man has cultivated it for more than 4000 years. However, only a few varieties are commercially cultivated throughout the country. More than 90 countries in the world grow mangoes. Global production of the mango has doubled in the past thirty years. Mango is native to Asia and it is the largest producer, representing 77% of global production, followed by America with 13% and Africa with 10%. India is a front runner in the production of fresh mango with a share in more than 44 per cent area under mango cultivation and 38 per cent in production. The world productivity of mango is 20.9 M.T. per hectare.

The mango productivity in India is estimated at 6.92 metric tons per hectare, while that of Brazil is 16 metric tons per hectare (Shyam Prakash Singh 2018). The raw mango and mango products earn foreign exchanges as well as a source of food & household income to the growers and help in poverty reduction by providing employment opportunities to the rural peoples, both male and female, for the non-growers through various activities such as marketability of raw mangos and processing of different products, both in raw and ripe stages. Therefore, it is considered a fruit with significant potential for employment generation. Despite this, India (80.27%) takes the third position in an increase in total production, followed by Indonesia (130.88%) and Pakistan (90.71%). The average yield per hectare of mangoes in India is one of the lowest globally - even behind countries like Bangladesh and Pakistan. The mango productivity in India was estimated at 8.56 MT/ ha, while that of Brazil was 16 M.T. / ha (2014-2015) (NHB 2015). The area and production of mango in the global market share have declined. This present study discussed India's current state of mango production and its growth rate.

MATERIAL AND METHODS

Data Analysis

The time-series data on mango area, production and productivity were collected for 2003- 2021. Hence the analysis was covered for the 2003- 2021 period. Data used for the study was collected from various published sources such as the National Horticulture Board, Agricultural and Processed Food Product Export Development Authority (APEDA) (New Delhi), Directorate General of Commercial Intelligence and Statistics, Annual Export Report and other sources.

Analysis of data

The annual report of mango area, production and productivity data has been collected and analyzed for 18 years, for 2003 - 2021. It has been collected and analyzed for compound mean, standard deviation (S.D.), coefficient of variation (C.V) and Compound Annual Growth Rate (CAGR).

Compound annual growth rate Analysis (CAGR)

The CAGR of area, production and productivity were worked out using an exponential form of the equation and modeling the time trend used for this study. The exponential trend or log-linear employed by Ahmed *et al.*, 2015 Nmadu *et al.,* 2009 and Samuel *et al.,* 2013 was used. The exponential trend equation for production is specified as follows

Consider the non-linear relationship between a study variable (Y) and time variable (X) as

Y=ab^t ……..(1)

By taking logarithm on both side, it may be written as

Log y = log a + t × log b……..(2)

Y = A + B × t

\Where,

Y = log y, A= log a, B= log b

Y= area (ha) / production (tons) and productivity (Kg / ha)

t = time elements which takes the value 1,2…n for various years

A= intercept, B= regression co- efficient

Compound growth rate

= (Antilog of B- 1) × 100

t = r/ SE (r)

Where,

r = Compound Growth Rate,

SE= Standard Error

Co-efficient of Variation (C.V)

In order to study the variability in the time series data, coefficient of variation (CV) was used as an index of consistency.

Tabular Analysis

In the present study, tabular analysis was used to determine the mean, standard deviation, coefficient variance and percentage of total area under cultivation, production and productivity.

Cuddy - Della Valle Instability Index (CDVI)

Cuddy - Della Valle Instability Index (%) = C.V × $\sqrt{(1-\bar{R})^2}$

Where,

C.V is the Coefficient of Variation in per cent,

$\bar{R}^2$ is the coefficient of determination from a time trend regression adjusted for its degrees of freedom.

Instability

Production instability has been measured by using Cuddy-Della Valle (CDV) index. We have divided the production instability into three classes namely, low instability, medium sized instability and high instability.

Coppock Index

Instability was also analyzed using Coppock's index which is calculated as the antilog of the square root of the logarithmic variance using the following formula (Coppock, 1962).

Coppock Index = (Antilog) $\sqrt{(V\log-1)}$ × 100

V log = $\frac{1}{(N-1)}\sum(\log p_{t+1} - \log p_t - M)^2$

M = $\frac{1}{(N-1)}\sum(\log p_{t+1} - \log p_t)$

Coppock instability index is a close approximation of the average year to-year percentage variation adjusted for trend and the advantage is that it measures the instability in relation to the trend in area, production and productivity. A higher numerical value for the index represents greater instability.

RESULTS AND DISCUSSION

State-wise distribution in area, production and productivity of mango in India

The state-wise data on mango area, production and productivity for 2003-2004 to 2020- 2021 has been collected and percentage shares have been worked out and given in Table-1. India's average area, production and productivity of mango were 2314.74 hectares, 20899.24 million tons and 9.03 million tons hectares, respectively, for 2020-2021 (APEDA). The top 10 states under mango cultivation in India are Andhra Pradesh (16.37%), Uttar Pradesh (12.06%), Odisha (9.44%), Tamil Nadu (7.28%), Karnataka (7.27%), Bihar (6.92%), Gujarat (6.97%), Maharashtra (6.30%) and Telangana (5.37%) respectively (2020-2021). The average production of major mango producing states in India in Andhra Pradesh, Uttar Pradesh, Maharashtra, Bihar, Karnataka, Odisha, Tamil Nadu, Gujarat and West Bengal. Andhra Pradesh rank first in average area and production of mango in India. It has occupied almost one-fifth area of the total area under mango cultivation in the country (India stat report 2020-2021) and contributed 23.57 per cent of total production, followed by Uttar Pradesh (23.00%), Karnataka (7.97%), Bihar (7.38%), Gujarat (5.67%), Telangana (5.50%), West Bengal (4.59%), Odisha (3.67%), Tamil Nadu (2.78%) and Maharashtra (2.02%) respectively. Hence the productivity (9.03Mt ha.$^{-1}$) was the lowest so that it ranked fourth, next only Uttar Pradesh, Karnataka and Bihar state. The productivity of mango was satisfactory in Northern states like Uttar Pradesh (17.21 Mt ha^{-1}.), Bihar (9.63 Mt ha^{-1}.) and southern states like Andhra Pradesh (13 Mt ha^{-1}.) and Karnataka (9.91 Mt ha^{-1}). Similar study on mango's area, production, productivity concluded the same trend, which revealed that mango's productivity was satisfactory in Northern states like Uttar Pradesh, Bihar and Jharkhand then the Southern states like Andhra Pradesh, Telangana and Karnataka. The productivity of these states increases in constant order. The productivity of the remaining major mango producing states of India was less than the average productivity of the states like Gujarat (7.35 Mt ha. $^{-1}$), Kerala (6.22 Mt ha. $^{-1}$), Odisha (3.51 Mt ha. $^{-1}$), Tamil Nadu (3.45 Mt ha. $^{-1}$) and Maharashtra (2.89 Mt ha. $^{-1}$).

State-wise growth rate of Area, Production and Productivity of Mango in India

India is found to be a leader in mango production in the world. India is one of the largest producers of fruits and ranked second in fruit production next to China (Karthick *et al.* 2013).Among the 28 states in India, one should be known which state is the leader in fruit production for making appropriate investment decisions. The details are analyzed and presented in Table 1. Table 1 shows the details of the state-wise area, production and productivity of fruits in India. In that, Maharashtra is the leader in the area under fruits. The trend and growth in the area, production and productivity of mango in India are analyzed and the results are presented in Table 1. It is understood from the table that the area and production of mango with an average of 2242.883 per thousand hectare and16688.92778 per thousand hectares have reached. During 2020-2021 the total value of production is 2315 thousand hectares and 20899 MT. Comparatively, the data were shown the year 2013-2014 is high in area whereas the production has low (2516.00 thousand hectare and 18431 MT respectively) during the year. It could be seen from the above table that the compound growth rate for mango area, production and productivity were 0.735838 Ha, 3.952633 MT/ ha and 3.156336 MT respectively. It is inferred that the compound annual growth rate is positive for area, production and productivity, indicating an increase in mango area by 40371.9 hectare.

Tamil Nadu

State-wise productivity of fruits revealed that Tamil Nadu is the second largest productivity earner next to Himachal Pradesh (Sekhar 2013). Mango is one of the trinity of fruits in Tamil Nadu and is a seasonal one. It is generally grown under rainfed conditions in the state. In this context, detailed analysis with special reference to mango in Tamil Nadu was analyzed, and the details are presented in Table 2. The area under cultivation of mango is 0.169533 Ha in Tamil Nadu. Mango is generally grown all over the state, but the cultivated area and quantity produced are explicitly concentrated in Krishnagiri, Dharmapuri, Dindigul, Theni, Vellore, Thiruvallur and Madurai. The table shows the mango production in Tamil Nadu and its share in total production for 17 years for 2003-2004 to 2019 - 2020. It could

be seen from the above table that the CAGR for mango area, production and productivity were 1.233307, -0.01831981 and -1.236378463 respectively. It is inferred that the CAGR is negative for production, i.e.,-0.01832 which shows a declining trend in production and productivity where area under mango cultivation is positive for 1.233307 area, indicating an increase in mango area 1.233307 ha.

Krishnagiri

The secondary data regarding mango area, production and productivity in the Krishnagiri (2003-2004 to 2019-2020) were collected and analyzed using growth rate and trend analysis and presented in this section. Table 3 Revealed that there is a negative trend over the area (-0.48293), production (-1.9933342), productivity (-1.517730839) of krishnagiri district of Tamil Nadu even though it stands 1st position in Tamil Nadu. Negative trend is due to the shift in cropping pattern followed nowadays by the local farmers of Krishnagiri district. Farmers changed their concern towards other crops like flower crops, vegetables, ragi, pulses, paddy and maize due to change in climatic condition and scarcity of water.

Measures of Instability of Area, production, and productivity of Mango

Instability analysis on the area, production and productivity of mango for a period of 18 years was carried out. Instability measures such as coefficient of variation, Cuddy-Della Valle index, and Coppock index were determined and presented in below table 4. The results of the analysis indicated that Cuddy-Della Valle index provides best estimates and instability was found to be more in mango productivity of Krishnagiri (31.47) followed by Tamil Nadu (24.51) and India (7.12). To be more in mango production of Krishnagiri (30.32) followed by Tamil Nadu (27.97) and India (5.38). To be cultivated high in area of mango in India (6.53) followed by Krishnagiri (5.53) and, Tamil Nadu (4.14). The results of the analysis indicated that Cuddy-Della Valle index shows production instability of Mango. As per CDVI production instability is high instability in Krishnagiri (30.35) and Tamil Nadu (27.97), low instability of production is found in India (5.38). The results of the Copock instability analysis is used to measure the instability to the trend in area, production and productivity. A higher numerical value for the index represents greater instability. Copock instability index value of Area was high in Tamil Nadu (39.56), followed by India (39.49) and Krishnagiri (38.97). The high value of Production were estimated in Krishnagiri (49.57) followed by Tamil Nadu (47.12) and India (45.54). Productivity of Copock value were high in Krishnagiri (49.68) followed by Tamil Nadu (46.51) and India (44.08).

CONCLUSION

The study revealed that the area, production and productivity of mango for the year 2003-2021 show that the CAGR is positive for area production and productivity, indicating an increase in mango area in India. In Tamil Nadu, (CAGR) is negative for production which shows a declining trend in production and productivity and it is positive for area indicates increase in mango area. There is no significant change in the area in the Krishnagiri district throughout 2003-2020. The production and productivity of mango tend to be maximum in 2008-09 followed by year 2010 - 11, 2003-04 and 2009-10, 2018-19 and 2015-16. Overall report for the production and productivity of mango is increased in the year of 2003- 04. In 2019-2020 CAGR is in declining trend in area production and productivity due to climate condition and new plantation, young bears. This negative growth in productivity of mango may be due to the adoption of poor cultivation management practices, method and system of orcharding by cultivation. Cuddy-Della Valle index provides best estimates and instability was found to be more in production (30.32) and productivity (31.47) of mango is Krishnagiri district and Area was high in India (6.53). Coppock Index values are high in area Tamil Nadu (39.56), Krishnagiri has high in production (49.57) and productivity (49.68). High coppock index values are represents greater instability.

SUGGESTIONS

In the Krishnagiri district total area, production and productivity was decreasing. Thus, there is a need to take productivity-enhancing measures in mango cultivation like the adoption of high-density planting techniques, pit size, split application of fertilizers and bio-fertilizers. Unseasoned rains leading to extended winter and continuous

rains in the mango growing areas did not allow adequate and timely flowering. Thereafter, the rise in temperature and rains resulting in the increasing humidity levels which lead to the breeding of insects, proved harmful to the mango crop, according to experts in the field. Hence, adopting the recommended level of plant protection measures would enhance the flowering and increase mango yield. The productivity is found to be stagnant over a period of time called for effective implementation of transfer of technology and demonstration of technologies at field with the motivation of price bonanza to the farmers and agri-preneurs.

REFERENCES

1. Agricultural and Processed Food Product Export Development Authority (APEDA).2021. http:agriexchange.apeda.gov.in / index/ report list.aspx.
2. Ahmad,I.M.,Samuel,E., Makama, S.A. and Kiresur V.R. 2015.Trend of area, production and Productivity of Major Cereals: India and Nigeria Scenario. *Research Journal of Agricultural and Forestry Scinces,*3(2):10-15.
3. Coppock. J.D. 1962. International Economic Instability. McGrew-Hill, New York, pp 523-525.
4. Horticultural Data Base. 2020. Directorate of Horticulture and Plantation Crop: Chennai Government of Tamil Nadu.
5. Indiastat Report. 2021. https://www.indiastat.com.
6. Nmadu, J.N., Ojo,M.A. and Ibrahim F.D. 2009. Prospects of Sugar Production and Imports: Metting the Sugar Demand of Nigeria by year 2020, *Russian Journal of Agricultural Scio-Economic Science,* 2(14): 15-25.
7. Samuel,E. and Patil, B. 2013.Trend Analysis of Area, Production and Productivity of Major Cereals in Ethiopia. *International Journal of Agricultural Economics and Management.,* 3(2):19-27.
8. Shyam Prakash Singh,Adarsha,L.K. A.K. Nandi and OmeJopir. 2018.Production Performance of Fresh Mango in India: A Growth and Variability Analysis. *International Journal Pure Applied Bioscience,* 6(2):935-941.
9. Karthick,V.,K. Manii and A.Anbarasan. 2013. Mango Pulp Processing Industry in Tamil Nadu –An Economics Analysis. *American International Journal of Research in Humanities, Arts and Social Sciences,* 2(1): 48-52.
10. Sekhar, C M.Selvarajan, A. Pounraj, M. Prahadeeswaran.2013. Production and Export of Mango in India – A paradigm to The Developing Nations. *American International Journal of Research in Humanities, Arts and Social Sciences,* 4(1):78-87.
11. Paulmurugan, S. A Study on-trend and growth of mango in Tamil Nadu. 2021. *Psychology and Education,* 58(2): 6593-6600.

Table 1. Area, Production and Productivity of Mango India for 2003-2021

Year	Area (000'Ha.)	Production (000'MT.)	Productivity (M.T.)
2003-04	1906.7	11490	6
2004-05	1970.4	11829.7	6
2005-06	2080.7	12663.1	6.1
2006-07	2153.7	13734.1	6.4
2007-08	2201	13997	6.4
2008-09	2309	12750	5.5
2009-10	2312.3	15026.7	6.5
2010-11	2297	15188	6.6
2011-12	2378.1	16196.4	6.8
2012-13	2500	18002.4	7.2
2013-14	2516	18431.3	7.3
2014-15	2163	18527	8.6
2015-16	2209	18643	8
2016-17	2212	19506	8.8
2017-18	2258	21822	9.7
2018-19	2296	21378	9.3

2019-20	2294	20317	8.9
2020-21	2315	20899	9
Total	40371.9	300400.7	133.1
Mean	2242.883	16688.92778	7.394444444
SD	156.0031	3453.187412	1.340532038
CV (%)	6.955471	20.69148754	18.1289081
LONGEST	1.007358	1.039526331	1.031563357
CAGR	0.735838	3.952633	3.156336

Source: India stat Report (2021-2021)

Table No .2 Area, Production and Productivity of Mango Tamil Nadu for 2003-2020

Year	Area (Ha.)	Production (MT.)	Productivity (M.T.)
2003-04	114926	615370	5.354489
2004-05	118444	539404	4.554085
2005-06	125104	537780	4.298664
2006-07	125856	694554	5.51864
2007-08	128221	702260	5.47695
2008-09	130012	644626	4.958204
2009-10	132697	636330	4.795361
2010-11	139496	957979	6.86743
2011-12	141140	626392	4.43809
2012-13	144509	1189270	8.22973
2013-14	143177	830291	5.799053
2014-15	140367	894869	6.375209
2015-16	139142	869881	6.25175
2016-17	134934	550019	4.076208
2017-18	136172	579929	4.258798
2018-19	147162	524229	3.562258
2019-20	145378	494478	3.401326
Total	2286737	11887661	88.21624315
Mean	134513.9	699274.176	5.189190774
SD	9516.061	189014.937	1.252606633
CV	0.070744	0.27030161	0.241387663
CV%	7.074405	27.0301612	24.13876628
LONGEST	1.012333	0.9998168	0.987636215
CAGR	1.233307	-0.01831981	-1.236378463

Source: Season and Crop Report 2019-2020

Table 3. Area, Production and Productivity of Mango Krishnagiri for 2003-2020

Year	Area (Ha.)	Production (MT.)	Productivity (M.T.)
2003-04	34626	252436	7.29
2004-05	34483	139809	4.05
2005-06	34780	101560	2.92
2006-07	33616	157509	4.69
2007-08	33083	163681	4.95
2008-09	32221	249184	7.73
2009-10	32208	223621	6.94

2010-11	33298	244109	7.33
2011-12	34164	136539	4.00
2012-13	37029	161953	4.37
2013-14	36889	141619	3.84
2014-15	35607	150417	4.22
2015-16	33052	170317	5.15
2016-17	30914	113415	3.67
2017-18	30976	138432	4.47
2018-19	31652	190629	6.02
2019-20	30806	93773	3.04
Total	569404	2829002.74	84.6967
Mean	33494.35	166411.926	5
SD	1939.011	49883.4454	1.531783386
CV	0.057891	0.29975884	0.307453795
CV%	5.789067	29.9758837	30.74537947
LONGEST	0.995171	0.98006666	0.984822692
CAGR	-0.48293	-1.9933342	-1.517730839

Source: Season and Crop Report 2019-2020

Table 4. Measures of Instability of Area, production, and productivity of Mango

Measures of instability	Area	Production	Productivity
India			
CV	6.955471	20.69149	18.12891
Copock Index	39.49175	45.54044	44.00805
CDVI	6.531267	5.388618	7.126648
Tamil Nadu			
CV	7.074405	27.03016	24.13877
Copock Index	39.56	47.12	46.51
CDVI	4.141947	27.97548	24.51228
Krishnagiri			
CV	5.789067	29.97589	30.74096
Coper Index	38.97107	49.57716	49.68254
CDVI	5.538605	30.32523	31.47527

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RESEARCH ARTICLE

Preparation and Evaluation of Gastro Retentive Floating Tablets of Felodipine

Jadav Subhash[1]*, P.Dwarakanadha Reddy[2] and S.V. Satyanarayana[3]

[1]Research Scholar, Department of Pharmaceutics, Jawaharlal Nehru Technology University, Ananthapur, Andhrapradesh, India
[2]Professor, Department of Pharmaceutics, Annamacharya College of Pharmacy, Rajampet, Andhrapradesh, India
[3]Principal, College of Engineering, Jawaharlal Nehru Technology University Kalkiri, Andhrapradesh, India

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***Address for Correspondence**
Jadav Subhash
Research Scholar,
Department of Pharmaceutics,
Jawaharlal Nehru Technology University,
Ananthapur, Andhrapradesh, India
Email: subhashspips@gmail.com

ABSTRACT

Felodipine (FD) has a poor bioavailability and a low obsorption window, and it is stable in acidic pH after oral administration, allowing for the development of a gastro retentive formulation. FD gastro-retentive floating tablets were made with hydrophilic polymers such as HPMC K 100M and CARBOPOL 940, as well as standard excipients including microcrystalline cellulose, sodium bicarbonate, magnesium stearate, and talc, utilizing the direct compression process. The physical characteristics of tablets, such as hardness, friability, floating time, thickness, and weight variation were assessed. The impact of polymer concentration on drug release and buoyancy was investigated. The drug-polymer compatability experiments revealed no issues with the polymers utilized in the study and the improved formulation (FDFA7) maintained drug release for 12 hours, as well as exhibiting non-fickian diffusion and adhering to the zero order, higuchi model. *In- vivo* x-ray studies of optimized formulation was shown gastric residence time of 4 hours. It showed no changes after three months of storage at 45^{0}C with 75% RH. The floating concept increased gastro retention time, which was through to be beneficial for prolonging the drug absorption window.

Keywords: Felodipine, gastric residence time, floating tablets, *In-vitro* buoyancy.

INTRODUCTION

The fact that not all drug candidates are absorbed uniformly throughout the gastrointestinal system complicates the distribution of oral sustained release drugs. Due to partial drug release and short residence time at the absorption site, GIT is often limited by poor bioavailability with conventional dosage forms. To address these concerns, gastro retentive dosage forms have been developed over the last three decades. Some of the current technical approaches intended to enhance stomach residence time include high density, swelling and expanding, polymeric mucoadhesive, ion exchange, raft forming, magnetic and floating drug delivery systems. To increase the drug's residence length and control its release behaviour, a gastro retentive sustained release system of FD was developed. FD is an anti hypertensive agent, when administered orally, FD is effectively absorbed from the gastrointestinal system .It has an elimination half-life of 6.4 hours and a low absorption window [1.2].

MATERIALS AND METHODS

Materials

FD was obtained as a gift sample, from Micro labs Bangalore, Carbopol 940,HPMC K100M, microcrystalline cellulose, sodium bicarbonate, magnesuim stearate, talc were obtained from S.D fine chemicals Mumbai, all other chemicals and solvents used were analytical grade.

Fourier transforms infrared spectroscopy (FTIR)

The infrared spectrum of the pure drug, each retardant, and the physical mixture of the optimal formulation was recorded using a BRUKER FTIR Spectrophotometer. The IR spectra of the samples were obtained using the KBr disc method, and the scanning range was 500-4000 cm^{1}.Any change in the drug's spectrum pattern attributed to the presence of polymers was studied to see if there were any chemical interactions [3].

Pre-compression parameters

The powder's angle of repose, bulk density, tapped density, compressibility index (Carr's index), and Hausner's ratio were all determined employing standardized [4].

Preparation of FD floating tablets

CARBOPOL 940, HPMC K100 M, and MCC were triturated in a mortar to obtain fine powder for FD tablets. A precisely weighed quantity of sodium bicarbonate was taken separately in a mortar and pestle, and the pulverized powder was passed through sieve# 40 and blended with the drug blend, which was then passed through sieve # 40 in a plastic bag for 5 minutes to achieve uniform mixing, then magnesium stearate was added and mixed for 5 minutes, and finally Talc was added and mixed for 2 minutes. After that, a 16- station punching machine equipped with an 8-mm flat-faced round punch was used to compress the homogenous mixture into 200-mg tablets(Cadmach, Ahmadabad, India) [5]. The drug was combined with various concentrations of polymers ratios to produce floating tablets were shown in Table 1, Table 2 .

Post compression parameters

The produced tablets were tested for physical factors such as weight variation and thickness uniformity, friability was determined using a ruche type friabilator and hardness was determined using a Monsanto hardness tester. To determine the drug concentration in each formulation, 6 tablets were triturated in a mortar and powder equivalent to average weight in 100 ml of 0.1 N HCl was added, and then shaken overnight. The solution was filtered via a 0.45 mm membrane filter, diluted appropriately, and measured at 234 nm against 0.1 N HCl using a UV/VIS double beam spectrophotometer(Elico SL 159 pvt ltd, Hyderabad) [6] .

***In-vitro* buoyancy of floating tablets in 0.1 N-HCl**

In a 100 ml glass beaker, the tablets were placed in 0.1N HCl.

1. Floating lag time: The time it takes for the tablet to rise to the surface of the medium and float is known as floating lag time.
2. Floating duration time: The floating duration time was calculated as the period of time, the tablet remained afloat on the surface of the medium.

***In- vitro* dissolution studies**

The USPXXIII dissolution test apparatus was used to determine the FD and marketed product dissolution profiles at 37±0.5^{0}c (LAB INDIA, Disso 2000).The dissolution medium was 900 ml of 0.1N HCl with a paddle stirrer spinning at 50 rpm. Samples (5ml) were extracted and filtered through a 0.45 mm pre filter at predetermined intervals. The absorbance of the filtered samples was measured at 234 nm, after they have been diluted with dissolution medium.

Drug release kinetics [7,8]

The purpose of this research is to explore into the kinetics of drug release from formulations.

Zero order kinetics

$C=K_0t$

It refers to a system in which the concentration of a drug has no effect on the rate at which it is released. The zero order release constant,K_0, t denotes the total amount of drug released during time .

First order kinetics

$\text{Log } C_t=\log C_0 \; K_1t/2.303$

It defines the drug release from the system in which the release rate is concentration dependent. Where C_t signifies the amount of drug released in time t, C_o is the initial concentration of the drug, and K_1 is the first order release constant .

Higuchi kinetics

$W=K_2t^{1/2}$

It describes of a solid drug dispersed in an insoluble matrix, where the rate of drug release is proportional to the rate of drug diffusion. W denotes the total amount of drug released over time t. K_2 is the dissolution constant .

Korsmeyer Peppas equation

$K_4t^n= M_t=M\infty$

It deviates from Fickian diffusion and describes drug release from a polymeric system, as seen in the equation. Where K_4 denotes the drug release constantan denoted the drug release exponent, and M denotes the total amount of drug dissolved over time t. In the case of matrix tablets, the drug release mechanism is Fickian diffusion, if the release exponent n= 0.45,if it is 0.45-0.89, it is non Fickian or anomalous diffusion. An exponent value of 0.89 is used to indicate case-II .

***In -vivo* (x-ray) studies**

For in-*vivo* experiments, 200 mg tablets were made with x-ray opaque ($BaSO_4$) included to make tablet opaque, and the drug and part of the MCC were replaced $BaSO_4$ [9].

***In -vivo* buoyancy by using radiographic studies**

Human volunteers were given FD floating tablets and were monitored using a radiological approach during the *in-vivo* investigation. After obtaining informed consent, three healthy male individuals (mean age 26 years, mean weight 60±10 kg) took part. The Human Ethical Committee (Reference no-IEC/2021/I/09) at St.Peters Institute of Pharmaceutical Sciences, Warangal, accepted the study, which was carried out by giving each subject one tablet. The prepared tablets were taken orally with a glass of water, during the experiment, volunteers were not allowed to eat but were permitted to drink water, and they were required to remain seated upright positions. X-ray photos were taken at various time intervals to monitor the position of the floating tablet in each patient, including 1, 2, and 4 hours [10].

Stability studies

The optimized formulation(FDFA7) was packed in silver foil for studying stability parameters at 40^0C± 2 /75 ± 5% RH, withdrawn samples at pre determined time intervals of 0(initial),and the tablet was then characterized for various physicochemical parameters such as appearance, weight variations, thickness, hardness, friability and drug content after 30,60,and 90 days [11] .

RESULTS

Fourier transforms infrared spectroscopy (FTIR)

The infrared spectrum of a physical mixture with an optimal formulation was assessed in Figures 1 and 2, and it was concluded that there is no interaction. All of the drug's and polymers prominent peaks were seen in the spectra. The IR spectra revealed distinct peaks associated with the observation. As a result, there were no obvious variations in the physical mixing of FD and Carbopol 940.

Pre compression parameters

Formulations FDFA1-FDFA14, Pre-compression factors results were suggesting that the flow properties were good and acceptable according to IP.

Post compression parameters

Table.3 shown. Post compression parameters of Felodipine with Carbopol 940 and HPMC K 100M formulations

***In-Vivo* Evaluation (X-Ray Studies)**

The activity of the floating tablet in the human stomach was observed in real time using a radiographic imaging approach (Figure 6) .The tablets were seen in the human stomach on radiographic pictures taken at 1 hr after ingestion. Significant changes were found in the following images, which were collected at 2 hr, and 4 hours.

Stability studies

Stability tests were carried out for an optimized formulation in accordance with ICH criteria. The tests lasted three months.

DISCUSSION

The IR spectra revealed distinct peaks associated with the observation. As a result, there were no obvious variations in the physical mixing of FD and carbopol 940. According to IP, The pre compression parameters values indicating the flow parameters were good and acceptable. The post compression parameters indicating good mechanical strength and all of the values were within Indian pharmacopeia specifications (IP) shown inTable.3. All of the formulations were found to be buoyant, optimized formulation buoyancy shown in Figure 3. They floated for 12 hours after being immersed in the media, with a lag time of 40 seconds to 59 seconds. The use of sodium bicarbonate dispersed in the matrix as a gas generating agent was shown to be successful in producing the requisite buoyancy

properties. Rapid floating commences a reaction with the acidic dissolution media as quickly as sodium bi carbonate is added, generating enough co_2 to be obtained and protected within the gel layer generated by hydration of carbopol 940 .As a result, the density of tablet decreases (reported as 1.004 to 1.010 g/cm^3).The tablet becomes buoyant as a consequence. The comparatively higher acid solubility of FD improves buoyancy by allowing dissolution media to penetrate the matrix more quickly. As a result of the faster start of the reaction and the rapid release of CO_2, the tablets become more buoyant. From FDFA1 to FDFA7 shown in figure 4, the release of formulations was continuously prolonged, which is due to the high amount of polymer used, increasing amount of polymer used decreases the release rate, which is due to increased hydrogel of formulation, which precludes the escape of entrapped generated gas from formulation, resuting in decreased density and increased total floating time. Because carbopol 940 remains unionized in acidic dissolution medium and acts as a physical barrier for extended release, it has higher sustained release profile. Among the various formulations, FDFA7 was chosen as the optimal formulation because of its long lasting drug release. Low amount of polymer utilized in formulations FDFA8 and FDFA9, rapid release occurred after 5-6 hours, this is due to the low amount of polymer used. From FDFA10 to FDFA14, formulations release was continuously prolonged due to the high amount of polymer used, increasing the amount of polymer used decreases the release rate. The optimized (FDFA7) formulation dissolution profile was compared to the marketed product, HPMC K100M formulations and FDFA7 drug released by 12 hours over the marketed product shown in figure 5.

Fitting dissolution data into different kinetic models including zero- order, first–order, higuchi and korsmeyer-peppas, according to the release data of formulations was used to establish the mechanism of release for the optimized formulations. The values of K and R^2 (regression analysis correlation coefficient) for zero order, first order, and higuchi models of developed formulations. The computed n values for various formulations were in the range of 0.48 to 0.69.The drug is released according to the korsemeyer-peppas and zero-order and higuchi model mechanisms in majority of the formulations, and the R^2 value is quite close to 1 compared to the R^2 values of other kinetic models. The drug release kinetics studies indicate that the drug release from FD gastro retentive floating tablets followed a zero order profile, indicating that the drug release followed a zero order profile. The high regression value of the Higuchi model ensured that drug release from matrix tablets followed a diffusion process, with the n values indicating non-fickian or anomalous diffusion. *In –vivo* x-ray scans revealed that the tablet had rotated and shifted its position. This demonstrated that the tablets did not stick to the stomach mucosa and instead floated on the gastric juice. In fact, the swelling of the tablet, as well as the white dry core and thin swelling layer around it, was plainly evident shown in figure 6. The glassy core of the floating tablets shrunk as the swelling progressed. Stability tests showed no significant changes in parameters.

CONCLUSION

Two polymers, CARBOPOL 940 and HPMC K100M were used to make FD floating tablets in variable concentrations. The optimized formulation (FDFA7) showed good *in-vitro* buoyancy lag time of 34 seconds and floated continuously for 12 hours, FTIR studies indicated that there is no interaction between drug and polymer, *in-vitro* dissolution studies of optimized formulation and marketed product was compared, and FDFA7 formulation was retarded the drug release over the marketed product and HPMC K100 M formulations. The drug release mechanism is non-fickian diffusion or anomalous diffusion, and the optimal formulation follows zero order, higuchi model and korsemeyer-peppas. Non- fickian diffusion or anomalous diffusion is the mechanism of drug release, and *in-vivo* x-ray investigations revealed a stomach residence period of 4 hours. Stability experiments found that there is negligible change in parameters.

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CONFLICT OF INTEREST

No conflict of interest are declared

ABBREVATIONS USED:

FD: Felodipine; HPMC: Hydroxy propyl methyl cellulose; GIT: Gastro intestinal tract; MCC:Micro crystalline cellulose; HCL: Hydrochloric acid;%: Percentage; hrs: Hours; ml:Milli liter; mg: Milli grams;nm: Nano meter;RH: Relative humidity;^{0}C: Degree celcius.

REFERENCES

1. Baumgartner S, Kristl J, Vrecer F, Vodopivec P, Zorko B: Optimisation of floating matrix tablets and evaluation of their gastric residence time.Int J Pharm 2000;195:125–135.
2. Uttam Kumar Mandal,,Faria Gias Senjoti, Gastro-retentive drug delivery systems and their *in-vivo* success: A recent update, Asian Journal of Pharmaceutical Sciences 2016;11 (5): 575-584.
3. D. Saritha, D. Sathish and Y. Madhusudan Rao : Formulation and Evaluation of Gastroretentive Floating Tablets of Domperidone Maleate, Journal of Applied Pharmaceutical Science 2012;02 (03) : 68-73.
4. .S.Madaka, K. Sanka, P.R. Veerareddy, Design and evaluation of hydrochlorothiazide gastro retentive floating drug delivery system, Asian Journal of Pharmaceutical Sciences 2011; 6 :208-17.
5. S. Umadevi, S. Vaghani, Floating matrix dosage form for propranolol hydrochloride based on gas formation technique: development and *in-vitro* evaluation, Sci. Pharm 2010; 78 :927-39.
6. Bomma R, Swamy Naidu RA, Yamsani MR, Veerabrahma K: Development and evaluation of gastroretentive norfloxacin floating tablets,Acta Pharm.2009;59:211–221
7. Higuchi T: Mechanism of sustained action medication, theoretical analysis of rate of release of solid drugs dispersed in solid matrices. J Pharm Sci1963; 52:1145-49.
8. Korsmeyer RW, Gumy R, Doelker E, Buri P, Peppas NA: Mechanism of solute release from porous hydrophilic polymer,Int J Pharm1983;15:25-35.
9. Shehzad MQ, Nazir T, Nazir SU, Taha N, Jamil T, Akram MA, Formulation, evaluation and *in-vitro* characterization of gastro retentive floating tablet of diclofenac sodium,Pak J Pharm Sci 2019; 32(6): 2573-2581.
10. Bomma R, Veerabrahma K, Development of gastroretentive drug delivery system forcefuroxime axetil: in vitro and *in-vivo* evaluation in human volunteers,Pharm Dev Technol2013; 5 :1230-7.
11. Harshal Ashok Pawa, Rachana Dhavale: Development and evaluation of gastro retentive floating tablets of an antidepressant drug by thermoplastic granulation technique. beni - suef university journal of basic and applied sciences 2014;3 :122-132.

Table. 1. Formulation of Floating tablets of FD with Carbopol 940

Ingredients	Formulations Codes						
	FDFA1	FDFA2	FDFA3	FDFA4	FDFA5	FDFA6	FDFA7
Felodipine	10	10	10	10	10	10	10
Carbopol 940	20	30	60	90	100	110	120
MCC	137	127	97	67	57	47	37

$NaHCO_3$	30	30	30	30	30	30	30
Talc	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Mg.Stearate	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Total weight	200	200	200	200	200	200	200

All weights in mgs

Table .2. Formulation of Floating tablets of FD with HPMC K100M

Ingredients	Formulations Codes						
	FDFA8	FDFA9	FDFA10	FDFA11	FDFA12	FDFA13	FDFA14
Felodipine	10	10	10	10	10	10	10
HPMC K100M	20	30	60	90	100	110	120
MCC	137	127	97	67	57	47	37
$NaHCO_3$	30	30	30	30	30	30	30
Talc	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Mg.Stearate	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Total weight	200mg	200mg	200mg	200mg	200mg	200mg	200mg

All weights in mgs

Table.3.Post Compression parameters of Felodipine with Carbopol 940 and HPMC K 100M formulations

Formulation code	Hardness (kg/cm²)	Friability (%loss)	Thickness (mm)	Drug content (%)	Total Floating Time (hr)	Floating Lag Time (sec)	Weight Variation (%)
FDFA1	3.4 ±0.07	0.26±0.02	3.06±0.03	98.2±0.02	6 .5	42	200±0.01
FDFA2	3.7±0.03	0.28 ±0.01	3.10±0.01	97.8±0.04	7	40	199±0.02
FDFA3	3.8±0.06	0.31 ±0.03	3.04±0.02	98.7±0.02	7	41	199±0.04
FDFA4	4.1±0.06	0.44±0.02	3.14±0.03	98.8±0.01	7.5	38	199±0.03
FDFA5	4.2±0.07	0.53±0.01	3.16±0.01	97.8±0.02	9	39	200±0.05
FDFA6	3.7±0.09	0.39±0.01	3.31±0.02	99.5±0.03	10	37	199±0.08
FDFA7	3.8±0.06	0.28±0.02	3.97±0.01	98.2±0.04	12	34	200±0.07
FDFA8	3.8 ±0.02	0.29±0.04	3.06±0.08	98.7±0.06	5 .5	51	199±0.05
FDFA9	3.6±0.04	0.30±0.07	3.10±0.02	98.2±0.02	6	52	201±0.02
FDFA10	3.9±0.09	0.33±0.08	3.04±0.05	97.8±0.07	7	50	200±0.08
FDFA11	4.0±0.09	0.44±0.03	3.14±0.06	98.5±0.06	7	48	199±0.04
FDFA12	4.1±0.01	0.49±0.03	3.16±0.06	97.9±0.07	9	46	200±0.07
FDFA13	4.0±0.07	0.37±0.04	3.31±0.05	99.4±0.01	10 .5	42	200±0.02
FDFA14	4.1±0.06	0.31±0.03	3.97±0.06	98.8±0.03	11	39	200±0.05

Mean±SD,(n=6)

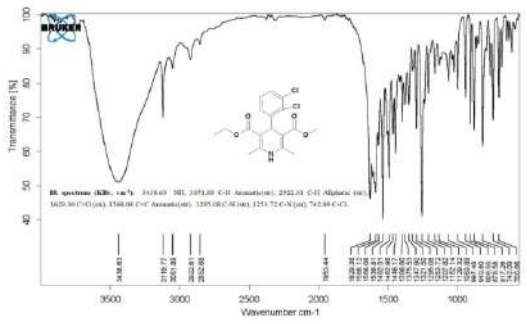


Figure. 1. FTIR spectra of FD pure drug

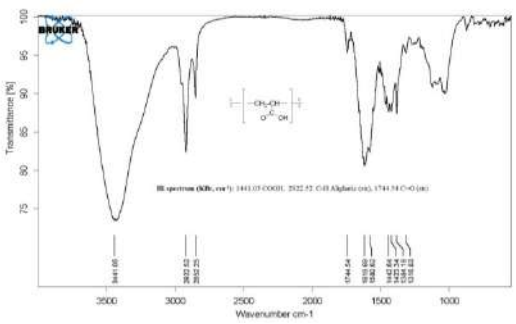
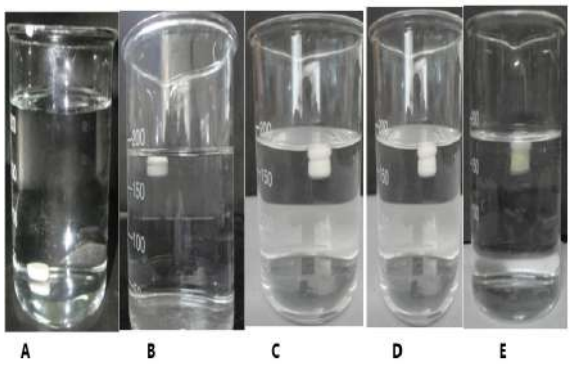
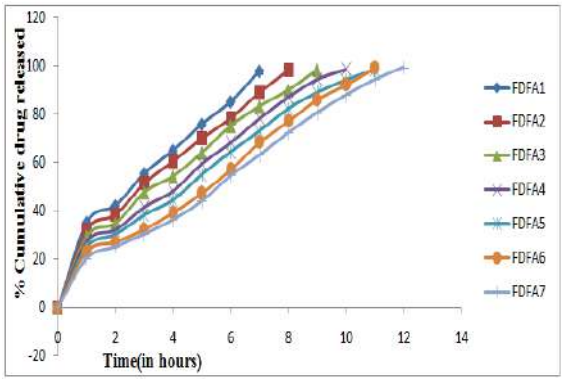


Figure. 2. FTIR spectra of optimized formulation (FDFA7)



***In-vitro* buoyancy of floating tablets in 0.1 N -HCl**

Figure. 3. *In-vitro* buoyancy of floating tablets of FD optimized formulation in 0.1 N -HCl



***In-vitro* dissolution studies**

Figure. 4. *In-vitro* dissolution profile of FDFA1-FDFA7 formulations

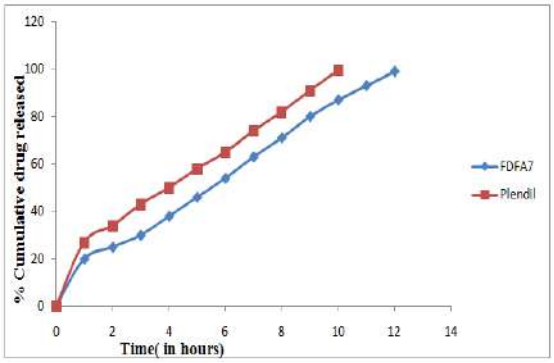


Figure. 5. *In-vitro* dissolution profile of FDFA7 formulation and marketed product.

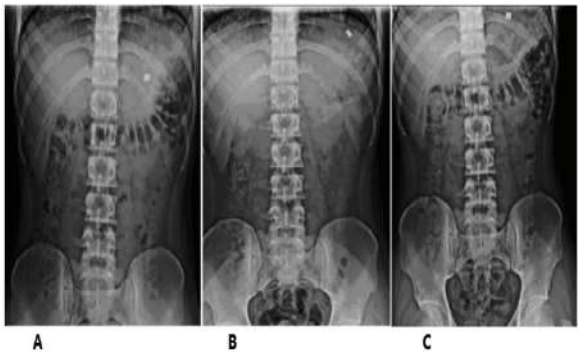


Figure.6 .*In-Vivo* Evaluation of FD optimized formulation (X-Ray Studies, A) After 1 hour, B) After 2 hours, C) After 4 hours.

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RESEARCH ARTICLE

Role of Osmolytes in Alleviation of NaCl Stress in *Arachis hypogaea* L. by Exogenous Application of Brassinolide and Paclobutrazol

Aryendu[1], Reyaz Ahmad Mir[1], M.Kathiravan[1] and R. Somasundaram[2]*

[1]Ph.D., Research Scholar, Stress Physiology Lab, Department of Botany, Annamalai University, Annamalai Nagar, Chidambaram, Cuddalore, Tamil Nadu, India.
[2]Professor, Stress Physiology Lab, Department of Botany, Annamalai University, Annamalai Nagar, Chidambaram, Cuddalore, Tamil Nadu, India.

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***Address for Correspondence**
R. Somasundaram
Professor, Stress Physiology Lab,
Department of Botany, Annamalai University,
Annamalai Nagar, Chidambaram,
Cuddalore, Tamil Nadu, India.
Email: botanysundaram@gmail.com

ABSTRACT

Due to the increasing use of water of low quality for irrigation and soil salinization, salinity is a significant abiotic stress that restricts plant development and productivity in many parts of the world. Peanut (*Arachis hypogaea* L.) is one of the most important legume crops grown worldwide as a source of edible oil and vegetable protein. Arid and semi-arid regions constitute more than half of the peanut production area and 15% of these regions are salt-affected. In this following study the effects of NaCl, NaCl+Brassinolide, NaCl+ Paclobutrazol, Brassinolide (BL) and Paclobutrazol (PBZ) treatments were investigated in osmoprotectant contents, the plants of BL and PBZ demonstrated recovery in salt. Osmolytes such proline and glycine betaine exhibit impressive recovery by BL under salinity in this study.

Keywords: Salt stress, Brassinolide (BL), Paclobutrazol (PBZ), Osmolytes

INTRODUCTION

Plants are continuously under exposureto many kinds of abiotic and biotic stresses starting from vegetative to reproductive stages [1]; [2]. Among abiotic stresses, soil salinity occurs to climate changes has become a major agricultural problem in the modern world [3]. Soil salinity is one of the major factors of soil degradation and is recorded in 19.5% of the irrigated land and 2.1% of the dry land agriculture existing on the globe [4]. Salinity is conspicuousin arid and semi-arid areas where 25% of the irrigated land is affected by salt [5]. Abiotic stresses on plants have been rising frequently and intensely due to rapid global climate change. Plant cells usually permit the

influx, sequestering, and synthesis of various solutes and accumulate them for maintaining homeostasis status and keeping the cell turgid for the growth and development of plants during abiotic stress conditions [6];[7]. In plants, osmotic adjustment mediated by the production of osmolytes has been found to protect the cellular machinery from stresses which could confer abiotic stress tolerance [8]; [9]. The term osmolytes refers to various low molecular weight compounds or metabolites namely, sugars, polyamines, secondary metabolites, amino acids, and polyols [10]. These molecules are also known as cytoprotectants due to their ability to protect cell contents against abiotic stresses [11], [12]. To cope with different stresses and protect themselves, plants have evolved complex and well–organized mechanisms[13].Biosynthesis and accumulation of various osmolytes are considered one of the paramount responses of host plants for combating oxidative as well as osmotic stress caused by various stressors. The increasing uncertainty of climate change definitely intensifies various abiotic stresses, in turn affecting considerable damage to the agricultural world. Consequently, precise environmental adaptation approaches to target plants were developed to address the problems [14]. The overproduction of osmolytes is the result of various stress signaling pathways mitogen-activated protein (MAP) kinase, phytohormones, and calcium-signaling pathways).Several osmolytes and their biosynthesis have been studied indetail, such as proline, glycine betaine (GB), and mannitol [15]. Therefore, controlling cellular osmotic balance and ionhomeostasis, i.e., intracellular transportation of water, transportation of toxic ions inside the vacuole, synthesizing osmolytes in the cytoplasm, heat shock proteins, and activation of the enzymatic as well as non–enzymatic antioxidant systems are the most important conserved mechanism that confers stress tolerance in plants.Peanut (*Arachis hypogaea* L.) is an important leguminous crop cultivated in arid and semi-arid regions [16] and also a major oilseed crop in India. However, the crop is susceptible to salinity, and productivity is severely affected [17]. The application of BL and PBZ to peanut (*Arachis hypogaea* L.) in this study results in reduced salinity stress and improved plant growth and yield.

MATERIAL AND METHODS

Plant material and Experimental Design

The Tamil Nadu Agricultural University in India provided the peanut (*Arachis hypogaea* L.) seeds (VRI-2 variety). The current investigation was carried out at the Botanical Garden, Annamalai University's Department of Botany, in Tamil Nadu. The experimental area's geographic coordinates were 11°23'23.1"N&79°43'05.3"E. Healthy Seeds were thoroughly washed in sterile double-distilled water after being surface sterilized for two minutes with a 0.2 percent mercuric chloride ($HgCl_2$) solution (ddH_2O). Six groups of 60 pots each included a VRI-2 cultivar. Ten replicates of each group received fertilizer that had been combined once with manure. Red dirt made up the terrain: Sand: Farmyard manure in a 1:1:1 ratio.Plants were exposed to Control, 100mM NaCl, 100mM NaCl + BL (3µM/L), 100mM NaCl + PBZ (20mg/L), 100mM NaCl + BL (3µM/L), and 100mM NaCl + PBZ (20mg/L). The soil samples from each pot were periodically examined using an electrical conductivity meter in order to maintain a specific salt level. At 30, 40, and 50 days following planting, plants were taken for osmolytes content estimation.

Determination of Free Proline Content

The Bates et al. method was used to extract and assess the proline content in the fresh plant materials [18]. Liquid nitrogen was used to grind fresh leaf materials weighing 50 mg in a mortar. The homogenate powder was combined with 1 mL of 3 percent w/v aqueous sulfosalicylic acid before being filtered using (Whatman #1) filter paper. The extracted solution was combined with an equivalent amount of Glacial acetic acid and the ninhydrin reagent (1.25 mg Ninhydrin in 30 mL of Glacial acetic acid and 20 mL of 6 M H_3PO_4), and the reaction was then conducted at 95°C for one hour. Placing the reaction in an ice bath caused it to stop. 2 mL of toluene was aggressively incorporated into the reaction mixture. The chromophore was detected at 520 nm after warming to 25 °C. Using L-proline as a standard.

Determination of Glycine Betaine

Plant material was frozen in liquid nitrogen immediately after harvesting, and grinded and the pestle was pre-chilled in liquid nitrogen. The frozen samples were placed in the mortar and pulverized to a fine powder. The

powder was transferred / weighted to several pre-cooled 1.5 mL tubes (eppis) and stored at –80 °C. The samples (40-50 mg FW) were suspended in 1 ml of ddH_2O water, subjected to a freeze-thaw cycle by freezing in liquid nitrogen and thawing at 40°C for20 min and left overnight at 4°C. Samples were then centrifuged at 14000 g, 4°C for 5 minutes. The clear supernatants were separated from the pellets. The eluted GB (retention time 4-5 min) was detected by measuring the absorbance at 200 nm using a diode-array spectrophotometer and quantified by a comparison of peak surface areas with those obtained with pure GB standard solutions in the range 0.05-4 mM [19]. GB content was calculated as follows:

GB content = Abs peak area extract ÷ slope × Vol. extract÷ Vol.aliquote × Conc. Factor

Data Analysis

Data obtained were expressed as the mean of three replicates and data were subjected to one-way analysis of variance (ANOVA) test. Differences between means were determined by least significance difference at $P< 0.05$, using SPSS Statistical Package version 20.

RESULT AND DISCUSSION

Proline

To protect plant cells during osmotic stress situations, proline serves as a compatible solute [20],and being a molecular chaperone, proline also acts as an antioxidant as well as ROS scavenger [21].

Leaf

Sodium chloride caused increased Proline content in plant leaves of salt-stressed *A. hypogaea* on sampling days and it was recorded at 255.5, 226.08, and 231.4 percent over control on 30, 40, and 50 DAS respectively. However, individual exogenous application of BL and PBZ to salt-stressed plants have lower the proline content in leaves when compared to the NaCl stressed plant, but higher than control and it was 211.4 and 217.1 percent over control respectively on 50 DAS. Further, the application of BL and PBZ caused an increase in proline content in leaves in unstressed plants compared to control and it was 137.1 and 162.8 percent over control on 50 DAS [Fig. 1]. Proline accumulation in salt-stressed plants is a primary defense response to maintain the osmotic pressure in a cell, which is reported in salt-tolerant and salt-sensitive cultivars of many crops[22]. Leaf proline content increased with increasing salinityin *Portulaca oleracea* L. [23], *Spinacia oleracea* L. [24], *Vigna unguiculata* L. [25] and *Durantaerecta* L[26].Exogenous application of BL alleviates Salt Stress by higher proline accumulation in *Malus hupehensis* Rehd [27] and *Brassica junce*aL. [28]. The triazole compounds like PBZ inhibit GA production and raise ABA and cytokinin levels, which ultimately aid in maintaining better osmolytes in the plants[29]. As a result, the results showed that PBZ causes the accumulation of proline content higher in leaves similar to the *Mangifera indica* L.[30] and *Sorghum bicolor* [L.] Moench [31].

Stem

Proline content in plant stems of salt-stressed *A. hypogaea* on sampling days and it was recorded at 316.6, 244.44, and 184.61 percent over control on 30, 40, and 5o DAS respectively due to Sodium chloride stress. However, individual exogenous application of BL and PBZ to salt-stressed plants have lower the proline content in the stem when compared to the NaCl stressed plant, but higher than control and it was 138.46 and 161.53percentover control respectively on 50 DAS. Further, the application of BL and PBZ caused an increase in proline content in stem in unstressed plants compared to control and it was 107.69 and 123.07percent over control on 50 DAS [Fig. 2]. Proline is an amino acid that builds up as a result of many types of abiotic stress [32]. Salinity stress increases in stem proline content in *Satureja rechingeri* Jamzad [33], *Cucumis sativus* L. [34],and *Aspergillus oryzae* [35]. Salt Stress is lessened by exogenous BL application because there is more proline formationin *Xanthoceras sorbifolium* Bunge [36] and *Zea mays*L. [37].Plants treated with NaCl and PBZ might cause a transient rise in ABA content, which could be a reason

for increasing proline [38]. The findings indicated that PBZ causes a buildup of increased proline content in leaves similar to *Myrica rubra* Siebold & Zucc. [39] and *Citrus karna* Raf. [40].

Root

Proline concentration was detected at 192.84, 136.11 and 123.40 percent higher than control in salt-stressed *A. hypogaea* plant roots on sampling days, respectively on 30, 40, and 50 DAS. However, individual exogenous application of BL and PBZ to salt-stressed plants have lower the proline content in root when compared to the NaCl stressed plant, but higher than control and it was 117.02 and 106.38percent over control respectively on 50 DAS. Further, the application of BL and PBZ caused an increase in proline content in roots in unstressed plants compared to control and it was 102.12 and 104.68percent over control on 50 DAS [Fig. 3]. Proline accumulation in stems under salt stress is involved in protective mechanisms such as restoration of cell volume and turgor, protection and stabilization of enzymes and membrane structures, and regulation of cellular acidity and ROS scavenging [41].Root proline content increased with increasing salinity in *Cucumis sativus* L. [42], *Triticum aestivum* L. [43], *Oryza australiensis* Domin [44], and *Glycine max* L. [45].In the present study, plants treated with NaCl gave plants with enhanced proline contents as compared to the control. Maximum accumulation of proline in plant roots due to BL under stress may be associated with an enhancement inproline formation due to the hydrolysis of proteins [46]. Exogenous BL application reduces salt stress because more proline is formed in *Solanum tuberosum*L. [47] and *Vigna radiata* L. [48]. Proline is the key osmolyte contributing toward osmotic adjustment and an increased level of proline with PBZ might have helped in better osmotic adjustment by stabilizing membrane and enzymes during stress conditions [49]. Identical outcomes of substantial proline increase were also seen in *Chenopodium quinoa* Willd. [50] and *Citrus aurantium*L. [51].

Glycine betaine

Glycine betaine, an important osmolyte, has widely been accumulated in plants and other microorganisms [52]. Physiological studies have demonstrated that the increased level of accumulation of GB is linked with the degree of to lerance [53].

Leaf

Sodium chloride caused increased Glycine betaine in plant leaves of salt-stressed *A.hypogaea* on sampling days and it was recorded at 350, 261.84 and 227.34 percent over control on 30, 40, and 50 DAS respectively. However, individual exogenous application of BL and PBZ to salt-stressed plants have lower the proline content in leaves when compared to the NaCl stressed plant, but higher than control and it was 192.57 and 217.18 percent over control respectively on 50 DAS. Additionally, the treatment of BL and PBZ increased the proline content in leaves of unstressed plants when compared to control, and on 50 DAS, it was 180.03 and 181.25% over control, respectively [Fig. 4]. In general, plants produce organic substances like proline and soluble sugars to control their cell osmotic potential when under stress [54]. Many plant species employ the accumulation of soluble organic molecules and low-mass organic solutes, including osmoregulators, to lessen the deleterious consequences of water deficiency and salt stress [55]. Under salt stress, over producing osmolytes like GB and proline protected thylakoid membranes and improved the stability of several cytoplasmic and mitochondrial enzymes [56]. Glycine betaine is a major organic osmolyte that accumulates in a variety of plant species in response to environmental stress like salinity [57], [58]. Leaf Glycine betaine content increased with increasing salinity in *Gossypium hirsutum* L. [59], *Vigna radiata* L. [60], *Tritcumae sativum* L. [61] and *Oryza sativa* L.[62]. Exogenous application of BL alleviates Salt Stress by higher glycine betaine accumulation in *Glycine max* (L.) Merr.[63] and *Solanum lycopersicum* L.[64]. The findings also indicated that PBZ causes the buildup of increased glycine betaine content in leaves similar to *Pisum sativum* L.[65] and *Phyllanthus amarus* Schumach. &Thonn [66].

Stem

Proline content in plant stems of salt-stressed *A. hypogaea* on sampling days and it was recorded at 152.04, 173.96, and 174.43 percent over control on 30, 40, and 5o DAS respectively due to Sodium chloride stress. However, individual exogenous application of BL and PBZ to salt-stressed plants has lower the proline content in the stem when

compared to the NaCl stressed plant, but higher than control and it was 132.73 and 158.74 percent over control respectively on 50 DAS. Further, the application of BL and PBZ caused an increase in proline content in stem in unstressed plants compared to control and it was 110.31 and 128.69 percent over control on 50 DAS [Fig. 5]. In response to environmental challenges like salt, a number of plant species synthesize glycine betaine, an organic osmolyte [67]. Salinity stress increases in stem Glycine betaine content in *Gossypium hirsutum* L. [68], *Vigna unguiculata* L. Walp. [69], *Spinacia oleracea* L.[70] and *Sorghum bicolor* L.[71]. Salt Stress is lessened by exogenous BL application because there is more glycine betaine formation in *Oryza sativa* L. [72] and *Zea mays* L. [73]. The findings indicated that PBZ causes buildup of increased glycine betaine content in stems similar to *Sorghum bicolor* [L.] Moench [74].

Root

Proline concentration was detected at 229.36, 211.82 and 174.27 percent higher than control in salt-stressed *A.hypogaea* plant roots on sampling days, respectively on 30, 40, and 50 DAS. The proline content in the roots of salt-stressed plants was lower when compared to NaCl-stressed plants, but greater than control, and it was 134.78 and 115.21 percent higher than control, respectively, on 50 DAS. Further, the application of BL and PBZ caused an increase in proline content in roots in unstressed plants in compared to control and it was 105.07 and 107.60 percent over control on 50 DAS [Fig. 3]. Compatible solutes, such as proline, and glycine betaine are known to accumulate in response to environmental challenges and to play a role in the process of osmotic adjustment in many crops [75]. Salinity stress increases in root Glycine betaine content in *Gossypium hirsutum* L. [76], *Zea mays*L. [77], *Phaseolus vulgaris* L. [78], and *Solanum lycopersicum* L.[79].Salt Stress is lessened by exogenous BL application because there is more glycine betaine formation in*Allium cepa*L. [80]. The findings indicated that PBZ causes a buildup of increased glycine betaine content in roots similar to *Sesamum indicum* L.[81].

CONCLUSION

Peanut is a major leguminous crop grown in arid and semi-arid locations where adverse circumstances like high salinity predominate and have a detrimental impact on productivity. Osmoprotectants like proline and glycine betaine are affected by salinity. Overall, the plants respond well to the exogenous application of BL and PBZ. Plants exposed to BL and PBZ under saline conditions showed substantial improvements in contents of proline and glycine betaine when under saline stress. However, the molecular mechanism involved in the function of stress protection remains to be explored.

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REFERENCES

1. Sahitya, U.L., Krishna, M.S.R., Deepthi, R. et al. (2018). Seed antioxidants interplay with drought stress tolerance indices in chilli (*Capsicum annuum* L) seedlings. *BioMed Research International* 2018, 1605096: 1–14.
2. Paul, S. and Roychoudhury, A. (2019). Transcript analysis of abscisic acid-inducible genes in response to different abiotic disturbances in two indica rice varieties. *Theoretical and Experimental Plant Physiology* 31 (1): 249–272.
3. Mir, R. A., & Somasundaram, R. (2021). Salicylic Acid and Salt Stress Tolerance in Plants: A Review. *Journal of Stress Physiology & Biochemistry*, *17*(3), 32-50.
4. Prăvălie, R., Patriche, C., Borrelli, P., Panagos, P., Roșca, B., Dumitraşcu, M., ... &Bandoc, G. (2021). Arable lands under the pressure of multiple land degradation processes. A global perspective. *Environmental Research*, *194*, 110697.
5. Dadkhah, A., &Rassam, G. (2015). Effect of salt stress on growth and essential oil of Matricaria chamomilla. *Z ArzneiGewurzpfla*, *20*, 131-133.

6. Sharma SS, Dietz K-J. The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. J Exp Bot. 2006;57(4):711–726. doi:10.1093/jxb/erj073.
7. Burg MB, Ferraris JD. Intracellular organic osmolytes: function and regulation. J Biol Chem. 2008;283(12):7309–7313.doi:10.1074/jbc.R700042200.
8. Chen H, Jiang J-G. Osmotic adjustment and plant adaptation to environmental changes related to drought and salinity. Environl Rev. 2010;18(NA):309–319. doi:10.1139/a10-014.
9. Liang X, Zhang L, Natarajan SK, Becker DF. Proline mechanisms of stress survival. Antioxid Redox Signal. 2013;19(9):998–1011. doi:10.1089/ars.2012.5074.
10. Hussain TM, Hazara M, Sultan Z, Saleh BK, Gopal GR. Recent advances in salt stress biology a review. Biotechnol Mol Biol Rev. 2008;3:8–13.
11. Khan SH, Ahmad N, Ahmad F, Kumar R. Naturally occurring organic osmolytes: from cell physiology to disease prevention. IUBMB Life. 2010;62(12):891–895. doi:10.1002/iub.406.
12. Groppa M, Benavides M. Polyamines and abiotic stress: recent advances. Amino Acids. 2008;34(1):35. doi:10.1007/s00726-007-0501-8.
13. Munns R, Tester M. Mechanisms of Salinity Tolerance. Annu Rev Plant Biol. 2008;59(1):651–681. doi: 10.1146/annurev.arplant.59.032607.092911.
14. Wu X, Jia Q, Ji S, Gong B, Li J, Lu G, Gao H. Gamma-aminobutyric acid (GABA) alleviates salt damage in tomato by modulating Na+ uptake, the GAD gene, amino acid synthesis and reactive oxygen species metabolism. BMC Plant Biol. 2020;20(1):1–21. doi:10.1186/s12870-020-02669-w.
15. Yang Q, Chen -Z-Z, Zhou X-F, Yin H-B, Li X, Xin X-F, Hong X-H, Zhu J-K, Gong Z. Overexpression of SOS (Salt Overly Sensitive) genes increases salt tolerance in transgenic Arabidopsis. Mol Plant. 2009;2(1):22–31. doi:10.1093/mp/ssn058.
16. Kumari A, Kumar A, Wany A, Prajapati GK, Pandey DM (2012). Identification and annotation of abiotic stress responsive candidate genes in peanut ESTs. Bioinformation 8: 1211-1219.
17. Meena, Hari &Bhalodia, PK &Jat, R.s &Vekaria, Lalit. (2012). Prospects of using saline water for irrigation in groundnut (Arachis hypogea)-Pearl millet (Pennisetum glaucum) cropping system in saline black soil of Saurashtra. Indian Journal of Agronomy. 57. 122-126.
18. Bates, L. S., Waldren, R. P., &Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. *Plant and soil*, *39*(1), 205-207.
19. P. Carillo et al., Nitrogen metabolism in durum wheat under salinity: accumulation of proline and glycine betaine, *Functional Plant Biology. 35 (2008)* 412–426.
20. KAVI KISHOR, P. B., &Sreenivasulu, N. E. S. E. (2014). Is proline accumulation per se correlated with stress tolerance or is proline homeostasis a more critical issue?. *Plant, cell & environment*, *37*(2), 300-311.
21. Matysik J, Alia BB, Mohanty P. Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. Curr Sci. 2002;525–532.
22. Hayat S, Hayat Q, Alyemeni MN, Wani AS, Pichtel J, Ahmad A. Role of proline under changing environments: a review. Plant Signal Behav. 2012 Nov;7(11):1456-66. doi: 10.4161/psb.21949. Epub 2012 Sep 5. PMID: 22951402; PMCID: PMC3548871.
23. Kafi, M., & Rahimi, Z. (2011). Effect of salinity and silicon on root characteristics, growth, water status, proline content and ion accumulation of purslane (Portulaca oleracea L.). *Soil Science and Plant Nutrition*, *57*(2), 341-347.
24. Yavuz, D., Kılıç, E., Seymen, M., Dal, Y., Kayak, N., Kal, Ü., & Yavuz, N. (2022). The effect of irrigation water salinity on the morph-physiological and biochemical properties of spinach under deficit irrigation conditions. *Scientia Horticulturae*, *304*, 111272.
25. Abiala, M. A., & Sahoo, L. Bacillus aryabhattai enhanced proline content, stabilized membrane and improved growth of cowpea under NaCl-induced salinity stress. *Journal of Applied Microbiology*.
26. Mostafa, G. G., Hamoud, M., El-Banna, S., Hassan, M., & Mohamed, Y. (2022). Response of Durantaerecta L. Var. Variegata plants to the proline and ascorbic acid under irrigation by seawater. *Journal of Desert and Environmental Agriculture*, *2*(1), 28-41.

27. Su Q, Zheng X, Tian Y, Wang C. Exogenous Brassinolide Alleviates Salt Stress in Malus hupehensisRehd. by Regulating the Transcription of NHX-Type Na+(K+)/H+ Antiporters. Front Plant Sci. 2020 Feb 6;11:38. doi: 10.3389/fpls.2020.00038. PMID: 32117377; PMCID: PMC7016215.
28. Naveen, Naveen & Kumari, Nisha & Avtar, Ram &Jattan, Minakshi& Ahlawat, Sushil & Rani, Babita& Malik, Kamla& Sharma, Anubhuti& Singh, Manjeet. (2021). Evaluation of Effect of Brassinolide in Brassica juncea Leaves under Drought Stress in Field Conditions. Horticulturae. 7. 514. 10.3390/horticulturae7110514.
29. Fletcher, R. A., Gilley, A., Sankhla, N., & Davis, T. D. (2000). Triazoles as plant growth regulators and stress protectants. Horticultural reviews, 24, 55-138.
30. Srivastav, M., Kishor, A., Dahuja, A., & Sharma, R. R. (2010). Effect of paclobutrazol and salinity on ion leakage, proline content and activities of antioxidant enzymes in mango (Mangifera indica L.). Scientia Horticulturae, 125(4), 785-788.
31. Forghani, A. H., Almodares, A., &Ehsanpour, A. A. (2020). The role of gibberellic acid and paclobutrazol on oxidative stress responses induced by in vitro salt stress in sweet sorghum. Russian journal of plant physiology, 67(3), 555-563.
32. Verbruggen N, Hermans C (2008) Proline accumulation in plants: a review. Amino Acids 35:753–759
33. Tabrizi, L., Alemardan, A., &Shokrpour, M. (2022). Quantification of changes in morphophysiological and biochemical criteria of SaturejarechingeriJamzad under salinity stress using nonlinear models. *Journal of Plant Production Research*, *29*(1), 39-59.
34. Zhou, Y., Sang, T., Tian, M., Jahan, M. S., Wang, J., Li, X., ... & Shu, S. (2022). Effects of Bacillus cereus on Photosynthesis and Antioxidant Metabolism of Cucumber Seedlings under Salt Stress. *Horticulturae, 8*(5), 463.
35. Jan, F. G., Hamayun, M., Moon, Y. S., Jan, G., Shafique, M., & Ali, S. (2022). Endophytic aspergillus oryzae reprograms Abelmoschus esculentus L. to higher growth under salt stress via regulation of physiochemical attributes and antioxidant system. *Biologia*, 1-14.
36. Li, K. R., & Feng, C. H. (2011). Effects of brassinolide on drought resistance of Xanthocerassorbifolia seedlings under water stress. Acta Physiologiae Plantarum, 33(4), 1293-1300.
37. El-Khallal, S. M., Hathout, T. A., Ashour, A. A., &Kerrit, A. A. (2009). Brassinolide and salicylic acid induced growth, biochemical activities and productivity of maize plants grown under salt stress. Res J Agric Biol Sci, 5(4), 380-390.
38. Gopi, R., Jaleel, C. A., Sairam, R., Lakshmanan, G. M. A., Gomathinayagam, M., &Panneerselvam, R. (2007). Differential effects of hexaconazole and paclobutrazol on biomass, electrolyte leakage, lipid peroxidation and antioxidant potential of Daucus carota L. Colloids and Surfaces B: Biointerfaces, 60(2), 180-186.
39. Hu, Y., Yu, W., Liu, T., Shafi, M., Song, L., Du, X., ... & Wu, J. (2017). Effects of paclobutrazol on cultivars of Chinese bayberry (Myrica rubra) under salinity stress. Photosynthetica, 55(3), 443-453.
40. Sharma, D. K., Dubey, A. K., Srivastav, M., Singh, A. K., Sairam, R. K., Pandey, R. N., ... & Kaur, C. (2011). Effect of putrescine and paclobutrazol on growth, physiochemical parameters, and nutrient acquisition of salt-sensitive citrus rootstock Karna khatta (Citrus karna Raf.) under NaCl stress. Journal of Plant Growth Regulation, 30(3), 301-311.
41. Bandeh-Hagh, A., Toorchi, M., Mohammadi, A., Chaparzadeh, N., Salekdeh, G. H., &Kazemnia, H. (2008). Growth and osmotic adjustment of canola genotypes in response to salinity. Journal of Food Agriculture and Environment, 6(2), 201.
42. Zhou, Y., Sang, T., Tian, M., Jahan, M. S., Wang, J., Li, X., ... & Shu, S. (2022). Effects of Bacillus cereus on Photosynthesis and Antioxidant Metabolism of Cucumber Seedlings under Salt Stress.*Horticulturae, 8*(5), 463.
43. Singh, R. P., Pandey, D. M., Jha, P. N., & Ma, Y. (2022). ACC deaminase producing rhizobacterium Enterobacter cloacae ZNP-4 enhance abiotic stress tolerance in wheat plant. *PloS one*, *17*(5), e0267127.
44. Nguyen, H. T. T., Bhowmik, S. D., Long, H., Cheng, Y., Mundree, S., & Hoang, L. T. M. (2021). Rapid Accumulation of Proline Enhances Salinity Tolerance in Australian Wild Rice Oryza australiensisDomin. *Plants*, *10*(10), 2044.
45. Afrin, S., Akhtar, N., Khanam, T., & Hossain, F. (2021). Alleviative effects of zinc on biomass yield and antioxidative enzymes activity in leaves of soybean (Glycine max L.) under salt stress. *Am. J. Agric. For*, *9*, 147-155.

46. Viegas RA, Silveira JAG (1999) Ammonia assimilation and proline accumulation in young cashew plants during longterm exposure to NaCl-salinity. Braz J Plant Physiol 11:153–159
47. Hu, Y., Xia, S., Su, Y., Wang, H., Luo, W., Su, S., & Xiao, L. (2016). Brassinolide increases potato root growth in vitro in a dose-dependent way and alleviates salinity stress. BioMed Research International, 2016.
48. Li, K. R., Wang, H. H., Han, G., Wang, Q. J., & Fan, J. (2008). Effects of brassinolide on the survival, growth and drought resistance of Robiniapseudoacacia seedlings under water-stress. New Forests, 35(3), 255-266.
49. Yoshiba, Y., Kiyosue, T., Nakashima, K., Yamaguchi-Shinozaki, K., & Shinozaki, K. (1997). Regulation of levels of proline as an osmolyte in plants under water stress. Plant and cell physiology, 38(10), 1095-1102.
50. Waqas, M., Yaning, C., Iqbal, H., Shareef, M., Rehman, H., & Yang, Y. (2017). Paclobutrazol improves salt tolerance in quinoa: Beyond the stomatal and biochemical interventions. Journal of Agronomy and Crop Science, 203(4), 315-322.
51. Sharma, D. K., Dubey, A. K., Srivastav, M., Singh, A. K., Pandey, R. N., &Dahuja, A. (2013). Effect of paclobutrazol and putrescine on antioxidant enzymes activity and nutrients content in salt tolerant citrus rootstock sour orange under sodium chloride stress. Journal of plant nutrition, 36(11), 1765-1779.
52. Chen THH, Murata N. Glycinebetaine: an effective protectant against abiotic stress in plants. Trends Plant Sci. 2008;13 (9):499–505. doi:10.1016/j.tplants.2008.06.007.
53. Saneoka H, Nagasaka C, Hahn DT, Yang WJ, Premachandra GS, Joly RJ, Rhodes D. Salt Tolerance of Glycinebetaine-Deficient and - Containing Maize Lines. Plant Physiol. 1995;107(2):631–638. doi:10.1104/pp.107.2.631.
54. Ashraf, M., & McNeilly, T. (2004). Salinity tolerance in Brassica oilseeds. Critical reviews in plant Sciences, 23(2), 157-174.
55. Chaves M.M., Maroco J.P., Pereira J.S.: Understanding plant 760 responses to drought – From genes to the whole plant. – Funct. Plant Biol. 30: 239-264, 2003.
56. Genard H., Le Saos J., Hillard J. et al.: Effect of salinity on lipid composition, glycine betaine content and photosynthetic activity in chloroplasts of Suaeda maritima. – Plant Physiol. Bioch. 29: 421-427, 1991.
57. Ashraf M, Foolad MR (2007) Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ Exp Bot 59:206–216
58. Sakr M.T., El-Sarkassy N.M., Fuller M.P.: Osmoregulators proline and glycine betaine counteract salinity stress in canola. – Agron. Sustain. Dev. 32: 747-754, 2012.
59. Hamani, A.K.M., Li, S., Chen, J., Amin, A.S., Wang, G., Xiaojun, S., Zain, M. and Gao, Y., 2021. Linking exogenous foliar application of glycine betaine and stomatal characteristics with salinity stress tolerance in cotton (Gossypium hirsutum L.) seedlings. *BMC Plant Biology, 21*(1), pp.1-12.
60. Syeed, S., Sehar, Z., Masood, A., Anjum, N. A., & Khan, N. A. (2021). Control of elevated ion accumulation, oxidative stress, and lipid peroxidation with salicylic acid-induced accumulation of glycine betaine in salinity-exposed Vigna radiata L. *Applied Biochemistry and Biotechnology*, *193*(10), 3301-3320.
61. Khedr, R. A., Sorour, S. G. R., Aboukhadrah, S. H., El Shafey, N. M., Abd Elsalam, H. E., El-Sharnouby, M. E., & El-Tahan, A. M. (2022). Alleviation of salinity stress effects on agro-physiological traits of wheat by auxin, glycine betaine, and soil additives. *Saudi Journal of Biological Sciences, 29*(1), 534-540.
62. Hafez, E.M., Gowayed, S.M., Nehela, Y., Sakran, R.M., Rady, A.M., Awadalla, A., Omara, A.E.D. and Alowaiesh, B.F., 2021. Incorporated biochar-based soil amendment and exogenous glycine betaine foliar application ameliorate rice (Oryza sativa L.) tolerance and resilience to osmotic stress. *Plants*, *10*(9), p.1930.
63. Alam, P., Albalawi, T. H., Altalayan, F. H., Bakht, M. A., Ahanger, M. A., Raja, V., ... & Ahmad, P. (2019). 24-Epibrassinolide (EBR) confers tolerance against NaCl stress in soybean plants by up-regulating antioxidant system, ascorbate-glutathione cycle, and glyoxalase system. Biomolecules, 9(11), 640.
64. Ahanger, M. A., Mir, R. A., Alyemeni, M. N., & Ahmad, P. (2020). Combined effects of brassinosteroid and kinetin mitigates salinity stress in tomato through the modulation of antioxidant and osmolyte metabolism. Plant Physiology and Biochemistry, 147, 31-42.
65. Sofy, M. R., Elhindi, K. M., Farouk, S., & Alotaibi, M. A. (2020). Zinc and paclobutrazol mediated regulation of growth, upregulating antioxidant aptitude and plant productivity of pea plants under salinity. Plants, 9(9), 1197.

66. Jaleel, C. A., Manivannan, P., Lakshmanan, G. M. A., Sridharan, R., &Panneerselvam, R. (2007). NaCl as a physiological modulator of proline metabolism and antioxidant potential in Phyllanthus amarus. ComptesRendusBiologies, 330(11), 806-813.
67. Wani SH, Singh NB, Haribhushan A, Mir JI. Compatible solute engineering in plants for abiotic stress tolerance - role of glycine betaine. Curr Genomics. 2013 May;14(3):157-65. doi: 10.2174/1389202911314030001. PMID: 24179438; PMCID: PMC3664465.
68. Hamani, A. K. M., Chen, J., Soothar, M. K., Wang, G., Shen, X., Gao, Y., &Qiu, R. (2021). Application of exogenous protectants mitigates salt-induced Na+ toxicity and sustains cotton (Gossypium hirsutum L.) seedling growth: Comparison of glycine betaine and salicylic acid. *Plants*, *10*(2), 380.
69. Miri, M., Ghooshchi, F., Tohidi-Moghadam, H. R., Larijani, H. R., &Kasraie, P. (2021). Ameliorative effects of foliar spray of glycine betaine and gibberellic acid on cowpea (Vigna unguiculata L. Walp.) yield affected by drought stress. *Arabian Journal of Geosciences*, *14*(10), 1-9.
70. Kayak, N., Kal, Ü., Dal, Y., Yavuz, D., &Seymen, M. (2022). Do Proline and Glycine Betaine Mitigate the Adverse Effects of Water Stress in Spinach?.*GesundePflanzen*, 1-17.
71. Jangra, M., Devi, S., Kumar, N., Goyal, V., & Mehrotra, S. (2022). Amelioration Effect of Salicylic Acid Under Salt Stress in Sorghum bicolor L. *Applied biochemistry and biotechnology*, 1-24.
72. Shahbaz, M., & Zia, B. (2011). Does exogenous application of glycinebetaine through rooting medium alter rice (Oryza sativa L.) mineral nutrient status under saline conditions. J. Appl. Bot. Food Qual, 84(1), 54Á60.
73. Rattan, A., Kapoor, D., Kapoor, N., Bhardwaj, R., & Sharma, A. (2020). Brassinosteroids regulate functional components of antioxidative defense system in salt stressed maize seedlings. Journal of Plant Growth Regulation, 39(4), 1465-1475.
74. Forghani, A. H., Almodares, A., &Ehsanpour, A. A. (2020). The role of gibberellic acid and paclobutrazol on oxidative stress responses induced by in vitro salt stress in sweet sorghum. Russian Journal of plant physiology, 67(3), 555-563.
75. Giri J. Glycinebetaine and abiotic stress tolerance in plants. Plant Signal Behav. 2011 Nov;6(11):1746-51. DOI: 10.4161/psb.6.11.17801. Epub 2011 Nov 1. PMID: 22057338; PMCID: PMC3329348.
76. Hamani, A. K. M., Chen, J., Soothar, M. K., Wang, G., Shen, X., Gao, Y., &Qiu, R. (2021). Application of exogenous protectants mitigates salt-induced Na+ toxicity and sustains cotton (Gossypium hirsutum L.) seedling growth: Comparison of glycine betaine and salicylic acid. *Plants, 10(2)*, 380.
77. Dustgeer, Z., Seleiman, M. F., Imran, K. H. A. N., Chattha, M. U., ALHAMMAD, B. A., JALAL, R. S., ... & HASSAN, M. U. (2021). Glycine-betaine induced salinity tolerance in maize by regulating the physiological attributes, antioxidant defense system and ionic homeostasis. *NotulaeBotanicaeHortiAgrobotanici Cluj-Napoca*, *49*(1), 12248-12248.
78. Desoky, E. S., Ibrahim, S. A., &Merwad, A. R. (2019). Mitigation of salinity stress effects on growth, physio-chemical parameters and yield of snapbean (Phaseolus vulgaris L.) by exogenous application of glycine betaine. *International Letters of Natural Sciences*, *76*.
79. Sajyan, T. K., Shaban, N., Rizkallah, J., & Sassine, Y. N. (2019). Performance of salt-stressed tomato crop as affected by nano-CaCO 3, glycine betaine, MKP fertilizer and aspirin application. *PoljoprivredaiSumarstvo*, *65*(1), 19-27.
80. Rady, M. O., Semida, W. M., Abd El-Mageed, T. A., Hemida, K. A., & Rady, M. M. (2018). Up-regulation of antioxidative defense systems by glycine betaine foliar application in onion plants confers tolerance to salinity stress. Scientia Horticulturae, 240, 614-622.
81. Abraham, S. S., Jaleel, C. A., Chang-Xing, Z., Somasundaram, R., Azooz, M. M., Manivannan, P., &Panneerselvam, R. (2008). Regulation of growth and metabolism by paclobutrazol and ABA in Sesamum indicum L. under drought condition. Global Journal of Molecular Sciences, 3(2), 57-66.

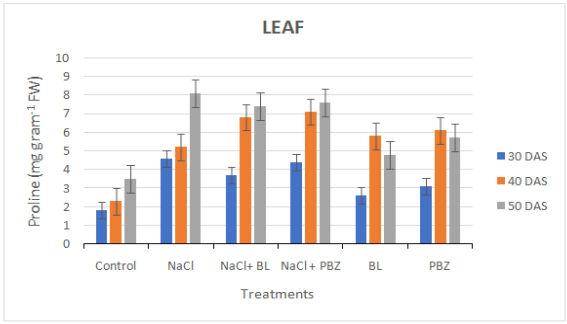

Fig. 1. Effect of exogenous application of BL and PBZ on Proline content in leaf of *A. hypogaea* (VRI-2 variety) under 100mM NaCl stress. Values represented in Bars are mean of three replicates (n=3) and (±) standard error.

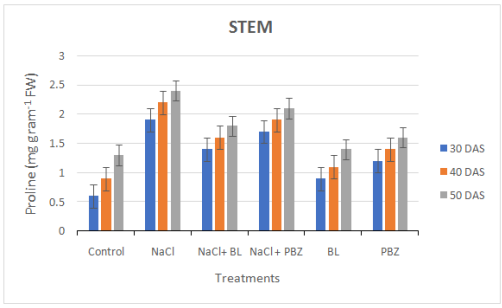

Fig. 2. Effect of exogenous application of BL and PBZ on Proline content in stem of *A. hypogaea* (VRI-2 variety) under 100mM NaCl stress. Values represented in Bars are mean of three replicates (n=3) and (±) standard error.

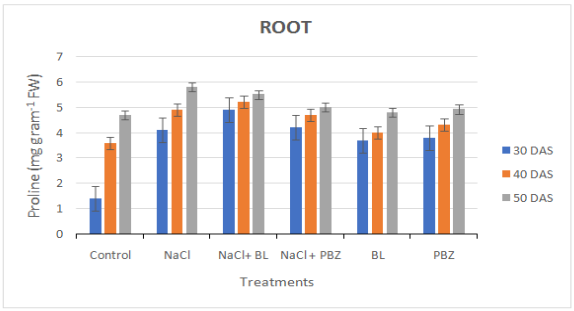

Fig. 3. Effect of exogenous application of BL and PBZ on Proline content in leaf of*A. hypogaea* (VRI-2 variety) under 100mM NaCl stress. Values represented in Bars are mean of three replicates (n=3) and (±) standard error.

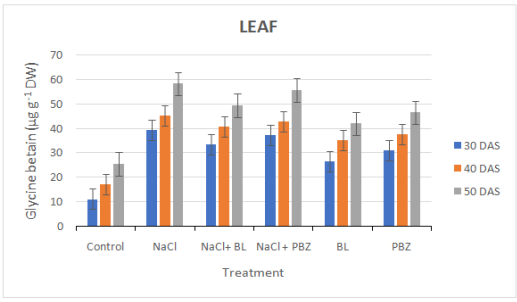

Fig. 4. Effect of exogenous application of BL and PBZ on Glycine betaine content in leaf of *A. hypogaea* (VRI-2 variety) under 100mM NaCl stress. Values represented in Bars are the mean of three replicates (n=3) and (±) standard error.

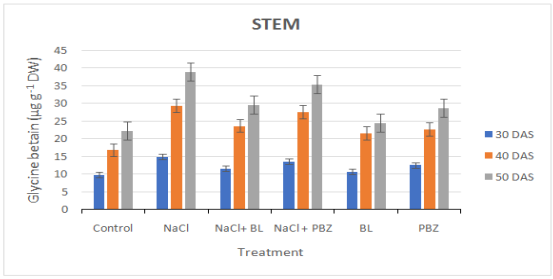

Fig. 5. Effect of exogenous application of BL and PBZ on Glycine betaine content in stem of *A. hypogaea* (VRI-2 variety) under 100mM NaCl stress. Values represented in Bars are mean of three replicates (n=3) and (±) standard error.

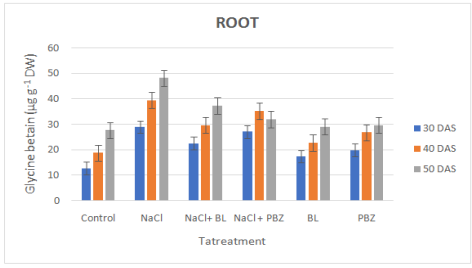

Fig. 6. Effect of exogenous application of BL and PBZ on Glycine betaine content in root of *A. hypogaea* (VRI-2 variety) under 100mM NaCl stress. Values represented in Bars are mean of three replicates (n=3) and (±) standard error.

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RESEARCH ARTICLE

Green Energy Production of *Chlorella vulgaris* and *Spirulina platensis* using Algal Fuel Cells – A Comparative Study under Laboratory and Solar Condition

Lavanyasri Rathinavel[1], Deepika Jothinathan[2], Paul Agastian[3] and Prabhakaran Mylsamy[4]*

[1]Research Scholar, Department of Botany, Pachaiyappas College, (Affiliated to University of Madras, Chennai-600005) Chennai-600030, Tamil Nadu, India.
[2]Assistant Professor, Department of Biotechnology, SRM Arts and Science College (Affiliated to University of Madras, Chennai -600005), Chengalpattu 603203, Tamil Nadu, India
[3]Associate Professor, Department of Plant Biology and Biotechnology, Loyola College, Chennai-600034, Tamil Nadu, India.
[4]Assistant Professor, Department of Botany, Pachaiyappas College, (Affiliated to University of Madras, Chennai -600005) Chennai 600030, Tamil Nadu, India

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***Address for Correspondence**
Prabhakaran Mylsamy
Assistant Professor,
Department of Botany, Pachaiyappas College,
(Affiliated to University of Madras, Chennai -600005),
Chennai 600030, Tamil Nadu, India
Email: prabhasnc@gmail.com

ABSTRACT

In day today's arena, the electrical energy consumption from various renewable sources, have reached the maximum leading to a great energy demand across worldwide. Similarly, the demand for petroleum products has also increased leading to environmental pollution. Day by day the situation gets worsen due to the rate of emission of Carbon-di-oxide keeps increasing. Biofuel cells are the emerging trend for the production for bioenergy. Electricity production using algae has greater advantages when they are coupled with Microbial Fuel Cells by which sustainable green energy can be produced. During Photosynthesis, algae tend to develop the capacity to utilize the carbon-di-oxide and convert into a potential biomass, by this biomass cultivation of algae can also be achieved. Dual chambered Algal fuel cell (DCAFC) was constructed to carry out the study under laboratory condition. For our research we have selected green microalgae *Chlorella vulgaris* and Blue green microalgae *Spirulina platensis*. Electrodes such as Carbon Rod, Carbon membrane and Carbon cloth were selected for optimization where carbon rod was found to be efficient. Three different Catholytes such as Potassium permanganate, Ferric nitrate and Air cathodes were also used for optimization, among which air cathode was efficient. From the above study, *Spirulina platensis* was the potential candidate, which was subjected to Single electrode and

multiple electrode analysis for voltage and current production. Multiple electrodes system showed a maximum voltage of 0.72 V against single electrode with 0.55 V. Further studies were carried out in solar condition similar to open pond cultivation, single chambered Algal Fuel cells (SCAFC) were constructed and Multiple SCAFC units were connected in Series and parallel where, high voltage of 3.2 V was achieved on 13th day when compared to 0.42 V in parallel connection. This research evidently proves that *Spirulina platensis* is a potent candidate in power production in SCAFC under solar conditions.

Keywords: Microalgae, *Chlorella vulgaris, Spirulina platensis,* Microbial Fuel Cell, Energy production, Algal Fuel cells.

INTRODUCTION

Over decades there has been a tremendous increase in the amount of emissions, thereby resulting in greenhouse effect that has drifted the focus towards cleaner and environmental friendly bioenergy alternatives. Microbial fuel cell (MFCs) is a promising renewable energy option where the photosynthetic microorganisms harnessing solar power could be utilized to generate electricity [1-2]. The algal biomass obtained during the growth phase could thereby be converted into high-value products making the overall process sustainable. Utilization of microalgae in MFC (MAFC) is one promising technology since the microalgae could generate oxygen in-situ to promote the reactions inside the cathode chamber. Along with the generation of bioelectricity, MAFC leads to sequestration of CO2 and removal of nitrogen contaminants from wastewater. It results in positive energy generation which in turn constraints related to environmental and cost could be resolved with integrated wastewater treatment and generation of electricity [3]. Many researchers have explored the cost-effective ways for integrated wastewater treatment and electricity generation using MAFC. Keeping microalgae in cathodic chamber, Gajda et al. (2015) produced electricity of 128 µW and significant biomass productivity has been achieved [4]. Yang et al. (2018) has operated the MAFC system for nutrient removal and bioenergy generation from domestic wastewater and achieved maximum removal efficiency of 96% and 91.5% for total nitrate and phosphate respectively [5]. Characterization of the MFC systems containing complex microbial assembly would aid in understanding the role of microalgae in functioning of MFC [6]. Bazdar et al. (2018) developed photosynthetic MAFC containing *Chlorella vulgaris* to analyse the effect of intensities of light and illumination regimes on simultaneous electricity generation and wastewater treatment [7].

Maximum power density of 126 mW m-3 and chemical oxygen demand removal of 5.47% was achieved. The electrodes made of carbon-based materials are the cost-effective candidates for MAFC and have characteristic feature of higher electrical conductivity, specific surface area and facilitate the growth of exoelectrogenic microorganism for integrated electricity and wastewater treatment [1].Algae play a dynamic role in converting the solar energy into various forms of biochemical energy by its photosynthetic process[8]. The photosynthetic redox reaction occurring in algae is exploited in the microbial fuel cell chambers for driving energy. Here, the solar energy is used to synthesize carbohydrates, oxygen and other compounds. Henceforth, an added advantage of biomass production is achieved in the system [9].*Microcystis aeruginosa, Chlorella vulgaris, Dunaliella tertiolecta, Scenedesmus obliquus, Arthrospira maxima, Ulvalactuca, Laminaria saccharin, Chlamydomonas reinhardtii, Cyanobacteria* and mixed algae are some of the algal species used in MFC [10-15]**.** The algal biomass produced in the MFC is often not in a measurable way and it is mainly concentrated on the power production accompanied with/without wastewater treatment. Cao et al., 2009 observed a high lipid production in algae [16]. Gouveia et al., 2014 extracted Pigments from the algal Biomass that is very rich in carotenoids [17]. The choice of electrode material plays a key role in the power production. Carbon based materials such as carbon rod/fibers/cloth and graphite fibers/pates/granules/foam are the commonly used in any MFC. The microbes readily adhere to the electrode and aid in transfer of bioelectricity [18]**.** Though many studies have concentrated on the power production of algae, the electrode variation in single algae, MFC under solar

conditions in environment is still under-examined. The present study aims to investigate the a) power production of *Chlorella vulgaris* and *Spirulina platensis* with different electrode systems, b) Under solar conditions and c) with single and multiple electrodes.

MATERIALS AND METHODS

Microalgae Axenic culture preparation

Axenic cultures of *Chlorella vulgaris* were prepared using Bold's Basal Medium (BBM) and *Spirulina platensis* were prepared using Zarrouk's medium. The growth of microalgae was monitored by measuring the optical density of the microalgae culture medium using spectrophotometer at a wavelength of 650 nm for *Chlorella vulgaris* and *Spirulina platensis* was measured at wavelength of 450 nm. The Axenic cultures were maintained in 12:12 light and dark regime under laboratory condition.

Construction of MFC

Microbial fuel cell was constructed in single-chambered and dual chambered mode consisting of interconnected anode and cathode separated by a proton permeable membrane. The MFC was utilized to grow two different microalgal species *Chlorella sp*., and *Spirulina sp*. The chambers had a capacity to hold 450 ml volume. SCMAFCs contained highly concentrated algal culture with the electrodes. DCMAFCs contained distilled water in the cathodic chamber while concentrated microalgae in the anodic chamber. Proton permeable membrane had circular openings and permitted the anodic and cathodic chamber to converge without any leakage facilitating the exchange process. The isolation of cultures was maintained and the architectural framework of the MAFC chambers ensured not only the flow of ions, but also the biological utilization of medium and growth of microalgae. Further, the anodic chamber was fabricated to mimic the operational condition of the photobioreactor, and maintain controlled operational conditions ensuring stabilization to facilitate the growth of microalgae.

Variation in performance of MAFCs with different electrodes and catholytes

The performance of MAFCs was evaluated by calculating the electricity generated in terms of the voltage and current produced by using multimeter for two different microalgal strains. Variation in the electrical performance was evaluated based on the changes in electrodes i.e. carbon rod, carbon cloth and carbon membrane used as cathode. The catholyte i.e. potassium permanganate, ferric nitrate and air cathode were also varied to evaluate its effect on voltage and current generation. The growth of both microalgal strains were accessed in terms of the optical density with the use of optimized electrode and the catholyte.

Effect of operational conditions on MAFC performance

Operational conditions impact the performance of MAFCs. The variation in the voltage and current was studied with respect to the light source (solar, artificial) to assess the efficiency of photosynthesis by microalgae and thereby the electricity generation potential. Changes in electricity generation also vary with the number and arrangement of electrodes as well as the chambers (single/dual) utilized during MAFCs operation. Thus, to access the electricity generated due to the operational variability the above-mentioned parameters were also accessed.

RESULTS AND DISCUSSION

Effect of carbon electrodes on MFCs performance with microalgae

The effect of electrode material and surface on MFC performance was evaluated using two different algae namely *Spirulina platensis* and *Chlorella vulgaris* in Microbial fuel cell. Among the three electrodes used, Carbon cloth aided in the maximum voltage production of 0.26V and carbon rod gave maximum current production of 30 µA. On the other hand, with different algae the overall power production varied with electrodes. Though both algae showed prominent bioelectricity production in MAFC, *Spirulina platensis* showed the maximum of 0.26V with carbon rod, 0.22V with carbon cloth and 0.25V with carbon membrane. *Chlorella vulgaris* showed 0.07 with carbon cloth, 0.16V

with carbon rod and 0.05V with carbon membrane. These results show that electrodes significantly affect the performance of microalgae in DCMAFC and should be optimised to check their maximum power output before proceeding for further experiments. Each algae respond in a different way with electrodes in MFC. Selecting an ideal electrode is important for the high efficiency of MFC [19]. In a recent study, Graphite Felt (GF) and Indium Tin Oxide coated on Polyethylene Terephthalate (ITO/PET) electrodes were used in algal MFC with *Scenedesmus obliquus.* Graphite felt generated 10 times larger current than the ITO/PET electrodes [20]. Similarly fine versions of carbon like multi-walled carbon nanotubes (MWCNTs) and Pt/rGO/carbon were used in MFC to analyse their performance [21]. Further research is required in electrodes system to favour the maximum power production in algal MFC.

Effect of catholytes in MFC performance with microalgae

Though air cathode works well in MAFC as an oxidising agent, the efficiency of the MFC can be further improved by adding strong oxidising agents like potassium permanganate, ferric nitrate, ferricyanide, persulfate, Manganese dioxide, Mercury, Copper, Chromium, Triiodide, Hydrogen peroxide, perchlorates, vanadium etc [22]. In this study, potassium permanganate and ferric nitrate are compared with air cathode in DCMAFC. Both *Spirulina platensis* and *Chlorella vulgaris* showed excellent results in terms of voltage and current with potassium permanganate. *Spirulina platensis* gave the maximum of 0.40V and 0.60µA and *Chlorella vulgaris* showed 0.35V and 0.45µA. Ferric nitrate produced good power production for *Spirulina platensis* but was not a suitable agent for *Chlorella vulgaris.* Air cathode was the least oxidising agent with both the algal DCMAFC. In a recent study, Potassium ferricyanide effectively enhanced the power generation of DCMAFC, and also acted as an electron acceptor in the cathode chamber to develop oxygen reduction rate [23]. Many MFC models employed algae in cathode where it efficiently produced oxygen via photosynthesis that served as electron acceptors [24]. Air cathode still serves to be the inexpensive catholyte in both bacterial and algal MFC's.

MAFC performance with single and multiple electrodes

Based on the above results it is evident that *Spirulina platensis* performed well in DCMAFC with high power production when compared to *Chlorella vulgaris.* Henceforth, *Spirulina platensis* was exposed to both single and multiple electrodes in a DCMAFC system to compare their power production. From the figures 3 a) it is observed that multiple electrodes system showed a maximum voltage of 0.72 V against single electrode with 0.55 V. There was a steep increase of current of 30 mA in DCMFC with multiple electrodes when compared to single electrode DCMAFC. These results show that algae MFC needs more surface area of electrodes to boost up the power production. In 2020, a unique tubular algal MFC was operated with multiple electrodes in cathode and 315 mV of electricity was generated in the system [25].

Power production of Multiple SCMAFC with *Spirulina platensis* in solar condition connected in Series and Parallel

Many SCMAFC models are extended to stacked version both in series or parallel connection to achieve maximum power output and also for the treatment of wastewater in field level [26]. In this note, MAFC prefer more environmental conditions against laboratory conditions. Henceforth, multiple SCMAFC inoculated with *Spirulina platensis* was kept in solar condition for the effective performance. They were connected in series and parallel to check the difference in power production. As a result, SCMAFC's in series showed a high voltage of 3.2 V on 13th day when compared to 0.42 V in parallel connection. However, SCMAFC's in parallel showed a maximum of 7.9 mA in 11th day against 2.34 mA on the same day in series connection. Figure 4 a) and 4 b) showed the voltage and current production of algal MFC in series and parallel connection. These results have proven that algal SCMAFC in stacked version performs better in solar condition. It is also obvious that maximum voltage is obtained in series connection with reference to the previous literature. In 2020, Amitap Khandelwal et al developed a low cost MFC of 10 L capacity with rock phosphate blended clayware and polyethylene bags as anode and cathode chambers and operated them in outdoor conditions. *Chlorella vulgaris* produced oxygen in cathode chamber [27]. A recent study by Nguyen and Min (2020) emphasized the treatment of leachate wastewater with high levels of N and P in MFC in continuous mode. Algal MFC was able to generate 303 mV using the effluent as a source at a hydraulic retention time of 20 h. In another study, stacked AFC's showed similar power production of 310 mV in a dual chambered MFC [28]. Similarly,

Yang et al. (2019) demonstrated a multiple anode MFC in series connection which yielded a 77% increase in power production with capacitors [29]. Walter et al, 2020 took this technology to the next level by applying the bioelectricity from bacterial MFC to power the screen of a microcomputer. The four cascading series MFC were able to effectively run the screen [30]. These studies prove that MFC's can be scaled up and successfully used in appliances in near future.

CONCLUSION

The current study has optimized electrodes and catholytes for the increased performance of MAFC with *Spirulina platensis and Chlorella vulgaris.* Since *Spirulina platensis* gave better bioelectricity production, it was further evaluated in outdoor conditions with multiple stacked SCMAFC. In series connection, the algae produced 3.2 V when compared to 0.42 V in parallel connection. In contrary, SCMAFC's in parallel showed a maximum of 7.9 mA in 11th day against 2.34 mA on the same day in series connection. This work has proven that *Spirulina platensis* is a potent candidate in power production in SCMAFC in solar conditions. Further research is desirable in improvising the culture conditions, electrode and limiting the losses in the system.

ABBREVIATIONS

MFC – Microbial Fuel Cell
MAFC -Microalgal Fuel cell
DCMAFC – Dual Chambered Microalgal Fuel Cell
SCMAFC – Single Chambered Microalgal Fuel cell

REFERENCES

1. Palanisamy G, Jung HY, Sadhasivam T, Kurkuri MD, Kim SC, Roh SH. A comprehensive review on microbial fuel cell technologies: processes, utilization and advanced developments in electrodes and membranes. J Clean Prod 2019; 221: 598–621. https://doi.org/10.1016/j.jclepro.2019.02.172.
2. Munoz-Cupa C, Hu Y, Xu CC, Bassi A. An overview of microbial fuel cell usage in wastewater treatment, resource recovery and energy production. Sci Total Environ 2021, Article 142429, 10.1016/j.scitotenv.2020.142429
3. Jaiswal KK, Kumar V, Vlaskin MS, Sharma N, Rautela I, Nanda M, Arora N, Singh A, Chauhan PK. Microalgae fuel cell for wastewater treatment: Recent advances and challenges. J. Water Process Eng., 38: 2020, Article 101549, 10.1016/j.jwpe.2020.101549
4. Gajda I, Greenman J, Melhuish C, Ieropoulos I. Self-sustainable electricity production from algae grown in a microbial fuel cell system. Biomass Bioenergy. 2015 1;82:87-93. https://doi.org/10.1016/j.biombioe.2015.05.017
5. Yang Z, Pei H, Hou Q, Jiang L, Zhang L, Nie C. Algal biofilm-assisted microbial fuel cell to enhance domestic wastewater treatment: nutrient, organics removal and bioenergy production. Chem Eng J 2018; 332:277-85. https://doi.org/10.1016/j.cej.2017.09.096
6. Uria N, Ferrera I, Mas J. Electrochemical performance and microbial community profiles in microbial fuel cells in relation to electron transfer mechanisms. BMC Microbiol. 2017 Dec;17(1):1-2. DOI:10.1186/s12866-017-1115-2
7. Bazdar E, Roshandel R, Yaghmaei S, Mardanpour MM. The effect of different light intensities and light/dark regimes on the performance of photosynthetic microalgae microbial fuel cell. Bioresour Technol 2018; 261, 350–360. https://doi.org/10.1016/j.biortech.2018.04.026
8. Mohan SV, Devi MP, Mohanakrishna G, Amarnath N, Babu ML, Sarma PN. Potential of mixed microalgae to harness biodiesel from ecological water- bodies with simultaneous treatment. Bio resour Technol 2011; 102:1109–17. doi: 10.1016/j.biortech.2010.08.103.
9. Karube I, Takeuchi T, Barnes DJ. Modern biochemical engineering. Berlin/Heidelberg: 1992; Springer-Verlag: vol.46

10. Wang HY, Bernarda A, Huang CY, Lee DJ, Chang JS. Micro-sized microbial fuel cell: a mini-review. Bioresour Technol 2011; 102(1):235. doi: 10.1016/j.biortech.2010.07.007
11. Velasquez-Orta SB, Curtis TP, Logan BE. Energy from algae using microbial fuel cells. Biotechnol. Bioeng 2009; 103 (6), 1068–1076. doi: 10.1002/bit.22346.
12. Nishio K, HashimotoK, WatanabeK. Light/electricity conversion by defined co cultures of *Chlamydomonas* and *Geobacter*. J Biosci Bioeng 2013; 115:412–7. doi: 10.1016/j.jbiosc.2012.10.015
13. Lakaniemi AM, Tuovinen OH, Puhakka JA. Production of electricity and butanol from microalgal biomass in microbial fuel cells. BioEnerg Res 2012; 5:481–491. 10.1007/s12155-012-9186-2
14. Cui Y, Rashid N, Hu N, Rehman MSU, Han J. Electricity generation and microalgae cultivation in microbial fuel cell using microalgae-enriched anode and bio-cathode. Energy Convers Manage 2014; 79: 674–80.558 L. http://dx.doi.org/10.1016/j.enconman.2013.12.032
15. Inglesby AE, Fisher AC. Enhanced methane yields from an aerobic digestion of *Arthro spiramaxima* biomass in an advanced flow-through reactor with an integrated re circulation loop microbial fuel cell. Energy Environ Sci 2012; 5:7996–8006. DOI https://doi.org/10.1039/C2EE21659K
16. Cao X, Huang X, Liang P, Boon N, Fan M, Zhang L. A completely anoxic microbial fuel cell using a photo-biocathode for cathodic carbon-di-oxide reduction. Energy Environ Sci 2009; 2:498–501. https://doi.org/10.1039/B901069F
17. Gouveia L, Neves C, Sebastião D, Nobre BP, Matos CT. Effect of light on the production of bioelectricity and added-value micro algae biomass in a Photo- synthetic Alga Microbial Fuel Cell. Bioresour Technol 2014; 154:171–7. doi: 10.1016/j.biortech.2013.12.049.
18. Mustakeem et al., (2015) Electrode materials for microbial fuel cells: nanomaterial approach. Mater Renew Sustain Energy 4:22
19. Ma CH, Yu TL, Lin HL, Huang YT, Chen YL, Jeng US, Lai YH, Sun YS. Morphology and properties of Nafion membranes prepared by solution casting. Polymer. 2009; 20;50(7):1764-77. 10.1016/j.polymer.2009.01.060
20. Gonzalez Olias L, Cameron PJ and Di Lorenzo M. Effect of Electrode Properties on the Performance of a Photosynthetic Microbial Fuel Cell for Atrazine Detection. Front. Energy Res. 2019; 7:105. doi: 10.3389/fenrg.2019.00105
21. Mahmoud RH, Samhan FA, Ibrahim MK, Ali GH, Hassan RY. Waste to energy conversion utilizing nanostructured Algal-based microbial fuel cells. Electrochemical Science Advances. 2021; https://doi.org/10.1002/elsa.202100071
22. Ucar D, Zhang Y, Angelidaki I. An Overview of Electron Acceptors in Microbial Fuel Cells. Frontiers in microbiology, 2017; 8, 643. https://doi.org/10.3389/fmicb.2017.00643
23. Lay CH, Kokko ME, Puhakka JA. Power generation in fed-batch and continuous up-flow microbial fuel cell from synthetic wastewater. Energy 2015; 91, 235–241. doi: 10.1016/j.energy.2015.08.029
24. Yabe K, Shinoda Y, Seki T, Tanaka H, Akisawa A. Market penetration speed and effects on CO_2 reduction of electric vehicles and plug-in hybrid electric vehicles in Japan. Energy Policy 2012; 45, 529–540. doi: 10.1016/j.enpol.2012.02.068
25. Nguyen HT, Min B. Using multiple carbon brush cathode in a novel tubular photosynthetic microbial fuel cell for enhancing bioenergy generation and advanced wastewater treatment. Bioresour Technol. 2020; 1;316:123928. DOI: 10.1016/j.biortech.2020.123928
26. Gajda I, Greenman J, Ieropoulos IA. Recent advancements in real-world microbial fuel cell applications. Curr Opin Electrochem 2018; Oct 1;11:78-83. 10.1016/j.coelec.2018.09.006
27. Khandelwal A, Chhabra M, Yadav P. Performance evaluation of algae assisted microbial fuel cell under outdoor conditions. Bioresour Technol. 2020 1;310:123418. https://doi.org/10.1016/j.biortech.2020.123418
28. Nguyen HT, Min B. Using multiple carbon brush cathode in a novel tubular photosynthetic microbial fuel cell for enhancing bioenergy generation and advanced wastewater treatment. Bioresour Technol. 2020; 1;316:123928. doi: 10.1016/j.biortech.2020.123928.
29. Yang Z, Zhang L, Nie C, Hou Q, Zhang S, Pei H. Multiple anodic chambers sharing an algal raceway pond to establish a photosynthetic microbial fuel cell stack: voltage boosting accompany wastewater treatment. Water Res. 2019;1;164:114955. https://doi.org/10.1016/j.watres.2019.114955

30. Walter XA, Greenman J, Ieropoulos IA. Microbial fuel cells directly powering a microcomputer. J. Power Sources. 2020; 15;446:227328. 10.1016/j.jpowsour.2019.227328

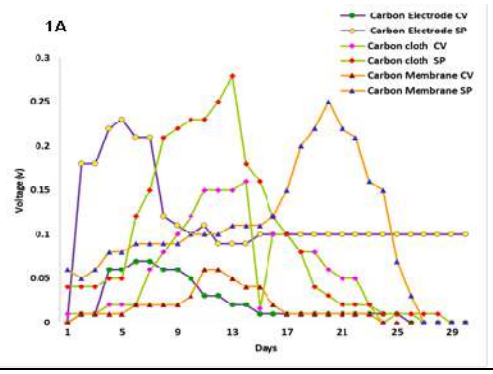

Figure 1: a) Voltage production of DCMAFC by two algae with three different electrodes

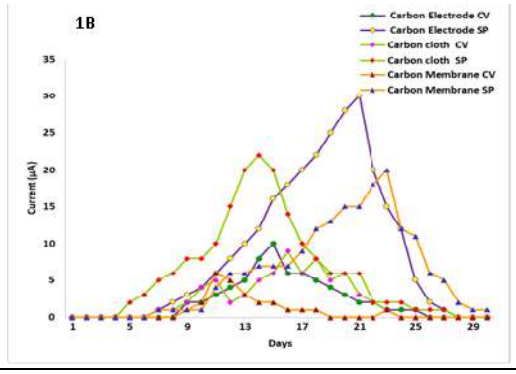

Figure 1: b) Current production of DCMAFC by two algae with three different electrodes

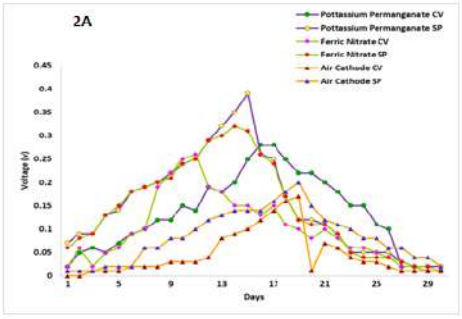

Figure 2: a) Voltage production of DCMAFC by two algae with three different catholytes

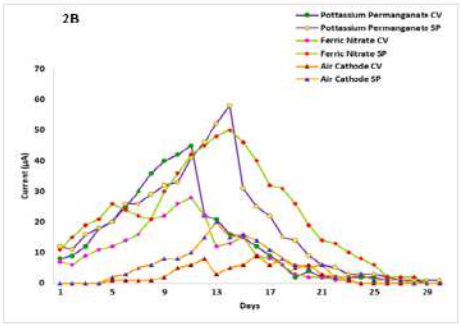

Figure 2: b) Current production of DCMAFC by two algae with three different catholytes

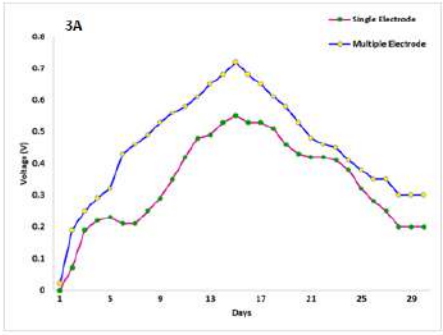

Figure 3 :a) Voltage production of DCMAFC by *Spirulina platensis* with single and multiple electrodes

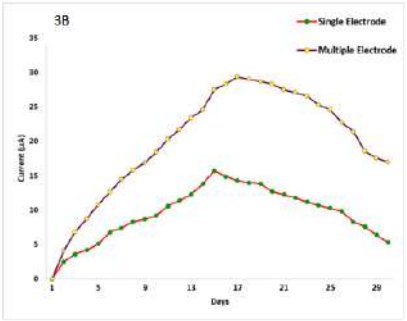

Figure 3: b) Current production of DCMAFC by *Spirulina platensis* with single and multiple electrodes

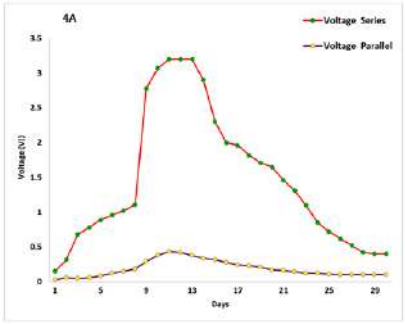

Figure 4 a) Voltage production of SCMAFC's connected in Series and Parallel under solar Condition

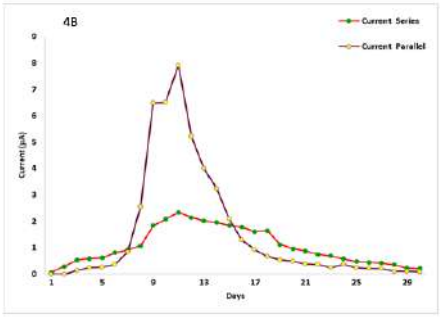

Figure 4 b) Current production of SCMAFC's connected in Series and Parallel under solar condition

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RESEARCH ARTICLE

Effect of Sources of Nitrogen on the Reduction of Hg(II) in An Aqueous Medium Through A Bioreactor Approach using Potential Bacterial Strains

Sreevidya Jayaram[1], P.M.Ayysamy[2], S Rajakumar[3]* and M. Prashanthi Devi[4]

[1]Research Scholar, Department of Marine Biotechnology, Bharathidasan University, Tiruchirapalli, Tamil Nadu, India
[2]Assistant Professor, Department, of Microbiology, Periyar University, Salem, Tamil Nadu, India.
[3]Associate Professor, Department of Marine Biotechnology, Bharathidasan University, Tiruchirapalli, Tamil Nadu, India
[4]Assistant Professor, Department of Environmental Science and Management, Bharathidasan University, Tiruchirapalli, Tamil Nadu, India.

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***Address for Correspondence**
S Rajakumar
Associate Professor,
Department of Marine Biotechnology,
Bharathidasan University,
Tiruchirapalli, Tamil Nadu, India
Email: kodairaj@gmail.com

ABSTRACT

Bioremediation is economical and eco-friendly and requires less expensive techniques for water and soil pollution. The chemical nature of the pollutants and their concentrations, the physicochemical characteristics of the environment and the availability of the chemical to the microorganisms determine the efficiency of the microbial remediation. The present study is aimed toward the assessment of the Hg(II) reduction capability of two bacterial strains, *Alcaligenes* sp. (S11) and *Listeria* sp. (S18) isolated from the Periyar river of Kerala, India. Batch mode studies were carried out by live bacterial cells and an integrated study was carried out to test the bacterial strains on mercury-contaminated water through bioreactor study under optimized conditions. The results proved that the nitrogen is an essential nutrient factor that is required for the removal of Hg(II). The Hg(II) was reduced by the selected bacterial strains thus confirming the possibility of their application to decontaminate the Hg present in the aqueous medium.

Keywords: Mercury (II), Sediments, Biotransformation, Growth rate, Reduction,

INTRODUCTION

Microorganisms are widely distributed in the biosphere because of their metabolic ability and they can easily grow in a wide range of environmental conditions. The nutritional versatility of microorganisms is exploited for

biodegradation of pollutants, based on the ability of certain microorganisms to convert, modify and utilize toxic pollutants to produce biomass and obtain energy in the process [1]. Microbial cells tend to react differently under different stress conditions. The efficiency of bioremediation depends on many factors, including the chemical nature and concentration of pollutants, the physicochemical characteristics of the environment, and their availability to microorganisms [2]. Therefore, it is very essential to screen mercury-reducing microorganisms and optimize microbial degradation rate with environmental parameters such as the effect of co-substrate, pH, and temperature. Earlier studies stated that the microorganisms require additional carbon and nitrogen sources for dual functions, such as growth and biodegradation [3]. The controlling and optimizing of bioremediation processes is a complex system due to many factors namely, the existence of a microbial population capable of degrading the pollutants, the availability of contaminants to the microbial population, and environmental factors like the type of soil, temperature, pH, the presence of oxygen or other electron acceptors and nutrients [4]. According to Aziz *et al.* [5], the microbial reduction of mercury is a detoxification reaction that requires energy rather than producing it. Thus, in any treatment medium, the bacteria have to be supplied with nutrients, which are their most essential response to their physiochemical environment [6]. In the soil, usually the organic substrates coupled with ionic substances act as electron donors [7, 8]. The objective of this paper is to find out the effect of nitrogen sources on the removal of Hg(II) in a synthetic medium using potential bacterial strains and to study the removal of Hg(II) in an aqueous medium as well as river sediments through a bioreactor and column approach.

MATERIALS AND METHODS

Preparation of bacterial inoculums

The potential isolates were cultured in a nutrient broth medium for 18 hrs at 37°C and the broth was centrifuged at 5000 rpm for 20 min. The bacterial suspensions were adjusted to 1.0 Optical Density (OD) at 600 nm using a UV-Vis spectrophotometer (Model: Cyber Lab UV 100, USA) by diluting with sterile distilled water to get an approximate cell density of 10 x 10^7 CFU/ml. About 1% of the above suspension was used as inoculum for the optimization of various parameters.

Effect of different nitrogen sources at various concentrations on the reduction of Hg(II)

Nitrogen is an important nutrient required for the growth of microorganisms. Bio-reduction of Hg(II) ion by the individual bacterial strain using synthetic defined medium (DM) with different nitrogen sources, namely, peptone and ammonium chloride at various concentrations (0.2%, 0.4%, 0.6%, 0.8%, 1.0% and 1.2%) was studied respectively. 1% glucose was incorporated as a carbon source along with a medium containing nitrogen source. The medium was prepared with 100ppm of Hg and sterilized. To it, about 1% of 1 OD of potential bacterial cultures individually (S11 and S18) was inoculated. The test flasks were kept in a shaker (120 rpm) at room temperature for 168 hrs. The samples were drawn aseptically at every 24hrs intervals and the bacterial growth was analyzed using UV-VIS spectrophotometer at 600nm. The reduction of Hg(II) was determined by the standard dithizone method using UV-VIS spectrophotometer at 495 nm [9].

Removal of Hg(II) in synthetic water through a bioreactor approach

The removal of Hg(II) from synthetic water was carried out through a bioreactor design. This set-up consisted of a reservoir, reactor tank, settling tank, filtration tank and collection tank. All the tanks are of 10-liter capacity and are made up of tarson. The reactor and settling tank was operated with a mechanical stirrer. The filtration tank was a cylindrical glass vessel 30 cm in height and 10 cm in width. To this, large pebbles were packed in the bottom of the vessel followed by small pebbles, gravel, coarse sand, fine sand and activated carbon. The depth of the packing material was divided into 6 portions. In the bioreactors, about 10 liters of synthetically contaminated Hg(II) water (100ppm) was taken and processed with bacterial strains for 10 days. Four reactor set-ups were studied as shown in the following

protocols
Reactor 1: Synthetic Hg(II) water + 1% glucose + 0.2% NH_4Cl + 1% S11
Reactor 2: Synthetic Hg(II) water + 1% glucose + 0.6% peptone + 1% S11
Reactor 3: Synthetic Hg(II) water + 1% glucose + 0.2% NH_4Cl + 1% S18
Reactor 4: Synthetic Hg(II) water + 1% glucose + 0.6% peptone + 1% S18

In each reactor containing synthetic Hg(II) water, the Hg-resistant bacterial strains (S11 and S18) individually were added aseptically with the addition of 1% glucose as a sole carbon source and different nitrogen sources. The control bioreactor served as those with bacterial inoculums but without the addition of any carbon source. After the addition of nutrients and the inoculums, the Hg spiked sample was subjected to aeration by an air pump. The experiments were carried out at room temperature and every 24 hours of incubation the samples were collected aseptically by using a sterile syringe. Hg(II) and bacterial count in the samples were analyzed using UV-VIS spectrophotometer [10]. Usually, a culture medium is important for the remediation of any contaminants. Similarly, the carbon and nitrogen sources generally play a significant role because the nutrients are directly linked with cell proliferation and biosynthesis of metabolites. In this study, synthetic Hg(II) spiked water at the concentration of 100 ppm was filled in the bioreactor with a working volume of approximately 10 L. The Hg resistant strains, S11 and S18 bacterial inoculums (1OD) were added aseptically at respective bioreactor tanks with the addition of 0.6% peptone and 0.2% ammonium chloride as the nitrogen source which was abundantly previously optimized.

RESULTS AND DISCUSSION

Effect of various nitrogen sources on the removal of Hg(II) by S11 in synthetic medium

The reduction of Hg(II) by the bacterial strains were evaluated using synthetic defined medium (DM) with different nitrogen sources, namely peptone, yeast extract and ammonium chloride at various concentrations (0.2%, 0.4%, 0.6%, 0.8%, 1.0% and 1.5%). In this study, the synthetic medium with 1% glucose was used since glucose is the representative carbon source. In addition, varying concentrations of nitrogen sources were used to promote the efficiency of Hg(II) removal. The results obtained are presented in Fig. 1. The removal of Hg(II) was higher which was from 100 ppm to 0.88 ppm in the medium supplemented with 0.6% of peptone. There were no major variations of Hg(II) removal noted when the study was carried out with other concentrations of peptone. During this study, the growth of the S11 bacterial strain was assessed and it was found to increase in number. In the study carried out with yeast extract, the removal of Hg(II) was higher from 100 ppm to 1.1 ppm in the medium supplemented with 0.6% of yeast extract. In the case of inorganic nitrogen sources like NH_4Cl, the removal of Hg(II) was insignificant which was from 100 ppm to 1.6 ppm in the medium supplemented with 0.2% NH_4Cl. From this study, 0.6% peptone was selected as the suitable nitrogen source since it is an organic source of nitrogen and produced significant removal of Hg(II) in the synthetic medium.

Effect of various nitrogen sources on the removal of Hg(II) by S18 in synthetic medium

The reduction of Hg(II) by the bacterial strains S18 was evaluated using synthetic defined medium (DM) with different nitrogen sources, namely peptone, yeast extract and ammonium chloride at various concentrations (0.2%, 0.4%, 0.6%, 0.8%, 1.0% and 1.5%). The results obtained are presented in Fig. 2. When the medium was amended with 0.6% of peptone, the removal of Hg(II) was higher which was from 100 ppm to 2.36 ppm by the strain S18. In the study of Hg(II) removal using other concentrations of peptone, no major significant variations were noticed. However, 0.6% peptone demonstrated a higher percentage of Hg(II) removal. In the study carried out with yeast extract, the removal of Hg(II) was noted to reduce from 100 ppm to 2.9 ppm in the medium supplemented with 1% of yeast extract. In the case of NH_4Cl used as an inorganic nitrogen source, the removal of Hg(II) was insignificant which was from 100 ppm to 12.56 ppm in the medium supplemented with 0.2% NH_4Cl. The peptone at 0.6% exhibits maximum removal of Hg(II) and hence this concentration was selected as the suitable nitrogen source in the synthetic medium.

Bioreactor approach to the removal of Hg(II) in synthetic water

Four different aspects (Treatments 1 to 4) were studied for the removal of Hg(II). Reactor 1 with synthetic Hg(II) water containing 1% glucose, 0.2% NH_4Cl and 1OD of 1% S11 bacterial strain showed an initial concentration of Hg(II) to be 100 ppm. It was drastically reduced to 1.18 ppm after 240 hours (Fig. 3). Similarly, Reactor 2 showed a significant level of Hg(II) reduction from 100 ppm to 1.183 ppm. It was almost the same efficiency as in Reactor 1. During the treatment, the bacterial biomass (S11) was determined and expressed as optical density (OD). The growth of S11 gradually increased up to 1.15 OD after 240 hours. Reactor 3 with synthetic Hg(II) water containing 1% glucose, 0.2% NH_4Cl and 1OD of 1% S18 bacterial strain significantly reduced the Hg(II) up to 4.85 ppm from the initial concentration of 100 ppm (Fig. 4). Reactor 4 with synthetic Hg(II) water containing 1% glucose, 0.6% peptone and 1OD of 1% S18 bacterial strain also showed a significant level of Hg(II) reduction from 100 ppm to 12.9 ppm. The bacterial biomass (S18) was determined in each treatment during the incubation period and expressed as optical density (OD). The growth of S18 was gradually increased up to 1.66 OD in Reactor 3 and up to 1.23 OD in Reactor 4. Of the four different aspects (Reactors 1 to 4) which were studied for the removal of Hg(II), all showed Hg(II) reduction.

Similar to this present investigation, a packed-bed bioreactor was designed to remove elemental mercury from wastewater streams. The accumulated mercury content produced by microbial reduction concentrates within the bioreactor [13, 14, 15]. This bioreactor removed mercury both from mercury cell wastewater as well as synthetic mercury chloride solutions [16].Ayyasamy *et al*. [17] reported that *Shewanella* sp. (HN 41) is capable of gaining energy from glucose as an electron donor and ferric iron as an electron acceptor. Ferric ion can be reduced to ferrous ion in low concentrations of oxygen. In this present study, the individual isolates themselves showed higher reduction as compared to the rate of reduction by the consortia at 1% glucose. Our results are in good conformity with the results of another study [18].Among various concentrations of varying nitrogen sources used in this study, the peptone at 0.6% peptone and ammonium chloride at 0.2% favored the removal of Hg(II) and the growth of bacterial strains. Nitrogen sources such as peptone at studied concentrations (0.2% to 1.5%) were found to reduce more than 50% Cr in the broth. Hence, peptone as a nitrogen source at a concentration of 0.6% was found to be most suitable for the reduction of Hg(II).The collected sediment samples after dismantling the columns were subjected to microwave oven digestion to find out the presence of Hg(II) by the AAS method. The initial concentration of Hg(II) was 100 ppm. The Hg(II) in sediment from Column 1 decreased to 12.41 ppm while in Column 2, it was noted to be 8.11 ppm.

CONCLUSION

In this study, the bacterial strains of S11 and S18 were isolated from metal contaminated samples obtained from different sites of Periyar river, Kerala. The reduction of Hg(II) by the selected bacterial strains S11 and S18 was evaluated using a synthetic defined medium (DM) with different nitrogen sources at various concentrations. The resulting outcome demonstrated that peptone at 0.6% and ammonium chloride at 0.2% favored the removal of Hg(II) and the growth of bacterial strains among various concentrations of varying nitrogen sources used in this study. In the bioreactor study, the results proved that the nitrogen sources are the essential nutrient factors that are required for the removal of Hg(II). The Hg(II) was reduced by the selected bacterial strains. From the above study, it could be concluded that strains S11 and S18 can be applied to decontaminate the Hg present in an aqueous medium.

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REFERENCES

1. Tang CY, Criddle CS, Leckie JO. Effect of flux (trans membrane pressure) and membranes properties on fouling and rejection of reverse osmosis and nano filtration membranes treating perfluorooctane sulfonate containing waste water. Environ Sci Technol. 2007; 41: 2008-2014. Link: https://goo.gl/JyEVBn
2. El Fantroussi S, Agathos SN. Is bioaugmentation a feasible strategy for pollutant removal and site remediation? Current Opinion in Microbiology. 2005; 8: 268-275. Link: https://goo.gl/y6kLsc
3. Jin, XC, Liu GQ, Xu ZH, Tao WY. Decolorization of a dye industry effluent by Aspergillus fumigatus XC6. Appl. Microbiol. Biotechnol. 2006; 74: 239-243.
4. Abatenh E, Gizaw B, Tsegaye Z, Wassie M. The Role of Microorganisms in Bioremediation- A Review. Open J Environ Biol. 2017; 2(1): 38-46. DOI: 10.17352/ojeb.000007
5. Aziz A, Azoddein M, Bakar RA, Meriam N, SulaimanN, Bazli A *et al*. *Mercury Removal from Actual Petroleum Based Industries Wastewater by P. putida in Membrane Bioreactor.* In: New Directions in Multidisciplinary Research & Practice (NDMRP 2015), 12-13 May 2015, Istanbul, Turkey; 2015. p. 313-330.
6. Volesky B. 2001. Detoxification of metal-bearing effluents: biosorption for the next century. Hydrometallurgy. 2001; 59: 203–216.
7. Coates JD, Ellis DJ, Roden E, Gaw K, Blunt-Harris EL, Lovley DR. Recovery of humics-reducing bacteria from a diversity of sedimentary environment. Appl Environ Microbiol 1998; 64:1504–1509.
8. Marsh TL, Leon NC, McInerney MJ. Physiochemical factors affecting chromate reduction by aquifer materials. Geomicrobiol. J. 2007; 17: 291– 303.
9. Elly CT. Dithizone procedure for mercury analysis. J. Water Pollut. Contr. Fed. 1973; 45: 940-945.
10. APHA. Standard Methods for the Examination of Water and Wastewater. 21st ed. American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC; 2005
11. Bonneville SC, Cappellen PV, Behrends T. Microbial Reduction of Iron(III) Oxyhydroxides: Effects of Mineral Solubility and Availability. Chemical Geology. 2004; 212: 255-268. DOI: 10.1016/j.chemgeo.2004.08.015.
12. Ayyasamy PM, Lee S. Redox transformation and biogeochemical interaction of heavy metals in Korean soil using different treatment columns in the presence of Shewanella sp. Chemosphere. 2009; 77: 501–509.
13. Brunke M, Deckwer WD, Frischmuth A, Horn JM, Lunsdorf H, Rhode M *et al*. Microbial retention of mercury from waste streams in laboratory columns containing merA gene bacteria. FEMS Microbiol. Rev. 1993; 11: 145–152.
14. Wagner-Dobler I. Pilot plant for bioremediation of mercury containing industrial wastewater. Appl. Microbiol. Biotechnol. 2003; 62(2–3): 124–133.
15. Deckwer WD, Becker FU, Ledakowicz S, Wagner-Dobler I. Microbial removal of ionic mercury in a three-phase fluidized bioreactor. Environ. Sci. Technol. 2004; 38(6): 1858–1865.
16. Von Canstein H, Li Y, Timmis KN, Deckwer WD, Wagner-Döbler I. Removal of mercury from chloralkali electrolysis wastewater by a mercury-resistant Pseudomonas putida strain. Appl. Environ. Microbiol. 1999; 65(12): 5279-5284.
17. Ayyasamy PM, Chun S, Lee S. Desorption and dissolution of heavy metals from contaminated soil using Shewanella sp. (HN-41) amended with various carbon sources and synthetic soil organic matters. J. Hazard. Mater. 2009; 161: 1095 – 1102.
18. Oehmen A, Zeng RJ, Saunders AM, Blackall LL, Keller J, Yuan ZG. Anaerobic and aerobic metabolism of glycogen accumulating organisms selected with propionate as the sole carbon source. Microbiology. 2006; 152:2767–2778.

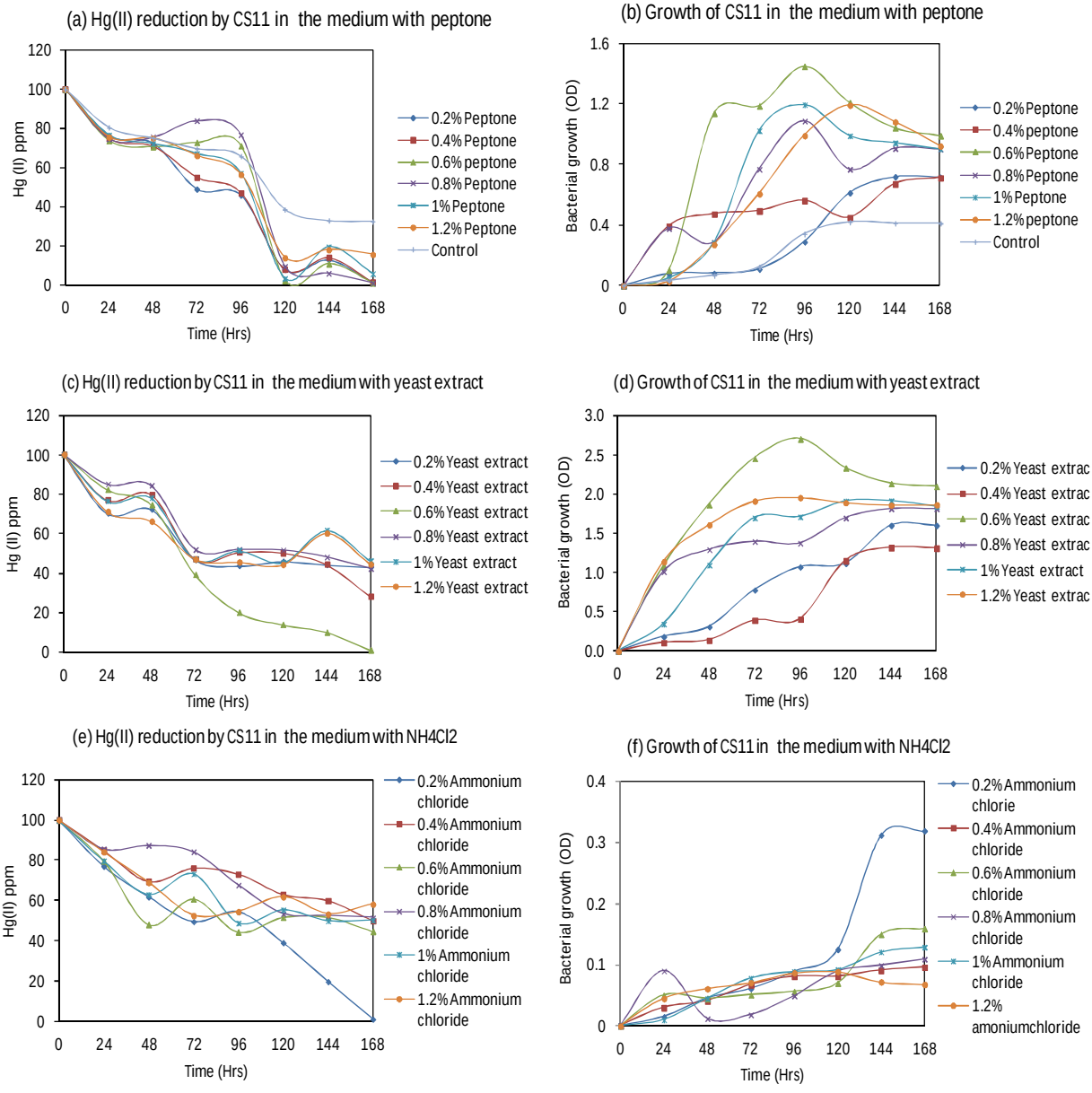


Fig. 1: Effect of various nitrogen sources on the removal of Hg(II) and the bacterial growth of S11 in synthetic medium

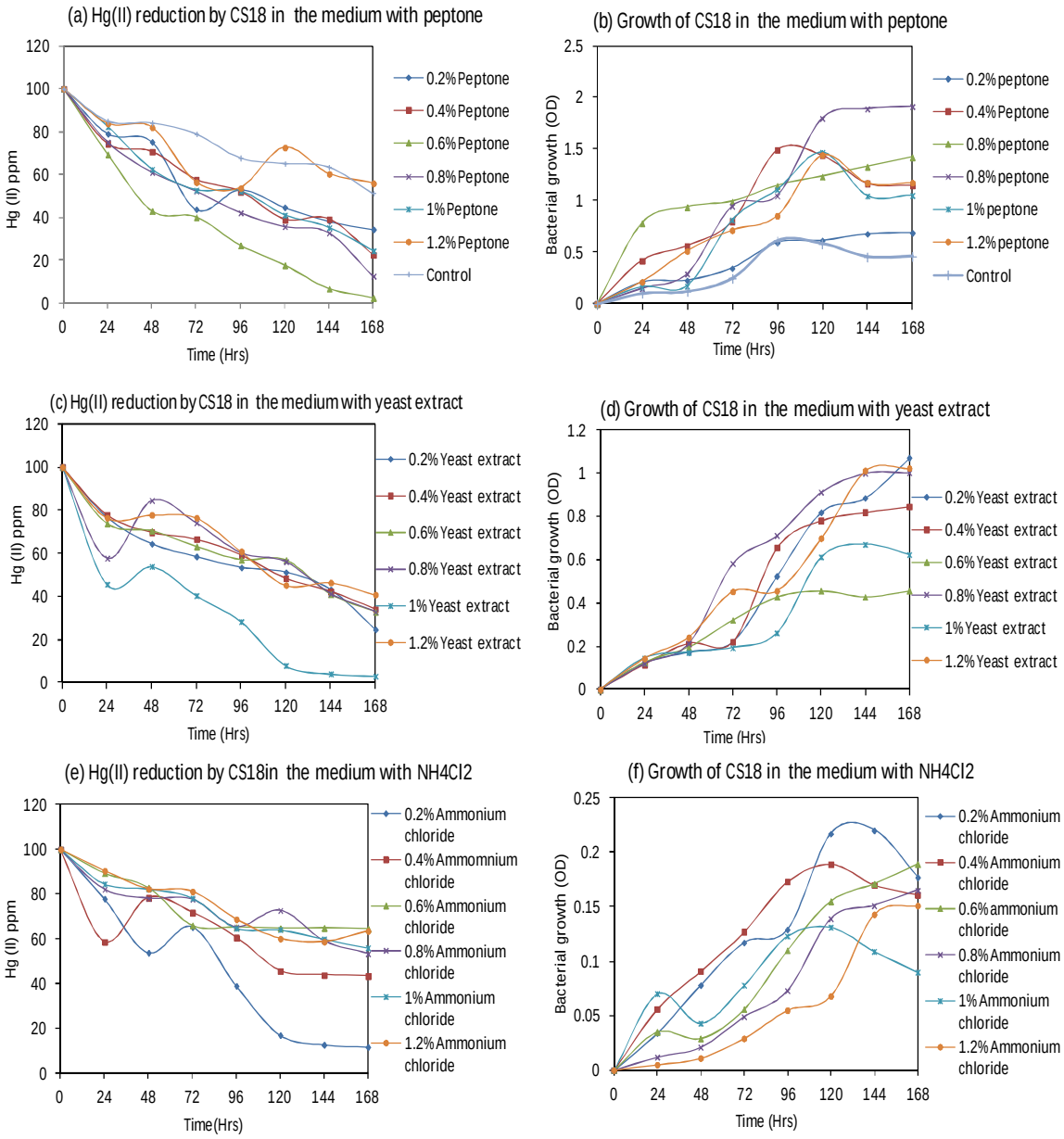
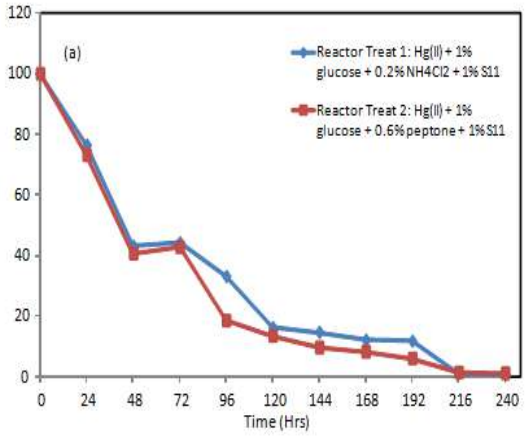


Fig. 2: Effect of various nitrogen sources on the removal of Hg(II) and the bacterial growth of S18 in synthetic medium



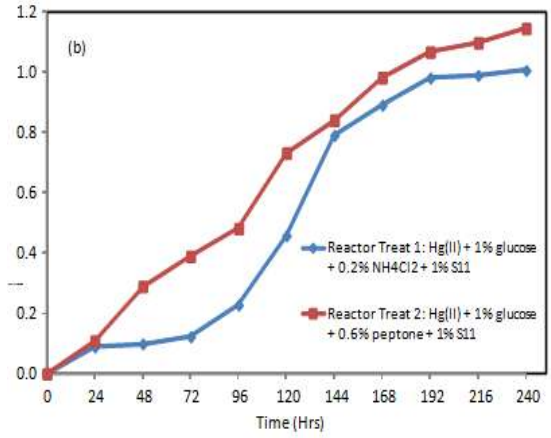
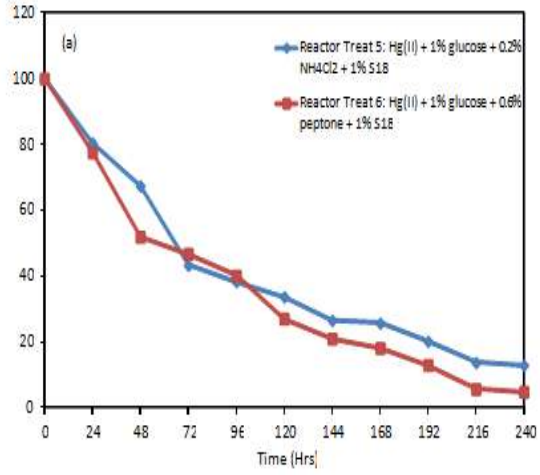


Fig. 3: Bioreactor study on the removal of Hg(II) and the growth of S11 in synthetic medium amended with carbon and nitrogen source



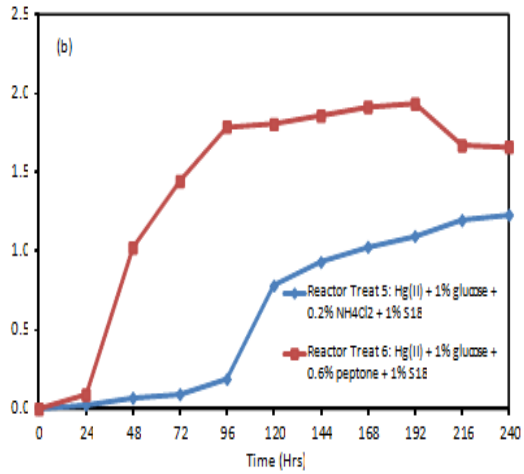


Fig. 4: Bioreactor study on the removal of Hg(II) and the growth of S18 in synthetic medium amended with carbon and nitrogen source

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RESEARCH ARTICLE

Unsupervised Hyperspectral Image Classification

A.Gokilavani*

Assistant Professor and Head, Department of Computer Science, KG College of Arts and Science, Coimbatore, Tamil Nadu, India.

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***Address for Correspondence**
A.Gokilavani,
Assistant Professor and Head,
Department of Computer Science,
KG College of Arts and Science,
Coimbatore, Tamil Nadu, India.
Email: a.gokilavani@kgcas.com

ABSTRACT

Out of all the data sources accessible to geographic information systems (GIS), remote sensing is one of the most crucial ones. Remote sensing is the process of gathering data about the surface of the Earth without physically being there. It detects radiations that are emitted and reflected and are normally captured by sensors that are installed on an aircraft or a satellite. Modeling and monitoring activities on the Earth's surface as well as identifying elements in the land cover by analysing spectral characteristics collected by sensors are the two main goals of remote sensing. When a specific sensor device collects and processes information from the electromagnetic spectrum, it is known as Hyperspectral Imaging (HSI). The data it generates is a goldmine of information. Use this data to solve a variety of problems in a variety of applications. A digital image's pixels are divided up into groups using hyperspectral imaging classification. These techniques were used to classify hyperspectral images using unsupervised hyperspectral image classification algorithms. K-Means and ISODATA algorithms are employed. ENVI is used to apply two algorithms to a hyperspectral image of Washington DC, USA. The accuracy of the procedure was assessed using Principle Component Analysis (PCA) and K-Means or ISODATA algorithm in this paper. The ISO-DATA algorithm outperforms the K-Means algorithm in terms of precision. Since The K-Means algorithm has a classification accuracy of 78.3398 percent, whereas the ISODATA algorithm has a classification accuracy of 81.7696 percent. When the number of classification iterations increased, so did the processing time.

Keywords: Unsupervised classification; K-Means algorithm; ISODATA algorithm; ENVI.

INTRODUCTION

Hundreds of continuous spectral bands are often present in hyperspectral remote sensing - based pictures, allowing for the exact discrimination of the many spectrally comparable land cover classifications. The "curse of dimensionality" (also known as the "Hughes effect") is caused by the fact that such high-dimensional data also contains strongly correlated and useless band information. The classification accuracy of the hyperspectral pictures is significantly decreased by the presence of unimportant and highly correlated spectral bands. Therefore, dimensionality reduction is a more difficult method to improve the hyperspectral image's accuracy of classification.
The art and science of gathering data from a distance is known as remote sensing. For the purpose of interpreting and managing the Earth's resources and surrounding environment it is seen as the measurement and analysis of electromagnetic radiation that is transmitted through, reflected from, or absorbed and dissipated by the atmosphere, the hydrosphere, and materials on or near the land surface It is possible to take images of the Earth's surface using optical remote sensing, which uses visible, near infrared and short-wave infrared sensors to observe the solar radiation reflected from targets on the background. At different wavelengths, different materials reflect and absorb differently. Spectral reflectance fingerprints in remote sensing photos can so distinguish the targets [1][2][3]. Airborne Imaging Spectro-radiometer for Applications (AISA) and other hyperspectral sensors made it possible to create a continuous reflectance spectrum for each pixel in the picture. It is possible to use these methods to make distinctions between various types of earth surface features. While humans can only perceive visible light in three bands (red, green, and blue), hyperspectral imaging divides the spectrum into more than a dozen different bands (infrared, RGB, and UV). This analogy is useful in understanding the concept of hyperspectral imaging better (see Fig. 2) [5][6].

If you look at the definition of hyperspectral, you'll see that it refers to the fact that there are a lot of wavelength bands. Spectral specificity means hyperspectral imaging gives extensive spectrum information to help distinguish and identify materials that are unique in spectral make-up. When compared to other remote sensing data types, hyperspectral imaging offers the potential for more precise and comprehensive information extraction [7]. As a result of its improved capabilities, it is better able to detect potentially hazardous materials and to provide the additional data required to identify and classify these substances. Material's spectral properties are shown by the HSI pixels, which form spectral vectors [10]. Several drawbacks to hyperspectral imagery include a lack of precision in attributes such as directions and distances, due to the earth's sphere. For example, shadows may obscure a particular area to be studied, or the brilliance of the light may be exaggerated in a particular region [11]. An important constraint of hyperspectral imaging is the pixel size, which might be large enough to include many properties but be difficult to categorise or small enough to have no features that can be classified [12] [13, 14].

CLASSIFICATION

The primary goal of satellite imagery categorization is to accurately assess terrain features and extract relevant information [13]. It's important to note that unsupervised and supervised algorithms are the two most used categorization methods. Fig. 3 shows an example of unsupervised classification. K-Means method and ISODATA are employed in an unsupervised classification chain. Unsupervised Classification is depicted in the following figure

K-Means Classification

Using the K-means method is a simple way to get the average of a collection of K-sets. The K-Means algorithm's goal is to minimise cluster variation [15][16][17]. The pseudo-code for the K-means method can be found here [18].

Algorithm for Iterative Self-Organizing Data Analysis (ISODATA)

As one of the most commonly employed algorithms for unsupervised classification, the ISODATA algorithm (see Fig. 5). [19][20][21]: The ISODATA clustering steps are as follows: This is a screenshot of the ISODATA Classifier.

ASSESSMENT OF THE RESULTS AND CONCLUSION

ENVI was used to do an unsupervised classification on a hyperspectral image. The image of Washington, DC that was used in the application of hyperspectral imaging is the hyperspectral dataset that was applied to the image. Principle Component Analysis (PCA) and K-Means or ISODATA methods are applied to the hyperspectral image. A categorised image is the outcome of applying the K-Means algorithm and ISODATA algorithm. When the number of iterations to get the categorised image grew, the process duration rose. Since each pixel in the image has been classified into a category other than "Unclassified Class," statistical information derived from the image data and verified using the K-Means and ISODATA algorithms is reliable. Because ISODATA algorithm has an overall classification accuracy of 81.7696 percent whereas K-Means algorithm has an overall classification accuracy of 78.3398 percent, ISODATA algorithm is superior in terms of accuracy.

An introduction to ENVI (Environment of Visualizing Images)

Images are processed using ENVI. It was made to process data collected by remote sensing. It is capable of seeing and analysing large amounts of image data in great detail. In terms of scientific data formats, it can handle a wide range [22][23].

Cases and Research

• Comparison of the hyperspectral image's outcomes after applying various RGB bands. Classification accuracy can be improved by increasing or decreasing the number of classification iterations.

• A comparison of the first time using K-Means and the second time using ISODATA.A comparison is made between the first time the PCA algorithm was used and the second time the K-Means algorithm was used, with results from both methods being used.

PCA and K-means were applied to a hyperspectral image (Washington, DC) using distinct RGB bands (see Table I) for each band. Fig. 3 displays the PCA results obtained using the image and the test values from Table I. The total number of courses. Figure 4 depicts the results of applying K-Means method on the output pictures of PCA, where the number of iterations is 3 and the number of clusters is 6.A hyperspectral image's classification accuracy rises as the number of iterations is raised; conversely, it falls as the number of iterations is decreased (Case Study 2). Based on the results of the tests described above, we can say that this is correct. Table II summarises the experiment's findings. A hyperspectral image of Washington, DC was subjected to the K-means and Iterative Self-Organizing Data Analysis Technique Algorithm algorithms in Case Study 3. (ISODATA). There are two ways to do this: first using PCA, and then using either K- Means or ISODATA as a second stage in the PCA process. PC Band 172 for R, PC Band 86 for G, and PC Band 24 for B have been selected. K-Means parameters include the number of classes (six) and the number of iterations (three). K-Means method results are shown in Fig. 8-a. The ISODATA settings specify a class size of 4–6 and a maximum iteration count of three. Using ISODATA yields the results shown in Fig. 8-b. A 4) Case Study 4: It's all about comparing the results of implementing PCA and K-Means, as demonstrated in Fig. 9-a and Fig. 9-b, in terms of classification accuracy. The hyperspectral image is used in this investigation (Washington DC).

The statistics of the C class

1) Using the K-means technique to compute class statistics: For the Washington DC picture results, the Means and Standard Deviation for all classes are shown in Fig. 10-a and Fig. 10-b, respectively, which illustrate a correlation between band number and value. This graph illustrates the minimum, maximum, and average values for each band of the Tree class. The standard deviation for the Tree class is shown in Figure 10-d. Table III provides a description of the class distribution, and Table IV presents the ground truth image-based confusion matrices. Figure 11 shows a total class mistake.

2) Applying the ISODATA technique to calculate class statistics: Analyze Washington DC hyperspectral image data using the ISODATA technique, which is shown in Fig. 12-a. Means for all classes are shown, as well as their standard deviations (Fig. 12-b). Fig. 12-c displays the minimum, maximum, and mean values for each band in the Tree class, as well as the standard deviation. The standard deviation for the Tree class is shown in Fig. 12-d. Table VI displays the

degree of misunderstanding among the various classes, as seen in Table V. Matrixes based on the original image's data. A complete error of classification is shown in Figure 13.

CONCLUSION

Hyperspectral images contain a wide range of spectral information that can be used to detect and separate materials with a distinct spectral fingerprint. In order to classify a hyperspectral image, it is necessary to identify items with similar features. After doing Principle Component Analysis (PCA) using ENVI, unsupervised classification algorithms (K-Means algorithm and ISODATA algorithm) are utilised. PCA is used in data analysis before classification to minimise the dimensionality of hyperspectral images. A typical site from the research area in the United States capital of Washington DC is used to test these methods. The K-Means classification strategy had an overall accuracy of 78.3398 percent, whereas the ISODATA classification approach had an accuracy of 81.7696 percent. Both the K-Means and ISODATA algorithms produce reliable findings, however ISODATA performs better on images of the test region.

REFERENCES

1. Sachin T. Jadhav and Mandar M. Kolap, "A Review on Methodologiesof Remote sensing, GPS And GIS", IOSR Journal of Electronics &Communication Engineering (IOSR-JECE), ISSN : 2278-2834, ISBN :2278-8735,PP.1-6.
2. FoudanSalem,TarekEl-Ghazawi,andMenasKafatos,"RemoteSensingAndImageAnalysisForOilSpill Mitigation In The Red. Sea",2ndBiennialCoastalGeoToolsConferenceCharleston,SC,January2001.
3. Principles Of Remote Sensing, ITC Educational Textbook series, ©ITC2001. [Online]. Available: http://www.gdmc.nl/oosterom/PoRSHyperlinked.pdf
4. Foudan Salem and MenasKafatos, "Hyperspectral Image Analysis For Oil Spill Mitigation ",22nd Asian Conference on RemoteSensing,Singapore, November2001.
5. "Hyperspectral imaging",Fleurus (Municipality, Province of Hainaut,Belgium):CRWFlagsInc.,2010.
6. "Hyper Spectral Image: How it happens" http://www2.brgm.fr/mineo/RapportFinal/Image16 .gif
7. S. Peg, "Introduction to Hyperspectral Image Analysis", Earth Science Applications Specialist Research Systems, Inc.
8. P.Shipper,"IntroductiontoHyperspectralImageAnalysis," AnInternationalElectronicJournal,2003.[Online].Availa ble:http://spacejournal.ohio.edu/pdf/shippert.pdf.[Accessed10october2013].
9. SpecTIR, "Hyperspectral Image," SpecTIR, 2012. [Online]. Available: http://www.spectir.com/technology /hyperspectral-imaging/.[Accessed10Nov.2013].
10. G. Shaw and H. Burke, "Spectral imaging for remote sensing," LincolnLaboratoryJ,p.3–28,2003.
11. S. Dr Ropert, "Introduction To Remote Sensing," New Mexico StateUniversity.
12. J.B.Campbell,"IntroductiontoRemoteSensing"(3rdEdition),London:TaylorandFrancis,2002.
13. [Online].Available:http://disi.unitn.it/seminars/281.[Accessed22September2013].
14. Tan,"TheK-Meansalgorithm, "December 2006. [Online]. Available http://www.cs.uvm.edu/~xwu/kdd/ Slides/Kmeans- ICDM06.pdf.[Accessed 2013].
15. A.Villa, J. Chanussot, J.A. Benediktsson, C. Jutten c, R.Dambreville,"Unsupervised methods for the classification of hyperspectral imageswith low spatial resolution", Journal of Pattern Recognition, Volume 46Issue6,pp.1556-1568,June2013.
16. S.PraveenaS.P.SinghI.V.MuraliKrishna,"AnApproachfortheSegmentationofSatelliteImagesusingK- means",KFCM,MovingKFCM and Naive Bayes Classifier. International Journal of ComputerApplications, Volume65,No.20,2013.
17. M.Priyadharshini, R.Karthi, S.N.Sangeethaa, Premalatha, k.S.Tamilselvan, "Implementation of Fuzzy Logic for the High-Resolution Remote Sensing Images with Improved Accuracy", IOSRJournal of Electrical and Electronics Engineering (IOSR-JEEE) e-ISSN:2278-1676,p-ISSN:2320-3331, Volume5, Issue3,2013,PP13-17.

18. "K-MeansClustering, "Home. deib. polimi. it, [Online]. Available : http://home.deib.polimi.it/matteucc/Clustering/tutorial_html/kmeans.htm l.
19. "ERDAS Field Guide", 2005 Leica. Geosystems Geospatial Imaging,LLC, 2005.
20. Mather, Paul, "Computer Processing of Remotely-Sensed Images", 2004JohnWiley&Sons,Ltd
21. Memarsadeghi,N.,Netanyahu,N.S.,LeMoigne,J.,"AFastImplementation of theISODATA Clustering Algorithm", InternationalJournal of Computational Geometry and Applications, IJCGA, Vol. 17,No. 1,PP.71-103,2007.
22. "Multispectral Processing using ENVI's Hyperspectral Tools, "10 December 1997. [Online]. Available: http://www.ltid.inpe.br/tutorial/tut14.htm.[Accessed6November2013].
23. ENVI,"ENVIUser's Guide",ResearchSystems,Inc.,2004

Table.1. The total number of courses

Experiment Description	Over all Accuracy	Class Percentage						
		Unclassified	Roof	Grass	Land	Trail	Road	Water
Comparison between the result of a classified image with only one iteration(Ground Truth)and a classified image with three iterations.	62.7079%	0.0	100	100	100	100	83.29	21.93
Comparison between the result of a classified image with three iterations (Ground Truth)and a classified image with ten iterations.	87.6110%	0.0	100	100	100	100	90.34	8.87
Comparison between the result of a classified image with only one iteration(Ground Truth) and a classified image with ten iterations.	57.8171%	0.0	100	100	100	100	89.59	27.06
Comparison between the result of a classified image with three iterations (Ground Truth) and a classified image with fifteen iterations.	87.6110%	0.0	100	100	100	100	90.34	8.87

Table. 2 . Class distribution summary (K-Means algorithm)

Unclassified	0points(0.000%)
Roof	51,689points(13.154%)
Grass	98,094points(24.963%)
Tree	56,529points(14.385%)
Trail	58,722points(14.944%)
Road	69,678points(17.732%)
Water	58,248points(14.823%)

Table.3. Accuracy Class Percentage of Experiment Description

Over all Accuracy=(307844/392960)78.3398%								
Kappa Coefficient=0.7373								
Ground Truth(Percent)								
Class	**Unclas-sified**	**Roof**	**Grass**	**Tree**	**Trail**	**Road**	**Water**	**Total**
Unclassified	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Roof	0.00	100.00	1.59	0.00	14.92	0.00	0.00	13.15
Grass	0.00	0.00	93.34	17.02	0.15	0.00	0.00	24.96
Tree	0.00	0.00	5.07	46.06	32.74	0.00	0.00	14.39
Trail	0.00	0.00	0.00	33.14	51.08	6.26	0.00	14.94
Road	0.00	0.00	0.00	3.77	1.11	84.79	0.00	17.73
Water	0.00	0.00	0.00	0.00	0.00	8.95	100.00	14.82
Total	0.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Table.4.Confusion matrix using isodata algorithm

Overall Accuracy=(321322/392960)81.7696%								
Kappa Coefficient=0.7726								
Ground Truth (Percent)								
Class	**Unclas-sified**	**Trail**	**Grass**	**Tree**	**Road**	**Water**	**Roof**	**Total**
Unclassified	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Trail	0.00	36.54	0.10	0.00	0.00	0.00	2.32	1.70
Grass	0.00	0.00	99.81	21.07	0.00	0.00	10.95	30.40
Tree	0.00	0.00	0.09	60.90	14.40	0.00	8.88	16.02
Road	0.00	0.00	0.00	18.03	78.73	4.96	0.00	19.40
Water	0.00	0.00	0.00	0.00	1.53	95.04	0.00	17.85
Roof	0.00	63.46	0.00	0.00	5.34	0.00	77.85	14.62
Total	0.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

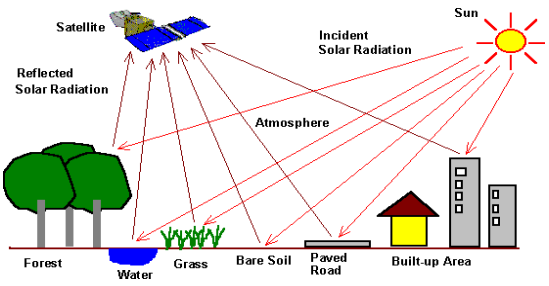

Figure 1: Remote sensing using optical and infrared methods

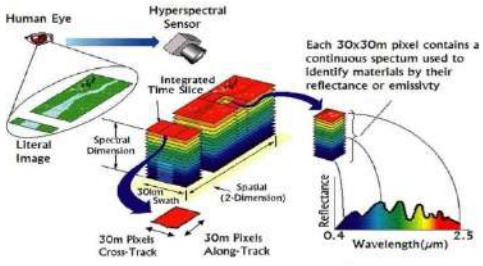

Figure 2. Hyper spectral imaging

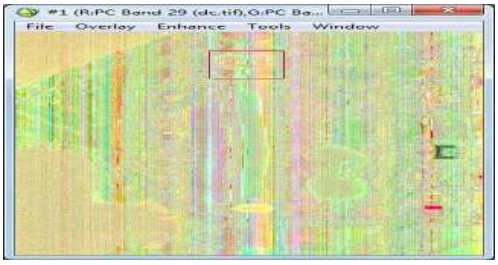

Figure 3. K-MEANS CLUSTERING

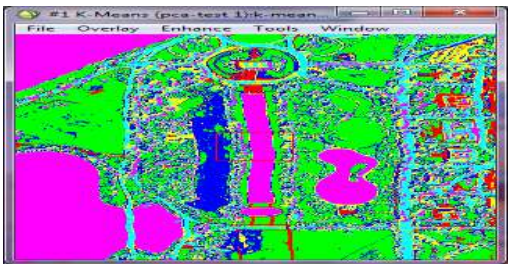

Figure.4

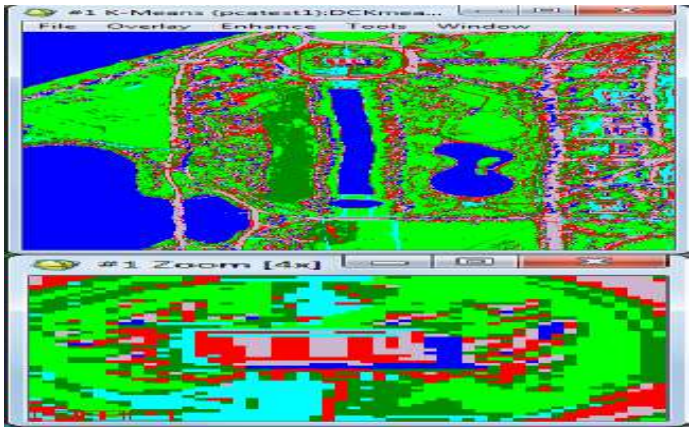

Figure.5. Result for K-means

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RESEARCH ARTICLE

A Fuzzy Retrial Queue with Bernoulli Schedule using Hexagonal Fuzzy Numbers

G.Kannadasan[1]* and V.Padmavathi[2]

[1]Assistant Professor, Department of Mathematics, Annamalai University, Annamalai Nagar, Chidambaram, Cuddalore, Tamil Nadu, India.
[2]Research Scholar, Department of Mathematics, Annamalai University, Annamalai Nagar, Chidambaram, Cuddalore, Tamil Nadu, India.

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***Address for Correspondence**
G.Kannadasan
Assistant Professor,
Department of Mathematics,
Annamalai University,
Annamalai Nagar, Chidambaram, Cuddalore,
Tamil Nadu, India.

ABSTRACT

Fuzzy techniques to analyze of "A Fuzzy Retrial Queue with Bernoulli Schedule using Hexagonal fuzzy Numbers" is discussed in this paper. We acquire model in fuzzy environment as the average number of customers in the system and the sojourn time of the customer. The effects of the parameters in the system are presented numerically.

Keywords: Hexagonal fuzzy, average number, techniques to analyze.

INTRODUCTION

RQS is that if the server is busy when a customer arrives, joins the retrial group so that service can be obtained after a certain amount of time. Do [1] , G.I. Falin [14] and Tuan Phung-Duc [12] are studied M/M/1 retrial queues. Pakkirisamy Rajadurai et al. [8] and Ivan Atencia, Pilar Moreno [10] are investigated retrial queue with Bernoulli schedule. Fuzzy queueing system is based on Zadeh's extension principle [7]. George Klir, Bo yuan [11] studied Fuzzy sets and fuzzy logic. Seyed Behrouz et al. [2] analyzed A fuzzy based threshold policy for a single server retrial queue with vacations. S. Upadhyaya[3] and Gautam Choudhury, Jau-Chuan Ke [4] are studied fuzzy queues with Bernoulli schedule. G.kannadasan , N.sathiyamoorthi [13] analyzed fuzzy queues with triangular fuzzy numbers. Dhurai.K, Karpagam. A [6] , Z Mueen et al., [5] and S.Narayanamoorthy, L Ramya [9] are analyzed hexagonal fuzzy queues. In this paper the Part 2 express the fuzzy model. In Part 3 the average number of customers in the system, the sojourn time of the customer are studied in fuzzy model. In part 4 some numerical results are discussed. Finally conclusion are gained in part 5.

The fuzzy model

The paper as M/M/1 retrial queue and We consider fuzzy arrival time $\bar{\lambda}$ then fuzzy service times $\overline{\beta_1}$, $\overline{\beta_2}$ the fuzzy retrial time $\bar{\alpha}$ are presume to be fuzzy numbers respectively.

Now

$\bar{\lambda} = \{(s, \mu_{\bar{\lambda}}(s)), (s) \in s(\bar{\lambda})\}$

$\overline{\beta_1} = \{(t, \mu_{\overline{\beta_1}}(t)), (t) \in s(\overline{\beta_1})\}$

$\overline{\beta_2} = \{(u, \mu_{\overline{\beta_2}}(u)), (u) \in s(\overline{\beta_2})\}$

$\bar{\alpha} = \{(v, \mu_{\bar{\alpha}}(v)), (v) \in s(\bar{\alpha})\}$

Where $S(\bar{\lambda})$, $S(\overline{\beta_1})$, $S(\overline{\beta_2}), S(\bar{\alpha})$ are the universal sets of the arrival time, service times, retrial time respectively. Applying Zadeh's extension principle (1978), the membership functions of the performance of measure $\bar{\lambda}$, $\overline{\beta_1}$, $\overline{\beta_2}$, $\bar{\alpha}$ can be given as.

$\mu_{f(\bar{\lambda},\overline{\beta_1},\overline{\beta_2},\bar{\alpha})}(H) = Sup_{\substack{s\in s(\bar{\lambda}) \\ t\in s(\overline{\beta_1}) \\ u\in s(\overline{\beta_2}) \\ v\in s(\bar{\alpha})}}$ {min [$\mu_{\bar{\lambda}}(s)$, $\mu_{\overline{\beta_1}}$(t) , $\mu_{\overline{\beta_2}}$(u), $\mu_{\bar{\alpha}}$ (v) / H = f (s,t,u,v)] } -------(1)

If the α-cuts of f ($\bar{\lambda}$, $\overline{\beta_1}$, $\overline{\beta_2}$, $\bar{\alpha}$) degenerate to some fixed value, then the system performance is a crisp number, otherwise it is a fuzzy number.

We acquire the membership function of some performance of measure as follows,

$\mu_{\overline{E(S)}}$ (Y) = $Sup_{\substack{s\in s(\bar{\lambda}) \\ t\in s(\overline{\beta_1}) \\ u\in s(\overline{\beta_2}) \\ v\in s(\bar{\alpha})}}$ {min [$\mu_{\bar{\lambda}}(s)$, $\mu_{\overline{\beta_1}}$(t) , $\mu_{\overline{\beta_2}}$(u), $\mu_{\bar{\alpha}}$ (v)/ Y = f (s,t,u,v)] } ---------- (2)`

Where

Y= $st + \frac{s^2u}{2(1-st)} + \frac{p[1-v]}{v-[p+qv]st}\{st + \frac{s^2pu}{2(1-st)(1-stq)}\}$

$\mu_{\overline{E(W)}}$ (Z) = $Sup_{\substack{s\in s(\bar{\lambda}) \\ t\in s(\overline{\beta_1}) \\ u\in s(\overline{\beta_2}) \\ v\in s(\bar{\alpha})}}$ {min [$\mu_{\bar{\lambda}}(s)$, $\mu_{\overline{\beta_1}}$(t) , $\mu_{\overline{\beta_2}}$(u), $\mu_{\bar{\alpha}}$ (v)/ Z = f (s,t,u,v)] } ----------(3)

Where

$$Z = t + \frac{su}{2(1-st)} + \frac{p[1-v]}{v-[p+qv]st}\{t + \frac{spu}{2(1-st)(1-stq)}\}$$

Using the above principle we are developing the membership values of $\mu_{E[\bar{S}]}$, $\mu_{E[\bar{W}]}$ as a function of the parameter α.

Performance of measure

The average number of customers in the system

Based on above principle $\mu_{\overline{E(S)}}(Y)$ is supermum of infimum over { $\mu_{\bar{\lambda}}(s)$, $\mu_{\overline{\beta_1}}$(t), $\mu_{\overline{\beta_2}}$(u), $\mu_{\bar{\alpha}}$ (v)}

Y =$st + \frac{s^2u}{2(1-st)} + \frac{p[1-v]}{v-[p+qv]st}\{st + \frac{s^2pu}{2(1-st)(1-stq)}\}$

to satisfying $\mu_{\overline{E(S)}}(Y) = \alpha,\ 0 \leq \alpha \leq 1$

We have four types:

Type (i) : $\mu_{\bar{\lambda}}(s) = \alpha$, $\mu_{\overline{\beta_1}}(t) \geq \alpha$, $\mu_{\overline{\beta_2}}(u) \geq \alpha$, $\mu_{\bar{\alpha}}(v) \geq \alpha$

Type (ii) : $\mu_{\bar{\lambda}}(s) \geq \alpha$, $\mu_{\overline{\beta_1}}(t) = \alpha$, $\mu_{\overline{\beta_2}}(u) \geq \alpha$, $\mu_{\bar{\alpha}}(v) \geq \alpha$

Type (iii) : $\mu_{\bar{\lambda}}(s) \geq \alpha$, $\mu_{\overline{\beta_1}}(t) \geq \alpha$, $\mu_{\overline{\beta_2}}(u) = \alpha$, $\mu_{\bar{\alpha}}(v) \geq \alpha$

Type (iv) : $\mu_{\bar{\lambda}}(s) \geq \alpha$, $\mu_{\overline{\beta_1}}(t) \geq \alpha$, $\mu_{\overline{\beta_2}}(u) \geq \alpha$, $\mu_{\bar{\alpha}}(v) = \alpha$

For type (i) the lower and upper bounds of the α-cuts of $\mu_{\overline{E(S)}}$ can be calculated through the corresponding parametric non-linear programme.
It can be written as

$E[S]_\alpha^L = \min_{\Omega}\{[Y]\}$ and $E[S]_\alpha^U = \max_{\Omega}\{[Y]\}$

Such like

$[s]_\alpha^L \leq s \leq [s]_\alpha^U$, $[t]_\alpha^L \leq t \leq [t]_\alpha^U$, $[u]_\alpha^L \leq u \leq [u]_\alpha^U$, $[v]_\alpha^L \leq v \leq [v]_\alpha^U$

Now,

$E[S]_\alpha^L$ and $E[S]_\alpha^U$ are inverse w. r. to α, the both side shape function, L(Y) = $[E[S]_\alpha^L]^{-1}$ and R(Y) = $[E[S]_\alpha^U]^{-1}$ can be derived from $\mu_{\overline{E(S)}}(Y)$ and it given by,

$$\mu_{\overline{E(S)}}(Y) = \begin{cases} L(Y), & E[S]_{\alpha=0}^L \leq Y \leq E[S]_{\alpha=0}^U \\ 1, & E[S]_{\alpha=1}^L \leq Y \leq E[S]_{\alpha=1}^U \\ R(Y), & E[S]_{\alpha=1}^L \leq Y \leq E[S]_{\alpha=0}^U \end{cases} \quad \text{---------- (4)}$$

Using the above method , the upcoming outcomes.

The sojourn time of the customer

$$\mu_{\overline{E(W)}}(Z) = \begin{cases} L(Z), & E[W]_{\alpha=0}^L \leq Z \leq E[W]_{\alpha=0}^U \\ 1, & E[W]_{\alpha=1}^L \leq Z \leq E[W]_{\alpha=1}^U \\ R(Z), & E[W]_{\alpha=1}^L \leq Z \leq E[W]_{\alpha=0}^U \end{cases} \quad \text{---------- (5)}$$

Numerical Study

The average number of customers in the system

Suppose the arrival time $\bar{\lambda}$, service times $\overline{\beta_1}, \overline{\beta_2}$ retrial time $\bar{\alpha}$ are presumed to be hexagonal fuzzy numbers expressed by:
$\bar{\lambda}$ = {1,2,3,4,5,6} and $\overline{\beta_1}$ = {9,10,11,12,13,14} and $\overline{\beta_2}$ = {18,19,20,21,22,23} and $\bar{\alpha}$ = {31,32,33,34,35,36} respectively.
Next

$\lambda(\alpha) = [\min_{x \in s(\bar{\lambda})}\{x \in s(\bar{\lambda}), G(x) \geq \alpha\}, \max_{x \in s(\bar{\lambda})}\{x \in s(\bar{\lambda}), G(x) \geq \alpha\}]$

Where

$$G(x) = \begin{cases} \frac{1}{2}\left(\frac{x-v_1}{v_2-v_1}\right), & for\ v_1 \le x \le v_2 \\ \frac{1}{2} + \frac{1}{2}\left(\frac{x-v_1}{v_3-v_2}\right), & for\ v_2 \le x \le v_3 \\ 1, & for\ v_3 \le x \le v_4 \\ 1 - \frac{1}{2}\left(\frac{x-v_4}{v_3-v_4}\right), & for\ v_4 \le x \le v_5 \\ \frac{1}{2}\left(\frac{v_6-x}{v_6-v_5}\right), & for\ v_5 \le x \le v_6 \\ 0, & for\ otherwise \end{cases} \quad \text{---------- (6)}$$

(i.e), $\lambda(\alpha)$ = [1 + α], [6 - α], $\beta_1(\alpha)$ = [9 + α], [14 - α], $\beta_2(\alpha)$ = [18 + α], [23 - α], (α) = [31 + α], [36 - α].

It is clear that , when $s = s_\alpha^U$, t = t_α^U, $u = u_\alpha^U$, $v = v_\alpha^U$, Y achieve its supremum value and when $s = s_\alpha^L$, t = t_α^L, $u = u_\alpha^L$, $v = v_\alpha^L$, Y achieve its infimum value

The given input values of $\bar{\lambda}$, $\overline{\beta_1}$, $\overline{\beta_2}$, $\bar{\alpha}$

(i) If Y decrease as s increases then for the fixed value t,u,v.

(ii) If Y decrease as t increases then for the fixed value s,u,v.

(iii)If Y decrease as u increases then for the fixed value s,t,v.

(iv)If Y decrease as v increases then for the fixed value s,t,u.

i.e., s = 1+ α, t ,u,v their upper bounds given by t= 14−α, u = 23−α, v = 36− α. The supremum value of E[S] occurs when s = 6- α , t = 9+ α , u = 18+ α , v = 31+ α.

$$\mu_{\overline{E(S)}}(Y) = \begin{cases} 0.5(x-1), & for\ y_1 \le y \le y_2 \\ 0.5 + 0.5(x-1), & for\ y_2 \le y \le y_3 \\ 1, & for\ y_3 \le y \le y_4 \\ 1 + 0.5(x-4), & for\ y_4 \le y \le y_5 \\ 0.5(6-x), & for\ y_5 \le y \le y_6 \\ 0, & for\ otherwise \end{cases} \quad \text{---------- (7)}$$

The values of $y_1, y_2, y_3, y_4, y_5, y_6$ get from (7) are:

$$\mu_{\overline{E(S)}}(Y) = \begin{cases} 0.5(x-1), & for\ 0.000 \le y \le 16.265 \\ 0.5 + 0.5(x-1), & for\ 16.265 \le y \le 18.769 \\ 1, & for\ 18.769 \le y \le 19.758 \\ 1 + 0.5(x-4), & for\ 19.758 \le y \le 16.952 \\ 0.5(6-x), & for\ 16.952 \le y \le 0.000 \\ 0, & for\ otherwise \end{cases}$$

In likewise we arrived the successive outcomes.

The sojourn time of the customer:

$$\mu_{\overline{E(W)}}(Z) = \begin{cases} 0.5(x-1), & for\ z_1 \le z \le z_2 \\ 0.5 + 0.5(x-1), & for\ z_2 \le z \le z_3 \\ 1, & for\ z_3 \le z \le z_4 \\ 1 + 0.5(x-4), & for\ z_4 \le z \le z_5 \\ 0.5(6-x), & for\ z_5 \le z \le z_6 \\ 0, & for\ otherwise \end{cases} \quad \text{---------- (8)}$$

The values of $y_1, y_2, y_3, y_4, y_5, y_6$ get from [8] are:

$$\mu_{\widetilde{E(W)}}(Z) = \begin{cases} 0.5(x-1), & for \quad 0.000 \le z \le 8.281 \\ 0.5 + 0.5(x-1), & for \quad 8.281 \le z \le 10.148 \\ 1, & for \quad 10.148 \le z \le 11.653 \\ 1 + 0.5(x-4), & for \quad 11.653 \le z \le 8.390 \\ 0.5(6-x), & for \quad 8.390 \le z \le 0.000 \\ 0, & for \quad otherwise \end{cases}$$

CONCLUSION

Here, A Fuzzy Retrial Queue with Bernoulli Schedule using Hexagonal Fuzzy Numbers is discussed. Also, the average number of customers in the system, the sojourn time of the customer are derived. The numerical results show the effective of performance measure. Fuzzy retrial queues are mainly used in communication systems, call center and telephone systems.

REFERENCES

1. T.V. Do, M/M/1 retrial queue with working vacations Acta Informatica, vol.47, no.1, PP. 67-75, 2010.
2. Seyed Behrouz Khodadadi, Fariborz Jolai , A fuzzy based threshold policy for a single server retrial queue with vacations, Central Europen Journal of Operations Research, Vol. 20(2), PP. 281-297, 2012.
3. S. Upadhyaya, Bernoulli vacation policy for a bulk retrial queue with fuzzy parameters , International Journal of Applied Operational Research, Vol. 3(3), PP.1-4, 2013.
4. Gautam Choudhury, Jau-Chuan Ke, A unreliable retrial queue with delaying repair and general retrial times under Bernoulli vacation schedule , Applied Mathematics and Computation, Vol.230, PP. 436-450, 2014.
5. Z Mueen, R Ramli, N Zaibidi Parametric nonlinear programming approach with fuzzy queues using hexagonal membership functions, Journal of Computational and Theoretical Nanoscience Vol. 14(10), PP. 4979-4985, 2017.
6. K.Dhurai , A Karpagam Fuzzy optimal solution for fully fuzzy linear programming problems using hexagonal fuzzy numbers, International journal of fuzzy mathematical archive, Vol. 10(2), PP. 117-123, 2016.
7. Zadeh.L.A, Fuzzy sets as a basis a theory of possibility, Fuzzy sets and systems, Vol.1, PP.3-28, 1978.
8. Pakkirisamy Rajadurai, M Varalakshmi, MC Saravanarajan, VM Chandrasekaran, Analysis of $M^{[X]}$/G/1 retrial queue with two phase service under Bernoulli vacation schedule and random breakdown, International Journal of Mathematics in Operational Research, Vol. 7(1), PP. 19-41, 2015.
9. S.Narayanamoorthy, L Ramya, Multi server fuzzy queueing model using DSW algorithm with hexagonal fuzzy number, International Journal of Pure and Applied Mathematics , Vol. 117(11), PP. 385-392, 2017.
10. Ivan Atencia, Pilar Moreno, A single server retrial queue with general retrial times and Bernoulli schedule, Applied Mathematics and Computation, Vol. 162(2), PP. 855-880, 2005.
11. George Klir, Bo yuan, Fuzzy sets and fuzzy logic, Prentice hall, 1995.
12. Tuan Phung-Duc, M/M/1/1 retrial queues with setup time, Advances in Intelligent Systems and Computing, Vol. 383, PP. 93-104.2015.
13. G.Kannadasan, N.Sathiyamoorthi, Analysis for the FM/FM/1 queue with multiple working vacation with N-policy, Annals of Pure and Applied Mathematics, Vol. 14(1), PP133-141, 2017.
14. G.I. Falin, A survey of retrial queues, Queueing systems, Vol. 2, no.3, PP.201-233, 1987.

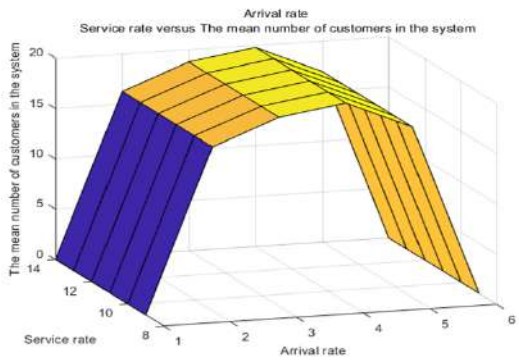

Fig.1: The upcoming two graphs are constitute the measure of efficiency

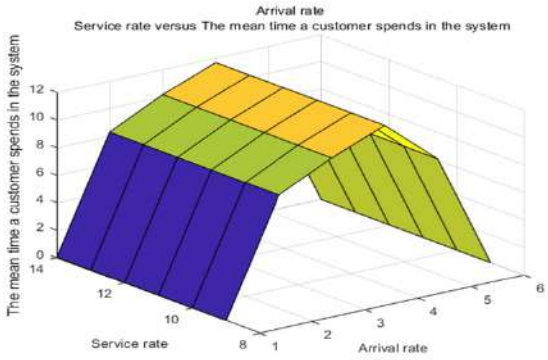

Fig. 2: Service rate versus the mean time a customer spends in the system

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REVIEW ARTICLE

A Review on the Traditional Preparation and its Biochemical Analysis of Rice Beer Northeast, India

Rosamund Jyrwa[1], Ikram Hussain[2], Lunasmrita Saikia[3], Gaurav Kakati[4], Md Rejwan Ahmed Choudhury[1], Ireenia Warjri[1], Biplab Kumar Dey[5] and Ananta Choudhury[6]*

[1]Assistant Professor, Department of Pharmacy, Assam down town University, Guwahati, India.
[2]Assistant Professor, Department of Pharmacy, School of Pharmacy, Kaziranga University, Assam, India.
[3]M.Pharm, Department of Pharmacy, Assam down town, University, Guwahati, India.
[4]B.Pharm, Department of Pharmacy, Assam down town, University, Guwahati, India.
[5]Professor, Department of Pharmacy, Assam down town, University, Guwahati, India.
[6]Associate Professor, Department of Pharmacy, Assam down town, University, Guwahati, India.

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***Address for Correspondence**
Ananta Choudhury,
Associate Professor,
Department of Pharmacy,
Assam down town, University,
Guwahati, India.
Email: anantachoudhury@gmail.com

ABSTRACT

Most of the ethnic communities residing in northeastern state, India have their own practices and habitudes. It also includes differences of preparing and consuming fermented food. Traditional alcoholic beverages (rice beer) are one of the most well- known beverages which are regularly utilized by ancestral networks living in North eastern region of India. Rice beer is an ethnic symbol of North-East India's rich and diverse culture. These fermented beverages go by various names in different tribal communities, for example, it is called 'Choko' by the Rabha tribe, 'Jou bishi' by the bodo tribe and 'Xaj pani or koloh pani' by the Ahom tribe of Assam to name a few. It is believed that the traditional rice beer was first acquired by the 'Missing' people of Assam. Thus the etymology of the word refers to its Assamese origins. Across the different types of Assam, the method of preparation of traditional rice beer has definite differences, but the substrates used for fermentation are almost the same. The biochemical analysis of rice beer of Assam consist of analysis of moisture content, ash content, crude fiber , crude fat, soluble sugar. These properties show the nature of the beverage and all the important nutrients. This review paper reflects the up-to-date dates of the biochemical study of few traditional beverages prepared by the tribes of Assam.

Determining these values can increase the nutritional value; also help in creating public awareness and modifying the therapeutic properties with an enhanced health benefit.

Keywords: Traditional beverage, biochemical study, Choko, Rabha, etymology, Assam.

INTRODUCTION

India is wealthy in biodiversity, ethnic variety with enormous number of customary information and practices. The north-eastern region is an integral part of India consisting of mixture of slope and plain regions. The region is incorporated with 8 different states Assam, Meghalaya, Manipur, Mizoram, Nagaland, Tripura, Arunachal Pradesh and Sikkim [1] popularly known as seven sisters prior to the insertion of Sikkim. The area is covering almost 2,62,379 km sq. that has been partitioned into two biogeography zones Eastern Himalaya and Upper East India. The area lies somewhere in the range of 22°N and 29°5 N scope and 88°E and 97°30"E longitude and shares international border line with Bangladesh, Bhutan, China, Myanmar [2]. The states shows tropical climate to a significant degree, particularly in the lowlands and has a rainstorm environment with weighty to exceptionally substantial rainfall, bound to at least for four months from June to September. The southwest storm is the primary wellspring of downpour, and June is the rainiest of all months. There are three seasons mainly winter, summer and stormy season [3].

Northeasern states comprises of more than 100 of ethnic groups and the equal number of vernacular in which the Bodo form is the largest indigenous ethnic group among all. The ways of life of people living here get reflected in customary works of art through the portrayal of native games, hunting horticulture and fishing style.[4]Many ethnic communities living in northeastern region have their own customs and habits, including differences in how they prepare and consume fermented rice beer. Each tribe has its own set of long-held beliefs and customs, which they adhere to when producing rice beer starting cultures during fermentation [5]. Rice beer is a traditional homemade drink that plays a significant part in the social and cultural existences of many tribal people of northeast India which is produced from rice through fermentation process utilizing yeast and different plant materials. Raw substance and organisms engaged with the interaction have wide changeability in better place. Individuals of northeast drink beer on standard premise especially on their celebrations in various festivals and it is accepted too have numerous medicinal and therapeutic properties[6] It is also considered as an source of income for the livelihoods of the tribes. The traditional alcoholic beverages have different composition and are also named differently by different tribal groups like Xajpani, Jou bishi, Sujen, Hor, Apong, Opo, Mingri, Kaid, Bitchi, Chuwak etc.

Preparation of Rice Beer

North eastern people prepare the beer completely on the knowledge and traditional manner which has been passed from one generation to another. Although the ingredients used by the various communities varies but the basic method of preparation tends to remain nearly same among all the tribes. For preparing the plants which are mostly used are *Accacia pennata* (Mimosaceae), *Piper longum* (Piperaceae), *Drymaria cordata* (Caryophyllaceae), *Artocarpus heterophyllus* (Moraceae), *Cyclosorus exlensa* (Thelypteridaceae), *Saccharum officinarum* (Poaceae), *Ananas comosus* (Bromeliaceae), *Clerodendrum viscosum* (Verbenaceae), *Hydrocotyle sibthorpioides* (Apiaceae), *Croton joufra* (Euphorbiaceae) etc. Sticky rice which is commonly known as Bora rice is a commonly used variety of rice for brewing. For the process of ready to drink beer, rice is used which can be both glutinous or non- glutinous. Rice is boiled and kept untilled cooled down. Fermentation is done on the traditional earthen pot. The cooked rice is mixed with their particular starter cake and kept in pot with required quantity of water. It is covered with leaves of different plants like Banana leaf (*Musa paradisiacal* L.) and stored over the fire place maintaining the required distance for incubation. The period of incubation varies for different tribes and also varies depending on the season of incubation but period generally ranges from 1day to 10day. The mixture which is fermented is filtered to get the beer which is used for drinking [1].

Biochemical Analysis

Biochemical is related to the chemical processes involved in any living organism. Biochemical examination strategies refers to a bunch of techniques, tests, and systems that empower researchers to investigate the substances found in living organic entities and the compound responses hidden life processes. With the biochemical analysis of rice beer we can we can have the scope to validate the various important properties present in it as most of the domestic beer are not only used for commercial, cultural or socio-cultural purposes, but also because they have some medicinal values. Understanding the changes in physico-synthetic properties is fundamental for redesigning the conventional handling to business scale and furthermore would uncover realities about various dietary part present in the drink. Biochemical properties of drinks are assume to play an great role in the human physiology when taken [6].

MATERIALS AND METHODS

Assam

Assam is the second biggest condition of North-eastern area of India. The topographical area and climatic states of this state upholds the act of conventional preparing among various ethnic gatherings of Assam. The technique of planning of rice brew and related starter culture is nearly same among various clans, however the plant materials used to get ready starter culture is unique. Some of the significant clans of Assam are Boro, Karbi, Deori, Ahom, Rabha, Mising and Sonowal [4].

Jou Bishi

The Bodos are one of the biggest linguistic gatherings in North-East India and among the soonest pioneers of Assam. The beer prepared by the people is known as Jou bishi and the culture is called Angkor. For the starter culture, preparation rice grains must be soaked in water for around 5 to 6 hours. The plant's elements required for the cake are rinsed and mashed along with the soaked rice grains. Grinding of the plants and rice grains is done with wooden traditional mortar and pestle. The batter is ready when a small amount of water is added to the mixture, and round cakes of about 5 cm in breadth and 1 cm in thickness are formed. This is followed by covering it with paddy straw and allowing it to dry for 3–4 days. These can be stored in a damp-free environment for up to a year. For the preparation of beer, either glutinous or nonglutinous rice can be utilized. When glutinous rice is utilized the item is known as maibra jou bishi and when non-glutinous rice is used it is known as matha jou bishi. The rice is first cooked. It is then cooled and permitted to dry. The above mention mixture of starter cake and plant elements is mixed again with the cooked rice and blended well. This blend is put inside a plastic pack and kept shut overnight. Later this a little water is added to it and left in a pot covered with banana leaves for a few days. The mass is blended in with water and stressed to get the fluid jou bishi [7,8].

Apong

Mising, the second biggest ethnic gathering of Assam and are the foundation of helpful financial advancement of Assam [9]. The Mising public generally live in house made of bamboo and wooden posts, settle themselves on the waterway side especially along the stream Brahmaputra and its feeders. They are living with the nature are as yet occupied with fishing and occasional development, and are familiar to a huge assortment of wild plants utilized generally in their everyday life. As ethnic clans, the Mising have their own social associations, strict convictions and lifestyle [10]. Apong is a traditional drink of the Mising people group which is created by fermented rice with natural based starter cakes called E'pob. Two types of Apong are created by the Tribe. The Apong which is created by aging of cooked rice with privately pre-arranged E'pob is called Nogin Apong [11].The leaves of the plant required for starter is gathered and cleaned. Then, at that point, they are dried by putting on a bamboo mat. Rice which are soaked are mixed with the leaves independently and they are combined as one in a vessel with water. From the batter, small dough are made and kept in the sun. For the liquid beer the rice is appropriately cooked and permitted to cool. This is trailed by mixing the rice with the culture cake and kept in an earthen pot which is covered with banana leaf and kept inside the ferment or. It is left for at least 4-5 days. Water is added and it is filtered to get apong [11,12].

Xaj

The Ahoms are individuals from the Tai gathering known to move from China and build up their capital in Sivasagar. Today the Ahoms are an unmistakable ethnic gathering of Assam having their own customs and native information [13]. "Xaj", the well known rice based cocktail is delivered by the Ahom people group of Assam utilizing maturation starter, Xaj pitha. The strategy of Xaj preparing is practically comparative among the Ahom people group living in various areas of Assam [14]. The preparation of xaj is done by mixing the steamed rice with culture cake known as xaj pitha. For the culture cake preparation rice grains are kept to be absorbed in water. The diverse plant parts required for starter cake are washed appropriately and crushed along with the soaked rice grain. Crushing is done in wooden mortar and pestle. Small dough of are made and put on banana leaves and permitted to dry for 3 to 5 days [8].For the beer Glutinous rice is steamed and allow cool down and inoculated with the cake and kept in the earthern pot for few days. The initial product is known Rohi [15]. The prepared mass is mixed water and strained to get Xaj [12].

Meghalaya

Meghalaya is located in the northeastern part of India lies between 25000 N to 26100 N and 89450 E to 92470 E. The altitude goes from 50 to 1950 m asl, the Shillong Peak is the highest among the region, which is arranged midway in the level of the Khasi Hills. The region experienced one of the highest rainfall in the entire country, and the environment shifts essentially with differing elevation and geography [16]. There are three distinct tribes found in the state; Khasis, Garos, and Jaintia.

Kiad

The Jaintia hill is one of the seven areas of Meghalaya which lies between 25.5'N to 25.4'N latitude and 91.51'E to 92.45'E longitude. The area is bound by the territory of Assam on the north and east, the East Khasi Hills on the west, and Bangladesh in the south. The region covers an space of 3819 km2 comprising 17.03% of the complete space of the state [17]. Pnar is the most prevalent and most established ethnic local area in the West Jaintia Hills of Meghalaya; they have confidence in the therapeutic plants for their essential medical care[18]. Kiad is likewise normally utilized by individuals of Khasi (Khasi Hills) and Pnar (Jaintia slopes) in Meghalaya during different religious celebrations and festivals. Least consumption is viewed as really great for wellbeing and goes about as a solution for different diseases or illnesses such as urinary difficulty and dysentery however abundance utilization might be harmful and intoxicated [19]. For the preparation of the starter cake powdered local red rice is mixed with spring water and powdered plant part to get a sticky paste [18]. Small doughs are prepared from the paste and kept until dried properly. For brewing the beer is mixed with spring water and cooked in a metallic vessel. After the rice is cooled and dried it is mixed with the yeast cake and the mixture is fermented for 3 to 4 days and boiled in a special vessel known as set-kiad to produce kiad [20,21].

Chubitchi

The Garos are of Tibeto-Burman stock which floated into eastern India and Burma across Tibet. Their language holds closeness with Tibetan [22]. Garo people group prevails, is arranged in the western piece of the state. The district known as the Garo Hills is partitioned into the accompanying regions—west and East Garo Hills, North-South Garo Hills, and South West Garo Hills. The Garo community like other tribal people has their traditional beverage known as Chubitchi. [23]. For the preparation of starter culture local sticky rice known as Menti is taken, 2-3 red chilies are added (optional step) and soaked for a few hours. After the water is drained the rice is ground together with leaves of plants and red chilies in a wooden mortar pestle. Water is added to the mixture of powdered rice and chilies. The mixture is rolled into small round doughs. Charcoal blocks are added from the fireplace into the rice dough to get a smoky flavor (optional step). The doughs which were made are packed tightly with the leaves of jackfruit (*Artocarpus heterophyllus*) and kept in bamboo baskets for 6-7days under room temperature. Dried starter cultures which are locally known as Wanti is produced [23,24]. For the beer, rice is cooked and kept until, after cooling of the rice is transferred in banana leaves and a few amounts of starter culture is also added to the rice and mixed uniformly. The rice starter mixture is kept in a bamboo basket which is tied and sealed properly with ficus / banana leaves and kept above a fireplace for fermentation to take place. Some charcoal and chilies are put on the top of the basket after

sealing as a belief that it keeps away evil spirits. The fermented rice drink is considered to be ready within 6 to 15 days depending on the temperature condition [23, 24].

Tripura

Tripura is the second smallest state after Sikkim in the northeastern area having a geological region of 10491 km2 sharing borderline with Bangladesh on three sides and provinces of Assam and Mizoram on the fourth side. Its capital city Agartala and the other significant urban communities of Khowai, Udaipur furthermore Kailashahar are significant metropolitan places [25]The territory comprises overwhelmingly of slope ranges and undulating uplands, the fields being restricted to 19.8 percent of the geological region. Around 60 percent of the space of the state (6292.681 sq. km) is government woodland. The region enjoys a typical rainfall with yearly precipitation between 2250 mm to 2500 mm [26].

Gora

According to custom from many years, the Koloi community of Tripura is preparing "Gora"- the traditional beer which is excellent in taste and smell applying their conventional native fermenting strategies.[27] Koloi ethnic community of Tripura belongs to the Tibeto-Burmese group and they have their ethnic beer. The process of rice brew making includes entirety rice grains which are cleaned and absorbed water for a couple of hours followed by cooking and is spread over a banana leaf or a mat for a few hours for cooling. After cooling, a proper measure of starter culture is blended in with cold cooked rice. The combination is kept in a container and covered with banana leaves so that fumes can escape out of the container, covered with some mat or old clean material, and saved for 3 days. Following 3 days, water is added and kept again for 2 days. The water is extracted out and drank as an undistilled rice bee. For refined rice brew, the combination is warmed and the fume is gathered in one more holder put on the highest point of the compartment the fumes from the compartment is permitted to go through a bamboo into another compartment containing cold water for refined rice and kept for almost a week for consumption [28].

Langi

It is a herbal rice beer known for its pleasant taste and non-aggressive aroma. Chuwan is the yeast utilized for blending Langi. It is a dry cake made of various plant/homegrown products and crude rice. The plant fixings utilized for the planning of starter cake vary from one local area to another, and now and again from one region to another.
Crude rice is absorbed water for 4 to 6 hours and plant fixings like, leaves, barks, roots, and so forth are washed, dried, and finely hacked. It is added in the extent to the amount of rice utilized, however, it might vary from processer to processer. Excess water from doused rice is drained. The following stage is to add all the slashed plant/homegrown elements for crushing and blending in with the ground rice. The combination, later it is diminished to a fine powder, is moved to a huge container. Water is sprinkled on the combination and it is made into a mash or batter manually. Bundles of around 100 grams (dry weight) are then carried out from and squeezed tenderly between palms, hence leveling the cakes to 1 cm thickness. A couple of smoothed cakes of around 200 grams are carried out and leveled into lengthened oval shapes. These are known as Chuwan chwla . These smoothed cakes are then kept on perfect and dry paddy straws spread on a round bamboo mat for slow expulsion of the overabundance dampness. The cakes are saved for 3 days under shade and after that kept on to the open sun for 2-3 days for additional drying. Chuwan is then ready for use, and it is put away in a cool and dry place [26].

Manipur

Manipur is one of the seven northeastern states that fall in the Himalaya and Indo-Burma worldwide biodiversity area of interest. The state displays a rich and unique diversity of verdure and the wealth of the plant variety is additionally apparent from the employment of an enormous number of plants species by the native networks of the state. The 'Meitei,' is the major native local area of the state for the most part living in the valley district. Moreover, there are more than 32 ethnic tribes in the state living for the most part in the slope areas [29]. Topographically, the province of Manipur is situated at the 23.80°-25.68°N scope and 93.03°-94.78°E longitude covering absolute geological space of 22,327 km^2.

What's more, arranged in the remote Eastern Hilly Region of India. The state is encircled by Nagaland on the North, Assam on the West, Mizoram on the South, and Myanmar on the East [30].

Yu

There are different methods for developing Yu, which are prepared from Hamei. Hamei is prepared by soaking the rice for a few hours and drying it until excess water is removed. Freshly chopped barks of Yanglee (*Albizia Myriophylla* Benth) are mixed with the required amount of water and filtered. The brown filtrate is obtained and mixed with the white rice and a paste is made. From the paste, small cakes are made of different shapes and kept for a few days by covering properly with straw and finally by cloth. After a few days, fine droplets of water appear on the surface. The entire process is done in dark light. It can be consumed when an alcoholic smell comes from the drink [31].

Nagaland

Nagaland is situated in the North East of India, limited by the territories of Assam, Arunachal Pradesh, and Manipur on the west, north, and south, individually, and Myanmar on the east. Its landscape is rocky (95%) and forested (87%). This little (16,579 sq km) yet scantily populated state is home to fourteen Naga and four non-Naga native clans perceived by the state government. The native and non-native clans represented around 86% of Nagaland's populace in 2011. These clans are for the most part Christian and talk more than twenty commonly indiscernible dialects. Around one-6th of the dialects with more than 10,000 speakers revealed in the 2001 Census of India are native to Nagaland, which represents under 0.2 percent of India's populace [32].

Zutho

The Angami is one of the biggest Naga bunches in Nagaland, numbering some 68,552 in 1971, and are the predominant gathering in the area of Kohima. They are essential for the Tengimae gathering comprised of seven clans sharing a solitary beginning and relocation legend, and a typical ancestor. Tenyidae is the term utilized to distinguish the formally normalized and perceived language of the Angami, generally dependent on the Kohima vernacular. Based on contrasts of dialect and custom practice, the Angami perceive three groupings which are named in English as Northern, Western and Southern Angami [33]. Zutho is a conventional rice refreshment arranged by the Angami clan that is generally situated in Kohima and Dimapur regions in Nagaland. This native rice mix frames a significant piece of the way of life and legacy of the ethnic clans of Nagaland like the Ao, Lotha, Angami, Khiamniungan, and Sümi Naga is called by various names with slight varieties in its arrangement. Zutho had a unique aroma that is similar to those of Japanese sake and sprouted rice sake [34]. Zutho' (rice brew) is a conventional cocktail-ready from rice (*Oryza sativa* L.), named by the Angami Naga dialect. It is prepared in two sections.

Preparation of malt

Malt is ready by drenching the unhulled rice grains in water for around 2-3 h and permitted to sprout. The sprouted grains are then spread on bamboo mats and left to dry in the sun followed by beating it into powder.

Preparation of Zutho

Polished rice grains are first soaked in water for 30 min, after which the overabundance water is depleted off. It is then spread over bamboo mats and permitted to air dry. It is beat into powder and hot bubbling water is added to the rice powder one small step at a time and saved side for quite a while to permit it to chill off. The powder of malt and cleaned rice grain powder are combined as one in the proportion of 3:7. After appropriate blending, it is saved at room temperature and permitted to age for around 4-5 days. The first stock in quite a while unadulterated structure is called 'Thutshe' and after it is weakened with some measure of water it is called 'Zutho'. It is burned-through as a well-known cocktail in Nagaland. These are by and large made during social celebrations and relationships [35].

Arunachal Pradesh

Arunachal Pradesh is the geologically biggest state among the North East states. It is a piece of Eastern Himalayan reach arranged betweenb26°28' to 29°31' N longitude and 91°30' E longitude. This state has tremendous ancestral variety, possessed by 26 significant clans and 105 sub clans [36]. The states share its borderline with Assam on south and west with China on the north and Myanmar on the east.

Jumin

In the preparation of 'Jumin', various processes are involved.

Preparation of 'Bichhi'

'Bichhi' is a starter culture cake that is prepared from rice and mixes different plant materials. For getting ready starter cake, rice grains are absorbed water for a few hours and dried in daylight, which later on beat into flour. This flour is blended in with a plant-based item (restorative arrangement) known as 'Sala', which contains a combination of grounded plant portions of different therapeutic spices and honey. Water is included in this combination to make a sticky paste and afterward, cakes are ready from the stock item which is later on kept in a dull space for around seven days for commencement of the fermentation process. The cakes are little in size, unpleasant in surface, and dark or dull white in appearance.

Cooking of rice grains fermentation

The rice grains are cooked in a pot. A piece of prior made cake (a source of yeast) is blended in with recently pre-arranged starter culture cakes (according to prerequisite) which then, at that point, added into cooked grains. Presently the entire item is wrapped by a fabric or by banana (*Musa paradisiaca* L.) leaves, which then, at that point, kept in a dim room in a pot and is covered with leaves / material for an entire day in summer and as long as two days in winter season. Assuming this item is kept consistently for over 15 days then it should be really great for extraction of cocktail.

Extraction of beer

For extracting the beer, 'Chhoa'— a utensil comprised of bamboo is utilized. The utensil is dipped inside the item and after thirty minutes the utensil gets filled with the white shaded alcohol 'Jumin'. It is for the most part ready during neighborhood celebrations, family capacity, ceremonies, and in other socio and cultural activities.

Utilization of staying result

After extraction of 'Jumin', the leftover side-effect privately known as 'Kham' or 'Khemyang' having nutritive worth is also utilized as beer (weakened structure) by adding the required amount of water.[37]

The methods for biochemical analysis are

1. Total acidity and volatile activity: Titrating the sample against NaOh solution.
2. Alcohol percentage: Titration of sample against sodium thiosulfate using Dichromate Oxidation.
3. Bitterness: Measured using spectrophotometry
4. Volatile analysis: Done by using gas chromatography.
5. Colour: Determined by using UV spectrophotometer and is expressed as colour unit EBC.
6. PH: By using glass tip electrode of calibrated PH meter
7. Nitrogen Content: By Micro-kjeldahl method
8. Total soluble sugar: Determined by Anthrone method
9. Alcohol content: determined by either pycnometer method or distillation method.
10. Determination total polyphenol: It is done using spectrophotometer, where the absorbance is measure at 760nm.

RESULT AND DISCUSSION

The values are expressed as mean±SEM. Values within a column with the same letters (a, b, c, d, e, f, g, h, and i) are not signifcantly diferent ($p<0.05$). (Table.1). DEB-Debbarma; JAM-Jamatia; KAL-Kalai; MOL- Molsom.).(Table.2). AP- Arunachal Pradesh, AS- Assam, ML- Meghalaya, NL-Nagaland. (Table.3). AP- Arunachal Pradesh, AS- Assam, ML- Meghalaya, NL-Nagaland. (Table.4). ASSC: Assam Starter Culture; ARSC : Arunchal Pradesh Starter Culture. Figure.1.

CONCLUSION

Northeast, India is blessed with many ethnic tribes and rice beer is an essential cocktail among them and is consumed by almost all the tribes of the region. It is observed that the process of brewing is almost similar but varies in their ingredients used for preparing the beer. The nutritional values present in the beer could provide a health benefit to the consumers .Therefore it is necessary to have the idea of the biochemical and nutritional values of these rice beer as people of these states consumed it on their daily basis.

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Authors' Contributions
Paper design was made by RJ. Manuscript was written by RJ, LS, GK and IH. Manuscript was critically revised by AC and finally approved by all the authors for further publication.

REFERENCES

1. Kalita P, Devi C, Konwar D, Kiranmai C, Tamuli AK, Allam US, Manikanta KN, Reddy PV. Traditional Rice Beer of Assam, North East India: Traditionalism, Ethnobiology and its Pharmacomedicinal Trends. Annals of the Romanian Society for Cell Biology. 2021 Jun 28;25(6):14276-93.
2. Chakravarty S, Suresh CP, Puri A, Shukla G. North-east India, the geographical gateway of India's phytodiversity. Indian Forester. 2012 Aug 1;138(8):702.
3. Dikshit KR, Dikshit JK. Weather and climate of north-east India. In North-East India: land, people and economy 2014 (pp. 149-173). Springer, Dordrecht.
4. Nath N, Ghosh S, Rahaman L, Kaipeng DL, Sharma BK. An overview of traditional rice beer of North-east India: ethnic preparation, challenges and prospects.
5. Bhuyan DJ, Barooah MS, Bora SS, Singaravadivel K. Biochemical and nutritional analysis of rice beer of North East India.
6. Basumatary TK, Terangpi R, Brahma C, Roy P. Jou: the traditional drink of the Boro tribe of Assam and North East India. J Sci Innov Res. 2014;3(2):239-43.

7. Das AJ, Deka SC, Miyaji T. Methodology of rice beer preparation and various plant materials used in starter culture preparation by some tribal communities of North-East India: A survey. International Food Research Journal. 2012;19(1):101.
8. Bhuyan B, Baishya K. Ethno medicinal value of various plants used in the preparation of traditional rice beer by different tribes of Assam, India. Drug Invention Today. 2013 Dec 1;5(4):335-41.
9. Morang J. A Review on the Economy of the Mising Tribe of Assam.
10. Gam NK. Ethno-medicinal claims existing among mising tribes of Assam. IJSIT. 2013;2(4):284-91.
11. Pegu R, Gogoi J, Tamuli AK, Teron R. Apong, an alcoholic beverage of cultural significance of the Mising community of Northeast India. Global Journal of Interdisciplinary Social Sciences. 2013;2(6):12-7.
12. Gogoi B, Dutta M, Mondal P. Various ethno medicinal plants used in the preparation of Apong, a traditional beverage use by mising tribe of upper Assam. Journal of Applied Pharmaceutical Science. 2013 May 1;3(4):S85.
13. Bailung B, Puzari M. Traditional use of plants by the Ahoms in human health management in upper Assam, India. Journal of Medicinal Plants Studies. 2016;4(2):48-51.
14. Keot J. Optimization of ethnic fermentaed Rice beer (xaj) production of Assam India. (Doctoral dissertation, AAU, Jorhat).
15. Tamang JP, Thapa N, Bhalla TC. Ethnic fermented foods and beverages of India. InEthnic Fermented Foods and Alcoholic Beverages of Asia 2016 (pp. 17-72). Springer, New Delhi.
16. Behera RN, Nayak DK, Andersen P, Måren IE. From jhum to broom: Agricultural land-use change and food security implications on the Meghalaya Plateau, India. Ambio. 2016 Feb 1;45(1):63-77.
17. Swer S, Singh OP. Coal mining impacting water quality and aquatic biodiversity in Jaintia Hills District of Meghalaya. Himalayan Ecology. 2003;11(2):29.
18. Debnath B, Langshiang AS, Debnath A, Bhattacharjee A, Paul C. Traditional healing practices of Pnar and War communities in West Jaintia Hills district of Meghalaya, Northeast India. Indian Journal of Traditional Knowledge (IJTK). 2020 Dec 30;19(4):776-87.
19. Sawian P, Nongkynrih KJ, Anand U, Charan AA. Biochemical tests performed for the identification of the isolates collected from local rice beer (Kiad). Journal of Pharmacognosy and Phytochemistry. 2018;7(1):395-7.
20. Jaiswal V. Culture and ethnobotany of Jaintia tribal community of Meghalaya, Northeast India-A mini review.
21. Samati H, Begum SS. Kiad-a popular local liquor of Pnar tribe of Jaintia hills district, Meghalaya.
22. Maikhuri RK, Gangwar AK. Ethnobiological notes on the Khasi and Garo tribes of Meghalaya, Northeast India. Economic Botany. 1993 Oct;47(4):345-57.
23. Marak SR, Sharma D, Sarma H. Ethnic preparation of Chubitchi, an alcoholic beverage of the Garo tribe of Meghalaya: a sociocultural analysis. Journal of Ethnic Foods. 2021 Dec;8(1):1-9.
24. Mishra BK, Das S, Prajapati JB, Hati S. Bio-functional properties and storage study of 'Chubitchi'-a fermented rice beverage of Garo Hills, Meghalaya. Indian Journal of Traditional Knowledge (IJTK). 2021 Apr 6;20(2):498-511.
25. Singh R. The Unexplored Paradise of Tripura: An exploration into trourism geography.
26. Sinha AK, Deb S. 'Langi', The Magnificent Herbal Rice Beer of Tripura.
27. Ghosh S, De Mandal S, Nath N, Kaipeng DL, Rahaman L, Nachimuthu SK, Sharma BK. Isolation and Partial Evaluation of a Potential Indigenous Yeast Strain Pichia kudriavzevii from a Traditional Rice Beer—"Gora" Prepared by the Koloi Tribes of Tripura. Advances in Microbiology. 2019 Sep 3;9(9):824-41.
28. Ghosh S, Rahaman L, Kaipeng DL, Deb D, Nath N, Tribedi P, Sharma BK. Community-wise evaluation of rice beer prepared by some ethnic tribes of Tripura. Journal of Ethnic Foods. 2016 Dec 1;3(4):251-6.
29. Panmei R, Gajurel PR, Singh B. Ethnobotany of medicinal plants used by the Zeliangrong ethnic group of Manipur, northeast India. Journal of ethnopharmacology. 2019 May 10;235:164-82.
30. Singh SR, Phurailatpam AK, Wangchu L, Ngangbam P, Chanu TM. Traditional medicinal knowledge of underutilized minor fruits as medicine in Manipur. International Journal of Agricultural Science. 2014 Aug 1;4(8):241-7.
31. Singh PK, Singh KI. Traditional alcoholic beverage, Yu of Meitei communities of Manipur.
32. Agrawal A, Kumar V. Cartographic conflicts within a union: Finding land for Nagaland in India. Political Geography. 2017 Nov 1;61:123-47.

33. Joshi V. The birth of Christian enthusiasm among the Angami of Nagaland. South Asia: Journal of South Asian Studies. 2007 Dec 1;30(3):541-57.
34. Teramoto Y, Yoshida S, Ueda S. Characteristics of a rice beer (zutho) and a yeast isolated from the fermented product in Nagaland, India. World Journal of Microbiology and Biotechnology. 2002 Dec;18(9):813-6.
35. Deb CR, Bendangnaro J. Ethnic Fermented Food Products of Nagaland, India. J Food Chem Nanotechnol. 2020 Feb 8;6(1):18-27.
36. Solanki GS, Chutia P. Ethno zoological and socio-cultural aspects of Monpas of Arunachal Pradesh. Journal of Human Ecology. 2004 Apr 1;15(4):251-4.
37. Bhatt KC, Malav PK, Ahlawat SP. 'Jumin'a traditional beverage of Nocte tribe in Arunachal Pradesh: an ethnobotanical survey. Genetic resources and crop evolution. 2018 Feb;65(2):671-7.
38. Das S, Deb D, Adak A, Khan MR. Exploring the microbiota and metabolites of traditional rice beer varieties of Assam and their functionalities. 3 Biotech. 2019 May;9(5):1-0.
39. Ghosh S, Rahaman L, Kaipeng DL, Deb D, Nath N, Tribedi P, Sharma BK. Community-wise evaluation of rice beer prepared by some ethnic tribes of Tripura. Journal of Ethnic Foods. 2016 Dec 1;3(4):251-6.
40. Bhuyan DJ, Barooah MS, Bora SS, Singaravadivel K. Biochemical and nutritional analysis of rice beer of North East India.

Table.1.Biochemical analysis of different ethnic group of Assam, Northeast, India

Sample Community	Sample Code	Alcohol Content(% v/v)	equivalent mg/ml)Antioxidant activity(ascorbic acid		Phenolic (gallic acid equivalent mg/ml)
			DPPH	ABTS+	
Ahom	A1	14.82±0.25hf	3.19±0.14a	2.85±0.06d	4.14±0.38a
Bodo	B1	12.26±0.74bf	3.60±0.02ac	3.48±0.07b	3.43±0.48a
Mishing	M1	10.67±1.02e	3.01±0.18c	2.04±0.06f	2.99±1.05

The values are expressed as mean±SEM. Values within a column with the same letters (a, b, c, d, e, f, g, h, and i) are not signifcantly diferent ($p<0.05$).

Table.2.Quantitative biochemical analysis of rice beer of some of the tribe of Tripura , Northeast, India

Parameter	Sample Code			
	DEB	JAM	KAL	MOL
pH	3.64 ± 0.003	3.61 ± 0.003	3.7 ± 0.004	3.52 ± 0.004
Moisture(%)	89.33 ± 0.56	6.0 ± 0.52	85.5 ± 0.62	86.5 ± 0.43
Alcohol % (% v/v)	6.13 ± 0.072	9.48 ± 0.021	10.06 ± 0.018	7.38 ± 0.128
Protein (mg/mL)	9.63 ± 0.088	11.43 ± 0.117	11.45 ± 0.085	12.42 ± 0.125
Non reducing sugar(mg/mL)	0.355 ± 0.006	0.392 ± 0.009	0.483 ± 0.008	0.784 ± 0.002
Reducing sugar (mg/mL)	0.055 ± 0.004	1.09 ± 0.026	0.046 ± 0.00	0.068 ± 0.001
Volatile acidity (gm/100mL)	0.352 ± 0.005	0.026 ± 0.001	0.020 ± 0.001	0.020 ± 0.001
Carbohydrate(mg/mL)	0.425 ± 0.007	0.51 ± 0.010	0.505 ± 0.008	0.83 ± 0.009
Alcohol % of distillate (% v/v)	28.14 ± 0.23	30.41 ± 0.32	35.18 ± 0.14	26.38 ± 0.23
Total acidity (g/ 100 mL)	0.193 ± 0.006	0.127 ± 0.010	0.059 ± 0.001	0.217 0.009

DEB-Debbarma; JAM-Jamatia; KAL-Kalai; MOL- Molsom.

Table.3 Biochemical analysis of different rice varieties of Northeast, India

Rice Sample Code	Moisture%	Crude Fat%	Amylose %	Amylopectin %	Starch	Crude Protein %	Non Reducing Sugar	Reducing Sugar	Total Soluble Sugar
AP1	10.94	2.85	3.42	96.58	68.34	5.91	0.836	0.703	1.539
AP2	12.24	1.0	3.72	96.28	75.39	7.581	0.77	0.344	1.114
AS1	11.54	1.20	1.03	98.97	71.29	6.32	0.922	0.138	1.06
ML1	10.63	0.72	2.68	97.32	71.13	5.833	0.224	0.690	0.914
NL1	11.64	1.16	12.30	87.7	76.38	9.428	0.783	0.087	0.87

AP- Arunachal Pradesh, AS- Assam, ML- Meghalaya, NL-Nagaland

Table 4 Alcohol, mineral, soluble protein and crude protein contents of rice beer samples of Northeast, India

sample code	Alcohol %	Calcium	Phosphorous	Sodium	Iron (mg/100ml)	Crude protein%	Soluble Protein	Potassium (mg/100ml)
AP1	12	12.5	78.108	1.90	0.296	1.95	1.163	4.78
AP2	12.5	10.93	23.563	1.27	0.497	2.33	1.397	4.76
AS1	13	26.5	19.123	1.91	3.069	2.51	1.675	6.67
ML1	12.8	34.37	39.948	3.49	1.4	1.85	1.359	9.63
NL1	12	15.625	33.711	2.22	0.489	2.07	1.315	6.43

AP- Arunachal Pradesh, AS- Assam, ML- Meghalaya, NL-Nagaland

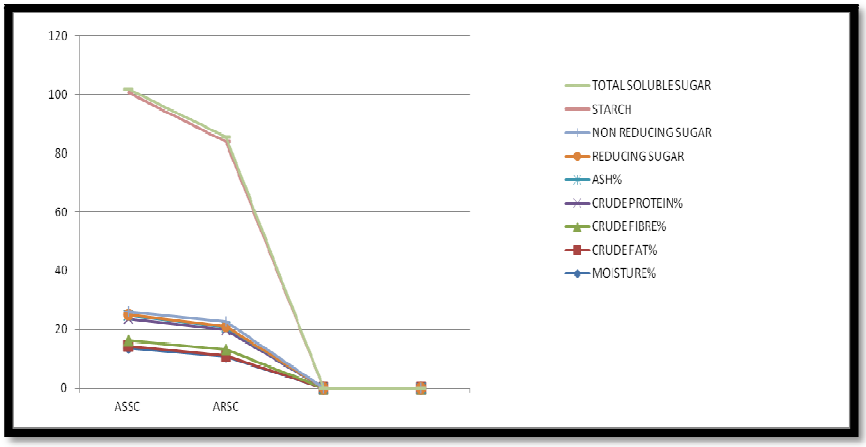

Figure.1.Biochemical composition of starter culture of Northeastern States
ASSC: Assam Starter Culture; ARSC : Arunchal Pradesh Starter Culture

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RESEARCH ARTICLE

Histological and Enzymatic Analysis in the Muscles Tissue of Mud Crab *Scylla olivacea* Exposed to Cadmium Nanoparticle

R. Kavitha*[1], A. Mohamed Niyaz[2] and T. Sheela[3]

[1]Department of Biotechnology, Jaya College of Arts and Science, Chennai, Tamil Nadu, India.
[2]Department of Botany, Presidency College (Autonomous), Chennai, Tamil Nadu, India.
[3]Department of Biotechnology, Dhanalakshmi Srinivasan College of Arts and Science for Women (Autonomous), Perambalur, Tamil Nadu, India.

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***Address for Correspondence**
R. Kavitha,
Department of Biotechnology,
Jaya College of Arts and Science,
Chennai, Tamil Nadu, India.
Email: dr.kavithasathya@gmail.com

ABSTRACT

Cadmium (Cd) is one of the toxic heavy metal that accumulates easily in organisms and causes numerous harmful effects, including tissue damage. To study the toxic effects of Cd on crustaceans, we exposed the mud crab *Scylla olivacea* to various Cd concentrations. In muscle tissues, CdNP induced the changes were slight necrosis followed by appearance of granular material in between the muscle fibers, disorganisation of the muscle fibers and fragmentation of the muscle fibers, absence of wavy appearance of basophilic deposits, but focal disappearance of the muscle fibers were marked, multi nucleate cells that assembled into fibers were evident, complete loss of musc1e structure and basophilic granules were also visible. Overall, the activities of Protease, Catalase, Glutathione peroxidase, Superoxide dismutase, Succinate dehydrogenase, Malate dehydrogenase, Lactate dehydrogenase and Alkaline phosphatase were higher / altered in tissues of *S. olivacea* following CdNP exposure.

Keywords: Mud crab, *Scylla olivacea*, cadmium nanoparticles, histological changes, biochemical modification

INTRODUCTION

Mud crabs are also called as mangrove crabs, which are wide distributed in mangrove ecosystem. Mud crabs are utilized widely due to their high nutritional quality for marine lives as well as for human. Mud crabs from genus *Scylla* have been extensively exploited worldwide, thus causing them to be cultured in many Asian countries which include Malaysia, Indonesia, Philippines, Taiwan, SriLanka, Vietnam, India, and China (Azra and Ikhwanuddin, 2016).The morphological and molecular characters revealed that *Scylla* has four distinct species, namely, *Scylla paramamosain*, *Scylla serrata*, *Scylla olivacea*, and *Scylla tranquebarica*. Trace metals plays an important role in the biochemical process in these organisms and they are significantly contribute to growth, development and physiological activities (Shanker, 2008). Among various trace elements, Cadmium (Cd) is one of the most toxic heavy metals for humans; the main source of non-occupational exposure to Cd includes smoking, air, and food and water contaminated by Cd (Nagata et al., 2005). Cd is a common inorganic contaminant of coastal sediments and waters due to anthropogenic pollution and natural sources (Ivanina *et al.,* 2010; Sokolova *et al.,* 2004). It can be accumulated in aquatic animals including, mud crabs after entering through different way such as respiratory tract, digestive tract, surface penetration etc. (Dailianis & Kaloyianni, 2004; Ivanina *et al.,* 2010). It is seriously harmful to the growth of aquatic life and survival, resulting in decline of their populations. At the same time, as aquatic food products, these animals exposed to Cd might threaten human health. Cd in waters can be absorbed by aquatic organisms via respiratory system, digestive system and body surface without significant excretion (Rainbow & White, 1989). So, it is necessary to evaluate nature of marine ecosystem which exposed to environmental stress including trace element contamination.

Ecotoxicology is the evaluation of risk for an ecosystem exposed to contamination. Although physicochemical parameters are essential for risk determination, during the past decade the results of biological response to chemical stress have been used as references to determine the expected biological damage (Axiak, 1991). Estuaries and coastal zones receive pollutant inputs from both specific and nonspecific sources, especially such ecosystems as seaports, cities, or other industrialized coastal areas that receive chronic inputs of metals. Since many species of crustaceans inhabit estuaries, numerous studies have aimed at examining the bioaccumulation and effects of various toxicants in these animals (Weis et al., 1992; Weis and Weis, 1994). Acute lethal toxicity bioassays are useful for providing a measurement of the relative toxicity of substances, for assessing the sensitivity of the species different stages of life to a particular substance, and for determining concentrations of chronic toxicity so as to assess water quality criteria. Moreover, mixed toxicity bioassays provide information on the global effects of mixtures present in environments and allow evaluation of the magnitude of the effects by determining additive, synergistic, and/ or antagonistic responses. The present study, aimed to determine the toxicity of cadmium nanoparticles (CdNP) on muscle tissue of mud crab *S. olivacea* using histological and biochemical analysis.

MATERIALS AND METHODS

Kavitha *et al. (*2013) and Rani *et al.* (2016) method was followed for the present work

Animal Collection

Fresh samples of *Scylla olivaceai.e,* both male and female species of were collected from Pulicate Lake, Tamil Nadu, India. Both male and female crabs were maintained separately in tanks with aerator which was (capacity of 1000 litres) filled with filtered sea water. The sea water was changed periodically and crabs were fed with commercial fish feed. During the acclimatization period, the specimens were fed twice a day. Naturally aged estuarine water was used after being shifted through a 0.45 mm pore filter and activated charcoal to remove dissolved organic matter and trace metals. Water temperature was maintained within a range (27.5± 0.5°C) as recommended for optimal growth of mud crabs (Chen and Jeng, 1980).

Cadmium nanoparticle treatment

After, standardization of LD_{50} value, a single concentration of 20ppm/kg of body weight was used for further experiments. Mud crab, *S. olivacea* was acclimatized in tanks and the temperature was maintained at 27˚C. Water was changed daily and aquaria were cleaned thoroughly, and crab were fed commercial fish feed. After acclimatization, healthy adult male and female crabs with a homogeneous size (carapace width 14-16cm, weight 200-300g) were selected for control and Cadmium nanoparticle (20 ppm/kg of crab weight) treatment. The acute exposure lasted for 8 days. During the experiment, crabs were fed and dead animals were removed in time.

Histological analysis

Cadmium nanoparticle treated and control crabs of both male and female *S. olivacea* are taken from the tank, anaesthetized in ice water for five minutes and sacrificed at every 2 day interval up to 8 days. Gut tissues are removed and then fixed by direct immersion in a 0.1 M, pH 7.4 phosphate buffer with 4% formaldehyde for 24 h at room temperature. Samples are dehydrated with ethanol and toluene series and embedded in paraffin. Serial sections (4 mm) are mounted on gelatin-coated glass slides and stained with hematoxylin and eosin.

Antioxidant enzymes analysis

The antioxidant enzymes such as catalase (CAT), Superoxide dismutase (SOD) and Glutathione peroxidase (GPx) is estimated in the muscle tissue to determine the free radical formation in the tissues. Antioxidant activity of gut tissues of *S. olivacea* after exposed to CdNP exposure was assayed colorimetrically. The Assays of Catalase, Superoxide Dismutase (SOD) is performed by following the procedures of Beers and Sizer (1952), Beyer and Fridovich (1987) and Glutathione Peroxidase by Lawrence and Burk (1976). Similarly, the tissue damaging enzymes in muscle tissues were also colorimetrically assayed. The procedure of Schirawski and Unden**(**1998) is followed for Succinate Dehydrogenase (SDH) and Gloster and Harris (1962) procedure for Lactate Dehydrogenase (LDH) is followed for the estimation of the enzymes.

RESULTS AND DISCUSSION

Muscles represent a major proportion of total tissue in mud crabs. Cadmium is a toxic pollutant that can disturb cell functions and even lead to cell death. Ariano *et al*. (2015), reported that the Cd concentrations in all samples of white crab meat, were found to be very low, although brown crab meat showed significantly higher Cd concentrations. Meanwhile, the group Lavradas *et al.* (2014) reported the presence of metals (Cu, Pb, Zn and Cd) in muscles, gills, soft tissues and eggs in specimens of male crabs; as well as ovigerous and non-ovigerous female specimens. In the present study, muscle tissue of the control crab was made up of muscle cells containing contractile filaments that move each other and change the size of the cell. Muscle tissue derived from mesoderm contains protein, and myosin filament (thread-like) form multi nucleate cells that assemble into fibers called myofibrils. On day 2, the changes were necrosis and appearance of granular material in between the muscle fibers. On day 4, atrophy and wavy appearance of the muscle fibers, fragmentation of the muscle fibers, and intermuscluar areas with granular exudates were observed. On day 6, wavy appearance of basophilic deposits, and atrophy and focal disappearance of the muscle fibers were marked. On day 8, muscle tissue derived from mesoderm contained protein, and myosin filaments (thread- like) formed multi nucleate cells that assembled into fibers called myofibrils, atrophy and necrosis, loss of muscle structure and necrosis were observed (Fig. 1,2,3,4). In female *S.olivacea*, control crabs showed ordered structure of contractile filaments. On day 2, the changes were slight necrosis followed by appearance of granular material in between the muscle fiber.

On day 4, disorganisation of the muscle fibers and fragmentation of the muscle fibers. On day 6, absence of wavy appearance of basophilic deposits, but focal disappearance of the muscle fibers were marked. On day 8, multi nucleate cells that assembled into fibers were evident, complete loss of muscle structure and basophilic granules were visible (Fig.1,2,3,4). Turoczy *et al.* (2001) states that, as Cd concentrations are unregulated and do not increase with size of crab, the concentrations in the muscle tissues reflect relatively short-term exposure. This type of

exposure may be expected to vary between individuals, depending on recent diet items or habitat. However, relatively fixed proportions of the recent dose seem to appear in the muscle tissues. With reference to the previous findings, the present results also suggested that tissues in the muscle of *S. olivacea* on exposure to CdNP show deterioration in the level of biochemical constituent. On exposure to CdNP toxicity the organs exposed to toxicant show declined enzyme activity against the physiological and biochemical reactions. Valarmathi&Azariah, (2003) reported that in the organs affected by toxicant, the enzyme activity may be increased or inhibited to balance energy demand of the organ when it is in denatured or distorted conditions.

CdNP (20ppm) exposure resulted in increased Catalase (CAT) activity in the muscles of *S. olivacea* than in control crabs. In male, catalase activity started increasing on day 2 and reached peak on day 10 of exposure. Similarly, in the case of female also catalase activity started increasing on day 2 and reached peak on day 10 of exposure. (Fig. 5a and b).CdNP exposure resulted in increased Glutathione Peroxidase (GPx) activity in the muscles of *S. olivacea* than in control crabs. In general, CdNP showed increased GPx activity than in control crabs. In males, GPx activity started increasing on day 2 and reached peak on day 10 of exposure. Similarly, in the case of female also GPx activity started increasing on day 2 and reached peak on day 10 of exposure (Fig. 6a and b). CdNPexposure resulted in increased in SOD activity in the muscles of *S. olivacea* than in control crabs. In males, SOD activity started increasing on day 2 and reached peak on day 10 of exposure. Similarly, in the case of female also SOD activity started increasing on day 2 and reached peak on day 10 of exposure. (Fig.7a and b).SOD and CAT are the two primary enzymes for radical scavenging, which are involved in protective mechanisms within tissue injury following oxidative process and phagocytosis and their activities are related to the status of the organisms affected by different factors including dietary nutrition, environmental factors etc. CdNP (20ppm) exposure resulted in increased alkaline phosphatase activity in the muscles of *S. olivacea* than in control crabs. In the case of male, alkaline phosphatase activity started increasing on day 2 and reached maximum on day 10 of exposure. A maximum of upto two fold increases in ALP activity was recorded in male crab compared to control. Similarly, in the case of female also alkaline phosphatase activity started increasing on day 2 and reached maximum on day 6 and increased up to on day 10 of exposure compared to their control. A maximum of up to 1.5 fold increase in ALP activity was recorded in male crab compared to control (Fig. 8a and b).

Schrauzer (2006) suggested that the enzymatic antioxidant are considered as the defence mechanisms of the organisms against oxidative stress and plays an important role in reducing toxicity of heavy metals. In the present investigations significant increment in the enzyme activity of antioxidant enzymes during the treatment of crabs with CdNP were observed which suggested that on exposure to heavy metals either in bulk or nanoparticle form would resulted in oxidative stress in the organism which in turn increases the enzyme activities to balance the energy requirement of the organism on exposure to toxicant. Results of LDH activity of *S. olivacea* exposed to CdNP(20ppm) were presented in Fig.9a and b. In general, CdNPresulted increased LDH activity in muscles than in control crabs. In males, SDH activity started increasing in exposed crabs right on day 2 and reached maximum on day 10 of exposure. Similarly, in the case of female LDH activity started decreasing in exposed crabs right on day 2 and reached lowest on day 10 of exposure. A maximum of up to five fold and three fold decrease in LDH activity was recorded in male and female crabs respectively compared to control. With reference to LDH activity, both male and female crab showed susceptibility to CdNP. Results of MDH activity in the muscles of *S. olivacea* exposed to CdNP(20ppm) were presented in Fig. 10a and b. In general, CdNP resulted in decreased MDH activity than in control crabs. In males, MDH activity started decreasing right on day 2 compared to control and reached lowest compared to control on day 10 of exposure. Similarly, in the case of female also MDH activity started decreasing on day 2 compared to control and reached lowest compared to control on day 10 of exposure.

Results of SDH activity of *S. olivacea* exposed to Cadmium nanoparticle (20ppm) were presented Fig. 11a and b. In general, CdNPresulted decreased SDH activity in the muscles than in control crabs. In males, SDH activity started decreasing in exposed crabs right on day 2 and reached lowest on day 10 of exposure. A maximum of up to two fold decreases in SDH activity was recorded in male crab compared to control on day 10 after exposure (Fig.11a). Similarly, in the case of female SDH activity started increasing on day 2 and decreased on day 10 of exposure. A

maximum of upto two and three fold decrease in SDH activity was recorded in male and female crabs respectively compared to control (Fig.11b). With reference to SDH activity, female crab showed more susceptibility to CdNP compared to male crabs.

LDH activity is usually seen as the organism's energy requirement under anaerobic conditions (Moreira et al., 2006), it was proved in *S. plana* in water contaminated by CdNPs and results in significant elevation of LDH level. Devi et al. (1993) and Reddy and Bhagyalaxmi (1994) reported that declined LDH activity in the hepatopancreas of fiddler crab, *Ucapugilator* as a result of exposure of CdCl2.The work of Valarmathi and Azariah, (2002) indicated that LDH levels were significantly elevated and SDH activity was suppressed in the muscle, gill and hepatopancreas tissues of the crab *S. quadratum* when exposed to two sublethal concentrations of chlorine. Mayekar *et al.(*2012) studied high SDH activity and declined LDH activity in the female Crab *Scylla serrata* on treatment with sublethal dose of nickel. From the previous evident the present results also revealed that under exposure to CdNP toxicity the mud crab, *S.olivacea* showed increased dehydrogenase activity to balance the metabolic stress induced by the CdNP.

CONCLUSION

The present study concluded that exposer of CdNP to the muscles of mud crab *S.olivacea* revealed negative effect of cadmium to the aquatic system especially mud crabs.

REFERENCES

1. Ariano A, Voi AL, D'Ambola M, Marrone R, Cacace D, Severino L. Levels of Cadmium in White and Brown Meat of Warty Crab (*Eriphiaver rucosa*). J Food Prot 2015; 78: 2253-6.
2. APHA.Standard Methods for the Examination of Water and Wastewater. APHA (American Public Health Association), AWWA (American Water Works Association) & WPCF (Water Pollution Control Federation), 18th ed., Washington, DC; 1992. 1200pp.
3. Axiak V. Sublethal toxicity tests: physiological responses. In: Abel, P., Axiak, P. (Eds.), Ecotoxicology and the MARINE Environment; 1991.133–146.
4. AzraMN, and Ikhwanuddin M.A review of maturation diets for mud crab genus Scylla broodstock: Present research, problems and future perspective.Saudi J. Biol. Sci2016; 23(2): 257–267.
5. Beers RF, Jr and Sizer IW.A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase.J Biolchem1952; 195: 133-140.
6. Beyer WF, Fridovich I. Assaying for superoxide disniutase activity: some large consequences of minor changes in conditions. Anal Biochem 1987; 161:559-566.
7. Chen CS, and HY Chen.Purification and properties of carboxymethylcellulase from Antarctic krill *Euphausiasuperba*.J. Chinese Biochem. Soc 1983;12(2): 61-70.
8. Dailianis S, Kaloyianni M. Cadmium induces both pyruvate kinase and Na+ /H exchanger activity through protein kinase C mediated signal transduction, in isolated digestive gland cells of *Mytilusgalloprovincialis* (L.). J. Exp. Biol2004; 207:1665-1674.
9. Devi M, Reddy PS, Fingerman M. Effect of cadmium exposure on lactate-Dehydrogenase activity in hepatopancreas and abdominal muscle in fiddler crab, *Ucapugilator*. Comp BiochemPhysiol 1993; 106C: 739-742.
10. Gloster JA, Harris P. Observations on an enzymatic method for the estimation of pyruvate in blood.Clin.Chim.Acta, 1962; 7:206.
11. Ivanina AV, Eilers S, Kurochkin IO. Effects of cadmium exposure and intermittent anoxia on nitric oxide metabolism in eastern oysters, *Crassostreavirginica*.J. Exp. Biol 2010; 213: 433-444.
12. Kavitha R, Deepa Rani S, Sivagnanam S, & Padmaja M. Cadmium nanoparticle induced histological and biochemical changes in hepatopancreas of mudcrab *Scylla olivacea* (Herbst, 1796). JAIR2013;2(3): 205.

13. Lavradas RT, Hauser-Davis, RA, Lavandier RC, Rocha RC, Saint' Pierre TD, Seixas T, Kehrig HA, & Moreira I. Metal, metallothionein and glutathione levels in blue crab (*Callinectes* sp.) specimens from southeastern Brazil. Ecotoxicol. Environ. Saf*2014; 107: 55-60.*
14. Lawrence RA, Burk RF. Glutathione peroxidase activity in selenium-deficient rat liver. Biochem.Biophys.Res. Comm 1976; 71:952-958.
15. Moreira SM, Limaa I, Ribeiro R, Guilhermino L. Effects of estuarine sediment contamination on feeding and on key physiological functions of the polychaete*Hedistediversicolor*: laboratory and *in situ* assays. Aquat.Toxicol 2006; 78:186-201.
16. Nagata C, Nagao Y, Shibuya C. Urinary Cadmium and Serum Levels of Estrogens and Androgens in Postmenopausal Japanese Women. Cancer Epidemiol.Biomark.Prev 2005; 14: 705-708.
17. Rani SD, Kavitha R and Padmaja M. Histological and biochemical changes in reproductive organs of mud crab *Scyllaolivacea*(Herbst, 1976) exposed to cadmium nanoparticle.JAIR 1976;2(7): 391–396.
18. Reddy SP, Bhagyalaxmi A. Changes in oxidative metabolism in selected tissues of crab, *Scylla serrata*in response to cadmium toxicity. Ecotox Environ Saf 1994; 29:255-264.
19. Reish D, Oshida P. Short-term static bioassays. Part 10.FAO,Doc.Tecn. Pesca; 1987. 247- 62
20. Schrauzer GN. Selenium yeast: composition, quality, analysis, and safety. Pure Appl. Chem 2006; 78(1):105-109.
21. Shanker AK.Mode of action and toxicity of trace elements. Trace elements: Nutritional benefits. Environ Contam Health;2008;21: 525-555.
22. Sokolova IM, Evans S, Hughes FM. Cadmium-induced apoptosis in oyster hemocytes involves disturbance of cellular energy balance but no mitochondrial permeability transition. J. Exp. Biol1952; 207:3369-3380.
23. Turoczy NJ, Mitchell BD, Levings AH, &Rajendram VS. Cadmium, copper, mercury, and zinc concentrations in tissues of the King Crab (*Pseudo carcinusgigas*) from southeast Australian waters. Environ. Int2001;27(4): 327-334.
24. Valarmathi S, Azariah J. Effect of Copper Chloride on the enzyme activities of the crab *Sesarmaquadratum* (Fabricius). Turk J Zool 2003; 27:253-256.
25. Ward G, Parrish P. Toxicity Tests, Part 6. FAO, Doc.Tecn.Pesca; 1982.185: 23.
26. Weis JS, Cristini A and Rao KR. Effects of pollutants on molting and regeneration incrustacea. Am. Zool1992; 32:495-500.
27. Weis J, Weis P. Effects of contaminants from chromate copper arsenate-treated lumber on benthos. Arch. Environ. Contam.Toxicol1994; 26: 103–109.
28. White SL and Rainbow PS. Regulation and accumulation of copper, zinc and cadmium by the shrimp *Palaemonelegans*.Mar. Ecol. Prog. Ser1992; 8:95-101.

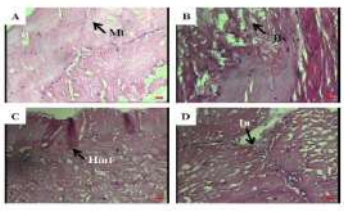

Fig. 1. Effects of CdNP (20ppm) on the anatomy of muscles in *Scylla olivacea by* light microscope on day 2. Scale bar, 10µm. Mt- Muscle Tissue; Bs- Basal surface; In- Intima; Hmt- Hyalinised muscle tissue;

A) Control (Male) B) CdNP treated (Male)
C) Control (Female) D) CdNP treated (Female)

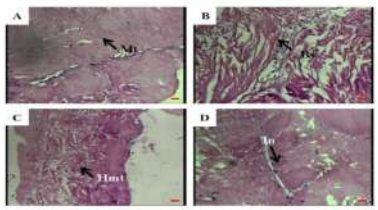

Fig. 2. Effects of CdNP on the anatomy of muscles in *Scylla olivacea* by light microscope on day 4. Scale bar, 10µm. Mt- Muscle Tissue; Bs- Basal surface; In- Intima; Hmt- Hyalinised muscle tissue;

A) Control (Male) B) CdNP treated (Male)
C) Control (Female) D) CdNP treated (Female)

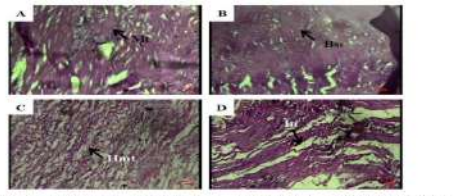

Fig. 3. Effects of CdNP on the anatomy of muscles in *Scylla olivacea* by light microscope on day 6. Scale bar, 10µm. Mt- Muscle Tissue; Bs- Basal surface; In- Intima; Hmt- Hyalinised muscle tissue;

A) Control (Male) B) CdNP treated (Male)
C) Control (Female) D) CdNP treated (Female)

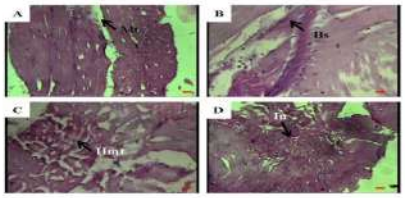

Fig. 4. Effects of CdNP on the anatomy of muscles in *Scylla olivacea* by light microscope on day 8. Scale bar, 10µm. Mt- Muscle Tissue; Bs- Basal surface; In- Intima; Hmt- Hyalinised muscle tissue;

A) Control (Male) B) CdNP treated (Male)
C) Control (Female) D) CdNP treated (Female)

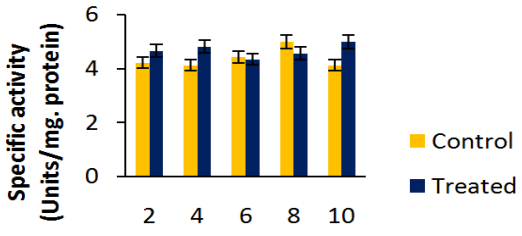

Fig. 5a

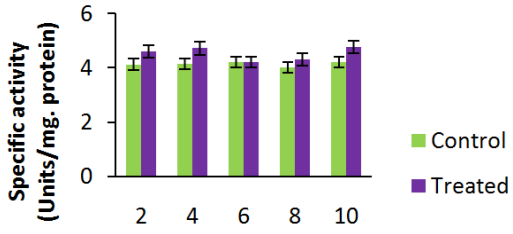

Fig. 5b

Fig. 5. a and b. Catalase activity (CAT) in muscle of *S. olivacea* after exposure of CdNP. a) male; b) Female

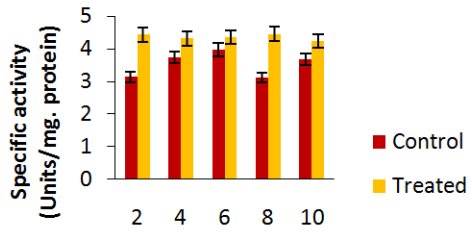

Fig. 6a

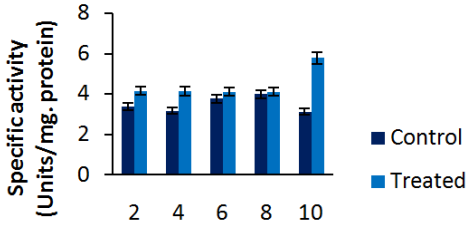

Fig. 6b

Fig. 6. a and b.Glutathione peroxidase (GPx) activity in muscle of *S. olivacea* after exposure of CdNP. a) male; b) female

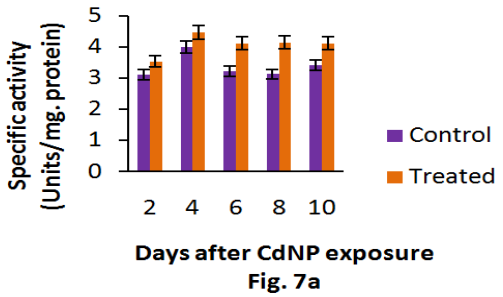

Fig. 7a

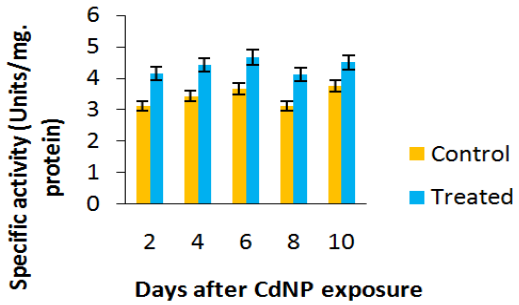

Fig. 7b

Fig. 7. a and b. Superoxide dismutase (SOD) activity in muscle of *S. olivacea* after exposure of CdNP. a) male; b) female

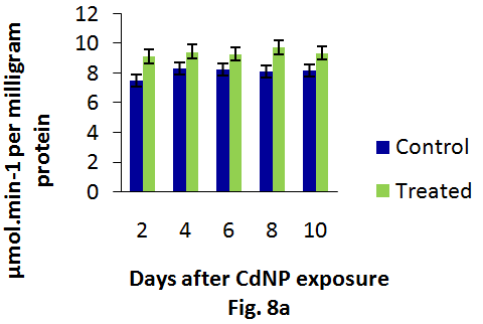

Fig. 8a

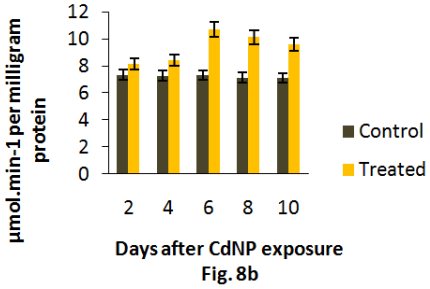

Fig. 8b

Fig. 8. a and b. Alkaline phosphatase (ALP) activity in muscle of *S. olivacea* after exposure of CdNP. a) male; b) female

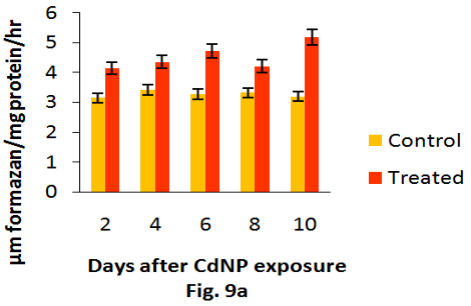

Fig. 9a

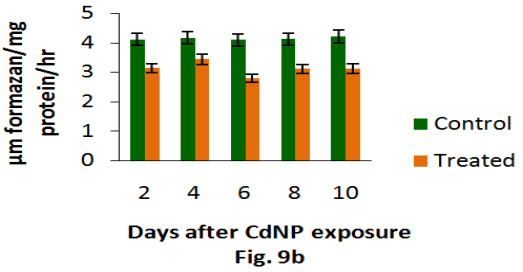

Fig. 9b

Fig .9. a and b. Lactate dehydrogenase (LDH) activity in muscle of *S. olivacea* after exposure of CdNP. a) male; b) female

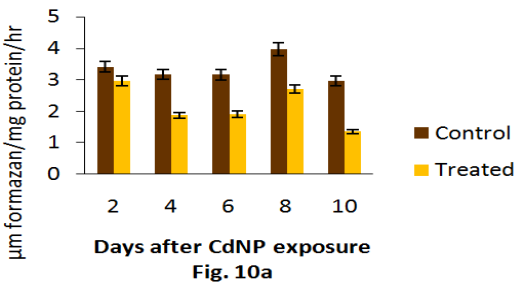

Fig. 10a

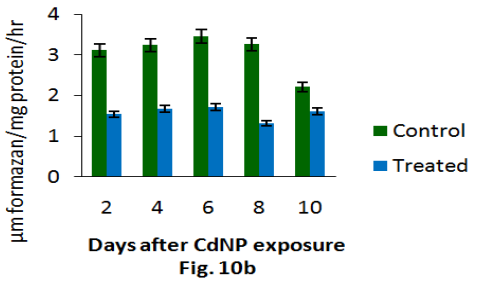

Fig. 10b

Fig.10. a and b. Malate dehydrogenase (MDH) activity in muscle of*S. olivacea* after exposure of CdNP. a) male; b) female

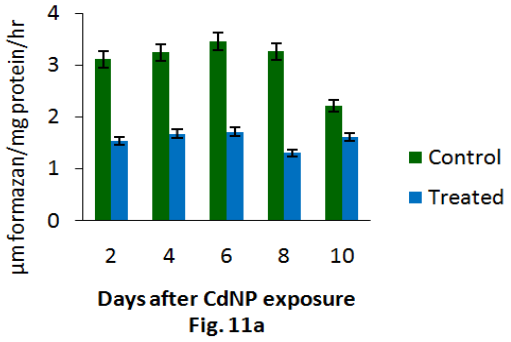

Fig. 11a

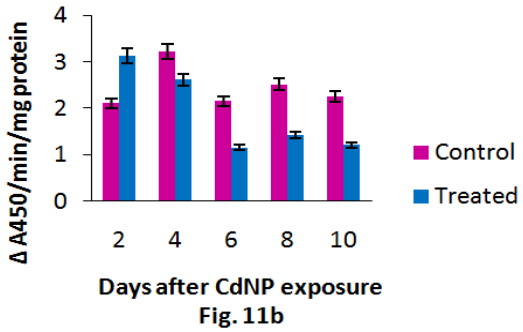

Fig. 11b

Fig.11. a and b. Succinate dehydrogenase (SDH) activity in muscle of *S. olivacea* after exposure of CdNP. a) male; b) female

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RESEARCH ARTICLE

Performance of Consumers While Shopping for Grocery Online

Satya Kishan[1]* and Sunny Motwani[2]

[1]Assistant Professor, Research Guide, MSBS Department, MATS University, Raipur, Chhattisgarh, India.
[2]Research Scholar, MSBS Department, MATS University, Raipur, Chhattisgarh, India.

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***Address for Correspondence**
Satya Kishan,
Assistant Professor,
Research Guide, MSBS Department,
MATS University, Raipur,
Chhattisgarh, India.
Email: imsatyakishan@gmail.com

ABSTRACT

Nowadays, people may purchase practically any goods online. Online grocery and nutritionist shopping is becoming popularity. The purpose of this essay is to discuss online grocery shopping practices and customer perceptions of online food purchases. The evaluation is supported by secondary data sources. Online grocery shops allow customers to access a wide variety of stores and goods from across the world without being constrained by location or business hours. Convenience and time savings are the two biggest benefits of online shopping, while the danger of improperly valuing some items and concern about the choice and handling of perishables, such vegetables, fruits etc are the two biggest drawbacks for customers.

Keywords: Customer Perception, Grocery, Online, Shopping

INTRODUCTION

With market development, consumers' position in the market is evolving. The Latin term consumens, which meant to buy (goods or services) for immediate use or possession, is where the English word consumer first appeared (Rybowska, 2010). Particular focus is placed on "consumer behaviour" and its determinants in the discipline of consumption theory and practise study. "Consumer behaviour is a process that comprises pre-, during-, and post-consumption phases," claims Salomon. Consumer behaviour is tied to contacts with charitable groups and governmental entities in addition to commercial concerns. Consumers choose, purchase, and use products, services, and experiences in a variety of ways (Solomon, 2013, p.2). E-commerce is growing in popularity among customers looking for the best ways to acquire products and have access to information about them as well as among businesses interested in expanding their market share (Karpiska-Krakowiak, 2014).According to European Union's statistical office reports that from 30% in 2007 to 53% in 2015, customers between the ages of 16 and 74 have made online purchases of products and services for their own use (Eurostat, 2015). The majority of internet shoppers are

happy with their purchases, with 70% claiming there are no issues. The top three online purchases in 2015 were lodging and travel (selected by 52% of e-buyers), domestic goods and toys (selected by 60% of e-buyers), and clothing and athletic goods (41 percent). The countries with the highest percentages of internet shoppers include the United Kingdom (81% of those aged 16 to 74 do so), Denmark (79%) and Luxemburg (79%). (78 percent).According to Eurostat (2015), younger generations (16–44 years old) prefer online purchasing over older generations (45–74 years old).Customers develop their skills in the virtual world. Many new items that were previously only partially accessible, including fresh food products, are now widely available online. Several agencies have predicted that people are increasingly likely to purchase for groceries online. Numerous online supermarkets exist, including Alma, Piotr I Pawe, Tesco, Auchan, E. Leclerc, frisko.pl, and dodomku.pl, where customers may obtain practically all of the nutritious items they want (Karpiska-Krakowiak, 2014).With research based on secondary information sources, the purpose of this study is to illustrate the circumstances of online grocery shopping and customers' attitudes regarding purchasing food online. Therefore, particular contrasts between online and traditional food shopping are discussed in this article, along with the benefits and drawbacks of doing so from the perspective of the customer.

The Characteristics of Online Groceries Shopping

One of the modes of trade that is most rapidly increasing is online purchasing (Ramus and Nielsen, 2005). Consumers in industrialised nations, like those in the USA or portions of Europe, have utilised the Internet for daily shopping, and both those regions of the world are seeing a yearly rise in the popularity of online grocery shopping (Bianchi and Andrews, 2012)As a result, there is growing curiosity about how consumers behave when they purchase for groceries online. In order to build an effective design and marketing strategy in the online shopping environment and to gain a competitive advantage, managers of Internet grocery shops search for answers to a number of problems related to customer behaviour (Andrews and Currim, 2004; Rohm and Swaminathan, 2004**)**. The characteristics of online grocery shopping are remarkable, with consumer attitudes and behaviour in this sector being distinct from those displayed while purchasing more complex items. Consumers are less engaged while buying nutrition articles than they are when buying other items. Consumers have greater opportunity to verify and evaluate items since routine, regularity, and repeatability of nutrition purchases are higher while the social and financial risk is reduced.

These circumstances frequently lead to spontaneous and unforeseen purchases (Walters *et al.,* 2005). The marketing experts employ a variety of techniques to persuade customers to purchase their goods, particularly when customers don't actually need them. The conditions that affect customer purchase decisions are significantly influenced by electronic food distribution systems. Customers can purchase anything from supermarket e-stores at any time and from any location; there are no restrictions based on location or business hours. Compared to conventional supermarkets, the purchase procedure is quicker. Compared to their initial visit to a typical shop, customers who have some familiarity with traditional shops do not need to spend as much time "getting to know" the range. Online grocery store selections are comparable to those seen in conventional markets. Sometimes a product category has a smaller selection of brands, which encourages customers to purchase store own-brand items. The pricing at different electronic supermarkets are not much different (about 7 percent including delivery costs). The delivery scheduling procedures vary depending on the specific establishments. For instance, some businesses do not allow customers to purchase a product and then get it the same day. In addition, during the holiday season, customers must request delivery service at least two weeks in advance (Karpiska-Krakowiak, 2014).

The attitude of consumers toward online grocery shopping

For customers to purchase food online, they must make a number of choices, such as the choice of the shopping network and location, budgetary considerations, creating shopping lists, or selecting product brands. There are two methods in literature for examining consumer choices. The first is related to how logically consumers behave. This theory contends that customers act rationally and want to maximise their utility function or pleasure (in economic terms). Sociologically speaking, they follow the hierarchy established by the social group to which they belong, and philosophically and psychologically, they seek rational justifications for their own acts**.** Wisdom, resoluteness, internal reliability, and consideration of alternatives all define their behaviour. The second set of theoretical ideas has

to do with the irrationality of human decision-making. The framework of this theory identifies a number of elements, such as uncertainty, risk, time constraints, and information access that restrict the most advantageous purchase decision. The consumers' actions are unpredictable, emotionally charged, impulsive, careless, or hurried. According to several studies, consumers purchase frequently used commodities, such as food, with a relatively large proportion of impulse and emotion. However, they make sensible selections when it comes to high interest goods**.** Many of the customers who responded to the survey confess that they often make rash, emotional, and impulsive food purchases. Various sales tactics, such as sales, coupons, additional discounts with a quick expiration date, or free services like delivery, might cause instant impulsive buying. (Karpiska-Krakowiak,2014) An e-profile shopper's has been defined in several research, particularly in terms of their demographic and psychographic characteristics.

There have been hints that online customers are wealthier, less risk-averse, and more impulsive and inventive than non-online shoppers. These customers prefer that online stores are available 24/7 and think that doing their shopping from home is enjoyable, simple, and time-saving. (Ramus and Nielsen ,2005) The convenience of online purchasing, according to customers, is the key factor influencing and benefiting from the success of online businesses. Online buying convenience comes in a variety of forms, including those for access, search, assessment, transaction, and possession. Consumers may buy whenever they want and from any location, including their home or place of business; they don't even need to leave their rooms. They get access to goods that aren't often offered at regular stores and can bypass lines and wait periods. The ability to explore and compare items without physically visiting establishments is connected to search convenience. Recently, there have been a number of advancements achieved in the evaluation of the ease of online buying. They get access to goods that aren't often offered at regular stores and can bypass lines and wait periods. The ability to explore and compare items without physically visiting establishments is connected to search convenience. Recently, there have been a number of advancements achieved in the evaluation of the ease of online buying. The majority of the adjustments centre on improving product descriptions by using better presentation tools, such images and videos. Peer review systems also increase the effectiveness of online goods purchases. Such consumer evaluation platforms facilitate quick, efficient, and painless purchasing decisions. Convenience of transactions is related to simplicity and ease of online payment. Online retailers are aware that convoluted payment processes frequently lead to consumers cancelling last-minute purchases. Because internet buying is more convenient, people spend less time and effort making purchases. Customers do not need to leave their homes or wait in large lines to shop (Jiang, Yang & Jun 2013). Customers complain that it can be challenging to shop at a regular grocery store sometimes due to factors like not having a car, not having the time, or not having the physical stamina to handle large products (Huang and Oppewal, 2006).According to the research, the ability to purchase groceries online and have them delivered saves consumers the most time because they have to visit traditional retail outlets less frequently (Verhoef and Langerak, 2001; Ramus & Nielsen, 2005). Customers who buy for groceries online benefit from having access to a variety of merchants and goods from across the globe (Ramus and Nielsen, 2005). It is important to keep in mind that the ability to save time travelling may have a higher influence on customer preferences for online grocery shopping than the amount of delivery fees (Huang and Oppewal, 2006).

However, there are certain drawbacks to doing your food shopping online. One is connected to the possibility that some items will be incorrectly assessed. It can be challenging to determine a product's real size, weight, and value when online product photos are of poor quality or lack altogether. Due to the lack of a chance to taste and smell a product during store presentation, consumers are less likely to purchase new items online (Karpiska-Krakowiak, 2014). It is also highlighted that several personal demands, such as consumers' desire for sensory stimulation and physical activity and their interest of learning while buying, are limited when conducting online purchasing. Similar circumstances apply to social requirements, such as the need for customers to engage with others, enjoy haggling while purchasing, and communicate with other consumers. However, there is the opportunity to join online forums where e-buyers may interact with others, which can serve to partially replace the joy of conventional shopping. Other drawbacks of online shopping for groceries are the high expenses of searching and the lengthy delivery wait times (Verhoef and Langerak, 2001). Concerns concerning the selection and handling of perishables, such as produce, eggs, and meat items, play a significant role in determining customers' inclination to purchase groceries online.

Knowing a product's expiration date is necessary for purchasing fresh goods, but doing so online is impossible (Galante, *et al*., 2013. Toomey and Wysocki, 2009).Prices on the internet are typically thought to be lower, however e-consumers may lose out on exclusive discounts in brick and mortar stores. Online buyers are likewise concerned with the issue of returning goods that are damaged or fall short of expectations. It is important to recognise that many customers prefer traditional shopping. They recognise that going grocery shopping has a social draw. Shopping is a popular activity for groups of friends and family to do together According to the research of non-Internet buyers, some people have reservations about using online payment methods. These are sceptical of the security of payment systems and do not wish to use their credit card information or other private information to make purchases online (Ramus and Nielsen, 2005).

The majority of survey respondents are generally happy with their experiences buying groceries online, according to various surveys. Many different customer demographics purchase online for various reasons. Mothers with small children in the home are one such category. Online shopping enables people to get wholesome items without having to bring small children to the store or find babysitters for their kids while they shop. Customers who shop for groceries online can avoid being persuaded by their kids to buy sweets. Consumers with physical limitations are the next category that has a lot of nice things to say about online grocery shopping because they like not having to lift and carry heavy products. It should be emphasised that many nations have an ageing population, which will likely lead to a rise in minor physical limits and an increase in the need for home delivery and shopping services (Morganosky and Cude, 2000).

CONCLUSION

One of the business sectors with the fastest growth is e-commerce. Nearly everything may be purchased online by consumers. According to several research, internet grocery shopping is becoming increasingly popular. Online shopping and conventional shopping differ greatly in this regard. Customers who purchase at supermarket e-stores do not have to contend with geographic or business-hour restrictions. More quickly and easily, they may purchase goods. The ability to save time (by not having to leave the house and stand in lengthy lines) is, in the perspective of customers, the most significant benefit of online grocery shopping. However, because of the subpar website presentation, there is a chance that you may overestimate the value of some goods when doing your grocery shopping online. Additionally, online buyers miss out on the chance to adjust their selections in accordance with product expiration dates. It should be recognised that certain demands, such as those for sensory stimuli or social interaction, are constrained while purchasing online. According to studies, people generally have good sentiments regarding doing their grocery shopping online and are happy with the overall experience. However, many customers still have reservations about making electronic payments and disclosing their personal information.

REFRENCES

1. Andrews, R. L., & Currim, I. S. (2004). Behavioural differences between consumers attracted to shopping online versus traditional supermarkets: implications for enterprise design and marketing strategy. Int. J. Internet Marketing and Advertising, 1(1), 38-61.
2. Bianchi, C., & Andrews, L. (2012). Risk, trust, and consumer online purchasing behaviour: A Chilean perspective. International Marketing Review, 29 (3), 253-276
3. Eurostat (2015). E-commerce by individuals, Retrieved from http://ec.europa.eu/eurostat/documents/2995521/ 7103356 /4- 11122015-AP-EN.pdf/276b6a7c-69a6-45ce-b6bf-488e975a8f5d (19.02.2016)
4. Galante, N., López, E. G., & Monroe, S. (2013). The future of online grocery in Europe. McKinsey & Company, 22-31.
5. Huang, Y., &Oppewal, H. (2006). Why consumers hesitate to shop online. An experimental choice analysis of grocery shopping and the role of delivery fees. International Journal of Retail and Distribution Management 34 (4/5), 334-353.

6. Karpińska-Krakowiak, M. (2014). Consumer behavior while grocery shopping on-line — research propositions. Marketing i Rynek 6, 20-26. Jiang, L., Yang, Z., & Jun, M. (2013). Measuring consumer perceptions of online shopping convenience. Journal of Service Management, 24 (2), 191-214.
7. Morganosky, M. &Cude, B. (2000). Consumer response to online grocery shopping. International Journal of Retail & Distribution Management, 28(1), 17-26.
8. Ramus, K., & Nielsen, N. (2005). Online grocery retailing: what do consumers think? Internet Research 15(3), 335-352. Rohm, A. J., & Swaminathan, V. (2004). A Typology of Online Shoppers Based on Shopping Motivations. Journal of Business Research, 57, 748–757.
9. Rybowska, A. (2010). Internet shops client's shopping behaviour. Research Journal, Gdynia Maritime University, 65, 23.
10. Solomon, M. R. (2013). Consumer Behavior, Buying, having being, 2. Toomey, A. C., &Wysocki, A.F. (2009). Distinguishing between Traditional and Online Retailing: Evaluating E-commerce with Respect to the Food System. Food and Resource Economics Department, 1-4.
11. Walters, D. M., Toase, M. C., Hong, P., &Mackel, M. (2005). A Survey into Consumer Experiences and Attitudes Towards Online Grocery Shopping in The UK. IADIS International Conference on WWW/Internet 2005, 236-244.
12. Verhoef, P., &Langerak, F. (2001). Possible determinants of consumers' adoption of electronic grocery shopping in the Netherlands. Journal of Retailing and Consumer Services, 8, 275-285

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RESEARCH ARTICLE

Microbiological Analysis of Drinking Water from the Villages of Anand District of Gujarat State in India

Navneet Kumar Singh[1], Dharti Parmar[2] and Riteshkumar Arya[3]*

[1]Associate Professor, Department of Paramedical Sciences, Sumandeep Vidyapeeth Deemed University, Gujarat, India
[2]Dr. Indravadan P. Patel Institute of Medical Technology and Research, Anand, Gujarat, India
[3]Assistant Professor, Department of Microbiology, Mehsana Urban Institute of Sciences, Ganpat University, Gujarat, India.

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***Address for Correspondence**
Riteshkumar Arya,
Assistant Professor,
Department of Microbiology,
Mehsana Urban Institute of Sciences,
Ganpat University, Gujarat, India
Email: rra02@ganpatuniversity.ac.in

ABSTRACT

Water is transparent, colorless, tasteless inorganic vital chemical for all forms of life, and used widely by everyone as their routine life practices. Ground water maintains its viability by natural filtering mechanism and used for drinking purpose but it can be contaminated by pathogens, due to sewage contact, therefore Assured drinking water is very important for prevention of water borne diseases. In this study we analyzed drinking water specimens for Bacteriological analysis from different villages of Anand district, Gujarat, India. Total 50 water samples in sterile container were collected from rural areas randomly with precaution from the tap water for analysis. For the Identification of the bacterial population from the collected drinking water samples we have used most probable number count (MPN) method. Along with it the commercial available testing kit from Hi Media were also used, it is the modified testing procedure which gives the presence of bacterial pathogen in a single step procedure with color change to black, it will further cultured for further step analysis. It is advantageous as it provides easy processing of sample.
Out of 50 specimens 35 (70%) samples are contaminated from the bacteria. 8 different bacterial species were isolated, *E. coli, Shigella, Proteus*, *Klebsiella*, *Serratia*, *Pseudomonas*, *S. aureus* & Enterobacter were identified from the water specimens. The maximum presence in this study shows bacteria belonging to the family enterobacteriaceae. Presence of these pathogenic contaminants in water may lead to illness & related diseases. The study finding suggest that poor sanitization &

unawareness to hygiene leads to contamination of water in rural habitations regions in Anand District

Keywords: Bacteriological analysis, MPN count, Anand, *E.coli, Pseudomonas* species

INTRODUCTION

Water borne ailments are critical concern in developing countries like India and others, here water resource and its sanitation amenities do not attentively examined in accordance to the population increase, development and industrial development. According to the New York Times, one-half of India's population excretes outdoors, which is a major cause of young children's stunted growth due to poor sanitation and hygiene conditions, which also contribute to chronic bacterial infections caused by coliforms and further results in poor nutrient absorption from food sources [1]. Total water usable on our earth is fresh water, of which 68.0% is groundwater and 30.0% surface water, In developing countries, 90.0% to 95.0% of all sewage and 70.0% of all industrial wastes are dumped untreated into surface waters. Approximately 22% of the fresh water found on the Earth's global surface is stored as groundwater. Ground water maintains its quality by the natural filtering mechanisms [2].

Pathogen free drinking water may control the waterborne infections, but in India safe water supply is very difficult. As per the World resources report s 70.0% of Indian water is polluted due to sewage contamination. As per the United Nations report quality of water in India is very poor and positioned 120/122 in nationwide data. Bacterial contamination are most significant problem correlated with sewage contamination, [2] Which leads to eruptions of water related illness, a leading cause of common water borne infections. Microbiological examinations play a very important roles in the screening of waterborne outbreaks. 60% water born transmitted diseases are due to insufficient water supply which indicate effects on our health status and life quality greatly impact the socioeconomic development of the nation[3]. Sewer water characterization has been done routinely, conducted by various scientific groups across the world. The present work is a attempt to analyze the water quality of potable water sources used in Anand district for drinking purpose[5]. Hence, Present study will show the quality of water portability and the contaminants in Anand district.

METHODS AND MATERIALS

Study Locality: in western India of Gujarat state, Anand district is popular area known as Milk city of Gujarat state and is identified as Charotar Pradesh. It is fulfilled with major and useful natural resources. It is surrounded by Kheda district in north, Vadodara district in east, Ahmadabad in the west, and the gulf of Khambhat in south. Anand district has the population of 20, 92,745 approx. give it a ranking of 219 in India out of a 640. Population growth rate over the decade was 12.57%. It Is administratively divided in eight separate talukas which are Anklav, Anand, Borsad, Petlad, Tarapur Khambhat, Sojitra, and Umrethh. In the present study water samples are collected from the Petlad and Anand regions as shown in the map. Total 50 drinking water specimens from different localities (like tap water, well water and bore well water) were aseptically and precautionary measurements were collected in sterilized containers from the different areas of villages of Petlad and Anand district. The water samples were processed within maximum limit of an hour for microbiological processing. The collected water was processed with extreme care to avoid contamination from the environment. The process is done by the tap water is voided first 2 to 3 min. and then the free flow of water is collected in the collection bottles. All the aseptic measurements were carried during collection of samples. The water containers bottles were kept in airtight containers with tight lead with ice. Water samples were transported immediately to laboratory for the early processing.

There are two methods are available for examination
1. Membrane filtration Technique and
2. Multiple tube test (M.P.N) method

M.P.N. is a method that is frequently used to evaluate the quality of water samples. It checks the bacterial count to see if the water is safe or not. In this study, the M.P. N approach is employed. After sub culturing on the broth medium with tenfold dilution, it is a technique used to determine the concentration of bacterial pollutants in a particular water sample.

Principle

Testing Water samples are diluted, then inoculated to the culture medium lactose broth, if bacteria grows they use the nutrients in the broth medium and produces acid with gas as metabolite. Due to acid production broth color is changed and gas is detected as air gaps in the durham's vial kept inverted position in bottommost of test tube. The total no. of *coli* forms is identified by the calculating the tubes giving positive changes with acid and gas formation and it is compared to the standard statistical tables for interpretation. M.P.N test is three step process including first Presumptive test followed by Confirmatory test and finally Completed test.

Presumptive test

It is a screening test used to determine whether *coli*form is present in samples of drinking water. Some prerequisites are Lactose broth, Mac-conkey broth, and Lauryl tryptose lactose broth are used as the culture medium (any one). Different-sized test tubes are utilised. sterilised pipettes/Micropippetes, Durham vials, and 5 ml, 10 ml, and 20 ml tubes are utilised.

METHODOLOGY

Single (N) and double strength (2N) concentrations of broth are made for use as a culture medium. Ten identically sized tubes (10 ml each) of double strength (2N) broth and five identically sized tubes (10 ml each) of single medium (N) are used for inoculation. The Durham vial is kept inverted in all of the broth medium. Durham's vial must be examined to ensure there are no air bubbles inside. When necessary, autoclaving is done for sterilization purpose.
1. To test water samples, 5 sets of double-strength test tubes (2N) and 10 sets of single-strength test tubes (1N) are utilised.
2. To each of the five test tubes containing the 10 ml (2N) medium, 10.0 ml of the testing water sample is added using a sterile pipette or micropipette.
3. The remaining 5 tubes, each holding 10 ml of (2N) medium, get 0.1 ml of water and the 5 tubes containing 1 ml of the testing water sample.
4. 24-48 hours are spent incubating the test tubes at 37°C.

Confirmed test

After lactose fermentation, a few additional bacteria besides coliforms are also creating acid and gas. This test is frequently carried out to confirm the presence of *coli* form bacteria. One wire loopful of suspension is added to the growth medium from each of the tubes that produced favorable findings. One wire loopful of suspension is added to the growth medium from each of the tubes that produced favourable findings. 3 ml of lactose broth in a brilliant green lactose broth tube, N. Agar slant, and tryptone water medium. .All the inoculated test tubes and agar slants are checked for colour change, gas generation and colony formation on the slant after incubated at 37°C for 24-48 hours. Gram staining must be done from the growth on the N. Agar slants for observing gram positive or gram negative bacteria. A member of the coliform group from the tested water sample is detected by Gram-staining revealing Gram negative rods on the appropriate agar slant while lactose broth medium is being tested for gas formation. Gram-negative bacteria are evident in lactose broth because there is gas and colour change, If the test is positive, it verifies the presence of coliform bacilli in the provided water sample. Additional confirmation is done from the gas positive slants by biochemical assays such the indole formation test.

Completed test

Since false positive findings have been noted occasionally, completed tests must be carried out. On a solid plate of Eosin Methylene Blue (E.M.B. Agar medium or Endo agar), the test inoculum from positive tubes of the confirmatory test is streaked. A wireloopful of sample from each positive Mac-conkey lactose broth tube is streaked onto a selective medium, such as an Eosin Methylene Blue agar plate, for this procedure. For the Eijkman's Test, the first plate is incubated for 24 hours at 37°C and the second at 44.0°C. After 24-48 hours, all plates are checked for the presence of characteristic bacterial colonies. Bacterial growth with a greenish metallic sheen is produced by coliforms. It is simple to distinguish it from colonies of non-coliforms without a metallic shine. When normal colonies grow at a high temperature (44.0 °C), Escherichia coli is present and thermotolerant (Eijkmen's test is positive).

Commercial kit technique

"Hi water testing kits" from the Hi Media company were used for this procedure. Rapid H2S producers can be found with this kit in a single step. It is a dependable procedure that is also quite simple. The kit test bottles are made up of rolled filter buds that have been dried, transferred to glass bottles, and sterilised. Testing water sample is placed in the vial to the designated level, and the rolled filter buds are allowed to soak. If necessary, the vial is then gently shaken. The water will turn from yellow to brown upon discharge of the blossom. For 24 to 48 hours, keep it at 35 to 37 degrees. After incubation, look for blackening of the contents. Water is not considered potable if it becomes black. After the autoclaving discard the testing water if it has coliform.

Principle and applications

Fecal pollution is typically to blame for the widespread outbreak of water-borne sickness. The World Health Organization advises routine testing of drinking water for salmonella spp. bacteria and thermotolerant coliforms. Contaminant analysis using culture methods is a four stage process that takes days to complete in order to confirm its total absence. As a result, this method was discovered utilising the H_2S strip (K055) testing kit, which is based on comparable lines for the identification of coliforms that produce H2S.

Observation chart

After incubation for 24-48 hrs. If color of the medium is changed to black color with its intensity. The color change with different shades of blackness indicates the presence of bacterial contaminant in the drinking water like salmonella sp. and citrobacter sp. Tested water samples are not safe for drinking purpose & this finding is to be further reported & confirmed from the nearest authorized district public health laboratory. interpretation is done as, Negative = no H_2S production, + = poor H_2S production, ++= fair H_2S production, +++ = good H_2S production, ++++ = heavy H_2S production Limitations: Presence of proteus spp. in water can exhibit blackening of water also,

Characterization of isolates: By observing following characteristics.

1. colony morphology, isolates shows different type of colonies on plate's surface observing their size, shape, texture, elevation & optical density.
2. Gram stain & motility were performed as primary identification.
3. Biochemical characterization-

As the secondary identification MR-VP, TSI test, citrate, PPA, oxidase, catalase, indole production test, urease test, etc. were performed according to Bergey's manual literature.

RESULTS

The results of drinking water microbial testing were presented in given table no.1. It shows that water contamination is find in the present analysis. Total 50 water samples were collected from the different villages and important locality of Anand district from protected wells and water reservoir used for drinking purpose. Out of all 50 analyzed water samples, 35 of them contain the different contaminant microbes. The isolates are *E.*

coli (28%), *Pseudomonas aeruginosa* (26%), *Shigella* Spp. (2%), Enterobacter (6%), *Klebsiella* (16%), *Proteus* sp. (2%), *Serratia* sp. (2%) and *S. aureus* (2%) were identified from the contaminated samples. *E. coli* and *Pseudomonas* showed higher occurrence whereas *Klebsiella* spp. also showed significant presence in our study. *E. coli* is responsible for UTI, traveler's diarrhoea, food borne disease, vomiting, pulmonary infections, abscesses etc. Supply of water shows that health depends on routine consumption and use of water in everyone's life style.

So, It is of utmost importance for priority must be given to assortment of the pure source of drinking water. Bacteriological quality of drinking water and its distribution is essential to public health for their good health. The study also comparatively analyses the municipal or gram panchayat supply water for qualitative estimation of the respective or particulate organism. According to the Indian standards IS – 1622 (1981), not a single sample should contain *E. coli* as well as other coliform organism more than 10 per 100ml. The presence of bacteriological quality of all the samples is shown in table 1.In present study the water samples showed higher significant percentage of *E. coli* and the *Pseudomonas* species. Where the presence of E.coli and *Pseudomonas* species (28%) and *Klebsiella* spp. (16%) shows the fecal contamination and is majorly responsible for the endemic outbreaks whereas the *Pseudomonas* species is the normal flora but is also an opportunistic pathogen. In our study *Pseudomonas* aeruginosa and *Pseudomonas* otitidis is identified. The presence of *S. aureus* and *Shigella* species may also be pathogenic responsible for several health issues, food spoilage, abscesses etc.

DISCUSSION

A significant global health issue is the bacterial contamination of drinking water in the water distribution system. Because of this, study academics have recently shown a great deal of interest in the correct examination and administration of water distributing systems. According to Ambilli M & Denoj Sebastian carried out the microbiological analysis of potable water in Kerala. The majority of the water-borne microorganisms they discovered were from a variety of sources with various samples, including Escherichia coli, *Klebsiella* species, *Enterobacter* species, *Citrobacter* species, *Shigella* species, and *Pseudomonas* species. Another study conducted in rural areas of northern Rajasthan by Surinder Suthar and Sushma Singh discovered a wide range of enteropathogenic bacteria, including *E. coli*, *E. aerogenes,* and *Klebsiella* species, among others, measured with standard plate counts ranging from 8.3x104 to 28.3x104 cfu/ml. All over the other region this type of examination should have to be done and reported study reveals about the various causative agents and presence of endemics and their respective pathogen. According to earlier investigations, *E. coli* is the primary, significant risk factor, and causal agent, similar finding is observed in the current study. High bacteria counts were discovered in various ground water samples as a result of the microbial load, particularly the coliforms present in water. These results concur with those of Esharegoma *et al.* (2018), who discovered that microbial counts were higher during the rainy season than during the dry season.

High bacterial counts were found throughout our analysis, which may be a sign that water contamination occurs more frequently during the rainy season than the dry season. Many investigations have revealed the presence of various bacterial populations. Members of the Enterobacteriaceae family, which includes *E. coli* and *Klebsiella* species, are recognised to be pathogens that can be found in soil and drinking water. According to various papers, *Pseudomonas* spp. in drinking water is also a serious concern. *E. coli* is a known bacteria that causes bloody diarrhoea in humans and is a common cause of UTI in females. In this investigation, *P. otitidis*, a member of the *Pseudomonadaceae* family, was found to induce otitis media in patients. (Rajanbir Kaur and others) Other pathogens, such as *Klebsiella* species, Proteus species, *S. aureus*, and others, are major concerns today because they can cause infections in humans as a opportunistic infection. Such study is very important study revealing proper control of water borne pathogens is necessary in view after COVID pandemic in Anand district situated in Gujarat state.

CONCLUSION

This study concluded that 70% water sources out of 50 water sample process containing the variable enteric pathogens from the 35 contaminated samples as the *E. coli* (28%) is present in significant amount precise the fecal contamination. Other bacterial flora is also identified which are *Pseudomonas* species *Klebsiella* species, *Enterobacter* species, and *Shigella* species, etc. are the predominant water borne microbes may be cause serious infection as opportunistic infections. Public awareness and precautionary measurements among the Anand population for the sanitation and hygienic condition for storage drinking water is needed to encourage the use of contamination free water in Anand District.

REFERENCES

1. Worley H. Water, sanitation, hygiene, and malnutrition in India. Washington, DC: Population Reference Bureau; 2014.
2. R.K. Mishra, S.C. Dubey, Fresh water availability and its global challenge, International Journal of Engineering Science Invention Research & Development; Vol. II Issue VI December 2015.
3. Karnwal, A., Dohroo, A., & Mannan, M. A. U. (2017). Microbial analysis of potable water and its management through useful plant extracts. International Journal of Science and Research, 73, 44-49.
4. Suthar, S., Chhimpa, V. & Singh, S. Bacterial contamination in drinking water: a case study in rural areas of northern Rajasthan, India. Environ Monit Assess 159, 43 (2009).
5. Kaur R, Singh D, Kesavan AK, Kaur R. Molecular characterization and antimicrobial susceptibility of bacterial isolates present in tap water of public toilets. Int Health. 2020 Sep 1;12(5):472-483.
6. Nazira S. et. al. , "The Efficacy of Treated Water from Water Filtration Machines for Safe Drinking Water Supply in Bandar Baru Bangi and Kajang, Selangor, Journal of Food Quality, vol. 2020, Article ID 3536420, 9 pages, 2020.
7. Sebastian, Denoj & Monacha, Ambili. (2015). MICROBIOLOGICAL ANALYSIS OF DRINKING WATER FROM MALABAR REGION OF KERALA. Conference proceedings.
8. ER-World Health Organization. WHO Guidelines for Drinking-Water Quality, 4th ed.; WHO Library Cataloguing-in-Publication Data, Malta Publisher: Gutenberg, Salt Lake City, UT, USA, 2011; pp. 1–541. ISBN 978924154815 1. Available online: http://www.who.int (accessed on 18th June 2022).
9. Bhat, B., Parveen, S., & Hassan, T. (2018). Seasonal assessment of physicochemical parameters and evaluation of water quality of river Yamuna, India. Advances in environmental technology, 4(1), 41-49.
10. Esharegoma O.S. *et al*. (2018) Microbiological and Physiochemical analysis of orogodo river, agbor, delta state, Nigeria. International journal of ecological sciences and environmental engineering 5 (2) 34-42

Table 1: Finding of Bacterial Species in Villages of Anand District

S. No.	Village/ town with code	Bacterial species							
		E.C	P.S (P.A+P.O)	K.P	P.M	S.S	S.A	E.A	S.S
1	IP-01	+	-	-	-	-	-	-	+
2	IP-02	-	-	-	-	-	-	-	-
3	IP-03	-	-	-	-	-	-	-	-
4	IP-04	-	-	-	-	-	-	-	-
5	IP-05	+	-	-	-	-	-	-	-
6	IP-06	+	-	-	+	-	-	-	-
7	IP-07	-	-	+	-	-	-	-	-
8	IP-08	-	-	-	-	-	-	-	-
9	IP-09	-	-	-	-	-	-	-	-
10	IP-10	-	-	-	-	+	-	-	-

11	IP-11	+	-	-	-	-	-	-	-
12	IP-12	-	-	-	-	-	-	-	-
13	IP-13	+	-	-	-	-	-	-	-
14	IP-14	-	+	-	-	-	-	-	-
15	IP-15	+	+	-	-	-	-	-	-
16	IP-16	-	-	-	-	-	-	-	-
17	IP-17	-	+	-	-	-	-	+	-
18	IP-18	+	-	-	-	-	-	-	-
19	IP-19	-	-	-	-	-	-	-	-
20	IP-20	-	-	-	-	-	-	-	-
21	IP-21	+	+	-	-	-	-	-	-
22	IP-22	-	+	-	-	-	-	-	-
23	IP-23	-	-	+	-	-	-	-	-
24	IP-24	-	-	+	-	-	-	-	-
25	IP-25	-	-	-	-	-	-	-	-
26	IP-26	-	-	+	-	-	-	-	-
27	IP-27	-	+	-	-	-	-	-	-
28	IP-28	-	+	-	-	-	-	-	-
29	IP-29	+	+	-	-	-	-	-	-
30	IP-30	+	-	-	-	-	-	-	-
31	IP-31	-	+	-	-	-	-	-	-
32	IP-32	-	-	-	-	-	-	-	-
33	IP-33	-	-	-	-	-	-	-	-
34	IP-34	-	+	-	-	-	-	-	-
35	IP-35	+	+	-	-	-	-	-	-
36	IP-36	-	+	-	-	-	-	+	-
37	IP-37	-	-	-	-	-	-	-	-
38	IP-38	-	-	-	-	-	-	-	-
39	IP-39	-	-	+	-	-	-	-	-
40	IP-40	-	-	-	-	-	+	-	-
41	IP-41	-	+	-	-	-	-	-	-
42	IP-42	-	-	-	-	-	-	-	-
43	IP-43	-	-	-	-	-	-	+	-
44	IP-44	+	-	-	-	-	-	-	-
45	IP-45	-	-	+	-	-	-	-	-
46	IP-46	+	-	-	-	-	-	-	-
47	IP-47	-	-	-	-	-	-	-	-
48	IP-48	-	-	+	-	-	-	-	-
49	IP-49	+	+	-	-	-	-	-	-
50	IP-50	-	-	+	-	-	-	-	-
Total		14	14	8	1	1	1	3	1

Short forms: E.C- *Escherichia coli*, P.A- *Pseudomonas aeruginosa*, K.P-*Klebsiella pneuomoniae*, P.M-*Proteus mirabilis*, S.S-*Serratia species*., S.A- *Staphylococcus aureus*, E.A-*Enterobacter aerogens*., S.S- *Shigella species*, P.O-*Pseudomonas otitidis*

Table:2 Bacterial Species from different water samples(in Percentage %)

Bacterial Isolates	Percentage
Escherichia coli	28%
Pseudomonas aeruginosa	26%
Klebsiella spp.	16%
Proteus mirabilis	2%
Serrati spp.	2%
Staphylococcus aureus	2%
Enterobacter spp.	6%
Shigella spp.	2%
Pseudomonas otitidis	2%

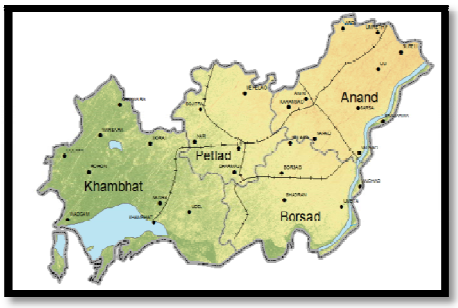

Figure 1: Selected area (Anand district) for water collection and Bacteriological analysis

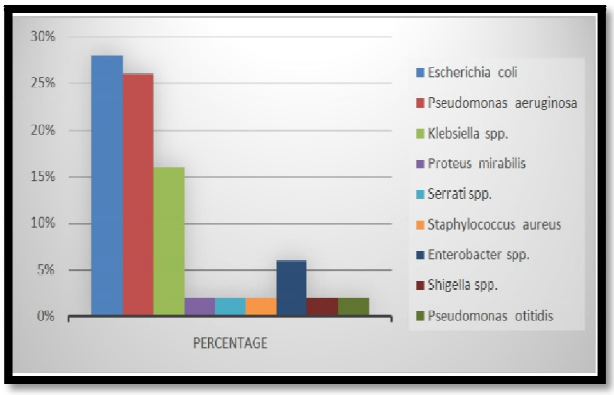

Figure: 2. Graphical representation of Bacterial Isolates from Drinking Water collected from Anand district

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REVIEW ARTICLE

Drug Review on Siddha Herbal Formulation – Nelli Mulli Ilagam

M G Chithra[1]*, Lakshmi KanthamT[2] and MeenaKumari R[3]

[1]PG Scholar, Department of Maruthuvam, National Institute of Siddha, Tambaram Sanatorium, Chennai, Tamil Nadu, India.
[2]Associate Professor**,** Department of Maruthuvam, National Institute of Siddha, Tambaram Sanatorium, Chennai ,Tamil Nadu, India.
[3]Director, National Institute of Siddha, Tambaram Sanatorium, Chennai, Tamil Nadu, India.

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***Address for Correspondence**
M.G.Chithra,
PG Scholar,
Department of Maruthuvam,
National Institute of Siddha,
Tambaram Sanatorium, Chennai,
Tamil Nadu, India.
Email: chithramanibsms@gmail.com

ABSTRACT

In siddha System of medicine the diseases are categorized into 4448 types on the basis of Mukkutram. *In* Yugi vaithiya chinthamani text Yugi munivar classified Veluppunoi into 5 types and AZHAL VELUPPU NOI is one among them. In Siddha system, many of the formulations are used to treating veluppunoi. The current review aims to explore about Siddha formulation Nelli Mulli Ilagam for the treatment of AzhalVeluppuNoi and their associated symptoms. Nelli Mulli Ilagam is one of the poly herbal formulation used to treat Anaemia. Ingredients of the siddha herbal formulation Nelli Mulli Ilagam and their pharmacological action, medicinal uses, and their scientific review in various research studies are talk through in this review. In conclusion, the results of the drug review disclose that the pharmacological action and medicinal uses of drug were equal to all ingredients of formulation. According to the results, all the ingredients of this preparation have the potency of relieving the symptoms of Azhalveluppunoi (Iron Deficiency Anaemia).

Keywords: Siddha system, Herbal Formulation, *Nelli MulliIlagam*, Review

INTRODUCTION

Siddha system is an earliest and distinctive system which defines health as a prefect state of physical, psychological, social and spiritual well-being of an individual. In Siddha system of medicine, the three Dhosam namely Vatham, Pitham, Kapam are the essential constituents of living body which are responsible for controlling all the body functions. Siddhars have classified the disease into 4448 types. Out of these types, Velupunoi or Paandu noi is one of the major disease. According to Yugi Vaidya Chindamani there are 6 types of Paandu. According to Siddha literature Azhalveluppunoi include the symptoms like pallor of skin and mucus membrane, fatigue, lassitude, chest discomfort, breathlessness, pica, giddiness, dizziness, angular stomatitis, pungent or bitter taste and they can be correlated well with iron deficiency anaemia (IDA) in Bio medicine. In Siddha, various herbal drugs are mentioned for the management of Pitha Paandu or Azhal veluppu noi(Iron Deficiency Anaemia). Number of herbs and herbo – mineral formulations are available in Siddha system of medicine in treating anaemia. So, I have gone through various siddha literatures to find an acceptable, cost effective, safely haematinic for anaemic patients.And I have marked to take "NELLI MULLI ILAGAM" which is indicated Pitha Paandu (Iron Deficiency Anaemia). This article reveals the detailed review of the Siddha herbal formulation *Nelli Mulli Ilagam*, which is mentioned in the Siddha classical text Agasthiyar Vaidhya Vallathi 600 indicated for PithaPaandu(Azhal veluppu noi). Hence, the current study has been carried out to explained the pharmacological action, medicinal uses and scientific review of the Nelli Mulli Ilagam.

Required raw drugs

1.Nellimulli (*Phyllanthus emblica.* Linn)
2.Athimadhuram (*Glycyrrhiza glabra*. Linn)
3.KoogaiNeeru (*Maranta arundinacea.* Linn)
4.Thiratchai (*Vitis vinifera.* Linn)
5.Thippili (*Piper longum.* Linn)
6.Perinthu (*Phonex dactilifera.* Linn)
7.Naatusarkarai (Brown Sugar from Sugar cane)
8.Honey

Nelli Mulli

Botanical Name: *Phyllanthus emblica.* Linn
Synonyms: Aamalagam, Aalagam, Aambal, Aamarigam, Thathari, Thathari, Korangam, Miruthubala, Meethunthu [1]
Vernacular Names
English : Indian Goseberry
Sanskrit : Amalaki
Taxonomical Classification
Kingdom : Plantae
Division : Tracheophyta
Order : Euphorbialess
Family : Euphorbiaceae
Genus :*Phyllanthus*
Species : *emblica*
Botanical Name : *Phyllanthus emblica* Linn.
Habitat: Mixed forests, Drier forests, Dry open sparse forests or scrub, village groves at elevations of 200 -2,300 meters in southern china
Parts Used: Leaf, Flower, Bark, Root, Fruit, Seeds
Organoleptic characters:
Taste : Sour, Astringent, Sweet

Character : Coolent
Division : Sweet
Actions :
Leaf, Bark, Dried fruit - Astringent
Flower - Refrigerant, Laxative
Fruit - Refrigerant, Diuretic, Laxative

General Characters of NelliMulli [1]

ஆகவன லஞ்சகிஅ சிர்க்கென்பு ருக்கிகண்னோய்
தாக முதிரவித்தந் தாது நஷ்டம் -மேகனத்தின்
இல்லிமுள்ளி போலரூகல் எண்கா மியவியங்கம்
நெல்லிமுள்ளி யார்போ நினை.

நல்லநெல்லி முள்ளியது நாக்குக் குருசிதரும்
அல்லல்விரி பித்தம் அகற்றுமதை மெல்லத் -
தலை முழுகக் கண்குளிருந் தாவுபித்த வாந்தி
இலையிழிமே கங்களும் போம் எண்.

- (தேரையர் குணவாகடம்)

Phytochemical Constituents [2]
- Alkaloids, Glyceroids, Carbohydrates, Phenolics, Tannins
- Lignin, Saponins, Flavonoids, Terpinoids

Pharmacological Activities
- Antimicrobial, Antioxidant,Anti- Inflammatory, Analgesic
- Antipyretic, Adaptogenic, Hepatoprotective, Antitumor

Medicinal Uses
- *Phyllanthus emblica.* Linn also enhances white blood cells which help move out the toxins from the body. It improves the body's immune system.
- *Phyllanthus emblica* Linn is a rich source of iron, deficiency of which causes anaemia.

Scientific Review [3]
The aqueous extract of *Phyllanthus emblica* Linn. enhances the RBC count, Haemoglobin level, Haematocrit, Mean Cell Volume, Mean Cell Haemoglobin, Mean Cell Haemoglobin Concentration and Red cell distribution width also improved the haemolytic anaemia induced by Streptozotocin in test animals, when compared with the untreated animals in the negative control group.

ATIMADHURAM
Botanical Name: *Glycyrrhiza glabra* Linn.
Synonyms: Athingam, Atti, Mathoogam, Kuntriver [1]
Vernacular Names
English : Jequitity: Indian or Jamaica liquorice
Sanskrit : Yashti - Madhukam
Taxonomical Classification:
Kingdom : Plantae
Division : Magnoliophyta
Order : Fabales
Family : Fabaceae

Genus : *Glycyrrhiza*
Species :*glabra*
Botanical Name : *Glycyrrhiza glabra* Linn.
Habitat: It is an under shrub growing up to a height of 2 meter. In India it is reported to be cultivated in Delhi, Jammu, Srinagar and South India.
Parts Used: Roots
Organoleptic Characters:
Taste: Sweet
Character: Coolent
Division: Sweet
Actions: Emolient, Demulcent, Mild expectorant, Laxative, Topic

General Characters of Atimaduram [1]

கத்தியரி முப்பிணியால் வருபுண் தாகங்
கண்ணோய்உன் மாதம்விக்கல் வலிவெண் குட்டம்
பித்தமெலும் புருக்கி கிரிச்சரம் ஆவர்த்த
பித்தமத மூர்ச்சை விட பாகம் வெப்பந்

தத்திவரு வாதசோ ணிதங்கா மாலை
சருவவிடங் காமியநோய் தாது நட்டங்
குத்திருமல் ஆசியங்கம் இதழ்நோய் இந்து
குயப்புண்ணும்போம் மதூகமெனக் கூறுங் காலே

(தேரையர் குணவாகடம்)

Phytochemical Constituents [4]
- Glycyrrhizin, Licoagnone, Glucoside, Liquoric acid, Glycyrretol
- Glabrone, Glyzarin, Glabridin, Liqcoumarin

Pharmacological Activity
- Anti – Inflammatory, Anti-oxidant, Expectorant, Hepatoprotective
- Anti – bacterial, Anti carcinogenic, Anti – allergic, Anti malaria

Mediucinal Use
- It is also useful in gout, asthma, sore throat, tonsillitis, cough, leucorrhoea, bleeding, jaundice.
- It is an important ingredient in rheumatism, paralysis and haemorrhagic diseases.

Scientific Review [5]
The number of WBC, RBC, platelet the percentage of lymphocyte and neutrophil and the levels of Hb, PCV, MCV, MCH and MCHC were significantly improved in the treated group by anaemia induced by Phenylhydrazinein test animals, when compared with untreated animals in the negative control group. The treatment with aqueous extract of *G.glabra* significantly enhanced the above parameters.

THIPPILI
Botanical Name: *Piper longum* Linn
Synonyms: Aarkathi, Uncharam, Ulavainaasi, Kaaman, Kudaari, Kolagam, Koli, Kolaiyarukki, Saram, Saadi, Thulavi, Maagathi, Kanai, Soundi, Thanduli, Kanam, Kalini, Paanam, Pippili, Vaithegi, Ambu, Aathimarunthu [1]
Vernacular Names:
English : Long pepper
Sanskrit : Pippali
Taxonomical Classification

Kingdom : Plantaes
Division : Magnoliophyta
Order : Piperales
Family : Piperaceae
Genus : *Piper*
Species : *longum*
Botanical Name: *Piper longum.* Linn

Habitat: Piper longum is a herb found growing in the hotter parts of india, from central Himalayas to Assam, Assam, Tamil Nadu and Andhra Pradesh.

Parts Used: Fruit

Organoleptic Characters:

Taste : Sweet
Character : Hot
Division : Sweet

Actions:

- Stimulant
- Carminative

General Characters of Thippili [1]

இருமல் குன்மம் இரைப்பு கயப்பிணி
ஈளை பாண்டு சந்யாசம் அரோசகம்
பொருமல் ஊதை சிரப்பிணி மூர்ச்சைநோய்
பூரிக் குஞ்சல தோடம் பீலிகமும்
வரும லப்பெருக்க கோடு மகோதரம்
வாதம் ஆதிமுத் தோடஞ் சுரங்குளிர்
பெருமாலைப்புரி மேகப் பிடகமும்
பேருந் திப்பிலிப் பேரங்குரைக்கவே.

(கட்டளைக்கலிப்பா)

Phytochemicals Constituents [6]

- Piperine, Piperlongumine, Sylvatin, Sesamine, Pipermonaline
- Piperundecalidine, Diaeudesminpiperlongumine

Pharmacological Activity

- Antioxidant, Hepatoprotective, Anti – tumor, Antiplatelet
- Anti - diabetic, Antiamoebic, Immuno modulatory
- Anti – inflammatory, Cardioprotective, Anti - apoptosis

Medicinal Uses

- It cures peptic ulcer and tumor in spleen.
- It is most frequently to treat chronic bronchitis, asthma, constipation, gonorrhoea, diarrhoea, cholera, chronic malaria, cough, paralysis of the tongue, tumors, viral hepatitis

Scientific Review [7]

The effects of piperine on albino mice induced by paracetamol hepatotoxicity were observed in serum. A significant change in the levels of SGPT and SGOT were observed when compared with untreated animals. So, the piperine enhances the hepatoprotective activity and reduces the liver toxicity.

KUVAIK – KIZHANGU

Botanical Name: *Maranta arundinacea.* Linn

Synonyms: Arrow root kizhangu, Koovamaakkizhangu, Koogaikkizhangu [1]

Vernacular Names:

English : East Indian Arrow root

Malayalam : Kuva, Kuva - kizhanna

Taxonomical Classification

Kingdom : Plantae

Division : Magnoliophyta

Order : Zingiberales

Family : Marantaceae

Genus :*Maranta*

Species :*arundinacea*

Botanical Name: *Maranta arundinacea.* Linn

Habitat: It has been extensively scattered throughout tropical countries. In India, it is distributed in Uttar Pradesh, Assam and Kerala.

Parts Used: Kizhangu

Organoleptic Characters

Taste : Sweet

Character: Coolent

Division : Sweet

Actions:

- Refrigerent
- Demulcent
- Nutrient

General Characters of Kuvaik – kizhangu [1]

மேனியிடும் வாய்க்கு மிருதுவாம் ஆக்கியுண்ணத்
தானிருமல் வெப்பதிக தாகமிவை ஏனிருக்கும் -
அம்பே றிளங்கிழங்கி தியாவர்க்கு மாமணப்பூங்
கொம்பே கூகைக்கிழங்கைக் கூறு.

(அகத்தியர் குணவாகடம்)

Phytochemical Constituents[8]

- Phenols, Flavonoids, Tannins, Alkaloids, Steroids
- Terpenoids, Glycosides, Saponins

Pharmacological Activities

- Antidiarrheal, Antiulcerogenic, Antioxidant, Antidysenteric
- Anti – inflammatory, Immunostimulatory, Vibriocidal
- Antimicrobial, Antispasmodic, Antihyperglycemic

Medicinal Uses

- Arrowroot is a soothing demulcent and nutritive food, easily digested
- An infusion of the root has traditionally been used to treat urinary infections.

Scientific Review [8]

The ethanolic extract of *M.arundinacea* was established to be aneffective scavengers of ABTS, DPPH, Hydrogen peroxide, Nitric and also possessed a good reducing power. The results give out indicate that *M.arundinacea*extract diminished oxidative stress via its antioxidant properties.

5.THRAKSHI:

Botanical Name: *Vitis vinifera.* Linn

Synonyms: Araavaaram, Kodimunthiri, Kodimunthirigai, Munthirigai, Thiraakshai, Madhurasam, Kothirigai, Thiraakkam, Palothamai [1]

Vernacular Names

English : Grapes, common grape – vine, Wine – grape, European grape

Sanscrit : Draksha

Taxonomical Classification

Kingdom : Plantae

Division : Magnoliophyta

Order : Vitales

Family : Vitaceae

Genus :*Vitis*

Species :*vinifera*

Botanical Name: *Vitis vinifera.* Linn

Habitat: It present in Central and southern Europe; Northern Africa; Western Asia and the Caucasus. It grows in Riverside and damp woods.

Parts Used: Leaf, Fruits

Organoleptic Characters

Taste : Sweet

Character: Coolent

Division : Sweet

Actions:

- Laxative
- Refrigerent
- Diuretic — Fruit
- Nutritive
- Astringent = Leaf
- Demulcent
- Laxative — Dried fruit

General Characters of Thrakshi [1]

- It acts as a laxative and increases haemoglobin production
- It cures stomach ache and stop bleeding

Phytochemical Constituents [9]

- Anthocyanins, Hydroxycinnamic acids, Proanthocyanidins, Pectin
- Vitamin C (Ascorbic Acid), Carotenoids, Lignin,Tannins
- Hemicellulose, Stilbenoids, Xylan, Tocopherols, dietary fiber

Pharmacological Activities

- Antioxidant, Antiviral, Antiplatelet, Hepatoprotective, Anticataract
- Anticholinergic, Anti-obesity, Anti – inflammatory, Neuroprotective

Medicinal Uses
- The nutrient content of *V.vinifera* is close to that of blood plasma, grape fasts are recommended for detoxification
- The fruit is also helpful in the treatment of varicose veins, haemorrhoids and capillary fragility

Scientific Review[10]
The measurement of Haemoglobin, Total Iron Binding Capacity (TIBC), Serum Ferritin, Mean Corpuscular Volume (MCV) and Serum Ironby assessing a venous blood sample when after received 20 days of oral dried raisins by seven healthy female volunteers. The results showed up mild increase in Hb level of the subjects after taking Raisins and the level of TIBC were decreased for all subjects in relation to pre - test levels.

PERICHCHU
Botanical Name: *Phonex dactilifera*.Linn
Synonyms : Karchoorakkai [1]
Vernacular Names:
English : Date palm
Sanscrit : Kharjjuram
Taxonomical Classification
Kingdom : Plantae
Division : Tracheophyta
Order : Arecales
Family : ArecaceSae
Genus : *Phonex*
Species : *dactilifera*
Botanical Name: *Phonex dactilifera.* Linn
Habitat: This palm grows around the world in dry, hot, frost-free regions.
Parts Used: Fruit, Seed, Gums, Wine, Stem, Jaggery, Palm candy crystals
Organoleptic Characters:
Taste : Sweet
Character: Hot
Division : Spicy
Actions:
- Febrifuge, Refrigerant, Laxative, Stomachic, Diuretic
- Nutritive, Tonic, Expectorant, Aphrodisia

General Characters of Perichehu [1]
பேரிந்தெனுங்கனிக்கு பித்தமத மூர்ச்சைசுரம்
நீரார்ந்த ஐயம் நெடுந்தாகம் பேரர -
இரத்தபித்த நீரிழிவி லைப்பறும் அரோசி
உரத்தமலக் கட்டுமறும் ஓது .

(அகத்தியர் குணவாகடம்)

Phytochemical Constituents[11]
- Alkaloids, Flavonoids, Tannins, Phenols, Saponins,

Pharmacological Activities
- Antioxidant, Anti – inflammatory, Anti mutagenic
- Anticancer, Antibacterial, Antipyretic , Anti analgesic

Medicinal Uses

- Have a variety of antioxidants and having amino acids and fiber content. These help in clear out the bad bacteria loaded in the intestines and to enhances the growth of good bacteria.
- Fresh date fruits are an excellent remedy for alcoholic intoxication

Scientific Review [12,13]

31 female primary school children and with IDA were fed with dates *(Phoenix dactlifera)* for 2 months. Blood parameters were calculated before the intervention and after 2 months of date consumption. The result of that study showed that the consumption of date fruit enhanced Hb, Hct and serum ferritin levels in school children.

Mel Honey

Synonyms: SegappuMadhu, Savuthidam, Maaniramaasitham, Mezhugu Sappirasam, Amaasanam, Pitparasam, Thoonithayilam, Varnthuppu, Kabilingam, Miruthavarnam,

General Characters [14]

ஐயிருமை லீளைவிக்க லக்கிப்புண் வெப்புடல்நோய்
பையவொழியும் பசியுமுறும் - வையகத்தி
லெண்ணுமிசையாமருந்திற் கேற்ற வனுபான
நண்ணு மலைத்தே னொன்றினால்

Actions

- Demulcent, Laxative, Astringent, Expectorant, Nutritive
- Stomachic, Soporific, Antiseptic

Phytochemical constituents

- Minerals - Calcium, iron, potassium, phosphorous, magnesium, selenium, Chromium, manganese
- Vitamins - Riboflavin, niacin, folic acid, pathothenic acid, vitamin B6

Pharmacological Activities

- Antibacterial, Antioxidant, Anticancer
- Anti-melanogenic, Anti – malarial, Antifungal

Medicinal Uses

- A daily intake of honey build up the immune system in children thus developing their disease resistance capacity.
- Asafoetida fried in ghee and mixed with a table spoon of honey can be taken thrice a day for heavy painful menstrual periods and leucorrhea.

Scientific Review [15]

The measurement of Haemoglobin, Blood indices and cells, serum ferritin, serum iron and iron binding capacity by assessing a venous blood sample when after received 2 weeks of regular diet supplemented with daily consumption of 1.2 g/kg body weight honey dissolved in 250 ml of water. The results showed up increased in Hb, Blood indices, serum iron and serum ferritin level for all subjects in relation to pre-test levels. Honey also has a strong antioxidant potential.

NAATU SARKARAI

English Name : Brown Sugar

Common Name : Cane sugar, raw sugar, whole cane sugar

Chemical Composition [16]

A. Minerals (%)-0.6 – 2.6

- Sodium - 0.006 – 0.025
- **Iron - 0.005 – 0.020**
- Magnesium - 0.008 – 0.105

B. Vitamins (mg/100 gm)

- Thiamin - 0.018 – 0.030
- Riboflavin - 0.042 – 0.046
- Nicotinic Acid - 3.92 – 4.50
- **Vitamin C - 5.20 - 30.00**

Medicinal Uses

- It helps in preventing obesity and helps in improving digestion
- It helps in the treatment of uterine contraction

Phytochemical Activity

Antioxidant activity

Scientific review [16]

Sugar is considered an effective medium for iron, because it is popularly gobbled and does not disturb iron absorption. For iron to be absorbed from the intestines, it must be decreased by intraluminal factors. It has been elucidated that brown sugar has strong anti-oxidative activity and accommodates a significant proportion of the ferrous ion.

REFERENCES

1. Vaidhya Rathnam Ka.sa.Murugesa Mudhaliyar, Gunapaadam Mooligaivaguppu; Edition 2006; Page no: (620-622, 13-14, 514-515, 376, 519,127-128)
2. Gaire,B.P., Subedi,L.Phyto chemistry, pharmacology and medicinal properties of PhyllanthusemblicaLinn..Chin.J.Integr.Med.(2014).https://doi.org/10.1007/s11655-014-1984-2
3. Maran,C M, Evaluation of Anti – Anaemic Activity of *Phyllanthus emblica* Linn., on Streptozotocin induced Rats. Masters thesis, college of Pharmacy, Madras medical college. April 2016.
4. Kaur R, Kaur H and Dhindsa AS: *GLYCYRRHIZA GLABRA*: A PhytopharmacologicalReview.Int J Pharm Sci Res 2013: 4(7); 2470-2477. Doi: 10.13040/IJPSR.0975-8232.4(7).2470-77
5. M.Zangeneh,M.pooyanmehr, A.Zangeneh; Evaluation of the anti – anaemic potential of Glycyrrhizaglabra aqueous extract in Phenylhydrazine – treated rats; Indian journal of Pharmacology and Therapeutics; 2017.
6. Hakim Md. Osman gani, Md. ObydulHoq and TahminaTamanna, Ethnomedicinal , phytochemical and pharmacological properties of *Piper longum*(Linn);Asian J. Med.Biol.Res.2019,5 (1), 1-7; doi: 10.3329/ ajmbr.v 5i1.41038
7. Prashant Sahu, Abhishek, Bhatt, AnandChaurasia, VirendraGajbhiye," Enhanced Hepatoprotective activity of Piperine Loaded Chitosan Microspheres" Int. J. drug Dev. & Res., October – December 2012, 4(4): 229 – 233.
8. NowfaFiroskhan, RagunathanMuthusawmy; Review on Marantaarundinacea L. (Marantacea); International journal of Pharmacognosy and Pharmaceutical Research; Volume 3, Issue1, 2021, page no : 01 – 04
9. Muhamad Insanu, Hana Karimah, HegarPramastya, IrdaFidrianny; Phytochemical Compounds and Pharmacological activities of VitisVinifera L.: an Updated Review. Biointerface Research in Applied Chemistry . March 2021. 11 (5): 13829 – 13849
10. Al - Aboud N. Effect of red raisins (VitisVinifera L.) Intake on the Level of Some Hematological Tests in a group of Female Volunteers. Biomed J Sci& Tech res 2 (3) – 2018. BJSTR.MS.ID.000764. DOI: 10.26717/BJSTR.2018.02.000764

11. Amanat Ali, Mohamed EssaMusthafa, MostafaIwaly, SankarDevarajan; Nutritional and MedicinalValue of Date Fruit, https://www.researchgate.net/publications/257416165
12. IrandeganiF, Arbabisarjou A, Ghaljaei F, Navidian A, Karajibani M. The Effects of a Date Consumption – Based Nutritional Program on Iron Deficiency Anaemia in Primary School Girls aged 8 to 10 Years Old in Zahedan (Iron). Pediatric Health, Mdicine and Threapeutics 2019; 10: 183-188. Doi:10.2147/PHMT.S225816
13. SN Onuh, EO Ukaejiofo, PU Achukwu, SA Ufelle, CN Okwuosa, CJ Chukwuk. Haemopoietic activity and effect of Crude Fruit Extract of *Phoneixdactylifera* on Peripheral Blood Parameters. International Journal of Biological & Medical Research. 2012; 3(2): 1720 – 1723.
14. .R.Thiyagarajan, GunapaadamThaathu&Jeevamvaguppu; 8th Edition 2013, Page No: 662
15. Al-Waili NS. Effects of daily consumption of honey solution on haematological indices and blood levels of minerals and enzymes in normal individuals. J Med Food. 2003;l6(2): 135-140.
16. AzrinaAzlan, Hock EngKhoo, Azliana Abu Bakar Sajak, Noor AtiqahAizan Abdul Kadir, BarakatunNisakMohdyusof, Zhafarina Mahmood and Sharmin Sultana; Antioxidant activity, nutritional and physicochemical characteristics, and toxicity of minimally refined brown sugar and other sugars. Food Science & Nutrition 2020Sep; 8 (9): 5048 – 5062.

		
Figure 1. NelliMullilIagam	**Figure 2. Nelli Mulli**	**Figure 3.Atimadhuram**
		
Figure. 4. Thippili	**Figure 5. Kuvaik – Kizhangu**	**Figure 6.Thrakshi**
		
Figure.7. Perichehu	**Figure 8. Mel Honey**	**Figure 9. Naatu Sarkarai**

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RESEARCH ARTICLE

A Novel Generalized Handwritten Character Recognition Model using Few-Shot Learning

Prabavathy Balasundaram[1]*, Lekshmi Kalinathan[1], Sushaanth Srinivasan[2], Sharvesh Shankar[2] and W Angeline Hilda[3]

[1]Associate Professor, Department of Computer Science and Engineering, Sri Sivasubramania Nadar College of Engineering, Chennai, Tamil Nadu, India.
[2]UG Student, Department of Computer Science and Engineering, Sri Sivasubramania Nadar College of Engineering, Chennai, Tamil Nadu, India.
[3]PG Student, Department of Computer Science and Engineering, Sri Sivasubramania Nadar College of Engineering, Chennai, Tamil Nadu, India.

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***Address for Correspondence**
Prabavathy Balasundaram
Associate Professor,
Department of Computer Science and Engineering,
Sri Sivasubramania Nadar College of Engineering,
Chennai, Tamil Nadu, India.
Email: prabavathyb@ssn.edu.in

ABSTRACT

Handwritten character recognition is a fundamental and most challenging research area with many useful applications. Many research works have been conducted with the use of deep learning networks in developing handwritten recognition systems that are focused on building language-specific models. However, building language-specific hand-written character recognition models is a time-consuming process. Furthermore, deep learning models require a lot of labeled data and are incapable of identifying samples of unseen classes. To address the issues of building time-constrained models and building models with few samples, this paper proposes a generalized model to recognize the characters of multiple languages using few-shot learning which can train models with few samples and generalize for samples of unseen classes.

Keywords: Handwritten character recognition, Few shot learning, Siamese Network, Triplet Network, Convolutional Neural Network

INTRODUCTION

Handwritten character recognition is a field of research in Artificial Intelligence, computer vision, and pattern recognition. It is required for many diverse applications such as digitizing historical documents, processing bank cheques and huge documents in legal, healthcare, and finance. Humans can easily understand different handwriting using their intelligence and learning. The same ability is induced into machines using Deep Learning. However, it requires a large amount of labeled data which is expensive to produce and requires high-end infrastructure to train in a reasonable time. Samples of handwritten characters corresponding to alphabets of multiple languages are limited. Hence, it is necessary to use the Few-Shot Learning technique which has the capability to learn from a small number of samples. The problem of handwritten character recognition has been approached from multiple angles in the field of deep learning. Frequent works have been done only on specific languages such as Arabic, Chinese, Devanagari characters, and numbers. A few works exist on specific Indian languages and Greek [1][2][3]. Many handwritten character recognition models were built using Convolutional Neural Networks (CNNs) which uses a lot of data for training and testing and thus makes it time consuming. A large amount of existing work regarding Few-Shot Learning is related to datasets other than Handwritten Characters such as Leaf [4], Plant Disease [5], Face [6][7], Palm Vein [8] and Metal Defect Detection [9]. Few works on Handwritten Character Recognition using Few-Shot Learning supports only the alphabets in Omniglot dataset. If the same model is used for Tamil or English, it might still recognize the characters, but with low accuracy. Building language specific models [10][11][12][13][14][15] for this purpose is tiresome as it demands high computational time and heavy domain knowledge. Therefore it necessitates a more generalized model, which supports the recognition of the languages in Omniglot, Tamil, the universal language English and the digits in MNIST.

Hence the objectives of the proposed work are as follows:

- To build a generalized model to recognize handwritten characters using a Few-Shot learning approach that could identify characters from other languages including Omniglot.
- To evaluate the built model with datasets of different alphabets and analyze its results.

Literature Survey

Koch et al., [3] have first proposed a siamese neural network for one shot image recognition. The Omniglot dataset was augmented using small affine distortions and the model and an accuracy of 92% was achieved. Wang B. *et al*., [4] have proposed a few shot learning methods based on the Siamese network for leaf classification. Feature extraction and spatial structure optimization were used. Experimental results show that with 20 training samples, classification accuracies are 95.32%, 91.31%, and 91.75% for Flavia, Swedish and Leaf snap datasets, respectively. Argueso D. *et al*. [5] have proposed a Siamese network based few shot learning with triplet loss using pretrained Inception-v4 for plant disease classification. The network is fine-tuned for plant leaf classification of the Plant Village dataset images. SVM classifier is used to classify the plant disease based on the distance between the feature vectors with an accuracy of 90%.Devi P. R. *et al*., [6] have proposed Few-Shot learning based on the Siamese network for facial recognition. Feature vectors were extracted using CNNs and clustering was performed with the K Means algorithm. The model was able to predict well for ATT and Yale datasets. Heidari M. *et al*., [7] have proposed face recognition using a Siamese network with contrastive loss. A pre-trained VGG16 model is used to extract the feature and Euclidean distance is calculated between the feature vectors. Experimental results show an accuracy of 95.62%. Marattukalam F. *et al*., [8] have proposed Siamese network based few shot learning for contactless biometric systems.

The features of both the palm vein images are extracted using CNN and concatenated to obtain a feature vector. Contrastive loss with a 2-way 5-shot learning setting obtained an accuracy of 90.5%. A feature fusion model utilizes the focus-area location and high-order integration to generate the feature representation for the few-shot tasks. Kim M. S. *et al.,* [9] have proposed a few-shot learning method with a Siamese network with contrastive loss to classify the steel surface defects. Li H. *et al.,* [12] have proposed a Siamese network based one shot Chinese character recognition. CNN with multilayer feature extraction and batch normalization was used to construct the siamese network. An accuracy of 95% is achieved for five-way one-shot learning. Chakrapani G. V. A. *et al*., [16] have proposed a solution

with one shot learning for handwritten word recognition. Experiments were conducted on the George Washington dataset and the Indian City Names dataset. Data augmentation techniques were applied to avoid over fitting. An accuracy of 92.5% was achieved on five-way one-shot tasks. Mukhtar *et al*., [17] have proposed one-shot learning based on the Siamese network to recognize wheat diseases. MobileNetv3 is used to extract features that are fine-tuned with the Plant Village dataset. The last two dense layers are fine-tuned with the Computer Vision for Crop Disease (CGIAR) dataset. The extracted feature vector's absolute difference is found, and a dense layer with a sigmoid function is used to generate the similarity score. The MobileNetv3 model has achieved 98% and 96% accuracy for training and validation. The one-shot network has achieved more than 92% accuracy, 84% precision, and 85% recall.

MATERIALS AND METHODS

This section describes in detail the Few-Shot learning technique and the proposed methods to create a generalized model.

Few-Shot Learning

Traditionally, a deep learning model considers the same categories of data for both train and test datasets. For example, to build a model to classify dogs and otters, firstly, the model is trained with a massive amount of labeled data of dogs and otters, and it is tested with unseen images of the trained class. If a classification of a tiger is to be included, then the model has to be trained again with a massive amount of labeled classes of dog, otter, and tiger. Whereas in Few-Shot learning, the model is trained with limited data and also used to classify the data samples of unseen classes. This is due to the fact that the Few-Shot learning model learns to find the similarity and dissimilarity between the images given to it. A Few-Shot learning model can have different categories of data in training and test sets. For example, to build a model to classify dogs and otters, firstly, the model is trained to identify the similarities and dissimilarities between the labeled data of dogs and otters, and it is tested with images of unseen class which may or may not be of the trained class. The model built using Few-Shot learning is tested with a support set and a query set. The support set contains N classes, in which each class has K samples. Query set is nothing but a test set consisting of a set of images to be tested. If the model is queried with an image of a squirrel, a support set consisting of an image of a squirrel has to be given. Since the model is capable of identifying similar and dissimilar images, it finds the similarity scores between every query image and the images present in the support set as shown in Figure 1. Further, the model reports the class of the query image based on the highest similarity score.

Proposed Siamese Neural Network

The proposed Siamese Neural Network (SNN) shown in Figure 2. is composed of identical twin networks, which are CNNs. These networks have the same configuration with the same parameters and weights. SNN is built to learn the similarity between the handwritten characters. Hence in order to build the model, Algorithm 1 has been used to construct similar and dissimilar pairs of characters. Figure 3 shows the procedure in which similar and dissimilar pairs are constructed for a batch size of 10. Initially, ten random class numbers of characters have been generated and stored in Input_1 & Input_2. It is seen that five class numbers across Input_1 & Input_2 are to be the same. Random samples for every class are generated and stored in sample_1 & sample_2. For example, 411 from Input_1 and 728 from Input_2 represent different classes. Sample 0 from the class 411 and sample 4 from class 728 are chosen at random to generate a dissimilar pair which is represented by 0 in the targets array. Similarly, samples 8 and 11 from class 919 are considered as a similar pair which is represented by 1 in the targets array. Figures 4 and 5 show the generated similar and dissimilar image samples.

In order to train the proposed network, similar and dissimilar pairs of images x1 and x2 are fed into the twin networks. Each CNN layer utilizes filters of increasing complexity. Every layer contributes to the learning of consequent image parts throughout. The first few layers learn basic features such as horizontal lines, vertical lines, edges and corners. The middle layers detect more specific features of the handwritten character such as its curves

and shapes. The last few layers learn the higher representations to recognize the full handwritten character, in different shapes and positions. Dropout layers are introduced to avoid over fitting by randomly dropping the connections between neurons. After the sequence of convolutional layers, the output is flattened into a single dimension vector and brought down into a feature vector of size 1 x 512. The feature vectors f(x1) and f(x2) are passed into the differencing layer to find the distance between them using the Euclidean Distance metric. This distance value is passed to the output layer where a sigmoid function is applied on the distance value which returns a number between 0 and 1. Based on the target and the output values, the loss will be calculated and will be used for back propagation to update the weights during training.

Proposed Triplet Neural Network:

The Triplet Neural Network (TNN) shown in Figure 6 utilizes three images as an input. These three images are a combination of an anchor image, a positive image, and a negative image. The image from the same class as the anchor image is considered to be positive and a different class is considered to be negative. For example, considering the image of a tiger to be an anchor image, any image from the tiger class is considered as a positive image. Any image other than a tiger is considered to be the negative image. Algorithm 2 has been used to construct triplets. Figure 7 shows the procedure in which the triplets are constructed for a batch size of 10. Initially, ten different random class numbers of characters have been generated and stored in Anchor & Input_2. The arrays Anchor and Input_3 are the same. Random samples for every class are generated and stored in sample_1, sample_2 and sample_3. An example of one of the triplets formed from Figure 7 consists of 411 from Anchor, 919 from Input_2 and 411 from Input_3 and are considered to be the anchor, negative and positive images respectively. Figure 8 shows the generated sample triplets. These images are fed into the respective neural network to extract the corresponding features vectors. These feature vectors of the positive and the anchor image are passed into one differencing layer to find the distance between them using the Euclidean Distance metric. Similarly the feature vectors of the anchor and the negative image are parallely passed to another differencing layer. The outputs of both the differencing layers are used to find the triplet loss. Based on the target and the output values, the loss will be calculated and will be used for back propagation to update the weights during training.

RESULTS AND DISCUSSION

This section describes in detail the datasets used for experimentation and discusses the results of the proposed methods.

Description of Omniglot Dataset

It contains 1,623 different handwritten characters from 50 different languages with 20 samples for each character.

Description Tamil Dataset

It consists of 156 characters, and each character contains approximately 300 samples written by native Tamil writers including school children, university graduates, and adults from the cities across Tamil Nadu, India. The images are in TIFF format, and the dimensions of each image are different.

MNIST dataset

The MNIST handwritten digits dataset consists of 60,000 images of handwritten single digits between 0 and 9. MNIST Fashion dataset consists of 60,000 images with 10 different classes.

Performance metric used

Accuracy - Metric for evaluating classification models. It is the fraction of correct predictions our model out of total predictions. Formally, accuracy has the following definition:

Implementation

The Omniglot dataset was used to build SNN and TNN. Among 50 languages in Omniglot, 30 languages are selected for training and 20 for testing. The train set contains 964 characters of different languages and a total of 19,280 samples. Among that, 20% of the data, i.e., 192 characters, is considered for validation. The test set contains 659 characters and a total of 13,180 samples. Pair of images or triplets were passed to SNN and TNN respectively. In both the networks, L1-distance was used to find the distances between similar and dissimilar pairs. This is fed into the dense layer with sigmoid activation and the final layer outputs the value 0 to 1, where 0 represents no similarity and 1 represents full similarity. Binary cross entropy and Triplet loss functions were used in SNN and TNN respectively. The Adam optimizer updates the weights to reduce the loss incurred.

Experiments on the proposed SNN and TNN models

The proposed SNN was evaluated by generating a random test set and the corresponding support set from the Omniglot dataset. The test set consists of classes that are not present in the train set. Figure 9 shows the test image and the support set containing 20 characters with one image per character including the test image. N such test samples are generated, and the model's accuracy is measured by the number of correct predictions made. Several variants of the proposed SNN models were built and training and testing accuracies have been measured. These models have been built with 128 and 256 image pairs, in which the model with 256 image pairs performed better compared to the other, with an improvement in accuracy of 3.2% as shown in Table 1. With the fixation of 256 image pairs, the variants of the model with 3000 and 7000 iterations have been built. The model trained for 7000 iterations performed better with an improvement in accuracy of 1.834%. With the fixation of 7000 iterations, the variants of the models with contrastive loss and binary cross entropy loss function have been built. The model with binary cross entropy loss function performed better with an improvement of 10.659%. With the fixation of the loss function as binary cross entropy, the number of classes in the support set has been varied from 10 and 20 classes. The variant model with ten classes in the support set performed better, with an improvement of 9.359%. Finally, the proposed model with 256 image pairs, 7000 iterations, binary cross entropy loss and a support set with 10 classes achieved an accuracy of 88.8%, which is shown in bold in the Table 2. Further, data in Omniglot has been augmented and its characters have been downscaled to achieve an accuracy of 92.8% for 10-way 1-shot.

With the same configuration, the proposed TNN model was tested for the Omniglot dataset. Further, these models were also tested for Tamil and MNIST datasets to observe the generalization of the built models. Handwritten characters of both the Tamil and MNIST datasets were resized into 64×64 pixels. Further, they were pre-processed with image dilation and thresholding. Figures 10 and 11 show the character before and after pre-processing. Same experiments have been conducted for the existing work of Koch et al.[3]for Omniglot, Tamil and MNIST datasets. Table 3 shows the performance comparison of the existing work, proposed SNN and TNN models for different datasets and it is inferred that the accuracy of Existing work, SNN and TNN performs better for 5-way 1-shot when compared to its counterparts. Further, it is also clear that for every dataset, TNN performs better when compared to Existing work and SNN. The performance of TNN over SNN for Omniglot is 2.2%. The performance of TNN over SNN for Tamil characters is 6.4%. The performance of TNN over SNN for MNIST is 12.2%. An improvement of 12.2% is seen in MNIST as all the 10 digits are distinct in its structure. In the Tamil character dataset, few characters are similar in structure. So there is relatively less improvement. In the Omniglot dataset, some of the characters across different languages are similar. So, there is less improvement. The performance comparison of existing work, proposed SNN and TNN models for different datasets are elucidated in Figures 13, 14 and 15.

Digitization of Handwritten English Characters

The built generalized model was tested for recognizing the characters from the handwritten English documents. A written document is scanned with a mobile camera under suitable illumination for clear processing of the image. The captured image as shown in Figure 12.a, may contain unwanted noise and it undergoes various stages of pre-processing. In pre-processing, the image is resized, converted into grayscale, and the noise is removed by dilation followed by erosion. Figures 12.b and 12.c show the gray scaled and cleaned image. The cleaned image is segmented into different logical parts, like lines of the paragraph, words of the line, and characters of the word as shown in

Figure 12.d. The segmented individual characters are shown in Figure 12.d and 12.e. A support set which consists of all 26 handwritten characters of English in lower case is generated. The segmented characters are recognized using the trained TNN and SNN models with the support set. An accuracy of 81.25% and 73.22% are obtained using TNN and SNN respectively. In addition, the characters are also recognized using Pytesseract, which is an open-source OCR engine. The performance of the digitization of the handwritten document has been tested using Pytesseract API, SNN, and TNN. For testing, four different documents were prepared with increased complexities in handwriting. The accuracies for each of the documents were measured and plotted as shown in Figure 13 and it is inferred that as the complexity of the handwriting increases, TNN performs better when compared to Pytesseract and SNN.

REFERENCES

1. Sparsh K., Nitesh P., and Vijaypal S.D. (2021). Handwritten Character Recognition using Deep Neural Networks. In proceedings of the International Conference on Data Science, Machine Learning and Artificial Intelligence. pp. 167–170. ACM.Pareek J., Singhania D., Kumari R. R., and Purohit S. (2020). Gujarati handwritten character recognition from text images. Procedia Computer Science. Vol. 171:514-523.
2. Sonawane P. K., and Shelke S. (2018). Handwritten Devanagari Character Classification using Deep Learning. In proceedings of International Conference on Information, Communication, Engineering and Technology (ICICET) pp. 1-4. IEEE.
3. Koch G., Zemel R., and Salakhutdinov R. (2015). Siamese neural networks for one-shot image recognition. ICML deep learning workshop. Vol. 2:10-16.
4. Wang B., and Wang D. (2019). Plant leaves classification: A few-shot learning method based on siamese network. IEEE Access. Vol. 7:151754-151763.
5. Argueso D., Picon A., Irusta U., Medela A., San-Emeterio M. G., Bereciartua A., Alvarez-Gila A. (2020). Few-Shot Learning approach for plant disease classification using images taken in the field. Computers and Electronics in Agriculture Journal. Vol. 175:650-657.
6. Devi P. R., Yashashvini R., Navyadhara G., and Ruchitha M. (2021). Similar Face Detection for Indian Faces using Siamese Neural Networks. In proceedings of 2nd International Conference for Emerging Technology. pp. 1-5. IEEE.
7. Heidari M. and Fouladi-Ghaleh K. (2020). Using Siamese Networks with Transfer Learning for Face Recognition on Small-Samples Datasets. In proceedings of International Conference on Machine Vision and Image Processing. pp. 1-4. IEEE.
8. Marattukalam F., Abdulla W. H., and Swain A. (2021). N-shot Palm Vein Verification Using Siamese Networks. In proceedings of International Conference of the Biometrics Special Interest Group. pp. 1-5. IEEE.
9. Kim M. S., Park T., and Park P. (2019). Classification of steel surface defect using convolutional neural network with few images. In proceedings of 12th Asian Control Conference. pp. 1398-1401. IEEE.
10. Balaha H. M., Ali H. A., Saraya M., and Badawy M. (2021). A new Arabic handwritten character recognition deep learning system (AHCRDLS). Neural Computing and Applications. Vol. 33(11):6325-6367.
11. Ashiquzzaman A., Tushar A. K., Rahman A., and Mohsin F. (2019). An efficient recognition method for handwritten arabic numerals using CNN with data augmentation and dropout. In proceedings of Data management, analytics and innovation. pp. 299-309. Springer.
12. Li H., Ren X., and Lv Y. (2019). One-shot chinese character recognition based on deep siamese networks. In proceedings of Chinese Intelligent Systems Conference. pp. 742-750. Springer.
13. Ahlawat S., Choudhary A., Nayyar A., Singh S., and Yoon B. (2020). Improved handwritten digit recognition using convolutional neural networks (CNN). Sensors. Vol. 12:3344-3350.
14. Naidu D. S., and Rafi T. M. (2021). Handwritten Character Recognition using Convolutional Neural Networks. International Journal of Computer Science and Mobile Computing. Vol. 10(8):41–45.

15. Bai J., Chen Z., Feng B., and Xu B. (2014). Image character recognition using deep convolutional neural network learned from different languages. In proceedings of International Conference on Image Processing. pp. 2560–2564. IEEE.
16. Chakrapani G. V. A., Chanda S., Pal U., and Doermann D. (2019). One-shot learning-based handwritten word recognition. In proceedings of Asian Conference on Pattern Recognition. pp. 210-223. Springer.
17. Mukhtar H., Khan M. Z., Khan M. U., and Younis H. (2021). Wheat Disease Recognition through One-shot Learning using Fields Images. In proceedings of International Conference on Artificial Intelligence. Pp. 229-233. IEEE.

Algorithm 1: Construction of Similar and Dissimilar pairs for training the Siamese Neural Network

```
Input: K - array of classes, N - array of samples for each class
Output: A set of similar and dissimilar pairs along with associated targets (0: dissimilar,
        1: similar)
targets = [ -1 for i in range(len(k))] ;
for i in range(len(k)) do
    if i >= len(k)/2 then
        targets[i] = 1 ;
    else
        targets[i] = 0 ;
    end
end
Input_1 = [] ;
Input_2 = [] ;
sample1= [] ;
sample2= [] ;
for i in range(len(k)) do
    Input_1.append(randint(0, len(k)-1)) ;
end
for j in range(len(k)/2), len(k)) do
    Input_2.append(Input_1[j]) ;
end
for j in range(len(k)/2), len(k)) do
    Input_2.append(Input_1[j]) ;
end
for i in range(len(k)) do
    sample1.append(N[Input_1[i]][randint(0, 19)]) ;
end
for i in range(len(k)) do
    sample2.append(N[Input_2[i]][randint(0, 19)]) ;
end
return sample1, sample2, targets
```

Algorithm 2: Construction of Anchor, Positive and Negative images Triplet Neural Network

```
Input: K - array of classes, N - array of samples for each class
Output: A set of triplets
anchor = [] ;
Input_2 = [] ;
sample1 = [] ;
sample2 = [] ;
sample3 = [] ;
for i in range(len(k)) do
    anchor.append(randint(0, len(k)-1)) ;
end
for j in range(len(k)/2), len(k)) do
    Input_2.append(Input_1[j]) ;
end
for j in range(len(k)/2), len(k)) do
    Input_2.append(Input_1[j]) ;
end
Input_3 = anchor.copy() ;
for i in range(len(k)) do
    sample1.append(N[anchor[i]][randint(0, 19)]) ;
end
for i in range(len(k)) do
    sample2.append(N[Input_2[i]][randint(0, 19)]) ;
end
for i in range(len(k)) do
    sample3.append(N[Input_3[i]][randint(0, 19)]) ;
end
return sample1, sample2, sample3
```

Table 1. Performance of variants of SNN Models

Loss Function	Interation	Batch size	Training Accuracy	Test Accuracy 20-way 1-shot	Test Accuracy 10-way 1- shot
Binary Cross Entropy	3000	128	78.8%	71.8%	86%
		256	86.4%	74.4%	87.2%
	7000	128	82.8%	80.8%	86%
		256	90%	81.2%	88.8%
Contrastive loss	3000	128	56.4%	51.6%	60.8%
		256	76.0%	78.8%	78.8%

Table 2. Inference on variants of SNN Models

S.No	Change factor	%improvement in Accuracy
1	Model trained with 256 pairs over 128 pair	3.2%
2	Model built with 7000 iterations over 3000 iterations	1.834%
3	Model built with binary cross entropy over contrastive loss	10.659%
4	Model tested with 10-way one shot over 20-way one shot	9.359%

Table 3. Performance of Existing (Koch et al), Proposed SNN and TNN models

Dataset	Evaluation Metric	Models		
		Existing work	SNN	TNN
Omniglot	Test Accuracy 5-way 1-shot	86%	94%	**96%**
	Test Accuracy 10-way 1-shot	84.9%	92.8%	93%
	Test Accuracy 20-way 1-shot	76.8%	84%	86%
Tamil	Test Accuracy 5-way 1-shot	80%	88%	**93.6%**
	Test Accuracy 10-way 1-shot	76%	83.6%	88.8%
	Test Accuracy 20-way 1-shot	68%	74.8%	80%
MNIST	Test Accuracy 5-way 1-shot	57.2%	64.4%	**73.6%**
	Test Accuracy 10-way 1-shot	49%	55.2%	62.8%
	Test Accuracy 20-way 1-shot	43.8%	49.3%	56.5%

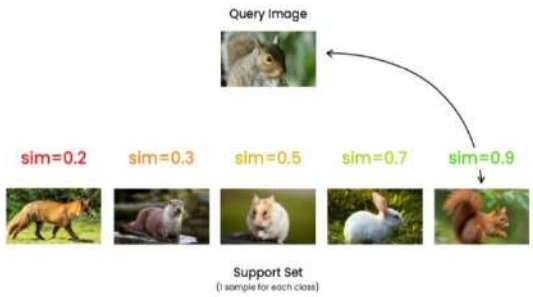


Figure 1. Identification of class of the query image in Few-Shot Learning

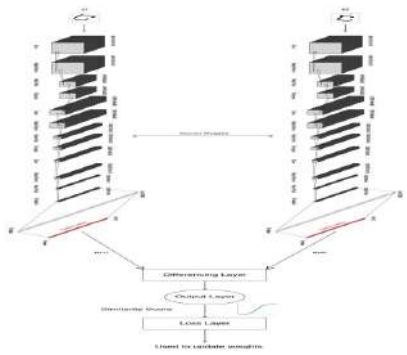


Figure 2. Design of Proposed Siamese Neural Network

```
Input_1 [411 685  14 720  53 919 700 927 229 177]
Input_2 [728, 6, 422, 842, 884, 919, 700, 927, 229, 177]
targets [0. 0. 0. 0. 0. 1. 1. 1. 1. 1.]
sample_1 [0, 13, 16, 0, 15, 8, 18, 14, 14, 9]
sample_2 [4, 8, 15, 4, 13, 11, 7, 10, 0, 3]
```

Figure 3. Construction of similar and dissimilar pairs

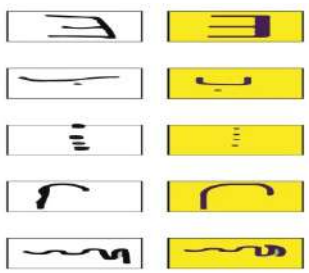


Figure 4. Generated similar pairs of characters

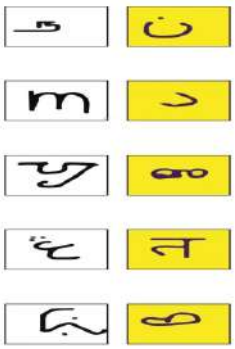


Figure 5. Generated dissimilar pairs of characters

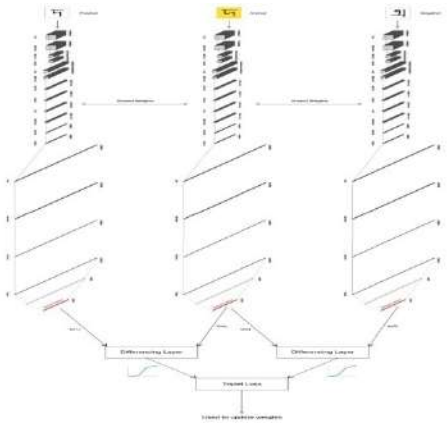


Figure 6. Design of Proposed Triplet Neural Network

```
Anchor [411 685 14 720 53 919 700 927 229 177]
Input_2 [919 700 927 229 177 411 685 14 720 53]
Input_3 [411 685 14 720 53 919 700 927 229 177]
Sample_1 [0 13 16 0 15 8 18 14 14 9]
Sample_2 [2 6 17 1 15 8 19 11 5 0]
Sample_3 [3 5 1 17 15 2 12 9 0 10]
```

Figure 7. Construction of triplets

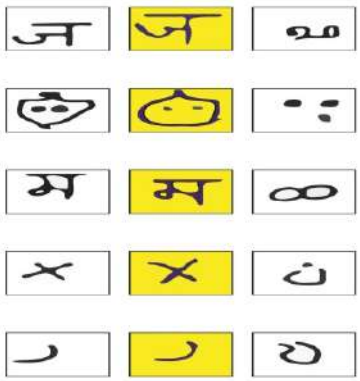


Figure 8. Generated triplets

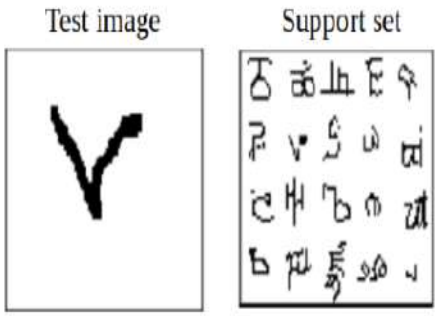


Figure 9. Test image and Support set

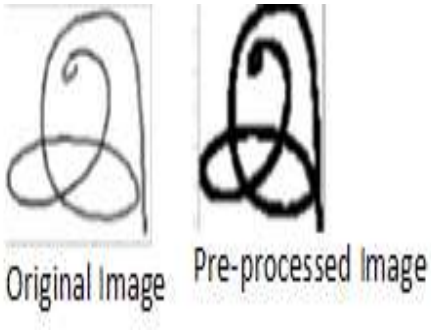


Figure 10. Original and Pre-processed images of Tamil dataset

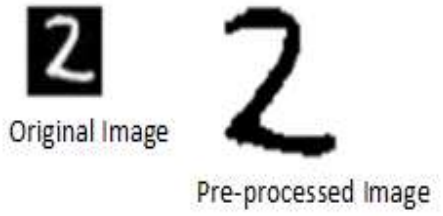
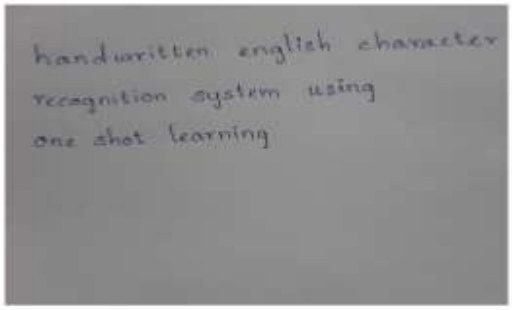
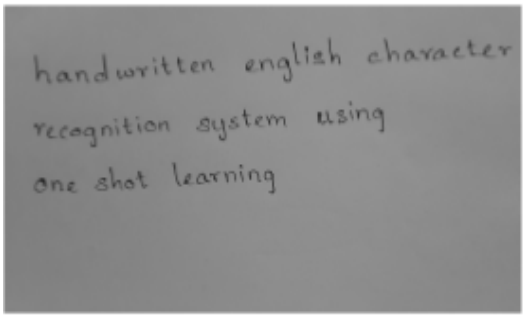


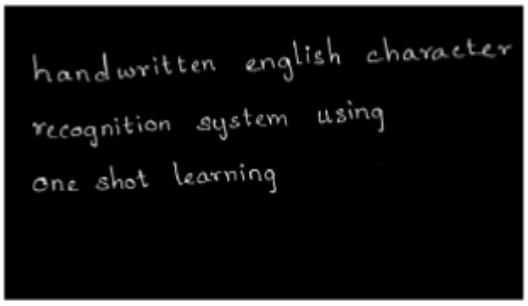
Figure 11. Original and Pre-processed images of MNIST dataset



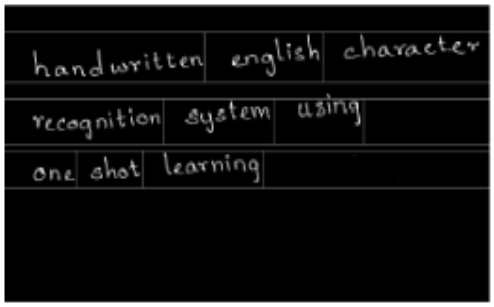
A. Handwritten English Text



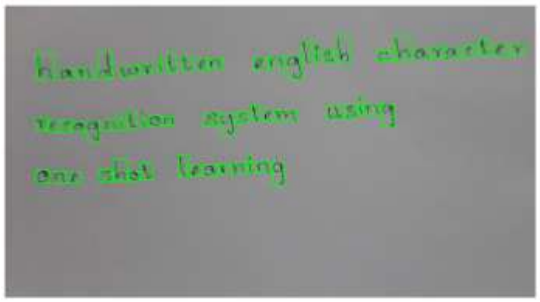
B. Gray scale Image



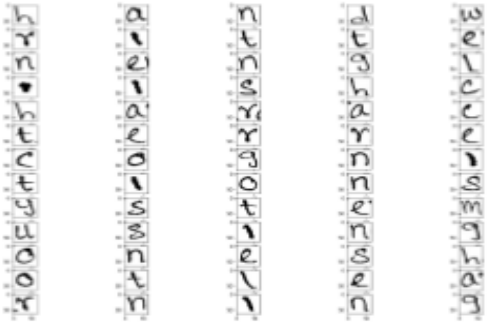
C. Image after binarization and noise removal



D. Image after line and word segmentation



E. Image after character detection



F. Individual segmented characters

Figure 12. Recognition of English characters

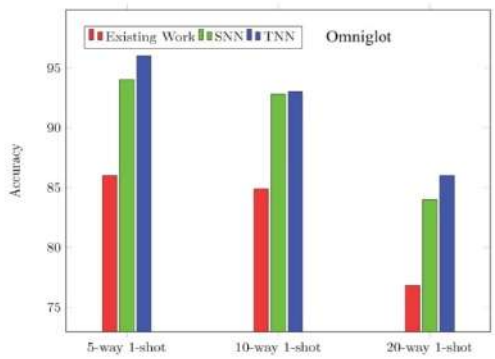


Figure 13. Performance of Existing Work, SNN, TNN for Omniglot Dataset

Figure 14. Performance of Existing Work, SNN, TNN for Tamil Dataset

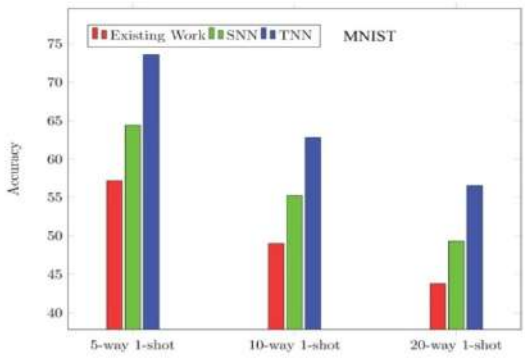


Figure 15. Performance of Existing Work, SNN, TNN for MNIST Dataset

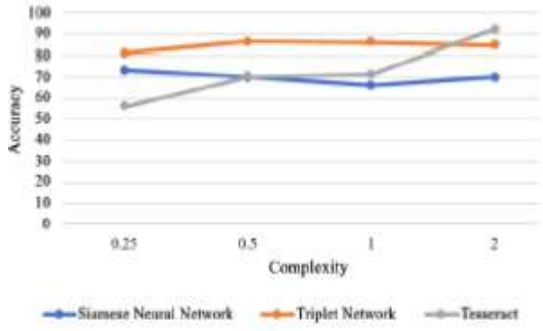


Figure 16. Performance comparison of OCR for English Text with the proposed models

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REVIEW ARTICLE

Overview of Numerous Therapeutic Properties of *Selenicereus undatus* Plant Sections with a Focus on Available Therapies: A Succinct Perspective

Koppala RVS Chaitanya[1]*, Abhishek Yekkaladev[2] and Santosh Kumar Ranajit[3]

[1]Assistant Professor, Department of Pharmacology, Vignan Institute of Pharmaceutical Technology, Kapujaggarajupeta, Duvvada, Visakhapatham, India.
[2]Department of Pharmacology, Vignan Institute of Pharmaceutical Technology, Kapujaggarajupeta, Duvvada, Visakhapatham, India.
[3]Associate Professor, School of Pharmacy, Centurion University of Technology and Management, Odisha, Bolangir, India.

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***Address for Correspondence**
Koppala RVS Chaitanya,
Assistant Professor,
Department of Pharmacology,
Vignan Institute of Pharmaceutical Technology,
Kapujaggarajupeta, Duvvada, Visakhapatham, India.
Email: koppala526@gmail.com

ABSTRACT

The current discussion focuses on *Selenicereus undatus* pharmacological effects on various physiological systems. This plant contains high amount of polyphenols thus showing antioxidant activity, influences the p-450 enzyme mechanism either by inhibiting or inducing the metabolic activity of enzymes thus, used for the treatment of hepatotoxicity. Also, *Selenicereus undatus* is known for its radical scavenging activity due to the presence of phenolic content. Onitin and luteolin isolated from the methanolic extract of *Selenicereus undatus* showed superoxide scavenging effects and DPPH free radical scavenging activity hence can also be used in cancer treatment. The antioxidant activity and phenolic composition of three different extracts (ethanol, n-butanol and water) of *Selenicereus undatus* were investigated by measuring the total reducing power expressed by ascorbate equivalent antioxidant capacity-AEAC, inhibition of lipid peroxidation, and free radical scavenging capacity (RSC) towards 2,2-diphenyl-1- picrylhydrazyl (DPPH radical) and nitric oxide (NO).The influence of different extracts during lipid peroxidation of sunflower oil induced by the lipophilic azo-initiator 4,4'-azobis(4- cyanovaleric acid) and soybean phosphatidylcholine liposomes induced by the hydrophilic azo-initiator 2,2'-azobis(2-amidinopropane) dihydrochloride was investigated in this study. Hepatoprotective activity-guided fractionation of the methanol extract of *Selenicereus undatus* showed that Onitin and luteolin possessed Hepatoprotective activities on tacrine-induced cytotoxicity in human liver-derived Hep G2 cells. This plant also possesses

sedative and anticonvulsant activity. The hydro alcoholic extract of stem from *Selenicereus undatus* shows antinociceptive and anti-inflammatory effects. Studies says that *Selenicereus undatus* can also produce a diuretic effect. The extract of *Selenicereus undatus* produced a dose-dependent inhibition of thrombin and ADP-induced platelet aggregation with in fact proofs that plant has many pharmacological activities.

Keywords: *Selenicereus undatus*, radical scavenging, Hepatoprotective, cytotoxicity, antinociceptive

INTRODUCTION

The process of evolution has made the survival of organisms by regulating metabolism and various other functions by an organ called liver. The basic functional unit of liver is called as hepatocytes which deals with reactive oxygen species and its effects [1,2]. Among the functions of liver include stimulation of antioxidant proteins: superoxide dismutase, glutathione peroxidase and catalase. There is also an enzyme associated antioxidant system (Cu–Zn, Mn, catalase, and glutathione reductase) that functions by terminating reactive oxygen species. A disproportion between oxidative and antioxidant defense systems causes an oxidative injury implicating various diseases: atherosclerosis, cancer, diabetes, liver cirrhosis, etc. [2, 3] The reactive oxygen species which are generated daily as a part of physiological functions are removed by intracellular and extracellular antioxidant mechanisms [2,4]. An unregulated reactive oxygen species production can damage macromolecules like DNA, proteins and antioxidant molecules [5]. The superoxide anion radical O_2^-, hydrogen peroxide (H_2O_2), alkoxyl (RO), peroxyl (ROO), hydroxyl radical (OH), hypochlorous acid (HOCl) and some non-oxygen species like nitric oxide (NO) and peroxynitrite form a part of ROS [6,2]. Liver plays a pivotal role in the substance metabolism, removal and is susceptible to drug toxicity, oxidative stress. The liver metabolism occur via two pathways i.e., cytochrome p-450 and glutathione- peroxidase. Cytochrome p-450 enzymes are vital for the metabolism of all the foreign chemical constituents as well as the natural products that organism consumes [7]. These enzymes are specific to several moieties that enter the body and helps in specific metabolism. However, many times the presence of other drugs will affect the metabolic process either by increasing the metabolism like potentiating process or by decreasing the metabolism like inhibiting the metabolic process. An example is the presence of grape fruit juice will inhibit the metabolism of other drugs and similarly, metabolism inhibition by warfarin kind of compounds on others. The hepatotoxicity treatment includes drugs that either inhibit or induce p-450 enzyme which includes amiodarone, cimetidine, ciprofloxacin, rifampicin, carbamazepine, phenobarbital, phenytoin[8]. In absence of dependable synthetic hepatoprotective drugs, hepatoprotective action of natural compounds have gained interest in recent times.

Many herbs such as *Silybum marianum*, *Tridax procumbens*, and *Andrographis paniculata* are reported for their hepatoprotective activity [9]. Plants contain wide variety of bioactive molecules including. Adding to their nutritional value plants contain several phytoconstituents (terpenoids, steroids, phenols, and flavonoids) exhibiting a wide array of pharmacological properties. Phenolic, flavonoid, and polyphenolic plant constituents contribute to the prevention of diseases associated with oxidative stress [2, 9]. HepG2 cells serves as an apt *in vitro* model for assessment of drug hepatoprotective activity by cytotoxic endpoint analysis. These cells retain specialized features characteristic to human hepatocytes. through analysis of different cytotoxic endpoints [2,10]. Hepatoprotective activity of natural herbal extracts namely *Phyllanthus emblica* Linn. (Euphorbiaceae), *Camellia sinensis* Linn. (Theaceae), *Punica granatum* Linn. (Punicaceae), *Mangifera indica* Linn. (Anacardiaceae), and *Acacia catechu* Linn.(Mimosaceae) are investigated on *t*-BH induced toxicity using HepG2 cells [11].Dragon fruit stems are scan dent, creeping, and branch profusely. They can 16-33 feet tall with joints of 12-47 inches and 3.9-4.5 inches thickness. Margins are corneous with age, and undulate. [12] Areoles are 0.079 inches across with internodes of 0.39-1.57 inches. Spines are acicular to conical, and are grayish brown to black in colour with a dark green epidermis.[13,14] The bear 9.8-11.8 inches long nocturnal flowers with a width of 5.9-6.7 inches and pericarpel of 0.98-1.97 inches long, 0.98 inches thickness, bracteoles are acute, ovate with 1.6 inches length. Receptacle are 1.2 inches thick and has linear-lamceolate bracteoles are linear-lanceolate. Outer tepals lanceolate-linear to linear, acuminate with 3.9-5.9 inches length, 0.39-0.59 inches width and

mucronate. Their colour is greenish-yellow or whitish, rarely rose-tinged; inner tepals are lanceolate to oblanceolate, 3.9-5.9 inches long 1.6 inches wide and mucronate, unbroken, sharp to acuminate, and white. Stamens 2.0-3.9 inches long, are declinate, inserted in one continuous zone from throat 1.4 inches above the pericarpel and cream. The style (bearing the stigma) to 17, they are 2.0-9.6 inches long, stout, 0.24-0.31 inches thick, cream, and up to 26 stigma lobes, they can be whole or sometimes split at the top, cream, about 0.98 inches long. Nectar chambers are 1.2 inches long. The fruit is oblong to oval, 2.4-4.7 inches long, 1.6 to 3.5 inches thick, red with large bracteoles, with white pulp and edible black seeds.[14,15] *Selenicereus undatus* is lithophytic or hemiepiphytic. It is widely distributed through the tropics in cultivation. Like all true cacti, the genus originates in the Americas, but the precise origin of the species *S. undatus* is uncertain and it may be a hybrid. It is a sprawling or vining, terrestrial or epiphytic cactus. They climb by use of aerial roots and can reach a height of 10 meters (32.8 feet) or more growing on rocks and trees. This species is closely related to *S. ocamponis* and *S. escuintlensis*. *Selenicereus undatus* was described by (Haw.) Britton & Rose and published in Flora of Bermuda 256. 1918. In 2017, D. R. Hunt groups the genus *Hylocereus* within the genus *Selenicereus*. This has been supported by a phylogenetic analysis of the *Hylocereeae* tribe, therefore this species is consigned under the name *Selenicereus undatus* [14,16].

HEPATIC DISORDERS

Liver

The liver is the largest human organ weighing 1.5 kg. It occupies the upper right and slightly the left abdominal quadrants with flour lobes (right, left, quadrate and caudate).[17] The liver receives blood from hepatic portal vein (75%) and heapatic artery (25%).Blood from hepatic portal vein is rich in nutrients, broken erythrocytes, endocrine secretions and ingested toxins. While liver receives oxygenatede blood through hepatic artery. Portal triad supplies blood to sinusoids, hepatocytes and finally draining into central vein and sub-lobular veins. The alternative drainage is through hepatic veins moving to inferior vena cava. The endothelial cells of the central veins are surrounded by connective tissue fibres [18]. Histologicaly, liver comprises of several microscopic physiological and functional units that unitely work to ensure the proper activity of liver.

Hepatocytes

Hepatocytes are large polyhedral cells that account for 80% of total liver cells. They contain two to four large spherical nuclei at the centre of the cell. Bile canaliculi, the small narrow space between the adjacent hepatocytes is of 1.0-2.0 μm in diameter. The cell membranes near bile canaliculi shows tight junctions. The hepatocyte have a lifespan of five months in average.

Hepatic cirrhosis

This condition is characterised by aggregates of regenerated hepatic cells that are separated by bands of scar tissue (deposited collagen tissue). These two processes take place in response to some extent of hepatocyte destruction, resulting in their damage and subsequent death. Some causes include: Chronic alcoholism, Hepatitis B or Confection, Certain autoimmuneconditionsSomegeneticmetabolicdiseasesthatresultinanexcessivestorageofcopperandiron. The scar tissue negatively impacts the blood flow from the sinusoids to the hepatocytes, resulting in a decrease in function. As a result, portal hypertension develops because the blood cannot drain from the portal vein [19]. Cirrhosis often has no signs or symptoms until liver damage is extensive. When signs and symptoms do occur, they may include: Fatigue, easily bleeding or bruising, loss of appetite, swelling in your legs, feet or ankles(edema), weight loss, itchy skin, yellow discoloration in the skin and eyes(jaundice), Fluid accumulation in your abdomen (ascites), spiderlike blood vessels on your skin, redness in the palms of the hands, confusion, and slurred speech (hepatic encephalopathy) [20]. Hepatic cirrhosis may result from different diseases and conditions as

- Iron build up in the body (hemochromatosis)
- Cystic fibrosis
- Copper accumulated in the liver (Wilson's disease)

- Poorly formed bile ducts (biliary atresia)
- Alpha-1 antitrypsin deficiency
- Inherited disorders of sugar metabolism (galactosemia or glycogen storage disease)
- Genetic digestive disorder (Alagille syndrome)
- Liver disease caused by your body's immune system (autoimmune hepatitis)
- Destruction of the bile ducts (primary biliarycirrhosis)
- Hardening and scarring of the bile ducts (primary sclerosing cholangitis)
- Infection, such as syphilis or brucellosis
- Medications, including methotrexate or isoniazid

Risk factors

- Excessive alcohol consumption
- Obesity associated non-alcoholic fatty liver disease and non-alcoholic steato-hepatitis
- Viral hepatitis can also lead to liver cirrosis

Jaundice

It is the yellow discoloration of tissues by excessive bilirubin and bile pigment deposition. Jaundice can be mainly generated by hepatic and biliary tract diseases. Diseases like sickle cell anaemia causes excessive RBC breakdown, thereby exceeding bilirubin secretion, a condition called as pre-hepatic jaundice. While hepatic jaundice is a condition where metabolism is impaired due to liver damage releasing excess bilirubin. This jaundice is associated with both conjugated and unconjugated hyperbilirubinaemia. Post-hepatic (obstructive) jaundice is due to a chemical blockage in the biliary system, mostly due to gallstones [19,21].

Hepatitis

It is a common condition of liver inflammation. The major reason being the viral infections are hepatitis A, B, C, D, and E. These can be sexually transmitted. The viruses in the family Herpesviridae such as the herpes simplex virus may cause hepatitis. Chronic (rather than acute) infection with hepatitis B virus or hepatitis C virus is the main cause of liver cancer. Globally, about 248 million individuals are chronically infected with hepatitis B (with 843,724 in the U.S.), and 142 million are chronically infected with hepatitis C (with 2.7 million in the U.S.). Globally there are about 114 million and 20 million cases of hepatitis A and hepatitis E respectively, but these generally resolve and do not become chronic. Hepatitis D virus is a "satellite" of hepatitis B virus (can only infect in the presence of hepatitis B), and co-infects nearly 20 million people with hepatitis B, globally [14]. Hepatic encephalopathy is caused by an accumulation of toxins in the bloodstream that are normally removed by the liver. This condition can result in coma and can prove fatal. Budd–Chiari syndrome is a condition caused by blockage of the hepatic veins (including thrombosis) that drain the liver. It presents with the classical triad of abdominal pain, as cites and liver enlargement. Many diseases of the liver are accompanied by jaundice caused by increased levels of bilirubin in the system.

The bilirubin results from the breakup of the hemoglobin of dead red blood cells; normally, the liver removes bilirubin from the blood and excretes it through bile.[14] Other disorders caused by excessive alcohol consumption are grouped under alcoholic liver diseases and these include alcoholic hepatitis, fatty liver, and cirrhosis. Factors contributing to the development of alcoholic liver diseases are not only the quantity and frequency of alcohol consumption, but can also include gender, genetics, and liver insult. Liver damage can also be caused by drugs, particularly paracetamol and drugs used to treat cancer. A rupture of the liver can be caused by a liver shot used in combat sports [14]. Primary biliary cholangitis is an autoimmune disease of the liver. It is marked by slow progressive destruction of the small bile ducts of the liver, with the intralobular ducts (Canals of Hering) affected early in the disease. When these ducts are damaged, bile and other toxins build up in the liver (cholestasis) and over time damages the liver tissue in combination with ongoing immune related damage. This can lead to scarring (fibrosis) and cirrhosis. Cirrhosis increases the resistance to blood flow in the liver, and can result in portal hypertension. Congested anastomoses between the portal venous system and the systemic circulation, can be a subsequent condition.[14] There are also many pediatric liver diseases, including biliary atresia, alpha-1 antitrypsin

deficiency, alagille syndrome, progressive familial intrahepatic cholestasis, Langerhans cell histiocytosis and hepatic hemangioma a benign tumour the most common type of liver tumour, thought to be congenital. A genetic disorder causing multiple cysts to form in the liver tissue, usually in later life, and usually asymptomatic, is polycystic liver disease. Diseases that interfere with liver function will lead to derangement of these processes. However, the liver has a great capacity to regenerate and has a large reserve capacity. In most cases, the liver only produces symptoms after extensive damage [14]. The liver is the only human internal organ capable of natural regeneration of lost tissue; as little as 25% of a liver can regenerate into a whole liver. This is, however, not true regeneration but rather compensatory growth in mammals. The lobes that are removed do not regrow and the growth of the liver is a restoration of function, not original form.

This contrasts with true regeneration where both original function and form are restored. In some other species, such as zebra fish, the liver undergoes true regeneration by restoring both shape and size of the organ. In the liver, large areas of the tissues are formed but for the formation of new cells there must be sufficient amount of material so the circulation of the blood becomes more active.[14] This is predominantly due to the hepatocytes re-entering the cell cycle. That is, the hepatocytes go from the quiescent G_0 phase to the G_1 phase and undergo mitosis. This process is activated by the p75 receptors. There is also some evidence of bipotential stem cells, called hepatic oval cells or ovalocytes, which are thought to reside in the canals of Hering. These cells can differentiate into either hepatocytes or cholangiocytes. Cholangiocytes are the epithelial lining cells of the bile ducts. They are cuboidal epithelium in the small interlobular bile ducts, but become columnar and mucus secreting in larger bile ducts approaching the portahepatis and the extra hepatic ducts. Research is being carried out on the use of stem cells for the generation of an artificial liver [14,22]. The preliminary phytochemical analysis showed that the plant contained alkaloids, carbohydrate, proteins and amino acids, phytosterols, saponins, sterols, ascorbic acid, silicic acid, phenol, tannin flavonoids and triterpenoids. The plant contained silicic acid, tartaric acid, methyl esters of protocatechuic, caffeic acids isoquercitrin, apigenin and kaempferol as phenolic compounds. Stem contained silicic acid and silicates (5-8%), calcium (1.3%), potassium (1.8%) and other minerals such as aluminium, sulphur, phosphorus, sodium, zinc, magnesium and manganese.

Alkaloids such as nicotine, palustrine and palustrinine were isolated from the plant. The total phenolic content of n-butanol, ethyl acetate and water extracts were 96.4, 26.4 and 15.4 mg/g of dry extracts, respectively. The plant contained 0.6 to 0.9% flavonoids including apigenin-5-0-glucoside, genkwanin-5-O-glucoside, kaempferol-3,7-di-0-glucoside, kaempferol- 3-0-(6'-0- malonylgluco side)-7-0-glucoside, kaempferol-3-O-sophoroside, luteolin-5-O-glucoside, quercetin-3-O-glucoside. It was also contained caffeic acid ester (up to1%including chlorogenic acid, dicoffeoyl-meso-tartaric acid), 5-7.7% silicic acid and pyridine alkaloids, and styrolpyrone glucosides [56, 60, 63,69-73]. Equisetumo side A (3- methoxy-11,12-dihydroxy-phenylhexane-9-one-4-O-β-D-glucopyranoside), equisetumo side B(3-methoxy-4,11- dihydroxy-phenylhexane-9-one-12-O-β-D- glucopyranoside), equisetumoside C (cis -ferulic acid potassium salt 4- O-β-D- glucopyranoside), uridine, inosine, 2′-deoxyinosine, 2′-deoxycytidine, tryptophan, thymidine, 5-carboxy-2′- deoxyuridine, coniferin, and kaempferol 3-O-β-D-sophoroside-7- O-β-D-glucopyrano side were isolated from the water-soluble extract of fertile sprouts of Selenicereus undatus. The volatile constituents of the sterile stems of Selenicereus undatus were investigated using GC, GC/MS and 13C-NMR. Twenty-five compounds were identified. Hexahydrofarnesyl acetone (18.34%), cis-geranyl acetone (13.74%), thymol (12.09%) and trans-phytol (10.06%) were the major constituents. [23, 24]

Pharmacological Actions

The plant contained high amount of polyphenols. Antioxidant activity (ABTS assay) was estimated to be 98.13± 3.84 (μM Trolox equivalents/g dry weight). The total phenol content, total antioxidant capacity and silicic acid amount were found to be 18.67 %, 123 mg gallic acid/g dry weight extract, 1608 μM TEAC/mg dry weight extract and 0.0049 mg silicic acid/mg dry weight extract, respectively. Aqueous and ethanol extract from top and body portions of field horsetail were tested for antioxidative activity using four different methods. The ethanol extract fractions of each portion were richer in total phenolic components than water extracts. These fractions had remarkable antioxidative activities, similar to that of 5 mm ascorbic acid. Water extracts of both portions showed high superoxide anion

radical-scavenging activities. Hydroxyl radicals were effectively scavenged by ethanol extracts. rich in vitamins C and E. and contained high levels of copper and zinc. These were essential elements, for superoxide dismutase to act against active oxygen species [23,24] . The antioxidative activity of different horsetail (*Selenicereus undatus*) extracts was studied by the electron spin resonance spectroscopy–spin trapping method. The influence of different horsetail extracts during lipid peroxidation of sunflower oil induced by the lipophilic azo-initiator 4,4'-azobis(4- cyanovaleric acid) and soybean phosphatidylcholine liposomes induced by the hydrophilic azo-initiator 2,2'-azobis (2-amidinopropane) dihydrochloride was investigated. The results of electron spin resonance analysis confirmed that the extracts suppressed the formation of lipid peroxyl radicals in both systems investigated, in a dose- dependent manner.

The results indicate that n-butanol, methanol, ethyl acetate, and water extracts had significant peroxyl radical scavenging activity[23,24]. The antioxidant activity and phenolic composition of three different extracts (ethanol, nbutanol and water) of *Selenicereus undatus* were investigated by measuring the total reducing power (expressed by ascorbate equivalent antioxidant capacity-AEAC), inhibition of lipid per oxidation, and free radical scavenging capacity (RSC) towards 2,2-diphenyl-1- picrylhydrazyl (DPPH radical) and nitric oxide (NO). The anti-oxidative activity of horsetail extracts was tested by measuring their ability to scavenge stable 2,2-diphenyl-1- picrylhydrazyl (DPPH) and reactive hydroxyl radicals by electron spin resonance spectroscopy. The results demonstrated that the free radical scavenging activity (versus both DPPH and hydroxyl radicals) depended on the type and concentration of applied extracts; the highest DPPH (EC50 = 0.65 mg/ ml) and hydroxyl radical scavenging activities (EC50 = 0.74 mg/ ml) were obtained in the case of n-butanol extract. The radical scavenging activity of extracts significantly correlated with total phenolic content. Onitin and luteolin from *Selenicereus undatus* methanolic extract showed superoxide and DPPH free radical scavenging activity with IC50 = 35.3 ±0.2 microM and 5.9 ± 0.3 microM and IC50 of 35.8 ±0.4 microM and22.7 ±2.8 microM, respectively.[25]

Anticancer effect

The antiproliferative activity of different horsetail (*Selenicereus undatus*) extracts was studied using the sulforhodamine B colorimetric assay on the human cancer cell lines HeLa, HT-29, and MCF7. The antiproliferative of the extracts was depended on cell line, type of extract, and extract concentration. Ethyl acetate extract exhibited the most prominent antiproliferative effect, without inducing any cell growth stimulation on human tumor cell lines. Mouse fibroblasts cell culture (NCTC cell line clone L929) was used to study the effect of polyherbal extract (70% ethanolic extract: 4 g *Selenicereu sundatus*, 3 g *Achillea millefolium*, 2.5 g *Echinacea purpurea* and 0.5 g *Hyssopus officinalis*) on collagen secretion. Cells were supplemented with 5% FCS, containing different concentrations of polyherbal extract (35-140 μg/ml). The results showed a significantly ($P< 0.05$) increase of collagen synthesis in the culture medium of fibroblasts treated with 70 and 140 μg/ml polyherbal extract, after 48 h and 72 h of cultivation. It was observed that the collagen synthesis was almost 2 times higher in cultures treated with 140 μg/ml polyherbal extract, for 72 h, compared to the value obtained in the control group.

The water extract from sterile stems of *Selenicereus undatus* exerted dose dependent cytotoxic effects on human leukemic U 937 cells. DNA fragmentation, externalisation of phosphatidilserine, the colapse of mithocondrial transmembrane potential, were all observed in cells cultured for 48 h with the herb extract. The authors concluded that the cytotoxicity of *Selenicereus undatus* water extract against U 93 7 cells was due to apoptosis. The antiproliferative effect of *Selenicereus undatus* extract was tested on melanoma B16cells. At a concentration of > 0.5mg/ml, it showed significant antiproliferative effect. The cytotoxicity of the methanolic extract of the dried aerial part of *Selenicereu sundatus* was tested against various cancer cell lines including cervical adenocarcinoma, lung fibroblast, breast adenocarcinoma, and human embryonic kidney cells. After 72 hours treatment, the cells were assayed to determine the relative percentages of dead and live cells. The extract induced death on the four tested cell lines with the greatest effect on human embryonic kidney cells followed by breast adenocarcinoma. However, the extent of toxicity varied depending on the cell type and the concentration of the used extract. Compared to untreated cells, the plant extract had a profound cytotoxic effect on the breast cancer cell line. This effect was concentration-dependent, where 50 μg/ml had a larger effect than 20 μg/ml. A cytotoxic effect was also observed on the embryonic

kidney cell line, 50 μg/ml showed more activity than 20 μg/m,. On HeLa cells, only a very slight difference was observed when extract-treated cells were compared to untreated cells [23, 27]. The crude *Selenicereus undatus* protein extract inhibited cancer cell proliferation in cell culture of L-1210 (mouse derived leukemia cells), 3T3 (mouse derived SV-transformed fibroblasts) and HMV-1 (human derived melanin producing melanoma cells). It also caused life prolongation in mice in an in vivo study using L-1210 and B16F1 (mouse melanoma cells).Concentrations range between 100-3000 μg/ml were tested for the first trial to determine IC50 value, which was appeared as 500 μg/ml in 48 hour. For this concentration, viability was determined as 49.61%. Cytotoxic evaluation of IC50 for 24, 48 and 72 hour was compared with total phenol content and antioxidant activity of the extracts.

Strong correlation was recorded between cytotoxic activity and antioxidant activity and total phenol content. A significantly higher cytotoxic activity was processed with extraction medium containing 90% ethanol for 12 hour, while extracts obtained with 10% ethanol for 2 hour did not decrease the viability upon exposure to fibroblast cells. Antimicrobial effect: The methanolic extract of the aerial parts of *Selenicereus undatus* displayed antibacterial activity against Escherichia coli at high concentration (1g/ml).[23,28] *Selenicereus undatus* extracts showed antimicrobial activity against Staphylococcus epidermidis and Escherichia coli, but it possessed no effect against *Candida albicans*. A disk diffusion method was used for the evaluation of the antimicrobial activity of volatile constituents of *Selenicereus undatus* against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Salmonella enteritidis*. The antifungal activity of the oil was studied against *Aspergillus niger* and *Candida albicans*. The 1:10 dilution of the essential oil of *Selenicereus undatus* possessed a broad spectrum and very strong antimicrobial activity against all the tested bacteria and fungi. The antibacterial activity of ethanolic and aqueous extract of *Selenicereus undatus* was screened against selected urinary tract pathogens (*E.coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus saprophyticus* and *Enterococcus faecalis*) using disc diffusion technique. Both the extracts at different concentration exhibited antibacterial activity against all the tested bacterial strains. Ethanolic extract exhibited comparably a high degree of activity than the aqueous extract. The ethanolic extract was more effective against *E.coli*, *Proteus mirabilis* and *Staphylococcus saprophyticus* with a zone of inhibition of 24mm, 23mm and 24 mm diameter (at concentration of 1000μg) respectively and was least effective against *Pseudomonas aeruginosa* with zone of inhibition of 11mm (at concentration of 1000μg). Among the other studied bacterial species, *Klebsiella pneumoniae* and *Enterococcus faecalis* showed a zone of inhibition of 18mm diameter (at concentration of 1000μg) and *Staphylococcus aureus* showed inhibition zone of 14mm diameter (at concentration of 1000μg) [29].

The *in vitro* antibacterial activity of ethanol stem extract (50-400μg/ml) of *Selenicereus undatus* was studied against two Gram positive (*Bacillus subtilis* and *Micrococcus luteus*) and four Gram negative (Vibrio cholerae, Escherichia coli, *Shigella flexneri* and *Shigell adysenteriae*) bacteria. Out of six bacterial species (except *Shigell adysenteriae* and *Vibrio cholera*), four were found to be very sensitive to plant extract at all concentrations. The mean zone of inhibition for the extract against Gram positive and Gram negative bacteria increased with the increasing concentration of the extract. The highest mean zone of inhibition (32 mm) was recorded against *Escherichia coli*. The water extract of aerial parts of *Selenicereus undatus* possesses inhibitory effect on HIV-1 induced cytopathy. Effect on smooth muscles: The vasorelaxant activities of dicaffeoyl- meso-tartaric acid from *Selenicereus undatus* was studied in isolated rat aorta strips. It showed slow relaxation activity against norepinephrine (NE)-induced contraction of rat aorta with/without endothelium. This compound did not affect contraction induced by a high concentration of potassium (60 mM K+), while it inhibited NE-induced vasoconstriction in the presence of nicardipine. The results showed that the inhibition of NE-induced vasoconstriction was due to a decrease in calcium influx from the extracellular space caused by NE. In addition, dicaffeoyl tartaric acids showed vaso relaxant activity, regardless of their stereochemistry. Dried powdered plant material was extracted with alcohol. The extract obtained after the removal of the alcohol was triturated with petroleum-ether (40-60°) and then charcoaled, filtered and dried under vacuum. A 10 mg/ml solution/suspension of the extract (in distilled water) was added to the bath in 100-800 ug/ml concentrations to study its effect on isolated guinea-pig ileum. The extract of *Selenicereus undatus* antagonized the effect of acetylcholine on the isolated guinea-pig ileum preparation [23,30].

Central nervous effects

In studying of sedative and anticonvulsant effects of *Selenicereus undatus,* hydro alcoholic extract of *Selenicereus undatus* (200 and 400 mg/kg), it appeared that the extract possessed significant activity on the open field, enhanced the number of falls in the rota-rod reducing the time of permanence in the bar and increased the sleeping time (46% and 74% respectively) in the barbiturate-induced sleeping time. In the pentylenetetrazole seizure, it increased the first convulsion latency, diminished the severity of convulsions, reduced the percentage of animals which developed convulsion (50% and 25% respectively) and protected animals from death. However, in the elevated plus maze, the doses 50, 100 and 150 mg/kg did not affect the evaluated parameters. The ethanolic extract of *Selenicereus undatus* (50 and 100 mg/kg) significantly increased the time-spent and the percentage of the open arm entries in the elevated plus-maze model, the effect was comparable to diazepam. Ethanolic extract (100 mg/kg) prolonged the ketamine-induced total sleeping time and decreased the locomotor activity in mice [31]. The sedative, pre-anesthetic and anti-anxiety effects of *Selenicereus undatus* were studied in rats. The extract of *Selenicereus undatu*s was given at doses of (100, 200, 400 mg/kg, ip) and Diazepam with dose of (0.5 mg/kg, ip). The hydro alcoholic extract of *Selenicereus undatus* caused a significant increase in ketamine induced sleep and showed anxiolytic, sedative and preanesthetic effects at a dose of 200 mg/kg ip. The chronic administration of the hydro alcoholic extract of stems of *Selenicereus undatus* (HAE) reversed the cognitive impairment in aged rats. Chronic administration of HAE at dose of 50 mg/kg, ip, improved both short- and longterm retention of inhibitory avoidance task and ameliorated the cognitive performance in reference and working memory version of the Morris Water Maze. No differences were found between all three groups of young controls, aged controls and EHA-treated animals with regard to the open field and elevated plus maze tests. *In vitro* assays revealed that HAE diminished the thiobarbituric acid reactive substances as well as nitrite formation, but did not alter catalase activity. The authors concluded that the cognitive enhancement effects of the HAE may be attributed, at least in part, to its antioxidant action [23].

Effect on immune system

The influence of crude *Selenicereus undatus* protein on immune responses was investigated by measuring interleukin-2 (IL-2) and interferon-ϒ (IFN- ϒ) produced by Th1 cells. After 24-hour culture with 0.2 mg/ml of crude *Selenicereus undatus* protein in the presence of 5μg/ml ConA, 1,434.5 pg/ml of IL-2 was produced, showing 1.7 times greater production than that in the control. In cells cultured for 48 hours, 2,130.9 pg/ml was produced by cells treated with 0.2 mg/ml of crude *Selenicereus undatus* protein in the presence of 10μg/ml ConA, showing 1.9 times greater production than that in the control. Regarding the IFN- ϒ production-enhancing effect, 929.3 pg/ml was produced by cells cultured for 24 hours with 0.2 mg/ml of crude *Selenicereus undatus* protein in the presence of 5μg/ml ConA, suggesting that Th1 cells were activated [32]. Antidiabetic effect: The methanolic extract of *c*(50, 100, 250 and 500 mg/ kg daily for 5 weeks) was investigated for antidiabetic activity in streptozotocin-induced diabetic rats. The results showed that different doses of methanolic extract significantly lowered blood glucose. Also the weights of methanolic-extract treatment group were significantly higher. Concurrent histological studies of the pancreas of these animals showed comparable regeneration by methanolic extract which were earlier, necrosed by streptozotocin [23].

Antinociceptive and anti-inflammatory effects

The antinociceptive and anti-inflammatory effects of hydro alcoholic extract of stem from *Selenicereus undatus* were studied in mice. The extract 10, 25, 50 and 100mg/kg, ip, reduced the writhing induced by acetic acid in 49, 57, 93 and 98%, respectively. In the formalin test, 50 and 100mg/kg, ip, reduced in 80 and 95% the licking activity in the first phase, but in the second phase only the latter dose diminished the licking time (35%). In both phases, naloxone failed to revert the analgesic effect of the extract. In the hot-plate test, the extract at 100 and 200mg/kg does not change the latency to licking or jumping. In the carrageen an-induced paw oedema, the extract at 50mg/kg, reduced the paw oedema 2h (25%) and 4h (30%) after carrageen an administration. The dose of 100mg/kg caused reduction of the paw oedema (29%) only 4h after carrageen an administration. Effect on urinary system: The diuretic effect of EADE was assessed clinically by monitoring the volunteers' water balance over a 24 h period. The dried extract of *Selenicereus undatus* (900mg/day) produced a diuretic effect that was stronger than that of the negative control and was equivalent to that of hydrochlorothiazide without causing significant changes in the elimination of electrolytes. Only

rare minor adverse events were reported. The mechanism of action by which ethanol root extract of *Selenicereus undatus* (EA) influences urinary bladder activity in rats was studied. The plant was extracted by hot ethanol (95 %). Rats in EA group were treated with a standard diet containing 0.2 % of the extract, while rats in the control group were fed with the diet only. After 3 weeks, cystometry with 0.2% acetic acid solution and bladder activity was recorded, blood pressure, body weight and adenosine triphosphate were measuredand0.2 % acetic acid solution was infused into the bladder and urinary adenosine triphosphate was determined before and after the stimulation. The results showed that during cystometry with acetic acid, the time interval between urinary bladder contractions was shorter and maximum bladder contraction pressure was much greater in rats in the control group, but in the *Selenicereus undatus* group, the changes were much lower. Furthermore, in the *Selenicereus undatus* group, plasma adrenaline and nor adrenaline levels were lower than for the control group. In addition, increase in the levels of urinary adenosine triphosphate was smaller in *Selenicereus undatus* group than in control group. The authors concluded that *Selenicereus undatus* ethanol root extract influences urinary bladder activity by decreasing adenosine triphosphate release [23,33].

Inhibition of platelet aggregation

The extract of *Selenicereus undatus* produced a dose-dependent inhibition of thrombin and ADP-induced platelet aggregation. The effect of the plant could be related in part to the polyphenolic compounds present in the extract suggesting their involvement in the treatment or prevention of platelet aggregation complications linked to cardiovascular diseases [23].

Hepatoprotective effect

Hepatoprotective activity-guided fractionation of the methanol extract of *Selenicereus undatus* showed that onitin and luteolin isolated from the methanolic extract of *Selenicereus undatus* possessed hepatoprotective activities on tacrine-induced cytotoxicity in human liver-derived Hep G2 cells, displaying EC50 values of 85.8 ±9.3 microM and20.2 ± 1.4 microM, respectively, while, Silybin, used as a positive control, showed EC50 value of 69.0 ±3.3 microM [23].

Side effects, contraindications and toxicity

In acute toxicity the various plant extracts showed no side effects and mortalities in rats. In subacute toxicity study, no body weights changes, cumulative body weight gains, biochemical and hematological side effects were recorded in rats consume 0.3, 1 and 3% *Selenicereus undatus* powder in diet. In a reverse mutation test, the number of revertant colonies on the plates treated with *Selenicereus undatus* was not increased for *S. typhimurium*. The test substance was not found to have mutagenic potential. In a chromosomal aberration test with Chinese hamster lung cells, the incidence of cells with chromosomal aberrations was lower than 5% both by the short treatment method and the continuous treatment method; the test substance was not found to have chromosomal aberration potential. In the micronucleus test in rats, the incidence of micronucleus was not significantly increased: the test substance was not found to have mutagenicity potential *in vivo*. However, the plant was possibly unsafe when taken by mouth long-term. It contained thiaminase, which breaks down the vitamin thiamine. This effect could lead to thiamine deficiency. Some products were labeled (thiaminase-free), but there was no enough information available for their safety [34].

There was no enough information about the safety of taking horsetail in pregnant or breast-feeding woman. It was contraindicated in alcoholic people who, they were generally also thiamine deficient. Therefore, taking horsetail might make thiamine deficiency worse. Horsetail lowered blood sugar levels in people with diabetes. Horsetail might flush potassium out of the body, possibly leading to decrease potassium levels. It used with caution in patient at risk for potassium deficiency. Horsetail was also contraindicated in patients who have edema due to impaired heart and kidney function. A doctor should be consulted when the drug is utilized as a bath additive in cases of major skin lesions, acute skin lesions of unknown origin, major feverish and infectious diseases, cardiac insufficiency and hypertonia. Toxicity was recorded in animals, symptoms of *Selenicereus undatus* poisoning were seen primarily in young, rapidly growing horses, cows and sheep. The symptoms of *Selenicereus undatus* poisoning developed slowly. These included scruffy physical appearance, diarrhea and slight in coordination. Untreated poisoning will

developed to loss of muscular control, staggering gait and nervousness. Animals may lie down and not be able to get up, may complain seizure and die within 1-2 weeks. Treatment should be oriented to remove the source of poisoning, Equisetum should not present in hay. Thiamine (vitamin B1) may be administered initially intravenously, then intramuscularly for several days. The acute hepatotoxicity of *Selenicereus undatus* (30, 50, and 100mg/kg for 14 days) was evaluated in rats. Blood samples were obtained to determine TGO, TGP, FA, DHL and GT-gamma activities. Hepatic tissue samples were collected for the anatomopathologic analysis. The anatomopathologic exam of the hepatic tissue showed lobular structure, however, there was no significant change in the activities of the hepatic enzymes when compared to control group [23,24].

CONCLUSION

The hepatoprotective activity of *Selenicereus undatus* was studied in albino wistar rats by inducing hepatotoxicity using paracetamol. The haematological parameters have shown a increase in liver enzymes in blood after liver damage and become to normal after giving the treatment. A high dose compared to low dose was found more effective in attaining hepatoprotection. The his to pathological studies were observed in microscopic examination, which confirmed the liver damage with paracetamol and a recovery of healthy hepatocytes after the treatment for 7 days. Thus it is concluded that the plant powder from *Selenicereus undatus* may be consisting of an active constituents which can improvise the health status of hepatocytes in damaged liver and also can protect the liver from damaging. Still further confirming studies are required for identifying the exact mechanism of action as well as identifying the lead molecule from the plant that is essential for hepatoprotective activity.

Conflict of interest: authors report no conflict of interest

REFERENCES

1. Giby Abraham (2014), A review on hepato-protective herbs usedinayurveda,*Global J Res. Med. Plants &Indigen. Med.*, Volume 3(7): 303–311
2. Hiraganahalli BD, Chinampudur VC, Dethe S, Mundkinajeddu D, Pandre MK, Balachandran J, Agarwal A. Hepatoprotective and antioxidant activity of standardized herbal extracts. Pharmacognosy magazine. 2012 Apr;8(30):116.
3. Editorial Committee of Chinese Medicinal Herbs, Chinese Medicinal Herbs, Shanghai Science and Technology Publishing House, Shanghai. 2, 1999;56–58.
4. Halliwell B, Gutteridge JM. Oxygen toxicity, oxygen radicals, transition metalsand disease. Biochem J.1984;219:1–14.
5. Jaeschke H, Gores GJ, Cederbaum AI, Hinson JA, Pessayre D, Lemasters JJ. Mechanisms of hepatotoxicity. Toxicol Sci.2002;65:166–76.
6. Jakus V. The role of free radicals, oxidative stress and antioxidant systems indiabetic vascular disease. BratislLekListy.2000;101:541–51.
7. Xu J, Ma M, Purcell WM. Characterization of some cytotoxic endpoints using rat liver and HepG2 spheroids as in vitro models and their application in hepatotoxicitystudies.
8. Glucose metabolism and enzyme release as cytotoxic markers. Toxicol App Pharmacol.2003;189:100–11.
9. D'Agostino M, Dini A, Pizza C, Senatore F, Aquino R. Sterols from Selenicereusundatus. Boll SocItalBiolSper 60, 1984;(12):2241–5.
10. HyuncheolOha,Do-HoonKimb,Jung-HeeChob,Youn-ChulKimc.Hepatoprotective and free radical scavenging activities of phenolic petrosins and flavonoids isolated from *Selenicereus undatus*, J. Ethnopharmacol. 95, 2004; 421– 424.
11. Malar HL, Bai SM. Hepatoprotective activity of *Phyllanthus emblica* against paracetamol induced hepatic damage in Wister Albino Rats. Afr J Basic Appl Sci. 2009;1:21–5.
12. SantosAR,NieroR,FilhoVC,YunesRA,PizzolattiMG,DelleMonacheF,etal.Antinociceptive properties of steroids

isolated from *Hyllanthus corcovadensis* in mice. Planta Med 61, 1995;(4):329–32.

13. Galisteo M, Rissel M, Sergent O, Chevanne M et al. Hepatotoxicity of tacrine: occurrence of membrane fluidity alterations without involvement of lipid peroxidation. J. Pharmacol. and Experimental Therapeutics 294, 2000;160–167.
14. *Selenicereus undatus*. Wikipedia, the free encyclopedia. Available at: https://en.wikipedia.org/w/index.php?title=Selenicereus_undatus&oldid=1083861931
15. Hepatitis A, B, and C Center: Symptoms, Causes, Tests, Transmission, and TreatmentsArchived 2016-01-31 Webmd.com(2005-08-19).
16. Schweitzer A, Horn J, Mikolajczyk RT, Krause G, Ott JJ (2015). "Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013". Lancet. 386 (10003):1546–1555.
17. Dény P (2006). "Hepatitis delta virus genetic variability: from genotypes I, II, III to eight major clades?".Curr. Top. Microbiol. Immunol. Current Topics in Microbiology and Immunology. 307:151–171.
18. Hirschfield, GM; Gershwin, ME (Jan 24, 2013). "The immunobiology and pathophysiology of primary biliary cirrhosis". Annual Review of Pathology. 8: 303– 330.
19. Adrian Rad. Liver histology. 2022; Available at: https://www.kenhub.com/en/library/anatomy/liver-histology
20. MFMER. Cirrosis. Symptoms, causes, diagnosis, treatment. 2021 Jan 25. Available at: https://www.mayoclinic.org/diseases-conditions/cirrhosis/symptoms-causes/syc-20351487#:~:text=Cirrhosis%20slows%20the%20normal%20flow,in%20the%20abdome
21. Chu, Jaime; Sadler, Kirsten C. (2009). "New school in liver development: Lessons from zebrafish". Hepatology. 50 (5): 1656–1663.
22. Suzuki K, Tanaka M, Watanabe N, Saito S, Nonaka H, Miyajima A (2008). "p75Neurotrophin receptor is a marker for precursors of stellate cells and portal fibroblasts in mouse fetal liver". Gastroenterology. 135 (1): 270–281.
23. Al-Snafi AE. The pharmacology of Equisetum arvense-A review. IOSR Journal of Pharmacy. 2017;7(2):31-42
24. Tietz PS, Larusso NF (May 2006). "Cholangiocyte biology". Current Opinion in Gastroenterology. 22 (3):279–287.
25. Čanadanović-Brunet JM, Ćetković GS, Djilas SM, Tumbas VT, Savatović SS, Mandić AI, Markov SL, Cvetković DD. Radical scavenging and antimicrobial activity of horsetail (Equisetum arvense L.) extracts. International journal of food science & technology. 2009 Feb;44(2):269-78.
26. Kawarada, Y; Das, BC; Taoka, H (2000). "Anatomy of the hepatic hilar area: the plate system". Journal of Hepato-Biliary-Pancreatic Surgery. 7 (6):580–586.
27. Clemens, J (2003). "In Memory of Ella O. Campbell, DNZM, FRIH" (PDF). Royal New Zealand Institute of Horticulture. 6 (1):2.
28. Philip D Mayne "Plasma enzymes in Diagnosis in Clinical Chemistry in Diagnosis and treatment ELBS 1994 Chapter15:299-313.
29. Tietz. N.W., Fundamentals of Clinical Chemistry, W.B. Saunders and Company, Philadelphia, PA (1976)602.
30. Henderson, A.R., Moss, D.W., Enzymes Tietz Fundamentals of Clinical Chemistry, 5th Ed., Burtis, C.A. &Ashwood, E.R. (W.B. Saunders eds. Philadelphia USA), (2001), 352.
31. Tietz,N.W.,Clinicalguidetolaboratorytests,3rdEd.,(W.B.Saunderseds.Philadelphia USA), (1995), 76.
32. U.S. National Plant Germplasm System, Selenicereusundatus, https://npgsweb. arsgrin. gov/gringlobal/taxonomydetail.aspx.400386.
33. Flora of North America, Selenicereusundatus, http://www.efloras.org/florataxon.Aspx flora &taxon 233500616.
34. Asgarpanah J and Roohi E. Phytochemistry and pharmacological propertiesofSelenicereusundatusL. Journal of Medicinal Plants Research 20112; 6(21): 3689- 3693.

Figure 1: various parts of *Selenicereu sundatus* plant (https://www.nparks.gov.sg/florafaunaweb/flora/1/4/1419)

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RESEARCH ARTICLE

Adsorption of Crystal Violet Dye on Biomass from *Bauhinia tomentosa* Seed Pod Powder: Equilibrium and Kinetic Studies

K. Poonkodi[1]*, V. Anitha[2], R. Chitradevi[2], M. Suganthi[3], K. Vimaladevi[4], V. Prabhu[4], R. Mini[4] and M. Anusuya[4]

[1]Assistant Professor and Head, Post Graduate Department of Chemistry, Nallamuthu Gounder Mahalingam College, Pollachi, Coimbatore, Tamil Nadu, India
[2]Assistant Professor, Department of Chemistry, Sri G.V.G Visalakshi College for Women, Udumalpet, Tiruppur, Tamil Nadu, India
[3]Assistant Professor and Head, Department of Chemistry, Dr.N.G.P Arts and Science College, Coimbatore, Tamil Nadu, India
[4]Assistant Professor, Department of Chemistry, Nallamuthu Gounder Mahalingam College, Pollachi, Coimbatore, Tamil Nadu, India

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***Address for Correspondence**
K. Poonkodi
Assistant Professor and Head,
Post Graduate Department of Chemistry,
Nallamuthu Gounder Mahalingam College,
Pollachi, Coimbatore, Tamil Nadu, India
Email: Poonks.che@gmail.com

ABSTRACT

The purpose of the current work is to explore the adsorptive removal of Crystal violet dye using a green adsorbent prepared from *Bauhinia tomentosa* seed pods (BTSP). The adsorbent was characterized by the use of FT-IR, XRD, EDAX and SEM studies. To evaluate the synthesized activated carbon's adsorption effectiveness, batch mode adsorption was used. The effect of pH, contact time, reaction temperature and adsorption dosage were tested. The experimental data were evaluated using Freundlich, Langmuir and Tempkin adsorption isotherms. Pseudo-first-order, pseudo-second-order, Elovich, and intraparticle diffusion models were used to assess the adsorption reaction's kinetics. The percentage removal of CV dye increases with the increase of pH, an increase in time, an increase in temperature and an increase in adsorbent dosage. The data were well fitted with Langmuir adsorption, which demonstrated that the elimination of CV dye followed the monolayer adsorption isotherm. The ideal pH range was 4 to 9, and the adsorption equilibrium was attained in 70 minutes. Pseudo-second-order and Elovich models successfully predicted the Crystal violet dye removal procedure. According to the results, the low-cost adsorbent made from *B. tomentosa* shows a high potential for removing dye from an aqueous solution.

Waste seed pods can be utilized in large-scale dye removal since they are an excellent, low-cost alternative to commercial adsorbents.

Keywords: Adsorption, Crystal violet, *Bauhinia tomentosa*, Equilibrium and kinetics.

INTRODUCTION

In recent years, man-made synthetic chemicals have become more prevalent in the environment. Poor disposal practises and insufficient measures to manage harmful effluents from various sectors have resulted in widespread contamination of surface water and ground water. Synthetic dyes are used by numerous major industries, like paper, plastic, cosmetics, textiles, and food, to colour their products [1]. The most evident sign of water pollution is colour; when coloured debris is dumped into streams, it not only detracts from their aesthetic appeal but also prevents sunlight from reaching the streams, which affects photosynthesis [2]. It is highly noticeable and undesirable when relatively minute levels of dyes are present in water [3]. Some colours are mutagenic and can cause cancer [4]. The Proper treatment of waste water is crucial due to the numerous negative impacts, and while many effective technologies are already in use, alternative low-cost and readily accessible adsorbents are still required. One of the most widely distributed plants, *Bauhinia tomentosa* is utilized as ornamental purpose and has many medicinal uses. The seed pods can be employed as a green adsorbent for the treatment of waste water and are freely available. From the literature, there is no report on the green adsorbent prepared from *B. tomentosa* seed pod, the present study aimed to evaluate the adsorptive removal of Crystal violet by green adsorbent prepared from *B. tomentosa* seed pods and its equilibrium and kinetics studies.

MATERIALS AND METHODS

Preparation of adsorbent

Ripened *B.Tomentosa* seed pods (BTSP) were collected in Samathur, Tamilnadu, India. The pods were cleaned with water to remove any dust particles, cut into small pieces, and then dried for a total of 10 days in the sun and another 24 hours in a hot air oven at 60°C. The material powdered up well after being fully dried. The powdered raw material was chemically activated by treating it with Conc. Sulphuric acid (1:2) with constant stirring and kept it for 24 hours. The resulting carbonized material was thoroughly cleaned with plenty of water, rinsed multiple times with distilled water to bring the pH level down to 7 and then dried at 105°C to 110°C in a hot air oven. The resulting adsorbent was thoroughly crushed, sieved through 210 mesh, labelled (BTSP), and stored in an airtight container for later use.

Adsorbate Solution

The adsorbate, Crystal Violet is a monovalent cationic triphenyl methane dye,the stock solution was prepared by dissolving 1g of Crystal Violet dye (AR) in distilled water and diluted to 1000 ml (1ppm). The stock solution was diluted to appropriate concentrations. The initial pH was adjusted with 0.1 M HCl or 0.1M NaOH.

Adsorbent Characterization

Scanning Electron Microscopy (SEM) and Energy Dispersive Analysis of X-Ray (EDAX) were used to evaluate the surface morphology of BTSP at the microscopic level. BTSP's crystalline structure was assessed using an X-ray diffract meter (XRD). Fourier Transform Infrared Spectroscopy was used to examine the functional groups that were present on the surface of BTSP.

Adsorption experiments

Batch mode experiments were performed to study the effect of various parameters such as contact time, pH, adsorbent dose, and temperature affecting the adsorptive removal of CV dye. In the adsorption experiments, 100 ml

of the dye solutions of the desired concentration and pH were taken in a conical flask containing pre-determined weighed amounts of adsorbent (50mg). The conical flask containing adsorbent and adsorbate was equilibrated by shaking the contents at room temperature using a thermostat coupled rotary shaker (120 rpm) for different time intervals (10, 20, 30, 40, 50, 60, and 70 minutes). Then the solutions were filtered using filter paper and the filtrates were analyzed for the residual CV dye concentration using a UV spectrometer at a wavelength of 591 nm.

$$\% \text{ removal of CV dye} = \frac{C_o - C_e}{C_0} \times 100 \quad (1)$$

Were, C_o and C_e (mg/L) are the initial and equilibrium concentration of CV dye respectively. The amount of dye adsorbed at equilibrium (q_e) was calculated from the following equation.

$$q_e = \frac{(C_0 - C_e)}{M} X\ V \quad (2)$$

Where q_e is the amount of dye adsorbed at equilibrium (mg/g). C_0 and C_e (mg/L) are the initial and equilibrium concentration of CV dye respectively. V is the volume of the solution (L) and M is the mass of the adsorbent used (g).

Adsorption isotherm studies

The adsorption isotherm models generally design and comprehend the mechanism of interaction between adsorbate and the adsorbent at equilibrium (Hameed et al, 2008). The Freundlich model implies heterogeneous energy distribution of active sites, while the Langmuir model assumes monolayer adsorption on a physically homogeneous adsorbent.

Langmuir equation

$$\frac{C_e}{q_e} = \frac{1}{q_m K_L} + \frac{C_e}{q_m} \quad (3)$$

Freundlich equation

$$\log q_e = \log k_f + \frac{1}{n} \log C_e \quad (4)$$

Tempkin isotherm

$$q_e = B\ln A + B\ln C_e \quad (5)$$

Where, q_e (mg/g) is the amount of dye adsorbed per unit weight of adsorbent, Ce (mg/L) is the concentration of dye solution at equilibrium, k_f (mg/g) and n are the Freundlich constants that indicate the adsorption capacity and intensity of adsorption respectively.

Adsorption kinetics

Kinetic studies are carried out to provide information about the mechanism of adsorption.

Pseudo- first –order kinetic [5]

$$\text{Log}(q_e - q_t) = \log q_e - k_1 t / 2.303 \quad (6)$$

Where q_e and q_t (mg/g) are the amounts of dye adsorbed at equilibrium and at time t and k_1 (1/min) are the rate constant of the pseudo -first order adsorption

Pseudo- second – order kinetic [6]

$$t/q_t = 1/K_2 q_e^2 + t/q_e \quad (7)$$

K_2 (g mg^{-1} min^{-1}) is the rate constant of pseudo -second order adsorption

Elovich kinetic model

$q_t = 1/\beta \ln(\alpha\beta) + 1/\beta \ln t$ (8)

The parameter β (g mg $^{-1}$) is related to the extent of the surface coverage and activation energy for chemisorptions [7,8]. The values of α and β can be calculated from the plot of q_t against 1/ ln t.

RESULTS AND DISCUSSION

FT-IR Analysis

The functional groups present in the raw powder, before and adsorption of CV dye onto BTSP were presented in Fig.1a,b & c.The broad peak appeared in raw powder responsible for -OH and –NH stretching at 3200-3500cm^{-1}, a sharp peak at 2800cm^{-1} was attributed to C=C symmetrical stretching, another sharp peak at 1670 cm^{-1} was due to C=O carbonyl stretching frequencies. From the IR results the *B.Tomentosa* seed pod contains many phytochemicals especially carboxylic acids, phenolic compounds, alcohol or amine compounds [9]. After carbonization, the peak intensity of –OH and C=C stretching was reduced. But After the adsorption of CV dye onto BTSP, the –OH and –C=O stretching disappeared, this may be due to the adsorption of dye molecule by these two active functional groups.

Scanning Electron Microscopic (SEM) studies

Figure 2 displays scanning electron microscopic images of the adsorbent BTSP both before and after Crystal violet dye adsorption. The image demonstrated the huge active cavities in the activated carbon made from *B. tomentosa* plant seed pods. These cavities are particularly helpful for adsorbing the dye molecules, as shown by the SEM image after adsorption, which revealed a reduction in cavity size from 50 to 5 m. This illustrates the efficiency of a prepared adsorbent.

EDAX Analysis

Based on the EDAX results, the elementary analysis of the BTSP fruit shell before adsorption are presented in Fig.3. The elements and their % mass were listed in Table 1. High levels of carbon served as an effective adsorbent.

X-ray diffraction analysis

The adsorbent can be crystallographically characterized by means of XRD. The XRD pattern of the adsorbents showed the typical spectrum having main and secondary peaks at around (2Θ=24°, 64°) respectively. The results showed that the adsorbent has more pore structures and resembles with graphitic carbon [10].

BATCH MODE ADSORPTION STUDIES

Effect of contact time on the adsorption of CV on to BTSP

As reaction time increased, the amount of dyes adsorbed also increased [11]. At an ideal initial concentration (100ppm and 150ppm), studies were conducted to determine the impact of contact time on the removal of CV dye [12]. The elimination of CV was 98.42% in 100 ppm and 99.06 % in 150 ppm. Fig. 4a shows the impact of the length of time the adsorbent and adsorbate were in contact. According to the findings, the adsorption of dye increased with longer shaking times and reached equilibrium at a constant value after a certain amount of time (70min). Additionally, it was shown that the dye's uptake began quickly during the first few minutes of contact time, increased gradually until it reached equilibrium, and then stabilized there. In addition, Fig. 4a probable monolayer covering of dye on the carbon surface was suggested by continual increase in adsorption, which eventually reached saturation [3].

Effect of adsorbent dose on the adsorption of CV onto BTSP

The effect of adsorbent dose was also tested for the removal of all the dyes from the aqueous solution. 100 ml of an aqueous dye solution and an adsorbent dosage ranging from (10 mg to 70 mg) were used to conduct the experiments [13]. The results, which are displayed in Fig.4b, demonstrated that dye removal efficacy increased with increasing

amount of BTSP. To put it another way, dye removal was dose-dependent. In 70 mg of BTSP, the elimination of CV was 100% for 100ppm and 98.77% for 150ppm. The increase in adsorption with the addition of more adsorbent can be attributed to the availability of more adsorption sites and an increase in adsorption surface[14,15].The addition of more binding sites for adsorption led to an increase in the elimination of dyes with increased adsorbent dose[13]. Beyond 70 mg/l, increasing the adsorbent dosage didn't significantly improve the efficiency.

Effect of pH on the adsorption of CV onto BTSP

In both chemical and biological interactions, the pH factor is extremely important. The amount of electrostatic charges that are imparted by the ionised dye molecules and the charges on the surface of the adsorbent are controlled by the pH of a medium [16]. Fig. 5a evaluated how pH affected the adsorptive removal of CV dye from BTSP. At pH 9, the greatest amount of elimination was seen (92.75 for 100 ppm & 94.36 for 150 ppm). Due to the large concentration of H^+ ions, which create repulsion and hence diminish biosorption at lower pH levels, CV biosorption was low at lower pH levels [17]. There are more negatively charged surfaces accessible when the pH rises, which causes the attraction between the positively charged dye molecule and the biosorbent to weaken [5].

Effect of temperature on the adsorption of CV onto BTSP

In order to determine the impact of temperature on the percentage of CV dye removed by BTSP adsorbent, experiments were performed at various temperatures for 100ppm and 150ppm dye concentrations (35.5 to 43.5°C). The findings are shown in Fig. 5b.As the temperature increased, the rate of absorption removal also increased, reaching a maximum at 97.9° C for 100 ppm and 99.53° C for 150 ppm [13]. This can be explained by the fact that the reduction in solution viscosity caused by an increase in temperature speeds up the diffusion of adsorbate molecules through the exterior boundary layer and into the internal pores of the adsorbent particles [18].Furthermore, the mobility or collision of the adsorbate support molecules increases with temperature, which also raises the quantity of active sites on the adsorbent [22].

Adsorption kinetics for the adsorption of CV onto BTSP

Pesudo first order kinetic model

For various dye concentrations, linear plots of log (qe-qt) vs. t were obtained (100 and 150 ppm). As a result, the adsorption process followed the first order rate expression (Fig.6a). The basic dye adsorption rate constants were observed to be (0.0112 and 0.0017 1/min). The regression coefficient (R2) of linear plots of pseudo-first order values was chosen as (0.944 & 0.939) for 100 &150 ppm, respectively. When pore diffusion limits the adsorption process, However the relationship between initial solute concentration and rate of adsorption will not be linear.

Pseudo- second –order kinetic model

If second-order kinetics is used, the plot of t/qt versus t shows a linear relationship. As shown in Fig. 6b, (t/ qt vs. t) is a straight line. For 100 and 150ppm, the K2 values are (0.004 & 0.002), respectively. The plot's slopes and intercepts determine the k2 and qe values. The best fit model was chosen because the values of the regression coefficient (R2) of linear plots of pseudo-second order are (0.999 & 0.973) for 100 and 150ppm, respectively. The linear plot revealed a high degree of agreement between the experimental and calculated qe values for CV adsorption onto BTSP. The best correlation for the system was provided by the pseudo-second-order model, which suggested that chemical adsorption might involve valancy forces via electron sharing or exchange between adsorbent and adsorbate [4]. This phenomenon could be explained in two stages: first, the concentration gradient drove the CV ions to access or enter the pores of the adsorbent; second, the CV ions did not diffuse into the pores of pirina for further reactions until the surface functional sites were fully occupied [19].

Elovich kinetic model

Adsorption also followed the Elovich model, a plot of qt vs lnt should yield a linear relationship with slope of (1/b) and an intercept of (1/b) ln (a b) [20]. The Elovich plot ln T vs qt was shown in fig 6c. The R^2 values are (0.973 & 0.944) for 100 and 150 ppm respectively. Pseudo second order results are in similar to that of Elovich model, thus indicating that the dynamic uptake of crystal violet can be best interpreted by the pseudo second-order model. It can

also be seen that the experimental equilibrium sorption values, obtained for 100 ppm &150 ppm dye solutions are in close agreement with the theoretical values. The corresponding correlation coefficient (R^2) values for the pseudo-second –order kinetic model was 0.999 in 100ppm & 0.973 150 ppm, indicated the applicability of the pseudo- second –order kinetic model which described the adsorption process of CV onto BTSP. It revealed that the pseudo- second – order kinetic model provided good correlation for the adsorption. The higher R^2 values confirmed that the sorption process of CV onto BTSP follow a pseudo- second –order kinetic model. Similar trends were observed for dye adsorption onto *Zea mays* [21]. It was suggested that the adsorption was controlled by chemisorptions [22]. Comparison of the correlation coefficients of kinetic parameters for the adsorption of CV onto BTSP was given in table. 2

Adsorption Isotherms for the Adsorption of CV on to BTSP

Langmuir Adsorption Isotherm

The values of K_L and b were determined from the slope and intercept of the plot. The K_L values were found in the range (0.2032 & 0.1459) for 100 ppm and 150 ppm, respectively indicated favourable adsorption of CV onto BTSP given the Table 3. When showed the values of Ce and Ce/qe. The graph is plotted between Ce vs Ce/ qe the linear line show the figure 7a & b. The maximum adsorption corresponds to a saturated monolayer of adsorbate on the adsorbent surface with constant energy.

Freundlich Adsorption Isotherm

A plot of log qe versus log Ce(Fig 8a & b) enabled us to determine the constant K_f and 1/n. $K_f$is the indicator of the adsorption capacity, related to the bond energy and 1/n is the adsorption intensity of dye onto the adsorbent or surface heterogeneity. The magnitude of the exponent, 1/n, gives an indication of favourable adsorption. Value of 1/n <1 represent a favourable adsorption condition while 1n>1 is indicative of cooperative adsorption[6]. The K_f values were found in the range (1.8757& 2.0218) for 100 ppm and 150 ppm and 1/n values are (0.1270& 0.1830) for 100 ppm and 150 ppm. The graph is plotted log Cevs logCe/qe the linear line was obtained and given in the (Fig 8 a & b).

Tempkin isotherm

Temkin isotherm takes into account adsorbing species- adsorbent interactions [24]. Isotherm constants A and B can be determined from a plot of qevs in Ce shown in Fig 9a & b. The correlation coefficients R2 value of (0.821 & 0. 781) for 100 ppm and 150 ppm respectively. The comparison of the linear form of Langmuir, Freundlish and Tempkin isotherms on the adsorption of CV onto BTSP can be made with the help of Table 3. It showed that the correlation Langmuir isotherm is much better (0.999 & 0.997) for 100 ppm and 150 ppm than that of Freundlish isotherm model (0.832 & 0.848) for 100 ppm and 150 ppm and Tempkin model (0.821 & 0.781) for 100 ppm and 150 ppm. Moreover Langmuir isotherm was the best fitted than other models. It proved the mono layer adsorption takes place in the adsorption of CV onto BTSP.

CONCLUSION

The results of the present investigation suggested that the BTSP might be promising low cost adsorbent for the removal of Crystal violet from waste water. There is no report on the adsorptive removal of dye solution by activated carbon prepared from *Bauhenia tomentosa*. The results proved that it can be utilized as a natural adsorbent in wastewater treatment as an alternate for commercial adsorbent.

Authors declares no conflict of interest

REFERENCES

1. R. Ahmad,"Studies on adsorption of crystal violet dye from aqueous solution onto coniferous pinus bark powder."J. Hazard, Mater.,171 (1), 767–773, 2009.

2. G. Annadurai,R.S. Juangand.J. Lee, "Use of cellulose based wastes for adsoption of dyes from aqueous solutions".J. Hazard mater.,.892, 263-274, 2002.
3. S.Arivoli S and M. "Hema Comparative Study on the Adsorption Kinetics and Thermodynamics of Dyes onto Acid Activated Low Cost Carbon"*Inter. J. Phy. Sci.,* 10 17 (2007).
4. Y.Benjelloun, Y.MiyahIdrissi, S. Boumchita S., A. El OualiLalami, and F.Zerrouq. "Removal of Crystal Violet by wet oxidation with H2O2 over an Iron oxide catalyst synthesized from Fly Ash." J Mater. Environ. Sci., 8 (7), 2259-2269 2017.
5. S.Chakraborty, S.Chowdhury, and P.D.Saha."Adsorption of crystal violet from aqueous solution onto NaOH-modified rice husk"Carbohydr. Polym. 86 (4), 1533–1541,2011.
6. Y .S.Ho, and G. Mckay." Pseudo second order model for sorption process."*Process Biochem.,*34, 451-465, 1999.
7. Y .S.Ho, and G. Mckay."Kinetic models for the sorption of dye from aqueous solution by wood."*Trans.I Chem. E. B,*76 183-191, 1998.
8. S.Lagergren, About the Theory of So-Called Adsorption of Soluble Substances. Kungliga Svenska Vetenskapsakademiens Handlingar, 24, 1-39,1898.
9. J.H.Elovich., andE.D Schulman."Proceedings of the Second International Congress on Surface Activity". Academic Press, Inc., New York, 11, pp.253, 1959**.**
10. E.H.Gürkan,S. Çoruhand S.Elevli."Adsorption of lead and copper using waste foundry sand: statistical evaluation."Int. J. Global Warming,14(2), 260-263,2018.
11. Y.Ming Chen, T.Tsao, M.Wang."Removal of Crystal Violet and Methylene Blue from Aqueous Solution using Soil Nano-Clays." IPCBEE, IACSIT Press **8,** 2011.
12. P.Priya, R.Nithya,R.Anuradha, T.Kamachi. "Removal of Colour from Crystal Violet Dye using Low Cost Adsorbents." International Journal of ChemTechResearch6(.9), 4346-4351, 2014.
13. V.K.Verma and A.K.Mishra, "Removal of dyes using low cost adsorbents." Indian J. Chem. technol., 15,140-145, 2008.
14. C.Namasivayam and S.Sumithra. "Removal of direct red 12B and methylene blue from water by adsorption onto Fe (III)/Cr (III) hydroxide, an industrial solid waste."*J. environ management*74(3), 207-215, 2005.
15. S.Senthilkumaar, P.Kalaamani and C.V.Subburaam. "Liquid phase adsorption of crystal violet onto activated carbons derived from male flowers of coconut tree."J. Hazard Mater, 136, 800–808,2006.
16. P.Mohandass and T. K.Ganesan."Removal of Dyes Using Leaves of Morinda Pubescensas a Low Cost Green Adsorbents."Int. J. Innovative Res. Sci. Eng. and Technol, 5(2), 1565-1571, 2016.
17. A. Saeed,M.Sharif andM.Iqbal."Application potential of grapefruit peel as dye sorbent: Kinetics, equilibrium and mechanism of crystal violet adsorption." J. hazard mater, 179(1), 564-572, 2010.
18. M.R.Unnithan, V.P.Vinodand T.S.Anirudhan. "Ability of iron(III) loaded carboxylated polyacrylamide-grafted sawdust to remove phosphate ions from aqueous solution and fertilizer industry wastewater: Adsorption kinetics and isotherm studies."J. Appl. Polym. Sci.,84, 2541–2553,2002.
19. S.Coruh, E.F.Gurkan. "Adsorption Of Crystal Violet From Aqueous Solutions Using Pirina." International Journal of Advances in Science Engineering and Technology, Vol-4,3, spl-2 , 2016.
20. K.Sakthivel, I.Arockiaraj, C. Kannanand S.Karthikeyan. "Film- Pore diffusion Modeling for Sorption of Azo Dye on to One and Three Dimensional Nano Structured Carbon Nano Materials from Jatropha Curcas."J. Environ. Nanotechnol,2(2), 66-75, 2013.
21. G.H.Sonawane andV.S. Shrivastava."Kinetics of decolourization of malachite green from aqueous medium by maize cob (Zea maize): an agricultural solid waste."Desalination, 247, 430-441,2009.
22. B.H. Hameed, A.T.M. Din, A.L. Ahmad. "Adsorption of methylene blue onto bamboo-based activated carbon: Kinetics and equilibrium studies."Journal of Hazardous Materials, 141(3), 819-825,2007.
23. M.Makeswari and T.Santhi. "Removal of Malachite Green dye from Aqueous Solutions onto microwave assisted zinc chloride chemical activated epicarp of RicinusCommunis."J. Water Res. Protect.,5, 222-238,2013.

Table 1: Data for the elements presented in BTSP

Element	App conc.	Intensity corrn.	Weight%	Weight% sigma	Atomic%
C	30.65	1.1308	60.75	1.16	67.75
O	7.84	0.4645	37.80	1.17	31.64
S	0.62	0.9634	1.45	0.18	0.61
Totals					100.00

Table 2: Comparison of the correlation coefficients of kinetic parameters for the adsorption of CV onto BTSP

Kinetic model	Concentration (ppm)	Parameters	BTSP
Pseudo first-order	100ppm	K_1(min-)	0.0112
		q_e(mg/g)	38.4615
		R^2	0.944
	150ppm	K_1(min-)	0.0104
		q_e(mg/g)	41.6666
		R^2	0.939
Pseudo second –order	100ppm	K_2(min-)	0.0017
		q_e(mg/g)	250
		R^2	0.999
	150ppm	K_2 (min-)	0.0008
		q_e(mg/g)	500
		R^2	0.973
Elovich model	100ppm	A_E (Mg/min)	0.0311
		b(g/mg)	68.36
		R^2	0.973
	150ppm	A_E (Mg/min)	0.0116
		B (g/mg)	63.80
		R^2	0.944
Intra particle diffusion	100ppm	K_{dif}	0.3825
		C	106.5
		R^2	0.915
	150ppm	K_{dif}	0.2490
		C	38.52
		R^2	0.960

Table 3: Comparison of the correlation coefficient parameters for the isotherm adsorption

Isotherms	Concentration(ppm)	Parameters	BTSP
Langmuir isotherm	100ppm	K_1 (Lmg-)	0.2032
		Q_m(mgg-1	40.00
			0.999
		Q_m(mgg-1)	43.47
		R^2	0.997
Freundlich isotherm	100ppm	1/n	01270
		K_f (mgg^{-1})	1.8757
		R^2	0.832
	150ppm	1/n	0.1830
		K_f (mgg^{-1})	2.0218
		R^2	0.848

Tempkin isotherm		α(Lg-1)	0.363
	100ppm	β (mgL-1)	4.107
		R^2	0.821
		β (g/mg)	4.838
		R^2	0.781

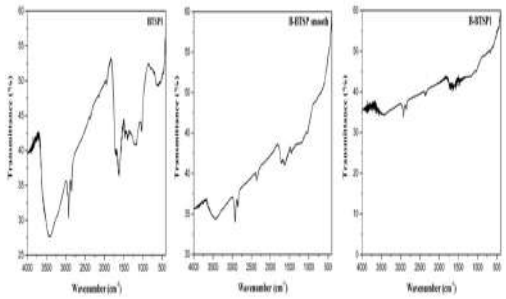

Fig.1.FT-IR analysis of BTSP adsorbent raw powder(1a), before(1b) and after (1c) adsorption of CV dye

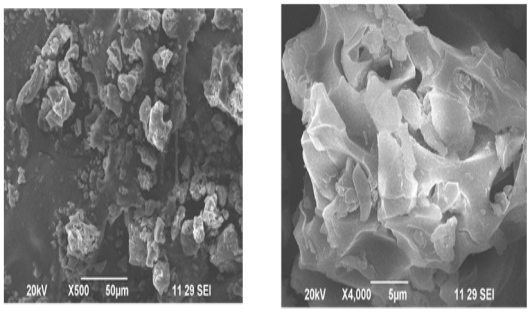

Fig. 2. SEM image of the BTSP before and after adsorption

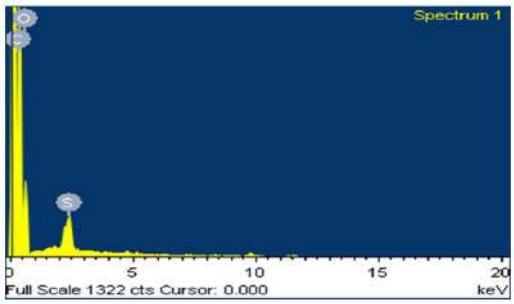

Fig. 3. EDX spectrum of BTSP

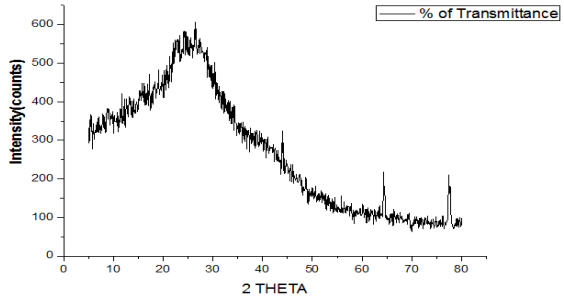

Fig. 4. XRD Pattern of BTSP

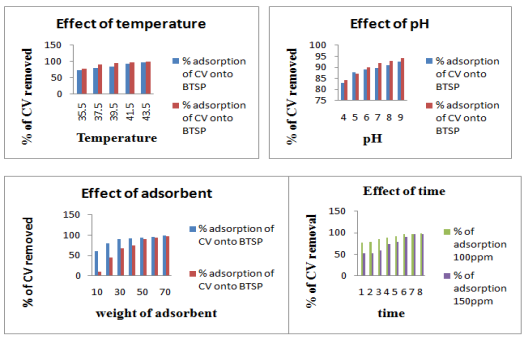

Fig. 5a, 5b,5c & 5d. Effect of pH, Temperature, dose and time on the adsorption of CV onto BTSP

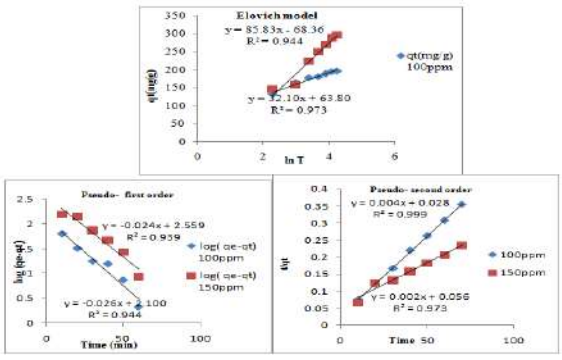

Fig. 6a, 6b & 6c. Pseudo First order, second order and Elovich model kinetics for CV onto BTSP

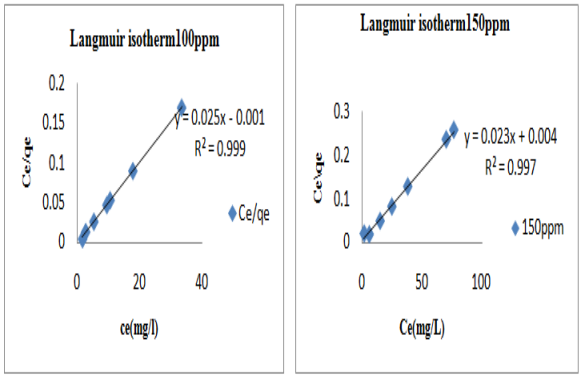

Fig. 7a.&b Langmuir isotherm plot for100 &150 ppm for adsorption CV onto BTSP

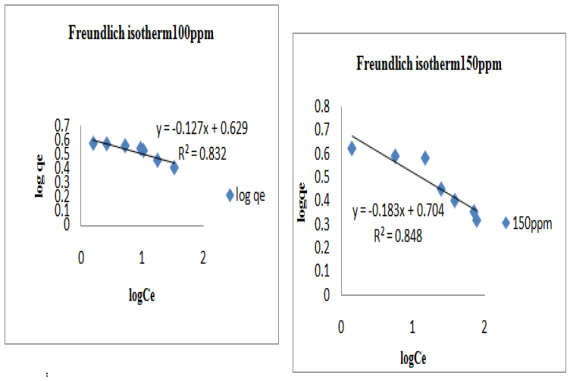

Fig. 8. a & b. Freundlich isotherm plot for100 & 150 ppm for adsorption CV onto BTSP fruit shell

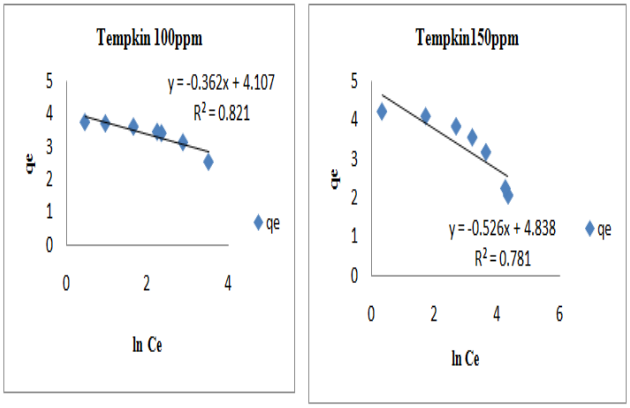

Fig. 9. a&b.Tempkin isotherm plot for100 &150 ppm for adsorption CV onto BTSP

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RESEARCH ARTICLE

Plotting the Mediasphere in Jammu and Kashmir

Nusratuel Islam Itoo[1]*, M.C. Raja[2], Ishfaq Bashir[3] and S.Thanigaivelan[4]

[1]Ph.D Research Scholar, Department of History, Annamalai University, Annamalai Nagar, Chidambaram, Cuddalore, Tamil Nadu, India.
[2]Professor, Department of History, Annamalai University, Annamalai Nagar, Chidambaram, Cuddalore, Tamil Nadu, India.
[3]Ph.D Research Scholar, Department of Philosophy, Annamalai University, Annamalai Nagar, Chidambaram, Cuddalore, Tamil Nadu, India.
[4]Assistant Professor, Department of Philosophy, Annamalai University, Annamalai Nagar, Chidambaram, Cuddalore, Tamil Nadu, India.

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***Address for Correspondence**
Nusratuel Islam Itoo
Ph.D Research Scholar,
Department of History, Annamalai University,
Annamalai Nagar, Chidambaram, Cuddalore,
Tamil Nadu, India.
Email: itoonusrat786@gmail.com

ABSTRACT

The article examines the situation of Jammu & Kashmir's media landscape (J&K). According to the data, the state of J&K has seen a huge growth in the number of publications over the period of time. Periodicals were also classified by district and area using information acquired from the Registrar of Newspapers for India (RNI) website. Additionally, linguistic research was carried out to assess the variations in the state's growth of press in different languages. Besides, the study also maps radio, TV, internet and mobile phones, media schools, and so on in the state.

Keywords: Multilinguistic, medias cape in J&K, media in Kashmir, Kashmir media sphere, press in Kashmir

INTRODUCTION

Jammu & Kashmir (J&K) is a picturesque province renowned for its breath-taking locales. Earlier, J&K state comprised three regions—Jammu, Kashmir valley, and Ladakh. It consisted of 22 districts (Jammu and Kashmir Official Portal, 2020). According to Census of India, 2011, J&K has a total population of 12,541,302. Press in this socially and culturally rich region plays a monumental role in extensively covering all the events of significance. The media here have always played a cardinal role in totality and prove to be an eye opener for the people. Press has

been instrumental in highlighting the progressive agenda and contributing to the society at large. It plays a cardinal role in public awakening and proves to be one of the most important social institutions of the people. History is witness to the fact that press in J&K has played an important role in the development of a public sphere at large. Historically, the development of journalism in Kashmir owes a lot to the Urdu press (Chandan, 2007). Urdu is one of the most read languages of the state of J&K. Being the official language of state, Urdu remains to be the lingua franca in this multilinguistic J&K. Urdu journalism actually paved the way for a more sensible and mature English press in the region. After doing wonders in the field of journalism, unfortunately, Urdu press couldn't match the speed of technological advancements lagging far behind the English press. Kashmir is a very difficult terrain to understand, discuss, and write. The situation here keeps on changing with time. The special status of the State was revoked in 2019 turning a new page in the history of J&K (CNN Editorial Research, 2020). With a minimal option of documenting and disseminating all these implicit and explicit cries, media has a cardinal role to play in totality in such a condition. But actually before studying the role of media, it essentially becomes paramount to map different mediums of mass communication in J&K. The first and foremost problem in J&K is that media here have not been mapped so far. So, before studying the role of media of this important region in detail, it becomes essentially important to map different mediums of mass communication in J&K. In this regard, this research article is an attempt of mapping all the media sphere in J&K. The study has been designed to gauge the growth of newspapers, radio, TV, cinema, internet, and other forms of media. The study will also help to get an idea about the media consumption or media diet of the citizens of the state at large.

Significance of Study

This study is an attempt to gauge the growth and development of various channels of mass communication in J&K. It will trace the development of newspapers, radio, TV, cinema, internet, and other forms of media in J&K. The study will also cover a brief history and will help to map the development of associations of journalists, media schools, and hybrid editions of the newspapers and national newspapers' J&K edition to get an idea about the media literacy and other journalistic activities in the J&K. While reviewing the literature, it was found that print media in J&K remains to be the main source of mass dissemination. The print media took birth in the region very late comparatively and no systematic evidences have been given till now regarding the development of media in this particular state. The study also intends to analyze the development of newspapers over the period of time. The study will further help to examine the developments of print media in view of periodicity, language, and region-wise and district-wise growth of newspapers in J&K

Research Objectives

Following research objectives have been formulated:

1. To map all the mediums of mass communication including newspapers, radio, TV, cinema, internet and mobile phones, telephone, and so on.
2. To analyze the growth of newspapers over the period of time.
3. To determine the frequency, language, and regional distribution of newspapers in J&K.

METHODOLOGY

The growth of the newspapers in the state will be assessed using information gathered from the Registrar of Newspapers for India's (RNI) official website. Additionally, periodicals would be recognized and arranged by district and by area. To assess the variations in the growth of press in different languages in the state, language-wise study was also done. Furthermore, the narrative analysis based on some primary and secondary sources is also the part of this mapping. The important newspapers currently in circulation have been considered for a historical narrative. Quantitatively, the periodicals of the state and their circulation would be further compared with other Indian states to get a rough idea of the share of the state in total. Using both qualitative and quantitative techniques, this study actually uses a triangulation method which is a mixture of quantitative and qualitative content analysis (mixed method) and historiography. Furthermore, it may be stated that this study does not actually fall under the

banner of either traditional history or critical history; this study using a top-down approach for studying the development of media institutions is actually a mixture of both the two methods and aims to give an evidence of the media institutions of the state in present. Besides that, keeping in mind the topic of study, Sociologist Micheal Schudson's (2002) first method, out of the three approaches, institutional history has been used to gauge the development of different media genres as medium of mass communication in the state. Nord (1989) refers to this approach as "hagiography." As the study has been taking into account all the media institution in totality instead of studying different media "saints," Nord's (1989) understanding of this approach of "institutional history" may not be applicable in this study. This methodology has been primarily used to gauge the development and growth of different media institutions as medium of mass communication in the state.

Data Sources and Collection

The data related to periodicals were retrieved from the official website of RNI, a statutory body of Government of India. RNI was established on the recommendations of First Press Commission of India on July 1, 1956. RNI (2017) issues certificates of registration to the newspapers and other periodicals published under valid declaration. The data about various other media genres were collected from different sources especially from government and private websites, state archives, books, research and newspaper articles, oral history, personal experience, and so on. These data were further substantiated with some other primary and secondary sources. The data were analyzed using statistical measures like percentage analysis and analysis of variance (ANOVA) test. ANOVA is a statistical method used to determine the significance of differences among the means of three or more than three groups. Furthermore, statistically significant ANOVA was followed by Tukey's HSD. Tukey's HSD is a post hoc test used for the further analysis of data. This test is applied after statistically significant ANOVA, to find means that are significantly different from each other. Furthermore, t-test was also used to analyze the levels of significance among the Jammu division and Kashmir division. Unlike ANOVA, a t-test is used to determine whether there is a significant difference between the means of two groups. So, the test was used to determine whether two divisions of the state—Jammu and Kashmir—are significantly different from each other on having total number of publications. All the aforementioned tools and techniques were used collectively to develop a broader understanding of growth of various media channels in the state.

LITERATURE AND DISCUSSION

Brief History of Press in J&K

Started in 1867, *Vida Vilas*, a weekly, is considered to be the first newspaper of the State of J&K. Published from *Vidya Vilas Press*, Jammu, a bilingual—Urdu & Hindi (Dev Nagri script), was an organ of *Vidya Vilas Sabha*, actually aimed to cover the proceedings of this sabha (Sharma, 2010). There were many ups and downs in the newspaper history of Kashmir till the Maharaja government permitted Mulk Raj Saraf to publish a regular Urdu newspaper *Ranbir* in 1924 (Sharma, 2010). This study focuses only on the newspapers registered by RNI, so its history in J&K begins 8 years after the establishment of RNI when noted Kashmiri writer Rasheed Taseer registered first ever publication from the state. To mark the beginning of registered publications in J&K, Taseer registered *Muhafiz*, an Urdu weekly from Srinagar in 1964. The very next year, 1965, witnessed the registration of 21 new publications. As per the data retrieved from the official website of the RNI on March 8, 2017, 1,326 titles have been verified from J&K till the aforementioned date. Out of a total of 1,326 titles, the year of verification of 13 titles remains to be unknown. The dates available with the RNI reveal that the first ever verification of a title from J&K was made on December 19, 1957. As per the record, there were some 15 verifications made on the same day. The data analysis shows that 1,176 titles have been registered so far from the state.

Registration of Publications

To gauge the growth of the newspapers and periodicals in J&K over the period of time, the discrete data available with the RNI was arranged year-wise and further decade-wise. For an easily understandable report of the growth of print media in the state, the data were shaped decade-wise with 1957 as the base year. 1957 was treated as base year

as the first ever verification from the state was made in this year. The time period was divided into a total of six decades from 1957 to 2016. Following this pattern, a group of following years was conceived: 1957–1966, 1967–1976, 1977–1986, 1987–1996, 1997–2006, and 2007–2016. These decades were further coded as D1, D2, D3, D4, D5, and D6, respectively. The total number of registered and verified titles was organized accordingly. It is important to mention that the 13 unknown titles, as mentioned above, have also been listed in D1. Many important newspapers currently in circulation like *Kashmir Times* and *Daily Excelsior* were registered during this decade. The foundation of J&K's first English newspaper, *Kashmir Times*, was laid by Baldev Prasad Sharma and Pandit Gawsha Lal Koul on November 26, 1934. *Kashmir Times,* a weekly, ceased its publication and was re-launched by Abdul Rehman Mitha, congress leader from Bombay, in 1943 (Mohi Ud Din, 2012). *Kashmir Times* was reviewed as a weekly from Jammu in 1954. The newspaper was converted into a daily in 1964 (*Kashmir Times*, 2017). Ved Bhasin, one of the renowned and oldest journalists of J&K, was the founding editor of the Kashmir Times in 1954. He remained to be the long-running editor of the newspaper from 1964 to 2000. *Kashmir Times* group also publishes *Dainik Kashmir Times*, a Hindi daily, *Jammu Prabhat*, a Dogri daily, and a monthly children's magazine, *Springer. Dainik Kashmir Times* was started in 1989 and *Jammu Prabhat* in 2008 (*Kashmir Times*, 2017).

One of the important and oldest English-language newspapers in the state registered during this period was *Daily Excelsior*. The newspaper started its publication from Jammu on January 1, 1965, as a weekly tabloid. SD Rohmetra is the founder-cum-editor in Chief of the newspaper actually owned by Excelsior House. It became a daily in 1967 (Daily Excelsior, 2017). Known for his satire "*Khazar Suchta Hai Wular Kay Kinaray*," Kh. Sanaullah Bhat switched back to Kashmir valley in 1957 and started publishing his newspaper, *Aftab* (Mohi Ud Din, 2012). Bhat registered the newspaper with RNI in 1965 as an Urdu daily from Srinagar. The newspaper in its 61st year of publication is considered as one of the reputed language newspapers of the valley. Ghulam Mohammad Mir has been registered as the publisher of the newspaper. *Aftab* in post 1950s is remembered as the representation of a nation speaking to itself and is considered as a watershed of struggle after the end of the feudal rule in the state. The range of issues that this newspaper would cover left hardly any segment—from culture to history, light read to literature, social issue to hard hitting political questions—untouched. With a distinct style, *Aftab* was the first newspaper with a home delivery service. Being a trend setter in many ways, the newspaper, unlike others, was also circulated to towns and rural areas apart from Srinagar city (Mohi Ud Din, 2012). *Roshni,* an Urdu daily from Srinagar, was started in 1943. Although *Roshni* claims to be in its 75th year of publication, the newspaper was for the first time registered with RNI in 1965 by Zahoor A. Shora. *Roshni* is currently printed and published by Shora. Aziz Kashmiri is the founding editor of the 8-page newspaper. AINA was started by Shamim Ahmad Shamim in 1965. AINA became so popular that it was converted into a daily newspaper in 1975. But after the Shamim's untimely death, paper could not survive long and thus it became an archival reference of great significance (Mohi Ud Din, 2012). A Kashmiri language monthly periodical *Kashur Adab* was registered by Ghulam Rasool Santosh in 1966. Many other publications registered during this decade include *Martand*, an Urdu daily started by Dina Nath Kotra from Srinagar in 1966, *Jammu Post* (1966), *Jahan-I-Nav* (1965) by Shamboo Nath Gurkha, *Imarat* (1965), *Humwatan* (1965) and *Hamara Kashmir* (1966), *Hamdard*, an Urdu weekly in 1965 by GR Arif, AINA (1965) by Shamim Ahmad Shamim and *Azad* to name a few.

D2 witnessed some five times increase in the number of titles as compared to D1. Some 169 titles have been registered during this decade. Some of the prominent newspapers of the state including *Srinagar Times* were started during this decade. Srinagar Times was started as an Urdu weekly by Sofi Ghulam Mohammad from Srinagar in 1969. This publication has been there for a very long time in the valley. The daily cartoon in the Srinagar Times is one of the unique elements readers get to see in the newspaper every morning, despite the fact that it may not be the most read publication in the valley. Bashir Ahmad Bashir (also known as BAB), who created the cartoons that have become synonymous with the Srinagar Times, made many a morning. As revealing as Kh. Sonaullah's "Khazar Suchta Hai Wular Kay Kinaray," his cartoons were. In 1969, BAB began working for the Srinagar Times, where she assisted in editing editorials and reporting. BAB is credited as being the father of political cartoons in Kashmir and is the brother of Sofi Ghulam Mohammad, who founded the newspaper. In 1971, the newspaper published its first ever cartoon in its mid page made by BAB on Sheikh Abdullah (Wani, 2014). *Rehbar* is credited to have started publishing of political cartoons in the State of J&K. First cartoon was made by Dost Mohammad Khan for Rehbar in 1934

(Taseer, 1989, pp. 140). Even though another newspaper *Hamdard* started publishing political cartoons in 1935 almost daily, but *Srinagar Times'* cartoons have been considered best ever political cartoons in the journalistic history of Kashmir. Surprisingly, D3 witnessed a decline in the number of registration of new titles in the state. Despite this, some most prominent newspapers currently in circulation like *Chattan and Afaq* Srinagar came into being. *Chattan* was registered as an Urdu newspaper from Srinagar by Tahir Mohi-ud-din in 1978. The newspaper was re-registered in 1983. Since then, Mohi-ud-din is the publisher, printer, owner, and Chief Editor of *Chattan.* The newspaper started its publication in 1985 as a weekly (Daily Chattan, 2017). The 8-page newspaper is printed at Abid Enterprises, Zainakote, Srinagar. The newspaper continuously updates news and views through popular social networking site Facebook. *Afaq Srinagar*, an Urdu daily, is also one of the important newspapers of the state started in 1985. Mohd. Yousuf Qadri is the founder of the newspaper. In threat of their life, many talented journalists left the valley during 1990s. On their departure, the baton of the journalism automatically passed into the hands of local editors. A serious challenge of manning local newspapers was thrown up for the local editors and journalists. Paving way for the growth of journalism, local editors and journalists had a series of new and important stories to report. This marked the beginning of the era of English journalism in the state of J&K. This was the period when one of the state's largest English daily, *Greater Kashmir*, hit the stands and filled the vacuum (Bukhari, 2012). After 6 years of its publication as a weekly, *Greater Kashmir* took on the daily garb from a weekly one in 1993. With this, valley got its first standard English daily owned by a Kashmiri Muslim, Rashid Makdoomi. Earlier, the journalists in the Kashmir valley were skeptical about the possibility of a local English-language daily in Kashmir that could sustain itself exclusively on readership and advertisements provided locally. On the other side, with growing literacy rate, even *Kashmir Times*, a Jammu-based newspaper, had a dependable readership in the valley in comparison to that in the Jammu region. Not many senior journalists and editors believed that the valley had readership or even the talent that could support and sustain a professionally edited daily English newspaper (Mohi Ud Din, 2012).

Fayaz Ahmad Kaloo is the Editor-in-Chief of the newspaper. *Kashmir Uzma*, the sister publication of *Greater Kashmir*, is one of the largest circulated Urdu dailies of the state. The Greater Kashmir Media Group is owned by Greater Kashmir Communications Pvt Ltd. The organisation also owns the monthly magazines Nawa e Jehlum in Urdu and Kashmir Ink in English. In reality, Kashmir Ink began as a monthly publication in 2014. Kashmir Ink then evolved into a weekly tabloid in 2016. It is currently published each week with the main book as a few extra pages. The newspaper Greater Kashmir has the distinction of being one of the valley's most popular publications. "Write Hand," a very short column written by Ajaz-Ul-Haque every Sunday is believed to be one of the most enjoyed columns of the newspaper. GK Communications Pvt. Ltd also uploads the latest videos on the GKTV, the audio-visual platform of the media group. After six months of continuous publication and a favourable reception from the educated Kashmiris, advertisers began to be drawn to Greater Kashmir. With time, this publication rose to prominence as Kashmir's leading source of English-language reporting. Zafar Aga was appointed as the newspaper's first bureau head in New Delhi in 1988. (Greater Kashmir, 2017). Asraf Shabab registered *Alsafa* News, another significant daily, in 1988. The Urdu newspaper Alsafa was well-known throughout the region. Alsafa once published half of its pages in Urdu and half in English, making it the valley's most widely read daily. Manzoor Ahmad Anjum from Srinagar founded *Uqab*, another Urdu newspaper, in 1994. In 1996, Kashmir Images, one of the best English publications in the valley, was registered by Bashir Ahmad Nayak (better known by his stage name, Bashir Manzar). Sneh Gupta also launched The *Himalayan Mail*, an English daily from Jammu, in 1996. The franchisee for The Indian Express' J&K edition was designated as the 12-page newspaper. In 1996, Raj Daluju registered State Times as a Jammu-based English daily newspaper. The newspaper publishes hybrid editions. The Hindi edition of State Times was also registered in 2007. Some of the important newspapers started in this decade currently in circulation include *The Kashmir Monitor, Tameel I Irshad, Early Times, Kashmir Observer, Kashmir Uzma, Rehbar, The Kashmir Convener, and The Rehmat.* One of the renowned journalists of J&K, Zafar Meraj started The Kashmir Monitor in 1998. Unlike the usual practice of publishing editorial on the left-hand column of the editorial page, The Kashmir Monitor publishes its editorial on the top of the editorial page. Zafar Meraj's son Shameem Meraj is the owner, publisher, and editor of the newspaper. Another important English daily, Early Times, was started by Bansi Lal Gupta in 2002 from Jammu. The newspaper also started a supplement Early Time Plus in 2010. The 12-page newspaper is being printed at Early Times Printing Press, Jammu. B.L. Gupta is the editor, printer, publisher, and owner of the newspaper. Kashmir

Observer is an English daily started in 1998 by Sajjad Haider from Srinagar. The newspaper allocates ample space to a wide range of issues prevailing in and outside the state. Currently, Sajjad Haider is the publisher and Editor in Chief of the newspaper. Another Urdu daily, Tameel I Irshad, was started by Aakash Amin Bhat in 2002. Rehbar, a weekly, was started in 1932 by Ghulam Mohi-ud-Din Rehbar. Later, the newspaper was registered with RNI by Shahid Iqbal Rehbar in 2002 as an Urdu weekly from Srinagar. The newspaper and Ghulam Mohi-ud-Din Rehbar's role resulted in his frequent arrests which affected the publication of paper. In 1933, Rehbar was stalled. Similarly, in 1936, 1938, and 1946, its publication was stopped (Rehbar, 2012). Some of the national newspapers including Amar Ujala, Dainik Jagran, and The Indian Express also registered their Jammu edition during this decade. Rising Kashmir was started by Syed Rafi-U-Din Bukhari in 2007. Prominent journalist and former Bureau-in-Chief of The Hindu in Srinagar, late Syed Shujaat Bukhari, was the Founding Editor of newspaper till he was assassinated on June 14, 2018. He was shot dead outside his newspaper's office in Press Enclave of Lal Chowk Srinagar. Bukhari's assassination was not a setback to the newspaper only but to whole journalist fraternity across the globe. Rising Kashmir didn't stop the print and hit the stand next day of Bukhari's assassination with a full-page photograph of newspaper's murdered editor-in-chief on front page against a black background and message that said it would not be cowed down ("Rising Kashmir Hits the Stands With Shujaat Bukhari Tribute on Front Page," 2018). Catering the needs of the people in the valley, the newspaper under his editorship had been covering the diverse issues of the state in general and valley in particular since the inception. *Rising Kashmir* is owned by the Kashmir Media Group. The group also owns an Urdu daily *Buland Kashmir* and a Kashmiri language daily *Sangarmal*. Setting a new benchmark in the field of journalism in the state of J&K, *Kashmir Reader* gained a lot of attention in the whole valley through its content and preference of the stories in a very short span of time. Registered in 2011, the newspaper was banned by the authorities for some 3 months in 2016.

On October 2, 2016, the birthday of Mahatma Gandhi, a proponent of free speech, five policemen entered the *Kashmir Reader's* office in Srinagar at 8:15 p.m., with an order in hand banning printing of the newspaper. The order, issued by the District Magistrate Srinagar, without mentioning a particular write-up, read publication of the newspaper can "easily incite acts of violence and disturb peace and tranquility." Invoking Section 144 Cr.P.C., Section 3 of News Papers Incitement of Offences Act, 1971, and Section 10 of Press and Publication Act, 1989, the printing of *Kashmir Reader* newspaper was stopped ("Govt Bans Publication of Kashmir Reader. . . ." 2016). This was a major setback to the press freedom in the valley. Started as an English daily in May 2012, Kashmir Reader is the only daily newspaper in the state of J&K which prefers offbeat human interest stories on its front page as the lead of the day. Kashmir Reader is to Kashmir what *New York Times* is to the world. The newspaper was started from Srinagar by Helpline Group, earlier the publisher of famous English magazine Conveyor. Haji Hayat Mohammad Bhat is the printer, publisher, owner, and Editor in Chief of the newspaper. Being a part of the mainstream media, *Kashmir Reader* seems to be fulfilling the duty of an alternative press in the valley. An important English weekly tabloid, *Kashmir Life* was started by Hilal Ahmad Dar in 2009. *Kashmir Life* is one of the finest examples of human interest and long-form journalism in the valley.

Decadal Growth of Publications

Furthermore, to shape the data in a more lucid form to increase the comprehensibility regarding the growth of print media, decade-wise percentage of the registered periodicals was gauged. The analysis shows the percentage share of each listed decade separately. The data reveal that the number of publications registered during D6 (46.85%) is almost equal to the sum total of all the titles registered during remaining all the decades. With a share of 2.90%, the D1 remains to be last in registering new titles. The data also reveal that during D3 an overall decline has been witnessed in the registration of new titles. Except this, there has been an overall increase during the rest of the decades. Apart from percentage analysis, the statistical test, ANOVA, was applied to see the significance of difference among the decades. ANOVA was applied to see the significance of difference among the decades. Table 8 shows the decade-wise descriptive statistics on the total number of registered publications. D6 scores highest mean (55.10) as compared to the other decades. Table 9 shows a significant difference among the decades. The F-value is 22.70 which is statistically significant at .01 level of significance. ANOVA test shows a statistically significant difference among the groups (Table 9). Furthermore, statistically significant ANOVA was followed by Tukey's HSD.

There was a statistically significant difference between groups as determined by one-way ANOVA ($F = 22.70$) ($p < .01$). A statistically significant ANOVA was followed by Tukey's post hoc test to see the significance of difference between the decades on total number of publications registered. Tukey's test shows a significant difference between D1 and D5 ($p < .05$), D1 and D6 ($p < .01$), D2 and D6 ($p < .01$), D3 and D6 ($p < .01$), D4 and D6 ($p < .01$), and D5 and D6 ($p < .01$). The analysis reveals a significant growth in the number of publications in J&K from D1 to D2. In the same way, a significant growth has been registered from D5 to D6. Analysis further reveals a significant difference between D1 and D6, D2 and D6, D3 and D6, D4 and D6, and D5 and D6. During the last decade (D6), an unprecedented growth in the number of publications has been recorded. With the findings of the study, it could be well established that there is a tremendous increase in the number of publications in the state of J&K over the period of time.

Total Percentage Share

Furthermore, an attempt has been made to gauge the share of publications registered in J&K in the total number of titles registered with RNI. It was found that there have been some 132,431 registered publications with RNI in India till March 8, 2017. The data reveal that the titles registered in J&K constitute only 0.89% of the total share. To further evaluate the current position of the press in J&K, the portion of the registered titles in J&K obtained through simple mathematical formulas was compared with the other Indian states. This comparison helps to understand the pinnacles in the development of press in the state of J&K. With 1,176 titles, J&K was found to have been contributing. a share of 0.89% to the total number of titles registered so far by the RNI. The data were arranged state-wise to analyze and compare the present state of publications among the different states and union territories. The analysis reveals that the state of J&K figures at 19th place in the list.

Circulation of the Newspapers

An attempt has been made to analyze the growth of press in J&K in terms of the total circulation of newspapers and periodicals in the state. The circulation of the newspapers and periodicals in the state was further compared with the circulation of the print media in other states. The data produced in the 58th Annual Report published by The RNI (2013–2014) was used. Findings reveal that a total of 99,660 titles were found to have been registered with RNI as on March 31, 2014. However, the circulation of newspapers and periodicals has been calculated on the basis of 19,755 publishers who have filed their annual statements online for the year 2013–2014. Furthermore, as per the data, there were 969 registered titles in J&K as on March 31, 2014. The total circulation claimed by the newspapers and periodicals in J&K was 9,627,424 copies as on March 31, 2014. According to the report, out of a total of 969, only 347 publications were found to have submitted their Annual Statements for the year 2013–2014. It comprised 230 dailies, 99 weeklies, 7 fortnightlies, 8 monthlies, 2 quarterlies, and 1 others. Further dividing these publications on the basis of language, it was found that 158 were English, 122 were Urdu, 29 Bilingual, 27 Hindi, 5 Kashmiri, 4 Multilingual, and 2 Dogri language publications. The report concluded that among the daily English newspapers, Daily Excelsior published from Jammu claimed to have the largest circulation of 308,988 copies a day and among the periodicals, Sharda, an Urdu weekly from Srinagar, was leading with a circulation of 60,500 copies per publishing day. According to RNI, Greater Kashmir has claimed a circulation of 137,305 copies, of both the editions for the year 2014–2015. In the same year, Kashmir Times has claimed a circulation of 390,634 copies for its three editions. Kashmir Uzma has claimed a circulation of 84,189 for its two editions. Rising Kashmir has claimed a circulation of 98,000 and 66,000 for its two editions while Sangarmal and Buland Kashmir claim to have a circulation of 66,000 and 72,000, respectively. On the basis of circulation claimed by newspapers and periodicals across India, J&K was compared with other states to analyze its share among total registered publications. The findings reveal that J&K constitutes 2.14% of the total circulation claimed by various publications across India.

Hybrid and Double Editions

Out of a list of 58 daily newspapers, only 21 (36.2%) newspapers of Kashmir are available online. Most of them have adopted social media technologies to some extent with Greater Kashmir, Rising Kashmir, and The Kashmir Monitor showing full visibility on the selected social media tools. The language newspapers seem to be quite at the stages of infancy in implementing these technologies (Gul & Islam, 2013). Few newspapers have also started publication of

two editions, separately from Jammu and Srinagar. Greater Kashmir publishes two editions—Greater Kashmir from Srinagar, the summer capital and Greater Kashmir from Jammu, the winter capital of the state, respectively. The Jammu edition of the newspaper is also printed with the same title "*Greater Kashmir*." *Rising Kashmir* has also started another edition namely Rising Jammu. Kashmir Times also publishes Srinagar edition of the newspaper in addition to main edition, Kashmir Times Jammu edition. Greater Kashmir's sister publication *Kashmir Uzma* also publishes Srinagar and Jammu editions of the newspaper. Few newspapers also publish two separate newspapers in two different languages with the same title.

National Newspapers' J&K Editions

On December 10, 2007, The Tribune, Chandigarh launched its Jammu edition in Jammu, becoming the third English daily from outside the state, after The Indian Express and Hindustan Times to have its separate edition. Apart from these three English dailies, Amar Ujala, Dainik Jagran, and *Punjab Kesri* have also launched their Jammu editions ("Tribune Launches Jammu Edition, Indicates Growing Prospects," 2007). *Hind Samachar* also registered the Jammu edition of the newspaper in 2007 (RNI, 2017). These Hindi newspapers couldn't extend their services to the valley due to language barriers. After *Jammu Tribune*, the newspaper also launched *Kashmir Tribune* on August 9, 2012 (Omar Launches Kashmir Tribune, 2012). Currently, the newspaper publishes a full J&K edition of the newspaper. Senior journalist, Ehsan Fazili, currently heads the Srinagar office of the newspaper. The Indian Express had also started its J&K edition in collaboration with The Himalayan Mail which lasted for 12 years. The Indian Express' J&K edition was available in the valley in coordination with The Himalayan Mail since 1998 (Rathore, 2010). My Times of Srinagar, a weekly supplement page of The Times of India, was also published for some time from Chandigarh. Apart from this, almost all the major national and international media organizations and news agencies have journalists, stringers, video-journalists, and photographers posted in J&K.

News Agencies

Kashmir News Service (KNS**)** is one of the major news regional agencies in the state. Current News Service (CNS), Global News Service (GNS), and few other local news agencies are also operational in the valley. Apart from this, ample newspapers have subscribed to Press Trust of India (PTI), United News of India (UNI), and IANS for national and other international news. Many newspapers reproduce the content of BBC, Aljazeera, Dawn, The Indian Express, Scroll.in, and other national and international media organizations.

Associations and Organizations

The journalists in State of J&K founded associations and actively participated in such activities since the Dogra period. The foundation of Kashmir Journalists Association was laid in the office of Vitasta in April 1933 under the president-ship of Sheikh Fazal Ahmad Kashmiri of Gulab. Bazaz was president while Baldev Prasad Sharma was its secretary. The foundation of Jammu chapter of the association Jammu Journalists Association was laid under the supervision of Lal Ram Saran Das Malhotra. Munshi Mehraj Uddin Ahmad of Pasban was nominated its first president while Lal Shiv Ram Gupta its first secretary (Taseer, 1989, pp. 179–180). The journalists of Kashmir were divided into three groups among which Kashmir Journalists' Association and Kashmir Journalists' Federation are important to mention. Initially, there was only one association in the valley, but few journalists under the influence of government broke up and started a new organization of journalists called Kashmir Journalists' Federation (The Khidmat Issue dated January 22, 1942). On May 19, 1942, under the leadership of Sadar Uddin Mujahid called upon all the members of Kashmir Journalists' Federation and Kashmir Journalists' Association and other non-affiliated journalists of the valley. After discussion, it was decided that the journalists of Kashmir will fight for their rights under one banner only.The new organization of the journalists would be formed. Two names, Kashmir Journalists' Federation and Kashmir Newspapers' Society, were proposed for the new organization. Resulting in a tie, both the names got nine votes each. Ending the confusion, the president of the meeting, Mujahid voted in favor of Kashmir Newspapers Society. A sub-committee was also nominated for the drafting rules and regulations (The Khidmat Issue dated May 20 1942). The Society organized a conference of all the journalists of the valley on April 10–11, 1943 (The Khidmat, Issue date January 29 1943). The Society also floated an idea of holding All Jammu and Kashmir Newspaper Editors' Conference in the month of June which was later conducted in August. Under the president-

ship of Mujahid, different committees were formed for the 3-day conference held between August 19 and 21, 1943 in Srinagar (The Khidmat Issue dated May 9 1943). Maharajas also organized various press and journalists' meets time to time in which reporters from different parts of the world were invited. Mulk Raj Saraf also invited Indian Federation of Working Journalists to organize an annual conference in Kashmir in 1964 which was later approved by the State government (Government of Jammu and Kashmir, Kashmir Bureau of Information, 1964). Currently, some of the associations and organizations of the journalists working in different towns and cities of the state are as follows:

- Kashmir Editors Guild (KEG);
- Aiwaan-e-Sahafat;
- Press Club of Jammu;
- Anjuman-e-Udru Shafat;
- Kashmir Working Journalists Association;
- Kashmir Press Photographers Association (KPPA);

and many others . .

Media Schools in J&K

Established in 1985, Media Education Research Centre (MERC), University of Kashmir (2017) is perhaps the first journalism school in the state. Offering a 2-year postgraduate course in Mass Communication and Journalism, MERC has nurtured a long list of journalists in Kashmir. MERC also publishes a fortnightly magazine, MERC Times. Apart from MERC, Government Degree College Boys Baramulla, Government College for Women, M.A. Road, Srinagar and Government Degree College Anantnag are three important institutions offering courses in media. Baramulla College is one of the important and oldest institutions of the state offering two different graduation level media courses—Mass Communication and Multimedia Production (MCMP) now B.A. (Honours) Multi Media and Mass Communication (BMMMC) since 2004 and Mass Communication and Video Production (MCVP) since 2002—of 3 years each (Govt. Degree College Boys Baramulla, 2017). For nurturing tall journalists in the valley, Baramulla College is known to be the bedrock of journalism in Kashmir. Islamic University of Science and Technology, Awantipora, Kashmir also offers a 2-year postgraduate course in Journalism and Mass Communication (Islamic University of Science & Technology, 2017). Department also publishes a tabloid, Echo. Central University of Kashmir also offers a postgraduate program in convergent journalism (Central University of Kashmir, 2017). In Jammu, the Central University of Jammu, Indian Institute of Mass Communication, Jammu (IIMC Jammu) and Gandhi Memorial College of Education, Jammu (GMCEJ) offer a course in mass communication. IIMC started its regional center at Jammu in 2012–2013. The University of Ladkah also started teaching media subject in 2020. Among all the aforementioned institutes, GMCEJ is the only private college offering the course in the state.

Cinema

The story of film-making in Kashmir begins in 1952 with a documentary Pamposh (Lotus). Made by Ezra Mir, Pamposh was screened at the Cannes Film Festival. The first Kashmiri feature film, Mehanzraat, was released in 1964. Directed by Jagjiram Pal, Mehanzraat was screened at a cinema hall in the main city of Kashmir and evoked tremendous response from the people. It was rewarded with president's award (Altaf, 2012). State particularly Kashmir valley also witnessed cinemagoing culture for long. Even before that, the Field Publicity Organisation of the J&K State Information Department organized 300 film shows during the year 1955–1956 only. The film shows were exhibited in schools, colleges, government offices, public places, hospitals, factories, and so on. The organizations also started exhibition of the film shows in the Srinagar Club weekly for the entertainment of visitors. They also exhibited films in the Central Jail, Srinagar, which was first of its kind in the history of Kashmir. The film shows were being exhibited fortnightly. The valley also witnessed a "Film Week" which commenced from March 24, 1956, was also organized by the Field Publicity Organisation of the J&K State Information Department. During this week, films of historical, cultural, and educational value were exhibited across the valley (Field Publicity Organisation, 1955–1956). There were more than 17 film theaters in the State in 1963 only which included three in Srinagar, one in Sopore, one in Anantnag, one in Baramulla, four in Jammu, one in Udhampur, one in Baderwah, two in Kathua, one in Samba, one in Ranbirsinghpura, and one in Leh (Information Department, Srinagar Archives, 1963). Despite the

ban, cinemas continued to operate in the valley till December 31, 1989 (Mir, 2012). Currently, no cinema hall in Kashmir valley is operational.

Radio

To serve the communication needs for the World War II, radio sets were sold in different parts of the world. As it was considered a luxury item, only some 22 radio sets had come to Kashmir for sale that time. These radio sets called "Sky Champion," made in Chicago, USA, were sold by Messrs Lyra & Co, Lal Chowk Srinagar (Ganjoo, 2001). Some of the buyers of the radio sets also include Maharaja Hari Singh, the management of the Amar Singh Club Srinagar, the then state physician, Dr S.K. Shangloo, Pandit Niranjan Nath Ganjoo and so on. Meanwhile, the "Air Messenger" brand radio sets were procured and issued for the community listening in various parts of Jammu and Srinagar (Ganjoo, 2001; Krishan, 2012). Soon after the India-Pakistan partition, the radio broadcasts from the Pakistani radio stations, mostly from Radio Lahore, had a direct bearing on J&K where this station was clearly heard (Krishan, 2012). Owing to Jammu's proximity to the border, the first radio station in J&K was commissioned by All India Radio (AIR) in Jammu (Radio Kashmir, Jammu) on December 1, 1947. The radio station was inaugurated by Maharaja Hari Singh in the presence of the then Union Home Minister of India, Sardar Valabh Bhai Patel (Bamzai, 1994). This station was operated in three classrooms of the Government Ranbir High School. Maharaja Hari Singh's speech was the first to broadcast at 6.30 p.m. As there were no recording facilities, this speech was aired live. Hari Singh's speech was followed by Maharani of Jammu and Kashmir's speech (Golden Jubilee Souvenir of Radio Kashmir Jammu on its 50 years—1947–1997, p. 9 (as cited in Krishan, 2012). Later, a station was started in Srinagar July 1, 1948. These two wings of Radio Kashmir were hurriedly set-up.These two stations began their operation under State Government's Information and Broadcasting Ministry and continued to do so till April 1954 when the stations were merged with AIR (Krishan, 2012). The first broadcast from Radio Kashmir, Srinagar was Yeh Radio Kashmir Hai (This is Radio Kashmir), made by Mir Ghulam Rasool Nazki was followed by the recitation from Holy Quran. Later, Sheikh Mohd Abdullah addressed the people of J&K (Hyderi, 2001, as cited in Krishan, 2012). The Radio Kashmir, Srinagar station was temporarily shifted near Government Arts Emporium Building after being functional at Polo Ground for 3 years. The station was shifted back to Polo Ground transmitters gutted in the fire earlier were repaired. Prime Minister Bakshi Ghulam Mohammad laid the foundation of the current building of Radio Kashmir Srinagar on June 1960. Both Radio Kashmir Jammu and Radio Kashmir Srinagar had initially transmission for a duration of 3 hr with rural program for 20 min and news for 30 min (Krishan, 2012). A new station was inaugurated on April 1, 1964. Another station was commissioned in Srinagar (Commercial Broadcasting Service, Radio Kashmir, Srinagar) on July 1, 1975. In 2015, government approved licenses to 15 new FM channels in J&K. Out of a total of 15 channels, three each will be in Kathua, Bhaderwah, Kargil, Leh, and Poonch ("Government Approves 18 New FM Channels for Northeast, 15 for Jammu and Kashmir," 2015). Big 92.7 FM, Red FM 93.5, Radio Tadka, Radio Mirchi, and others have also started services in Srinagar and Jammu. Besides that, there is also one community radio in the state at Islamic University of Science and Technology, Awantipora.

Television

In J&K, DD Kendras are stationed at Jammu, Srinagar, Rajouri, and Leh. In 1993, DDK Jammu got commissioned as PGF. DDK Jammu produces programs in eight different languages/dialects. DD Kashir channel was launched on June 26, 2000. It was converted into a 24-hr channel on August 15, 2003. This is primarily a satellite channel having a terrestrial support of 30 transmitters covering about 77% of the population in the Kashmir region (Bharati, 2015). Even though private cable operators air local cable channels like KBC, JK Channel, Insaaf TV, and Sen Channel, J&K has no private satellite TV channel or national channel of its own. There were only eight local TV channels which are registered and have a license to beam entertainment programs among total 37 channels working in the valley till January 2010 (Manzoor-Ul-Hassan, 2012). Few channels like Network 18 Urdu and Hyderabad-based Munsif TV, New Delhi based Gulistan TV continuously air Kashmir news and have dedicated special time slots to the valley.

Internet and Mobile Phones

The mobile phone service in J&K was for the first time launched on August 20, 2003. Former chief minister of the state late Mufti Mohammad Sayeed in Srinagar made the first call to Indian prime minister late Atal Bihari Vajpayee

in New Delhi ("PM Launches Mobile Phone Services for J&K," 2003). Government-owned Bharat Sanchar Nigam Ltd (BSNL) was the first cellular services company to provide service in the state. J&K had 5.2 million cellular subscribers as of June 2010. BSNL, Airtel, Vodafone, Idea, Jio, and so on provide wireless cellular services in the state. According to the latest figures available with Telecom Regulatory Authority of India (TRAI), quoted by Mirani (2015), J&K had some 35.3 lakh internet users till the end of June 2015. Another report by Malik (2017), quoting TRAI data published by Greater Kashmir, states that J&K crossed the mark of one crore mobile users at the end of October 2016. According to the TRAI data, J&K had 10,428,635 mobile users till the end of October 2016.

CONCLUSION

The analysis reveals a tremendous increase in the number of publications in the state of J&K over the period of time. Keeping in mind the world trends, D6 (2007–2016) is the time period when the print across the globe had been witnessing a decline in terms of readership due to the advent of technology and further growth of electronic and new media. Surprisingly, the J&K has been witnessing an unprecedented growth in the registration of new titles specifically during the aforementioned period. Furthermore, increase in the number of mobile phone and internet users has no bearing on the print. Even though some FM channels have started their services in Kashmir, no license has been allotted to start a private satellite TV channel in J&K. Cinema culture on the other side has been the worst hit in Kashmir. Kashmir's media industry is not developing on the same model as is witnessed currently in the other parts of the world. It may be well established that print seems to be the main supplier of media diet in the state.

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REFERENCES

1. Altaf, S. (2012, November). Kashmir's film industry longs for life.
2. The Dawn. https://www.dawn.com/news/766279/kashmirsfilm-industry-longs-for-life
3. Bamzai, P. N. K. (1994). Culture and political history of Kashmir. M.D. Publications.
4. Bharati, Prasar. (2015). Annual report 2014-15. http://prasarbharati.gov.in/Annual_ReportPDF/English_14-15.pdf
5. Bukhari, S. (2012, March 17). A success story amid all odds. The Hoot. http://asu.thehoot.org/story_popup/a-success-story-amidall-odds-5807
6. Census of India. (2011). http://censusindia.gov.in/2011-prov-results/ data_ files/india/Final_PPT_2011_chapter6.pdf
7. Central University of Kashmir. (2017). https://www.cukashmir. ac.in
8. Chandan, G. (2007). Jammu wa Kashmir ma Urdu Sahafat. Urdu Sahafat Ka Safar [Urdu journalism in Jammu and Kashmir.
9. The journey of Urdu journalism]. Educational Publishing House.
10. CNN Editorial Research. (2020, March 26). Kashmir fast facts.
11. CNN international. https://edition.cnn.com/2013/11/08/world/ kashmir-fast-facts/index.html
12. Daily Chattan. (2017). http://www.chattanonline.com/aboutus.aspx
13. Daily Excelsior. (2017). http://www.dailyexcelsior.com/about-us
14. Field Publicity Organisation. (1955–1956). Information department record, activities list of field publicity organization of the J&K state information department for the years 1955-56, Srinagar Aarchives.
15. Ganjoo, S. J. L. (2001, August). Koshur Samachar. New Delhi.
16. Golden Jubilee Souvenir of Radio Kashmir Jammu (1947 to 1997), p. 9.

17. Government approves 18 new FM channels for Northeast, 15 for Jammu and Kashmir. (2015, January 17). The Economic Times. http://economictimes.indiatimes.com/industry/media/ entertainment/media/government-approves-18-new-fmchannels-for-northeast-15-for-jammu-and-kashmir/articleshow/45925729.cms
18. Govt. bans publication of Kashmir Reader, says "its publication can incite violence and disturb peace." (2016, October 2). Kashmir Reader. https://kashmir.today/govt-bans-publication-kashmirreader-says-publication-can-incite-violence-disturb-peace/
19. Govt. Degree College Boys Baramulla. (2017). http://www.baramullacollege.net/mcmpdept.php
20. Government of Jammu and Kashmir, Kashmir Bureau of Information. (1964). D. O. No. KBI/1909
21. Greater Kashmir. (2017). https://www.greaterkashmir.com
22. Gul, S., & Islam, S. (2013). Adoption of social media by online newspapers of Kashmir. Annals of Library and Information Studies, 60, 56–63. http://nopr.niscair.res.in/bitstream/123456789/17696/3/ALIS%2060%281%29%2056-63.pdf
23. Hyderi, Z. (2001). Radio in Kashmir. Hyderi Publications.
24. Information Department, Srinagar Archives. (1963). Inf/st–2/63.
25. Islamic University of Science & Technology. (2017). http://www. iustlive.com/Index/Default.aspx
26. Jammu and Kashmir Official Portal. (2020). https://jk.gov.in/ jammukashmir/?q=divisions
27. Kashmir Times. (2017). http://www.kashmirtimes.com/aboutus. aspx
28. Krishan, B. R. (2012). Strategic interest and media a study of the strategic interest and media role of radio Kashmir since 1947. Department for Strategic and Regional Studies, University of Jammu. https://shodhganga.inflibnet.ac.in/bitstream/10603/105623/5/05_chapter.pdf
29. Malik, S. (2017, January 29). JK again crosses 1 cr-mobile subscriber base. Greater Kashmir. http://www.greaterkashmir. com/news/business/story/239949.html
30. Manzoor-Ul-Hassan. (2012, February 14). 69 valley newspapers have negligible circulation: Survey. Greater Kashmir. http:// www.greaterkashmir.com/news/news/69-valley-newspapershave-negligible-circulation-survey/113615.html
31. Mir, N. (2012). Forgotten, forbidden cinema culture of Kashmir. http://www.jammu-kashmir.com/archives/archives2012/kashmir20120429d.html
32. Mirani, H. (2015, December 9). JK has 35 lakh internet users, 96 lakh mobile phone subscribers. Greater Kashmir. http://www. greaterkashmir.com/news/business/jk-has-35-lakh-internetusers-96-lakh-mobilephone-subscribers/203716.html
33. Mohi Ud Din, K. (2012). Print journalism in Kashmir: Evolution & challenges [Unpublished Master's dissertation].

Table 1. Year-Wise Verification and Registration of Publications During D1 in J&K.

S.No	Year	Tittles verified	Tittles registered
1	1957	15	0
2	1958	0	0
3	1959	01	0
4	1960	0	0
5	1961	0	0
6	1962	0	0
7	1963	0	0
8	1964	02	01
9	1965	0	21
10	1966	05	12
Total		23	34

Table 2. Year-Wise Verification and Registration of Publications during D2 in J&K

S.No	Year	Titles verified	Titles registered
1	1967	34	15
2	1968	24	21
3	1969	6	23
4	1970	15	11
5	1971	13	21
6	1972	18	23
7	1973	15	24
8	1974	05	14
9	1975	10	10
10	1976	07	07
Total		147	169

Table 3. Year-Wise Verification and Registration of Publications during D3 in J&K.

S. no.	Year	Titles verified	Titles registered
1	1977	17	12
2	1978	04	11
3	1979	16	08
4	1980	11	07
5	1981	5	06
6	1982	5	08
7	1983	5	11
8	1984	14	14
9	1985	14	18
10	1986	11	10
Total		92	105

Table 4. Year-Wise Verification and Registration of Publications during D4 in J&K.

S. no	Year	Titles verified	Titles registered
1	1967	07	12
2	1988	06	10
3	1989	09	11
4	1990	03	05
5	1991	08	06
6	1992	13	15
7	1993	10	08
8	1994	22	13
9	1995	15	21
10	1996	13	16
Total		106	117

Table 5. Year-Wise Verification and Registration of Publications during D5 in J&K

S. no.	Year	Titles	verified Titles registered
1	1997	11	11
2	1998	7	14
3	1999	67	10
4	2000	25	19

5	2001	22	22
6	2002	22	29
7	2003	29	29
8	2004	13	18
9	2005	24	13
10	2006	35	35
Total		255	200

Table 6. Year-Wise Verification and Registration of Publications During D6 in J&K.

S. no	Year	Titles verified	Titles registered
1	2007	40	36
2	2008	44	32
3	2009	61	63
4	2010	56	48
5	2011	78	68
6	2012	137	107
7	2013	74	84
8	2014	56	50
9	2015	80	44
10	2016	64	19
Total		690	551

Table 7. Depicting Percentage Share of All the Decades.

S. no	Decade	Registered titles	% share
1	D1 (1957–1966)	34	2.90
2	D2 (1967–1976)	169	14.40
3	D3 (1977–1986)	105	8.93
4	D4 (1987–1996)	117	9.95
5	D5 (1997–2006)	200	17.00
6	D6 (2007–2016)	551	46.85
Total		1,176	100

Table 8. Mean and Standard Deviation of the Decades.

Descriptives			
Title registered	**N**	**M**	**SD**
D1	10	3.40	7.22
D2	10	16.90	6.24
D3	10	10.50	3.59
D4	10	11.70	4.85
D5	10	20.00	8.57
D6	10	55.10	26.15
Total	60	19.60	20.47

Table 9. Level of Significance between the Groups and Within the Groups.

ANOVA					
Title registered	**Sum of squares**	**df**	**Mean square**	**F**	**Sig.**
Between groups	16,753.60	5	3,350.72	22.70	001
Within groups	7,970.80	54	147.60		
Total	24,724.40	59			
ANOVA = Analysis of variance					

Table 10. Multiple Comparisons and Level of Significance Between the Selected Decades Using Tukey's HSD.

Multiple comparisons			
Tukey's HSD			
(I) Decades	**(J) Decades**	**Mean difference (I-J)**	**Sig**
D1	D2	−13.50	.147
	D3	−7.10	.780
	D4	− 8.30	.648
	D5	−16.60*	.039
	D6	−51.70*	.000
D2	D1	13.50	.147
	D3	6.40	.845
	D4	5.20	.929
	D5	−3.10	.993
	D6	−38.20*	.000
D3	D1	7.10	.780
	D2	-6.40	.845
	D4	-1.20	1.000
	D5	-9.500	.507
	D6	-44.60*	.000
D4	D1	8.30	.648
	D2	-5.20	.929
	D3	1.20	1.000
	D5	-8.30	.648
	D6	-43.40*	.000
D5	D1	16.60*	.039
	D2	3.10	.993
	D3	9.50	.507
	D4	8.30	.648
	D6	-35.10*	.000
D6	D1	51.70*	.000
	D2	38.20*	.000
	D3	44.60*	.000
	D4	43.40*	.000
	D5	35.10*	.000

HSD = honestly significant difference.

*The mean difference is significant at the .05 level

Table 11. Depicting J&K's Contribution to Total Circulation of Publications in India.

	India	J & K	Percentage
Circulation	450,586,212	9,627,424	2.14

Figure 1. Depicting difference in verified and registered titles.

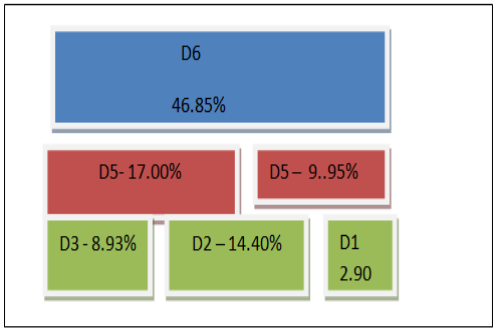

Figure 2. Depicting decade-wise increase in the number of publications.

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RESEARCH ARTICLE

Habitat Association of Grey Junglefowl (*Gallus sonneratii*) and Aravalli Red Spurfowl (*Galloperdix spadicea caurina*) with other Avian Species in Mount Abu Wildlife Sanctuary

Narayan Lal Choudhary[1] and Nadim Chishty[2]*

[1]Research scholar, Wildlife, Limnology and Toxicology Research Laboratory, Department of Zoology, Government Meera Girl's College (Mohanlal Sukhadia University) Udaipur, Rajasthan, India
[2]Associate Professor, Wildlife, Limnology and Toxicology Research Laboratory, Department of Zoology, Government Meera Girl's College (Mohanlal Sukhadia University) Udaipur, Rajasthan, India

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***Address for Correspondence**
Nadim Chishty
Associate Professor,
Wildlife, Limnology and Toxicology Research Laboratory,
Department of Zoology, Government Meera Girl's College,
(Mohanlal Sukhadia University),
Udaipur, Rajasthan, India
Email: narayanlalchoudhary1995@gmail.com

ABSTRACT

The study was conducted to analyse the habitat associations of grey junglefowl and Aravalli red spurfowl with other avian species in Mount Abu Wildlife Sanctuary. During the study, 24 bird species were found to have habitat associations with grey junglefowl (*Gallus sonneratii*) and 27 bird species with Aravalli red spurfowl (*Galloperdix spadicea caurina*). Grey junglefowl were more likely to share a habitat with *Galloperdix spadicea caurina* and *Pavo cristatus*, *Francolinus pondicerianus, Streptopelia chinensis* and *Columba livia*. Grey Jungle fowl share habitat with several dove species like *Streptopelia decaocto, Spilopelia senegalensis*, *Streptopelia orientalis* and *Streptopelia tranquebarica*. The Aravalli red spurfowl generally observed with *Gallus sonneratii* and *Pavo cristatus*, *Francolinus pondicerianus, Spilopelia senegalensis, Argya malcolmi, Argya striata* and *Streptopelia chinensis*. Both species of fowl have a close habitat association with Indian peafowl and grey francolin. Grey junglefowl and Aravalli red spurfowl forage and feed with peafowl, doves and babblers. Both species of fowls have poor habitat associations with Accipitridae and Falconidae family species.

Keywords: Grey junglefowl, Aravalli red spurfowl, habitat, association, Mount Abu Wildlife sanctuary

INTRODUCTION

There are many different types of interactions present between many species and they can found between closely related species or between species that aren't related at all. Several studies have been conducted on different species interactions in coral reef fishes, birds and mammals (FitzGibbon, 1990; Peres, 1992a &b and 1993; Chapman and Chapman, 2000; Corkeron, 1990; Weller *et al.,* 1996; Herzing and Johnson, 1997; Frantzis and Herzing, 2002). In a mixed species flock of birds, generally two species forage together in terrestrial habitat for food. These group show huge variation in size and association stability strength in various parts of the world, such as Campephagidae (Minivets), Timaliidae (babblers), Dicruidae (drongos) in the Palaeotropics region; Picidae (woodpeckers), Stittidae (nuthatches), Paridae (tits) in temperate regions and Thamnophilidae (antshrikes), Thraupidae (Tanagers) and antwrens (Thamnophilidae) in Neotropic regions (Moynihan, 1962; Terborgh, 1990; Greenberg, 2001). Two basic hypotheses have been given to describe why birds congregate in flocks; enhanced feeding effectiveness and decreased predation risk (Morse, 1977). Comparative analysis, experiments and observation techniques have all been used to find what benefits are present in mixed species flocks (Greenberg, 2001). Several studies have been found to support foraging and antipredatory benefits, but a general conclusion has not been obtained yet (Grubb, 1987; Cimprich and Grubb, 1994; Dolby and Grubb, 1998; Thiollay, 1999; Beauchamp, 2004). The sizes of flocks depend upon the availability of food resources, habitat types and seasons. Several species forage together when food resources are limited and less abundant. Mixed species foraging flocks are common in the tropics during the dry season and in woody habitats at high latitudes in winter (Morse, 1970; Croxall, 1976; Earle, 1983; Poulsen, 1996; Develey and Peres, 2000). In late summer and fall, more species are seen in flocks in high latitudes. That occurs due to the variety of migrant species that join the flocks during those times (Morse, 1970; Rodewald and Brittingham, 2002; Hobson and van Wilgenburg, 2006). The Grey junglefowl found in south and central India (Grimmett *et al.,* 1998; Rajasekaran *et al.,* 2002; Madge and McGowan, 2002; Joshi and Shrivastava, 2012) and is categorised as a least concern species by HBW and BirdLife International (2021). Grey jungle fowl commonly occur in the southern part of Rajasthan, mainly in Mount Abu, Sitamata, Phulwari-ki-nal and Kumbhalgarh wildlife sanctuaries (Tehsin, 1986; Devarshi, 2008; Sharma, 2014; Sen PK, 2017; Sharma, 2017). The species found in a diverse habitat including secondary forests, lowland forests and forest edge habitats (Collias and Collias, 1967; Madge and McGowan, 2002; Sharma, 2017). Very few studies have been done on habitat utilisation and abundance of grey jungle fowls (Tata and Gautam, 1993; Zacharias, 1997; Subramanian *et al.,* 2002; Satyakumar, 2006; Sathyanarayana, 2007; Sen PK, 2017).

Aravalli red-spur fowls is distributed in the southern part of Rajasthan, especially in protected areas like Kumbhalgarh, Rawali Todgarh, Sajjangarh, Sitamata, Mount Abu and Phulwari ki-nal sanctuaries and their peripheral areas and also distributed in Sundha mata conservation reserve of Jalore district (Tehsin, 1986; Sharma, 2007; F.E.S., 2010; Sharma, 2014; Sen PK, 2017; Sharma, 2017). They found in diverse forest habitat mainly in foothills but also in hilly forest altitudinal ranges (Jathar and Rahmani, 2006; Sharma, 2014; Sen PK, 2017). Red spurfowls is found in rocky hilly and thick bamboo forest habitats (Baker, 1920; Sahabbudin *et al.,* 2004; Sharma, 2014; Sharma, 2017), moist deciduous hilly forest habitat (Kukreja *et al.,* 2007) and dense forest at higher altitudes than Grey junglefowls (Sen PK, 2017). Very few studies have been undertaken on the grey junglefowl and Aravalli red spurfowl and those were on abundance (Ramesh *et al.,* 2011), distribution (Sharma, 2014; Sharma, 2017), habitat utilization, feeding, activity and behavioural pattern (Sen PK, 2017). Several bird species are associated with other birds and animals for enhanced foraging opportunities (Alcock, 1997). Birds and animals congregate for a variety of reasons, including protection from predators, food and foraging and reproductive benefits. The primary benefits of group formation for non-reproductive purposes are declined predation risk and enhanced foraging efficiency (Morse, 1977). A present study was conducted on the habitat association of grey junglefowl and Aravalli red spurfowl with other avian species in Mount Abu Wildlife sanctuary.

MATERIALS AND METHODS

The study was carried out in the Mount Abu Wildlife Sanctuary area and it situated (24°33′′-24°43′N and 72°38′ - 72°53′E) and altitude ranges from 300 m from MSL at the foothills to 1722 m at Guru Shikhar. Observations were made with the help of Nikon 8x40 binocular, Nikon P1000 (semi SLR) and Canon EOS 700D cameras with 150-500mm lens. Study was carried out from January, 2022 to June, 2022 and observation taken in early morning 6:00 am to 9:00 am and late evening 4:00 pm to 6:00pm. Observation of habitat association of grey junglefowl (*Gallus sonnerattii*) and Aravalli red spurfowl (*Galloperdix spadicea caurina*) with other avian species were observed using focal and scan sampling method(Altmann, 1974). Observation of grey junglefowl and Aravalli red spur fowl association with other species were taken during the foraging, feeding and roosting time. Observations were taken that which species were actively sharing habitat, especially during foraging and feeding near the grey jungle fowl and Aravalli red spurfowl. Avian species around grey jungle and Aravalli red spurfowls were recorded within a 50 meters radius at every location. The Bray-Curtis cluster neighbour joining analysis was done with the help of PAST 4.03 software for the association of grey junglefowl and Aravalli red spurfowl with other bird's species. The avian species were identified using standard field guide (Grimmett *et al.,* 1998 and Vyas, 2013).

RESULT AND DISCCUSION

During study, a total 24 species of birds associated with grey junglefowl and 27 species of birds associated with Aravalli red spurfowl were recorded in Mount Abu Wildlife Sanctuary area. Grey jungle fowl has maximum habitat association with Aravalli red spurfowl (*Galloperdix spadicea caurina*) and Indian peafowl (*Pavo cristatus*) followed by Indian grey francolin (*Francolinus pondicerianus*), spotted dove (*Streptopelia chinensis*) and rock pigeon (*Columba livia*). Grey junglefowl share habitat with several dove species namely Eurasian collared dove (*Streptopelia decaocto*), laughing dove (*Spilopelia senegalensis*), oriental turtle dove (*Streptopelia orientalis*) and red collared dove (*Streptopelia tranquebarica*). Further association declined with large grey babbler (*Argya malcolmi*) followed by common babbler (*Argya caudate*), jungle babbler (*Argya striata*), common myna (*Acridotheres tristis*), red-vented bulbul (*Pycnonotus cafer*), red-whiskered bulbul (*Pycnonotus jocosus*), yellow footed green pigeon (*Treron phoenicopterus*), house crow (*Corvus splendens*) and greater coucal (*Centropus sinensis*). Minimum or lower habitat association found with Falconidae family species common kestrel (*Falco tinnunculus*) and Accipitridae family species; shikra (*Accipiter badius)* and crested serpent eagle (*Spilornis cheela*) and black-shouldered kite (*Elanus axillaris).* Figure 1- Bray-Curtis cluster neighbour joining analysis show habitat association of grey junglefowl with other 24 avian species. Aravalli red spurfowls had stronger habitat association with grey junglefowl (*Gallus sonneratii*) and Indian peafowl (*Pavo cristatus*) followed by Indian grey francolin (*Francolinus pondicerianus*) and laughing dove (*Spilopelia senegalensis*), large grey babbler (*Argya malcolmi*) and jungle babbler (*Argya striata*), spotted dove (*Streptopelia chinensis*), Eurasian collared dove (*Streptopelia decaocto*), common babbler (*Argya caudate*) and red collared dove (*Streptopelia tranquebarica*), black lored tit (*Machlolophus xanthogenys*) and oriental turtle dove (*Streptopelia orientalis*).

Further association of Aravalli red spurfowl declined with other avian species and less habitat preference and similarity shown by common hoopoe (*Upupa epops*) followed by red-vented bulbul (*Pycnonotus cafer*), common myna (*Acridotheres tristis*),red-whiskered bulbul (*Pycnonotus jocosus*), red-wattled lapwing (*Vanellus indicus*), yellow footed green pigeon (*Treron phoenicopterus*), brahmin starling (*Sturnus pagodarum*), house sparrow (*Passer domesticus*) and greater coucal (*Centropus sinensis*). Lowest association found with house crow (*Corvus splendens*) and jungle crow (*Corvus macrorhynchos*) and very poor interaction found with Accipitridae family species; shikra (*Accipiter badius)*, crested serpent eagle (*Spilornis cheela*), black-shouldered kite (*Elanus axillaris)* and Falconidae species common kestrel (*Falco tinnunculus*). Figure 2- Bray-Curtis cluster neighbour joining analysis show habitat association of Aravalli red spurfowl with other 27 avian species. Both fowls (grey jungle and Aravalli red spurfowls) show very strong and closed habitat association with Indian peafowl and Indian grey francolin (Figure 1 and 2). During the foraging and feeding time, Aravalli red spurfowl also share feeding grounds with babblers and dove species, while Grey jungle

fowls feed alongside pea fowls and doves. Both species have poor associations with Accipitridae and Falconidae family species.

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REFERENCES

1. Alcock, J. (1997). Animal behavior: an evolutionary approach. Sinauer Associates, Inc. Publishers, Sunderland, Massachusetts, U.S.A.
2. Altmann, J. (1974). Observational study of behavior: sampling methods. *Behaviour*. 49:227-267.
3. Baker, E.C. (1920). Stuart, The game birds of India, Burma and Ceylon. *Journal of the Bombay Natural History Society*. 27:906-911.
4. Beauchamp, G. (2004). Reduced flocking of birds on islands with relaxed predation. *Proceedings of the Royal Society of London B: Biological Sciences*, 271:1039–1042.
5. Chapman, C.A. &Chapman, L.J.(2000). Interdemic variation in mixed-species association patterns: common diurnal primates of Kibale National Park, Uganda. *Behavioral Ecology and Socio-biology*. 47:129–139.
6. Cimprich, D.A. & Grubb, T.C. (1994). Consequences for Carolina chickadees of foraging with tufted titmice in winter. *Ecology*.75 1615–1625. Doi: 10.2307/1939622.
7. Collias, N.E.& Collias, E. C.(1967). A field study of the red jungle fowl in north central India. *The Condor*. 69:360–386.
8. Corkeron, P.J. (1990). Aspects of the Behavioural Ecology of Inshore Dolphins *Tursiops truncatus* and *Sousa chinensis* in Moreton Bay, Australia. In: Leatherwood, S and Reeves RR, Eds. *The Bottlenose Dolphin, Academic Press*, San Diego, 245-265.
9. Croxall, J.P. (1976). The composition and behavior of some mixed-species bird flocks in Sarawak. *Ibis*. 118 333–346, doi:10.1111/j.1474-919X.1976.tb02024.x.
10. Devarshi, D. (2008). Avifauna of Rajasthan: An overview. In Conserving Biodiversity of Rajasthan, with emphasis on Wild fauna and flora. Ed. Ashok Verma (*Himanshu Publications*)Udaipur.pp-193-210.
11. Develey, P.F & Stouffer, P.C. (2001). Effects of roads on movements by understory birds in mixed-species flocks in central Amazonian Brazil. *Conservation Biology*. 15 1416–1422.
12. Dolby, A.S. & Grubb, T.C. (1998). Benefits to satellite members in mixed-species foraging groups: an experimental analysis. *Animal Behaviour*. 56:501–509.
13. Earle, R.A. (1983). Notes on bird parties in some Transvaal indigenous forests. *Ostrich*. 54: 176-178.
14. F.E.S. (2010). Foundation for Ecological Security- Assessment of Biodiversity in Sitamata Wildlife Sanctuary. A Conservation Perspective. Report of Foundation for Ecological Security, Gujarat India.pp- 1-162.
15. FitzGibbon, C.D. (1990).Mixed-species grouping in Thomson's and Grant's gazelles: the antipredator benefits. *Animal Behaviour*. 39:1116–1126.
16. Frantzis, A. & Herzing, D.L. (2002). Mixed species associations of striped dolphins (*Stenella coeruleoalba*), short-beaked common dolphins (*Delphinus delphis*) and Risso's dolphins (*Grampus griseus*) in the Gulf of Corinth (Greece, Mediterranean Sea). *Aquatic Mammals*. 28:188–197.
17. Greenberg, R.S. (2001). Birds of many feathers: the formation and structure of mixed species flocks of forest birds. In *On the move: how and why animals travel in groups*, edited by Boinski S and Garber P (*University of Chicago Press*) Chicago.pp-521–558.
18. Grimmett, R., Inskipp, C. & Inskipp, T. (1998). Birds of the Indian subcontinent. *Oxford University Press,* Mumbai.pp- 888.

19. Grubb, T.C. (1987). Changes in the flocking behaviour of wintering English titmice with time, weather and supplementary food. *Animal Behaviour*. 35:794–806, doi:10.1016/S0003-3472(87)80116-1.
20. HBW and BirdLife International (2021). Handbook of the Birds of the World and BirdLife International digital checklist of the birds of the world. Version 6. Available at: http://datazone.birdlife.org/userfiles/file/ Species/ Taxonomy/HBW-BirdLife_Checklist_v6_Dec21.zip.
21. Herzing, D.L. & Johnson, C.M. (1997). Interspecific interactions between Atlantic spotted dolphins (*Stenella frontalis*) and bottlenose dolphins (*Tursiops truncatus*) in the Bahamas, 1985–1995. *Aquatic Mammals.* 23:85–99.
22. Hobson, K.A. & van Wilgenburg, S. (2006). Composition and timing of post-breeding multi-species feeding flocks of boreal forest passerines in Western Canada. *Wilson Journal of Ornithology*. 118:164-172.
23. Jathar, G.A. & Rahmani, A.R. (2006). Endemic birds of India. *Buceros.* 11:1-53.
24. Joshi, P. & Shrivastava, V.K. (2012). Ecological study and bird diversity of Tawa Reservoir and its surrounding areas of Hoshangabad district (Madhya Pradesh). *The Bioscan*. 7(1):129-133.
25. Madge, S. & McGowan, P.J.K. (2002). Pheasants, Partridges and Grouse. Christopher Helm A and Black C (Publishers) Ltd., London.pp-488.
26. Morse, D.H. (1970). Ecological aspects of some mixed-species foraging flocks of birds. *Ecological Monographs*. 40:119–168.
27. Morse, D.H. (1977). Feeding behavior and predator avoidance in heterospecific groups. *Bioscience*. 27:332–339.
28. Moynihan, M. (1962). The organization and probable evolution of some mixed species flocks of neotropical birds. *Smithsonian Miscellaneous Collections*. 143(7):1–140.
29. Peres, C.A. (1992a). Consequences of joint-territoriality in a mixed-species group of Tamarin monkeys. *Behaviour*. 123:220–240.
30. Peres, C.A. (1992b). Prey-capture benefits in a mixed group of *Amazonian tamarins*, *Saguinus fuscicollis* and *S. mystax*. *Behavioral Ecology and Sociobiology*. 31:339–347.
31. Peres, C.A. (1993).Diet and feeding ecology of saddle-back (*Saguinus fuscicollis*) and moustached (*S. mystax*) tamarins in an Amazonian terra firme forest. *Journal of Zoology*. 230:567–592.
32. Poulsen, B.O. (1996). Relationships between frequency of mixed-species flocks, weather and insect activity in a montane cloud forest in Ecuador. *Ibis*. 138:466-470.https://doi.org/10.1111/j.1474-919X.1996.tb08066.x.
33. Rajasekaran, A., Arun, P.R., Azeez, P.A. & Bhupathy, S. (2002). Ecological observations on Baphlimali Hill and its Environs, Orissa. National Seminar on Conservation of Eastern Ghats, At Triupati, Andra Pradesh, 212-222.
34. Ramesh, N., Sathyanarayana, M.C. & Lloyd, H. (2011). Abundance of grey junglefowl *Gallus sonneratiiat* Theni Forest Division, Western Ghats, India: implications for monitoring and conservation. *International Journal of Galliformes Conservation*. 2:14-21.
35. Rodewald, P.G. & Brittingham, M.C. (2002). Habitat use and behavior of mixed species landbird flocks during fall migration. *Wilson Bulletin of Ornithology*. 114:87-98.
36. Sathyakumar, S. (2006). Habitat use by Grey Junglefowl *Gallus sonneratii* Temminck at Mundanthurai Plateau, Tamil nadu. *Journal of the Bombay Natural History Society*. 103 (1): 57–61.
37. Sathyanarayana, M.C. (2007). Review of research on Pheasants in southern India. Galliformes of India. *Envis*. 10(1):105–108.
38. Sen, P.K. (2017). Study on The Biology and Behavioral Ecology of Grey Jungle Fowl *Gallus sonneratii* Temminck and Aravalli Red Spurfowl *Galloperdix spadiceacaurina* Blanford in The Deciduous Forests of Southern Rajasthan. Ph.D.thesis Mohanlal Sukhadia University, Udaipur, Rajasthan.
39. Shahabuddin, G., Verma, A. & Kumar, R. (2004). Birds, forests and conservation: Critical issues in Sariska Tiger Reserve, Rajasthan, India. *Newsletter for Ornithologists*.1 :(6) 82-84.
40. Sharma, S.K. (2007). Study of biodiversity and ethno biology of Phulwari Wildlife Sanctuary, Udaipur (Rajasthan). Ph. D. Thesis. Mohanlal Sukhadia University, Udaipur.
41. Sharma, S.K. (2014). Faunal And Floral Endemism in Rajasthan (With Notes on endemic biota of Adjoining states). *Himanshu publications*, Udaipur Rajasthan, 144.
42. Sharma, S. (2017). Distribution of wild fowls in Rajasthan: a study. *Anusandhaan - Vigyaan Shodh Patrika*. 5(01):01-09. https://doi.org/10.22445/avsp.v5i01.9838

43. Subramanian, C., Sathyanarayana, M.C. & Kambarajan, K. (2002). Habitat utilization by Grey Junglefowl (*Gallus sonneratii*) in Theni Forest Division, Megamalai. Tamilnadu. Proceedings of the National Symposium on Galliformes, A.V.C College, 28–36.
44. Tata, L.R.R. & Gautam, P. (1993). Habitat utilization by Grey Junglefowl in Bori Wildlife Sanctuary, India. Abstract. *World Pheasant Association Journal*. 17 & 18:91.
45. Tehsin, R. (1986). How Wild Dog (*Cuon alpinus*) use their chemical weapon in hunting. *Tiger Paper*. 13 (2):23.
46. Terborgh, J. (1990). Mixed flocks and polyspecific associations: costs and benefits of mixed groups to birds and monkeys. *American Journal of Primatology*. 21:87-100.
47. Thiollay, J.M. (1999).Frequency of mixed species flocking in tropical forest birds and correlates of predation risk: an intertropical comparison. *Journal of Avian Biology*. 30:282–294.
48. Vyas, R. (2013). Birds of Rajasthan. Bombay Natural History Society and Oxford University Press.Pp.xiv+236.
49. Weller, D.W., Wursig, B., Whitehead, H., Norris, J.C., Lynn, S.K., Davis, R.W., Clauss, N. & Brown, P. (1996). Observations of an interaction between sperm whales and short-finned pilot whales in the Gulf of Mexico. *Marine Mammal Science*. 12:588–594.
50. Zacharias, V.J. (1997). Status of Grey Jungle fowl in Periyar Tiger Reserve. Kerala. *Tragopan*. 7:13–14.

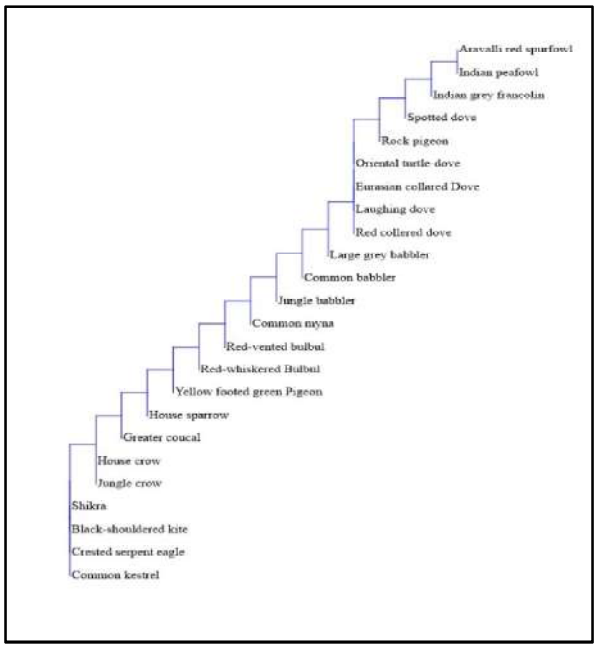

Figure 1. Habitat association of grey jungle fowl with other avian species

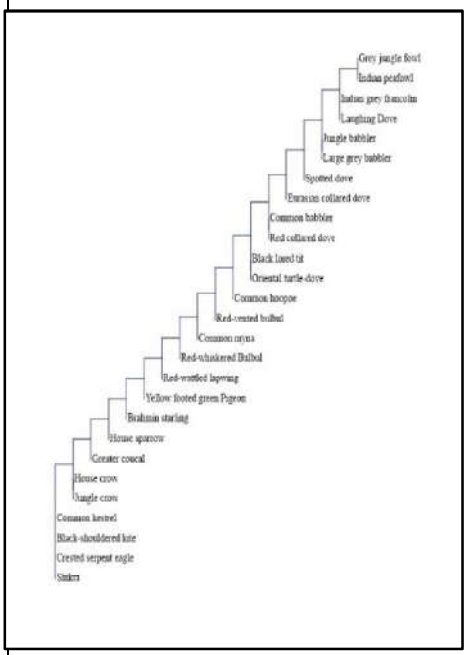

Figure 2. Habitat association of Aravalli red spurfowl with other avian species

Figure 3. Aravalli red spur fowl sharing feeding ground with jungle babbler

Figure 4. Aravalli red spur fowl forage and feeding roadside

Figure 5. Grey jungle fowl in *Lantana* dominated habitat

Figure 6. Grey jungle fowl sharing feeding ground with Indian peafowl and rock pigeon

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RESEARCH ARTICLE

Synthesis and *In vitro* Anticancer Evaluation of Novel Chalcones against MCF Cell Lines

Deepika P[1]*, D.Uma Maheswari[2], B.S.Venkateswarlu[3], M.Kumar[2] and Prema S[4]

[1]Research Scholar, Department of Pharmaceutical Chemistry, Vinayaka Mission's College of Pharmacy, Salem, Tamil Nadu, India
[2]Professor, Vinayaka Mission's College of pharmacy, Vinayaka Mission Research Foundation, Salem, Tamil Nadu, India
[3]Professor, Department of Pharmaceutics, Vinayaka Mission's College of Pharmacy, Vinayaka Mission Research Foundation, Salem, Tamil Nadu, India
[4]Professor, Department of Pharmaceutical Chemistry, Crescent school of Pharmacy, B.S. Abdur Rahman Crescent Institute of Science and Technology, Vandalur, Tamil Nadu, India

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***Address for Correspondence**
Deepika P
Research Scholar,
Department of Pharmaceutical Chemistry,
Vinayaka Mission's College of Pharmacy,
Salem, Tamil Nadu, India
Email: deepikaprasannakumar@gmail.com

ABSTRACT

The present study's primary aim was to perform chalcone synthesis and assess its anticancer activity. Substituted benzimidazole is prepared by combining O-phenlyne diamine with formic acid. The resultant benzimidazole was then refluxed with acetyl chloride to yield substituted 2-methoxy benzimidazole. The resulting mixture was then treated with 3-chloroquinoline carbaldehyde, which was prepared from acetanilide, yielding chalcone derivatives (C1=NO_2, C_2=Cl,C_3=Br , C_4=OCH_3 &C_5=Cl_2). All synthesised compounds were evaluated and confirmed by IR, H-NMR, C-NMR, and mass spectroscopy. The newly synthesised compounds that had been characterised were then tested using the MCF-7 breast cell line. The MTT assay was used to determine cell viability. The prepared chalcone derivative C1 was more effective and potent than the other chalcone derivatives with lower viability as measured by MTT assay, and the compound's IC 50 value was determined to be 24.2513 g/ml. As a result, it is suggested that chalcone derivate (C1) can be used effectively to treat breast cancer.

Keywords: Chalcones, O-phenlyene diamine, Anticancer, MCF-7, and IC_{50}.

INTRODUCTION

Cancer is caused by a series of subsequent gene mutations that alter cell functions [1]. There are many anticancer agents are available in the market but they are failed to differentiate the normal cell and cancerous cell. Breast cancer is estimated to be one of the most common cancers, and it is the most common malignant neoplasm in women. Metastasis is the leading cause of death in breast cancer, accounting for 90% of all deaths. Despite advancements in cancer treatment and detection techniques, breast cancer continues to be a leading cause of death in women. To control tumour growth, radiation, chemotherapy, and surgery can be used; however, these options must be effective in order to manage breast cancer metastases [2]. The aim of the present investigation is to prepare the novel chalcones. Chalcones are a type of polyphenolic compound derived from plants that belongs to the flavonoids family. According to research, some chalcones have a wide range of cytoprotective and modulatory functions that may have therapeutic potential for a variety of diseases [3]. Based on the chalcone skeleton, several lead compounds with various pharmacological properties have been developed [4]. Chalcones, which are considered precursors of flavonoids and isoflavonoids, are abundant in edible plants [5].They are composed of open-chain flavonoids with two aromatic rings linked by a three-carbon,-unsaturated carbonyl system. Among flavonoids, chalcones are an intriguing target class of compounds that have received a lot of attention due to their diverse biological activities, which include Anti-inflammatory [6], Anti-invasive, [7] Antitumor, [8] and Antibacterial [9] properties. They are thought to be promising anticancer agents against the majority of human cancers. Previous research suggests that chalcones can induce apoptosis [10 & 11] as well as uncouple mitochondrial respiration and thus collapse mitochondrial membrane potential [12]. Because a number of clinically useful anticancer drugs have genotoxic effects due to interactions with nucleic acid amino groups, chalcones may be free of this significant side effect [13]. Hence our aim is to synthesise chalcones and investigate their cytotoxic potential against breast cancer cell lines, as part of the ongoing search for potent and selective cytotoxic chalcones.

MATERIAL AND METHODS

MCF-7-554 cell line procured from NCCS pune, O-phenylene diamine, Formic acid, Nitric aicd, Ammonia, Acetanilide, Acetyl chloride, Phosphorous oxy chloride, Dimethyl formamide, Sodium hydroxide, 3-chloro quinoline carbaldehyde, Concentrated HCl and ethanol was procured from Merck.

Preparation of Chalcone Derivatives

Step 1: Synthesis of 5-substituted benzimidazole

6.75g of 5-substituted**-**o-phenylene diamine, 4.35 g of formic acid,30ml of ethanol was added in a round bottom flask and heated at 100^{0}C for 2 hrs, cooled and the crude benzimidazole was filtered and washed with 25ml of water. The crude product was dissolved in 400 mL of boiling water and 0.5g of decolouring carbon and digested for 15min,filtered rapidly at the pump through a preheated Buchner funnel. Filterate was cooled to 10^{0}C. benzimidazole was filtered and washed with 25ml of cold water and dried at 100^{0}C.

Step 2: Synthesis of 5-substituted-2-methoxy-1*H*-benzimidazole

Equimolar quantities of 5-substituted**-**benzimidazole 0.01 mol (3.5 g) and 0.01mol (2.37 g) of acetyl chloride were added, mixed and refluxed for 4hrs. Concentrated ammonia was added till alkalinity was achieved and the product obtained was recrystallized from 20% aqueous ethanol.

Step 3: Synthesis of 3- chloro quinoline carbaldehyde

To a solution of acetanilide (N-phenyl-acetamide) in dry DMF (15mmoles) at 50C, phosphorous oxychloride (60mmoles) was added drop wisely with stirring and reaction mixture was stirred at 80-1000C for a time ranging between 4-16 hours. Then mixture was pour on to crush ice, stirred for 50C minutes and resulting solid was filtered. Washed well, and dried .The compound was recrystallized from ethyl acetate.

Step 4: 5-substituted-1*H*-benzimidazol-2-yl-4-2-chloroquinolin-3-yl but-2-en-1-one

5-substituted-2-methoxy-1 *H*-benzimidazole. (0.01 mole,3.46g) was dissolved in 20ml ethanol and sodium hydroxide solution (60 ml, 40%) was added and the mixture was cooled. To this was added (0.01 mole, 3g) 3- chloroquinoline carbaldehyde dissolved in a minimum quantity of ethanol and then the reaction mixture was stirred for a period of 4-5 hours and was left overnight. Conc. HCl was added drop by drop till the solution was slightly acidic. The solid separated was filtered, washed with water and dried. The crude product was crystallized from aqueous ethanol. The yield of chalcone synthesis was depicted in table no 1. The scheme of chalcone synthesis was depicted in the figure no .1.

Characterisation of Chalcone Derivatives

All synthesised compounds were identified by using IR, H-NMR, C-NMR, and mass spectroscopy. MTT ASSAY [14]. Cytotoxicity of the C1-C5 samples on MCF-7 cell line was determined by MTT Assay. The cells (10000 cells/well) were cultured in 96 well plate for 24 h in RPMI 1640 medium supplemented with 10% FBS and 1% antibiotic solution at 37°C with 5% CO_2. Next day cells were treated from 10-320 µg/ml of the formulations (different concentrations were prepared in incomplete medium). After incubation for 24 h, MTT Solution (a final concentration of 250 µg/ml) was added to cell culture and further incubated for 2 hrs. At the end of the experiment, culture supernatant was removed and cell layer matrix was dissolved in 100 µl Dimethyl Sulfoxide (DMSO) and read in an Elisa plate reader (iMark, Biorad, USA) at 540 nm (660 nm as the reference wavelength) The results and figures of the MTT assay were mentioned in table no 2 and figure no 3.

RESULTS AND DISCUSSION

Chemotherapy efficacy has not improved significantly, and unwanted side effects remain unacceptably high. This emphasises the importance of developing novel chemotherapeutic agents for more effective cancer treatments [15]. Chalcones have been receiving a lot of attention not just because their broad range of activities, in addition to their ease of synthesis and alteration of the main core, as well as their good safety profile for oral administration [16]. The purpose of the research were to produce different chalcone derivatives by varying the substitutions. The prepared chalcone derivatives are then characterised, and the results are listed below:

IR Spectral values

IR spectra data for **C1** compound is NH strecth (3450cm^{-1}), C=O strecth (1680 cm^{-1}), C=N stretch(1615cm^{-1}), Aromatic C=C Stretch (1523cm^{-1}), C-Br stretch (700cm^{-1}),IR C_2:NH strecth (3341cm^{-1}), C=O strecth (1675 cm^{-1}), C=N stretch(1605cm^{-1}), Aromatic C=C Stretch (1503cm^{-1}), C-Br stretch (722cm^{-1}),C_4: compound is NH strecth (3441cm^{-1}), C=O strecth (1670 cm^{-1}), C=N stretch(1615cm^{-1}), Aromatic C=C Stretch (1513cm^{-1}), C-Br stretch (1322cm^{-1}),**C5:** NH strecth (3471cm^{-1}), C=O strecth (1675 cm^{-1}), C=N stretch(1613cm^{-1}), Aromatic C=C Stretch (1422cm^{-1}), C-Br stretch (2970 cm^{-1}).

NMR spectral values

H-NMR spectra data for C1 compound is 5.151 (s, 1H, NH - benzimidazole), 6.614 (s, 1H, ethylene), 7.442 (s, 1H, CH -ethylene), 7.562-7.693 (m, 3H, aromatic proton), 7.395-8.844 (m, 5H, aromatic proton), C_2: 5.131 (s, 1H, NH - benzimidazole), 6.604 (s, 1H, ethylene), 7.459 (s, 1H, CH -ethylene), 7.592-7.693 (m, 3H, aromatic proton), 7.735-8.844 (m, 5H, aromatic proton), C_4: 5.151 (s, 1H, NH - benzimidazole), 6.614 (s, 1H, ethylene), 7.442 (s, 1H, CH -ethylene), 7.562-7.693 (m, 3H, aromatic proton), 7.935-8.844 (m, 5H, aromatic proton),C5: 5.131 (s, 1H, NH - benzimidazole), 6.604 (s, 1H, ethylene), 7.509 (s, 1H, CH -ethylene), 7.582-7.793 (m, 3H, aromatic proton), 7.835-8.844 (m, 5H, aromatic proton), 2.310 (s, 3H, CH_3 methyl).

C-NMR spectra data for compound (Value in PPM)

C_1:111.50,115.93,125.57,127.40,127.42,127.45,128.18,130.57,130.65,131.11,135.36,138.55,141.10,145.67,147.93,148.07,187.30,C_2:115.93,126.17,127.17,127.45,127.40,127.45,128.42,130.1,132.11, 135.36,138.55, 141.10, 145.67, 147.93,149.67, 187.30, C_4:110.56,116.93,127.00,127.40,127.45,127.48,127.64,128.42,130.18,130.57,138.55,141.55,145.82,147.93,149.07,186.30,C5:115.33,126.51,127.00,127.40,127.42,127.45,127.64,128.42,110.57,131.05,132.11, 131.36, 138.55, 141.10, 145.67,147.93, 149.07, 187.30.

Mass spectral data:

C_1: base peak 194.20, molecular ion peak (m) at m/z 412.24, Moleculaer weight: 412.24, C_2: base peak 163.20, molecular ion peak (m) at m/z 351.21, Moleculaer weight: 351.21,C_4: base peak 190.23, molecular ion peak (m) at m/z 378.76, Moleculaer weight: 378.76, C_5: base peak 159.23, molecular ion peak (m) at m/z 347.79, Moleculaer weight: 347.79.The chalcones which have been confirmed by spectral studies are then tested for anticancer activity using the MCF -7 cell line. The prepared compounds were treated in cancer cells at various concentrations (0,10,20,40,80,160, and 320g/ml). Figure no 2 depicts cell inhibition at various concentrations. The C_1 compound inhibited cell growth effectively, and the minimum inhibitory concentration was determined to be 24.2513g/ml. Due to C_1's remarkable cell inhibition capacity, it can be used to effectively treat breast cancer. The previous literature suggested that presence of an electron-withdrawing nitro group was indeed found to be one of the essential requirements for niclosamide's anticancer effect in SAR studies [17]. Henceforth it was concluded that substituting a nitro moiety in chalcone was reasonable for the potent anticancer effect of newly prepared chalcones. However gene expression studies could be done to discover about the mechanism of action of the preapared chalcones.

CONCLUSION

MCF-7 breast cell lines are treated with the novel chalcone derivatives. The nitro substituted chalcone derivative (C_1) showed potent cytotoxicity against the MCF-7 breast cell line. The compound's IC 50 value was determined to be 24.2513g/ml. As a result, the prepared chalcone compound has the potential to be used as an anticancer agent against breast cancer cell lines. It has been concluded that the novel chalcone derivative can be used as an effective anticancer agent as an alternative to conventional anticancer agents. However, *in vivo* testing can be performed to the activity of the prepared chalcones for further confirmation.

REFERENCES

1. Seyed Hossein Hassanpour, Mohammadamin Dehghani. Review of cancer from perspective of molecular. Journal of Cancer Research and Practice 2017;4:127-129.
2. Firas Hassan , Ghufran Mohammed , Gamal A. El-Hiti , Ahemd Alshanon , Emad Yousif. Cytotoxic effects of tamoxifen in breast cancer cells. Cytotoxic effects of tamoxifen in breast cancer cells. J. Unexplored Med. Data 2018;3:3.
3. AthanasiosValavanidis,ThomaisVlachogianni.Stud. Nat. Prod. Chem. 2013;39:269-295.
4. Luisa Helena Cazarolli, Fátima Regina Mena Barreto Silva. Studies in Natural Products Chemistry. Volume 39, 2013, Pages 47-89 Chapter 2 - Natural and Synthetic Chalcones: Tools for the Study of Targets of Action—Insulin Secretagogue or Insulin Mimetic
5. Suvitha Syam, Siddig Ibrahim Abdelwahab, Mohammed Ali Al-Mamary and Syam Mohan. Synthesis of Chalcones with Anticancer Activities. Molecules 2012;17:6179-6195.
6. Nowakowska, Z. A review of anti-infective and anti-inflammatory chalcones. Eur. J. Med. Chem. 2007;42:125–137.
7. Go, M.L., Wu, X., Liu, X.L., Chalcones: An update on cytotoxic and chemoprotective properties. Curr. Med. Chem. 2005;12:483–499.
8. Kumar, D., Kumar, N.M., Akamatsu, K., Kusaka, E., Harada, H., Ito, T. Synthesis and biological evaluation of indolyl chalcones as antitumor agents. Bioorg. Med. Chem. Lett 2011;20:3916–3919.

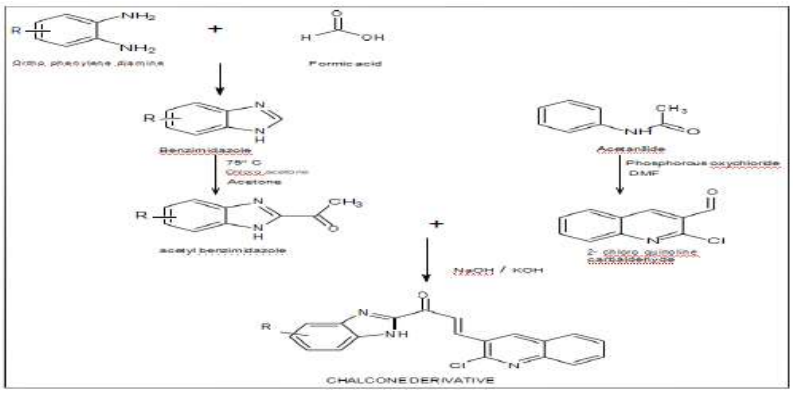
9. Sivakumar, P.M., Ganesan, S., Veluchamy, P., Doble, M. Novel chalcones and 1, 3, 5-triphenyl2-pyrazoline derivatives as antibacterial agents. Chem. Biol. Drug. Des 2010;76:407–411.
10. Sakai, T., Eskander, R.N., Guo, Y., Kim, K.J., Mefford, J., Hopkins, J., Bhatia, N.N., Zi, X., Hoang, B.H. Flavokawain B. A kava chalcone, induces apoptosis in synovial sarcoma cell lines. J. Orthop. Res 2011;30:1045–1050.
11. Nishimura, R., Tabata, K., Arakawa, M., Ito, Y., Kimura, Y., Akihisa, T., Nagai, H., Sakuma, A., Kohno, H. Suzuki, T. Isobavachalcone, a chalcone constituent of Angelica keiskei, induces apoptosis in neuroblastoma. Biol. Pharm. Bull 2007;30: 1878–1883.
12. Hijova, E. Bioavailability of chalcones. Bratisl. Lek. Listy 2006;107;80–84.
13. Zhang, H.J., Qian, Y., Zhu, D.D., Yang, X.G., Zhu, H.L. Synthesis, molecular modeling and biological evaluation of chalcone thiosemicarbazide derivatives as novel anticancer agents. Eur. J. Med. Chem 2011;46:4702–4708.
14. Atena Daneshforouz, Samad Nazemi, Omid Gholami, Marzieh Kafami & Bahareh Amin.The cytotoxicity and apoptotic effects of verbascoside on breast cancer 4T1 cell line. BMC Pharmacol. Toxicol 2021;22.
15. V.Raja, Solomonab HoyunLee. Anti-breast cancer activity of heteroaryl chalcone derivatives. Biomed. Pharmacother 2012;66(3):213-220.
16. Natalia Maciejewska, Mateusz Olszewski, Jakub Jurasz, Marcin Serocki, Maria Dzierzynska, Katarzyna Cekala, Ewa Wieczerzak & Maciej Baginski. Novel chalcone-derived pyrazoles as potential therapeutic agents for the treatment of non-small cell lung cancer. Nature 2022;12:3703.
17. Tereza Kauerova, Jiri Kos, Tomas Gonec, Josef Jampilek, and Peter Kollar. Antiproliferative and Pro-Apoptotic Effect of Novel Nitro-Substituted Hydroxynaphthanilides on Human Cancer Cell Lines. Int J Mol Sci 2016;17(8):3703.

Table 1: Yield of chalcone derivatives

S.No	Compound code	Physical apperance	% yield	Melting point	TLC solvent
1.	C1	Yellow in colour	45	226	Ethyl acetate:N-hexane (9:1)
2.	C2	Yellow in colour	60	22	Ethyl acetate:N-hexane (9:1)
4.	C4	Yellow in colour	53	224	Ethyl acetate:N-hexane (9:1)
5.	C5	Yellow in colour	51	218	Ethyl acetate:N-hexane (9:1)

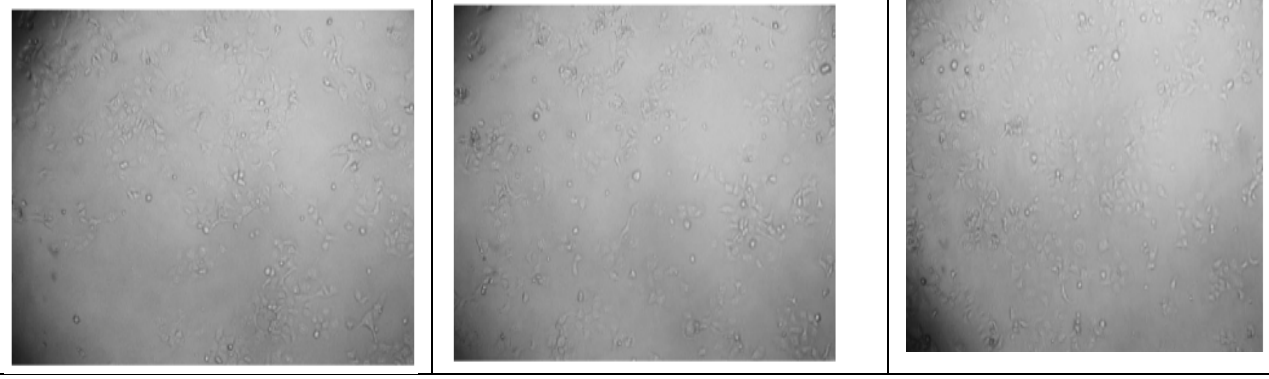
Table 2: Percentage inhibition of chalcones

S.No	Concentration(µg/ml)	Percentage inhibition
1	10	53.22
2	20	22.41
3	40	45.25
4	80	79.21
5	160	97.06
6	320	95.15

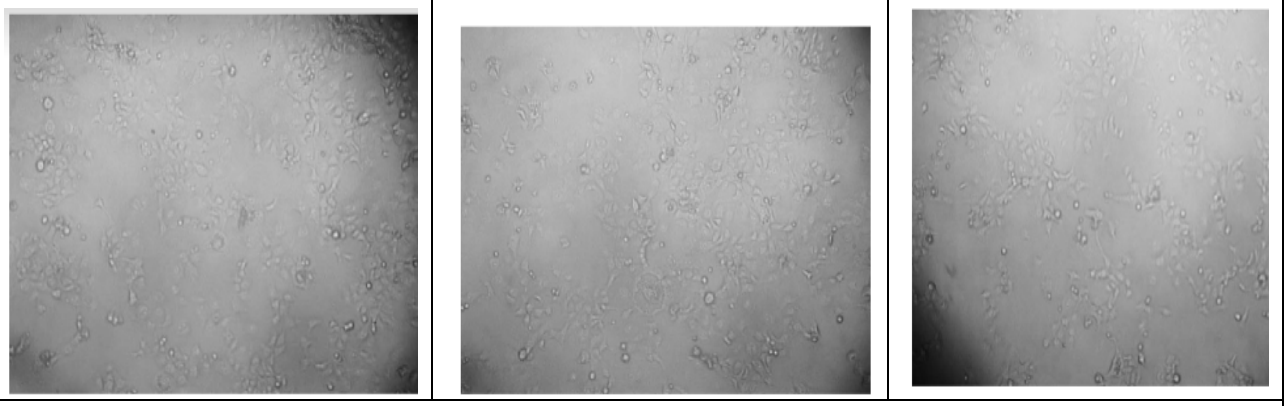


Where, R= NO_2(C1),Cl(C2),Br(C3) ,OCH3(C4) &Cl_2(C5)

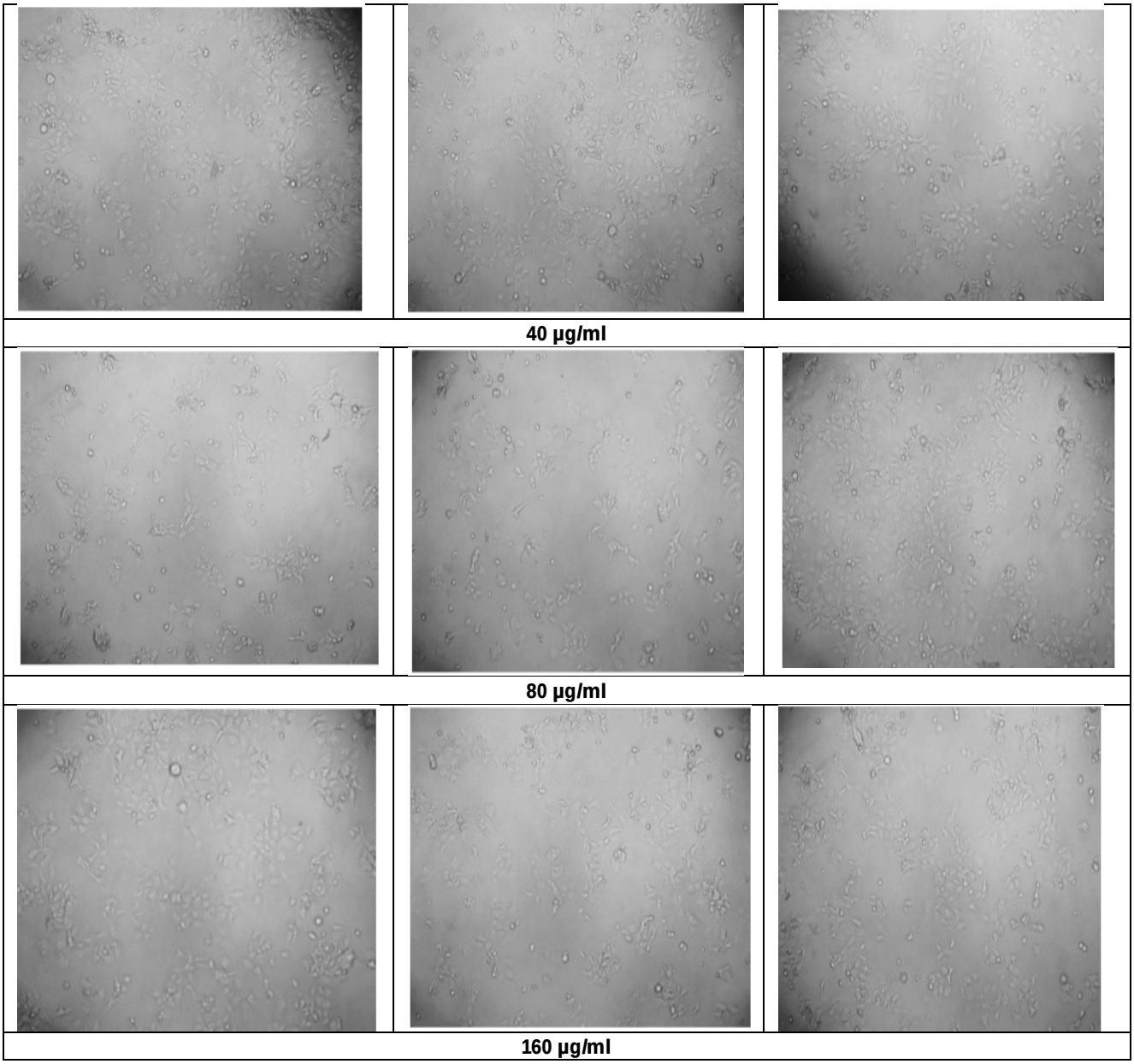
Figure 1: Synthesis of Chalcone Derivatives



10µg/ml



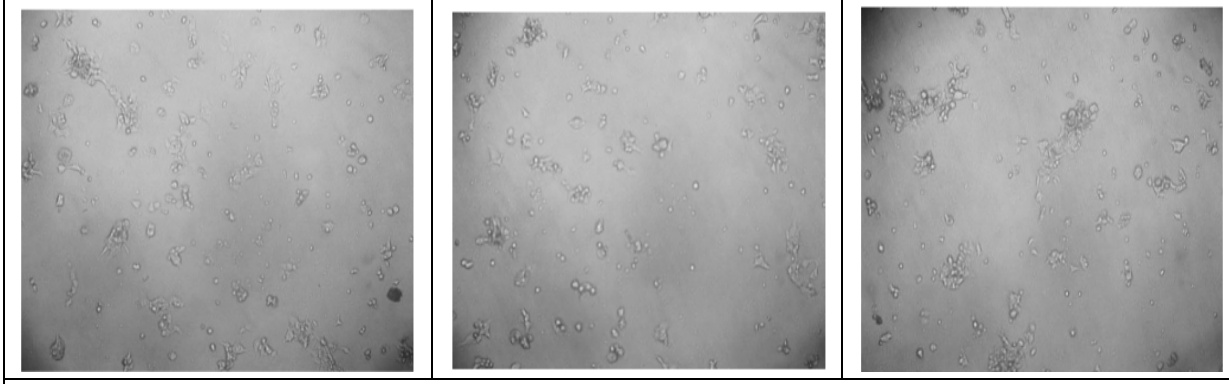
20µg/ml



40 µg/ml

80 µg/ml

160 µg/ml



320 µg/ml

Figure 2: Various concentration of chalcones in MTT assay

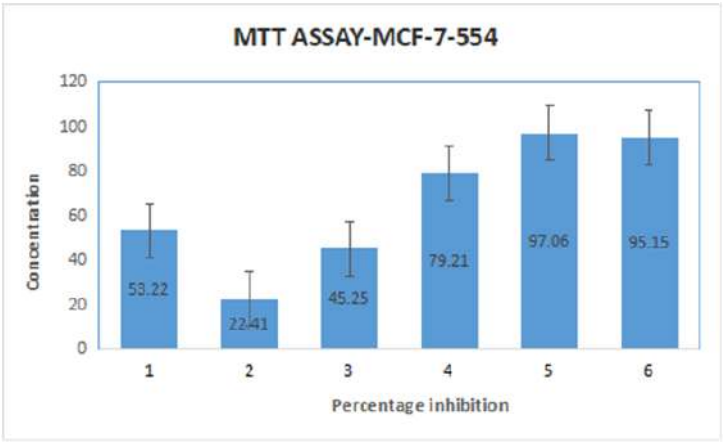


Figure 3: MTT assay of chalcones

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RESEARCH ARTICLE

Analytical Method Development and Validation of Taxol-Containing Drug by UV Spectrophotometric Method

Shreeshail Tumbagi[1]*, Bharath Srinivasan[2] and Pushpendu Gaurav[3]

[1]Research Scholar, Faculty of Pharmacy, M.S. Ramaiah University of Applied Sciences, Bengaluru, Karnataka, India
[2]Dean, Faculty of Pharmacy, M.S. Ramaiah University of Applied Sciences, Bengaluru, Karnataka, India
[3]Assistant Manager, Karnataka Antibiotics and Pharmaceuticals Limited, Bengaluru, Karnataka, India

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***Address for Correspondence**
Shreeshail Tumbagi
Research Scholar,
Faculty of Pharmacy,
M.S. Ramaiah University of Applied Sciences,
Bengaluru, Karnataka, India
Email: tumbagishreeshail@gmail.com

ABSTRACT

The present research aimed to develop a simple, precise, rapid and accurate UV spectrophotometric method for paclitaxel in bulk and marketed formulation. Various validation parameters have been carried out as per ICH Q2(R1) guidelines**.** Paclitaxel dissolved in methanol and PBS 7.4 (1:1) ratio showed the UV spectrum for maximum absorbance at 230 nm. Linearity for the concentration range 2.0 – 15.0 µg/ml showed a linear curve with a correlation coefficient value of R^2 0.999. Repeatability and intermediate precision results for % RSD showed that the method is precise for any concentration at different environmental conditions. The accuracy of the drug for % recovery studies was 99.13% compared to a standard limit of 98.0 – 100.0%, indicating that the method is accurate and reproducible.

Keywords: Paclitaxel, UV spectrophotometry, validation.

INTRODUCTION

Paclitaxel is a semi-synthetic drug belonging to the Taxaceae family, isolated from the bark of Pacific yew, *Taxus brevifolia*. It was discovered in early 1962 [1]. Paclitaxel is on the WHO list of essential medicines and is considered the most important medication needed in a basic health system [2]. It is also known by its original brand name Taxol. Paclitaxel, a taxane class of new anticancer agents, exert their cytotoxic effects through a unique mechanism[3,4]. Paclitaxel is chemically (2*AR* (2a alpha,4 beta,4a beta,6 beta,9 alpha (11 alpha,12 alpha,12b alpha))-beta-(benzoyl amino)-alpha-hydroxybenzene-propanoic acid 6,12b-bis(acetyloxy)-12-(benzyloxy) 2a,3,4,4a,5,6,9,10,11,12,112a,12b-dodecahydro-4,11dihydroxy-4a,8, 13, 13-tetramethyl-5-oxo-7, 11-methano-1H-cyclodeca (*3, 4*)benz (*1, 2-g*) oxet-9-yl

ester, an antineoplastic agent used in the treatment of breast, colon, head cancer and non-small cell lung cancer. The empirical molecular formula for paclitaxel is $C_{47}H_{51}NO_{14}$, with a molecular weight of 853Da. It is insoluble in water, very lipophilic, and easily soluble in methanol and ethanol. The standard melting point of the paclitaxel was reported to be 216-217°C.Several research articles have been published to determine paclitaxel in biological fluids using modern analytical techniques, including capillary electrophoresis [5], Liquid chromatography-Mass spectrometry [6] and HPLC [7-14]. Still, no reports have been reported for determining paclitaxel by a suitable UV spectrophotometric method and validation. Hence the present work is proposed for developing a simple, precise, suitable UV spectrophotometric method and its validation.

MATERIALS AND METHODOLOGY

MATERIALS

Paclitaxel standard bulk drug was procured from Shilpa pharmaceuticals Ltd, Raichur, Karnataka, India. Chemical like disodium hydrogen phosphate & potassium dihydrogen phosphate & sodium chloride was purchased from Rankem. The formulation was purchased locallyand manufactured by Cipla Limited, India. Methanol, and distilled water used for the work were of AR grade and purchased from Karnataka fine chemicals, Bengaluru, India.

Preparation of standard solution

Weigh about 10mg of paclitaxel accurately and transfer into a 10 ml volumetric flask. Dissolve the drug with methanol: PBS 7.4 (1:1) ratio to get a concentration of 1000 µg/ml as stock-I. The solution is now sonicated for about 5 min. From the above stock, I pipette out 1 ml and diluted it with 10 ml of methanol: PBS (1:1) to get a concentration of 100 µg/ml as stock-II. From the stock-II pipette, out 1 ml and make the final dilution to 10 ml with the methanol: PBS (1:1) to get a 10 µg/ml concentration.

Preparation of sample solution

Weight equivalent to 10mg of paclitaxel marketed powder accurately and transfer into a 10 ml volumetric flask. Dissolve the drug with methanol: PBS 7.4 (1:1) ratio to get a concentration of 1000 µg/ml as stock-I. The solution is now sonicated for about 5 min. From the above stock, I pipette out 1 ml and dilutedit with 10 ml of methanol: PBS 7.4 (1:1) to get a concentration of 100 µg/ml as stock-II. From the stock-II pipette, out 1 ml and make the final dilution to 10 ml with the methanol: PBS 7.4(1:1) to get a 10 µg/ml concentration.

Determination of λ_{max}

The standard stock solution of paclitaxel was scanned for UV spectrum from 400-200 nm.

Validation of the UV Method

As per guidelines ICH, Q2(R1), the method developed, is validated for determining Linearity, Range, Accuracy, Precision, LOD, LOQ and Specificity.

Linearity

The standard stock solution of 100 µg/ml of Paclitaxel, a series of dilutions were prepared for the concentrations of 2.0, 4.0, 6.0, 8.0, 10.0&15.0µg/mL, and the absorbance was measured at 230 nm. Linearity is determined for the above concentrations by plotting a calibration curve for paclitaxel.

Range

Each lower and higher concentration sample was performed in six duplicates, i.e. at 2.0 µg/mL and 15.0 µg/mL, respectively and absorbance was checked at 230nm. In addition, the mean and relative standard deviation were calculated.

LOD and LOQ

The LOD (Limit of Detection) and LOQ (Limit of Quantification) of paclitaxel were calculated using the equation LOD=3.3 σ/s and LOQ=10 σ/s, where σ is the standard deviation of 'y' intercept of the calibration curve (n=6), and s is the slope of the regression coefficient.

Precision

Precision for paclitaxel was carried out with repeatability and intermediate precision.

Repeatability

Six Sample preparation were analysed as per methodology, and % assay was relative to % RSD, and a 95% confidence interval of the results were calculated.

Intermediate Precision

The experiment was carried out similarly under repeatability, with the typical variations of days, analysts& instruments.

Accuracy

Recovery solutions were prepared to obtain the solutions by covering 80% of the lowest and 120% of the highest test concentration of the sample. Therefore, we prepared the recovery solutions at all three concentration levels in triplicate.

RESULTS AND DISCUSSION

Determination of λ_{max}

The standard stock solution of paclitaxel was scanned for UV spectrum from 400-200 nm, which covers the whole UV Spectrum reason. As a result, the highest absorption good range was obtained at a maximum wavelength of 230 nm, as shown in Fig. 2. A comparison was also made to check the market sample and standard stock solution absorption, shown in Fig. 3.

Linearity

A series of solutions were prepared by quantitative dilutions of the primary drug standard stock solution to obtain concentration at 20% to 150% of the working concentration sample. Then, each solution was checked with the absorbance, and the absorbance area was recorded as slope, Y-intercept and regression correlation. The value of concentration & absorbance area are presented below in table No.1. A graph of peak area v/s concentration (µg/ml) was plotted. The results showed linearity in the graph plot with an R^2 Value of 0.999.

Range

The mean and relative standard deviation were recorded at lower & higher concentration levels, i.e. at 2.0µg/ml and 15.0µg/ml, over which the results are linear and have shown reproducible results. The %RSD of 2µg/ml and 15µg/ml are 0.20 & 0.05 respectively.

Precision

Precision for paclitaxel market formulation was carried out with repeatability and intermediate precision. In repeatability & intermediate precision, absorbance for the sample is checked and reproducible. The same is seen in Table No. 3.

Accuracy

The analytical recovery method is employed by the standard addition method at 80, 100, and 150% levels to check the % recovery of the developed method and the interference of formulation excipients. First, the total amount of the

drug taken and the percentage recovery of the drug is calculated. The results were shown for no interference of excipients, and data are given below in Table No. 4.

Specificity

The obtained result concluded that no interference was observed due to the blank solution having the same absorbance as in the standard and sample solutions. The same is presented in Table No. 5.

LOD and LOQ

The LOD (Limit of Detection) and LOQ (Limit of Quantification) of paclitaxel were calculated using the equation LOD=3.3 σ/s and LOQ=10 σ/s, where σ is the standard deviation of 'y' intercept of the calibration curve (n=6), and s is the slope of the regression coefficient. The results are shown below in table No. 6.

CONCLUSION

The above-proposed paclitaxel method was simple, precise, accurate, and obeyed beer law within its concentration range of 2.0 – 15.0µg/ml. The results for Linearity calibrated in the concentration range of 2.0 – 15.0µg/ml showed a linear curve with the correlation coefficient R^2 value of 0.999. The precision carried out for inter day and intraday proved that the method is precise for developing a technique in different environmental conditions. The results for accuracy to determine the percentage recovery by the standard addition method of 80,100 &150% showed its accuracy for the limit 98-100%. The optimized parameters for beer law, LOD and LOQ, accuracy, specificity and precision A straightforward, exacting, and accurate method was devised to make the analytical approach acceptable and cost-effective with quality characteristics for routine laboratory analysis. The developed method has also been validated in accordance with ICH Q2 (R1) criteria.

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CONFLICT OF INTEREST

We have no Conflict of Interest

REFERENCES

1. The American Society of Health-System Pharmacists. Retrieved Jan 2, 2015.
2. WHO model list of essential medicines. World Health Organization. October 2013. Retrieved 22 April 2014
3. Liebmann, J.A. Cook, C. Lipschultz, D. Teagne, J.B. Mitchell, Cancer Chemother. Pharmacol. 33 (1994) 331–336
4. J. Vhweij, M. Clavel, B. Chevalier, Am. Oncol. 5 (1994) 495–498. J.
5. Hempel .G, Doris Lehmkuhl, Sebastian Krumpelmann, Gottfried Blaschke, Jochim Boss. Determination of paclitaxel in biological fluids by micellar electrokinetic chromatography. Journal of chromatography. A 745, 1996, 173-179.
6. Baldrey E.H, Bodric R.R, Morris G.R, Jenkins E.H, Brookes S.T. Comparision of LC-UV and LC-MS/MS for the determination of Taxol. Chromatographia supplement. Vol 55, 2002, 187-192.
7. Seshagiri Rao J.V.L.N, Mastanamma .SK, Prahlad .P, Anantha Kumar. A validated RP-HPLC method for determination of docetaxel in injectable dosage forms. Journal of research in pharmaceutical and biomedical sciences, Vol 2 (1), 2011, 182-186.

8. Sathyamoorty .N, Vijayalakshmi Rajendran, Naveena .V.S.H, Dasartha Dhanaraju .M. An approach for validated RP-HPLC method for the analysis of paclitaxel in rat plasma. Journal of applied pharmaceutical science. Vol 4 (9), 2014, 73-76.
9. Badea I, Dana Ciutaru, Lelia lazar, Daniela Nicolescu, AurelTudose. Rapid HPLC method for the determination of paclitaxel in pharmaceutical forms without separation. Journal of Pharmaceutical and biomedical analysis. Vol 34, 2004, 501-507.
10. Sanjay Jain .K, Ankit Jain, Arvind Gulbake, Ashish Jain, Pooja Hurkat, Sushil Kashaw et al., Development and validation of HPLC method for simultaneous estimation of paclitaxel and topotecan. Journal of Chromatographic Science. 2013, 1-7.
11. Sparreboom Alex, Peter de Bruijn, KeesNooter, Walter J.loos, Gerritstoter, JaapVerweij. Determination of paclitaxel in human plasma using single solvent extraction prior to isocratic RP-HPLC chromatography with ultra violet detection. Journal of Chromatography. B 705, 1998, 159-164.
12. Yoo Sun .D, Sang Heon Lee, Kyu Hyun Lee. Rapid and sensitive determination of paclitaxel in mouse plasma by HPLC. Journal of Chromatography. B 724, 1999, 357-363.
13. Jin Sun, Tianhongzhang, Helian. A rapid and sensitive determination of paclitaxel in rat lasma by UPLC-MS/MS method: Application to pharmacokinetic study. Journal of Pharmaceutical Sciences. 8, 2013, 199-205.
14. Jaynish Tailor, AnkurRaval, Cchaya Engineer, HareshKotadia, DeveshKotwala. Development and validation of RP-HPLC method for invitro loading and release analysis of paclitaxel coated steat. Trends Biomater of Artificial Organs. Vol 22 (2), 2008, 79-86.

Table. 1: Linearity results for paclitaxel

S.No.	Concentration (µg/ml)	Absorbance at 230 nm
1.	2.0	0.0585
2.	4.0	0.1185
3.	6.0	0.1777
4.	8.0	0.2287
5.	10.0	0.2858
6.	15.0	0.4235
Y-INTERCEPT		0.0285
Correlation Coefficient		0.999

Table. 2: Range Result of Paclitaxel

Sample No.	Absorbance	
	2.0 µg/ml	15.0µg/ml
1.	0.0582	0.4232
2.	0.0581	0.4233
3.	0.0584	0.4231
4.	0.0582	0.4235
5.	0.0583	0.4229
6.	0.0581	0.4231
Standard Deviation	0.0001	0.0002
Mean	0.0582	0.4232
% RSD	0.20	0.05

Table. 3: Precision results for paclitaxel market formulation

S.No.	Drug	Concentration(µg/ml)	% RSD for repeatability	% RSD for intermediate precision	Absolute Difference
1.	Paclitaxel	10 µg/mL	0.51	0.12	0.39

Table. 4 Accuracy results for paclitaxel

S.No.	Drug	% Amount added	Label claim (mg)	% Recovery	% Mean Recovery
1.	Paclitaxel	80	30	99.3	99.13
		100		98.3	
		150		99.8	

Table. 5: Specificity results concluding no interference of Placebo& Blank

Name of Solution	Absorbance
Blank	No interference
Placebo	No interference
Standard Solution	0.2890
Sample Solution	0.2895

Table. 6: Optimized, validated parameters for paclitaxel

S.No	Parameter	Results
1.	Beers range	2.0 – 15.0µg/ml
2.	Correlation coefficient R^2	0.999
3.	% RSD for range level 1(µg/ml)	0.20
	% RSD for range level 2 (µg/ml)	0.05
4.	% mean recovery	99.13 %
5.	LOD (Limit of Detection)	0.296 µg/ml
6.	LOQ (Limit of Quantification)	0.896 µg/ml
7.	Specificity	No interference due to blank and placebo
8.	% RSD for repeatability	0.51
9.	% RSD for intermediate precision	0.11

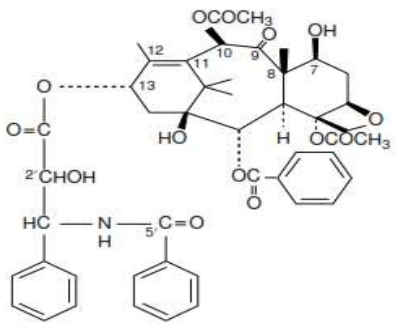

Fig. 1 Structure of paclitaxel

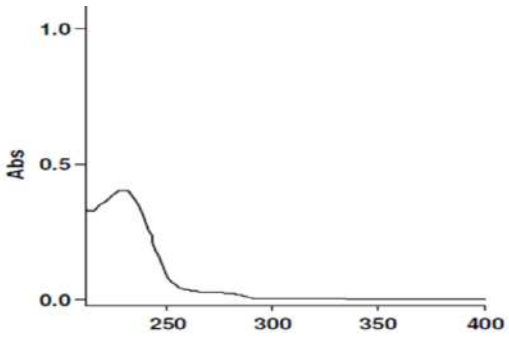

Fig. 2: UV spectrum of paclitaxel showing maximum absorbance at 230 nm

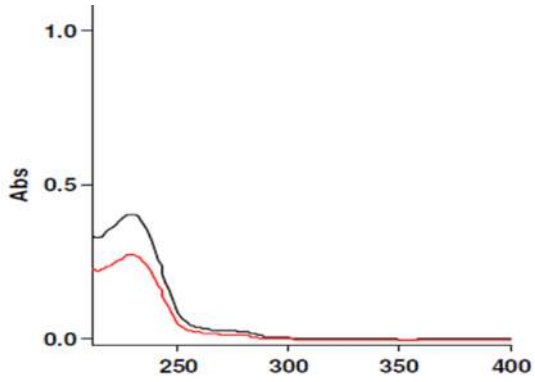

Fig. 3: UV spectrum of paclitaxel and sample showing maximum absorbance at 230 nm

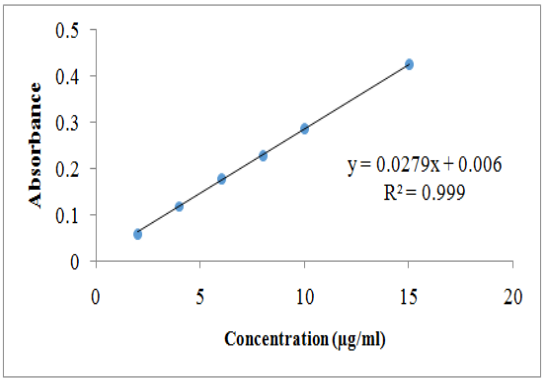

Fig. 4: Calibration curve for paclitaxel

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REVIEW ARTICLE

Plant Derived Essential Oils as Promising Antimicrobial Agents against Food Borne Pathogens

Surjya Loying[1], Rajeev Sarmah[2], Devabrata Saikia[1], Deep Prakash Parasar[1], Manash Pratim Kashyap[3] and Manash Pratim Sarma[3]*

[1]Assistant Professor, Assam down town University, Panikhaiti , Guwahati, Assam, India.
[2]Professor, Assam down town University, Panikhaiti, Guwahati, Assam, India.
[3]Associate Professor, Assam down town University, Panikhaiti, Guwahati, Assam, India.

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***Address for Correspondence**
Manash Pratim Sarma,
Associate Professor,
Assam down town University,
Panikhaiti, Guwahati, Assam, India.
Email: manash3268@gmail.com

ABSTRACT

For centuries, plants and their derivatives have been utilized in multifarious grounds, from food preservation to cosmetics production including folk medicine. Plant essential oils encompass a myriad of secondary metabolites that could decelerate or hinder the growth of several bacterial, fungal, and viral pathogens, thus have significant prospective in the sphere of biomedicine. On the other hand, pathogenic bacteria of food origin have been considered as the primary causes of food-borne diseases in both developed and developing countries. In this aspect, essential oil can be the alternative to these conventional antimicrobials as they can effectively destroy diverse pathogens because of the presence of group of *phenolics*, *terpenes*, *aldehydes*, as well as other antimicrobial compounds to the combat escalating resistance of microorganisms to the conventional antimicrobials. Thus, this review summarizes the antimicrobial and antibiofilm prospects of different plant essential oils including their mechanism of action against food borne pathogens.

Keywords: Essential oils, antimicrobial, antibiofilm, food borne pathogens, antimicrobial resistance

INTRODUCTION

Food borne pathogens have been considered as the primary causes of food-borne diseases in both developed and developing countries [62]. An estimated 100 million infection of food origin and 120,000 food borne disease-related deaths occur each year in India[31]. The discovery of antibiotics that changed medicine in the 20th century is now seems to be becoming ineffective day by day due to the indiscriminate and injudicious use of these antimicrobials

[13]. The development of antimicrobial drug resistance by different microorganisms such as fungi, bacteria, parasites, viruses etc. has become serious threat to the arena of therapeutic sector in managing the ailments caused by them, which prompted the researchers to find novel molecules as an alternative to the conventional antimicrobials. Plants have been used by humankind as fuel, clothing, shelter, and food even before the prehistoric ages. With the advent of civilization, human being started to explore the plants and thus medicinal plants as traditional medicine [26] **.**Even today, plant and its extracts are considered as a vital resource in diverse arena such as the pharmaceutical, culinary, cosmetic industries [36]. Globally, more than 60% population prefer traditional medicine as the first line of health care system; and it is around 80% from developing countries dependent on plants as medicine for various ailment [5,52] . Till now, over 9000 plants have been recognized for their therapeutic values [55] and 3000 essential oils have been identified [12]. Besides the wide-spectrum antimicrobial properties of the active compounds extracted from plant essential oils, another important attribute for which they are gaining increasing attention is that they are considered as safe to use [6,44,45] . In the last decade, researchers have discovered the chemical compounds present in the essential oil and their antimicrobial activities [6,15]. Also, due to the drug resistance, toxicity, carcinogenic effects and environmental hazard potential, the application of simulated chemical antimicrobials for the handling of pathogenic microorganisms is restricted. In this milieu, the utilization of plant essential oils to manage emerging multiple drug resistant pathogens can be beneficial to tackle several contagious diseases [64]. Hence, this review narrates the antimicrobial prospective of selective plant based essential oils along with their probable mechanisms entailed in the inhibition of food borne disease causing microorganisms.

Essential Oil-What it is?

Plant essential oils are complex compounds, which are inherently synthesized in various parts of plants during secondary metabolism. These are volatile in their nature. It has been reported that more than 17,500 species of plants that produce essential oil belong to several angiosperm families e.g., *Rutaceae*, *Lamiaceae*, *Zingiberaceae*, *Asteraceae* and *Myrtaceae* but merely, around 300 of them are commodified [35]. Due to the existence of native substances produced by distinct parts of the plant, essential oils have the potential to limit the growth of a wide range of pathogenic organisms. The aromatic nature of essential oil of plants is ascribed to the existence of heterogenous chemical compounds pertaining to various chemical groups together with aldehydes, *phenolic, terpenes, alcohols, ketones, ethers* etc [63]. Chemical interpretation of plant essential oils portrays the existence of merely two to three prime constituents at the peak concentration (approximately twenty to seventy percent) in comparison to the other constituents which are in minute quantities. Amidst those components, *terpenoids*; terpenes such as *p*-cymene, limonene, mycene, *terpinene, pipene*; and aromatic phenols such as *eugenol, safrole*, as well as other varied chemical components of low molecular weight are found to have played the key part in make-up of different plant essential oils. In addition, fatty acids, derivatives of sulphur and oxides may also be existed in them [30, 63].

These multifarious chemicals of essential oils are synthesized in plant cell's plastids and cytoplasm along different avenues such as mevalonic acid, malonic acid. These compounds are secreted and deposited in intricate structures like glands, resin conduits, cavities. Further, they are present in various parts (such as stems, roots, leaves, barks) of plants as fluid drops [56]. The chemical formula (C_5H_8) n represents the compound terpene. The major components of terpenes are isoprene units of mono, bi, tri or acyclic. Moreover, terpenes are structurally heterogeneousness, so they are categorized into varied groups such as mono, di, and tri terpenes. The monoterpenes are the major components of plant-based essential oil (around 90%) [14]. Various intrinsic and extrinsic aspects affecting these chemical elements include reciprocation with the plant environs like soil type, climate, maturity of the concerned plant, harvesting time during the day, extraction method of essential oil, geographical location etc [17]. Different pathogenic microorganisms show different antimicrobial activities due to their varied chemical compositions [37]. The antimicrobial properties of plant-based oils can differ in one way due to their varied chemical composition and on the other way the method of extraction and the chemicals used in the process [23].

Antimicrobial Effects of Essential Oil

Over the decade, scientists have studied the characteristics of different essential oils on antimicrobial properties. Till date, most essential oils contemplated include thyme, lavender, peppermint, eucalyptus, and cinnamon bark

essential oils [64]. Research on the antibacterial properties of various plant essential oils and their modes of action have been extensively performed; however, there is little detailed information on the activities against yeasts and molds or fungi [44]. The injudicious use of conventional antibiotics for human healthcare has led to the emergence of multidrug resistance [65] and due to the dearth of novel effective antimicrobials the situation is further aggravating [28]. This emergence of resistance mechanism in these microbial strains contributes to the ineffectiveness of currently used antimicrobials, perpetuate illness, and increase economic load [62]. Furthermore, the ability of bacteria to develop biofilm has further augmented the number of deadly microbial infections in humans as microbes residing in biofilm are irrepressible to the defense system and antibiotics [61]. Thus, arise of multiple drug resistant bacteria delineate a worldwide challenge owing to the lack of new antibacterial agents.

In this respect, plant essential oil can be the substitute for the existing drugs because of its useful antibacterial and antibiofilm characteristics. From bacteria to bacteria, the antibacterial properties of essential oil varied because of their diverse chemical structures. In comparison to gram positive and negative, the susceptibility of essential oil is more to gram positive bacteria [55,44]. It is because of the extra thick lipopolysaccharide (LPS) layer in cell wall which curbs the influx of essential oil into the inner cellular milieu.[58] The effect of antibacterial activity may be bacteriostatic (that prevent the bacterial growth) or bactericidal (that kills the bacteria). Because of the difficulty in distinguishing these two actions (bacteriostatic or bactericidal), antibacterial activity is often estimates as the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). The rapid antibacterial activity testis performed often by following the agar diffusion method. In this method, researcher put essential oils in filter paper or holes in agar that have been inoculated with bacterial strains. The antibacterial action is measured by the inhibition zone formed after the incubation [39]. Majority of the plant used in medicine or pharmaceutical, food and cosmetic industries belong to Lamiaceae family [56]. and essential oil extracted from *Mentha spicata* showed bioactive potential against commonly found food borne pathogenic bacteria such as *Bacillus subtilis, Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus, Escherichia coli* O157:H7 and *Salmonella typhimurium* [51]. The gram-positive bacteria are found more susceptible to *M. spicata* essential oil. Among the tested bacterial organisms, *L. monocytogenes* found the highest sensitive towards the *M. spicata* oil. The MIC and MBC were 2.5µL/mL and zone of inhibition was 22mm.

The antibacterial effect of this oil was correlated with the presence of the principal compounds like carvone (78.76%), limonene (11.50%) and β-bourbonene (11.23%) [51]. Antibacterial effect of another species of Mentha, *M. piperita* essential oil was reported upon *L. monocytogens* and *S. typhimurium.* The later was reported as more sensitive than the other. The antagonistic properties of 12 varied essential oils (thyme, eucalyptus, sage, pine, tea, orange, lavender, lemon, rosemary, juniper, laurel, and myrtle) were reported against various pathogens of food origin namely, *S. aureus, E. faecalis, S. paratyphi A, E. coli, K. pneumoniae, C. jejuni, Y. enterocolitica, A. hydrophila, P. aeruginosa.* According to them, though the antibacterial effects of these essential oils differ depending upon the chemical constituents and particular bacterial strains evaluated, most of these essential oils displayed antibiotic action upon one or more than one bacterial strain. Their research revealed that, pine and thyme essential oils are tremendously effective upon the pathogens of food origin. The inhibitory activity was correlated with the chemical compounds, such as carvacrol, limonene, linalool, monoterpene hydrocarbons, monoterpene phenol, oxygenated monoterpenes, α-pinene, eucalyptol ,linalyl acetate, camphor, and 1,8-cineole. [40] The antibacterial property of nano emulsion based upon thyme essential oil on diverse bacteria*(S. aureus, S. paratyphi A, K. pneumoniae,* and *E. faecalis*) and fish spoilage bacteria (*P. luteola, E. faecalis, P. damselae, S. liquefaciens, V. vulnificus* and *P. mirabilis*).

It was observed that non purified nano-emulsions had added effectiveness on pathogenic bacteria [41]. The antibiotic potential of thyme, basil, parsley and lovage essential oil were compared both individually and in combination of these (1:1, v/v), against gram positive bacteria such as *B. cereus, S. aureus, P. aeruginosa* and gram-negative bacteria such as *E. coli* and *Salmonella typhimurium*. It was reported that thyme essential oil has the high antibacterial potential among all estimated formulations. The formulation with combinations of essential oils showed a decline trend in antibacterial properties. [50] The antibacterial properties of thyme (*Thymus vulgaris*), Mexican oregano (*Lippia berlandieri*) and mustard (*Brassica nigra*) were studied singly and in duplex against *S. aureus,*

S. Enteritidis as well as *L monocytogenes* and identified allyl isothiocyanate to be the prime component for which mustard essential oil was proved to be the most active. In combination with thyme or *Mexican oregano* essential oil the mustard essential oil showed synergistic effects. Furthermore, an add-on effect was observed by mixing Mexican oregano or thyme essential oils [48]. The antimicrobial properties of a vast range of essential oils from multiple plant genera were observed upon the pathogenic bacteria of food origin *S. aureus* and *E. coli* and concluded that, though thyme and oregano were the most active, oils from *Azadirachta indica* and *Litsea cubeba* had the potential to be the promising candidates as antimicrobial agent [57]. The antibacterial action of lavender essential oil upon the bacterial strains *Pseudomonas aeruginosa*, *Salmonella enterica*, *Yersinia enterocolitica, Enterococcus faecalis*, *Staphylococcus aureus*, *Bacillus subtilis* showed linalool (32.7%), 1,8-cineole (8.1%) and linalool acetate (35.0%) as the primary components for the affectivity [60]. In latest research on the tea tree and rosemary oil showed effective antibiofilm activity to *S. aureus* and *E. coli*. It was reported that the content of Eucalyptol and α-pinene were high in rosemary essential oil whereas; 4-terpineol and terpinolene were major compounds in tea tree essential oil responsible for this inhibitory effect [33].

The studies on the inhibitory and antibiofilm properties of thyme essential oil upon the antibiotic resistant food borne *E. faecalis*. The major component was found to be thymol (70%). The study concluded that essential oil of thyme is effective antibiofilm or bactericidal agent for handling *E. faecalis* biofilms [32]. Another study also reported the antibacterial as well as antibiofilm potential of tea tree, thyme, lavender, eucalyptus, rosemary, mint, and basil oils against 16 biofilm forming strains of staphylococci. Among them, thyme essential oils displayed the highest antibiofilm properties against staphylococcal strains [11]. The investigation on the antibacterial as well as antibiofilm activities of clove oil against *Salmonella intrepidities* and *Listeria monocytogens*. The investigation found out that at the minimum inhibitory concentration and within 1 hour incubation with clove oil, the biofilms were decreased to 20.3% and 32.2% for *S. typhimurium* and *L. monocytogens,* respectively. The inhibitory effect of clove essential oil was attributed to the principal bioactive component eugenol (78.85%). The results showed that essential oil of clove can be a potent bactericidal and bacteriostatic agent to control biofilm formation in food related environments [54]. Another assay of biofilm inhibition and deactivation were evaluated and observed remarkable reductions of *S. Heidelberg* in 0.5% concentration of lemongrass essential oil and total inhibition of biofilm formation, motility, already molded biofilm in concentration of 0.15%. Thus, the study revealed the potentiality of lemongrass essential oil as antibacterial and antibiofilm agent in controlling the food borne pathogen [16]. The fungal genera such as, *Penicillium*, *Alternaria*, *Fusarium*, *Aspergillus* produce secondary metabolites called mycotoxins. The Food and Agricultural Organization (FAO), United Nations recognized fungi along its toxic metabolites as one of the chief reasons of global loss of agricultural foodstuffs such as cereals, nuts and rice [7]. Despite the facts that fungal growth and its toxic metabolites is potent agent of causing adverse health hazard, studies on antifungal properties of plant essential oil are very finite in comparison to the studies on their antibacterial potentiality [38].

Mechanism antimicrobial activities of plant based essential oils

Even though the antimicrobial activities of plant based essential oils have been studied extensively in the last decades, the detailed information on their chemical constituents along with the mechanism of action is still lacking. Notably, plants synthesize a broad array of auxiliary metabolites together with the primary metabolites including essential oils, primarily as e defense weapon against various microbial pathogens. Depending upon the chemical elements present in essential oils, the antimicrobial action is facilitated by a sequence of biochemical events in the microbial cells. Primarily, essential oil weakens the cellular organization and integrity of the cellular membrane, thus increases membrane porosity. This eventually disturbs activities of the cell like membrane transport, energy production and other functions related to metabolism. Furthermore, cellular organization of gram positive and gram-negative bacteria varies which affect the mechanism [44,55]. The essential oils of plants are hydrophobic in nature. For this property essential oil can move easily through the lipid layer of bacterial membranes. Thus, it disrupts the structure of the cell membrane which results in increasing permeability which leads to cell death.[6] There is a practice of cell-to-cell communication among bacteria known as quorum sensing, is one of the mechanisms by which bacteria show resistance to antimicrobials and form biofilms.

So, inhibition of bacterial quorum sensing is another way of inhibition of bacterial growth kinetic and resistance mechanism. The effect of various plant-based oils and their compounds on the reticence of bacterial biofilm formation that generates significant disintegration of their signaling communications was observed [10]. The studies also reported that essential oil of oregano increases membrane permeability and disrupt cell membrane integrity thereby interfere the ETS (electron transport system). This leads to the breakdown of proton pump exhausting ATP during its biosynthesis [10]. Another evaluation found that treatment of limonene (a monoterpenoid compound found in the essential oils of plants) upon the food originated pathogen *L. monocytogen*. They reported that limonene could deplete ATP concentration, performance of ATPase (Na^+K^+-ATPase, Ca^{2+}ATP-ase) the complexes of the respiratory chain. In the investigation, they showed that at concentration of 20ml/L of limonene, the cellular protein and nucleic acid content decline because of the disorientation of the cellular membrane accelerating outflow of primary cytoplasmic contents [25]. Although the numerous scientific reports published so far interpreting essential oil, definite mechanism regarding site of action has not yet been explicated [2]. Among various actions upon fungal pathogens of plant essential oils, one is affecting the synthesis of ergosterol in the plasma membrane of fungal cell [18,53]. The studies reported the inhibition of ergosterol synthesis in fungal cells by essential oil of *Cinnamomum cassia* and *M. cardiaca*.

The essential oils of these plants cause a sharp decline in ergosterol content enhancing the leakage of ions, like Ca^{2+}, K^+ and Mg^{2+} from the targeted cells and thus interpreted the plasma membrane of fungal cells as the locus of action of these oils. Besides the effect of essential oil on membrane ergosterol, effect on efflux of cellular ions is one of the fundamental modes of antifungal action. The cytoplasmic imbalance caused by efflux of crucial ions, like Ca^{2+}, K^+, and Mg^{2+}leads into metabolic disturbance and leading to cell death.[18,53] It is also depicted cell membrane to be the plausible locus of inhibition of toxic mode of fungal activity. They reported that *Cinnamomum cassia* essential oil significantly inhibited the biosynthesis of ergosterols well as disrupt cell membrane fluidity. Findings revealed notable enhancement of efflux of cellular ions in response to the increase in concentration of *C. cassia* essential oil. Another vital way of restraining fungal activity by plant essential oil is breakdown of the mitochondrial membranes. The modification of electron flow inward of electron transport system route is attributed to the disintegration of the mitochondrial membrane which can impair lipids and proteins components, and nucleic acid contents of the affected cells [53].

CONCLUSION

Worldwide, the emergence of multidrug resistance in the post antibiotic era is one of the primary health problems. Efforts are being made relentlessly to create novel and more effective antimicrobials diminish the intensity of multidrug resistance. Furthermore, there is an ever-increasing affinity of humankind towards natural antimicrobials compared with synthetic or chemical agents due to toxicity and potential side effects. Because of their myriad of biological features including antimicrobial potentiality, plant essential oils have been regarded as the focal point of further research as a substitute of currently used antimicrobials. Thus, plant essential oils might play a crucial role in developing innovative and improved antimicrobials combating food borne pathogens in the upcoming era. However, so far, the studies on plant essential oil pertaining to its antimicrobial potentiality have been unable to furnish adequate knowledge on their mechanism of action. The perception of antimicrobial functions of plant-based oils is pivotal in further approach of these essential oils in clinical practice.

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Conflict of interest

None

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Ethics statement
Ethical committee of Assam down town university, India, approved this study.

REFERENCES

1. Abdullah, Asghar, A., Algburi, A., Huang, Q., Ahmad, T., Zhong, H., Javed, H.U., Ermakov, A.M. & Chikindas, M.L. (2021). Anti-biofilm potential of elletaria cardamomum essential oil against Escherichia coli o157:h7 and *Salmonella Typhimurium* jsg 1748. Front. Microbiol.12: 620227.
2. Arasu, M. V., Viayaraghavan, P., Ilavenil, S., Al-Dhabi, N. A., & Choi, K. C. (2019). Essential oil of four medicinal plants and protective properties in plum fruits against the spoilage bacteria and fungi. Indus. Crops Products.133: 54–62.
3. Badr, M. M., Badawy, M. E.I. & Taktak N. E.M. (2021). Characterization, antimicrobial activity, and antioxidant activity of the nano-emulsions of Lavandula spica essential oil and its main monoterpenes. Journal of Drug Delivery Science and Technology 65: 102732
4. Battistini, R., Rossini, I., Ercolini, C., Goria, M., Callipo, M.R., Maurella, C., Pavoni, E. & Serracca, L. (2019). Antiviral Activity of Essential Oils Against Hepatitis A Virus in Soft Fruits. Food Environ Virol. 11(1): 90-95.
5. Bhatt H. B. & Patel N. B. (2020). Antimicrobial activity of ajwain oil, lemon peels oil and vetiver oil. In: P. Saranj. Compendium of "Research insights of life science students. (pp. 287-289).
6. Bhavaniramya, S., Vishnupriya, S., Al-Aboody, M. S., Vijayakumar, R. & Baskaran, D. (2019). Role of essential oils in food safety: Antimicrobial and antioxidant applications, Grain & Oil Science and Technology. 2(2) : 49–55.
7. Bhunia, A.K. (2018). Molds and mycotoxins. In Foodborne Microbial Pathogens: Mechanisms and Pathogenesis, 2nd ed.; Springer: New York, NY, USA, pp. 167–174
8. Bintsis T. Foodborne pathogens. AIMS Microbiol. 3(3): 529-563.
9. Bouyahya, A., Dakka, N., Et-Touys, A., Abrini, J. & Bakri, Y. (2017). Medicinal plant products targeting quorum sensing for combating bacterial infections. Asian Pac. J. Trop. Med. 10:729–743.
10. Bouyahya, A., Abrini, J., Dakka, N. and Bakri, Y. (2019). Essential oils of *Origanum compactum* increase membrane permeability, disturb cell membrane integrity, and suppress quorum-sensing phenotype in bacteria. J Pharm Anal. Oct. 9(5):301-311.
11. Brożyna, M., Paleczny, J., Kozłowska, W., Chodaczek, G., Dudek-Wicher, R., Felińczak, A., Gołębiewska, J., Górniak, A. & Junka, A. (2021). The antimicrobial and antibiofilm in vitro activity of liquid and vapour phases of selected essential oils against *Staphylococcus aureus*. *Pathogens. 10*:1207.
12. Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods-a review. Int J Food Microbiol. 94: 223-253.
13. Byrne, M.K., Miellet, S., McGlinn, A., Fish, J., Meedya, S., Reynolds, N. & vanOijen, A. M. (2019). The drivers of antibiotic use and misuse: the development and investigation of a theory driven community measure. BMC Public Health.19:1425.
14. Caputo, L., Reguilon, M. D., Mińarro, J., De Feo, V. & Rodriguez-Arias, M. (2018). *Lavandula angustifolia* essential oil and linalool counteract social aversion induced by social defeat. Molecules. 23: 2694.
15. Chouhan, S., Sharma, K., & Guleria, S. (2017). Antimicrobial Activity of Some Essential Oils-Present Status and Future Perspectives. Medicines (Basel, Switzerland). 4(3):58.
16. Dewi, G., Nair, D. V.T., Peichel, C, Johnson, T. J., Noll, S. & Johny A. K. (2021). Effect of lemongrass essential oil against multidrug-resistant Salmonella Heidelberg and its attachment to chicken skin and meat. Poultry Science.100 (7):101116.
17. Dhifi, W., Bellili, S., Jazi, S., Bahloul, N., & Mnif, W. (2016). Essential Oils Chemical Characterization and Investigation of Some Biological Activities: A Critical Review. Medicines. 3: 25.

18. Dwivedy, A. K., Prakash, B., Chanotiya, C. S., Bisht, D. & Dubey, N. K. (2017). Chemically characterized Mentha cardiaca L. essential oil as plant-based preservative in view of efficacy against biodeteriorating fungi of dry fruits, aflatoxin secretion, lipid peroxidation and safety profile assessment. Food Chem. Toxicol. 106 : 175–184.
19. Ebani, V.V., Nardoni, S., Bertelloni, F., Tosi, G., Massi, P., Pistelli, L. & Mancianti, F. (2019). In vitro antimicrobial activity of essential oils against *salmonella enterica* serotypes enteritidis and typhimurium strains isolated from poultry. Molecules. 24(5) : 900. .
20. FDA (U.S. Food and Drug Administration) Available from: What You Need to Know about Foodborne Illnesses | FDA
21. Gilling, D.H., Kitajima, M., Torrey, J.R. & Bright, K.R. (2014a). Antiviral efficacy and mechanisms of action of oregano essential oil and its primary component carvacrol against murine norovirus, J appl microbiol. 116(5) : 1149-1163.
22. Gilling, D.H., Kitajima, M., Torrey, J.R. & Bright, K.R. (2014b). Mechanisms of antiviral action of plant antimicrobials against murine norovirus. Appl Environ Microbiol. 80(16) :4898-4910.
23. Gonelimali, F.D., Lin, J., Miao, W., Xuan, J., Charles, F., Chen, M. & Hatab, S. R. (2018). Antimicrobial properties and mechanism of action of some plant extracts against food pathogen sand spoilage microorganisms. Front. Microbiol. 9: 1639.
24. Hall, C.W. & Mah, T.F. (2017). Molecular Mechanisms of Biofilm-Based Antibiotic Resistance and Tolerance in Pathogenic Bacteria. FEMS. Microbiol. Rev. 41: 276–301.
25. Han, Y., Sun, Z. & Chen, W. (2020). Antimicrobial susceptibility and antibacterial mechanism of limonene against *Listeria monocytogenes*. Molecules. 25(1) : 33.
26. Jamshidi-Kia, F., Lorigooini, Z. &Amini-Khoei, H. (2018). Medicinal plants: Past history and future perspective. J Herbmed Pharmacol. 7(1):1-7.
27. Ju, J., Xie, Y., Yu, H., Guo, Y., Cheng, Y., Zhang, R. & Yao, W. (2020). Major components in Lilac and *Litsea cubeba* essential oils kill *Penicillium roqueforti* through mitochondrial apoptosis pathway. Indus. Crops Prod. 149: 112349.
28. Khare, T., Mahalunkar, S., Shriram, V., Gosavi, S., & Kumar, V. (2021). Embelin loaded chitosan gold nanoparticles interact synergistically with ciprofloxacin by inhibiting efflux pumps in multidrug-resistant Pseudomonas aeruginosa and Escherichia coli. Environ. Res. 199:111321.
29. Kim, Y. W, You, H. J., Lee, S., Kim, B., Kim, D. K., Choi, J., Kim, J., Lee, H.J., Joo, I.S., Lee, J.S., Kang, D.H., Lee, G., Ko, G.P. & Lee S.J. (2017). Inactivation of Norovirus by lemongrass essential oil using a Norovirus surrogate system. Journal of Food Protection. 80(8) :1293–1302.
30. Koul, O., Walia, S., & Dhaliwal, G. S. (2008). Essential oils as green pesticides: potential and constraints. Biopestic.Int. 4(1) : 63–84.
31. Kristkova ZS, Grace D, Kuiper M. The Economics of Food Safety in India–A Rapid Assessment. Amsterdam, Wageningen: Wageningen University & Research; 2017. p. 51
32. Liu, F., Jin, P., Gong, H., Sun, Z., Du, L. & Wang, D. (2020a). Antibacterial and antibiofilm activities of thyme oil against foodborne multiple antibiotics-resistant *Enterococcus faecalis*. Poultry Science. 99(10) : 5127-5136.
33. Liu, T., Wang, J., Gong, X., Wu, X., Liu, L. & Chi, F. (2020b). Rosemary and tea tree essential oils exert antibiofilm activities in vitro against Staphylococcus aureus and Escherichia coli. J Food Prot. 83(7) : 1261-1267.
34. Marsh, Z., Shah, M.P., Wikswo, M.E., Barclay, L., Kisselburgh, H., Kambhampati, A., Cannon, J.L., Parashar, U.D., Vinje, J. & Hall, A.J. (2018). Epidemiology of Foodborne Norovirus Outbreaks - United States, 2009-2015. Food Saf. 6(2): 58-66.
35. Merillon, J.M. & Riviere, C. (2018). Natural Antimicrobial Agents; Springer International Publishing AG: Cham, Switzerland.
36. Millezi, A. F., Costa, K. A. D., Oliveira, J. M., Lopes, S. P., Pereira, M. O. & Piccoli, R. H. (2019). Antibacterial and anti-biofilm activity of cinnamon essential oil and eugenol. Ciencia Rural. 49(1).
37. Mostafa, A. A., Al-Askar, A. A., Almaary, K. S., Dawoud, T. M., Sholkamy, E. N., & Bakri, M. M. (2018). Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. Saudi J. Biol. Sci. 25: 361–366.

38. Mutlu-Ingok, A., Devecioglu, D., Dikmetas, D.N., Karbancioglu-Guler, F. & Capanoglu, E. (2020). Antibacterial, Antifungal, Antimycotoxigenic, and Antioxidant Activities of Essential Oils: An Updated Review. *Molecules.* 25(20): 4711.
39. Oulkheir, S., Aghrouch, M., Mourabit, F. El., Dalha, F., Graich, H., Amouch, F., Ouzaid, K., Moukale, A. & S. Chadli. (2017). Antibacterial Activity of Essential Oils Extracts from Cinnamon, Thyme, Clove and Geranium Against a Gram Negative and Gram-Positive Pathogenic Bacteria. *Journal of Diseases and Medicinal Plants*. Special Issue: New Vistas of Research in Ayurveda System of Medicine. *3*(2-1):1-5.
40. Ozogul, Y., Kuley, E., Ucar, Y. & Ozogul, F. (2015). Antimicrobial Impacts of Essential Oils on Food Borne-Pathogens. *Recent Pat Food Nutr Agric*, *7*(1), 53-61.DOI: 10.2174/2212798407666150615112153
41. Ozogul, Y., Boga, E. K., Akyol, I., Durmus, M., Ucar, Y., Regenstein, J. M. & Koşker, A. R. (2020). Antimicrobial activity of thyme essential oil nanoemulsions on spoilage bacteria of fish and food-borne pathogens. Food Bioscience. 36: 100635.
42. Oliveira, R. C., Carvajal-Moreno, M., Mercado-Ruaro, P., Rojo-Callejas, F., Correa, B. (2020a). Essential oils trigger an antifungal and anti-aflatoxigenic effect on *Aspergillus flavus* via the induction of apoptosis-like cell death and gene regulation. Food control. 110: 107038.
43. Oliveira, R.C., Carvajal-Moreno, M., Correa, B., Rojo-Callejas, F. (2020b). Cellular, physiological, and molecular approaches to investigate the antifungal and anti-aflatoxigenic effects of thyme essential oil on *Aspergillus flavus.* Food Chem. 315: 126096.
44. Pandey, A. K., Kumar, P., Singh, P., Tripathi, N. N. & Bajpai, V. K. (2016). Essential Oils: Sources of Antimicrobials and Food Preservatives. Front. Microbiol. 7: 2161.
45. Pisosch, i A.M., Pop, A., Georgescu, .C, Turcuş, V., Olah, N.K. & Mathe, E. (2018). An overview of natural antimicrobials role in food. *Eur J Med Chem*, *1*(143), 922-935.
46. Porter, J. A. &Monu, E, A. (2019). Evaluating the Antimicrobial Efficacy of White Mustard Essential Oil Alone and in Combination with Thymol and Carvacrol Against Salmonella. J Food Prot. 82(12):2038–2043.
47. Raeisi, M., Hashemi, M., Ansarian, E., Hejazi, J., Azar, H.H., Daneshamooz, S., Jannat, B. & Aminzare, M. (2019). Antibacterial effect of *mentha piperita* essential oil against food borne pathogens in minced meat during storage at abuse refrigeration temperature. Adv. Anim. Vet. Sci. 7(8): 720-726.
48. Reyes-Jurado, F., Malo, A. L., & PALOU, E. (2016). Antimicrobial activity of individual and combined essential oils against foodborne pathogenic bacteria. Journal of Food Protection. 79(2):309–315.
49. Sanchez, G. & Bosch, A. (2016). Survival of Enteric Viruses in the Environment and Food. Vir. Food. 26:367–392.
50. Semeniuc, C. A., Pop, C. R. & Rotar, A. M., (2017). Antibacterial activity and interactions of plant essential oil combinations against Gram-positive and Gram-negative bacteria. J Food Drug Anal. 25(2): 403-408.
51. Shahbazi, Y. (2015). Chemical Composition and In Vitro Antibacterial Activity of Mentha spicata Essential Oil against Common Food-Borne Pathogenic Bacteria. Journal of Pathogens. 2015.
52. Shrestha, P.M, Dhillion, S.S. (2003). Medicinal plant diversity and use in the highlands of Dolakha district, Nepal. Journal of Ethnopharmacolog. 86(1): 81-96.
53. Singh, A., Deepika, Chaudhari, A. K., Das, S., Prasad, J., Dwivedy, A. K. &Dubey, N. K. (2021). Efficacy of *Cinnamomum cassia* essential oil against food-borne molds and aflatoxin B_1 contamination. *Plant Biosystems* - An International Journal Dealing with all Aspects of Plant Biology. 155(4):899-907.
54. Somrani, M., Debbabi, H. & Palop, A. (2021). Antibacterial and antibiofilm activity of essential oil of clove against *Listeria monocytogenes* and *Salmonella Enteritidis*. Food Science and Technology International. 4:10820132211013273.
55. Swamy, M. K., Akhtar, M. S. & Sinniah, U. R. (2016). Antimicrobial Properties of Plant Essential Oils against Human Pathogens and Their Mode of Action: An Updated Review. *Evidence-Based Complementary and Alternative Medicine*, *2016*.
56. Stringaro, A., Colone, M. & Angiolella, L. Antioxidant, antifungal, antibiofilm, and cytotoxic activities of Mentha spp. essential oils. (2018). Medicines. 5: 112.
57. Thielmann, J., Muranyi, P. & Kazman, P. (2019). Screening essential oils for their antimicrobial activities against the foodborne pathogenic bacteria Escherichia coli and *Staphylococcus aureus*. Heliyon. 5(6): e01860

58. Trombetta, D., Castelli, F., Sarpietro, M. G., Venuti, V., Cristani, M., & Daniele, C., Saija, A., Mazzanti, G., & Bisignano, G. (2005). Mechanisms of antibacterial action of three monoterpenes. Antimicrob.AgentsChemother. 49(6) : 2474–2478.
59. Trudel-Ferland, M., Jubinville, E. & Jean, J. (2021). Persistence of Hepatitis A Virus RNA in Water, on Non-porous Surfaces, and on Blueberries. Front. Microbiol. 12: 618352.
60. Valkova, V. & Duranova, H., Galovicova, L., Ivanisova, E., Vukovic, N. & Kacaniova, M. (2021). Lavender essential oil: its chemical composition, and antimicrobial and antioxidant properties. Conference Paper
61. Vestby, L.K., Gronseth, T., Simm, R., Nesse, L.L. (2020). Bacterial Biofilm and its Role in the Pathogenesis of Disease. *Antibiotics*. 9(2):59.
62. WHO (2020). World Health Organization. Available from:https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance
63. Winska, K., Wanda, M., Łyczko, A. J., Grabarczyk, M., Czubaszek, A. and Szumny, A. (2019). Essential Oils as Antimicrobial Agents—Myth or Real Alternative? Molecules. 24(11) : 2130.
64. Yang, S-K., Tan, N-P, Chong, C-W., Abushelaibi, A., Lim, S-H-E. & Lai, K-S. (2021). The Missing Piece: Recent Approaches Investigating the Antimicrobial Mode of Action of Essential Oils. Evolutionary Bioinformatics.
65. Youssef, F.S., Eid, S.Y., Alshammari, E., Ashour, M.L., Wink, M., El-Readi, M.Z. (2020). *Chrysanthemum indicum* and *Chrysanthemum morifolium*: Chemical Composition of Their Essential Oils and Their Potential Use as Natural Preservatives with Antimicrobial and Antioxidant Activities. *Foods*. 9(10) : 1460.
66. Puvača, N , Milenković, J., Galonja Coghill, T., Bursić, V., Petrović, A., Tanasković, S. & Miljković, T. (2021). Antimicrobial activity of selected essential oils against selected pathogenic bacteria: In vitro study. Antibiotics. 10(5): 546.

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RESEARCH ARTICLE

DKBT: Decentralized Key based Trustworthy Computing in Vanet with Blockchain

N. Parthiban[1]* and G. Dheepa[2]

[1]Ph.D. Research Scholar, Department of Computer Science, P.K.R Arts College for Women, Gobichettipalayam, Erode, Tamil Nadu, India
[2]Associate Professor, Department of Computer Science, P.K.R Arts College for Women, Gobichettipalayam, Erode, Tamil Nadu, India

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***Address for Correspondence**
N. Parthiban
Ph.D. Research Scholar,
Department of Computer Science,
P.K.R Arts College for Women, Gobichettipalayam,
Erode, Tamil Nadu, India

ABSTRACT

The Internet of Things (IoT) will soon include intelligent cars, which might alter human existence in smart cities by providing important services and possibilities. An intelligent vehicle's foundation is the Vehicular Ad-hoc Network (VANET). Vehicle-to-vehicle and vehicle-to-infrastructure communication may be more accurate and secure, resulting in fewer accidents and gridlocks. Security flaws like denial-of-service (DoS), replay attacks, and Sybil attacks might compromise the security and privacy of VANET. An attack on a rogue node might result in other nodes in the network receiving inaccurate information. This paper proposes the DKBT framework for trust-based computing in VANET using blockchain technology (BT).In DKBT, as a replacement for third-party service providers; we use BT. User characteristics may also be used to set distinct VANET data access permissions. Data access may be made even more efficient by shifting encryption and decryption processes from the VANET devices to the more capable RSUs through a more robust Elliptic Curve Cryptography (ECC). Several simulation experiments and security assessments proved that the DKBT could provide good data security and low-performance overheads. We employ the Blockchain to replace the third-party service providers for user identity management and data storage. And different VANET data access rights can be established according to user attributes. By improving the ECC, the lightweight VANET devices can outsource complex encryption and decryption operations to powerful RSUs and improve data access efficiency. Finally, we conducted a series of simulation tests and security analyses, proving that the DKBT can provide effective data security and low-performance overhead.

Keywords: ECC, DKBT, Signature generation, VANET

INTRODUCTION

The number of intelligent cars on mobile ad-hoc networks has increased dramatically as smart cities have advanced rapidly. In the next ten years, 2 billion intelligent automobiles will be on the road [1-3]. As a result, a mobile ad hoc network (MANET) with onboard units (OBU) of wireless communication devices has been developed [4-7]. These gadgets are equipped with hardware security chips that save crucial data about the vehicle [8]. Two main types of VANET connections are vehicle-to-infrastructure and vehicle-to-vehicle (V2V/V2I). The Dedicated Short-Range Communication Radio (DSRC) [9-12] will be used to exchange crucial traffic information between the cars. Each car is a node in the network, and it may transmit and process information. Making VANET [13-16]] has numerous goals, including minimizing traffic accidents and improving traffic flow. Vehicles' mobility and volatility in VANET make them vulnerable to various assaults, which might significantly affect the network. VANET's decentralized structure makes it harder to track cars or misbehaving users [17-19]. The VANETs, on the other hand, do not have the same level of focus on all users as other networks [20]. Because of its unique properties, such as high mobility, integrity, and authenticity, VANETs must use digital signature techniques to meet fundamental security needs. On the other hand, the digital signature on each communication relates directly to the driver's identity and cannot be disputed. As a result of these characteristics, attackers may easily get confidential information about the driver. Instead of conveying a message with any credibility, vehicles that utilize absolute anonymity to safeguard privacy risk doing so. Privacy security in VANETs relies heavily on the ability of other cars and infrastructure to verify that a message sent from a vehicle is valid.

The primary contributions of the paper are

- Trustworthy computing in VANETs
- Securing the data using Blockchain
- ECC Cryptography for additional security
- Signature generation algorithm for verification

Everything after that is laid out as follows. Section II provides background information on VANET and BT. Section III presents the suggested system model, design objectives, and mechanisms. Sections IV includes the security analysis and performance evaluation. Section V brings this study to a conclusion and future scope.

BACKGROUND STUDY

Alharthi et al., [1] introduce a Biometrics Block Chain (BBC) to secure VANET data exchange. This BBC framework protects VANET communications in real-world applications. The suggested system allows secure and reliable vehicle communication while maintaining anonymity. Using biometrics and BT ensures safe data transmission, monitors data exchange and identifies the liable vehicle if erroneous signals are received. To demonstrate the feasibility of Operation and Maintenance New Equipment Training (OMNETCC), Veins and Simulation of Urban Mobility (SUMO) are used. Packet delivery rate, packet loss rate, and computational cost are all used to gauge system performance. Alfadhli, S.A., and others [2] Multi-factor authentication is performed using dynamic materials. It improves security and privacy by eliminating the need to keep sensitive information in a perfect Tamper Proof Device (TPD). Authors have shown that physical and cloning assaults do not affect the security of A Multi-Factor Secured and Lightweight Privacy-Preserving Authentication Scheme (MFSPV). The Road Side Unit (RSU) does not have access to any critical private data, and Physical Unclonable Funciton (PUFs) may be used to provide the needed functionality, security, and privacy. This method eliminates these difficulties, which does not bottleneck the Congestion Avoidance (CA) or Road Side Unit (RSU) and does not need a resource-intensive bilinear pairing technique. The true identity of a vehicle cannot be traced by any other party or hostile vehicle because of this. Studies have shown that this technique outperforms conventional multi-factor and non-multifactor systems in terms of performance and features. The suggested technique provides a VANET alternative. Jinarajadasa, G.M., and Liyange, S.R. [4] assess machine learning to increase MANET trustworthiness. Other approaches are available for future investigation. Machine learning has several advantages. Better methods exist. Swarm intelligence is another method.

Flexible for MANET mobile nodes. Energy-saving strategies are difficult to implement. Because of AI, MANETs use a lot of energy. When all factors are considered, machine learning comes out on top Problems. In mobile ad hoc networks, reinforcement learning yields more precise results. No prior data is required to predict the behavior of freshly connected network nodes because of the ease with which dynamic activity may be captured. Li H et al. [7] FADB is a unique data-sharing architecture introduced by the authors. The Fine-Grained Access Control Scheme (FADB) provides an encrypted data-sharing platform using Cipher text Policy Attribute Based Encryption (CPABE), Blockchain, and Interplanetary File System (IPFS). Combining data security and privacy access, FADB accesses VANET easily. Reliable data storage and sharing services. FADB features a blockchain network based on RSUs. FADB is compatible with two distributed ledgers (IC, DC). Smart contracts can read/write and manage user identity, data, and shared information. All RSUs offer block-level backups for user data. Data that is resistant to external dangers. Interplanetary File System (IPFS) is immune to Fine-Grained Access Control Scheme (FADB) Single Point of Failute (SPOFs). Ineffective in comparison to conventional cloud storage. Incentives result in replication-proof, erasure-coding, and improved dependability and availability.

R. Shrestha et al. [11] used the centralized server efficiently. In this concept, a centralized server specifies each car's confidence. Each receiving car queries the centralized server to authenticate the sender's reliability. By a selection system, the recipient vehicle influences the reliability of event communication. After demonstrating the validity of the message, it spread. There are vehicles around. The authors compared their approach to Waze regarding message quality, timeliness, credibility, and energy efficiency. Wei Z. et al. [14] offer an unforgeable Identity-Based Signature (IBS) without a public key encryption Pvt k—a random oracle. Two safe and economical outsourcing strategies are proposed to decrease exponential operating costs. These outsourcing procedures apply to Operations ecosystems with exponential development. The author built a VANET procedure that protects user privacy using outsourced computation. Authentications use proxy signatures and the IBS method. VANET offers time alone and track. Privacy Pairing and exponential approaches, according to the authors, are inefficient. Zhou et al. [17] Multi-key Secure Outsourced Computation scheme (MSOC) without public-key exploitation. Then fully homomorphic encryption (FHE). Based on MSOC, LSCP Lightweight Secure Comparison Protocol (LSCP) is constructed without considering server-user communication. Simple authentication that protects privacy Lightweight Privacy-Preserving Authentication protocol (LPPA) removes the requirement for authorization by duplicating encrypted Location-Based Service (LBS) communications before authentication. Zhang et al. [20] provide an anti-attack method for trust management. Anti-Attack Trust Management Scheme (AATMS) measures VANET vehicle reliability. Local automobile trust levels are determined via Bayesian inference. The authors provide a mechanism for calculating global trust based on Trust Rank. Trust in actual automobiles is explored. Choose automobiles that are resistant to collisions. Degradation and forgetfulness are also mentioned as considerations. Resist novice and sporadic assaults. Simulations demonstrate the authors' effective approach to managing the trust.

SYSTEM MODEL

We proposed DKBT architecture for decentralized key-based trustworthy computing in VANETs.

ARCHITECTURE

Network Model

As designed, an intelligent vehicle comprises several sensors (such as face radar, rear radar, and others) that collect data about the surrounding environment that the driver would not normally be able to see [12].

Onboard Unit (OBU)

OBUs exchange vehicle information with RSUs and other OBUs in other vehicles through GPS. When OBU is plugged into the car's battery, it receives electricity. A global positioning system-type sensor and an event data recorder are included in each vehicle (EDR) [13].

Trusted Authority (TA)

The trusted authority is responsible for registering RSUs, OBUs, and vehicle customers as part of the VANET device management. OBU ID and information on whether any malicious message or suspected activity is present are both exposed by TA, which consumes a significant amount of energy. TA also contains a method for identifying assaults.

Road Side Unit

The roadside machine is a computer connected next to or at a specified area, for example, parking or crossroads.

Overview of Elliptic Curve Cryptography

An abelian group is formed by a limited number of points on an elliptic curve on a finite field. It is called Elliptic Curve Scalar Multiplication (ECSM) for any integer and point P, representing DATA POURING (DP) for any integer and point. P + P +... (d 1) times) is represented by the operation dP, which signifies the sum of the preceding operations. Q = can be easily calculated if d and P are provided as inputs to the one-way function.

Digital Signature Algorithm

The Digital Signature Algorithm (DSA) is the standard digital signature used by the United States Federal Government. Since specified in FIPS 186, which was authorized in 1993 in the National Institute of Standards with Technology, it exists suggested meant for inclusion.

Key Generation

Key Generation consists of two parts. Before computing public and public key encryption Pvt k for a single user, the first step is to choose algorithm parameters that several system users may share.

Parameter Generation

- Select a valid cryptographic hash function T. T was always SHA-1 in the original DSS. However, the stronger SHA-2 hash algorithms are permitted in the recent Digital Signature Standard (DSS). The hash output may be shortened to a key pair's unique identifier.
- Choose L and N key lengths. This is the major indicator of the key's cryptographic strength. The original DSS required L to be a 64-bit multiple between 512 and 1024. NIST 800-
- Choose an N-bit prime q. N must be less than or equal to the output length of the hash function.
- Select an L-bit prime modulus p with p–1 being a multiple of q.
- Select the integer s, whose multiplicative order modulo p is q. This may be accomplished by setting s = t(p–1)/q mod p for an arbitrary t (1 t p1) and retrying with a different t if the result is 1. Most selections of t will result in a useful s; t=2 is typically utilized.

Several system users may share the algorithm parameters (p, q, s).

Accuracy of the algorithm

The cross technique is valid because the verifier forever accepts authentic signature. This may be revealed as follows:

First, if $s=t^{(p-1)/q} \bmod p$, it follows that *by Fermat's little theorem,* $sq \equiv tp - 1 \equiv 1 \ (mod\ p)$. Since $s > 1$ and q is prime, s must have order q.

The signer computes

$n = k^{-1}(H(l) + xr) mod q$ -------- (1)

so

$k \equiv H(m)n^{-1} + xrs^{-1}$ ---------- (2)

$\equiv H(l)w + xrw \ (modq)$---------- (3)

Since shasorder *q* (modp),

$g^k \equiv g^{H(m)w} g^{xrw}$ ---------- (4)

$\equiv g^{H(m)w} g^{xrw}$ ---------- (5)

$\equiv g^{u1} y^{u2} \quad (modp)$ ---------- (6)

In conclusion, the precision of DSA follows from

$r = (g^k modp) modq = (g^{u1} y^{u2} modp = u$---------- (7)

Sensitivity

The entropy, privacy, and individuality of the casual signature value k are essential for DSA. It is so crucial to breaching just one of these three conditions might expose your whole secret key to an aggressor. By the same price, double while trust km unseen with an anticipated price, or leak level k over several signatures, is sufficient to undermine DSA.

Signature Generation Algorithm

1. Choose achance figure *k* from [1, *n*–1].
2. Compute $r = m_1 \pmod n$, where $(m_1, n_1) = kS$. If $r=0$, go back to step 2.
3. Compute $s = k^{-1} (q + rd_A) \pmod n$. If $s=0$, go back to step 2.
4. The name is the pair (*r*,*s*).

To compute s, the string q returned by HASH(m) must be transformed to an integer. Q may be more than n, but not longer. It is essential to choose a unique k used for each name; if not, the equation in pace four be capably solve d for d_A, the person al key:

SIGNATURE VERIFICATIONALGORITHM

Bob must possess Alice's pk SA to authenticate Alice's signature. If he lacks confidence in the source of SA, he must verify the key. Here, O represents the identifier:

1. verify that S_A is not equal to *O* and its coordinates are otherwise valid
2. verify that S_A lies on the curve
3. verify that $NS_A = O$

After that, Bob follows these steps:

1. validate that *r* and *s* are integers in [1,*n*–1]. If not, the signature is invalid.
2. analyze *e* = HASH (*m*), where HASH is the same meaning use d in the name creation. Let *s* be the L_n left most bits of *e*.
3. analyze $w = s^{-1} \pmod n$.
4. analyze $u_1 = sw \pmod n$ and $u_2 = rw \pmod n$.
5. analyze $(m_1, n_1) = u_1 S + u_2 S_A$.
6. The signature invalidates if r = m1(mod n).

Straus's approach, sometimes known as Shamir's trick, the total of two scalar multiplications $u_1 S + u_2 S_A$ may be computed more quickly than with two scalar multiplications alone.

Algorithm for ECC

The information must be publicly available to all users, creating open key cryptography. The well-recognized entity include:-

- From the elliptic curve equation, we must determine:-
- These are the values of the constants a' also by
- The value of m where the elliptic curve over SF is defined. (2m).
- set associated with the elliptic curve. A base point B, or some list on the arc E, belongs to the set used as a bottom.

The algorithms for the special part of ECC are:-

Signature Generation and validation Algorithm

- Calculation of message digest with a HASH function, preferable SHA-1, where ‗e' is the message digest, mis the message such that e = HASH fun(m)
- Produce a random positive integer between 1 and n-1.
- Sign 1 is derived from sign1 = m mod n, where m is the product of B and rand, i.e., m = mood(rand × B), where the mood is a function for obtaining the m coordinate.
- But if sign1 is 0, then repeat the preceding operation.
- The second portion of the signature, sign2, is computed using the formula sign2 = rand -1(e + (Apriv×sign1)(modn)
- However, if sign2 is 0, re-generate r and repeat the method.
- The produced signature is a pair (sign1, sign 2).

Signature Validation Algorithm

Check if sign1 and sign2 fall inside the interval between 1 and n-1. Otherwise, the signature is invalid. Using the same hash algorithm, compute the message digest from the received message,

Block Chain Elliptic Curve Digital Signature Authentication

ECDSA is a variation of DSA based on elliptic curves. Scott Vanstone initially suggested it in 1992. The elliptic arc Discrete Logarithm Problem is a fundamental computationally challenging mathematics issue. Set an elliptic curve, E denied above Fs, an end P2E (Fs) of order n and point S2E(Fs) determine the integerl;0 In 1, such to S=IP. Assuming such a figure exists. This specific logarithm difficulty above the elliptic curve is substantially more difficult than the DLP over Sp, Typically, the curve is specified by its two parameters, a and b, and its equation (8). For a finite field, the curve equation is the equation that is the same for everyone.

$n^2 + mn = m^3 + am^2 + b$ ---------- (8)

Algorithm ECDSA signature and Routing algorithm

Input

$\sum^{k}_{i=1} m_i$ // m_i is a set of house-hold-bin moving from home to a meeting point.

$\sum^{l}_{j=1} n_j$ // NJ is a set of mobile garbage collectors moving on local street roads.

Output

r_m // Returns the direct path from m_i to meeting point m

r_n// Returns the direct path from NJ to meeting point m

while (true) do

if ($v_j \geq \omega$), then // Volume of n_j reaches the threshold ω

v_j=0 // n_j tends to be emptied

end if

if ($v_i \geq \omega$), then // Volume of m_i reaches the threshold ω

n_n=nearest(m_i,$\sum^{l}_{j=1} n_j$) // Identifies the nearest n_j to meeting point

m=point(m_i, n_n) // Identifies the meeting point m on the street or local road

r_m=route(m_i, m) //calculate the direct path from $m_i$toward m

r_n=route(n_n, m) // calculate the direct path from $n_n$toward m

v_m=0
end if
end for
The mathematical foundation for DSA. So, the strength-per-key-bit is much larger than in DSA, and fewer parameters (keys) may exist to provide equal levels of security using elliptic curve cryptosystems. The area parameter for ECDSA, which are the parameter of the arch E, the underlying limited field, and a stand spot G, must be discussed and agreed upon by the communication partners to allow interoperability.

RESULTS AND DISCUSSION

Using NS2 Simulator, our suggested protocol is simulated. We compare our DKBT Model to the Blockchain-based decentralized input organization technique for VANET (DB-KMM). The network is 550 x 480 meters in size. DB-KMM has mechanically performed the registration, inform, and revocation of the user's pk. Parallel, an un-important protocol for shared confirmation and key agreement is base on bivariate polynomial algorithms. Figure 1 represents the energy consumption Comparison chart using DB-KMM and DKBT models. In this diagram, the x-axis is time (seconds), while the y-axis is energy (joules). Figure 2 represents the routing overhead Comparison chart using DKBT and DB-KMM models to determine the accuracy results. The x-axis is time (seconds), while the y-axis is overhead (percentage). Figure 3 represents the data _set_ buffering comparison chart. The output evaluated by using DKBT and DB-KMM model used. Data sets are represented on the x-axis and the frame on the y-axis. Figure 4 denotes the delivery–rate comparison chart. This will be calculated by using DB-KMM and DKBT models. The delivery rate is shown by the y-axis, while the x-axis represents time. Figure 5provides details about the detection rate comparison chart. In that, DB-KMM and DKBT models were used. As you can see, time is shown on the X-axis, while the detection rate is plotted on the Y-axis. Figure 6 provides details about the accuracy_ rate comparison chart. In that DB-KMM and DKBT model. The X-axis represents data slots, and the y-axis represents the accuracy rate.

CONCLUSION

This paper proposes the DKBT framework for decentralized, trustworthy computing for VANETs. This model uses ECC, signature verification, and ECDA algorithm with BT. We could show that the DKBT system is impervious to physical and network intrusions. The RSU does not have access to any sensitive private information under this proposed plan. The inherent security characteristics of DKBT may be used to obtain the needed functionality, security, and privacy. Another benefit is that this system does not need resource-intensive bilinear pairing operations, which are common in other ID-based methods. It does not produce a bottleneck on the certificate authority (CA) or RSU. There are additional advantages as well, such as making sure that no other party or hostile vehicle may find out the true identity of a car. In-depth security and performance testing show that our scheme outperforms current multi-factor and newer intriguing non-multi-factor techniques in terms of performance and functionality. Consequently, the suggested method is a viable alternative for VANETs.

REFERENCES

1. Alharthi, A., Ni, Q., & Jiang, R. (2021). A Privacy-Preservation Framework Based on Biometrics Blockchain (BBC) to Prevent Attacks in VANET. IEEE Access, 9, 87299–87309. doi:10.1109/access.2021.3086225
2. Alfadhli, S. A., Lu, S., Chen, K., & Sebai, M. (2020). MFSPV: A Multi-Factor Secured and Lightweight Privacy-Preserving Authentication Scheme for VANETs. IEEE Access, 1–1. doi:10.1109/access.2020.3014038
3. Celes, C., Silva, F. A., Boukerche, A., Andrade, R. M. de C., & Loureiro, A. A. F. (2017). Improving VANET Simulation with Calibrated Vehicular Mobility Traces. IEEE Transactions on Mobile Computing, 16(12), 3376–3389. doi:10.1109/tmc.2017.2690636

4. Jinarajadasa, G. M., &Liyange, S. R. (2020). A survey on applying machine learning to enhance trust in mobile adhoc networks. 2020 International Research Conference on Smart Computing and Systems Engineering (SCSE). doi:10.1109/scse49731.2020.9313021
5. Jiang, Y., He, M., Zhang, X., &Xiong, L. (2021). Blockchain-Based Anonymous Authentication Mechanism with Semi-TTP for VANETs. 2021 IEEE 6th International Conference on Cloud Computing and Big Data Analytics (ICCCBDA). doi:10.1109/icccbda51879.2021.9442520
6. Lu, Z., Wang, Q., Qu, G., Zhang, H., & Liu, Z. (2019). A Blockchain-Based Privacy-Preserving Authentication Scheme for VANETs. IEEE Transactions on Very Large Scale Integration (VLSI) Systems, 1–10. doi:10.1109/tvlsi.2019.2929420
7. Li, H., Pei, L., Liao, D., Chen, S., Zhang, M., Zhang, M., & Xu, D. (2020). FADB: A Fine-Grained Access Control Scheme for VANET Data based on Blockchain. IEEE Access, 1–1. doi:10.1109/access.2020.2992203
8. Moussaoui, D., Kadri, B., Feham, M., & Ammar Bensaber, B. (2021). A Distributed Blockchain Based PKI (BCPKI) architecture to enhance privacy in VANET. 2020 2nd International Workshop on Human-Centric Smart Environments for Health and Well-Being (IHSH). doi:10.1109/ihsh51661.2021.9378727
9. Ma, Z., Zhang, J., Guo, Y., Liu, Y., Liu, X., & He, W. (2020). An Efficient Decentralized Key Management Mechanism for VANET with Blockchain. IEEE Transactions on Vehicular Technology, 1–1. doi:10.1109/tvt.2020.2972923
10. Ravi, N., & Kapoor, C. (2021).Bk Techniques to Detect Attacks on VANET System: A Survey. 2021 2nd International Conference on Intelligent Engineering and Management (ICIEM). doi:10.1109/iciem51511.2021.9445311
11. Shrestha, R., Bajracharya, R., & Nam, S. Y. (2018). Centralized approach for trustworthy message dissemination in VANET. NOMS 2018 - 2018 IEEE/IFIP Network Operations and Management Symposium. doi:10.1109/noms.2018.8406184
12. Sun, C., Liu, J., Jie, Y., Ma, Y., & Ma, J. (2018). Ridra: A Rigorous Decentralized Randomized Authentication in VANETs. IEEE Access, 1–1. doi:10.1109/access.2018.2868417
13. Wan, L., Eyers, D., & Zhang, H. (2019). Evaluating the Impact of Network Latency on the Safety of Blockchain Transactions. 2019 IEEE International Conference on Blockchain (Blockchain). doi:10.1109/blockchain.2019.00033
14. Wei, Z., Li, J., Wang, X., & Gao, C. (2019). A lightweight privacy-preserving protocol for VANETs based on secure outsourcing computing. IEEE Access, 1–1. doi:10.1109/access.2019.2915794
15. Wazid, M., Das, A. K., Kumar, N., Odelu, V., Goutham Reddy, A., Park, K., & Park, Y. (2017). Design of Lightweight Authentication and Key Agreement Protocol for Vehicular Ad Hoc Networks. IEEE Access, 5, 14966–14980. doi:10.1109/access.2017.2723265
16. Xie, L., Ding, Y., Yang, H., & Wang, X. (2019). Blockchain-based Secure and Trustworthy Internet of Things in SDN-Enabled 5G-VANETs. IEEE Access, 1–1. doi:10.1109/access.2019.2913682
17. Zhou, J., Cao, Z., Qin, Z., Dong, X., & Ren, K. (2019). LPPA: Lightweight Privacy-preserving Authentication from Efficient Multi-key Secure Outsourced Computation for Location-based Services in VANETs. IEEE Transactions on Information Forensics and Security, 1–1. doi:10.1109/tifs.2019.2923156
18. Zhang, X., Lyu, C., Shi, Z., Li, D., Xiong, N. N., & Chi, C. (2019). Reliable Multiservice Delivery in Fog-enabled VANETs: Integrated Misbehavior Detection and Tolerance. IEEE Access, 1–1. doi:10.1109/access.2019.2928365
19. Zhang, L., Wu, Q., Domingo-Ferrer, J., Qin, B., & Hu, C. (2017). Distributed Aggregate Privacy-Preserving Authentication in VANETs. IEEE Transactions on Intelligent Transportation Systems, 18(3), 516–526. doi:10.1109/tits.2016.2579162
20. Zhang, J., Zheng, K., Zhang, D., & Yan, B. (2020). AATMS: An Anti-Attack Trust Management Scheme in VANET. IEEE Access, 8, 21077–21090. doi:10.1109/access.2020.2966747

Table 1.Elliptic Curve Cryptography Parameters

Parameter	Definition
Q	Field size
Fr	The origin used
A b	ground essentials major the top of the arc
Dps	Domain parameter seed, possible
S	Base point
T	Cofactor

Table 2: Simulation Parameters

Parameters	Value
Simulation Time	500(s)
Number of Nodes	0to127
Mobility	10-50m/s
Routing Protocol	DSDV
Channel Type	wireless channel
Simulation Area	550x480 m
Transmission Range	250m
MAC type	Mac/802_11

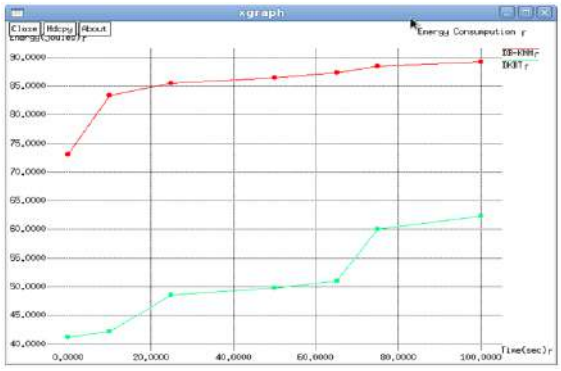

Fig. 1. Energy consumption comparison chart

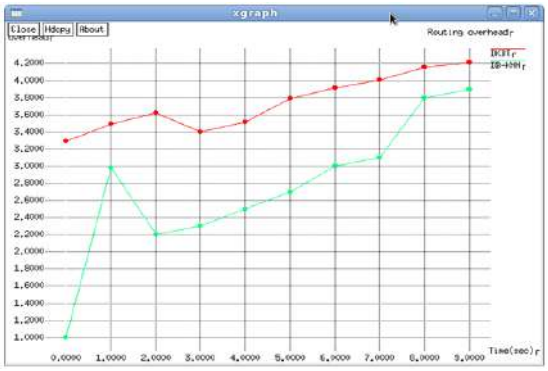

Fig.2. Routing overhead comparison chart

Fig. 3. Data_set_buffering comparison chart

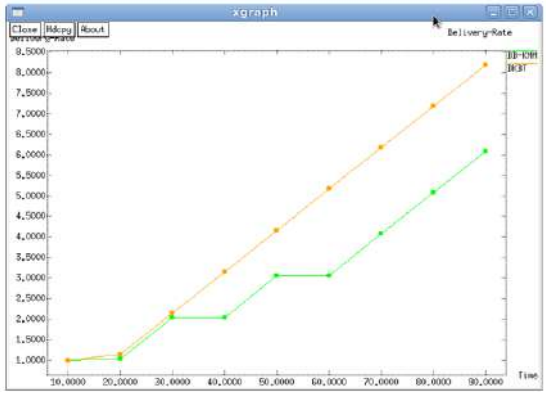

Fig. 4. Delivery- rate comparison chart

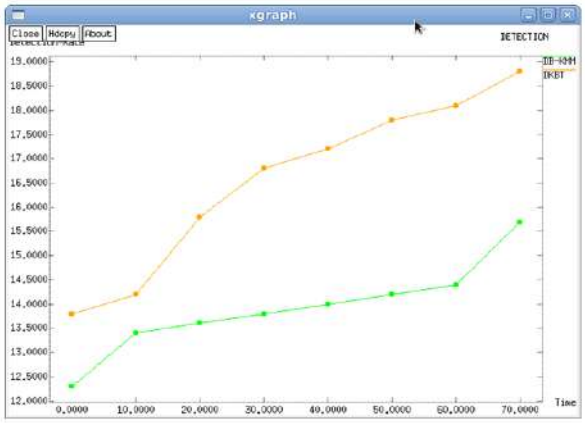

Fig. 5. Detection rate comparison chart

Fig.6. Accuracy-rate comparison chart

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RESEARCH ARTICLE

Effect of Nitrogen Limitation and Starvation on Microalgal Lipid Content

K.Sowmiya[1]* and S. Mahalakshmi[2]

[1]Ph.D., Research Scholar, Department of Agricultural Microbiology, Annamalai University, Chidambaram, Tamil Nadu, India
[2]Assistant Professor, Department of Agricultural Microbiology, Annamalai University, Chidambaram, Tamil Nadu, India.

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***Address for Correspondence**
K.Sowmiya
Ph.D., Research Scholar,
Department of Agricultural Microbiology,
Annamalai University, Chidambaram,
Tamil Nadu, India

ABSTRACT

Microalgae can be an alternative option to produce fuels because of their versatility as biomass source. Microalgae have higher photosynthetic efficiency, higher biomass productivities, and faster growth rates than higher plants, as well as the highest CO_2 fixation and O_2 production rates, positioning microalgae as one of the Earth's most important renewable fuel crops. Algae species such as *spirulina sp , chlorella vulgaris , Botryococcus sp* were isolated from fresh water and studied for the Effect of starvation of nitrogen sources on growth biomass and lipid content were studied with different time interval . From the result it revealed that nitrogen source (3.5g/L) produced the maximum lipid content but decrease comparatively when increase the concentration of nitrogen in algal represented as biomass. Among the different Algal species tested the chlorella showed more lipid. Then the best isolate *Chlorella* sp were sequenced based on 18s rRNA sequencing and identified as *chlorella vulgaris.*

Keywords: Microalgae, *Spirulina* sp*, Botryoccussp* ,*chlorella vulgaris,* lipid content, Biomass , starvation of nitrogen source.

INTRODUCTION

In aquatic environments, microalgae are at the base of the food chain. They have an innate ability to absorb water and carbon dioxide using sunlight and then convert them into complex organic compounds that are released from the cell. Heat, cold, drought, salt, photo-oxidation, anaerobiosis, osmotic pressure, UV exposure, and nutrients are only a few of the many environmental challenges that microalgae are well-adapted to withstand (Tandeau and Houmard 1993). The biomass and lipid productivities are important parameters to optimize in order to increase the

economic viability of microalgae culture for biodiesel production. According to reports, microalgae's biomass productivity and biochemical make-up (such lipid content) can be easily altered to provide a desired yield by modifying the medium's nutrients or physical culture conditions (Pancha*et al.*, 2014). The crucial function of nitrogen in promoting cell growth. Nitrogen is a crucial building block that microalgae can use to create a variety of compounds, including proteins, chlorophyll, and nucleic acids (Goncalves, et al., 2017). The main phospholipids in algae are phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, phosphatidylglycerol, phosphatidic acid, and diphosphatidytil glycerol. The major algal glycolipids are monogalactosyl dyglyceride, digalactosyl diglyceride, and sulphoquinovosyl diglyceride. A novel class of algal lipids are chlorosulpholipids which are derivatives of N-docosane-1, 14-diol and of N-tetracosane-1,15-diol disulphates found in *Chrysophyceae*, *Xanthophyceae*, *Chlorophyceae*, and *Cyanophyceae* (Pohl, 1982). After carbon, nitrogen (N) is the most important nutrient contributing to the production of biomass. The nitrogen content of microalgal biomass can range from 1% to more than 10%, and it can vary between different groups (e.g., low in diatoms) and within specific species depending on supply and availability (Grobbelaar, 2004).Discoloration of the cells is a frequent response to nitrogen limitation due to a decrease in chlorophyll content and an increase in carotenoids, as well as the accumulation of organic carbon compounds such as polysaccharides and certain oils like polyunsaturated fatty acids (PUFAs) (Becker, 1994b). Nitrogen deficiency, salt stress, and osmotic stress all increased lipid biosynthesis in microalgae. After three days of nitrogen deprivation, chloroplasts condensed and photosynthesis efficiency dropped by about 50%. Oil body formation was more efficient under non-saturating light intensity levels coupled with nitrogen starvation than under 100 mol/m2s light intensity. DCMU inhibited both photosynthesis and oil body formation, implying that during nitrogen deficiency, photosynthesis rather than autophagy provides the energy for oil body formation (Szu-Ting Wang et al., 2011). Green microalgae oil content increases significantly after a few days of nitrogen starvation treatment (Wang et al., 2009; Li et al., 2010; Moellering and Benning, 2010).

MATERIALS AND METHODS

Sampling of the microalgae

The microalgae used in our experiment were collected from a fresh water pond near Annamalai University in Tamil Nadu. The microalgae were isolated by separating single algal cells from the mixed algae suspension with capillary pipettes and cultivating them in culture dishes. Microalgae that grew successfully were transferred to separate tubes for routine maintenance. Following this direct isolation procedure, the cells are transferred to tubes containing BG11 medium and incubated under fluorescent light for 12 hours before being purified using the dilution method. (Su, 2015) .

Purification and identification

Purification was carried out according to the method described by Hutagalung (Hutagalung et al., 2014). Diluting the cell suspension to the appropriate concentration was the first step; aliquots of the diluted cell suspension were added to 10 reaction tubes containing BG 11 medium to purify and cultivate the algal cells of interest. This procedure was repeated several times until pure cultures of single cell isolates were obtained. The purified algae were then morphologically identified based on their size, shape, and colour.

Molecular identification of Algal species

Amplified PCR products were purified and prepared for Cycle sequencing using the Big Dye® Terminator 3.1 sequence kit (Applied Bio systems, Foster City, California, USA). After cycle sequencing, the products were purified using Ethanol-EDTA purification protocol to remove the un-incorporate ddNTP's, ddNTP's and primer dimer**.** The purified cycle sequencing products were dissolved in 12µl Hi-Diformammide and the samples were subjected for denaturation at 95^{0}C for 5 mins. Denatured products were subjected for sequencing in forward and reverse direction using Genetic Analyzer 3500 (Life Technologies Corporation, Applied Bio systems®, California 94404, USA) as per

manufacture's Instruction. Sequences were aligned and edited using Mega software version11 (Koichiro Tamura *et al*.2021) to confirm the species.

Effect of nitrogen limitation

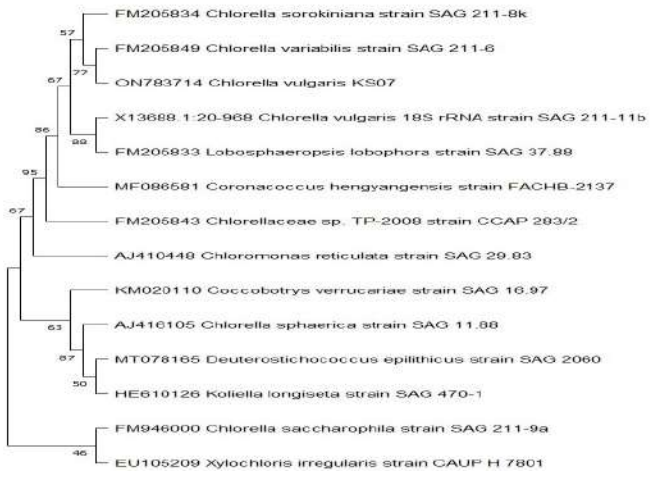
The isolates were inoculated in 50-mL Erlenmeyer flasks containing 20 mL modified basal medium, Chu 13 medium and Zarrouk's medium incubated with 125 rpm agitation at 25 ± 1°C with 16:8 h light and dark cycles for 7 days under nitrogen deficiency condition. The culture was then centrifuged at 1585g for 15 min. Cell pellets were re-suspended in 50 and 100% nitrogen depletion and starvation conditions provided in these Zarrouk's media, Chu – 13 and BBM media. The Zarrouk's medium, Chu – 13 and BBM medium were used as control. Consequently, all the applications were repeated in triplicate in 15 days' time intervals. After incubation, biomass was harvested and to analyze the biomass and dry weight of the micro algal isolates.

RESULT AND DISCUSTION

Effect of nitrogen limitation on growth of microalgae

The effect of nitrogen and limitation on growth and lipid content of microalgae strains *Spirulina* sp, *Chlorella vulgaris* and *Botryococcus* sp and data were given in Table- 1and fig-1. From these results, it seemed that nitrogen stress (3.5g/L) produced the maximum lipid content but decrease in algal growth represented as biomass. Under these conditions lipid content of micro algal strains higher when compared presence of nitrogen. The maximum lipid content was observed 59 per cent in *Chlorella vulgaris* when compared to other strains. The impact of nitrogen deficiency on algal growth and lipid production are showed. An increase in algal biomass was found under nitrogen-rich condition for all strains. In the absence of a nitrogen source, no growth was observed and the cells appeared bleached. Although some loss in algal biomass was found, the lipid contents of three strains increased. Similar results supported by Chittra and Benjamas (2011). Ahlgren and Hyenstrand (2003) reported that under nitrogen-deficient conditions, algal cells often accumulate a surplus of carbon metabolites as lipids. It was also reported that microalgae respond to the nitrogen starvation condition by degrading nitrogen containing macromolecules and accumulating carbon reserve compounds, such as polysaccharides and fats (Banerjee *et al*., 2002; Dayanand*et al*., 2005). Nitrogen is a key component of many biomolecules, such as protein, chlorophylls, and nucleic acids, all of which are required for algal growth (Li et al., 2016). Nitrogen deficiency or starvation frequently resulted in an increase in lipid and carbohydrate content at the expense of biomass productivity (Sun et al., 2018). Due to this trade-off, it is necessary to investigate the optimal nitrogen concentration and nitrogen starvation periods to increase the economic production of lipid-rich algal biomass.

Phylogenetic tree



REFERENCES

1. Banerjee, A., Sharma, R., Chisti, Y. and Banerjee, U. C. (2002). *Botryococcus braunii*: a renewable source of hydrocarbons and other chemicals. Crit. Rev. Biotechnol. 22: 245-279.
2. Becker, E. W. (1994b). Chemical Composition: Lipids. In: Microalgae: Biotechnology and microbiology. (Ed Becker, E. W.), 179-183. Cambridge University Press. Cambridge, UK.
3. Chittra Y, Benjamas, C. (2011) Effect of nitrogen, salt, and iron content in the growth medium and light intensity on lipid production by microalgae isolated from freshwater sources in Thailand. Bioresource Technology, 102: 3034-3040.
4. Dayananda C, Sarada R, Bhattacharya S, Ravishankar GA (2005). Effect of media and culture conditions on growth and hydrocarbon production by *Botryococcus braunii*.Pro.Biochem. 40: 3125-3131.
5. Gonçalves, A.L.; Pires, J.C.; Simões, M. A review on the use of microalgal consortia for wastewater treatment. Algal Res. 2017, 24, 403–415.
6. Grobbelaar, J. (2004). Mineral Nutrition. In: Handbook of microalgal culture (ed. Richmond A.), 104-106. Blackwell Publishing Company, 2121 State Avenue, Ames, Iowa, USA**.**
7. Hutagalung, R.A,,Sukoco, A.E, Soedharma, D. Goreti, L.M., Andrean, L., Bamma, E. &Noryawati M. (2014). Isolation, Identification and Growth Optimization of Microalgae Derived from Soft Coral Dendronephthya sp. ApcbeeProcedia, 10, 305-310.
8. KoichiroTamura , Glen stecher, Sudhirkumar. (2021) .MEG11: Molecular Evolutionary Genetic Analysis Version 11.Mplecular Biology and Evolution DOI:10.1093/molbev/msab120.
9. Li, T.; Xu, J.; Gao, B.; Xiang, W.; Li, A.; Zhang, C. Morphology, growth, biochemical composition and photosynthetic performance of *Chlorella vulgaris* (Trebouxiophyceae) under low and high nitrogen supplies. Algal Res. 2016, 16, 481–491.
10. Li, Y., D. Han, G. Hu, D. Dauvillee, M. Sommerfeld, S. Ball, and Q. Hu. (2010). Chlamydomonasstarchless mutant defective in ADP-glucose pyrophosphorylase hyper-accumulates triacylglycerol. Metab. Eng. 12: 387-391.
11. Moellering, E.R. and C. Benning. (2010). RNA interference silencing of a major lipid droplet protein affects lipid droplet size in Chlamydomonasreinhardtii. Eukaryot Cell. 9: 97- 106.
12. Pancha, I.; Chokshi, K.; George, B.; Ghosh, T.; Paliwal, C.; Maurya, R.; Mishra, S. Nitrogen stress triggered biochemical and morphological changes in the microalgae
13. Pohl, P**.** (1982). Lipids and fatty acids of microalgae. In: CRC handbook of biosolar resources. (Ed. Zaborsky, O. R.), 383-404. 1. CRC Press Inc., Boca Raton, FL, USA.
14. Scenedesmus sp. CCNM 1077. Bioresour. Technol. 2014, 156, 146–154.
15. Sun, Y.; Liao, Q.; Huang, Y.; Xia, A.; Fu, Q.; Zhu, X.; Fu, J.; Li, J. (2018) Application of growth-phase based light-feeding strategies to simultaneously enhance Chlorella vulgaris growth and lipid accumulation. Bioresour. Technol. 256, 421–430.
16. Szu-Ting WANG, Yi-Ying PAN, Chiu-Chin LIU, Lu-Te CHUANG, and Ching-Nen Nathan CHEN (2011). Physiology of micro algal oil body formation.Botanical Studies. 52: 305-312.
17. Tandeau-de-Marsac, N.; Houmard, J. (1993.) Adaptation of cyanobacteria to environmental stimuli: new steps towards molecular mechanisms. *FEMS Microb. Rev.*, *104*: 119–190.
18. Wang, Z.T., N. Ullrich, S. Joo, S. Waffenschmidt, and U. Goodenough. (2009). Algal lipid bodies: stress induction, purification, and biochemical characterization in wild-type and starchless Chlamydomonasreinhardtii. Eukaryot Cell 8: 1856-1868.

Table -1:Effect of nitrogen limitation on the growth and lipid content of Microalgae

Si. No	Different Sodium nitrate concentration	*Spirulina* sp						*Chlorella vulgaris*						*Botryococcus* sp					
		15th day		30th day		45th day		15th day		30th day		45thday		15th day		30th day		45th day	
		Biomass (g/L)	ipid(%)	Biomass (g/L)	Lipid (%)	Biomass (g/L)	Lipid (%)	Biomass (g/L)	Lipid (%)	Biomass (g/L)	Lipid (%)	Biomass (g/L)	Lipid (%)	Biomass (g/L)	Lipid (%)	Biomass (g/L)	Lipid (%)	Biomass (g/L)	Lipid (%)
1.	Control	4.0 ± 0.2$_{+}$	10.2 ± 0.1^{c}	5.2 ± 0.3^{d}	21.4 ± 0.2^{c}	9.3 ± 0.2^{e}	34.5 ± 0.2^{e}	4.5 ± 0.3^{c}	14.3 ± 0.4^{d}	7.2 ± 0.3^{e}	24.2 ± 0.2^{c}	10.3 ± 0.2^{d}	46.5 ± 0.1^{d}	1.9 ± 0.1^{c}	12.4 ± 0.2^{c}	3.9 ± 0.3^{e}	23.2 ± 0.1^{e}	5.7 ± 0.1^{e}	44.3 ± 0.1^{e}
2.	12.5g/l	3.6 ± 0.1^{c}	13.5 ± 0.2^{b}	4.8 ± 0.2^{d}	24.5 ± 0.2^{c}	8.1 ± 0.3^{d}	39.9 ± 0.4^{d}	3.9 ± 0.3^{b}	17.0 ± 0.2^{d}	6.8 ± 0.4^{d}	27.5 ± 0.4^{c}	9.5 ± 0.4^{c}	48.5 ± 0.1^{d}	1.4 ± 0.2^{b}	14.8 ± 0.2^{c}	3.0 ± 0.1^{d}	26.8 ± 0.3^{d}	4.9 ± 0.2^{d}	46.2 ± 0.3^{d}
3.	9.5g/l	3.2 ± 0.2^{c}	16 ± 0.4^{b}	4.4 ± 0.3^{c}	26.5 ± 0.2^{b}	6.4 ± 0.4^{c}	41.8 ± 0.3^{c}	3.3 ± 0.4^{b}	20.5 ± 0.2^{c}	6.4 ± 0.2^{c}	30.9 ± 0.2^{b}	8.9 ± 0.2^{b}	51.5 ± 0.4^{c}	1.0 ± 0.1^{b}	17.6 ± 0.1^{c}	2.9 ± 0.2^{c}	29.5 ± 0.4^{c}	2.4 ± 0.2^{c}	48.5 ± 0.4^{c}
4.	6.5g/l	2.8 ± 0.3^{b}	19.0 ± 0.1^{b}	3.8 ± 0.2^{b}	29.5 ± 0.2^{b}	5.2 ± 0.2^{b}	44 ± 0.2^{b}	2.9 ± 0.3^{a}	23.7 ± 0.4^{b}	5.2 ± 0.2^{b}	34.5 ± 0.3^{b}	8.2 ± 0.2^{b}	54.3 ± 0.3^{b}	0.9 ± 0.5^{a}	21.7 ± 0.2^{b}	2.2 ± 0.3^{b}	32.9 ± 0.4^{b}	2.3 ± 0.1^{b}	52.5 ± 0.3^{b}
5.	3.5g/l	2.4 ± 0.4^{a}	22.4 ± 0.3^{a}	3.3 ± 0.1^{a}	32.5 ± 0.2^{a}	4.8 ± 0.2^{a}	48 ± 0.1^{a}	2.3 ± 0.2^{a}	26.1 ± 0.3^{a}	4.7 ± 0.1^{a}	38.5 ± 0.3^{a}	7.4 ± 0.3^{a}	59.3 ± 0.2^{a}	0.7 ± 0.4^{a}	26.3 ± 0.2^{a}	1.7 ± 0.1^{a}	35.5 ± 0.1^{a}	1.9 ± 0.1^{a}	55.0 ± 0.4^{a}

Values are a mean of five determinants ± Standard deviation within a column different letters after values indicate that there is a significant difference at a P value of 0.05 as determined by Duncan multiple range tests.

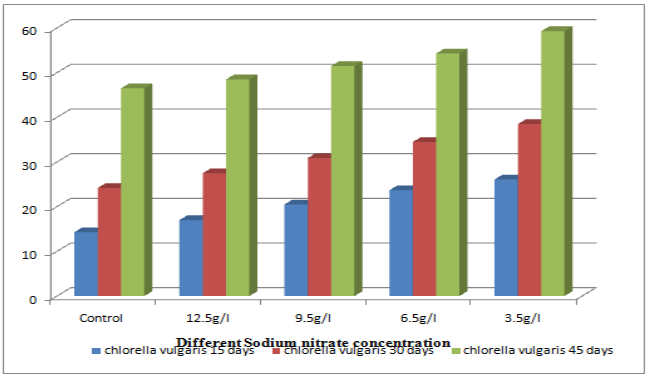


Fig.1: Effect of nitrogen limitation on the growth and lipid content of Microalgae

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RESEARCH ARTICLE

Formulation Optimazation and Characterization of an Inflammation Targeted Curcumin Loaded Nano Carriers for Ulcerative Colitis

B.Anish Babu[1]*, Margret Chandira Rajappa[2], Palanisamy Pethappachetty[2] and Sri Bendi Venkateswarlu[3]

[1]Research Scholar, Department of Pharmaceutics, Vinayaka Mission's Research Foundation, (Deemed to be University), Salem, Tamil Nadu, India.
[2]Professor, Department of Pharmaceutics, Vinayaka Mission's College of Pharmacy, Vinayaka Mission's Research Foundation, (Deemed to be University), Salem, Tamil Nadu, India.
[3]Professor and Principal, Department of Pharmaceutics, Vinayaka Mission's College of Pharmacy, Vinayaka Mission's Research Foundation, (Deemed to be University), Salem, Tamil Nadu, India.

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***Address for Correspondence**
B.Anish Babu,
Research Scholar,
Department of Pharmaceutics,
Vinayaka Mission's Research Foundation (Deemed to be University),
Salem, Tamil Nadu, India.
Email: bandreddyanesh92@gmail.com

ABSTRACT

The well-known polyphenol medicine curcumin exhibits excellent therapeutic potential. The limited bioavailability of this chemical is a significant issue, though. Mesoporous silica nanoparticles (MSN) have been used, as we recently demonstrated, to considerably increase curcumin bioavailability. In the current study, we compared curcumin and diclofenac sodium to curcumin to examine the anti-inflammatory benefits and associated adverse effects on the kidney and gastrointestinal system. By administering *carrageenan* to the feet of Wistar rats, experiments on the anti-inflammatory effects were carried out. Both macro- and microscopical observations of ulcerogenic processes were conducted. The average number of necrotic cells in the proximal tubule and distal tubules were examined using kidney histology. In the absence of substantial macroscopic and microscopic changes, the administration of peroral curcumin and curcumin-MSN resulted in anti-inflammatory action.

Keywords: curcumin, mesoporous silica nanoparticles (MSN), non-steroidal anti-inflammatory drugs, anti-inflammatory effects, kidney histopathology.

Classification numbers: 2.05, 4.02, 5.09

INTRODUCTION

Inflammation is the first response of the immune system to irritation or infection by germs. Inflammation has been considered as a major risk factor for cancer [1, 2]. Patients with inflammation, usually given treatment to slow or limit the process of tissue damage that occurs in the inflammatory area. The inflammatory mediator in the body is a prostaglandin, produced from arachidonic acid, has an essential role in the defense and repair of gastric epithelial cells, produces bicar- bonate mucus, inhibits parietal cell secretion, retains mucosal circulation, and restitution of epithelial cells [3]. The commonly used drug classes are non-steroidal anti- inflammatory drugs (NSAIDs). Diclofenac sodium is one of the most used NSAIDs. It was first introduced in the US in the 1990s and has been consumed by more than 1 billion patients. It is also one of the most prescribed NSAIDs [4]. These medications restrict COX enzyme activity, which has an impact on prostaglandin synthesis [1]. The COX enzymes come in two different varieties, COX-1 and COX-2 [5]. In addition to causing cardiovascular adverse effects, the inhibitory action mechanisms of COX-1 enzymes can produce gastrointestinal side effects. NSAIDs can have serious negative effects on the digestive system, including renal problems and stomach irritation that can result in peptic ulcers [5]. The negative consequences of NSAID usage have accelerated research into curcumin, a natural anti-inflammatory drug with fewer side effects. The active component of the dietary spice turmeric is called curcumin (Curcuma longa). The effectiveness of curcumin as a medicinal agent has been supported by several clinical trials and animal research [6]. Anti-inflammatory, antioxidant, and antiviral properties are acquired by curcumin. It has tremendous potential as a treatment for a number of pro-inflammatory chronic disorders, including AIDS, Parkinson's disease, multiple sclerosis, epilepsy, cardiovascular disease, cancer, allergy, asthma, bronchitis, colitis, rheumatoid arthritis, renal ischemia, and diabetes [7, 8].

According to Anand et al study's [9], oral administration of curcumin results in low blood and tissue concentrations as well as quick metabolism and elimination. The curcumin's low solubility is the main cause of this. There are numerous ways to deal with the solubility issue, including making nanoparticles [10] and adding a carrier component such mesoporous silica nanoparticles [11]. Numerous studies have demonstrated the advantages of employing mesoporous silica materials to boost the therapeutic effects of a variety of medications, including doxorubicin [12], paclitaxel [13, 14], and telmisartan (TEL) [15, 16]. Due to their great qualities, which include a very wide surface area, porous structure, ease of surface functionalization, and biodegradability, these materials have drawn a lot of attention as a drug delivery mechanism [16]. The oral medication delivery technique is also extremely promising according to these materials [15, 17, 18]. Curcumin drug delivery systems have been created utilizing mesoporous silica materials and a variety of strategies. These methods include the production of mesoporous silica nanoparticles (MSN) with guanidine functionalized PEGylation [19], curcumin-based MSN with lipid bilayer coating [20], curcumin-loaded silica encapsulated porous chitosan [21], mesoporous silica coated curcumin lipid core [22], curcumin silica composites with double functionalization [23], and composite hydrogel However, a lot of these synthesis techniques demand a difficult process, which restricts their usefulness. Curcumin-mesoporous silica materials must be produced using a straightforward process. In vitro test findings from the majority of investigations on curcumin-mesoporous silica composites were also encouraging. The therapeutic benefits of curcumin were improved by the drug delivery methods. Few in vivo studies have, however, been conducted to investigate the real consequences of the curcumin-mesoporous silica composite.

In order to demonstrate the notion of the composite's full potential, a thorough in vivo investigation on curcumin-mesoporous silica materials is urgently needed. In order to boost the bioavailability of curcumin, we recently published a simple approach for the manufacture of mesoporous silica nanoparticles (MSN). Curcumin content in mice's blood was three times greater after oral treatment of curcumin-MSN at an equal dosage of 10 mg kg-1 than after oral administration of curcumin alone (free curcumin). At all phases throughout the bioavailability test, the curcumin concentrations resulting from the administration of free curcumin were extremely low [25] The curcumin-MSN from our earlier studies was employed in the current investigation to assess the anti-inflammatory potential and associated biocompatibility. Carrageenan induction was used to test the curcumin-ability MSN's to reduce inflammation in white male Wistar rats. By comparing free curcumin and commercial diclofenac sodium to relevant

organs under microscopic and macroscopic inspection, the adverse effects of the curcumin-MSN to the digestive and renal system were evaluated (NSAIDs).

EXPERIMENTALS

MATERIAL AND METHODS

Aldrich supplied the 1,3,5-trimethylbenzene, 3-aminopropyl triethoxysilane, phosphate buffer tablet, triblock copolymer EO106PO70EO106 (Pluronic F127, MW = 13 400), Tween 80, diclofenac sodium, and carrageenan. It was decided to obtain a fluorocarbon surfactant (FC-4) from Yick-Vic Chemicals & Pharmaceuticals (HK) Ltd. Without purification, all compounds were utilized as obtained. 97% pure curcumin extracts were sourced from PT. Java plant in Solo, Indonesia.

Animals

White male Wistar rats, weighing 180–220 grams, were the experimental animals utilized in this investigation. They were Wistar rats at 6–8 weeks old. Animals were trained for one week under the same circumstances or treatment before being used in the study. Weight measurements and behavioral observations were made during this time. If an animal doesn't exhibit any signs of disease and loses no more than 10% of its starting weight, it is considered healthy and can be employed in research. The handling guidelines established by the ethics committee at Gadjah Mada University in Indonesia were strictly followed for all animal-related research (ethics committee approval number: 00094/04/LPPT/III/2017).

Synthesis of Curcumin loaded Nanoparticles

MSN were created by mixing 60 ml of HCl at a concentration of 0.02 M, 0.5 g of F127 as a main surfactant, and 1.4 g of FC-4. To get a clear solution, the three ingredients were thoroughly combined. Next, 0.5 g of TMB was added, and then 3 g of TEOS was added. 24 hours at 30 degrees Celsius were spent stirring. The solution was taken out of the stirring process and placed in an autoclave for a 24-hour hydrothermal treatment at 100°C. The result from the hydrothermal treatment was separated using a centrifuge technique, then washed and dried. Finally, the surfactants were eliminated using a calcination procedure. The calcination took place for 5 hours at 550 °C [26].

Amine functionalization

To create amine functionalized MSN, 3-aminopropyl triethoxysilane (APTES) was used to modify MSN. MSN was weighed up to 0.6 g, then 30 ml of toluene was added. The solution was heated while being agitated. 0.2 g of APTES was added once the solution's temperature reached 70 C. Another many hours went by as the stirring persisted. The product was then dried and sorted using a centrifugation process [25].

Curcumin loading

Curcumin-MSN was synthesised by using a rotary evapo- rator. Curcumin and MSN with ratio 1:4 (50 mg of curcumin and 200 mg of MSN) were mixed in 20 ml of ethanol. The mixture was first sonicated for 15 min. The solution was then heated and evaporated slowly at 55 ◦C. The process was per- formed under vacuum condition and continued until the dry curcumin-MSN was obtained [25].

Anti-inflammatory activity

18 hours were spent fasting in wistar rats. There were four groups of eight animals each among the animals. A control group makes up the first group. Water was administered for injection to the control group (Group-1) (WFI). The second group (Group-2) received a free dose of curcumin (10 mg kg1) in this instance. The curcumin-MSN concentration given to the third group (Group-3) was 50 mg kg1. (10 mg of curcumin and 40 mg of MSN are included in 50 mg of curcumin-MSN). Diclofenac sodium was administered to Group-4, the last group, at a dosage of 5 mg kg1. A 1% carrageenan intraplantar injection was used to test for inflammation in the rat's leg. The rats were

initially treated in accordance with the recommended group 60 minutes prior to the addition of induced carrageenan. The volume of the edema that was present was measured at 30, 60, 120, 180,mins using a mercury plethysmometer. The percentage of Edema Rate (%ER) and the percentage anti-inflammatory can be determined by using the following equations (1) and (2).

$$\%ER = \frac{Vt - V0}{V0} \times 100\%, \quad (1)$$

$$\text{Percentage of anti-inflammatory} = \frac{AUCp}{AUCk} \times 100\%. \quad (2)$$

Vt is the rat leg edema volume at time t, V0 is the initial volume of the rat leg, AUCk is the area under the curve for the control group, and AUCp is the area under the curve for the treatment group.

Ulcerogenic test

Wistar rats were fasted for 18 hours. The animals were divided into four groups each of 8. The first group is a control group. Water was administered for injection to the control group (Group-1) (WFI). The second group (Group-2) received a free dose of curcumin (10 mg kg1) in this instance. Group-3 got curcumin-MSN at a dosage of 50 mg kg1 (50 mg curcumin-MSN contains 10 mg curcumin and 40 mg MSN). Diclofenac sodium was administered to Group-4, the last group, at a dosage of 5 mg kg1. One treatment each day was given for seven days. Animals are dissected and removed out of their stomachs on the eighth day after being slaughtered and exposed on the operating table. The stomach is exposed over the white cork after being opened in a broad arch and cleaned/washed with 0.9% NaCl. The ulcerogenic effect was observed with gastric ulcer imaging, number of ulcers were calculated, and ulcer diameter was measured, then compared with the control group. The severity of the ulcer is expressed as the index of ulcer, which is calculated using equation (3). Ulcer index = A + B. (3) A is average number of ulcer, B is average diameter of ulcer [27]. The index data were analysed statistically, while the histopathology data of gastric ulcers were analysed descriptively.

Kidney histopathology observation

For 18 hours, Wistar rats were starved. There were four groups of eight animals each among the animals. A control group makes up the first group. Group-1, the control group, received water injections (WFI). The second group (Group-2) received a free dose of curcumin (10 mg kg1) in this instance. The curcumin-MSN concentration given to the third group (Group-3) was 50 mg kg1. (10 mg of curcumin and 40 mg of MSN are included in 50 mg of curcumin-MSN). The final group (Group-4) received a dose of 5 mg kg1 diclofenac sodium. One treatment each day was given for seven days. Animals are slaughtered on the eighth day, exposed on the operating table, the kidneys are removed, and the kidneys are deposited in organ pots with 10% formalin buffer. On renal preparations, hematoxylin-eosin (HE) staining was done before renal histopathologic observation, or counting the number of necrotic cells in the proximal tubes and distal contrast tubules. Five independent fields of vision were combined into one observation with a 1000 times magnification.

Statistical analysis

Data obtained from each group were analyzed using one-way ANOVA and when data had significant differences between the group of treatments, the analysis was followed by The Post Hoc Duncan test.

RESULT AND DISCUSSION

Similar to our earlier techniques, curcumin-MSN with ratios of 1 to 4 were prepared in this investigation. Before adding curcumin, MSN with particle sizes of around 100 nm, pores of 10 nm, and a cubic meso-structure were functionalized with the amine group (APTES) using rotary evaporator [25].

Anti-inflammatory activity

By comparing the percentage rate of edema development in rat foot with the percentage of anti-inflammatory qualities, researchers were able to show the anti-inflammatory test of free curcumin, curcumin-MSN, and diclofenac sodium against edema inhibition in male Wistar rats. Figure 1 demonstrates that for groups 2 and 3, the edema volume gradually grew, peaked at roughly 30–60 min, then started to decline. Group 4 had a very same pattern. It increased to a maximum of 30 minutes before decreasing. In comparison to the free curcumin group and diclofenac sodium, the curcumin-MSN therapy group (group-3) had the lowest percentage of the rate of edema development at the same time (180-240 min). Figure 1 demonstrates that for group 3, the rate of edema reached a minimum of three hours. This outcome is consistent with the curcumin release bioavailability test from MSN, which revealed a maximal concentration of three hours [25]. Our earlier research revealed a similar pattern between free curcumin and curcumin MSN. Both had a three-hour maximum concentration. While concentrations of free curcumin were never higher than 0.05 g ml1, those of curcumin from curcumin MSN grew from 0.019 to 0.029 g ml1. As a result, the curcumin concentrations from curcumin-MSN were 4 to 6 times greater [25].We believe the concentration of free curcumin was too small to generate the noticeable anti-inflam- matory effect.

That is why free curcumin did not show similar anti-inflammatory effects as curcumin-MSN. The best edema inhibition was produced at the highest concentration. For groups 3 and 4, the computed area under the curve (AUC04) values were quite close. Table 1 reveals that group 3 (curcumin-MSN) had a proportion of anti-inflammatory (21.06%), which was nearly identical to group 4 (diclofenac sodium): 23.33%. Group 2 (free curcumin) had the lowest proportion, which suggests that curcumin-MSN had a stronger anti-inflammatory impact than pure curcumin. According to Figure 1, diclofenac sodium and curcumin-MSN showed anti-inflammatory effects that were comparatively similar. Comparing curcumin-MSN to free curcumin, the performance was noticeably improved. We think this is because curcumin released from curcumin-MSN has a greater bioavailability than free curcumin, resulting in larger blood levels and stronger anti-inflammatory effects. Free cur- cumin has a rapid plasma clearance. It is important to make a complex of curcumin with other substance to increase the systemic bioavailability [28]. The oral medication delivery method called Curcumin-MSN enhanced the bioavailability of curcumin.

According to the bioavailability research, curcumin concentration from curcumin-MSN was three times greater in mice's blood than free curcumin. It was challenging to distinguish the curcumin concentration from free curcumin during the bioavailability test [25]. The curcumin particle size is constrained by the porous, nanoscale structure of MSN, which affects the solubility. The crystalline form of curcumin was transformed into an amorphous structure by loading it into the pores of MSN. Together, these elements improved curcumin's bioavailability. Additionally, MSN shields curcumin from the harshly acidic conditions found in the stomach [29]. There are several ways for curcumin to reduce inflammatory processes. It down-regulates cyclooxygenase-2 (COX-2), lipoxygenase, and inducible nitric oxide synthase (iNOS) enzymes. These conditions inhibit the inflammatory process and tumorigenesis [28]. However, curcumin alone had a lim- ited anti-inflammatory activity due to its low bioavailability. The encapsulation of curcumin into MSN is very important to increase curcumin bioavailability and finally revealed its true potency. Curcumin released from MSN had an equally potent of anti-inflammation compared to diclofenac sodium.

Ulcerogenic test

Rat's Stomach is the outcome of macroscopic observation. Counting the amount of ulcers in the rat's stomach allowed for macroscopic visualization of the ulcers. Table 2 in this study contains the findings of the index computation, and Figure 2 displays macroscopic photos of the examined animals. Based on the calculation of the ulcer index number (table 2), it was discovered that the ulcer index value in the curcumin-MSN group (group-3) was practically identical to the control group index (2.32 0.82). The value of group-3 was lower compared to the group given free curcumin. The largest ulcer index was in the group of diclofenac sodium which was 6.16 0.41. The ulcer index of group-4 was three times higher compared to group-3.The index number shows the severity of ulcers that occur in the stomach. The control group showed normal or absent gastric mucosal features as shown in figure 2(A). Figure 2. Macroscopic images of rat gastric mucosa. Group-1 (A), Group-2 (B), Group-3 (C) and Group-4 (D). The blue arrows point the ulcer (observed by magnifying glass). Note: Group-1: (control group) received WFI; Group-2:

received curcumin 10 mg kg−1; Group-3: received curcumin-MSN 50 mg kg−1 (curcumin 10 mg and MSN 40 mg); Group-4: received diclofenac sodium 5 mg kg−1. Because of this, sodium diclofenac had more negative effects than curcumin-MSN and free curcumin. The anti-inflammatory characteristics of curcumin result in the least amount of negative effects when used to treat stomach ulcers. A number of gastrointestinal diseases, including dyspepsia, Helicobacter pylori infection, peptic ulcer, irritable bowel syndrome, Crohn's disease, and ulcerative colitis, have been reported to respond well to curcumin [28]. Pruksunand *et al* study .'s [32] demonstrated the effectiveness of curcumin in curing ulcer.

Curcumin was administered orally for 12 weeks during phase II of a clinical trial including 45 patients, 25 of whom had varying sizes of ulcers between 0.5 and 1.5 cm in diameter. In 48% of individuals, the ulcers vanished after four weeks. For the other cases, it took 8–12 weeks to completely eradicate [32]. gastrointestinal examination of rats under a microscope. Microscopic findings comprised a descriptive descent on the rat stomach cross section by examining certain modifications (figure 3). The approach of computing five separate fields of view at around 100 cells was used to observe microscopic necrotic cells in the stomach under 1000 times magnification. Table 3 displays the findings of the examination of necrotic cells. The average number of necrotic cells was very low in Curcumin-MSN, and it was fairly similar in the control group. On the other hand, sodium diclofenac had the greatest quantity. Group 4 had twice as many necrotic cells as group 3 did. A depiction of the mucosal defect that extends into the submucosa, muscularis propria, or deeper is shown in Figure 3. Figure 3. Microscopic images of rat gastric mucosa in the control group and treatment groups were performed in 5 fields of view with 1000 magnification and eosin hematoxylin staining. Group-1 (A), Group-2 (B), Group-3 (C) and Group-4 (D). The yellow arrows point normal cells while the blue ones point necrotic cells. Note: Group-1: (control group) received WFI; Group-2: received curcumin 10 mg kg−1; Group-3: received curcumin-MSN 50 mg kg−1 (curcumin 10 mg and MSN 40 mg); Group-4: received diclofenac sodium 5 mg kg−1. Figure 3 displays the average number of necrotic cells in the control group, which averaged 7.30 3.06, in the stomach mucosa, which was nearly fully normal or did not exhibit a distinctive alteration (A). Compared to the group receiving 50 mg kg of curcumin-MSN, the group given 10 mg kg of free curcumin had more harm. According to figures 3(B), (C) and table 3, the average number of necrotic cells for curcumin and curcumin-MSN was 10.86 3.97 and 9.46 2.40, respectively. Comparatively, diclofenac sodium at a dosage of 5 mg kg1 caused the greatest amount of cell damage, 22.76 5.68. The computation of the ulcer index on the stomach mucosa for the group receiving diclofenac sodium was substantially different from the other treatment groups, according to the results of the ANOVA test (Fcount(25.698) >Ftable(3.10)).

In addition, the diclofenac sodium group had a higher mean number of stomach mucosal necrotic cells than the other treatment groups (Fcount(19.173) >Ftable(3.10)). According to the findings above, curcumin and curcumin-MSN exhibited less severe side effects than diclofenac sodium after being administered orally for seven days. The proportion of necrotic cells in groups 2 and 3 was comparable. Both were fairly similar to the control group in terms of having few necrotic cells. In contrast, group-4 had the highest number of necrotic cells. A number of necrotic cells in group-4 were double than that of group-2 and group-3. Many reasons cause necrotic cell death such as trauma, infec- tion, toxins, and others. Necrotic cells are related to the cell swelling and rapid loss of membrane integrity [33, 34]. Necrosis is the death of cells and tissues in the living body. The cell nucleus exhibits changes during necrosis, including the loss of the chromatin image, the core being wrinkled and no longer vesicular, appearing thicker and dark black (pyknosis), and the core being fragmented into fragments. The core no longer accepts many colors on the coloring due to its pale, unreal appearance, which gradually disappears (karyolysis). Because of its anti-inflammatory properties, curcumin has the unusual ability to destroy cancer cells while sparing healthy ones. However, curcumin does not cause normal cells to undergo apoptosis [34]. Curcumin was proven to have no effect on typical rat hepatocytes. High biocompatibility with healthy cells was demonstrated [35].Histopathology test on rat kidney.Group3. had a very low number of necrotic cells inprox -imaltubules at 4.83 similar to group 2 (table 4). In contrast, diclofenac sodium caused the highest necrotic cells at 10.67.

The cell nucleus undergoes changes during necrosis, including the loss of the chromatin image, the core becoming wrinkled, not vesicular anymore, looking denser and dark black (pyknosis), the core being divided into fragments,

the core being torn (karyorrhexis), and the core no longer taking many colors on the coloring because it appears pale and unreal before disappearing altogether (karyolysis). With its anti-inflammatory properties, curcumin has the rare ability to destroy cancer cells while sparing healthy cells. Curcumin causes cancer cells to undergo apoptosis but not healthy ones [34]. Curcumin was proven to have no effect on typical rat hepatocytes. High biocompatibility with healthy cells was demonstrated [35].Histopathology test on rat kidney Similar to group 2, group 3 exhibited a very low number of necrotic cells in proximal tubules at 4.83 (table). 4). In contrast, diclofenac sodium caused the highest necrotic cells at 10.67. Figure 4 shows the fundamental differences between normal cells and necrotic cells in the proximal tubule section. Figure 4. Microscopic overview of the Wistar rats in the proximal tubule section was performed in 5 fields of view with 1000magnificationandeosinhematoxylinstaining.Group-1(A),Group-2(B), Group-3 (C) and Group-4 (D). Note: Group-1: (control group)received WFI; Group-2: received curcumin 10 mg kg^{-1}; Group-3: received curcumin-MSN50mgkg^{-1}(curcumin10mgandMSN40mg);Group-4:received diclofenac sodium5mgkg^{-1}.

CONCLUSION

Peroral curcumin-MSN (50 mg kg1) dosing resulted in potent anti-inflammatory action that is essentially identical to diclofenac sodium. Free curcumin (10 mg/kg) and curcumin-MSN (50 mg/kg) supplementation did not appear to significantly alter the gastric organ's macroscopic or microscopic structure. Diclofenac sodium (5 mg kg1) when administered orally caused significant changes in the macroscopic and microscopic stomach, including the development of gastric ulcers and necrotic cells, indicating that curcumin and curcumin-MSN were more effective at treating inflammation while having fewer side effects than diclofenac sodium (NSAID group drugs). Peroral curcumin extract and curcumin-MSN treatment exhibited a minimal impact on the necrosis of proximal tubule cells and distal tubule cells. Therefore, curcumin-MSN can be regarded as a safe medicine as opposed to generic NSAIDs that may induce gastro-intestinal toxicity and associated harm to renal difficulties. Therefore, we created an oral curcumin-MSN drug delivery system with a high anti-inflammatory effectiveness and minimal side effects. The strong anti-inflammatory properties open the possibility.

REFERENCES

1. Hadisoewignyo L, Hartono SB, Kresnamurti A, Soeliono I, Nataline Y, Prakoso GA, Aulia DA. Evaluation of anti-inflammatory activity and biocompatibility of curcumin loaded mesoporous silica nanoparticles as an oral drug delivery system. Advances in Natural Sciences: Nanoscience and Nanotechnology. 2018 Aug 14;9(3):035007.
2. Isailovic N., Daigo K., Mantovani A., Selmi C. Interleukin-17 and innate immunity in infections and chronic inflammation. *J. Autoimmun.* 2015;60:1–11. doi: 10.1016/j.jaut.2015.04.006. [PubMed] [CrossRef] [Google Scholar]
3. Pedraza-Alva G., Pérez-Martínez L., Valdez-Hernández L., Meza-Sosa K.F., Ando-Kuri M. Negative regulation of the inflammasome: Keeping inflammation under control. *Immunol. Rev.* 2015;265:231–257. doi: 10.1111/imr.12294. [PubMed] [CrossRef] [Google Scholar]
4. Lucas S.M., Rothwell N.J., Gibson R.M. The role of inflammation in CNS injury and disease. *Br. J. Pharmacol.* 2006;147:S232–S240. doi: 10.1038/sj.bjp.0706400. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
5. Rock K.L., Lai J.J., Kono H. Innate and adaptive immune responses to cell death. *Immunol. Rev.* 2011;243:191–205. doi: 10.1111/j.1600-065X.2011.01040.x. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
6. Fernandes J.V., Cobucci R.N., Jatobá C.A., Fernandes T.A., de Azevedo J.W., de Araújo J.M. The role of the mediators of inflammation in cancer development. *Pathol. Oncol. Res.* 2015;21:527–534. doi: 10.1007/s12253-015-9913-z. [PubMed] [CrossRef] [Google Scholar]
7. Heppner F.L., Ransohoff R.M., Becher B. Immune attack: The role of inflammation in Alzheimer disease. *Nat. Rev. Neurosci.* 2015;16:358–372. doi: 10.1038/nrn3880. [PubMed] [CrossRef] [Google Scholar]
8. Loane D.J., Kumar A. Microglia in the TBI brain: The good, the bad, and the dysregulated. *Exp. Neurol.* 2016;275:316–327. doi: 10.1016/j.expneurol.2015.08.018. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

9. Waisman A., Liblau R.S., Becher B. Innate and adaptive immune responses in the CNS. *Lancet Neurol.* 2015;14:945–955. doi: 10.1016/S1474-4422(15)00141-6. [PubMed] [CrossRef] [Google Scholar]
10. Vignali D.A., Kuchroo V.K. IL-12 family cytokines: immunological playmakers. *Nat. Immun.* 2012;13:722–728. doi: 10.1038/ni.2366. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
11. Montgomery S.L., Bowers W.J. Tumor necrosis factor-alpha and the roles it plays in homeostatic and degenerative processes within the central nervous system. *J. Neuroimmune. Pharmacol.* 2012;7:42–59. doi: 10.1007/s11481-011-9287-2. [PubMed] [CrossRef] [Google Scholar]
12. Zelová H., Hošek J. TNF-α signalling and inflammation: interactions between old acquaintances. *Inflamm. Res.* 2013;62:641–651. doi: 10.1007/s00011-013-0633-0. [PubMed] [CrossRef] [Google Scholar]
13. Fenton M.J. Review: Transcriptional and post-transcriptional regulation of interleukin 1 gene expression. *Int. J. Immunopharmacol.* 1992;14:401–411. doi: 10.1016/0192-0561(92)90170-P. [PubMed] [CrossRef] [Google Scholar]
14. Rider P., Carmi Y., Voronov E., Apte R.N. Interleukin-1α *Semin. Immunol.* 2013;25:430–438. doi: 10.1016/j.smim.2013.10.005. [PubMed] [CrossRef] [Google Scholar]
15. Langrish C.L., McKenzie B.S., Wilson N.J., de Waal Malefyt R., Kastelein R.A., Cua D.J. IL-12 and IL-23: master regulators of innate and adaptive immunity. *Immunol. Rev.* 2004;202:96–105. doi: 10.1111/j.0105-2896.2004.00214.x. [PubMed] [CrossRef] [Google Scholar]
16. Duvallet E., Semerano L., Assier E., Falgarone G., Boissier M.C. Interleukin-23: A key cytokine in inflammatory diseases. *Ann. Med.* 2011;43:503–511. doi: 10.3109/07853890.2011.577093. [PubMed] [CrossRef] [Google Scholar]
17. Sabat R. IL-10 family of cytokines. *Cytokine Growth Factor Rev.* 2010;21:315–324. doi: 10.1016/j.cytogfr.2010.11.001. [PubMed] [CrossRef] [Google Scholar]
18. Ng T.H., Britton G.J., Hill E.V., Verhagen J., Burton B.R., Wraith D.C. Regulation of adaptive immunity; The role of interleukin-10. *Front. Immunol.* 2013;31:129. doi: 10.3389/fimmu.2013.00129. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
19. Kwilasz A.J., Grace P.M., Serbedzija P., Maier S.F., Watkins L.R. The therapeutic potential of interleukin-10 in neuroimmune diseases. *Neuropharmacology.* 2015;96:55–69. doi: 10.1016/j.neuropharm.2014.10.020. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
20. Goetzl E.J., An S., Smith W.L. Specificity of expression and effects of eicosanoid mediators in normal physiology and human diseases. *FASEB J.* 1995;9:1051–1058. [PubMed] [Google Scholar]
21. Leff J.A., Busse W.W., Pearlman D., Bronsky E.A., Kemp J., Hendeles L., Dockhorn R., Kundu S., Zhang J., Seidenberg B.C., et al. Montelukast, a leukotriene-receptor antagonist, for the treatment of mild asthma and exercise-induced bronchoconstriction. *N. Engl. J. Med.* 1998;339:147–152. doi: 10.1056/NEJM199807163390302. [PubMed] [CrossRef] [Google Scholar]
22. Peters-Golden M., Henderson W.R. Leukotrienes. *N. Engl. J. Med.* 2007;357:1841–1854. doi: 10.1056/NEJMra071371. [PubMed] [CrossRef] [Google Scholar]
23. Zhao J., Quyyumi A.A., Patel R., Zafari A.M., Veledar E., Onufrak S., Shallenberger L.H., Jones L., Vaccarino V. Sex-specific association of depression and a haplotype in leukotriene A4 hydrolase gene. *Psychosom. Med.* 2009;71:691–696. doi: 10.1097/PSY.0b013e3181b05c57. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
24. Moncada S., Bolanos J.P. Nitric oxide, cell bioenergetics and neurodegeneration. *J. Neurochem.* 2006;97:1676–1689. doi: 10.1111/j.1471-4159.2006.03988.x. [PubMed] [CrossRef] [Google Scholar]
25. Rayet B., Gélinas C. Aberrant rel/nfkb genes and activity in human cancer. *Oncogene.* 1999;18:6938–6947. doi: 10.1038/sj.onc.1203221. [PubMed] [CrossRef] [Google Scholar]
26. Oeckinghaus A., Hayden M.S., Ghosh S. Crosstalk in NF-κB signaling pathways. *Nat. Immunol.* 2011;12:695–708. doi: 10.1038/ni.2065. [PubMed] [CrossRef] [Google Scholar]
27. Ling J., Kumar R. Crosstalk between NFkB and glucocorticoid signaling: A potential target of breast cancer therapy. *Cancer Lett.* 2012;322:119–126. doi: 10.1016/j.canlet.2012.02.033. [PubMed] [CrossRef] [Google Scholar]
28. Khalifa A.B. *Herbs: Nature's Pharmacy.* 1st ed. Arab Cultural Center; Casablanca, Morocco: 2004. pp. 286–288. [Google Scholar]

29. Lindequist U., Niedermeyer T.H.J., Jülich W.D. The pharmacological potential of mushrooms. *Evid. Based Complement. Altern. Med.* 2005;2:285–299. doi: 10.1093/ecam/neh107. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
30. Elsayed E.A., El Enshasy H., Wadaan M.A.M., Aziz R. Mushrooms: A potential natural source of anti-inflammatory compounds for medical applications. *Mediat. Inflamm.* 2014 doi: 10.1155/2014/805841. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
31. Konturek SJ, Konturek PC, Pawlik T, Sliwowski Z, Ochmański W, Hahn EG. Duodenal mucosal protection by bicarbonate secretion and its mechanisms. Journal of physiology and pharmacology: an official journal of the Polish Physiological Society. 2004 Jul 1;55:5-17.
32. Takahashi M, Suzuki K, Kim HK, Otsuka Y, Imaizumi A, Miyashita M, Sakamoto S. Effects of curcumin supplementation on exercise-induced oxidative stress in humans. International journal of sports medicine. 2014 Jun;35(06):469-75.

Table 1. The value of AUC0–4 and percentage of anti-inflammatory

S. No	AUC0–4 (μg min ml–1)	Anti-inflammatory (%)
1	(WFI)	2.18 ±0.17
2	(Curcumin)	1.82±0.12
3	(Curcumin-MSN)	1.72±0.07
4	(Diclofenac-sodium)	1.67±0.11

Note. Group-1: (control group) received WFI; Group-2: received curcumin 10 mg kg–1; Group-3: received curcumin-MSN 50 mg kg–1 (curcumin 10 mg and MSN 40 mg); Group-4: received diclofenac sodium 5 mg kg–1.

Table 2. Ulcer index

		Mean ulcer score					
S. No	Group	ulcer	number	ulcer	diameter	ulcer	index
1	(WFI)	1.00	±0.00	1.00	±0.00	2.00	±0.0
2	(Curcumin)	1.33	±0.81	1.33	±0.81	2.66	±1.2
3	(Curcumin-MSN)	1.16	±0.41	1.16	±0.41	2.32	±0.2
4	(Diclofenac sodium)	3.16	±0.41	3.00	±0.00	6.16	±0.41

Note. Group-1: (control group) received WFI; Group-2: received curcumin 10 mg kg–1; Group-3: received curcumin-MSN 50 mg kg–1 (curcumin 10 mg and MSN 40 mg); Group-4: received diclofenac sodium 5 mg kg–1. (2.00 0.00).

Table 3.The average number of necrotic cells in rat stomach with five fields of view.

S. No	Group	Average	number of necrotic cells
1	(WFI)	7.30	3.06
2	(Curcumin)	10.86	3.97
3	(Curcumin-MSN)	9.46	2.40
4	(Diclofenac sodium)	22.76	5.68

Note.Group-1:(controlgroup)receivedWFI;Group-2:receivedcurcumin10mgkg^{-1};Group-3:receivedcurcumin-MSN50mgkg^{-1}(curcumin 10mgandMSN40mg);Group-4:received Diclofenacsodium5mgkg^{-1}.

Table 4.Numberof necrotic cells in proximal tubule.

1	(WFI)	3.50	1.3
2	(Curcumin)	4.83	0.75
3	(Curcumin-MSN)	4.83	1.94
4	Sodium diclofenac	10.67	4.50

Table5.Number of necrotic cells on distal tubule

1	(WFI)	4.67	1.6
2	(Curcumin)	6.33	3.5
3	(Curcumin-MSN)	7.67	6.28
4	(diclofenac sodium)	9.17	4.62

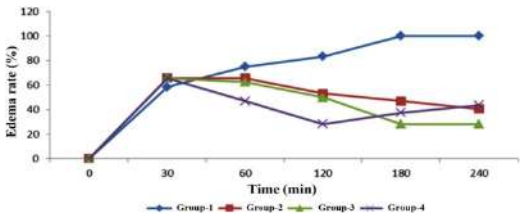

Figure 1. The relationship between the percentages of edema formation rate versus time (minutes). Note: Group-1: (control group) received WFI; Group-2: received curcumin 10 mg kg–1; Group-3: received curcumin-MSN 50 mg kg–1 (curcumin 10 mg and MSN 40 mg); Group-4: received diclofenac sodium 5 mg kg–1.

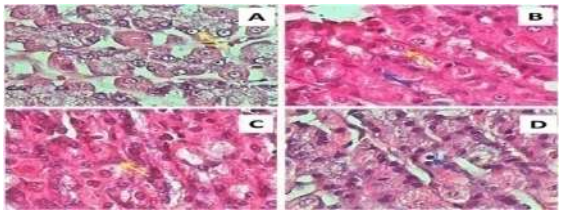

Figure 2. Macroscopic images of rat gastric mucosa. Group-1 (A), Group-2 (B), Group-3 (C) and Group-4 (D). The blue arrows point the ulcer (observed by magnifying glass). Note: Group-1: (control group) received WFI; Group-2: received curcumin 10 mg kg–1; Group-3: received curcumin-MSN 50 mg kg–1 (curcumin 10 mg and MSN 40 mg); Group-4: received diclofenac sodium 5 mg kg–1

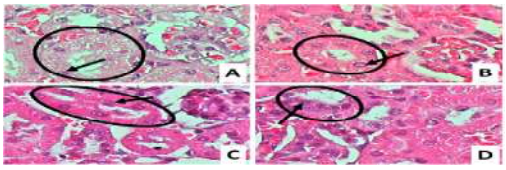

Figure 3. Microscopic images of rat gastric mucosa in the control group and treatment groups were performed in 5 fields of view with 1000 magnification and eosin hematoxylin staining. Group-1 (A), Group-2 (B), Group-3 (C) and Group-4 (D). The yellow arrows point normal cells while the blue ones point necrotic cells. Note: Group-1: (control group) received WFI; Group-2: received curcumin 10 mg kg–1; Group-3: received curcumin-MSN 50 mg kg–1 (curcumin 10 mg and MSN 40 mg); Group-4: received diclofenac sodium 5 mg kg–1.

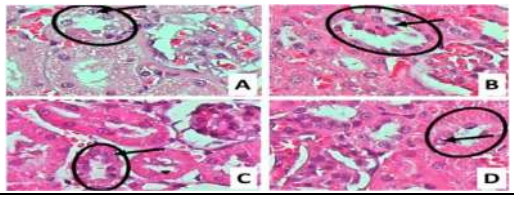

Figure 4. Microscopic overview of the Wistar rats in the proximal tubule section was performed in 5 fields of view with 1000 magnification and eosin hematoxylin staining. Group-1(A),Group-2(B), Group-3 (C) and Group-4 (D). Note: Group-1: (control group)received WFI; Group-2: received curcumin 10 mg kg^{-1}; Group-3:receivedcurcumin-MSN50mgkg^{-1}(curcumin 10mg and MSN 40mg);Group-4:received diclofenac sodium 5mg kg^{-1}.

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RESEARCH ARTICLE

Psychological Factors Affecting the Mental Health: A Study on Elite Wushu Athletes

Dilshith A. Kabeer[1]*, S. Binthu Mathavan[2], Vaishali Khatri[3], Nafih Cherappurath[4] and K. Binoy[5]

[1]Assistant Professor, Department of Physical Education and Sports, Pondicherry University, Kalapet, Puducherry, India.
[2]Assistant Professor, Department of Physical Education, Central University of Punjab, Bathinda, Punjab, India.
[3]Student, Department of Physical Education, Central University of Punjab, Bathinda, Punjab, India
[4]Assistant Professor, Department of Physical Education, Amal College of Advanced Studies, Nilambur, Kerala, India.
[5]Assistant Professor, Department of Physical Education, Government Arts and Science College, Calicut, Kozhikode, Kerala, India.

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***Address for Correspondence**
Dilshith A. Kabeer,
Assistant Professor,
Department of Physical Education and Sports,
Pondicherry University,
Kalapet, Puducherry, India.
Email: : dilshid7@gmail.com

ABSTRACT

The study aims to compare the psychological elements that affect the mental health of national and international wushu athletes. It includes the psychological characteristics such as sports competition anxiety, task, ego orientation, and mental health of athletes. In total, 37 Wushu athletes (International = 17 and National = 17) aged between (International: *M*age= 16.4 years; *SD* =1.14; National: *M*age= 15.8 years; *SD* =0.92) completed a questionnaire on Sports Competition Anxiety (SCAT), Assignment and Ego Orientation in the Sports Questionnaire (TEOSQ) and the Mental Health scale by Peter Becker. The study covers the demographic profiles and past playing experiences of athletes. Correlation and regression were used to identify factors that highly influence mental health. In comparison to national-level wushu players, international wushu players, both men and women, have greater mental health, according to this study. In both international and national level wushu players, the sports competition anxiety variable has a detrimental impact on mental health, whereas the other two variables, task and ego orientation, have a low-level beneficial impact. Furthermore, this study found that anxiety (SCAT) negatively impacted performance. Based on the results of this analysis, anxiety (SCAT) has a negative impact on sports performance; hence, anxiety among selected national and international level wushu players should be

reduced. In addition, while the other two tasks and ego orientation have a low positive correlation, they may have a high positive effect on performance. To do this, we need additional psychological training sessions to control the phenomena mentioned above to enrich performance.

Keywords: Sports Competition anxiety, Task and Ego Orientation, Mental health

INTRODUCTION

Wushu is a full-contact martial art that has been practiced in China for over 4,000 years. The biological sciences, literature, philosophy, ethics, and religion have influenced it (McAnulty *et al*., 2016). It now contains more ingredients and is better for your physical and mental wellbeing. It has become a well-known modern sport and type of physical activity that promotes health because of its different competitive characteristics and artistic manifestations. There are two types of wushu events: 'taolu' and 'sansoo'. Tai Chi, Nanquan (NQ) and Changquan (CQ) are among the Taolu events, while the sansoo tournament features eleven weight classes. Taolu, a set of effective movement patterns to strengthen the body's balance and stamina, forms CQ and NQ. CQ and NQ have many societal benefits, such as better health, willpower, personality development, and cultural enrichment (Tsang *et al*., 2010). Sports psychology is a fast-growing field that attempts to understand better, diagnose, treat and rehabilitate all types of athletes (Carr, 2006). There is little research on professional athletes' mental health and psychological well-being, although there is a growing interest in it (Baron *et al*., 2013; McDuff, 2012; Glick *et al*., 2012). Aspects of social, physical, spiritual, economic and mental life are balanced with mental health. People evaluate themselves, their lives, and others in various situations and consider options such as stress management and decision making (Bellenir, 2010). Mental health is an individual's ability to realize their potential, manage everyday life stressors, work efficiently, and give their best (Souter *et al.,* 2018).However, other research has suggested that because of their training and experience, athletes tend to display less anxiety(Gould *et al*., 1984). Concerns exist regarding how anxiety affects performance depending on the type of sport (individual or team), the athlete's gender, and other factors (Al-ansari, 2006; Lorimer, 2006; Halbreich & Kahn, 2007; Rudigers *et al*., 2007), as well as the athlete's experience in specific sports, and their abilities. Numerous studies on individual sports, including taekwondo and similar sport have been done (Finkerberg *et al*., 1992), golf (Pons *et al*., 1999), and swimming.

In addition to team sports like indoor soccer, futsal and basketball (Jones & Swain, 1992). Mental wellness depends on maintaining a sense of equilibrium (Bellenir, 2010). You may need to rebalance if you push the steadiness too far. Peluso *et al*. (2005) found that elite athletes experience unique mental and physical health obstacles, putting them at higher risk for mental health concerns and risk-taking. Athletes' reactions and perceptions of these stressors can substantially impact their mental health and athletic performance (Lazarus, 2000). Because of stigma, a lack of knowledge about mental health and how it may affect performance, as well as the athletes perceiving that seeking help is a sign of weakness, athletes do not seek care for mental health issues (Gulliver *et al*., 2012). Simultaneously, it is well acknowledged that physical activity benefits mental health (Hamer *et al*., 2009; Daley, 2008). Sports Competition anxiety is an emotion that arises due to the interpretation and evaluation of the situation encountered (Cox, 2007). It is one of the most critical and important areas of study in sports psychology, and it continues to attract much research attention (Weiss & Gill, 2005). In sports, especially among elite athletes, the ability to deal with stress and anxiety is important (Hardy *et al*., 1996; Orlick & Partington, 1988). Anxiety occurs when an athlete doubts their ability to cope with stress (Hardy *et al*., 1996). There are still many unknown concerns about how high-level competitive athletes deal with anxiety (Woodman & Hardy, 2001; Gould *et al*., 2002). Some researchers argue that low trait anxiety is vital for athletic achievement (Humara, 1999). Task orientation emphasizes improving one's skills, acquiring knowledge, and the belief that one must strive to understand the task and work with peers to achieve it.Ego orientation is the drive to the desire to do better than others and the belief that success requires more skills than external standards (Duda & Nicholls, 1992).

Duda (1989) developed the Task and Ego Orientation Questionnaire in Sport to measure whether a person is task-oriented or ego while performing sports (TEOSQ). Nicholls (1984, 1989) observed that task-oriented persons concentrate on learning and adopt more intrinsic motive schemes, such as ability growth and enjoyment, whereas ego-oriented people see their skills and triumphs from a recognized perspective (Papaioannou&Theodorakis, 1994). Diggelidis and Krommydas (2008) claim that task and ego have opposing viewpoints on competence and success. In contrast, ego-oriented athletes are more concerned with their interests, but task-oriented athletes exhibit pro-social behaviours and judgments. These people view achievement differently, with less effort and are influenced by extrinsic rewards. People with more ego will try to exceed their peers and believe it will lead to success. More task-oriented people believe success depends on new efforts, interests, and abilities (Treasure & Roberts, 1995). Most research suggests that task orientation is associated with moral behaviour, while ego orientation is associated to unsportsmanlike behaviour (Dunn & Dunn, 1999; Proios *et al.,* 2004).

In contrast to these findings, According to some of the researchers, task orientation and moral behaviour are unrelated. (Kavussanou, 1997; Stephens & Bredemeier, 1996). Developing a thorough grasp of athletes' mental health and psychological well-being could lead to better care, management, and performance gains. This information is required for sports experts such as coaches, doctors, and sports psychologists to assist elite athletes in learning how to deal with difficult situations and improve their emotional health (Nicholls & Polman, 2007). Knowing what elements affect an athlete's mental health can help them to enhance their performance by focusing on those factors. In the context of sports, several things may affect mental health. We will focus on sports competition anxiety, ego and task orientation in this study, all of which have been linked to mental health. More research across various sports and disciplines is needed for a better understanding. To the best of our knowledge, no studies have examined the psychological elements that affect the mental health of wushu athletes. In India, no study has been conducted to compare the differences between national and international wushu athletes, especially in psychological phenomena.

MATERIALS AND METHODS

Study Design

All data were obtained at once by using purposive random sampling. The study was approved by the research committee of the Central University of Punjab, Bathinda. For data collection, prior authorization was obtained from the organizing committee of the Junior and Youth National Wushu Championship-2020 in Fatehabad, Haryana, and informed consent was given by the participants. The participants were briefed on the study's purpose and requirements. The questionnaires were given within an instruction leaflet. After their completion, the questionnaires were collected to analyse the results.

Participants

The study sample consisted of 34 wushu athletes from various states in India who competed at national and international levels. A total of 17 international athletes aged (*M*age = 16.4 years; *SD* = 1.14) years and with playing experience of (6.85 years; *SD* = 1.84) years have participated. Similarly, 17 national athletes with (National: *M*age = 15.8 years; *SD* = 0.92) years of age and (4.94 years; *SD* = 1.74) years of experience have participated.

MATERIALS

Demographic Questionnaire

It contains gender, age, height, weight, sport, years of experience, top achievement in sport, number of competitions in a year, and competitive level.

Sport Competition Anxiety Test (SCAT) Questionnaire

The 15 questions on the Martens Sports Competition Anxiety and, Test range in score from 10 to 30. Higher scores indicate greater anxiety. The reliability of the Martens test is 0.77 (Martens *et al*., 1990). In this study, we described the SCAT questionnaire to athletes struggling with English. We tested the questionnaire on 40 master's degree students

from the Central University of Punjab, Bathinda, India, to ensure the reliability of the questionnaire. SCAT was reliable ($\alpha = 0.82$) in this sample. For this study's assessment, the questionnaire sports competition anxiety was used.

Task and Ego Orientation in Sport Questionnaire (TEOSQ)

The Task and Ego Orientation in Sports Questionnaire (TEOSQ), adapted from Nicholls (1989), was used to test the level of task and ego orientation. In addition to being asked for recollect a time when they felt most successful in their sport, the athletes were also asked to respond 13 questions based on ego- and task-oriented criteria. A 5-point Likert scale (1 =strongly disagree, 5 = strongly agree) was used to indicate how strongly one agreed or disagreed with a statement.

Mental Health Questionnaire

Peter Becker (1989) designed a mental health questionnaire and accessed based on item response theory with a statistical techniques conformation. It consists of 20 elements that measure the mental health of athletes. Each question contains four answers, one of which must be marked by the athlete. On a 4-point Likert scale, responses ranged from 1 (always) to 4 (never). The questionnaire proved validation with (r = 85), and the total score is derived from the total number of items and subscales.

Statistical Analysis

All of the data were examined by using SPSS 21. The reliability of each scale was ascertained, and all variables were standardized (i.e., mean centred) before proceeding to interpret. Descriptive statistics were performed to detect differences among national and international wushu athletes. The impact of task and ego orientation on mental health was investigated via correlation and regression analysis.

RESULTS

The correlation results (table 2) indicate that, there is a negative significant correlation, which means less anxiety and higher will be mental health. Whereas ego and task have positive correlation towards mental health. The correlation with mental health and ego shows a significant positive correlation. The lower correlation among independent variables indicates that, the multi collinearity issues are not affected. The results of regression analysis (table 3), mental health of the players is taken as dependent variables and anxiety, ego and task are taken as dependent variable. The results explain that anxiety of the players has a significant negative effect on the mental health of the players. It indicates that, if the anxiety is less the mental health of the players will be good and vice versa. Whereas the Ego of the players has significant negative influence on the mental health of the players. If means that, if the ego is high the mental health of the players also will be high. Similarly, the task variable also having a positive impact on the health, but the influence is not statistically significant. The chi-square value shows that, the model is fit and can be used for the further analysis. The R-square value shows that, nearly 25 percentage of the variance in the dependent variables is due to the independent variables.

DISCUSSION

The present study investigated the factors affecting the mental health of elite wushu athletes. Additionally, the national athletes were compared with international wushu athletes who represented India. In comparing both groups, it is evident that the international players had lower anxiety, especially for men, compared to women. Sports Competition Anxiety is a negative emotion that affects perceptions in sports competition. Most athletes perceive competition anxiety as destructive, leading to performance decline (Weinberg & Gould, 2019; Hanin, 2000). Heavy playing schedules, competition for team slots, the media, and trophy pressure lead the athletes to high anxiety levels (Heather, 2010). The researcher found that national players have more sports competition anxiety than international athletes due to their lack of playing experience. A similar study was found for gymnasts (Jones *et al*., 1993) and basketball players (Swain & Jones, 1996). Gould *et al*. (1984) found that the more experienced athletes have a lower level of anxiety. Perry and Williams (1998) also found that professional tennis players participating in the sport for

years have less anxiety than novice players. The most important finding was that the players competing at a higher level of competition had a low level of anxiety. Although athletes at a high level would logically seem more anxious, these findings suggest that elite athletes might have learned to control their anxiety than national athletes better.

Many studies revealed that succeeding in competition depends on how athletes handle their anxiety levels (Humara, 1999). According to Parnabas *et al*. (2000), competitive anxiety can lead to poor performance and dropout. It is crucial to recognise the level of anxiety and reduce it. Studies found that sports competition anxiety is higher for amateur athletes in individual sports compared to team sports (Simon & Martens, 1977). In addition, individual non-contact sports participants have lower anxiety levels than individual contact sports (Lowe & McGrath, 1971). According to the IZOFs (Individualized Zones of Optimal Functioning) theory (Hanin, 1980, 1986), each person has an optimal degree of pre-performance anxiety for peak performance. Performance suffers if pre-performance anxiety is too high or too low (Hanin, 1980, 1986). Cognitive and somatic anxiety can negatively impact athletes' performance (Singh & Gaurav, 2011; Martens, Vealey & Burton, 1990). Cognitive anxiety affects mentally like negative expectations about achievement or self-evaluation, negative self-talk, performance anxieties, and interrupted attention (Singh & Gaurav, 2011; Martens, Vealey & Burton, 1990). Somatic anxiety refers to a physiological component which leads to autonomic arousal, nervousness, elevated blood pressure, muscular tension, and rapid heart rate (Singh & Gaurav, 2011; Martens *et al*., 1990). A study found that male and female athletes have extreme anxiety, significantly affecting their mental health (Stevens *et al*.,2013). Anxiety can harm an athlete's performance because it is difficult to control the game's pace, react on time, and regulate muscle contraction. It impairs an athlete's ability to interpret their opponent's play accurately and encourages rash decisions and uncontrolled actions (Hasanah & Refanthira, 2020).

Athletes who concentrate on self-improvement (task orientation) typically achieve greater results than those who concentrate on winning (competition orientation) and measure their performance by comparing it to other athletes' performances (ego orientation). Sportsmanship, instrumental aggressiveness, and athletes' pro-social perceptions (Sage *et al*., 2006), antisocial behaviour toward opponents (Boardley & Kavussanu, 2010), sportsmanship and instrumental aggression (Yukhymenko-Lescroart, 2018), or psychological well-being and social connectedness (Yukhymenko-Lescroart, 2018) are all examples of these conflicting effects of task and ego orientations (Wayment & Walters, 2017). According to a systematic review conducted by Biddle *et al*. (2003), athletes' task orientation is linked to many positive outcomes, such as their perceptions of competence, their feelings of happiness, their lack of negative emotions, and their sense of ego orientation is linked to both positive and negative outcomes, such as their beliefs about their competence or their unsportsmanlike behaviour. Athletes can perform at their best if they have good mental health. Anxiety, ego-orientation, task-orientation, and other psychological elements influence achieving the goal. To perform at their highest level, one must keep their attitude in control.

The intensity of the competition necessitates a high level of mental health. Athletes' motor skill gaps are sometimes relatively small compared to one another. As a result, the mental preparation of athletes before and during competition is critical (Majzub & Muhammad, 2011). These results mainly support relationships between mental health, task and ego orientation in national and international athletes. Higher intrinsic motivation was significantly correlated with task orientation, but external regulation and competitive performance were positively correlated with ego orientation. Athletes who prioritized their tasks orientation played their sport because they like and enjoyed it.while ego-oriented athletes joined to fulfill social expectations. However, all the hypotheses about the impact of task and ego orientation on mental health are not accepted. When comparing task-oriented athletes versus ego-oriented athletes, task-oriented athletes had better mental health. In this way, we may argue that both task and ego orientations relate to different aspects of sports performance and mental health, which is further supported by the fact that both were uncorrelated.

CONCLUSION

The research revealed that athletes competing at national and international levels experience equivalent effects of sports competition anxiety, negatively affecting their overall performance. The competitive athlete’s ego and task

orientation have a beneficial impact on their mental health. This allowed us to validate several of our hypotheses and confirmed on psychological variables like sports competition anxiety, Ego and task orientation may impact sports performance and the mental health of the athletes. Lack of playing experience may be one of the significant reasons for sports competition anxiety and ego. We recommend coaches take these findings into account to improve the athletes' mental health; it can help competitive athletes to lower their degree of anxiousness which will lead to maximizing their performance in concern sportsata different level of competition. This study proposes that future research should combine qualitative and quantitative methodologies to thoroughly understand athletes' thoughts during competition. Future studies can take advantage of bigger samples of athletes with a variety of skills (amateur to professional).Additionally, experimentation can look into the effectiveness of psycho-therapeutic plans and athletic training too. To assess athletes' mental and psychological fortitude on a global level, comparative studies between local amateur and professional athletes as well as between nations are also may require.

REFERENCES

1. Alansari, B. M. (2006). Gender differences in anxiety among undergraduates from sixteen Islamic countries. Social Behavior and Personality: an international journal, 34(6), 651-660.
2. Baron, D. A., Reardon, C. L., & Baron, S. H. (Eds.). (2013). Clinical sports psychiatry: an international perspective. John Wiley & Sons.
3. Bellenir, K. (2010). Mental Health Information for Teens.
4. Biddle, S., Wang, C. J., Kavussanu, M., & Spray, C. (2003). Correlates of achievement goal orientations in physical activity: A systematic review of research. European Journal of sport science, 3(5), 1-20.
5. Boardley, I. D., & Kavussanu, M. (2010). Effects of goal orientation and perceived value of toughness on antisocial behavior in soccer: The mediating role of moral disengagement. Journal of sport and exercise psychology, 32(2), 176-192.
6. Carr, C. M. (2006). Sport Psychology: Psychologic Issues and Applications. *Physical Medicine and Rehabilitation Clinics of North America*, *17*(3), 519–535.
7. Cox, R. H. (2003). Sport psychology: Concepts and applications (sixth edit). Australia: Mc-Graw-Hill International edition.
8. Daley, A. (2008). Exercise and depression: a review of reviews. Journal of clinical psychology in medical settings, 15(2), 140-147.
9. Diggelidis, N., & Kromidas, C. (2008). Differences between pupils as fair play and satisfaction in physical education. Explorations in physical education & sports. 6 (2), 149, 161.
10. Duda, J. L. (1989). Relationship between task and ego orientation and the perceived purpose of sport among high school athletes. Journal of sport and exercise psychology, 11(3), 318-335.
11. Duda, J. L., & Nicholls, J. G. (1992). Dimensions of achievement motivation in schoolwork and sport. Journal of educational psychology, 84(3), 290.
12. Dunn, J. G., & Dunn, J. C. (1999). Goal orientations, perceptions of aggression, and sportspersonship in elite male youth ice hockey players. Sport Psychologist, 13, 183-200.
13. Finkerberg, M. E., DiNucci, J. M., McCune, E. D., & McCune, S. L. (1992) Analysis of the effect of competitive trait anxiety on performance in Taekwondo competition. Perceptual and Motor Skills, 75, 239-243.
14. Glick, I. D., Kamm, R., & Morse, E. (2009). The evolution of sport psychiatry, circa 2009. Sports Medicine, 39(8), 607-613.
15. Gould, D., Greenleaf, C. A., & Krane, V. (2002) Arousal-anxiety and sport behavior. In T. S. Horn (Ed.), Advances in sport psychology. (2nd ed.) Champaign, IL: Human Kinetics. Pp. 207-242.
16. Gould, D., Petlichkoff, L., & Weinberg, R. S. (1984). Antecedents of, temporal changes in, and relationships between CSAI-2 subcomponents. Journal of Sport and Exercise Psychology, 6(3), 289-304.
17. Gulliver, A., Griffiths, K. M., & Christensen, H. (2012). Barriers and facilitators to mental health help-seeking for young elite athletes: a qualitative study. BMC psychiatry, 12(1), 1-14.

18. Halbreich, U., & Kahn, L. S. (2007). Atypical depression, somatic depression and anxious depression in women: are they gender-preferred phenotypes?. Journal of affective disorders, 102(1-3), 245-258.
19. Hamer, M., Stamatakis, E., & Steptoe, A. (2009). Dose-response relationship between physical activity and mental health: the Scottish Health Survey. British journal of sports medicine, 43(14), 1111-1114.
20. Hanin, Y. (2000). Emotions in sport. Human Kinetics.
21. Hanin, Y. L. (1980). A study of anxiety in sport in WF Straub (Ed) Sport Psychology: An Analysis of Athletic Behaviour Movement Publications: NY.
22. Hanin, Y. L. (1986). STATE-TRAIT ANXIETY. Cross-cultural anxiety, 3, 45.
23. Hardy, L., Jones, G., & Gould, D. (1996). Understanding Psychological Preparation for Sport: Theory and Practice of Elite Performers. Wiley, Chichester.
24. Hasanah, U., & Refanthira, N. (2020, January). Human problems: Competitive anxiety in sport performer and various treatments to reduce it. In 5th ASEAN Conference on Psychology, Counseling, and Humanities (ACPH 2019) (Vol. 395, pp. 144-148).
25. Heather, B. (2010). Psychology: motivation, anxiety, confidence and goal setting.
26. Huang, H. C., Wu, W. L., Chang, Y. K., & Chu, I. H. (2016). Physical fitness characteristics of adolescent wushu athletes. The Journal of sports medicine and physical fitness, 58(4), 399-406.
27. Humara, M. (1999). The relationship between anxiety and performance: A cognitive behavioral perspective. Athletic Insight, 1(2), 1-14.
28. Jones, G., & Swain, A. (1992) Intensity and direction as dimensions of competitive state anxiety and relationships with competitiveness. Perceptual and Motor Skills, 74, 467-472.
29. Jones, G., Swain, A., & Hardy, L. (1993). Intensity and direction dimensions of competitive state anxiety and relationships with performance. Journal of sports sciences, 11(6), 525-532.
30. Kavussanu, M. (1997). Moral functioning in sport: An achievement goal perspective. University of Illinois at Urbana-Champaign.
31. Lazarus, R. S. (2000). How emotions influence performance in competitive sports. The sport psychologist.
32. Lorimer, R. (2006). The relationship between self-presentational concerns and competitive anxiety: the influence of gender. International Journal of Sport Psychology, 37(4), 317-329.
33. Lowe, R., & McGrath, J. E. (1971). Stress, Arousal and Performance: Some Findings Calling for a New Theory Lowe and McGrath. ILLINOIS UNIV AT URBANA.
34. Majzub, R., & Muhammad, T. A. (2011). Goal orientation and achievement of junior in golfers Malaysia. Procedia-Social and Behavioral Sciences, 15, 1644-1649.
35. Martens, R., Vealey, R. S., & Burton, D. (1990). Competitive anxiety in sport.
36. McAnulty S, McAnulty L, Collier S, Souza-Junior TP, McBride J. Tai Chi and Kung-Fu practice maintains physical performance but not vascular health in young versus old participants. Phys Sportsmed 2016;44: 184-189.
37. McDuff, D. R. (2012). Sports psychiatry: strategies for life balance and peak performance. American Psychiatric Pub.
38. Nicholls, A. R., & Polman, R. C. (2007). Coping in sport: A systematic review. Journal of sports sciences, 25(1), 11-31.
39. Nicholls, J. G. (1984). Achievement motivation: conceptions of ability, subjective experience, task choice, and performance. Psychological review, 91(3), 328.
40. Nicholls, J. G. (1989). The competitive ethos and democratic education. Harvard University Press.
41. Orlick, T., & Partington, J. (1988). Mental links to excellence. The Sport Psychologist, 2, 105-130.
42. Papaioanou, A., Theodorakis, I., & Goudas, M. (1994). The motivation in physical education. Sports & social, 8, 69-77.
43. Parnabas, V., Parnabas, J., & Parnabas, A. M. (2015). The effect of cognitive anxiety on sport performances among football players. The International Journal of Indian Psychology, 2, 1-8.
44. Peluso, M. A. M., & Andrade, L. H. S. G. D. (2005). Physical activity and mental health: the association between exercise and mood. *Clinics*, *60*(1), 61-70.
45. Perry, J. D., & Williams, J. M. (1998). Relationship of intensity and direction of competitive trait anxiety to skill level and gender in tennis. The Sport Psychologist, 12(2), 169-179.

46. Pons, D. (1991) Estado actual de la investigación sobre la ansiedad en los deportes de competición [State of the art in competitive sport anxiety]. Unpublished doctoral dissertation, Univer. of Valencia, Spain.
47. Proios, M., Athanailidis, I., & Zarotis, G. (2004). Relation motivation and fair play. Physical education, Sports, Health, 16-17, 117-125.
48. Ridgers, N. D., Fazey, D. M., & Fairclough, S. J. (2007). Perceptions of athletic competence and fear of negative evaluation during physical education. British Journal of Educational Psychology, 77(2), 339-349.
49. Sage, L., Kavussanu, M., & Duda, J. (2006). Goal orientations and moral identity as predictors of prosocial and antisocial functioning in male association football players. Journal of Sports Sciences, 24(05), 455-466.
50. Simon, J. A., & Martens, R. (1977). SCAT as a predictor of A-states in varying competitive situations. Psychology of motor behaviour and sport, 2, 146-156.
51. Singh, A., & Gaurav, V. (2011). A study of pre-competitive and post-competitive anxiety level of inter-collegiate volleyball players. International Journal of Sports Science and Engineering, 5(04), 237-241.
52. Souter, G., Lewis, R., & Serrant, L. (2018). Men, Mental Health and Elite Sport: a Narrative Review. Sports Medicine - Open, 4(1), 1–8.
53. Stephens, D. E., & Bredemeier, B. J. L. (1996). Moral atmosphere and judgments about aggression in girls' soccer: Relationships among moral and motivational variables. Journal of sport and exercise psychology, 18(2), 158-173.
54. Stevens, R. E., Loudon, D. L., Yow, D. A., Bowden, W. W., & Humphrey, J. H. (2013). Stress in college athletics: Causes, consequences, coping. Routledge.
55. Swain, A., & Jones, G. (1996). Explaining performance variance: The relative contribution of intensity and direction dimensions of competitive state anxiety. Anxiety, Stress, and Coping, 9(1), 1-18.
56. Treasure, D. C., & Roberts, G. C. (1998). Relationship between female adolescents' achievement goal orientations, perceptions of the motivational climate, belief about success and sources of satisfaction in basketball. International journal of sport psychology, 29(3), 211-230.
57. Tsang TW, Kohn MR, Chow CM, Fiatarone Singh MA. Kung fu training improves physical fitness measures in overweight/obese adolescents: the "martial fitness" study. J Obes 2010;2010. pii: 672751.
58. Wayment, H. A., & Walters, A. S. (2017). Goal orientation and well-being in college athletes: The importance of athletic social connectedness. Journal of Sports Sciences, 35(21), 2114-2120.
59. Weinberg, R. S., & Gould, D. (2019). Foundations of sport and exercise psychology, 7E. Human kinetics.
60. Weiss, M. R., & Gill, D. L. (2005) What goes around comes around: re-emerging themes in sport and exercise psychology. Research Quarterly for Exercise and Sport, 76, 71-87.
61. Woodman, T., & Hardy, L. (2001) Stress and anxiety. In R. N. Singer, H. A. Hausenblas, & C. M. Janelle (Eds.), Handbook of sport psychology. (2nd ed.) New York: Wiley. Pp. 290-318.
62. Yukhymenko-Lescroart, M. A. (2018). On identity and sport conduct of student-athletes: Considering athletic and academic contexts. Psychology of Sport and Exercise, 34, 10-19.

Table.1.Descriptive statistics of the demographic and study variables

	M & W International	M & W National	Men International	Men National	Women International	Women National
Age	16.40 ± 1.14	15.88 ± 0.92	16.46 ± 1.06	16.14 ± 0.89	16.2 ± 1.48	15.77 ± 0.97
Height (*ft.*)	5.44 ± 0.27	5.28 ± 0.33	5.49 ± 0.27	5.40 ± 0.27	5.3 ± 0.22	5.23 ± 0.38
Weight	58.65 ± 8.13	54.70 ± 7.44	60.93 ± 7.08	56.14 ± 9.47	51.8 ± 7.75	53.88 ± 56.33
BMI	21.27 ± 2.46	21.30 ± 3.99	21.77 ± 2.40	20.82 ± 3.99	19.78 ± 2.19	21.54 ± 4.42
Playing Experience	6.85 ± 1.84	4.94 ± 1.74	7.06 ± 1.98	4.71 ± 1.79	6.2 ± 1.30	5.22 ± 1.85
Anxiety	19.20 ± 3.59	20.47 ± 3.12	18.26 ± 0.27	20.14 ± 2.54	22 ± 2.91	20.77 ± 3.80
Ego	2.83 ± 0.71	3.04 ± 0.55	2.89 ± 0.77	3.30 ± 0.64	2.64 ± 0.51	2.80 ± 0.38
Task	4.31 ± 0.44	4.33 ± 0.46	4.39 ± 0.38	4.38 ± 0.42	4.08 ± 0.56	4.26 ± 0.52
Mental Health	60.90 ± 6.19	59.11 ± 7.67	62.53 ± 4.82	61.28 ± 9.89	56 ± 7.81	57.55 ± 6.10

Note: M = Men, W = Women.

Table.2.Analysis of Correlation for International and National level Wushu players

	Mental Health	Anxiety	Ego	Task
Mental Health	1			
Anxiety	-.346*	1		
Ego	.408*	-.123	1	
Task	.236	-.209	.317	1

Note. * Correlation is significant at the 0.05 level (2-tailed).

Table.3.Analysis of Regression on various parameters influence towards mental health

Variables	Beta	t-value	Sign
Constant	56.395	4.289	0.000
Anxiety	-.289	-1.885	0.068*
Ego	.352	2.226	0.033**
Task	.064	0.401	0.691

Note. Chi-Square: 3.851(0.018); R-Value: 0.509; R-Square: 0.259

Dependent Variables: Mental Health

* Significant at 10% and **Significant at 5% level of significance

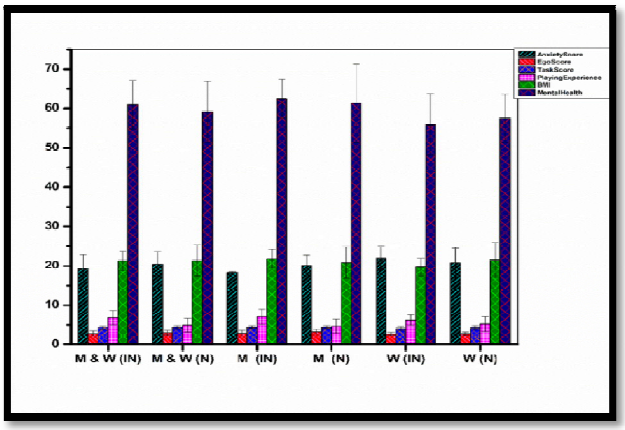

Figure 1. Level of changes in the psychological variables

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RESEARCH ARTICLE

Characterization of Epicuticular Wax from *Nerium oleander* L., *Calotropis procera* R. Br., *Colocasia esculenta* L. and *Alocasia indica* Schott. and its Application for Development of Hydrophobic Paper

Varsha Lazar[1], Arya P Mohan[2]* and Alphonsa Vijaya Joseph[3]

[1]M.Phil Student, St.Teresas (Autonomous) College, Ernakulam, Mahatma Gandhi University, Kottayam, Kerala, India.
[2]Guest lecturer, St.Teresas (Autonomous) College, Ernakulam, Mahatma Gandhi University, Kottayam, Kerala, India.
[3]Associate Professor, St.Teresas (Autonomous) College, Ernakulam, Mahatma Gandhi University, Kottayam, Kerala, India.

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***Address for Correspondence**
Arya P Mohan,
Guest lecturer,
St.Teresas (Autonomous) College,
Ernakulam, Mahatma Gandhi University,
Kottayam, Kerala, India.
Email: aryapmhn@gmail.com

ABSTRACT

Food safety standards are very critical when it comes to the packaging of foods eco-friendly packing materials are high in demand nowadays. The plant-derived packing materials are a good alternative to chemical and plastic packing materials. The leaf surface of some selected plants including *Nerium oleander* L., *Calotropis procera* R. Br., *Colocasia esculenta* L. and *Alocasia indica* Schott. are covered with a hydrophobic layer of bio-wax. This paper discusses the isolation of the bio-wax layer from the leaves by organic solvent extraction methods using Chloroform, Benzene and Hexane. The extracted wax is tested and made in the form of coating and applied on the surface of the paper to obtain hydrophobicity and that is identified as a potential component for making biodegradable hydrophobic paper bags. The isolated bio-wax was subjected to various tests like wax confirmatory test, quantitative analysis of bio-wax, hydrophobicity test, heat sensitivity test and antibacterial test. It is observed that the paper coated with the bio-wax attained hydrophobic properties in the experiments and the heat sensitivity test showed that the bio-wax retains hydrophobicity even at high temperatures. Chloroform leaf extracts of *Colocasia esculenta* L., and *Alocasia indica* Schott. showed longer period of hydrophobicity so, these plants are more suitable for the development of hydrophobic paper.

Keywords: *Nerium oleander* L., *Calotropis procera* R. Br., *Colocasia esculenta* L., *Alocasia indica* Schott., Hydrophobic paper

INTRODUCTION

Plastic bags are convenient for usage due to their properties like high durability, non-corrosiveness, lightweight, electrical and thermal insulation etc. Despite having a varying range of commercially useful properties, plastic bags have become a global concern. They possess a serious threat to the environment due to their non-biodegradable nature. Plastic bags produced which are non-biodegradable or biodegradable both are made of toxic ingredients and are harmful to the environment [12, 13]. The only way to get rid of plastic pollution is to create alternatives like biodegradable plastic bags. Hence to tackle these environmental issues, paper bags are encouraged, which are generally ecofriendly. Paper bags are not water resistant, therefore their use is limited. When these paper bags are coated with synthetic compounds like slimicides [17] is a chemical that prevents the growth of slime in paper stock and attains hydrophobicity and their degradation can lead to water and soil pollution. In an attempt to find suitable organic material to solve the above environmental concerns, plant extracted bio-wax is the solution available in nature. The aerial surface of plants contains hydrophobic waterproofing wax (bio-wax), which protects the plant cells against environmental stresses. Plants like *Nerium oleander* L., *Calotropis procera* R. Br., *Colocasia esculenta* L. and *Alocasia indica* Schott. produce this wax, which is highly hydrophobic.

Nerium oleander L. belongs to Apocynaceae family [6] and it is a small shrub with milky juice present. They are found mostly in seasonally dry rock watercourses, in high irradiance. It is a xerophytic plant that tolerant to both drought and inundation. Leaves are usually in groups of three and narrowly lanceolate. It is cultivated as an ornamental shrub in warm temperature, and dry subtropical regions and as a plant for conservatory in cooler climates. It has various medicinal properties; it is used for treating cardiac conditions in patients who cannot tolerate digitalis. In traditional medicine, the leaves have been used for a variety of medicinal purposes including the treatment of heart diseases, as a diuretic. *Calotropis procera* R. Br. belongs to the Asclepiadaceae family [6] and is a wild perennial shrub that grows in drought-prone areas. This plant grows as a weed in hot and arid environments and survives due to the presence of hairs and a thick layer of wax on the leaf surface. It has medicinal properties, so it is used in the treatment of several diseases and disorders including eczema, asthma, digestion problems, vomiting, diarrhea and coughing. The leaves of these plants are available throughout the year but are reduced during summer since leaves are shed as xerophytic adaptation. *Colocasia esculenta* L. and *Alocasia indica* Schott. belong to the Araceae family [6]. They are herbaceous perennial plants with a large underground corm. They are the most widely cultivated species of several plants in the family Araceae; that are used as vegetables for their corms, leaves, and petioles. They are used as staple food in many countries. They are widely used in India also. The hydrophobic surfaces have attracted considerable attention in the last two decades because of their various potential applications relevant to water repellents, self-cleaning and antifouling.

In 1997, it became clear that hydrophobicity in nature occurs on textured surfaces with hierarchical micrometer and nanometer sized structures in connection to hydrophobic surface components. For example, the surface of the *Nerium oleander* L., *Calotropis procera* R. Br. and Lotus leaf. Since then, significant research is focused on the fabrication of hydrophobic surfaces, mimicking the famous example of the surface of the lotus leaf. Microscopic textures play a huge role in hydrophobicity. The leaves of the *Colocasia* and *Alocasia* plants are covered with waxy, microscopic bumps that prevent water drops from being able to stick, or adhere to the leaf. Most surfaces pull down the liquid drop that rests on them. For example, if water sprinkles on a glass surface, the water droplets lie flat. However, on a hydrophobic surface, they tend to be rounder and barely touch the surface. When a drop of water was gently placed on a taro leaf, it sat on the flakes present on the sides of the honeycomb structured surface, rather than filling up space in these honeycomb structures. Since the water drop touches the surface at fewer points, it gets pulled down less and remains round. The flakes and honeycomb-like cavities, in essence, help in providing the water-repelling effect. Paper is an inherent hydrophilic material because of the hydroxyl groups contained in cellulose. The inherent hydrophilic nature of cellulose poses obvious limitations in the use of paper when hydrophobicity is highly demanded. For example, water resistance of paper is important for the packaging industry. Hydrophobic water repellent coatings can provide enhanced protection against water and moisture. Another

potential application of water repellent films on paper is related to the preservation of documents and books which is aided by the unique anti sticking and self-cleaning properties. The modification of the chemical composition of the surface is necessary to change the wettability of the water-loving cellulosic paper to being hydrophobic. *Nerium oleander* L., *Calotropis procera* R. Br., *Colocasia esculenta* L. and *Alocasia indica* Schott. are selected for the present investigation. It is well known that surface wax of plant leaves plays an important role in reducing the rate of transpiration. The leaf surface properties and composition may vary with environmental conditions [3].The present study is to characterize epicuticular wax from *Nerium oleander* L., *Calotropis procera*R. Br., *Colocasia esculenta* L. and *Alocasia indica* Schott. and its application for development of the hydrophobic paper. The study envisages the isolate and analyzing the epicuticular wax from *Nerium oleander* L., *Calotropis procera* R. Br., *Colocasia esculenta* L. and *Alocasia indica* Schott. using suitable solvents. The isolated waxes are quantified and characterized based on their hydrophobicity and heat sensitivity. To understand the antibacterial activity, the waxes are tested with gram-positive *Staphylococcus aureus* and gram-negative E. coli bacteria. The obtained epicuticular wax is tested for its application for the development of hydrophobic paper.

MATERIALS AND METHODS

Collection of Plant Sample

The leaves of *Nerium oleander* L., *Calotropis procera* R. Br., *Colocasia esculenta* L. and *Alocasia indica* Schott. were collected from Ernakulam.

Isolation of Wax from Surface of Leaves

Fresh leaves of *Nerium oleander* L., *Calotropis procera* R. Br., *Colocasia esculenta* L. and *Alocasia indica* Schott. were collected and the leaves of each plant were cut into small pieces. Two grams of each plant leaf were weighed. Then 15 ml of three solvents (Chloroform, Benzene and Hexane) were taken in separate beakers for *Nerium oleander* L., *Calotropis procera* R. Br., *Colocasia esculenta* L. and *Alocasia indica* Schott. The leaf fragments were immersed using a glass rod in the solvents for three minutes. Then three solvents were transferred into different petri dishes and allowed them to evaporate (20 minutes). A white cloudy layer of wax appeared on the surface of petri dishes.

Wax Confirmatory Test

Wax was extracted from the leaves of *Nerium oleander* L., *Calotropis procera* R. Br., *Colocasia esculenta* L. and *Alocasia indica* Schott. by solvent extraction method. The solvent (Chloroform) is evaporated and the wax was then dissolved in ethanol and transferred into a test tube. 10 ml of distilled water was added to the solution and shaken well and the appearance of milky white in the test tube confirms the presence wax.

Quantitative Analysis of Bio-Wax

Fresh leaves of *Nerium oleander* L., *Calotropis procera* R. Br., *Colocasia esculenta* L. and *Alocasia indica* Schott. were collected and cut into fragments. Two grams of these plant leaf fragments were measured and then the leaves of *Nerium oleander* L., *Calotropis procera* R. Br., *Colocasia esculenta* L. and *Alocasia indica* Schott. fragments were then dipped in 15 ml of each solvent for three minutes. The solvent was then discarded and the remaining solvents on the leaf fragments were then allowed to dry in open air condition and weighed. The amount of wax was calculated by subtracting the weight of the air-dried leaves from fresh leaves.

Test for Hydrophobicity

Bio-wax was isolated from leaves by solvent extraction method. The solvents such as Chloroform, Benzene and Hexane containing the wax were poured into a petri dish and the solvent was allowed to evaporate. After evaporation of solvents, the wax coating on the Petri plate was again dissolved in three ml of three different solvents to get high concentration of wax. The solvents containing wax were then poured on a rectangular piece of What man filter paper using a micropipette. The test for hydrophobicity was then done by dropping water on it using a pipette

and was compared to a filter paper without the wax coating. The water resistance was evaluated by observing the time until which the paper shows hydrophobicity.

Heat Sensitivity Test

Wax was extracted in different petriplates using Chloroform, Benzene and Hexane. The solvent was then allowed to evaporate at room temperature. Petri plates were then subjected to 70^{0}C for two minutes and then the retention of hydrophobic properties of the wax was studied.

Test for Antibacterial Property

The antibacterial activity of the wax extracts was studied using selected bacterial pathogens. Two pathogens, *Escherichia coli* and *Staphylococcus aureus*, were selected for the study. The following preparatory microbial culture activities have carried out for testing the antibacterial property of the wax extracts.

Sterilization of Glass wares

The materials needed were sterilized following the standard protocols [9]. The required number of Petri plates, measuring jars, beakers, conical flasks, and glass rods were washed using detergent and rinsed with distilled water and kept in the oven (60^{0}C) for drying and then autoclaved and kept in an inoculation hood for UV treatment for further sterilization.

Nutrient Broth Preparation

A growth medium or culture medium is a substance in which microorganisms or cells can grow. There are different types of media for growing different types of cells. The general purpose of the liquid medium is the cultivation of organisms that are not demanding in the nutritional requirements. For the preparation of nutrient broth medium, 1.3g nutrient broth was added and mixed with 100ml distilled water in a conical flask. Gently boil it using a water bath by slightly shaking it to dissolve in solvents. Dispensed 10 ml of broths to each culture tube and the prepared cotton plugs were inserted into the mouth of the test tube and covered with aluminum foil. Transfer all the broth tubes into a test tube stand. Test tubes were autoclaved and sterilized at 121^{o}C for 20 minutes. Broth tubes were taken out and then allowed to cool down. Test tubes were properly labelled and kept in the incubator at 37^{0}C overnight for attaining proper growth of *Escherichia coli* and *Staphylococcus aureus*.

Preparation of Inoculum

The permanent cultures of bacterial test strains were inoculated into five ml of nutrient broth medium and incubated at 37^{0}C for 18 to 24 hrs. The growth of bacteria was confirmed by observing the turbidity (spectrophotometer) of the medium.

Preparation of Agar Plates

Nutrient broth with two-grams agar-agar was prepared and autoclaved in a 500 ml of the conical flask. 20 ml of the NBA medium was poured into each of the sterile petri plates. The plates were left undisturbed in an incubator for eight hours and confirmed to be free of contamination.

Antibacterial Activity Test by Disc Diffusion Methods

The standard disc diffusion method was adopted for antibacterial activity tests. The sterile nutrient broth agar plates were inoculated with the test culture by surface spreading using a sterilized cotton swab and each bacterium was evenly spread on the entire surface of the plate to obtain uniformity of the inoculum. Sterile filter paper discs of 4mm diameter were immersed in the wax extracted from *Nerium oleander* L., *Calotropis procera* R. Br., *Colocasia esculenta* L. and *Alocasia indica* Schott. using the three solvents Chloroform, Benzene and Hexane and they were placed onto the agar medium with sterile forceps. Filter paper discs with Chloroform, Benzene and Hexane were used as the control. The inoculated plates were incubated for 24 hours at 37^{o}C. The plates were examined for the presence of a bacterial inhibition zone around each disc. Antibacterial activity was determined from the zone of inhibition around the discs. The zones of inhibition were measured using a ruler and results were reported in millimeters.

RESULTS

The plant material selected for the study were *Nerium oleander* L., *Calotropis procera* R. Br., *Colocasia esculenta* L. and *Alocasia indica* Schott., shown in Fig 1. They were collected from Ernakulam.

Isolation of Wax from Surface of Leaves

Isolation of wax from leaves was carried out by solvent extraction method. The solvents such as Chloroform, Benzene and Hexane were used. A whiter cloudy layer of wax was obtained by using the solvent Chloroform for leaves of *Nerium oleander* L., *Calotropis procera* R. Br., *Colocasia esculenta* L. and *Alocasia indica* Schott. as shown in Fig 2. The solvent Chloroform had given the maximum and the solvent Hexane had recorded the minimum yield of wax from the leaves of selected plants. The solvent benzene had given less amount of wax than chloroform and more wax than hexane. Therefore, Chloroform is the best-suited solvent compared to Benzene and Hexane for the extraction of wax from the leaves of *Nerium oleander* L., *Calotropis procera* R. Br., *Colocasia esculenta* L. and *Alocasia indica* Schott.

Wax Confirmatory Test

The wax obtained by solvent extraction method was dissolved in ethanol and 10 ml of distilled water was also added to solution and shaken well. The solution turning into milky white colour confirms the presence of wax. *Nerium oleander* L. and *Colocasia esculenta L.* wax extracts appeared milkier and more turbid than *Calotropis procera* R. Br. and *Alocasia indica* Schott. shown in Fig 3.

Quantitative Analysis of Bio-wax

In the quantitative analysis of bio-wax, the amount of wax was calculated by subtracting the weight of the leaves after the solvent treatment from the weight of fresh leaves. In *Nerium oleander* L. the initial weight in all three solvents was two-grams. In the case of Chloroform, the amount of wax obtained were 0.19 grams. In the solvent Benzene and Hexane, the amount of wax obtained were 0.17 grams and 0.1 grams respectively. Therefore, the wax yield is maximum for *Nerium oleander* L. with Chloroform as solvent. Similarly, in *Calotropis procera* R. Br. the initial weight of the leaves in all three solvents was two-grams. The wax yield for the solvent Chloroform, Benzene, and Hexane were 0.21 grams, 0.1 grams, and 0.05 grams, respectively. Here also the solvent Chloroform generated maximum wax compared to other two solvents. The initial weight of *Colocasia esculenta* L. leaves in all three solvents was two-grams. The amount of wax obtained for Chloroform solvent were 0.22 grams. In the solvent Benzene and Hexane, wax yield were 0.03 grams and 0.06 grams respectively. Therefore, in *Colocasia esculenta* L., the solvent Chloroform recorded maximum wax production than other two solvents, and the minimum wax was obtained from Hexane. The same set of measurements was repeated for *Alocasia indica* Schott. The wax yield for the solvents Chloroform, Benzene, and Hexane were 0.24 grams, 0.06 grams, and 0.04 grams respectively. It is evident from the measurements that the solvent Chloroform recorded maximum wax production compare to other two solvents for all the leaves studied. The extracted wax using three solvents from different leaves studied are shown in Table 1.

Test for Hydrophobicity

The hydrophobicity test was carried out by dropping water on wax coated What man filter paper and compared with a filter paper without the wax coating. The water resistance was evaluated by observing the time till which the paper shows hydrophobicity, shown in Table 2. *Nerium oleander* L. wax coated filter paper using the solvent Chloroform, showed hydrophobicity of 4 hours. The solvents Benzene and Hexane showed hydrophobicity only for 2 minutes and 1 minutes, respectively. For *Calotropis procera* R. Br, the solvent Chloroform, showed hydrophobicity of about 15 minutes and the other two solvents such as Benzene and Hexane showed hydrophobicity of about 1.5 minutes and 1 minutes respectively. In both *Colocasia esculenta* L. and *Alocasia indica* Schott. wax coated filter paper for both plants using the solvent Chloroform showed hydrophobicity for about 5 hours, benzene showed only 15 minutes and hexane showed 1 minute respectively as shown in Fig 4.

Heat Sensitivity Test

In the heat sensitivity test, the obtained wax was subjected to 70°C for two-minutes. The retention of hydrophobic property and integrity of wax was observed. It was observed that, the *Nerium oleander* L., *Calotropis procera* R. Br., *Colocasia esculenta* L., and *Alocasia indica* Schott. with solvent Chloroform retain its hydrophobic property even after subjected to elevated temperature for the prescribed time period. In other two solvents, Hexane and Benzene, the hydrophobic property was not retained when it subjected elevated temperature as shown in Fig 5.

Test for Antibacterial Property

Chloroform, Benzene and hexane were used as the control as shown in Fig 6. in the experiment. The antibacterial activity was analyzed by disc diffusion method. The following results were obtained.

Effect on *Escherichia coli (E. coli)* and *Staphylococcus aureus*

The antibacterial activity analyzed by disc diffusion method showed that *E. coli* was susceptible only to the wax obtained from *Colocasia esculenta*L. using the solvent Chloroform. An inhibition zone of 3mm was observed around this disc, which is circled in the fig 6. The wax from *Nerium oleander* L., *Calotropis procera* R. Br., *Colocasia esculenta* L. and *Alocasia indica* Schott. using the three solvents Chloroform, Benzene and Hexane did not have any antibacterial activity against the bacteria *E. coli* and *Staphylococcus aureus*, shown in the Fig 6.

DISCUSSION

Plant epicuticular waxes are complex mixtures of long-chain aliphatic compounds including primary alcohols, aldehydes, fatty acids, and alkyl esters. According to Sharma et al., (2019), Gas Chromatography Mass Spectrometry (GC-MS) analysis of wax extracted from *Calotropis procera* R. Br. revealed that the wax consists of mainly esters, alkane, and alkene. The wax composition of various plant species varies with the season and age of the plants [7]. Epicuticular wax present on the outer surface of plants acts as a protective barrier against biotic and a biotic loss of water against UV radiation, bacteria and fungi. The plant epicuticular wax from leaf sample can be effectively utilized for the development of hydrophobic coating on the paper. The hydrophobic nature of paper is due to the presence of hydroxyl (-OH) groups [16]. A study conducted by Mukherjee *et al.,* (2019) focus on the development of super hydrophobic layer containing chemically extracted bio-waxes from lotus and taro leaves on silicon substrates. The epicuticular wax of *Nerium oleander* L., *Calotropis procera* R. Br., *Colocasia esculenta* L. and *Alocasia indica* Schott. are used for development of hydrophobic paper with various solvent extraction methods are discussed further in detail. The quantitative analysis of *Colocasia esculenta* by Nayan *et al.,* (2018) showed that one gram of sample leaf contained 0.116 gram of wax. It was observed that the paper coated with the bio-wax attained hydrophobic property which was similar to the natural *Colocasia* leaf. Plant based taro wax can be a source of sustainable and renewable hydrophobic material for use in the Heating, Ventilation and Air Conditioning application system [16].

The present study deals with the antimicrobial evaluation and developing of hydrophobic paper using epicuticular wax of *Nerium oleander* L., (Apocynaceae), *Calotropis procera* R. Br. (Asclepiadaceae), *Colocasia esculenta* L. and *Alocasiaindica* Schott. (Araceae). These plants possess a highly hydrophobic layer of bio-wax on its leaves. The bio-wax was extracted from these leaves by organic solvent extraction method. The solvents used for solvent extraction are Chloroform, Benzene, and Hexane. Among the three, the Chloroform is the best suited solvent because it isolates more wax from the leaves of these plants than other two solvents. The wax confirmatory test is used to confirm the wax extracted by solvent extraction method that showed the milky white colour, confirming the presence of wax. The quantitative analysis of bio-wax revealed that the amount of wax obtained from the leaves of *Nerium oleander* L., *Calotropis procera* R. Br., *Colocasia esculenta* L., and *Alocasia indica* Schott. is higher when extracted with the solvent chloroform. The chloroform recorded maximum wax production because chloroform is frequently used as an extraction solvent, based on its ability to support high and reproducible lipid yields in numerous plant species [8], and has been shown as an effective solvent for lipid extraction from leaf surfaces of several plant species [5]. Since waxes are a type of long chain of non-polar lipid. Hexane solvent exhibited the lowest recovery at each extraction

time and it is an ideal non-polar solvent for extraction of hydrocarbons not for the extraction of non-polar lipids like bio-wax [10].

The extracted bio-wax from the leaves of *Nerium oleander* L., *Calotropis procera* R. Br., *Colocasia esculenta* L. and *Alocasia indica* Schott. showed hydrophobicity and detailed in the study. The water resistance was evaluated by observing the time till which the paper showed hydrophobicity. Alvarez *et al.,* (2019), developed hydrophobic paper containing wax of taro leaves and chitin from crab shells were developed and compared the physical and chemical properties of formulated hydrophobic paper with the properties of commercial photographic paper. In the present study, the developed hydrophobic paper is compared to the normal What man filter paper. The wax extracted using the solvent chloroform showed more hydrophobicity, than the other two solvents such as Benzene and Hexane. According to Yadav *et al.,* (2014), the highest hydrophobicity (29.57%) was found in paper discs coated with epicuticular wax extracted with benzene from the adaxial surface of *Calotropis procera*. Heat sensitivity study showed that hydrophobic property was retained even when it was exposed to high temperature, 70^{o}C.

These characteristics make the bio-wax from *Nerium oleander* L., *Calotropis procera* R. Br., *Colocasia esculenta* L. and *Alocasia indica* Schott. suitable substance for coating papers to make them hydrophobic. The hydrophobic papers thus created may then be used to make biodegradable hydrophobic paper bags. Elisha *et al.,* (2017) observed leaves of nine medicinal plant species with high antibacterial activity against *Escherichia coli* were extracted with acetone and their Minimal Inhibitory Concentration (MIC) values were determined using a micro plate serial dilution technique against Gram-positive (*Staphylococcus aureus, Enterococcus faecalis* and *Bacillus cereus*) and Gram-negative (*Escherichia coli, Salmonella Typhimurium* and *Pseudomonas aeruginosa*) bacteria. Analyzing the antibacterial activity of the waxes extracted from the leaves of *Nerium oleander* L., *Calotropis procera* R. Br., *Colocasia esculenta* L. and *Alocasiaindica* Schott. using the three solvents Chloroform, Benzene and Hexane, it was found that *E. coli* was susceptible only to the wax obtained from *Colocasia esculenta* L. using Chloroform as the solvent.

According to Mostafa et.al., (2018) ethanolic extracts of *Punica granatum*, *Syzygium aromaticum, Zingiber officinales* and *Thymus vulgaris* were potentially effective against *E. coli* and *Staphylococcus aureus. Nerium oleander* L., *Calotropis procera* R. Br. and *Alocasia indica* Schott. wax extracts did not have any kind of anti-bacterial activity against *E. coli* and *Staphylococcus aureus* as the bio-wax of both these plants did not inhibit the growth of *E. coli* and *Staphylococcus aureus* when incubated for 24 hours. Several properties such as anti-sticking, self-cleaning and anti-corrosion have been identified for these super hydrophobic surfaces and have vast application in the industry [11]. Although only few researches have been done in this field, still it is restricted to laboratory levels and not applied in real-life applications and commercial products. Although the mimicking part has been understood, still there are issues related to cost and durability which needs to be addressed by the researchers. It is a great challenge for scientists and researchers to develop permanent and semi-permanent super hydrophobic coatings for commercial use with all the desired properties on all the base materials. Also, the use of fluorine-based products and other non-biodegradable chemicals are dangerous to human as well as environmental health and need to be substituted with eco-friendly alternatives.

CONCLUSION

The extracted from *Nerium oleander* L., *Calotropis procera* R. Br., *Colocasia esculenta* L. and *Alocasia indica* Schott. can be used for making hydrophobic paper which can be used as an alternative for hazardous plastic coatings in papers. The current study concludes that the wax can be isolated from leaf surface by solvent extraction method by using the solvents such as Chloroform, Benzene, and Hexane. Among these solvents, chloroform is the most suitable solvents for wax isolation from leaves of these plants. The extracted bio-wax possess high hydrophobic property. One of the potential applications of hydrophobic paper is related to the preservation of documents and books which is aided by the unique anti-sticking and self-cleaning property. Further studies on developing hydrophobic paper by isolating epicuticular wax from various plants following same extraction method are promising.

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REFERENCES

1. Akash K and Nayan T, *Colocasia esculenta* (L.) leaf bio-wax as a hydrophobic surface coating substance or paper for preparing hydrophobic paper bags, International Journal of Pharmacy and Biological sciences2018; 583-590.
2. AlvarezMLC, Hydrophobic Paper from the Wax of *Colocasia esculenta* (taro leaf) and Chitin from Crab Shell, International Journal of Research Publications2019; 30: 1-7.
3. Dutta M and Laskar S, Hydrocarbons in the surface wax of the leaves of *Alstonia scholaris* (Linn.) R. Br., Orient. J. Chem2009; 25: 437-439.
4. ElishaIL, BothaSF, McGawLJ, and Eloff JN, The antibacterial activity of extracts of nine plant species with good activity against Escherichia coli against five other bacteria and cytotoxicity of extracts, BMC complementary and alternative medicine 2017; 17: 110.
5. Fernandez-Moreno JP, Malitsky S, Lashbrooke J, Biswal AK, Racovita RC and MellerowiczEJ, An efficient method for medium throughput screening of cuticular wax composition in different plant species, Metabolomics 2016; 12.
6. Gamble JS and Fischer CEC, Flora of the Presidency of Madras,1915-1935; 2: 802, 831-832.
7. Grunhofer P, Herzig L and Schreiber L, Leaf morphology, wax composition, and residual (cuticular) transpiration of four poplar clones. Tress 2022; 36: 645-658.
8. Holloway PJ, Surface lipids of plants and animals, CRC Handbook of chromatography, lipids1984; 1: 346-380.
9. Jain A, Jain R and Jain S, Sterilization of glassware; preparation and sterilization of media, In: Basic Techniques in Biochemistry, Microbiology and Molecular Biology2020; 93-99
10. Loneman DM, Peddicord L, Al-Rashid A, Nikolau BJ, Lauter N and Yandeau-Nelson MD, A robust and efficient method for the extraction of plant extracellular surface lipids as applied to the analysis of silks and seedling leaves of maize, PLoS one2017; 12
11. Manoharan K and Bhattacharya S, Superhydrophobic surfaces review: Functional application, fabrication techniques and limitations, Journal of Micromanufacturing 2019; 2: 259–278.
12. Matlack A, Introduction to Green Chemistry, CRC Press, 2001.
13. Miller GT, Sustaining the Earth: An Integrated Approach, Pacific Grove Press, 2005.
14. Mostafa AA, Al-Askar AA, Almaary KS, Dawoud TM, SholkamyEN and Bakri MM, Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases, Saudi Journal of Biological Sciences 2018;25: 361-366.
15. Mukherjee A, Chakraborty S, Das C, Karmakar A, and Chattopadhyay S, Study of Optical and Electrical Characteristics of chemically extracted Lotus and Taro BioWax for Hydrophobic Surface Engineering, International Conference on Opto Electronics and Applied Optics (Optronix) 2019; 1-4.
16. Nasri NS, Ahmed MM, Mohd Noor N, Mohammed J, Hamza UD and Mohd Zain H, Hydrophobicity Characterization of Bio-Wax Derived from Taro Leaf for Surface Coating Applications, In Advanced Materials Research2014; 1043: 184-188.
17. Pathak P, Kumar V, Bhardwaj NK and Sharma C, Slime control in paper mill using biological agents as biocides, Physical Science Reviews 2021; 6: 25.

18. Sharma P, Madhyastha H, Madhyastha R,Nakajima Y, Maruyama M, Verma KS and Gour VS, An appraisal of cuticular wax of Calotropis procera (Ait.) R. Br.: extraction, chemical composition, biosafety and application, Journal of hazardous materials 2019; 368, 397-403.
19. Yadav J, Datta M and Gour VS, Developing hydrophobic paper as a packaging material using epicuticular wax: A sustainable approach, BioResource 2014; 9: 5066-5072.

Table 1. The details of wax extracted using different solvents from the study leaves

Name of plant	Name of solvent	Initial weight (gm)	Final weight (gm)	Amount of wax obtained (gm)
a) *Nerium oleander* L.	Chloroform	2	1.81	0.19
	Benzene	2	1.83	0.17
	Hexane	2	1.90	0.1
b) *Calotropis procera* R. Br.	Chloroform	2	1.79	0.21
	Benzene	2	1.90	0.1
	Hexane	2	1.95	0.05
c) *Colocasia esculenta* L.	Chloroform	2	1.78	0.22
	Benzene	2	1.97	0.03
	Hexane	2	1.94	0.06
d) *Alocasia indica* Schott.	Chloroform	2	1.76	0.24
	Benzene	2	1.94	0.06
	Hexane	2	1.96	0.04

Table 2. Hydrophobicity test

Name of solvent	Time of hydrophobicity			
	***Nerium oleander* L.**	***Calotropis procera* R. Br.**	***Colocasia esculenta* L.**	***Alocasia indica* Schott.**
Chloroform	4 Hours	15 minutes	5 hours	5 hours
Benzene	2 minutes	1.5 minutes	15 minutes	15 minutes
Hexane	1 minute	1 minute	1 minute	1minute

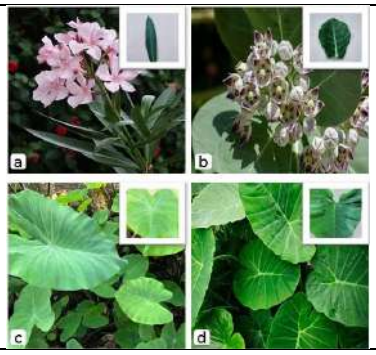

Fig. 1 Shows the study material- leaf of a) *Nerium oleander* L. b) *Calotropis procera* R. Br. c) *Colocasia esculenta* L. d) *Alocasia indica* Schott.

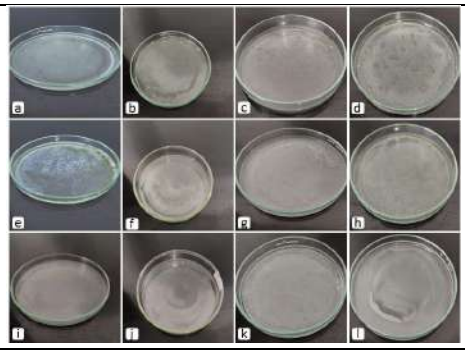

Fig. 2 The wax extracted from different plant material using: Solvent chloroform a) *Nerium oleander* L. b) *Calotropis procera* R. Br. c) *Colocasia esculenta* L. d) *Alocasia indica* Schott.; Solvent benzene e) *Nerium oleander* L. f) *Calotropis procera* R. Br. g) *Colocasia esculenta* L. h) *Alocasia indica* Schott.; Solvent hexane i) *Nerium oleander* L. j) *Calotropis procera* R. Br. k) *Colocasia esculenta* L. l) *Alocasia indica* Schott.

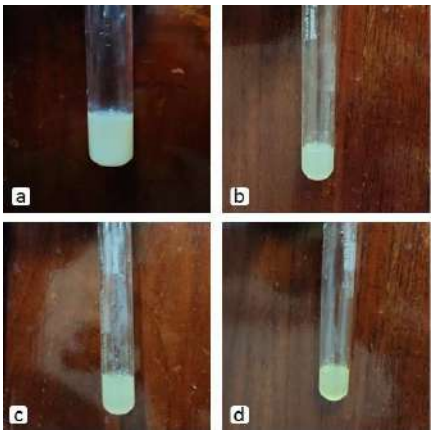

Fig. 3 Wax confirmatory test of different plant material a) *Nerium oleander* L.b) *Calotropis procera* R. Br. c) *Colocasia esculenta* L. d) *Alocasia indica* Schott.

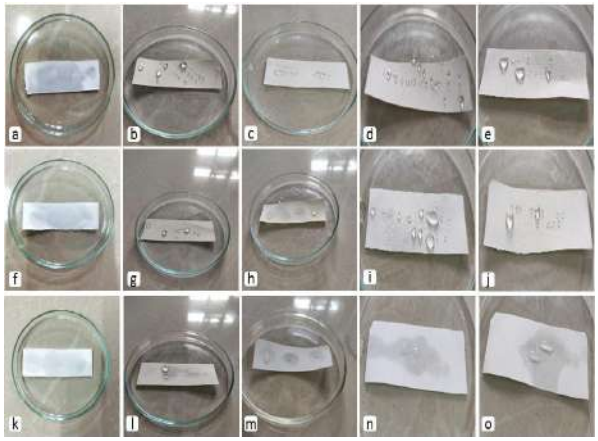

Fig. 4 The hydrophobicity of different plant material using: Solvent Chloroform a) Control b) *Nerium oleander* L. c) *Calotropis procera* R. Br. d) *Colocasia esculenta* L. e) *Alocasia indica* Schott.: Solvent Benzene f) Control g) *Nerium oleander* L. h) *Calotropis procera* R. Br. i) *Colocasia esculenta* L. j) *Alocasia indica* Schott.; Solvent hexane k) Control l) *Nerium oleander* L. m) *Calotropis procera* R. Br. n) *Colocasia esculenta* L. o) *Alocasia indica* Schott.

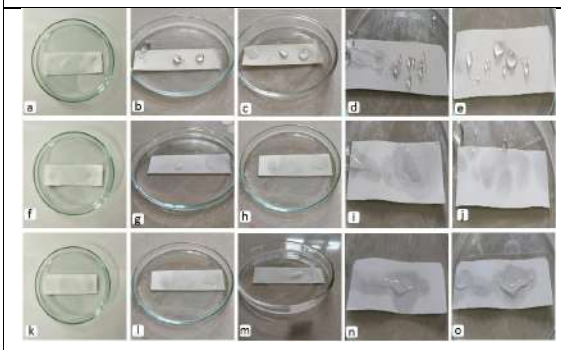

Fig. 5 Heat sensitivity test of different plant material using: Solvent chloroform a) Control b) *Nerium oleander* L. c) *Calotropis procera* R. Br. d) *Colocasia esculenta* L. e) *Alocasia indica* Schott.; Solvent benzene f) Control g) *Nerium oleander* L. h) *Calotropis procera* R. Br. i) *Colocasia esculenta*L. j) *Alocasia indica* Schott.; Solvent hexane k) Control l)*Nerium oleander* L. m) *Calotropis procera* R. Br. n) *Colocasia esculenta* L. o) *Alocasia indica* Schott.

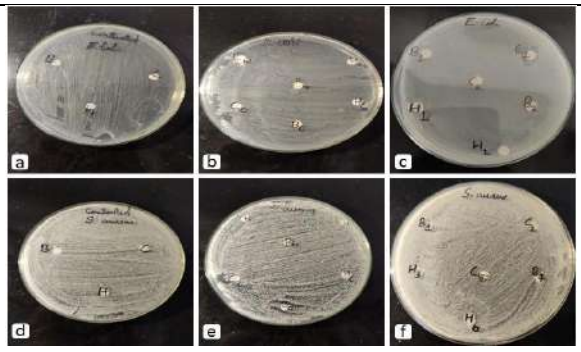

Fig. 6 Shows the antibacterial property of different bio-waxes against *E coli* a) Control b) Solvent chloroform, benzene, hexane extract of *Nerium oleander* L. and *Calotropis procera* R. Br. c) Solvent chloroform, benzene, hexane extract of *Colocasia esculenta* L. d) *Alocasia indica* Schott. *Staphylococcus aureus* d) Control b) Solvent chloroform, benzene, hexane extract of *Nerium oleander* L. and *Calotropis procera* R. Br. e) Solvent chloroform, benzene, hexane extract of *Nerium oleander* L. and *Calotropis procera* R. Br. f) Solvent chloroform, benzene, hexane extract of *Colocasia esculenta*L. and *Alocasia indica* Schott.

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REVIEW ARTICLE

Dynamical Behaviour of Selective Natural Systems using Vibrational Resonance: A Review

R.Kabilan[1]* and A.Venkatesan[2]

[1]Assistant Professor, Department of Physics, Nehru Memorial College (Autonomous), Affiliated to Bharathidasan University, Tiruchirappalli, Tamil Nadu, India.
[2]Associate Professor, Nehru Memorial College (Autonomous), Affiliated to Bharathidasan University, Tiruchirappalli, Tamil Nadu, India.

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***Address for Correspondence**
R.Kabilan,
Assistant Professor,
Department of Physics,
Nehru Memorial College (Autonomous),
Affiliated to Bharathidasan University,
Tiruchirappalli, Tamil Nadu, India.
Email: kabilanrajagopal82@gmail.com

ABSTRACT

Resonances have been intensively researched in a variety of natural systems because they can have harmful effects in real life. This article enables us to investigate nonlinear resonances regardless of application area. Though it does not provide all of the instant answers, this article does help us learn more about natural systems with nonlinear resonances. The phenomenon of vibrational resonance has received a great deal of study attention in the last two decades, and it has been intensively explored due to its numerous potential industrial and biomedical uses in a variety of scenarios. There have been reports of several intriguing features, unexpected phenomena, and prospective uses.

Keywords: intriguing features, unexpected phenomena, and prospective uses.

INTRODUCTION

In 2000, Landa and McClintock discovered that when a system was driven by a biharmonic signal, made up of both a high-frequency and a low-frequency signal, the high-frequency signal acted as stochastic resonance's noise and amplified the response to the low-frequency signal, known as vibrational resonance (VR). Nonlinear systems driven by low-frequency signals modulated by a high-frequency input signal are common in nature and have applications in neuroscience, laser physics, ionospheric physics, acoustics, and atomic physics. They are also frequently employed in communication technology. As a result, several theoretical and experimental works on VR have been reported

since the work of Landa and McClintock. The action of vibration in nonlinear systems frequently produces strange, and sometimes quite unexpected, results. On the one hand, these effects can be used in technology, as the operating principles of many of the most efficient machines are based on them. On the other hand, the same effects can be the source of unfavourable, even disastrous, situations. For example, planetary waves in the Earth's atmosphere (wavelengths 1000 km),Stokes edge waves in the coastal zone (wavelengths 10m). The central auditory nerve system uses nonlinear neural resonator networks to analyse sounds for temporal processing. Resonant response of biological tissue is used to analyse cases of suspected sickness, e.g., cancer and other illnesses.

In particular, the Rayleigh-Plesset oscillator for a gas bubble oscillating in an incompressible liquid, the Duffing oscillator with fractional order, bistable systems, multistable systems, asymmetric Duffing oscillator, neural models, oscillatory networks, biological nonlinear systems, parametrically excited systems, systems with nonlinear damping, and deformed potential, disordered systems, and quantum systems have all been studied. We believe that for resonances to have an impact on biology, the system must absorb more energy than thermal noise. That noise energy will be approximately equal to kT for elements acting incoherently, where k is Boltzmann's constant and T is the Kelvin temperature. Such a large energy transfer requirement necessitates long relaxation times and high coupling strengths to the electromagnetic field. There has been a lot of emphasis dedicated to dissipation processes, but less attention has been made to electromagnetic couplings (Grundler and Keilman; Dorfman and Van Zandt; Van Zandt, 1981, 1986). A system's ability to absorb energy depends on the field's ability to interact with the dipole moment charge distribution of the system, which is impossible because the wavelength of microwave radiation exceeds 1 mm.

Bi-harmonic signals and their importance

The fact that two-frequency signals typically modulate a high-frequency carrier signal makes them crucial for transmission. They are also of importance in a number of other disciplines, including acoustics, neurobiology, and laser physics. In physics, engineering, and biology, the investigation of the behaviour of two-frequency signals is crucial. The biharmonic signal has specifically been studied in relation to vibrational resonance. The importance of two-frequency bands in long-distance vocal communication in the green tree frog was noted by Gerhardt. The study of two-frequency signals is relevant to laser physics, acoustics, neurobiology, and ionosphere physics. High-frequency driving has already been used to good effect in the treatment of Parkinson's illness, accelerated bone and muscle healing, resonantly enhanced bio-gradation of microorganisms, and higher drug uptake by brain cells. High stability and efficiency were discovered in a two-frequency laser unit made up of a laser, a half-wave plate, and an electro-optic modulator. In the global positioning system, biharmonic signal transmission is a common technique for locating and navigation. Neurobiological reaction regulation by electrical stimulation at various frequencies has been explored in conjunction with the appearance of Faraday waves in a pattern formation with two-frequency excitation (the signal frequency and the sub harmonic frequency). High-frequency electromagnetic radiation may be safe for sensitive biomolecules like the DNA helix, but it can have an impact on the cell's physiological functions, according to researchers.

In addition to causing tissue damage, high-frequency electromagnetic waves can interfere with lower-frequency signals that regulate ion transport across cell membranes or information processing through neuron networks and synapse. Soliton ratchets have been created using biharmonic forces. The Antarctic ice sheet's health is being monitored with the help of multi-frequency signals. An oscillation of the beach water table caused by wave run-up and rundown has been documented. Evaporation, water inflow and outflow, and temperature fluctuations can all cause similar high-frequency oscillations in the underground water table, in addition to the low-frequency periodic oscillations caused by seasonal variation. Through irrigation or aquifer pumping, it can also be artificially achieved When vegetation is removed or planted, the water table can rise or fall, respectively. Neuronal bursts can occur at two different time points in the brain's dynamics. It is common for fast and slow variables, such as hares and tortoises, to interact in ecologies. There are both slow and fast cycles in plankton dynamics. The dynamics of many ecological systems can be both slow and fast. A longer time horizon than a single growing season and far beyond the frequency of disturbances caused by humans is required to understand vegetation dynamics.

It has been demonstrated that high-frequency alternating currents delivered to the nerve because reversible conduction block, which has clinical uses. High-frequency electrical simulation has been used clinically to treat movement disorders like Parkinson's disease and dystonia using deep-brain simulation electrodes implanted in specific parts of the brain. In a discrete FitzHugh-Nagumo model, high-frequency stimulation is found to both promote and suppress pulse propagation. By stimulating presynaptic terminals with high frequencies, the rate of replenishment of the pool of synaptic vesicles in the central nervous system is significantly increased. This can be utilised to pinpoint the short- and long-term synaptic alterations' underlying mechanisms. Using an acoustically driven gas bubble model submerged in an incompressible liquid, we investigated the VR phenomenon in acoustic cavitation, in which bubbles are excited by an acoustic sound field mostly consisting of single or dual frequencies, has drawn substantial interest to the dynamics of bubbles in the life sciences and natural sciences as well as technology. In addition to bifurcation structures and chaotic behaviour, bubble dynamics is rich in unique resonances, which have been a major focus of recent research. For example, Zhang *et al*. recently explored numerically across a wide range of parameters the nonlinear dynamics of bubbles under dual-frequency acoustic excitation. Combination resonance and simultaneous resonance were found to be important resonance properties.

Furthermore, it was found that variables including the radius of the bubble, the amplitude of the acoustic pressure, and the energy distribution between the wave components were critical in determining the nonlinear bubble oscillation. The dynamics of bubble oscillations under multi-frequency acoustic cavitation exhibit evidence of resonances. Gas bubble dynamics driven by dual-frequency forces have been studied extensively, however despite the large number of investigations and the wide range potential applications of multi-frequency excitation of gas bubbles, there is still much work to be done. There are numerous medical diagnostic and therapeutic applications where multi-frequency acoustic sound fields can be used to enhance system effectiveness and control the chaotic dynamics of bubble oscillations in particular. Dual-frequency approaches are also used to design sonochemical reactors, synthesise with nanoparticles and sonoluminescence, investigate bubble size distributions with void fraction in the open ocean, study dynamic variations in bubble radii during oscillations, and improve ultrasound accuracy for biomedical diagnosis and the efficiency of tumour therapy, including tumour ablation. Large bubbles can be generated using the dual-frequency approach because mass transfer can speed up bubble growth. We point out a recent study that used GPU-accelerated computations to investigate bubbles driven by two distinct frequencies in a high-dimensional parameter space. A comprehensive review of the synergetic effects of dual-frequency driving on sonochemical yields, efficiency, and other factors is provided, ranging from the most recent state of the art in dual-frequency irradiation applications to a wide range of previously unexplored possibilities.

Vibrational resonance analysis in bi-harmonically driven plasma

Only when particle kinetics is required, such as in reactive collisions, is the plasma treated as a fluid. Several models have been put out in this direction. When a high-frequency (HF) signal is imposed on the system, we will explore the occurrence of vibrational resonance (VR) using the extremely nonlinear dissipative magnetised plasma model, which characterises plasma as composed of electrons and ions. The potential for witnessing vibrational resonance (VR) in dissipative plasma models driven by two periodic pressures has yet to be investigated, despite the recent flurry of study and the importance of plasma in communication and human activities in general. Modeling plasma as two interpenetrating conducting fluids of positive ions I with charge +e and electrons (e) with -e is convenient for this study. Detecting, extracting, or separating signals, reducing noise, or accentuating certain features of the plasma signal are some of the direct industrial applications of this technology. It is important to note that plasma plays a significant role in the production of integrated circuits, which are made by a series of processes that include deposition, masking, etching, and stripping. As a result, dense plasma can be generated, which can increase the etching or deposition rate from a variety of source.

Straight ion orbits with variable energy define the ideal plasma source. The DC potential applied to the etched surface controls the voltage drop in a sheath used to accelerate ions for profile control. A radio-frequency (RF) voltage is used to charge the substrate negatively and then the unidirectional flow of electrons is used to establish the DC potential. Since increasing the applied RF voltage also increases density and other characteristics, it is impossible

to adjust this RF bias independently in parallel plate discharges. As the fast input signal, the plasma is ionised in an inductive or electron-cyclotron resonance (ECR) source independent of the substrate's radio frequency (RF) bias. In this method, desired plasma production responses can be enhanced, controlled, or filtered.

Vibrational Resonances in Biological Systems

The mechanical vibration of system components can be predicted to play a role in many biological systems. Resonances in the microwave region are the most common natural frequencies. If the charge distributions of these systems are linked to the electromagnetic field, then it is possible that microwave exposures could have a physiological effect on humans and other animals. Such microwave excitable resonances, however, are likely to be significantly dampened by their watery biological context. Low-intensity microwave fluxes may affect biology by excitation of elastic resonances in biological systems, according to theoretical conjectures (Fro lich, 1968; Van Zandt, 1986; Edwards *et al*., 1984; Grundler and Keilman, 1983) and experimental results (Edwards *et al*., 1984; Grundler and Keilman, 1983). Although the damping constraint may be somewhat relaxed, we suggest that biological fluids severely restrict such possibilities (Van Zandt, 1986) by showing that typical systems will not be coupled to electromagnetic fields with sufficient strength to allow significant energy transfers.

Proteins in living cells interact with one another in genetic networks in both direct and indirect ways. As a result, these interactions form a dynamic genetic regulatory network that serves as a complex dynamic system for controlling cell functions. These relationships between predators and prey in an ecosystem form the edges of an ecological network. A cell's chemical reactions are represented in molecular biology by means of networks. Neuronal networks depict the connections between brain cells. Receptors, messengers, and other molecules send signals to microorganisms and cells in response to their environments. Magnetometers, ferroelectric detectors for electric fields, and other nonlinear sensors can benefit from networks of flux gate magnetometers by increasing their utility and sensitivity.

Cylindrical systems exhibit longitudinal vibrations

DNA

Through viscous impedance, the molecules in their aqueous biological elements lose energy to the liquid around them. For elements in water, "Organ pipe" standing sound waves propagating longitudinally along cylindrical structures, such as DNA, seemed to have the longest relaxation times. However, Dorfman and Van Zandt (1983) demonstrated that such a resonance (and any other mode of resonance) would be over-damped with plausible assumptions about the viscosity's character. DNA in water cannot have microwave resonances, according to previous studies (Gabriel *et al*., 1987; Foster *et al*., 1987) using techniques designed to detect amplitudes less than one twentieth of what was reported by Edwards *et al*. (Gabriel *et al*., 1987). (1984).

Vibrational resonance in a synthetic gene network

Gene expression has a significant impact on a person's life. Gene expression must be extremely precise if it is to ensure a normal course of life. In spite of the fact that the theoretical basis for studying gene networks dates back nearly 30 years, experimental and theoretical advances have only recently made it possible to quantify genetic networks. If you want to see an example of how a synthetic gene network is linked to a cell's natural rhythms, Hasty *et al* papers is an excellent place to start. The protein concentrations in a synthetic gene network were found to oscillate in response to noise.

VR in a synthetic gene network has, as far as we know, received very little attention. It's possible that the switch will randomly switch between the "on" and "off" states due to the presence of gene signals. One of the primary goals of this study is to determine the effects of high- and low-frequency signals on vibrational resonance (VR) in an artificial gene network with reasonable control parameters. Studies of high-frequency and low-frequency effects on VR in the synthetic gene network revealed that VR is present in appropriate signal ranges and reasonable control parameters, suggesting that the synthetic gene network can act as a resonator. This could be a useful module for signal amplification in actual gene regulatory networks.

Time-Delayed Systems

Some physical and biological systems employ the use of local information to enhance signals. Because of this, it is essential to examine the possibility of improving the response of a single system by means of delayed feedback signals. It is therefore necessary to first investigate how vibrational resonance can be enhanced by the presence of a single periodic force and time-delayed feedback before looking into the possibility of vibrational resonance in a dynamic system that is time-delayed. Here are a few notable examples of time-delay in various scientific disciplines. Delays are incorporated into epidemic model systems to account for the time spent in various stages of the disease's progression. The reason is that it takes time to recover from a disease, and this is why this is the case. If the breakout of a disease brought on by an infection is brought on by biological and environmental processes, time-delay is incorporated into immune response models. Due to the restricted pace of data movement in the axons and dendrites and the processing lag in the synapses, neural networks may communicate with delays ranging from a few milliseconds to hundreds of milliseconds.

Theta and Gamma rhythms in the hippocampus are made possible by delayed feedback in neuronal networks, which fires basket cells precisely. Bubble transport is used in some medical applications to deliver drugs. The drug is delivered to a target using ultrasound-forced micro bubbles that are filled with the drug. The speed of sound in the liquid must be taken into account when studying the behaviour of interacting micro bubbles. Induced pressure waves between bubbles will be delayed as a result of this. In most cases, a disease's incubation period can be estimated. Prior to infection, parasites in the case of malaria would go through a series of stages in the host. When it comes to mosquitoes and their incubation period, which can last anywhere from 10 to 21 days, the range is wide. Between a few weeks and several months, the incubation period for rabies can be expected to occur. The incubation period is accounted for in disease models by including a time delay. The last step of a biochemical reaction can be a source of time delay.

Crayfish pacemaker cells show bursts and high-frequency discharges with relatively long periods of silence due to self-coupling. Networks, laser arrays, electronic circuits, and neural systems all have propagation delays. Due to climatic and weather changes, the gestation and incubation durations of organisms in ecological systems can be affected, resulting in a time delay. Populations can have growth delays as a result of age structure, such as the length of time spent in the larval stage prior to maturation into an adult animal or human. It's important to note that delayed self-communication has a regulatory mechanism in nature and technology, which is why it's important. Excitable gene regulatory systems, eye movements, human balance, and optically communicating semiconductor lasers are a few examples where time delay is unavoidable due to the slowness of transcription, translation, and translocation. In order to account for the time it takes for nutrients consumed to be converted into viable cells in a chemostat model (a device used for continuous microorganism culture), time-delay is introduced.

CONCLUSION

The efficiency of equipment can be improved by furthering our knowledge of the VR phenomenon. VR has the potential to shed light on the treatment of a wide range of conditions and the dynamics of disease because of the high-frequency signal's ability to affect a wide range of biological processes. It would also be fascinating to look into the parameter regimes for which VR can occur in various systems in more depth.

REFERENCES

1. M. Lakshmanan, S. Rajasekar, Nonlinear Dynamics: Integrability, Chaos and Patterns (Springer, Berlin, 2003)
2. Shanmuganathan Rajasekar Miguel A.F. Sanjuan,Nonlinear Resonances (Springer Series in Synergetics, Switzerland, 2016).
3. Two New Sciences, translated by H. Crew and A. DeSalvio (Dover, New York, 1954).
4. Robert K. Adair,BiophysicalJournal,82, 1147–1152(2002).

5. ElenaKartashova,Nonlinear Resonance Analysis, (Cambridge University Press, New York, 2010).
6. A.H. Nayfeh, D.T. Mook, Nonlinear Oscillations (Wiley, New York, 1979).
7. T.O.Roy-Layinde,U. E. Vincent,S. A. Abolade,O. O. Popoola,J. A. Laoyeand P. V. E. McClintock, Philosophical transactions A,Royalsocietypublishing(2020).
8. Jincheng Shi, Chusheng Huang, Tao Dong and Xiuzhi Zhang,Phys. Biol. 7 (2010).
9. F. Borgogno, P. D'odorico, F. Laio, L. Ridolfi, J. Theor. Biol. 293, 65 (2012).
10. V.N. Chizhevsky, G. Giacomelli, Phys. Rev. A 71, 011801(R) (2005).
11. Shepherd, G. M, Neurobiology (Oxford, New York) (1994).
12. Koch, C. Biophysics of Computation,Oxford, NY. 153(1999).
13. E.M. Izhikevich, Dynamical Systems in Neuroscience: The Geometry of Excitability and Bursting (The MIT Press, Cambridge, 2005).
14. S. Rajasekar, M. Lakshmanan, J. Theor. Biol. 166, 275 (1994).
15. M. Perc, Phys. Rev. E 76, 066203 (2007).
16. B.T. Ginn, B. Steinbock, M. Kahveci, O. Steinbock, J. Phys. Chem. A 108, 1325 (2004).

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RESEARCH ARTICLE

Influence of Seasoned Pressmud, Poultry Manure, $ZnSO_4$ and Borax on Yield Attributes in Brinjal

Anindita Majumder[1]* and D. Venkatakrishnan[2]

[1]Ph.D Research Scholar, Department of Soil Science and Agricultural Chemistry, Annamalai University, Annamalai Nagar, Chidambaram, Cuddalore, Tamil Nadu, India.
[2]Associate Professor, Department of Soil Science and Agricultural Chemistry, Annamalai University, Annamalai Nagar, Chidambaram, Cuddalore, Tamil Nadu, India.

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***Address for Correspondence**
Anindita Majumder
Ph.D Research Scholar,
Department of Soil Science and Agricultural Chemistry,
Annamalai University, Annamalai Nagar,
Chidambaram, Cuddalore, Tamil Nadu, India.
Email: aninditamajumder1905@gmail.com

ABSTRACT

Brinjal is one of the vegetable crop with high yield. Now-a-days demand for brinjal as a vegetable is increasing rapidly among the vegetable consumers in view of its better fruit colour and size. Pot experiments were carried out at Department of Soil Science and Agricultural Chemistry, Annamalai University to evaluate the effect of Seasoned Press mud, poultry manure, $ZnSO_4$ and Borax in two different soils (Neutral Soil and Coastal Saline Soil). The treatments include 100% RDF, 75% RDF with Poultry manure, Seasoned press mud soil application @ 6.25 tha^{-1} and 25 tha^{-1},$ZnSO_4$ and Borax soil application @ 25 kg ha^{-1} and 10 kg ha^{-1}. Foliar spray $ZnSO_4$ @ 0.5% and Borax @ 0.25%. There were 13 treatments combinations in FCRD with three replications. Among the treatments highest no. of fruits (24.62), fruit weight $plant^{-1}$ (42.1 g $plant^{-1}$) and fruit yield (1036.50 g $plant^{-1}$) were recorded in treatment (S_1T_{10}) receiving seasoned press mud@25kgha^{-1}+$ZnSO_4$@ 25 kg ha^{-1} + Borax @ 10 kg ha^{-1} in neutral soil

Keywords: Brinjal, Seasoned pressmud, $ZnSO_4$, Borax, Yield attributes.

INTRODUCTION

Soil fertility, compactability are the elements of soil quality. Among these nutrients, decline in soil fertility endangers the maximum in productivity (Reshma *et al.,* 2016). The indiscriminate use of fertilizers over a period of time has resulted in built-up of certain nutrient elements like phosphorus and deficiency of zinc in sandy loam soils. Depending upon the cropping pattern, considerable amount of nutrients are lost from soil every year. If intensive cropping is continued over a period of time without balanced fertilization and restoring of nutrients in soil,

reduction in soil fertility and loss in crop yields is inevitable. Hence for sustainability of the present day agricultural system and balanced management of soil resources, it is imperative to emphasize on the rational management of soil fertility (Chitdeshwari *et al.,* 2017). Coastal sandy soil has potential to be utilized in the biomass production process. In general, volume space of coastal sandy soil is dominated by the sand fraction. The soil texture ranges between sandy and loamy and does not form soil aggregates. The limitation of coastal sandy soil which does not form soil aggregates, causes this soil to have a high leaching capacity so that most of the nutrients can move downward through gravity water (Kumar *et al.,* 2017). India is known as a horticulture paradix as it produces a wide variety of vegetables. Brinjal is the second most important vegetable crop after tomato. It's also known as egg plant, and it's a major vegetable crop that originated in India (Mufti *et al.,* 2021). Being a hardy vegetable crop, the yield, DMP are largely influenced by application of fertilizers. Nutrient management is important for healthy crop lead to improved efficiency as well as system sustainability. Recent developments in intensive agriculture through contributed immensely towards surplus food, causes degradation of fertile land. Thus there is an increasing awareness through out the world about the sustainable agricultural practice (Prem Sekhar and Rajashree, 2009).

Use of organic manures of meet to nutrient requirement of crop would be an inevitable practices in the years to come for sustainable agriculture. Since organic manures improve the sol physical, chemical and biological properties along with conserving the moisture holding capacity and thus resulting in enhanced crop productivity. One source of organic material that can be used to improve coastal sandy soils, physical properties in Seasoned Press mud, which is a waste processing sugarcane stalks to sugars. This material is in the form of a blackish, brown solid can be used as a source of organic matter to increase the productivity of coastal sandy soil and neutral soil (Diaz, 2016). Poultry manure is resistant to microbial degradation. It is essential for establishing and maintaining the optimum soil physical condition for plant growth. The use of poultry manure increases the soil organic carbon content enhances the activities of soil microbes improve soil crops structure and nutrient status of the soil as well as yield compost plays a vital role in improving soil properties and sustaining nutrient status (Paul *et al.,* 2017).

Agricultural intensification resulted in a serious depletion in micronutrients reserve in soils and degradation of environment. Following such, intensification, widespread deficiencies of Zn and B are reported across the India (Bholantha Saha *et al.,* 2020). Zinc seems to affect the capacity of water uptake and transport soil pH, organic matter, available P clay content and temperature are some, important factor affect zinc availability in soil. Organic matter good sources of many essential plant nutrients, improves the availabilty of native soil nutrients organic matter is an important soil constituent originates from decomposition of plant and animal residues. Increase in availability of Zn with the application of organic manure had been reported by Nawal *et al.* (2010). Considering the emerging zinc deficiency in Indian soil, zinc deficiency and role of organic matter in increase in available zinc, it is necessary to improve the zinc content of vegetables with application of zinc sulphate and organic sources (Harshit Kumar *et al.,* 2021). For improving plant growth and yield application of organic and inorganic fertilizers is very important. It has emerged as an important micronutrient in Indian agriculture, next to zinc (Zn). Boron deficiency has been realized as the second most important micronutrient constraint in crops after zinc on global scale (Ahmad *et al.,* 2012). In India, boron deficiency is 52% (Singh, 2012). Soil application of B through borax has resulted in greater availability to crops (Altaf Kuntoju *et al.,* 2019).With the above materials including Seasoned Press mud, Poultry manure, Zinc Sulphate and Borax are involved to evaluate the effect of composts, Zinc sulphate and Borax in pot experiment for yield and yield attributes of brinjal.

MATERIALS AND METHODS

A pot culture study has been conducted to study at Department of Soil Science and Agricultural Chemistry, Annamalai University during July to October, 2021.

Pot experiment

Pot experiment were carried out to study the effect of Seasoned Press mud, Poultry manure, $ZnSO_4$ and Borax application in two different soils.

Treatment details

S_1 – Neutral soil

S_2 – Coastal saline soil

T_1 – Control (RDF 100:50:30 N,P_2O_5 and K_2O kg ha^{-1})

T_2 – 75% RDF + Poultry manure @ 6.25 t ha^{-1} + Soil application Zinc sulphate @ 25 kg ha^{-1}

T_3 – 75% RDF + Poultry manure @ 6.25 t ha^{-1} + Soil application Borax @ 10 kg ha^{-1}

T_4 – 75% RDF + Poultry manure @ 6.25 t ha^{-1} + Soil application Zinc sulphate @ 25 kg ha^{-1} + Borax @ 10 kg ha^{-1}

T_5 – 75% RDF + Poultry manure @ 6.25 tha^{-1} + Foliar spray @ 0.5% Zinc sulphate on 45 and 75 DAT

T_6 –75% RDF + Poultry manure @ 6.25 tha^{-1} + Foliar spray @ 0.2% Borax on 45 and 75 DAT

T_7 –75% RDF + Poultry manure @ 6.25 tha^{-1} + Foliar spray @ 0.5% Zinc sulphate + 0.2% Borax on 45 and 75 DAT

T_8 – 75% RDF + Seasoned Pressmud @ 25 t ha^{-1} + Soil application Zinc sulphate @ 25 kg ha^{-1}

T_9 – 75% RDF + Seasoned Pressmud @ 25 t ha^{-1} + Soil application Borax @ 10 kg ha^{-1}

T_{10} – 75% RDF + Seasoned Pressmud @ 25 t ha^{-1} + Soil application Zinc sulphate @ 25 kg ha^{-1} + Borax @ 10 kg ha^{-1}

T_{11} – 75% RDF + Seasoned Pressmud @ 25 t ha^{-1} + Foliar spray @ 0.5% Zinc sulphate on 45 and 75 DAT.

T_{12} – 75% RDF + Seasoned Pressmud @ 25 t ha^{-1} + Foliar spray @ 0.2% Borax on 45 and 75 DAT.

T_{13} – 75% RDF + Seasoned Pressmud @ 25 t ha^{-1} + Foliar spray @ 0.5% Zinc sulphate + 0.2% Borax on 45 and 75 DAT.

Each treatment is replicated three times in a Factorial Completely Randomized Design (FCRD) Soil samples were collected at the depths of 0-20 cm from 2 sites *i.e.* Vallampadugai, (Neutral soil), Therku Pichavaram (Coastal Saline Soil). Soil samples were air-dried and passed through a 2 mm sieve after removing coarse fragments and roots and stored at room temperature. 20kg of air-dried processed soil was filled in35×30 cm cement pot. The Seasoned pressmud, Poultry manure, $ZnSO_4$ and Borax were applied in soil basally. Foliar spray of $ZnSO_4$ and Borax were due to critical stages of brinjal crops. The chemical compositions of manures are presented in Table 2. Nutrient content of $ZnSO_4$ and Borax are mentioned in Table 3.

Yield attributes

Number of fruits $plant^{-1}$

Average of fruits of all pickings were counted from the total number of fruits harvested.

Average fruit weight $plant^{-1}$

After harvesting in the all pickings the brinjal were weighed with the help of electronic balance and average fruit weight in g was drawn. Then the average fruit weight $plant^{-1}$ was also calculated.

$$\text{Average fruit weight (g plant}^{-1}) = \frac{\text{Total weight of fruits plant}^{-1}\text{ (g)}}{\text{Total no. of fruits plant}^{-1}}$$

Fruit yield (g $plant^{-1}$)

The weight of the fruit harvested from each pot was recorded at different pickings were added and total fruit yield (g $plant^{-1}$) were recorded.

Analysis of soil sample

Soil samples were collected just before the start of the pot experiment. The collected soil samples were air dried in shade ground with wooden mallet, passed through 2 mm sieve and stored in polythene bags. Soil sample was collected and analyzed for various physico-chemical properties as per standard procedures and presented in Table 1.

RESULTS AND DISCUSSION

Yield attributes and yield

The data (Table 4) showed significant difference between two soil types, Seasoned Press mud, Poultry manure, $ZnSO_4$ and borax application. The highest average fruit weight (39.11 g plant^{-1}), number of fruits (19.87) and fruit yield (778.80 g plant^{-1}) was registered in neutral soil. Among the treatments yield attributes like fruit weight (41.79 g plant^{-1}),no. of fruits(23.15)and fruit yield(968.15gplant^{-1}).With the interaction of soil types and treatments, the maximum was recorded in treatment S_1 T_{10} (75% RDF + Seasoned Pressmud @ 25 t ha^{-1} + $ZnSO_4$ @ 25 kg ha^{-1} + Borax @ 10 kg ha^{-1}) registered fruit weight plant^{-1} (42.09 g plant^{-1}), no. of fruits plant^{-1} (24.62 g plant^{-1}) and fruit yield per plant (1036.50 g plant^{-1}). Among organic manures Seasoned Pressmud application along with fertilizers performed better. It resulted in enhanced fruit length, fruit girth and ultimately increased average fruit weight. Moreover application of seasoned pressmud leads to increased photosynthetic area and favourable physiological activities under higher nutrient levels would have resulted in more production and translocation of photosynthates in plants, which accelerated the formation of more number of large sized fruits resulting in higher yields (Vinodkumar and Chopra, 2016).With regard to poultry manure application, it facilitates fruit yield of brinjal. The crop yield improvement due to addition of poultry manure was attributed to the presence of both readily available and slow release nitrogen. The incorporated poultry manure pots suggests that early mineralization and release of nutrients from the poultry manure, hence their availability for adsorption for plant growth. It also showed that poultry manure application method determines fruit yield by influencing nutrient availability (Adekiya and Aghede, 2017). Among micronutrients application, $ZnSO_4$ incorporation performed better in yield attributes of brinjal. The application of $ZnSO_4$ in the rhizosphere with constant supply coupled with higher zinc uptake might have increased the fruit yield (Vinodhini and Baskar, 2018). With respect to boron application significantly influenced fruit yield of brinjal. This is due to the role of boron is cell division, sugar development and hormone development (Kalaiyarasan *et al.,* 2020).

CONCLUSION

From the above results, it may be concluded that application of seasoned pressmud @ 25 kg ha^{-1} + $ZnSO_4$ @ 25 kg ha^{-1} + Borax @ 10 kg ha^{-1} in neutral soil performed the best for enhancing yield and yield attributes of brinjal.

REFERENCES

1. Adekiya, A.O. and T.M. Agbede. 2017. Effect of methods and time of poultry manure application on soil and leaf nutrient concentration growth and fruit yield of tomato. Journal of the Saudi Society of Agricultural Sciences, 16: 383-388.
2. Ahmad, W., M.H. Zia, S.S. Mahi, A. Niaz and Saifullah. 2012. Boron deficiency in soils and crops: A review crop plant, pp. 65-67.
3. Altaf, K., C.T. Subbarayappa, P.K. Basavaraja and G.K. Mukunda. 2019. Effect of different sources and levels of boron on soil properties and uptake of nutrients in tomato. International Journal of Agriculture Sciences, 11(6): 8037-8040.
4. Bholanath Saha, Manikant Prabhakar, Sushanta Saha, Mahendra Pal, Jagati Mandal, Reshma Singh, Pratap Singh, Ashish Rajan, Md. Shamin and Pancham Kumar Singh. 2020. Could maneuvering the methods of zinc and boron application influences yield and agro-morphological traits of brinjal in inceptisols. International Journal of Chemical Studies, 8(2): 11-15.
5. Bouyoucos, G.J. 1962. Hydrometer method improved for making particle size analyses of soils. *Agronomy Journal,* 54: 464-465.
6. Bower, C.A. and L.A. Wilcox. 1965. Methods of soil analysis. Part 2 ASA Inc., Madison, Wisconsin, USA.
7. Chapman, H.D. 1965. Cation-exchange capacity. *In:* Black, C.A. (Ed.) Methods of soil analysis – chemical and microbiological properties. *Agronomy,* 9: 891-901.

8. Chitdeshwari, T., R. Santhi, K. Radhika, S. Sivaganesan, S. Hemalatha, Pradip Dey and A. Subha Rao. 2017. GPS and GIS based soil fertility mapping for Cuddalore district of Tamil Nadu. Madras Agricultural Journal, 104(7-9): 251-257.
9. Diaz, P.M.N. 2016. Consequences of composts pressmud as fertilizers. D.J. International Journal of Advances in Microbiology and Microbiological Research, 1(1): 28-32.
10. Hanway, J.J. and H. Heidel. 1952. Soil analysis methods as used in Lowa State College soil testing laboratory, Lowa State College. *Bulletin,* 57: 1-31.
11. Harshit Kumar, B.P. Dhyani, U.P. Shahi, Ashok Kumar, Vivek, Akash Tomar and Anand Singh. 2021. Effect of zinc and vermicompost application on zinc, content, uptake and yield of later grown wheat. *Journal of Indian Society of Soil Science,*69(3): 339-343.
12. Hatcher, J.T. and C.V. Wilcox. 1950. Colorimetric determination of boron using carmine. *Analytical Chemistry*, 22: 567-569.
13. Jackson, M.L. 1973. Soil chemical analysis. Prentice Hall of India Pvt. Ltd., New Delhi.
14. Kalaiyarasan, C.D., Tamizhselvan, S. Jawahar, S. Ezhilkumar, S. Suseendran, S. Madhavan and S. Ramesh. 2020. Effect of sulphur and boron on hybrid sunflower. *Plant Archives,* 20(1): 741-746.
15. Kumar, S., R.S. Meena, D. Jinger, H.S. Jatav and T. Banjara. 2017. Use of pressmud composts for improving crop productivity and soil health. International Journal of Chemical Studies, 5(2): 384-389.
16. Lindsay, W.L. and W.A. Norvell. 1978. Development of a DTPA soil test for zinc, iron, manganese and copper. Soil Science Society of America Journal, 42: 421-428.
17. Mutfti, S., M.A. Chattoo, R. Anaya, A. Malik, R. Rasool and F. Nisar. 2021. Effect of nutrient sources on quality attributes of brinjal-feeugreek cropping sequence. Journal of Crop and Weed, 17(3): 91-97.
18. Olsen, S.R., C.V. Cole, F.S. Watanabe and C.A. Dean. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. United Stated Department of Agriculture, Circular No. 939.
19. Paul, O.J., O.O. Frank and A.E. Ambrose. 2017. Effect of poultry manure and NPK fertilizer on growth and yield of egg plant. *Journal of Agriculture and Rural Research,* 1(II): 112-117.
20. Prem Sekhar, M. and V. Rajashree. 2009. Influence of organic manure an growth yield and quality of okra. *American Eurasian Journal of Sustainable Agriculture,* 3(1): 6-8.
21. Reshma, M.R., V.P. Duraisami, D. Muthumanickam, Soniya Purma and N. Jayasrorian. 2016. Delineation and mapping of soil available ironand copper status in soils of Salem district of Tamil Nadu using GIS and GPS techniques. International Journal of Agricultural Science and Research, 6(2): 295-300.
22. Singh, M.V. 2012. Spread of micronutrient deficiencies specially boron in India and response of field crops. "Brain storming workshop on soil test based nutrients including boron and other micronutrients" Organized by ICRISAI-Agriculture Directorate, Karnatka-Rio Tinto India at Bangalore, India.
23. Soil Survey Staff. 1966. Key to soil taxonomy 8th edition, USDA, Washington, DC, USA, p. 326.
24. Subbiah, A. and G.C. Asija. 1956. A rapid procedure for the estimation of available nitrogen in soils. *Current Science,* 25: 259-260.
25. Vinodkumar and Chopra (2016) effect of sugarcane pressmud on agronomical characteristics of hybrid cultivar of egg plant under field condition. International Journal of Recycling Organic Waste in Agriculture, 5: 149-162.
26. Vinothini, R. and M. Baskar. 2018. Influence of soil application of zinc sulphate along with Foliar spray on growth and yield of rice in sodic soil under different amendments. Madras Agricultural Journal, 105(10-15): 511-516.
27. Walkley, A. and I.A. Black. 1934. An examination of different method for determining soil organic matter and a proposed modification of chromic acid titration method. *Soil Science,* 37: 29-38.

Table 1. Physico-chemical properties of initial soil

S. No.	Parameters	Neutral soil	Coastal saline soil	Methods Employed
A	Physical properties			
1.	Mechanical Analysis			
	Sand (%)	65	76	
	Silt (%)	28	19	
	Clay (%)	6	4	
	Textural class	Sandy loam	Sandy	Bouyoucos (1962)
	Taxonomical class	*Typic Ustifluvents*	*Typic Ustipsamments*	
2	Bulk density (Mg m^{-3})	1.50	1.55	Soil Survey Staff (1966)
3	Particle density (Mg m^{-3})	2.62	2.61	Soil Survey Staff (1966)
B	Chemical Properties			
4	pH	7.2	8.4	Jackson (1973)
5	EC (dSm^{-1})	0.75	4.2	Bower and Wilcox (1965)
6	Organic carbon (g kg^{-1})	4.7 (Medium)	0.99 (Low)	Walkley and Black (1934)
7	CEC [(cmol (p+)) kg^{-1}]	16.8	2.9	Chapman (1965)
8	$KMnO_4$–N (kg ha^{-1})	159.6 (Low)	103.6 (Low)	Subbiah and Asija (1956)
9	Olsen–P (kg ha^{-1})	50 (High)	23 (High)	Olsen *et al.* (1954)
10	NH_4OAC-K (kg ha^{-1})	135.5 (Medium)	197 (Medium)	Hanway and Heidel (1952)
11	DTPA-Fe (mg kg^{-1})	25.13 (Sufficient)	19.13 (Sufficient)	Lindsay and Norvell (1978)
12	DTPA-Mn (mg kg^{-1})	12.56 (Sufficient)	9.56 (Sufficient)	Lindsay and Norvell (1978)
13	DTPA-Zn (mg kg^{-1})	0.28 (Deficient)	0.22 (Deficient)	Lindsay and Norvell (1978)
14	DTPA-Cu (mg kg^{-1})	1.53 (Sufficient)	0.55 (Sufficient)	Lindsay and Norvell (1978)
15	Hot water soluble B (mg kg^{-1})	0.47 (Deficient)	0.32 (Deficient)	Hatcher and Wilcox (1950)

Table 2. Nutrient content in Seasoned Pressmud and Poultry manure

Material	N (%)	P (%)	K (%)	OC (%)	Fe (ppm)	Mn (ppm)	Cu (ppm)	Zn (ppm)
Seasoned Pressmud	1.525	1.09	0.99	23.6	500	300	64	125
Poultry manure	3.03	1.14	1.16	3.87	930	210	24	25

Table 3. Nutrient content of $ZnSO_4$ and Borax

Micronutrient	Zn (%)	B (%)
Zinc sulphate ($ZnSO_4$)	36.5	–
Borax ($Na_2B_4O_7$)	–	11.36

Table 4. Yield attributes and yield of brinjal

Treatments	No. of fruits $plant^{-1}$			Fruit weight per plant (g $plant^{-1}$)			Fruit yield (g $plant^{-1}$)		
	S_1 (Neutral soil)	S_2 (Coastal saline soil)	Grand mean	S_1 (Neutral soil)	S_2 (Coastal saline soil)	Grand mean	S_1 (Neutral soil)	S_2 (Coastal saline soil)	Grand mean
T_1 - Control	16.30	14.11	15.21	36.00	34.01	35.01	587.16	480.07	533.61
T_2 - PM + $ZnSO_4$ @ 25 kg ha^{-1}	20.52	17.30	18.91	41.07	36.19	38.63	842.75	626.61	734.69
T_3 - PM + Borax @ 10 kg ha^{-1}	21.41	18.50	19.96	41.01	37.01	39.01	837.41	678.02	757.72
T_4 - PM +$ZnSO_4$@	22.07	19.03	20.55	42.19	38.89	40.54	929.23	740.67	834.95

25 kg ha^{-1} + Borax @ 10 kg ha^{-1}									
T_5 - PM + FS $ZnSO_4$ @ 0.5%	17.19	15.09	16.14	37.39	35.19	36.29	643.27	531.51	587.39
T_6 - PM + FS Borax @ 0.2%	16.49	14.79	15.64	36.19	34.08	35.14	597.29	504.68	550.98
T_7 - PM + FS $ZnSO_4$ @ 0.5% + Borax @ 0.2%	19.91	16.92	18.42	38.79	36.59	37.69	772.88	619.27	696.08
T_8 - SPM + $ZnSO_4$ @ 25 kg ha^{-1}	21.41	19.01	20.21	40.19	38.69	39.44	861.07	736.07	798.57
T_9 - SPM + Borax @ 10 kg ha^{-1}	23.51	20.41	21.96	39.79	37.59	38.69	963.08	767.79	851.93
T_{10} - SPM + $ZnSO_4$ @ 25 kg ha^{-1} + Borax @ 10 kg ha^{-1}	24.61	21.68	23.15	42.09	41.49	41.79	1036.16	900.13	968.15
T_{11} - SPM + FS $ZnSO_4$ @ 0.5%	18.30	16.11	17.21	37.79	35.59	36.69	629.11	573.86	632.99
T_{12} - SPM + FS Borax @ 0.2%	17.20	15.23	16.22	37.59	35.39	36.49	647.08	541.26	594.17
T_{13} - SPM + FS $ZnSO_4$ @ 0.5% + Borax @ 0.2%	19.31	17.11	18.21	38.39	36.39	37.39	741.88	623.15	682.52
Mean	19.87	17.33	18.60	39.11	36.70	37.91	778.80	640.24	709.52
	S	T	S × T	S	T	S × T	S	T	S × T
S.Ed.	0.04	0.11	0.16	0.8	0.21	0.22	1.63	4.18	5.91
CD (P=0.05)	0.08	0.22	0.32	0.22	0.62	0.82	3.29	8.38	11.86

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RESEARCH ARTICLE

Impact of Industrial Relations on the Performance of Employees

S.Babu Priya[1], M.Sankar[2]* and B.Jayalakshmi[3]

[1]Assistant Professor, Department of Business Administration, Indian Institute of Handloom Technology (IIHT), Salem, Tamil Nadu, India
[2]Assistant Professor, Department of Business Administration, Mahendra Arts and Science College (Autonomous), Namakkal, Tamil Nadu, India
[3]Associate Professor, Department of Management Studies, Vivekanandha College of Arts and Science for Women (Autonomous), Elayampalayam, Tiruchengode, Namakkal, Tamil Nadu, India

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***Address for Correspondence**
M.Sankar
Assistant Professor,
Department of Business Administration,
Mahendra Arts and Science College (Autonomous),
Namakkal, Tamil Nadu, India.
Email: sankarobu@yahoo.co.in

ABSTRACT

Industrial relations have enormous significance in industrial life. The term "industrial relations" refers to the interaction between the inside employees' organizations. Using labor management strategies, collective bargaining, and trade unionism often enables industries to maintain industrial peace. This study seeks to establish factors that have the most significant impact on labor relations and the impact of labor relations on employee performance. This study examined the operation of medical facilities in Tamil Nadu. The researcher took the following factors into account when conducting this study: financial incentives, labor welfare and safety measures, grievance procedures, dispute resolution techniques, trade union activities, leadership competency, working conditions, organizational policies, and individual policies. Five hundred healthcare professionals were given questionnaires to gather preliminary information. Secondary data were compiled from books, magazines, and academic publications. The researcher concludes that factors like financial incentives, labor welfare and safety measures, grievance procedures, dispute resolution techniques, trade union activities, leadership quality, working conditions, organizational policies, and personal policies influenced industrial relations and that industrial relations have a significant impact on an organization's workforce performance.

Keywords: Industrial relations, labor welfare measures, labor safety measures, grievance redressal, dispute settlement.

INTRODUCTION

Industrial relations are the relationships and associations in the industry, particularly between management and employees, because of their complex attitudes and approaches regarding controlling the industry's affairs for the benefit of management and employees. According to Dale Yoder, the process of management is interacting with one or more unions to bargain and later manage a collective bargaining agreement or labor agreement known as industrial relations. Good relationships boost staff morale, productivity, mutual interest, job quality, and product efficiency and reduce waste, poor quality, and production costs. Mr. Kirkaldy asserts that the goals of labor relations are to enhance workers' economic well-being under the current industrial management system. Inadequate wage fixing, unhealthy working conditions, indiscipline, a lack of supervisory and managerial people skills, the introduction of automation without creating the right environment, a heavy workload, inadequate employee welfare facilities, unfair labor practices, retrenchments, dismissals and lock-outs, strikes, and general economic and political environments are the causes of poor industrial relations. Poor labor relations bring on instability and industrial disputes. To foster positive working connections between employers and employees, each organization's human resources must be involved in everything that occurs within the organization.

The primary goal of maintaining good working relations is to
1. Protect the value of labor and management by ensuring mutual respect and goodwill.
2. Increase efficiency to a high level by combating the trend of absenteeism and labor turnover.
3. Prevent workplace disputes and foster amicable management-labor relations.
4. Reduce strikes, lock-outs, and other pressure techniques by enhancing pay and working conditions.

LITERATURE REVIEW

Armstrong (2005) defines employee motivation as proactively addressing problems that have a detrimental effect on the workplace. In contrast, employee relations refers to navigating the dynamic between management and staff to maximize output. According to Clarke, great connections between employers and workers are the product of deliberate strategies and activities taken by employee relations managers to improve two-way communication (2001). For managers to effectively manage their employees' relationships with one another, they must establish and maintain open lines of communication with their staff. Walton argues that an employer and employee should be able to work together because they share values. According to George and Jones, effective communication and connections among workers boost productivity, job satisfaction, motivation, and morale. According to Foot and Hook, productive workplaces have strong employer-employee connections because they facilitate trust and mutual respect and provide adequate and appropriate work for all parties involved.

On the other hand, employees follow legitimate and reasonable instructions, show loyalty, and put in the extra effort. Bernal J. Gargallo-Castel, Luis Marzo-Navarro, and Jose Rivera-Torres concluded that an unsatisfied workforce prevents a corporation from delivering on its quality promises. Work, pay, promotion, benefits, working conditions, supervision, coworkers, company, and management are all aspects of an employee's employment that must be understood to succeed, as outlined in Locke's handbook of industrial and organizational psychology. Work, promotion, recognition and benefits, working conditions, supervision, coworkers, organization, and administration are some of the most frequently recognized elements of job satisfaction, as found in research by Locke E. A., Witte H. D, M. E. Sempane, Rieger H S, and G. Roodt. Robbins (2001) identifies work, incentives, consistency in working circumstances, and great partners as four of the most critical aspects of job happiness.

Further, he argued that a person's upbringing and sense of self are crucial. Sequeira and Dhriti (2015) found that the effectiveness of a company's HR policies and practices can be directly traced back to the level of productivity achieved by its employees. Workers who are happy with how things are done at their firm are more productive and less likely to embrace change. Boosting employee morale and performance directly results from an organization's

efforts to strengthen its employee relations practices. Kuzu and Ozihan's (2014) research and Al-(2015) khozondar's study established a link between positive employee relations and increased productivity. Effective staff management is essential for every business if its objectives are to be realized, and employee relationship management is one method for doing just that (Lagergren and Anderson, 2013).

RATIONALE OF THE STUDY

Because it motivates workers to perform better and deliver more outcomes, a positive employer-employee connection is crucial to the victory of the business (Burns,2012). Effective communication between employees and their bosses is challenging to maintain—workplace relations impact employee happiness. If the effect is favorable, employees are content with the industrials of the organizations, which motivates them to perform well. When people perform well, it helps organizations advance in their field. Poor labor relations negatively impact customers, the government, employers, and industrialists. In-depth research is needed to fully comprehend factors that influence industrial relations and the effects such relations have on employees' performance. Emphasizing labor relations can promote and preserve workplace harmony and boost productivity for people and businesses. It has become difficult because of many firms' poor relationships between employers and employees (Kaliski,2007). Employee relations were defined by Bajaj R. (2013) as a relationship between workers and managers that aims to strengthen workers' morale, dedication, and trust as well as create an environment at work where they can give their all to achieving organizational objectives.

RESEARCH METHODOLOGY

The sample size in this descriptive study is the proportion of the total population drawn for the study. The primary purpose of the sample size calculation is to identify the number of respondents who fairly and adequately represent the total population. The number of samples used in this study was calculated using the Taro Yamane (1967) statistics technique. Five hundred people were selected as samples for this research. Both primary and secondary sources were used to compile this study's findings. Information was gathered from respondents using a standardized questionnaire. A sample of 10% of the entire piece was used to determine how well the data would hold up in more extensive research. Thus, the results may be deemed dependable. Cronbach's alpha was determined using SPSS for a study of internal consistency (SPSS). The pilot testing findings showed that the instrument was trustworthy, with an alpha value greater than 0.80. Chi-square analysis was used to specify the significance of observed differences between groups. Correlation analysis was utilized to assess the strength of the connection between "industrial relations" and "employee performance."

DISCUSSION

From Table 1, it is identified that percentages of males and females are25.8 % and74.2%, respectively, which shows female dominance; the percentages of age-wise categories 20 to 30, 31 to 40, 41 to 50 and above 50 years were 11.6%,39.2%,28.8%, and 20.4% respectively which shows 31 to 40 years age group playing majority in the industry. The percentages of the married and unmarried are 83.4 and 16.6 respectively, and those below five years of experience,5 to 10 years,11 to 15 years, and above 15 years were 12%, 33.6%,33.6%, and 20.8%,respectively. The percentages of the school level, diploma, graduate, and postgraduate are 6%, 33.4%, 37.4%, and 23.2%, respectively, and the percentages of the nuclear family and joint family are 83.2% and 16.8%,respectively, which shows the majority of the respondents belong to nuclear family structure.

From Table 2, it is identified that, among the factors of industrial relations, working conditions (23%) are among the most influential, followed by monetary benefits (20%) influencing industrial relations. Since the chi-square test was applied to measure the difference between the factors, the chi-square value was 186.280, and the p-value was .000.

So, the researcher concludes that the Null hypothesis 'There is no difference between the factor's influence and industrial relation' has been rejected. The alternative view, 'There is a difference between the factors influence and industrial relation,' has been accepted. From table 3, visible employees working in the organizations think that industrial relations are essential to the smooth functioning of an organization since the chi-square test was applied. The Chi-square value was 27.848, and the p-value was .000. So, the researcher. Concludes that the Null hypothesis 'There is no association among employee opinions that industrial relations are important for smooth running of industry' has been rejected. The alternative hypothesis. 'There is an association among the opinions of employees that industrial relations are important for smooth running of industry' has been accepted.

Correlation analysis has been applied to measure the relationship between' industrial relations and employees' performance' with the hypothesis that there is no relationship between 'industrial relations and employees' performance.' Table 4 shows a positive correlation between industrial relations and employee performance since the p-value was0.000. So, at this moment researcher concludes that the Null hypothesis 'there is no relationship between industrial relations and employee performance has been rejected. Alternative to the idea, 'there is a relationship between industrial relations and employee performance,' has been accepted.

RESULTS

In this research, the researcher found that from the following factors monetary benefits, welfare measures, welfare measures, grievance redressal methods, dispute settlement, dispute settlement, quality in leadership, working conditions, organizational policies, and personal policies, the working conditions and the monetary benefits are the most influencing factor of industrial relations. It is suggested that to concentrate the working conditions of the organization, keep healthy and favorable working conditions by adopting new and strategic working policies regarding the monetary benefits and revise the pay according to the cost of living and consumer price index value.

CONCLUSION

From the research, the researcher has concluded that working conditions and monetary benefits strongly influence industrial relations. Employees working in the industry passionately believe that industrial relations are most significant for the smooth functioning of the industry. Industrial relations are highly influenced by employee performance and are directly interconnected with employees' performance. It could not maximize employee performance without having good industrial relations.

REFERENCES

1. Al-Khozondar N. (2015). Employee Relationship Management and its effect on employee Performance in Telecommunication and Banking Sectors, Doctoral Thesis Submitted to The Islamic University- Gaza, Deanship of Graduates Studies, Faculty of Commerce, page-4.
2. Armstrong M. (2005), Armstrong's Handbook of Management and Leadership: Approaches to HRM and L&D. Kogan page Limited Publishing, United States.
3. Bajaj, R. (2013). Suggestions to Implement Human Relations and Its Determinants in Public Sectors. American Journal of Engineering Research, 02(12), 91-97.
4. Burns A. (2012). Employee Relations. In Prescott RK, Rothwell WJ (Eds.) Encyclopedia of Human Resource Management, Key Topics, and Issues, John Wiley & Sons. 1:186.
5. Clarke KF. (2001). What businesses are doing to attract and retain employees- becoming an employer of choice. Employee Benefits Journal 9(7): 34-37.
6. Daly Yoder: https://www.whatishumanresource.com/industrial-relations.
7. Foot M., Hook C. (2008). Introducing Human Resource Management. (5th ed.) Harlow (Essex). Prentice Hall.

8. George J M.& Jones G R. (2008)., Understanding and Managing Organizational behavior, (5th Ed.). Pearson/Prentice Hall, New Jersey.
9. Garcia-Bernal J., Gargallo-Castel A., Marzo-Navarro M., Rivera-Torres P. (2005). Job satisfaction: empirical evidence of gender differences. Women in Management Review 20(4): 279-288.
10. Kaliski B S. (2007). Encyclopedia of Business and Finance, (2nd ed.). Thompson Gale, Detroit.
11. Kuzua O H., Ozilhan D. (2014). The Effect of Employee Relationships and Knowledge Sharing on Employees' Performance: An Empirical Research on Service Industry: An Empirical Research on Service Industry. Social and Behavioral Sciences 109: 1370 – 1374.
12. LockeEA. (1976). Thenature andcausesof jobsatisfaction. In M.D.Dunnette(Ed.), Handbook of industrial and organizational psychology: 1297–1351.
13. Lagergren, N & Andersson, V (2013). I am becoming the CFO's best friend by gaining a deeper customer understanding. Master Thesis.
14. Robbins S P. (2001). Organizational behavior (9th ed.) New York. Prentice-Hall, Inc.
15. Sempane M E., Rieger H S., Roodt G. (2002). Job satisfaction about organizational culture. SA Journal of Industrial Psychology 28 (2): 23-30.
16. Sequeira, A and Dhriti,A.(2015). Employee Relations and its Impact on Employee Performance: Case Study.
17. Walton R E, From control to commitment in the workplace. Harvard business review 66(2):77-84.
18. Witte H D. (2005). Job insecurity: review of the international literature on definitions,Prevalence, antecedents and consequences. SA Journal of Industrial Psychology 31(4):1-6.
19. Yamane, Taro (1967). Statistics and Introductory Analysis. 2nd Edition, New York, Harper, and Row.
20. https://www.economicsdiscussion.net/industries/industrial-relations/32249

Table 1: Democratic Factors

Particulars	Basis	Frequency	Percentage
Gender	Male	129	25.8
	Female	371	74.2
Age	20-30	58	11.6
	31-40	196	39.2
	41-50	144	28.8
	Above 50	102	20.4
Marital Status	Married	417	83.4
	Unmarried	83	16.6
Experience	Below five years	60	12.0
	5 to 10 years	168	33.6
	11 to 15 years	168	33.6
	Above 15 years	104	20.8
Qualification	School level	30	6.0
	Diploma	167	33.4
	Graduate	187	37.4
	Post Graduate	116	23.2
Familytype	Nuclear family	416	83.2
	Joint family	84	16.8

Source: Primary Data

Table 2. Factors Influencing on Industrial Relations of an Organizations					
Factors	Frequency	Percent	Chi-Square	df	Asymp. Sig.
Monetary Benefits	100	20.0	186.280	9	.000
Welfare Measures	30	6.0			
Safety Measures	40	8.0			
Grievance Redressal Methods	46	9.2			
Dispute Settlement	32	6.4			
Trade Union Activities	57	11.4			
Quality in Leadership	30	6.0			
working conditions	115	23.0			
Organizational Policies	20	4.0			
Personal Policies	30	6.0			
Total	500	100.0			
x^2=186.280,df=9,P=.000 and p<0.05					

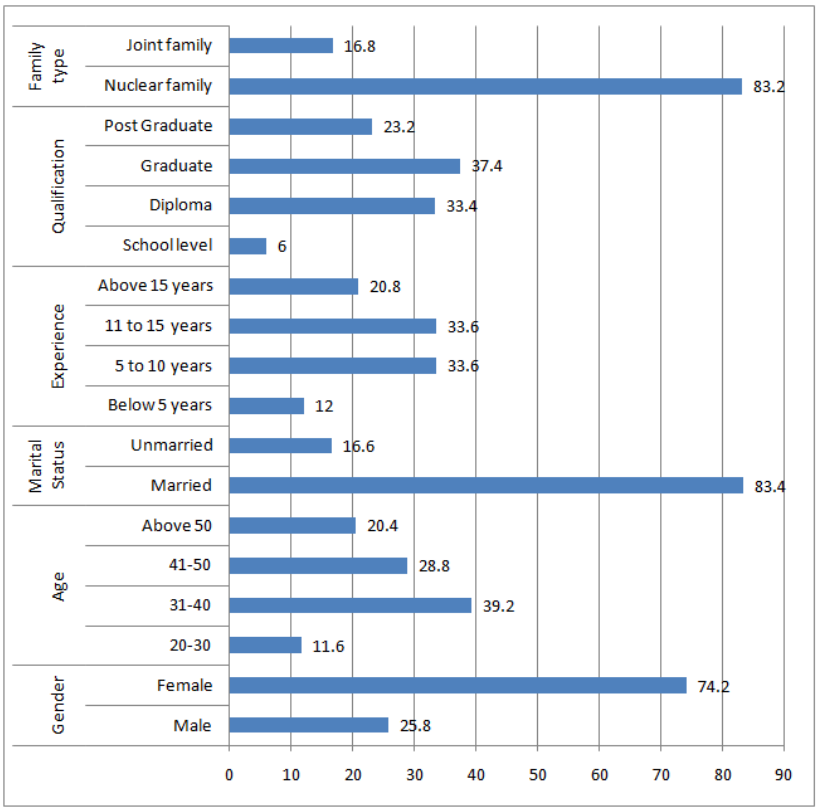
Source: Primary Data

Table. 3. Employee's opinion About industrial Relations are Important for Smooth Running of Industry				
Opinion of Employees	Frequency	Chi-Square	df	Asymp. Sig.
YES	309	27.848	1	.000
No	191			
x^2=27.848,df=1, P=.000 and p<0.05				

Source: Primary Data

Table 4. Correlationsamong Industrial Relations and Employee Performance

		Industrial relations	Employee Performance
Industrial relations	Pearson Correlation	1	1.000**
	Sig. (2-tailed)		.000
	N	500	500
Employee Performance	Pearson Correlation	1.000**	1
	Sig. (2-tailed)	.000	
	N	500	500



Source: Primary Data

Figure 1: **Democratic Factors**

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RESEARCH ARTICLE

Assessment of Trend, Growth and Instability of Cocoa Beans in Tamil Nadu

M. Sundariya[1]*, V. Banumathy[2] and S.Ravichandran[3]

[1]Ph.D Research Scholar, Department of Agricultural Economics, Annamalai University, Chidambaram, Tamil Nadu, India.
[2]Professor, Department of Agricultural Economics, Annamalai University, Chidambaram, Tamil Nadu, India.
[3]Associate Professor, Department of Agricultural Economics, Annamalai University, Chidambaram, Tamil Nadu, India.

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***Address for Correspondence**
M. Sundariya,
Ph.D Research Scholar,
Department of Agricultural Economics,
Annamalai University,
Chidambaram, Tamil Nadu, India.
Email: sundariyamegalai@gmail.com

ABSTRACT

The study focused on growth rate and instability of area, production and productivity of cocoa in Tamil Nadu and employed CAGR and Cuddy Della Valle Instability index using the time series data on area, production and productivity collected from DCCB for the period of 2011-12 to 2020-21. Tamil Nadu witnessed the positive growth rate of area (4.43%), production (13.03%) and productivity (8.93%) with increasing trend for last decade. The major 5 districts namely Coimbatore, Dharmapuri, Erode, Kanniyakumari and Salem were selected on the basis of higher production with cocoa produced area during 2020-21. The CDVI showed higher instability in Dharmapuri and low instability in Erode in case of production. The government enhance to stable cocoa production with area and increase the procurement centers, developing the crop insurance schemes during the uncertainty condition in the state which will increase the high production in the upcoming years.

Keywords: Area, Production, Tamil Nadu, Compound Annual Growth Rate and Cuddy Della Valle Instability

INTRODUCTION

Cocoa (*Theobroma cacao* L.) has a native of South America and it entered India during the 20th century. In India, early 1970's cocoa was started as cultivated as a commercial crop and also being a tropical crop. It was mostly grown as an intercrop (70% of the total cocoa area) with coconut and areca nut plantations rather than monocrop. The percentage share of cocoa produced by India among the total plantation crops in the nation was accounted to be 0.16 per cent. Cocoa is cultivated in the states of Andhra Pradesh, Kerala, Karnataka, and Tamil Nadu which combinedly produce 27072.15 MT of cocoa from 103376 ha in the year 2020-21. Tamil Nadu increases cocoa production three folds by increasing the area from 21 thousand hectares to 32 thousand hectares during the past decade [1]and increases its share from 1.1% to 10% of the nation's cocoa production. Tamil Nadu found to be ranks first in terms of production in 2020-21. India provides the scope for improving cocoa cultivation by implementing the Mission for the Integrated Development of Horticulture (MIDH) in the year 2014-15 with the objective of enhancing the farmer's income through the integrated innovations in the cultivation of horticultural and perennial crop plantations.

Despite of decreasing trend in Tamil Nadu during 2019-2020, due to the sudden drop of price in cocoa. The literature reviews of studies have been conducted on the growth and instability of agricultural commodities. [2]discussed the growth and instability of export of Indian groundnut and revealed that the negative growth in area was found at national level while production and productivity registered non-significant positive growth and the instability in export as well as in area, production and productivity at country level were low. [3]analysed growth and instability of Indian agriculture and revealed that instability of rice, sugarcane, pulses, cotton and oilseeds were increased in areas over the period of study. [4] found that positive and significant growth in area, production and

Productivity of mango. With this in mind, the study was conducted with following specific objectives
1.To analyses the growth rate of area, production, and productivity of cocoa in Tamil Nadu.
2.To know the instability of area, production and productivity of selected districts in Tamil Nadu.

DATA AND METHODOLOGY

The study was based on the time series data on area, production and productivity for the period of last 10 years (2011-12 to 2020-21) which were collected for four southern parts of India namely Andhra Pradesh, Karnataka, Kerala and Tamil Nadu. The major districts were selected on the basis of high production during 2020-21 among the 38 districts in state of Tamil Nadu. The state wise data were collected from the source of Directorate of Cashew nut and Cocoa Development Board (DCCB) and district wise data from Directorate of Statistical department, Chennai for the period of last decade.

Compound Annual Growth Rate (CAGR) analysis

The compound Annual Growth Rate analysis was carried out to determine the growth rates in area, production and productivity of cocoa in Andhra Pradesh, Karnataka, Kerala and Tamil Nadu of India for the period of last 10 years (2011-12 to 2020-21) using the exponential growth function of the form given by [5].

$$Y = ab^t u_t$$

Where,
Y= area, production and productivity of cocoa
a = Intercept
b = Regression coefficient
t = Time variable for last decade (2011-12 to 2020-21)
u_t= Error term
The equation was estimated after transforming the above equation as logarithmic form:

$\ln Y = \ln a + t \ln b + \ln u_t$
Then, the per cent CGR(g) was calculated using the relationship;

g = {antilog of (ln b)-1} *100

Cuddy Della Valle Instability
Instability analysis in area, production and yield of selected districts was estimated by Cuddy Della Valle Instability Index [6]. It is a modification of coefficient of variation to accommodate trend present in the data of last decade, a feature of economic time series data. This method is superior over the scale dependent measures such as standard deviation. The Cuddy Della Valle Index (CDVI) was calculated as follows:

Instability index (CDVI) = CV $(1-R^2)^{1/2}$
Where,
CV = Coefficient of Variation
R^2 = Coefficient of Determination adjusted for degrees of freedom
CV = (Standard Deviation/ Mean) *100

RESULT AND DISCUSSION

The results were obtained for growth rate analysis and Cuddy Della Valle index of selected districts and it was discussed as below. Table 1 showed state wise growth rate of cocoa over the period of last decade (2011-12 to 2020-21) revealed that Tamil Nadu found to be first rank with 13.03 per cent per annum followed by Andhra Pradesh with the growth rate of 12.19 per cent per annum. In case of area, the Andhra Pradesh found to be ranked as first and positive growth rate of 8.40 % followed by Tamil Nadu with 5.56 % per annum. Tamil Nadu increases the cocoa production to three folds by increases the area from 21 thousand hectare to 32 thousand hectare during the past decade [1] and increase its share from 1.1% to 10% in national cocoa production. During 2020-21, current country's production of 27072.15 MT recorded a growth rate of 8.19% in the area of 103376 ha with growth rate of 5.56 percent per annum over a period of time. The productivity has clambered up with a mere CAGR of 9.72 and 8.93 percent statistically significant at 5 % level in Karnataka and Tamil Nadu respectively during the period. The average productivity of cocoa in India was 230 kg/ha during last decade. The trend line was drawn for area and production of cocoa in Tamil Nadu and it was shown in figure 1.It could be seen that the production of cocoa was declined in the period of 2011-12 to 2013-14, after that government took step to increase the production of 1750 MT with the extent of 26959 ha through the implementation of Mission for Integrated Development of Horticulture (MIDH) which have a significant impact on the cocoa production in the year 2014-15. The trend line on cocoa production and area showed increasing trend in the year 2018-19, this is due to the establishment of new plantation under the programme of 3rd year maintenance of cashew and cocoa plantations was assisted as per the MIDH. In 2019-2020, the trend was slightly declined in area as well as production under cocoa due to the procurement of beans by chocolate companies at a lower rate of price in covid pandemic situation which resulted to uprooted cocoa in the state.

According to the trend, the government took place the procurement centers for cocoa beans and developing the crop insurance schemes for cocoa during the uncertainty condition in the state of Tamil Nadu will increase the high production with low extent of cocoa plantation in the upcoming years. District wise percentage change in area and production of cocoa in 2020-21is presented in table 2 and the major districts were shown in figure 2. The total production of cocoa in the year 2020-21 was arrived at 3361 MT with the extent of 2170 hain Tamil Nadu. Among the 38 districts of Tamil Nadu, Dharmapuri was found to secure higher production of cocoai.e., 1082.5 MT .with 32.20 per cent and followed by Coimbatore (31.15), Salem (9.91), Kanniyakumari (7.59) and Erode (3.00) in the year 2020-21. In terms of area, Coimbatore is found to be first i.e., 748 ha with 34.47 per cent of total cocoa produced area in the state followed by Dharmapuri, Salem, Kanniyakumari and Erode accounted for 19.95, 10.97, 5.02 and 3.87 per cent respectively to the total cocoa produced area in the state during last year. The percentage contribution of cocoa was found to be dismal and hence efforts to enhance the production with area under cocoa. The figure 3represented the

area and production of cocoa in major districts for the period of last 5 years. During 2016-17, the Kanniyakumari and Salem showed high production comparatively other districts due to the monopoly market of chocolate company in the districts resulted to decrease the production as well as income of cocoa growers in the study period of last 5 years in Tamil Nadu.

The graphical representation of figure 4 shows the instability index of area, production and productivity of cocoa for the period of 2011-12 to 2020-21 in selected districts. Dharmapuri district had higher instability on area (113.28)and production (106.73) followed by Coimbatore showed the instability index of 94.76 and 93.48 on area and production respectively in the period of last decade. The higher instability occurred due to the procuring the beans at low price leads to the unstable condition of growing cocoa in Coimbatore, farmers move to grow the alternative crops like nutmeg, areca nut commodities are procuring with high price. Erode, Kanniyakumari and Salem showed lesser instability in area and production of cocoa which may be due to lesser demand for procuring the cocoa beans in the particular districts. In case of productivity, Dharmapuri and Kanniyakumari accounted low instability index of 3.46 and 3.36ofrespectively and erode, Salem and Coimbatore have higher instability index of 19.78, 17.49 and 10.77 respectively in last decade.

CONCLUSION

The study analyzed the growth pattern of the Indian cocoa and the instability were measured by the compound growth rate and Cuddy Della Valle index. The growth rate of the area, production and productivity four states India and revealed that high cocoa production in Tamil Nadu followed by Andhra Pradesh. Indian cocoa shows the increasing trend of area, production and productivity in the state of Tamil Nadu due to the implementation of MIDH programme after 2014-15. The growth rate of productivity of cocoa has positive increase in the period of last decade. The growth rate has decreased in the year 2019-2020 due to chocolate companies procuring the beans at a lower rate of price in covid pandemic situation which resulted to uproot cocoa in the state. In the year 2020-21, the Coimbatore, Dharmapuri, Kanniyakumari, Salem and Erode were districts producing more cocoa among 38 districts in the state of Tamil Nadu. The reason of monopoly competition occurred in the district of Kanniyakumari, which lead to decline in production as well as area and the farmers are not cultivated the cocoa in the particular region. Coimbatore accounted the higher instability and Erode has lower instability of area, production and productivity of cocoa in the period of 2011-12 to 2020-21. The higher instability occurred due to the procuring the beans at low price leads to the unstable condition of growing cocoa in Coimbatore. Despite of decreasing trend in Tamil Nadu during 2019-2020, the farmers in the state are involving in intercropping cocoa in their farm which leads to increase production with extent of cocoa produced areas. The government take efforts to enhance the production with area of cocoa and increase the procurement centers for cocoa beans and developing the crop insurance schemes for cocoa during the uncertainty condition in the state of Tamil Nadu which will increase the high production with low extent of cocoa plantation in the upcoming years.

REFERENCES

1. Government of India.Directorate of Cashewnut and Cocoa Development Board 2020-21*. Department of Agriculture, Cooperation and Farmers Welfare, Ministry of Agriculture & Farmers Welfare, New Delhi.*2020.
2. Ranjana A, Thakar K P, Burark S S and Arti A. Production and Export performance of Indian groundnut. *International Journal of Agricultural Sciences.*2017. 9 (4): 3724-3727.
3. Anjum S and Madhulika. Growth and Instability Analysis in Indian agriculture.*International Journal of Multidisciplinary Research and Development.* 2018. 5 (11):119-125.
4. Patil R S, Deshmukh R G, Bhaskar K R and Jahagirdar S W. growth and export performance of mango in India.*International Journal of Current Microbiology and Applied Sciences.* 2018. 6: 2667-3673.
5. George A and Raju K V.An Empirical analysis of the Dynamics of Cocoa Cultivation in India. *Agricultural Situation in India.*2021.78: 27-34.

6. Cuddy JDA, Della Valle PA. Measuring the Instability of Time Series Data.*Oxford Bulletin of Economics and Statistics.* 2009; 40(10):79-84.

Table.1.state wise growth rate of area, production and productivity of cocoa in India(2011-12 to 2020-21)

S. No	States	CAGR per cent per annum		
		Area (ha)	Production (MT)	Productivity (kg/ha)
1	Andhra Pradesh	8.40	12.19	18.15
2	Kerala	4.19	5.93	5.53
3	Karnataka	3.45	3.77	9.72**
4	Tamil Nadu	4.43	13.03	8.93**
5	India	5.56	8.19	5.30

Source: Directorate of Cashew and Cocoa Development Board, 2020-21

CAGR: Compound Annual Growth Rate

**indicates the 5% level of significance

Table.2.District wise percentage change in area and production of cocoa in 2020-21

S. No	Districts	Area (ha)	Per cent in change	Production (MT)	Per cent in change
1	Ariyalur	0	0	0	0
2	**Coimbatore**	**748**	**34.47005**	**1047.2**	**31.15452**
3	Cuddalore	1	0.046083	1.5	0.044625
4	**Dharmapuri**	**433**	**19.95392**	**1082.5**	**32.20471**
5	Dindigul	135	6.221198	94.5	2.811404
6	**Erode**	**84**	**3.870968**	**100.8**	**2.998831**
7	Kancheepuram	0	0	0	0
8	Chengalpattu	0	0	0	0
9	**Kanniyakumari**	**109**	**5.023041**	**255.06**	**7.588113**
10	Karur	9	0.414747	7.2	0.214202
11	Krishnagiri	25	1.152074	25	0.743758
12	Madurai	10	0.460829	8	0.238002
13	Nagapattinam	0	0	0	0
14	Mayiladurai	0	0	0	0
15	Namakkal	49	2.258065	58.8	1.749318
16	Perambalur	0	0	0	0
17	Pudukottai	25	1.152074	17.5	0.52063
18	Ramanathpuram	0	0	0	0
19	**Salem**	**238**	**10.96774**	**333.2**	**9.912802**
20	Sivagangai	0	0	0	0
21	Thanjavur	63	2.903226	88.2	2.623977
22	Theni	69	3.179724	69	2.052771
23	The Niligiris	56	2.580645	56	1.666017
24	Thiruvallur	6	0.276498	6	0.178502
25	Thiruvanamalai	3	0.138249	3	0.089251
26	Thiruvarur	1	0.046083	1	0.02975
27	Thooothukudi	0	0	0	0
28	Thirupur	42	1.935484	42	1.249513
29	Trichy	3	0.138249	1.5	0.044625
30	Thirunelveli	14	0.645161	32.9	0.978785
31	Thenkasi	35	1.612903	19.95	0.593519

32	Vellore	7	0.322581	7	0.208252
33	Ranipettai	2	0.092166	2	0.059501
34	Thirupathur	0	0	0	0
35	Villupuram	0	0	0	0
36	Kallakurichi	0	0	0	0
37	Virudhunagar	3	0.138249	1.5	0.044625
38	Chennai	0	0	0	0
Total		**2170**	**100**	**3361.31**	**100**

Source: Directorate of Statistical Department, Chennai 2020-21

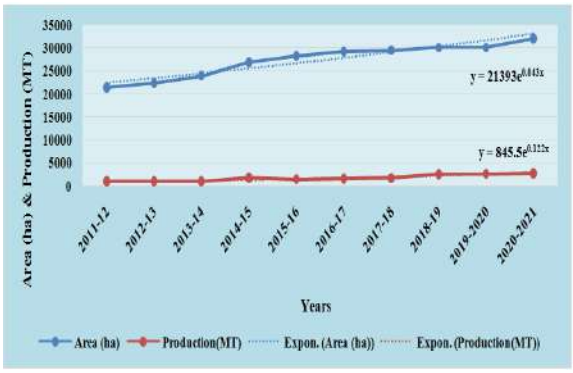

Figure 1. Trend in Area and Production of Cocoa in Tamil Nadu (2011-12 to 2020-21)

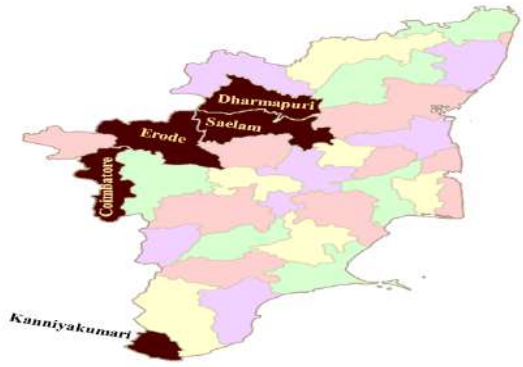

Figure 2. Major districts of cocoa cultivated areas during 2020-21

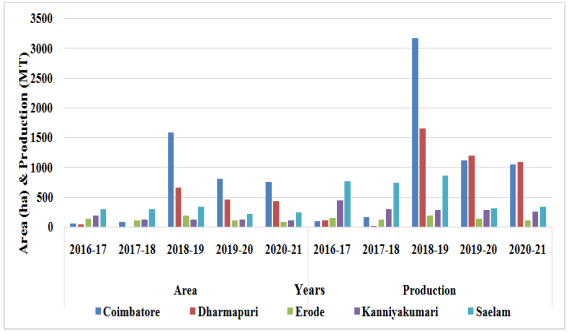

Figure 3. Area and Production of Cocoa in major districts in last 5 years (2016-17 to 2020-21)

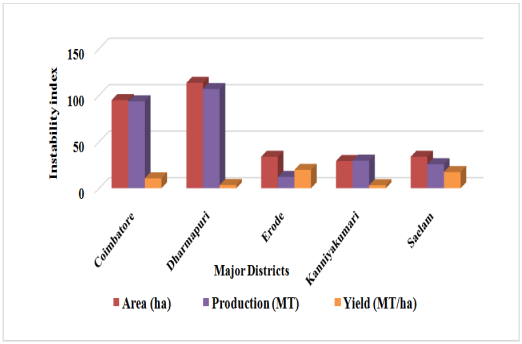

Figure 4. Cuddy Della Valle Index of major districts (2011-12 to 2020-21)

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RESEARCH ARTICLE

Assessment of Entrepreneurship Skills among Students Entrepreneurs

S.Balasaraswathi[1]* and T.Radha[2]

[1]Ph.D. Scholar, Department of Home Science Extension Education, Avinashlingam Institute for Higher Education for Women, Coimbatore, Tamil Nadu, India.
[2]Assistant Professor, Department of Home Science Extension Education, Avinashlingam Institute for Higher Education for Women, Coimbatore, Tamil Nadu, India.

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***Address for Correspondence**
S.Balasaraswathi
Ph.D. Scholar, Department of Home Science Extension Education,
Avinashlingam Institute for Higher Education for Women,
Coimbatore, Tamil Nadu, India.
Email: balasaraswathis@vvvcollege.org

ABSTRACT

Students are the future of our country and its leaders. They are the bedrock upon which our nation rests and the engine that drives its progress. The high unemployment rate is a significant problem and is one of the most contentious topics facing our country today. Not only has unemployment spread to the educationally illiterate, but it has also spread to the educated. India is a young nation that fully utilizes the potential of its young population. We need to provide people in our country with the tools and resources they need to thrive. The spirit of entrepreneurship is among the agencies at students' disposal for combating unemployment and empowering themselves. In their eagerness to seek out new experiences and overcome obstacles, today's students drive positive change in our nation. Successful people invest time and energy into building their businesses, which increases their output, marketability, and potential earnings. The link between employers and potential employees. These days, it is regarded as a crucial resource for every aspiring entrepreneur's toolkit. As a result, we are doing this study to determine how to measure entrepreneurial aptitude in student populations.

Keywords: Unemployment, Entrepreneurship, employability, and earning opportunities.

INTRODUCTION

Students are the future of any country, its bulwarks, the engine of growth, and future leaders. One in five Indians nowadays is between the ages of 10 and 19, and one in three is a young adult (10-24 years). The greatest way for the country to capitalize on its competitive edge — its demographic dividend — is to invest in this generation. According to the 2011 census, there are 253.2 million Indians between the ages of 10 and 19, making up 20.9% of the

total population, and 231.9 million Indians between the ages of 15 and 24, making up 19.2%. 3 Three hundred and sixty-four million individuals fall between the age bracket of ten to twenty-four (30.1 percent). (Indian Census & UNFPA, 2014). Abraham and Vinoj (2017). Over the previous two decades, unemployment has worsened in India's high-growth period. Just 140 million of India's 300 million increase in the working-age population between 1991 and 2013 were absorbed by the economy (UNDP 2016: 53). With 62% of the people in the prime working age range of 15-59 and a yearly increase in the labor force of 4-12 million people2, "jobless growth" has emerged as a defining feature of India's economy (India Skills Report 2018; Dewan 2018). It is expected that by 2050, the world's working-age population will have grown by 280 million (UNDP 2016: 6). While the prior two decades of substantial economic expansion did not result in sufficient job creation, the economy now needs to accommodate a larger employment market.

It is essential to recognize that recent college graduates frequently find themselves without work (Audretsch & Mahmood, 1994; Gürol & Atsan, 2006; Othman, Ghazali, & Sung, 2006; Koe, 2016). After finishing their degrees, the students actively seek positions in the business world. There is much competition for open jobs soon. Many factors might contribute to this, including badacademic performance, low self-esteem, a lack of communication skills, and a lack of relevant background information (Robinson, 2008; Ullal et al., 2019). One in five Indians nowadays is between the ages of 10 and 19, and one in three is a young adult (10-24 years). A nation's competitive advantage, which is segmented by age group, may be best exploited through investments in the cohort. According to the 2011 census, there are 253.2 million Indians between the ages of 10 and 19, making up 20.9% of the total population, and 231.9 million Indians between the ages of 15 and 24, making up 19.2%. 3 364.6 million individuals are between the ages of 10 and 24. (30.1 percent). When compared to the Census of 2001, there has been a decrease in the percentage of the teenage population and an increase in the young population. Students and young entrepreneurs are better able to compete for jobs and advance professionally even in the face of economic hardship because of the skills they learn in entrepreneurial programs. (Indian Census & UNFPA, 2014).

The worrying rise in the national unemployment rate has prompted a call for increased support for start-up businesses. Since its origin, entrepreneurship's influence on the economic growth of a region or country has led to widespread support for the concept (Crijns and Vermeulen, 2007; Karimi et al., 2016). Entrepreneurs are guardians who foster economic growth by conceptualizing and popularising new company concepts (Turker and Sonmez Selçuk, 2009). Growing businesses are vital to the economy because they create jobs, spur innovation, and boost productivity in various fields (Crijns and Vermeulen, 2007). Future economic expansion will depend on the development of entrepreneurial talent (Robinson, 2008; Pinto et al., 2019). Young people gain invaluable life skills through engaging in entrepreneurial activities, including emotional intelligence and the ability to take calculated risks. The experience also enhances their respect for the possibility of working for themselves. When faced with unemployment, young people don't give up; instead; theyuse their skills to forge new chances as entrepreneurs (Chen et al., 2015). Thus, the current study aims to assess the "Assessment of Entrepreneurial Skills among College Students."

OBJECTIVES

The objectives set forth for the study is

1. Toknow the socio-economic profile of the student's entrepreneur
2. Toanalyze the motivating factors to become a students entrepreneur
3.To assess the skill needed for students entrepreneur

Selection of the Area

The Selection of the Area was Virudhunagar District. In Virudhunagar district, six areas, namely Virudhunagar, Sivakasi, Srivilliputtur, Rajapalayam, Sattur, and Aruppukottai for taken to study.

Selection of the Sample

The sample study wastaken to final college year undergraduate and postgraduate students doing entrepreneurial activities.In the six areas of Virudhunagar district, 320 college final-year students weredoing entrepreneurial activities.

Selection of the Sampling Techniques

Convenience sampling techniques were adapted for this study.

Collection of the Data

This research used both primary and secondary sources for its data. All of the primary information was gathered using a well-designed questionnaire. The research questions were formulated into a questionnaire. The secondary data used to back up the study came from various print sources.

Data Analysis

Z test was used to analyze entrepreneurial skills.

FINDINGS AND DISCUSSION

The findings of the study were discussed under the following headings

Socio-Economic Profile (Table 1)

Out of 320 respondents

- 185 (57.81%) are in the age group of 15-18 years
- 178 (55.63%) are female
- 188 (58.75%) are Hindu
- 127 (39.69%) belong to the backward class
- 189 (59.06%) are graduates
- 176 (55%) come under rural area
- 178 (55.73%) belong to the nuclear family
- 174 (54.38%) have 4-8 members in their family
- 201 (62.81%) have earned a monthly income of Rs. 10,000 – Rs. 20,000
- 189 (59.06%) have lived in their own house.

Enterprise Profile (Table 2)

Out of 320 respondents

- 83 (25.94%) started their enterprise at the age of 20-25 years
- 175 (54.69%) are engaged in food manufacturing
- 47 (14.69%) run hotels, motels, restaurants, takeaway outlets, and fast food outlets
- 124 (38.75%) have put an investments of Rs.10,000- Rs.50,000 as capital
- 189 (59.06%) have started the business with their funds
- Out of 131 respondents who use borrowed funds for their business,
- 67 (51.15%) have approached moneylenders
- 62 (19.38%) are in the stage of established and growing

SKILLS NEEDED FOR AN ENTREPRENEUR

To become an entrepreneur, entrepreneurial skills, marketing skills, behavioral skills, business managerial skills, human resources management skills, economic resource management skills, and soft skills are essential for an entrepreneur. In this study, the respondents undertake food manufacturing and service units. This attempt was made to analyze the respondents' opinions regarding the nature of business units and the skills needed to become an entrepreneur.

Z test was used to analyze whether there is any significant difference between food manufacturing and service units regarding entrepreneurial skills. The null hypothesis was that there was no significant difference between food manufacturing and service units regarding entrepreneurial skills. The outcomes of the Z test are given in table- 3. For food manufacturing units, marketing skills followed by business managerial skills are highly essential as their mean scores are high when compared to other skills. For service units, behavioral skills (28.387) followed by soft skills (27.932) are highly essential as their mean scores are higher than other skills.

Regarding entrepreneurial skills, marketing skills, behavioral skills, business managerial skills, human resources management skills, economic resource management skills, and soft skills, the calculated value of the Z test is significant as its p-value is less than 0.05. Hence, the null hypothesis was rejected. Thus, it proved a significant difference between food manufacturing and servicing units regarding entrepreneurial skills.

CONCLUSION

Building student entrepreneurs has helped countries overcome unemployment, slow economic growth, and other challenges. Therefore, it is essential that students, who have been steadily increasing their knowledge base at each stage, pivot toward entrepreneurship and contribute to the expansion and improvement of the whole.

REFERENCES

1. Abraham, V. (2017). Stagnant Employment Growth: Last Three Years may have been the Worst. *Economic and Political Weekly*, *52*(38), 13-17.
2. Chen, S. C., Hsiao, H. C., Chang, J. C., Chou, C. M., Chen, C. P., & Shen, C. H. (2015). Can the Entrepreneurship Course Improve the Entrepreneurial Intentions of Students? *International Entrepreneurship and Management Journal*, *11*(3), 557-569.
3. Iqbal, H., & Vishal, S. (2019). Employee Relations in Micro Small and Medium Enterprises in India. *International Journal of Scientific and Technology Research*.
4. Jones, N., Presler-Marshall, E., & Thuy, D. B. (2014). Falling between the Cracks.
5. Ms.Sunitasanghi & Ms.A.Srija, (2014). "Youth Unemployment in India "Confederation of Indian Economy, P.33.
6. Pinto, S., Pinto, P., Hawaldar, I. T., & Sarea, A. M. (2019). Motivation and Blockades for Entrepreneurship among Graduates. *Hindu*, *124*, 28-6.
7. Pourraz, J. (2022). Making Medicines in Post-Colonial Ghana: State Policies, Technology Transfer and Pharmaceuticals Market. *Social Science & Medicine*, *311*, 115360.
8. Robinson, D. F. (2008). Planting the Seeds of Effective Entrepreneurship by Teaching Risk, Advising, and Design through Growth. *Journal of Technology Management & Innovation*, *3*(2), 29-35.

Table 1. Socio-Economic Profile

Socio-Economic Factors		No. of the Respondents	%
Age (in years)	15-18	185	57.81
	19-22	78	24.38
	22-26	57	17.81
	Total	320	100.00
Gender	Male	142	44.38
	Female	178	55.63
	Total	320	100.00
Religion	Hindu	188	58.75
	Christian	97	30.31
	Muslim	35	10.94

	Total	320	100.00
Community	General	50	15.63
	BC	127	39.69
	MBC	78	24.38
	SC/ST	65	20.31
	Total	320	100.00
Education	Graduation	189	59.06
	Post-Graduation	68	21.25
	Research Scholar	63	19.69
	Total	320	100.00
Area	Rural	176	55.00
	Urban	144	45.00
	Total	320	100.00
Type of family	Nuclear	178	55.63
	Joint	142	44.38
	Total	320	100.00
Marital Status	Married	35	10.94
	Unmarried	285	89.06
	Total	320	100.00
Family size	Below 4	93	29.06
	4-8	174	54.38
	Above 8	53	16.56
	Total	320	100.00
Monthly Income (in Rs.)	Below 10,000	43	13.44
	10,000-20,000	201	62.81
	Above 20,000	80	25.00
	Total	324	101.25
Nature of the house	Own house	189	59.06
	Rental house	131	40.94
	Total	320	100.00

Table 2. Enterprise Profile

Socio-Economic Factors		No. of the Respondents	%
Age at the time of starting the enterprise	below 10	57	17.81
	10-15	64	20.00
	15-20	79	24.69
	20-25	83	25.94
	25-30	37	11.56
	Total	320	100.00
Activity	Manufacturing	175	54.69
	Service	145	45.31
	Total	320	100.00
Type of Food Industry	Grain milling	27	8.44
	Fruits and Vegetable Processing	19	5.94
	Dairy (milk and milk products)	18	5.63
	Eggs and Poultry	24	7.50
	Meat and meat products	27	8.44

	Fish and other marine food processing	33	10.31
	Bakeries	12	3.75
	Beverages (tea/coffee/wineries)	17	5.31
	Convenience foods (ready-to-eat or ready-to-cook foods)	19	5.94
	Confectionery	23	7.19
	Snacks and sweet shop	16	5.00
	Peanut chikki business	18	5.63
	Hotels, Motels, Restaurants. Takeaway outlets. Fast food outlets	47	14.69
	Others	20	6.25
	Total	320	100.00
amount invested in capital	Below 5,000	33	10.31
	5,000 – 10,000	94	29.38
	10,000 – 50,000	124	38.75
	50,000-1,00,000	69	21.56
	Total	320	100.00
source of raising capital	Owned funds	189	59.06
	Borrowed funds	131	40.94
	Total	320	100.00
Sources of Borrowings	Banks	64	48.85
	Money lenders	67	51.15
	Total	131	40.94
Position of business	Start-up stage	43	13.44
	Pre-profit	56	17.50
	Profitable and growing	58	18.13
	Established and growing	62	19.38
	Established and stable	46	14.38
	Established but stressed	55	17.19
	Total	320	100.00

Source: Primary data

Table 3. Skills Needed for an Entrepreneur

Entrepreneurship Skills	Food Product Manufacturing		Service		Z test	p-value
	Mean	SD	Mean	SD		
Entrepreneurial Skills	24.198	3.457	19.732	2.178	32.83	0.000
Marketing Skills	31.012	2.841	17.041	1.937	44.99	0.002
Behavioral Skills	19.979	1.838	28.387	0.722	18.10	0.001
Business Managerial Skills	30.006	0.652	17.931	1.036	14.03	0.013
Human Resources Management Skills	11.049	4.357	16.921	2.682	26.04	0.000
Economic Resource Management Skills	17.108	2.851	15.937	5.034	21.50	0.021
Soft Skills	15.048	1.730	27.932	3.562	17.24	0.000

Source: Primary data

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RESEARCH ARTICLE

Hydrogeochemical Studies and Groundwater Quality Assessment of the Somwarpet Watershed, Kodagu District, Karnataka, India

Roja H D[1], D Nagaraju[2]*, Pradeepraju N[1] and Sudeep S R[1]

[1]Research Fellow, Department of Studies In Earth Science University of Mysore, Manasagangothri, Karnataka, India.
[2]Professor, Department of Studies In Earth Science University of Mysore, Manasagangothri, Karnataka, India.

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***Address for Correspondence**
D Nagaraju
Professor,
Department of Studies In Earth Science University of Mysore,
Manasagangothri, Karnataka, India.
Email: rojadharmappa23@gmail.com.

ABSTRACT

The Geochemical studies were carried out for analysis of Groundwater quality parameters of the Somwarpet watershed to analyse the quality for drinking purpose. A total of 30 groundwater samples were collected during pre-monsoon and post-monsoon seasons of the year 2020. The various physico-chemical parameters for the collected groundwater samples were determined. The parameters analysed are, pH, EC, TDS, Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, HCO_3^{-}, Cl^{-}, SO_4^{2-}, NO_3^{-}. In the majority of water samples, the analyzed physico-chemical parameters fall within the desirable limits and suggest their portability for drinking use as per the standards set by WHO (2011), BIS (2012), and ISI (1983) standards. The overall hydrochemistry of the groundwater samples in both seasons falls in the rock dominance field. The majority of the water samples analyzed from the pre-monsoon and the post-monsoon seasons have the percentage of Na, SAR, RSC, etc., values within the permissible limit. Hence, the studies carried out interprets that the groundwater is suitable for drinking and irrigational purpose in the study area expect few samples.

Keywords: Hydro geochemistry, Drinking Purposes, Rock dominance, irrigation Purposes, Permeable limit.

INTRODUCTION

With rapid increase in population groundwater is a major source of water for drinking purposes and to meet other domestic, Industrial and agricultural aspects in the country. Kodagu being the hub of coffee plantation it is very much essential to maintain the groundwater level throughout the year, After the recent landslides in the district which led to deformation of land use and land cover pattern in some parts of the district and also has experienced

decline in groundwater level inspite of good rainfall in the district, it was observed that water level at wells and rivers were decreasing which is leading to the shortage of potable water. In recent years it was observed that the groundwater quality is deteriorating due to over exploitation and land pollution, which in turn infiltrates the contaminated water during the rainy season, especially in the town area. Hence in order to understand water quality of the watershed the current study is carried out. Groundwater is a major natural resource for all purposes of water requirements in India and it is considered as the preferred source of water for meeting domestic, industrial, and agricultural requirements. In India, more than 90% is rural and nearly 30% of the urban population completely depends on it for drinking water. It accounts for nearly sixty percent of the total irrigation potential in the county. The dependency on groundwater is expected to increase in the future and the requirement in 2050. The rapid growth of the population enhanced the demand for freshwater. Besides, industrial development and agricultural evolution have imposed continuous escalated demand for freshwater, both on its quantity and quality. Thus it is imperative that a careful assessment and management of water resources becomes the utmost priority for a quality life.

Study area

Geological formations in the area of study falls within the Archean era which is about, 2,600 million years old. The early Archean era appears to be characterized by the development of shallow sub-watersheds of small size; the general appearance of the district varies considerably in its different parts. In the vicinity of Somwarpe**t** in the north of the district, the hills are generally rounded, altering with sloping grades. Near to Madikeri, the hills are closer together, and more abrupt and the ravines are deeper and wilder. Towards Kushalnagara, the area assumes the character of the Mysore plateau. The region of this study is located between the North latitudes of 12º03'00'' and the East longitudes of 75º04'00'' in the Western Ghats of South Karnataka (Fig.1). The study area is covered in the Survey of India Toposheets Numbers 48 P/14, 48P/15, 48P/10, and 48P/11. The study area comprises of hard rock terrain, predominantly consists of amphibolitic metapelitic schist, Gneisses, charnockites and migmatites, the common intrusive rock found in the study area are Pegmatite veins and dolerite dykes. The detached boulders of dolerite dykes are seen in some places where it can be seen as an intrusive structure in Granitic rocks. The study area consists of both hilly area as well as the flat and low-lying area. For example, the elevation ranges from 811meters to 1631mtrs above MSL.

MATERIALS AND METHODS

To carry out the hydrogeochemical studies of the groundwater for the year 2020 for pre monsoon and post monsoon season a total of 30 samples were collected from bore wells in the study area, Fig-1 depicts the sample collection locations. With reference to population and settlement in the study area sample locations were finalized. To collect the water sample 1 litre Sterilized and acid cleaned bottles were used. clean one-liter polythene bottles were used, which were pre-cleaned with the concentrated Water according to WHO standards. The P^H and EC values were determined in the field using standard equipment. The values of Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, HCO_3^{-}, Cl^{-}, SO_4^{2-}, NO_3^{-} ions were determined in the geochemical laboratory of the Department of Studies in Earth Science, Mysore University, Mysore. The digitized top sheets from Survey of India were used for preparation of base map of the study area using Arc Gis 10.8 and Groundwater sample locations were marked using GPS (Garmin). Spatial distribution maps of the pre monsoon and postmonsoon season were prepared using Arc GIS 10.8 software. The correlation matrix has been prepared to know the relationship between the various Physico-chemical parameters such as pH, EC, TDS, CO_3^{-}, HCO_3^{-}, Cl^{-}, SO_4^{2-}, Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+}. To evaluate the suitability of groundwater for irrigational purposes, and the parameters such as Sodium percentage (Na%), Residual Sodium Carbonate (RSC), Sodium Absorption Ratio (SAR) were derived by standard methods like ISI (1983), APHA (1995)..

RESULTS AND DISCUSSIONS

Drinking suitability of groundwater

By considering the chemical constituents of the water, the suitability of groundwater for driniking purpose was determined. For household purpose and other usages it is very much essential to know the quality of groundwater. The analysis of physio-chemical data of the grounwater of Somwarpet watershed for pre monsoon and post monsoon season for the year 2020.

Hydrogen ion concentration (pH)

The pH value of the water in the study area lies between 6 to 8.62 in pre-monsoon and post-monsoon seasons with an average of 6.9 for pre monsoon season and 7.7 for post monsoon season. This is due to poor to moderate rainfall during post-monsoon season pH has retained the same value. In pre-monsoon season 26.6% and in post-monsoon season 13.33% of the water samples from the study area are not within the prescribed limit by BIS (2012). Amongst the groundwater samples collected from the watershed majority of the water sample exhibits alkaline nature. This alkalinity nature of groundwater is attributed to the influence of weathered basalts observed in the present study area and the existence of weakly basic salts in soil attributed to anthropogenic activity and sewage disposal.

Electrical conductivity (EC)

In the present study area, the electrical conductivity of the groundwater varies from 66 to 1042 µS/cm at 25^{0}C, with an average of 422.12 µS/cm during pre-monsoon season and in post-monsoon season the value varies from 64.6 to 1079 µS/cm at 25^{0}C, with an average of 461.39 µS/cm. As per the prescribed limits set by BIS (2012) the water samples in pre-monsoon season and post-monsoon season are within the set limits. In the present study area, classification of water samples based on EC values (Sarma et al., 1982), 42 % of the water samples are permissible, 23 % are brackish, 19 % are excellent, 9 % are saline and 4% of the water samples are good in the pre-monsoon season. Whereas in the post-monsoon season 57 % are permissible, 14 % of water samples fall under excellent and brackish, 11 % are saline and 2 % are good. EC spatial distribution map has been prepared for both seasons (Fig. 2a and 2b).

Total dissolved solids (TDS)

The total dissolved solid in the present study area during pre-monsoon season varies from 51 to 1280 mg/l with an average of 552 mg/l and in post-monsoon season it varies from 51.2 to 1280 mg/l with an average of 566.09 mg/l. In the present study area, all the water samples for both seasons are within the prescribed limit (2000 mg/l) as per BIS (2012). The higher total dissolved solvent concentration is attributed to the dissolution of minerals from aquifer and agricultural activities and solid wastes. Generally, the higher concentration of total dissolved solvent in the groundwater is seen due to the contribution of salts from the soil, weathered parent rock materials, and also more residence time in an aquifer body (Dar et al., 2011; Tiwari & Singh, 2014). There is a marginal increase in the concentrations of ions during post-monsoon season due to the leaching of salts from the soil through the infiltration of agricultural activities and due to the weathering of basalts. In the present study area, water classification based on TDS, which is described by Freeze and Cherry (1979) has been adopted. The area showing the lowest TDS value may be considered for groundwater recharge. From the above classification, it is clear that in the present study area 93% of water samples belong to the freshwater category and 7% belongs to brackish in pre-monsoon season, whereas in post-monsoon season 89% of water samples belongs to the freshwater category and 11% to brackish. The TDS values for both seasons have been represented in the form of spatial maps (Fig. 3a and 3b). The many ionic contributing to TDS are sodium, potassium, calcium, magnesium, and chloride.

Cations

Calcium (Ca^{2+})

In the present study area during the pre-monsoon season, calcium ranges from 4.8 mg/l to 91.2 mg/l. The average value recorded is 34.95 mg/l during pre-monsoon season. During post-monsoon season the calcium in the study area

ranges from 3.2 mg/l to 100 mg/l with an average value of 37.186 mg/l. In pre-monsoon and post-monsoon seasons, a water sample does not exceed the permissible limit (200 mg/l) as per BIS (2012).

Magnesium (Mg^{2+})

Magnesium content in the present study area varies from 2.83 mg/l to 38.89 mg/l. The average value recorded during pre-monsoon season 20.02 mg/l. During the post-monsoon season, the magnesium concentration in the water samples of the study area ranges from 2.65 mg/l to 24.473 mg/l. The average value recorded during the post-monsoon season is 42.5 mg/l. groundwater samples do not exceed the desirable limit (100 mg/l) as per BIS (2012) for the pre-monsoon and post-monsoon season in the present study area.

Sodium (Na^{+})

During the pre-monsoon season, the sodium concentration in the present area varies from 6 mg/l to 56.4 mg/l. The average value recorded during pre-monsoon season is 17.723 mg/l. whereas; during the post-monsoon season sodium varies from 5 mg/l to 57.1 mg/l with an average value of 16.536 mg/l. The reason for this might be due to the silicate weathering or dissolution of soil salts stored by the influences of evaporation and anthropogenic activities (Stallard & Edmond, 1983; Meyback, 1987; Subba Rao et al., 2002). In the present study area, all the samples for both seasons are within the prescribed limit as per BIS (2012).

Potassium (K^{+})

The main sources of potassium in groundwater include weathering of minerals like orthoclase, microcline, mica, and nepheline which release potassium ions in groundwater and the use of potash fertilizer in agriculture also acts as the source of enrichment of potash. Potassium content in the study area varies from 0.1 mg/l to 23.5 mg/l for the pre-monsoon season. The average value recorded during the pre-monsoon season is 3.164 mg/l. Whereas, during post-monsoon season it varies from 0.5 mg/l to 10.8 mg/l. The average value recorded during the post-monsoon season is 4.05 mg/l. A high concentration of potassium in groundwater can be seen in irrigated areas where the potash fertilizers are used for agriculture (Harth, 1965). In the present study, all the samples from both seasons are within the prescribed limit of BIS (2012).

Anions

Carbonate and bicarbonate (CO_3^{-} and HCO_3^{-})

Dissolved carbon dioxide in rainwater is the main cause of carbonate and bicarbonate ions in groundwater, which infiltrates in soil and dissolves carbon dioxide. In the study area, the carbonate content varies from 39.6 mg/l to 388.08 mg/l with an average value of 169.752 mg/l in pre-monsoon season and during post-monsoon season it varies from 25.27 mg/l to 440.15mg/ with an average of 191.19 mg/l. The bicarbonate value varies from 54 mg/l to 316.8mg/l and with an average value of 144.48 mg/l in pre-monsoon season, whereas during post-monsoon season it varies from 31.04 mg/l to 278 mg/l and with an average value of 116.028 mg/l. An increase in the temperature or decrease in pressure causes a reduction in the solubility of carbon dioxide in water (Karanth, 1989). The carbonate and bicarbonate values for both seasons have been represented in the form of spatial maps (Fig. 4a and 4b).

Chloride (Cl^{-})

Chloride is mainly derived in groundwater by chloride-bearing minerals such as sodalite, chlorapatite. Chloride is present in the groundwater in the form of sodium chloride. Due to the Base Exchange process, the chloride content is exceeding the sodium. During the pre-monsoon season, chloride content in the study area varies from 13.69 mg/l to 150.67 mg/l. The average value recorded during the pre-monsoon season is 46.323 gm/l. Whereas, during the post-monsoon season the chloride concentration ranges from 14.21 mg/l to 109.38 mg/l with an average value of 42.895mg/l. According to BIS (2012) in the present study area, the water samples from both seasons do not exceed the prescribed limit (1000 mg/l). The chloride content in the groundwater may result from natural and anthropogenic sources such as agricultural runoff, use of fertilizers, animal feeds, and landfill leachate (Srinivasamoorthy et al., 2008, Taiwo et al., 2011).

Sulphate (SO_4^{2-})

Sulphur mineral is the main source of sulphate in the rock. The concentration of sulphate in groundwater is mainly due to precipitation and solution as water travels through the soil. If the concentration of sulphate is more than 400 mg/l then it reacts with the human organs. Sulphate content in the study area varies from 1 mg/l to 44.34 mg/l with an average value of 8.358 mg/l during pre-monsoon season and in the post-monsoon season, it varies from 0.2 mg/l to 50.69 mg/l with an average value of 10.733 mg/l. High sulphate content may be due to the breakdown of organic substances of weathered soils, human activities, use of fertilizers, and sulfate leaching. In the present study area, none of the samples during pre monsoon and post monsoon seasons do not exceed the prescribed limit set by BIS (2012).

Nitrate (NO_3^-)

Nitrogen is originally fixed from the atmosphere and then mineralized by soil bacteria into ammonium. As nitrate is soluble and mobile it is susceptible to leaching through the soil along with infiltrating water. The common sources of nitrate are decaying organic matter, domestic waste, etc. Nitrate content in the present study area during pre-monsoon season varies from 0.1 mg/l to 1.04 mg/l. The average value recorded during the pre-monsoon season is 0.150 gm/l. Nitrate concentration in the study area during post-monsoon season varies from 0.1 mg/l to 154.36 mg/l. The average value recorded during the post-monsoon season is 23.777 mg/l. 10.00% of the samples exceeds the prescribed limit of BIS (2012) during post-monsoon season. This is due to poor sanitary conditions and indiscriminate use of higher nitrogen-based fertilizers (such as NPK). Spatial distribution maps of nitrate have been illustrated for both seasons (Fig. 5a and 5b).

CONCLUSION

The present study focuses on the assessment of the groundwater quality by hydrochemical studies and the suitability for drinking purposes of the Somwarpet watershed, Kodagu district, Karnataka, India. The hydro geochemical study reveals that the pH values for both seasons indicate that groundwater in the study area is of alkaline nature. The EC values and TDS values of groundwater samples are all found to be within acceptable limits during both sampling seasons. Geologically, the study area is covered by extensive spreads of the hardrock terrain, geochemical weathering process, and evaporation procedures have played the main role in hydro-geochemical analysis impact for the concentration of major ions in groundwater quality. The major cations (Ca^{2+}, Mg^{2+}, Na^+, K^+) and major anions (HCO_3^-, Cl^-, SO_4^{2-}, NO_3^-) concentrations of the groundwater samples from the study area were compared with standards of BIS (2012) and WHO (2011), indicating that the majority of the groundwater samples are within permissible limits during both seasons and it can be used for drinking and domestic purposes. In the present study area, water samples show facies according to their dominance. During pre-monsoon season, 40.48 % of water samples belong to Ca-HCO_3 type, 30.95 % mixed Ca-Na-HCO_3, 14.29 % mixed Ca-Mg-Cl type, 7.14 % Na-HCO_3 type, 4.76 % Na-Cl type, and 2.38 % Ca-Cl type. Whereas during post-monsoon season 35.71 % of water samples belong to Ca-HCO_3 type, 28.57% mixed Ca-Na-HCO_3, 19.05% mixed Ca-Mg-Cl, 11.91% Na-Cl type, and 2.38 % Ca-Cl and Na-HCO_3 type respectively. The interaction of the rock–water includes the processes such as the chemical weathering of rocks, dissolution-precipitation of secondary carbonates, and ion exchange between water and clay minerals.

High positive correlation values of TDS with EC, HCO_3^- with Ca^{2+}, SO_4^{2-} with Na^+ and SO_4^{2-} with Cl^- for almost both seasons suggest the excess use of the fertilizers in the area, anthropogenic related activities, and over-exploitation of groundwater. The irrigation suitability was determined by the Sodium percentage (%Na), Sodium Absorption Ratio (SAR), Residual Sodium Carbonate (RSC). The SAR value depicts the excellent category and RSC value explained that the majority of the samples are suitable for irrigation. The percent sodium explained that most of the samples had >60 % of sodium which comes under the excellent, good and permissible category for irrigation purposes. Whereas during post-monsoon season 40.48 % of the groundwater samples belong to the good to the permissible category, 33.33 % of the groundwater samples come under excellent to permissible class and the rest of the 26.19 % of the groundwater samples fall under the permissible to good category. Hence, there is a requirement of adequate

drainage and proper management for salinity control, plants should be cultivated which are having high salt tolerance. Thus, the overall hydro geochemistry of groundwater of the study area in pre-monsoon and post-monsoon seasons are suitable for drinking and irrigation purposes except for few samples indicating signs of deterioration in the study area.

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REFERENCES

1. Arumugam M., Karthikeyan S., Thangaraj K., Kulandaisamy P., Sundaram B. P., Ramasamy K., Vallaikannu A., Velmayil P., (2021) Hydrochemical analysis for groundwater suitability appraisal in Sivagangai, an economically backward district of Tamil Nadu, Journal of Geological Society if India, V.97 pp-789-798.
2. Davithuraj, J. and Manjunatha, S., (2014). Assessment of Groundwater Quality for Drinking and Irrigation Purposes of Upper Bennihalla Basin, Karnataka. Nature environment and pollution technology, 13(1), pp.57-62.
3. Deshpande, S.M. and Aher, K.R., (2011). Quality of groundwater from Tribakeswar-Peth area of Nashik District and its suitability for domestic and irrigation purpose. Gond. geol. mag., 26(2): 157 – 162.
4. Elkrail, A.B. and Obied, B.A., (2012). Hydrochemical characterisation and groundwater quality in delta Tokor alluvial plain, Red sea Coast-Sudan. Arabian Journal of Geosciences.https://doi.org/10.1007/s 12517-012-0594-6.
5. Goyal, S.K., Chaudhary, B.S., Singh, O., Sethi, G.K. and Thakur, P.K., (2010). GIS based spatialdistribution mapping and suitability evaluation of groundwater quality for domestic and agricultural purpose in Kaithal district, Haryana state, India. Environmental Earth Sciences, 61(8), pp.1587-1597.
6. Khan, A et al., (2015). Hydrochemical characterization of groundwater in lower Kali watershed, Western Uttar Pradesh. Journal geological society of India 86, pp.195-210.
7. Khan, R. and Jhariya, D.C.,(2018). Hydrochemistry and groundwater quality assessment for drinking and irrigation purpose of Raipur city, Chhattisgarh. Journal geological society of India, 91, pp.475-482.
8. Manjunatha, S. and Devithuraj, J., (2014). Evaluation of groundwater quality in shallow and deep aquifer in parts of Uttatra Kannada district, Karnataka. International Journal of recent trends in science and technology, 11(1), pp.95-104.
9. Manjunatha, S. et al., (2016). Hydrochemical Facies and Assessment of Groundwater for Drinking Purpose in Goa, India. International Journal of Earth Sciences and Engineering 09, pp.35-42.
10. Nag, S.K. and Ghosh, P. (2013). Variation in groundwater levels and water quality in Chhatna Block, Bankura district, West Bengal - A GIS approach. Journal geological society of India, 81 (2), pp.261-280.
11. Nag, S. K., (2014). Evaluation of hydrochemical parameters and quality assessment of the groundwater in Gangajalghati Block Bankura district, West Bengal, India. Arabian journal of science and engineering 39, pp.5715-5727. https://doi.org/10.1007/s13369-014-1141-4.
12. Prasanth, S.S., Magesh, N.S., Jitheshlal, K.V., Chandrasekar, N. and Gangadhar, K.J.A.W.S., (2012). Evaluation of groundwater quality and its suitability for drinking and agricultural use in the coastal stretch of Alappuzha District, Kerala, India. Applied Water Science, 2(3), pp.165-175.
13. Purushotham, D., Rao, A.N., Prakash, M.R., Ahmed, S. and Babu, G.A., (2011). Environmental impact on groundwater of Maheshwaram watershed, Ranga Reddy district, Andhra Pradesh. Journal of the Geological Society of India, 77(6), pp.539-548.
14. Ravi Kumar, P. and Somashekar, R. K., (2010). Multivariate analysis to evaluate geochemistry of groundwater in Varahi river basin of Udupi in Karnataka (India). The Ecoscan 4 (2 & 3), pp.153-162.
15. Ravikumar, P., Venkatesharaju, K., Prakash, K.L. and Somashekar, R.K., (2011). Geochemistry of groundwater and groundwater prospects evaluation, Anekal Taluk, Bangalore urban district, Karnataka, India. Environmental monitoring and assessment, 179(1), pp.93-112.

16. Shreya, Das. and Nag, S. K., (2015). Deciphering groundwater quality for irrigation and domestic purpose - a case study in Suri I and II blocks, Birbhum District, West Bengal, India. Journal of earth system science, 124(5), pp.965-992.
17. Sreedevi, P.D., Ahmed, S. and Reddy, D.V., (2016). Assessment of groundwater quality for irrigation use in gooty Mandal, Andhra Pradesh, India. Journal of Applied Geochemistry, 18(3), pp.320-331.
18. Srivastava, A. K. and Parimal, P. S., (2015). Hydrochemistry of Purna Alluvial basin, central India. Journal geological society of India, 85, pp. 673-685.
19. Rao, N.S., Rao, J.P., Devadas, D.J. and Rao, K.S., (2002). Hydrogeochemistry and groundwater quality in a developing urban environment of a semi-arid region, Guntur, Andhra Pradesh. Journal of Geological Society of India (Online archive from Vol 1 to Vol 78), 59(2), pp.159-166.
20. Taiwo, A.M., Adeogun, A.O., Olatunde, K.A. and Adegbite, K.I., (2011). Analysis of groundwater quality of hand-dug wells in peri-urban area of Obantoko, Abeokuta, Nigeria for selected physico-chemical parameters. Pac J Sci Technol, 12(1), pp.527-534.
21. Tiwari, A.K., Singh, P.K. and Mahato, M.K., (2016). Hydrogeochemical investigation and qualitative assessment of surface water resources in West Bokaro coalfield, India. Journal of the Geological Society of India, 87(1), pp.85-96.
22. Verma, D.K., Bhunia, G.S., Shit, P.K. and Tiwari, A.K., (2018). Assessment of groundwater quality of the Central Gangetic Plain Area of India using Geospatial and WQI Techniques. Journal of the Geological Society of India, 92(6), pp.743-752.
23. Vijay, R., Sardar, V.K., Dhage, S.S., Kelkar, P.S. and Gupta, A., (2010). Hydrodynamic assessment of sewage impact on water quality of Malad Creek, Mumbai, India. Environmental monitoring and assessment, 165(1), pp.559-571.
24. WHO (2011). Guidelines for Drinking Water Quality. World Health Organization, Geneva, Switzerland.

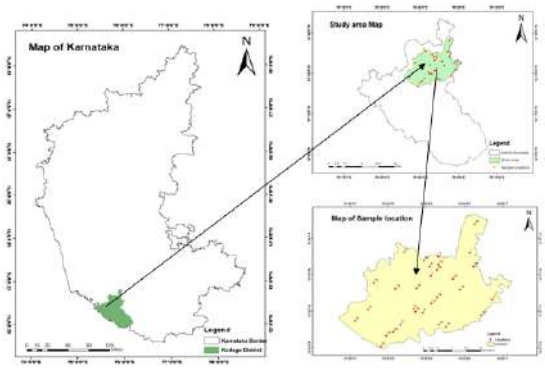

Fig. 1: Location map of the study area along with sample locations

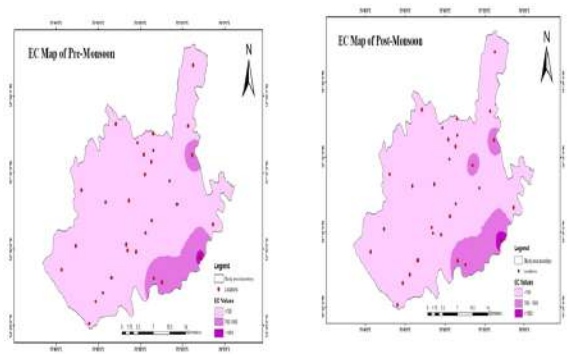

Fig. 2a and 2b spatial variation maps of physico-chemical parameters analyzed during pre-monsoon and post-monsoon seasons.

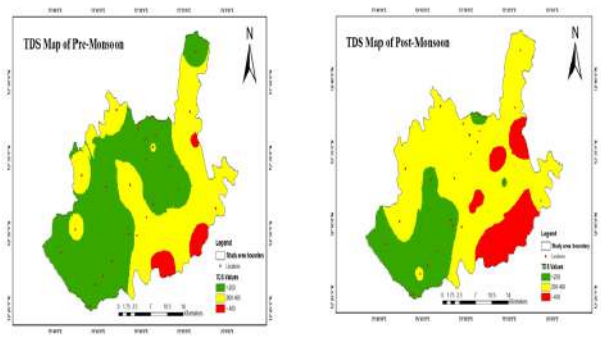

Fig. 3a and 3b Spatial variation maps of physico-chemical parameters analyzed during pre-monsoon and post-monsoon seasons.

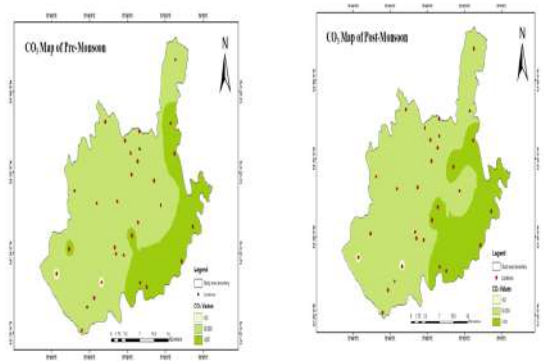

Fig. 4a and 4b Spatial variation maps of physico-chemical parameters analyzed during pre-monsoon and post-monsoon seasons.

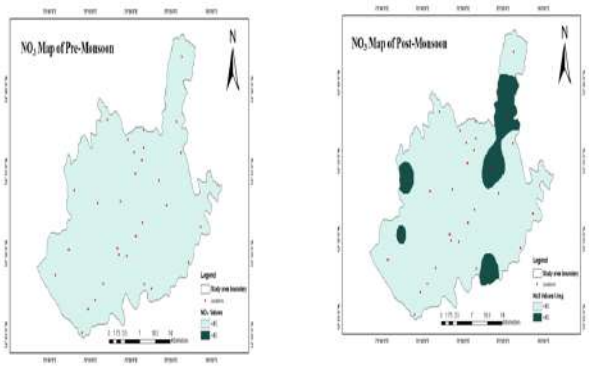

Fig. 5a and 5b Spatial variation maps of physico-chemical parameters analyzed during pre-monsoon and post-monsoon seasons.

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RESEARCH ARTICLE

Synthesis, Spectral Characterization, DNA Binding and Antibacterial Studies of Ternary Metal Complexes with 1, 10 Phenanthroline and 2-Acetylthiophene-4-Ethyl-3-Thiosemicarbazone

K.Srinivasulu[1]*, Shabana Palyam[2], Y.B .Nagamani[3] and K.Anuja[4]

[1]Academic Consultant, Sri Krishnadevaraya University, Ananthapuramu, Andhra Pradesh, India.
[2]Researcher, Sri Krishnadevaraya University, Ananthapuramu, Andhra Pradesh, India.
[3]SYTR Govt. Degree College, Madakasira, Andhra Pradesh, India.
[4]Lecturer, S.S.B.N Degree and PG. College, Ananthapuramu, Andhra Pradesh, India.

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***Address for Correspondence**
K.Srinivasulu,
Academic Consultant,
Sri Krishnadevaraya University,
Ananthapuramu, Andhra Pradesh, India.
Email: seenuptp@gmail.com

ABSTRACT

Divalent metal complexes having the components $M(Phen)Cl_2$ (where, M = Cu(II), Ni(II) and Co(II); Phen = one,10-Phenonthroline) composites reacted with 2-acetylthiophene-4-ethyl-3-thiosemicarbazone (ATET) to form ternary complexes with chemical formula $[M(Phen)(ATET)Cl_2]$ liquid. The resulted complexes were characterised through physic-chemical properties such as molar conductivity, mass spectra, infrared and electronic spectroscopies) strategies. chemical behavior of complexes were demonstrated using cyclic voltammetry. DNA binding properties of the complexes are determined by UV-Visible Absorption spectrophotometry. Metal complexes are monitored for medicinal activity by agar well diffusion methodology against infective microorganism strains viz. Gram–ve like Escherichia coli, enteric bacteria respiratory disorder and Gram+ve like cocci aureus, Bacillus caryophylloid dicot genus. The $[Cu(Phen)(ATET)Cl_2].H_2O$ advanced inhibits microorganism a lot of stronger than the other advanced.

Keywords: 2-Acetylthiophene-4-ethyl-3-thiosemicarbazone, Ternary transition metal complexes, cyclic voltammetry, DNA binding, antibacterial activity

INTRODUCTION

Sulphur has been used medicinally since archiasm. It is one of the main historic elements, and the Greek people recognised its recovering properties. The Food and Drug Administration (FDA) approved magnesium sulphate as the first sulphur-containing compound, and sulphur-containing compounds were the hallmarks of several antibiotic quantum leap that brought mankind into the new antibiotic era. Sulphur-containing drugs have received several Nobel Prizes for their research. Sulfur-containing compounds continue to account for a sizable part of new FDA assent. Thiosemicarbazones are a type of sulphur-containing ligand that is useful in the synthesis and characterization of transition metal complexes [1,2]. These ligands are widely used as chromogenic reagents for determining transition metal ions spectrophotometrically [3]. The stereo chemical, electrochemical, and electronic properties of transition metal complexes with heterocyclic thiosemicarbazones are known to be fascinating [4,5]. Metal complexes of Phen have been shown to be anti-tumor agents [6]. MLMC with Schiff bases as the main ligand and Phen as a co-ligand have recently been reviewed [7]. However, no report on mixed ligand metal complexes with Phen and ATET is available in the literature [8-10]. The ligand was synthesised, characterised [11,12], and used in our laboratory to determine copper (II) in amalgam, vegetable oils, and seeds [13]. Transition metal complexes with Phen as the primitive ligand and thiosemicarbazone as the secondary ligand, on the other hand, have not been investigated yet [14]. Mixed ligand complexes of biologically salient composites have the potential to serve as biochemical process models [15, 16]. In order to develop antimicrobial agents, we recently studied [17-19] deoxyribonucleic acid binding and antibacterial activity of various transition metal complexes. To rekindle our interest, we present our findings on the spectral characterization, DNA binding, and antibacterial activity of ternary metal complexes with Phen and ATET. The primary goal of this work is to bring correlation between D Mixed ligand metal complexes with Schiff bases as the main ligand and Phen as a co-ligand [7]. However, there is no report in the literature on mixed ligand metal complexes with Phen and ATET [8-10]. The ligand was synthesised, characterised [11, 12], and in our laboratory to determine copper (II) in alloys, edible oils, and seeds [13].. Transition metal complexes with Phen as the primary ligand and thiosemicarbazone as the secondary ligand, on the other hand, have not been studied [14].NA binding and complex bacterial inhibition.

MATERIALS AND METHODS

4-ethyl-3-thiosemicarbazide, 2-Acetylthiophene and Phen, were purchased from Sigma-Aldrich. Elemental analyses were carried out on a Heraeus Vario EL III Carlo Erba 1108 instrument. Molar conductivity measurements at 298 ± 2K in dry and purified DMF were carried out using a CM model 162 conductivity cell (ELICO). ESR spectra were recorded on a Varian E-112 X-band spectrophotometer at room temperature (RT) and liquid nitrogen temperature (LNT) in solution (DMF) state. Cyclic voltammetric measurements were taken on degassed (N_2 bubbling for 5 min) solutions (10^{-3} M) containing 0.1 M Bu_4NPF_6 as the supporting electrolyte.

Preparation of ATET Ligand

The primary ligand, ATET was prepared using 4-ethyl-3-thiosemicarbazide and 2-Acetylthiophene. Ethanolic solutions of 4-ethyl-3-thiosemicarbazide (5 mmol), and 2-Acetylthiophene (5 mmol) were combined in a RBF flask. Two drops of CH_3COOH were added and the reaction mixture was refluxed for 3 hrs and cooled to room temperature. The composite obtained was a shiny milk white crystalline product, which was eventually utilized in the synthesis of metal complexes. Yield 80 %, Melting Point 118 - 120 ºC, IR spectra (cm^{-1}) 3178, 1592, 1201 cm^{-1} are assigned to ν (N-H asym), ν (> C = N) and ν (> C = S) stretching vibrations respectively. NMR spectra (δ, in ppm) 8.77 (singlet 1H), 7.03 - 7.37 (multiplet 3H), 3.49 (quartet 2H), 1.00 (Triplet 3H) are assigned to > NH, ethyl, thiophene H and CH_3 protons respectively. In the mass spectrum of ATET, the molecular ion peak value concurred with its formula weight 227.

Preparation of mixed ligand metal complexes [M(Phen)(ATET)Cl_2]. H_2O

A 1.2 g of ATET ligand (0.006 moles) was solvated in 15 mL of 0.05 N NaOH in methanol solvent in 100 mL beaker. A 1.0 g Cu $(Phen)_2Cl_2$ (0.003 moles) was solvated in 15 mL of methanol solvent in 100 mL beaker. Ligand solution and $Cu(Phen)_2Cl_2$ solution were transmitted into 100 mL RBF flask and heated under reflux for 1 hr. On slow cooling the components of flask have shown light green coloured complex was formed. It was collected by filtration, washed several times with lesser abundance of methanol and dessicated in air. [Ni(Phen)(ATET)Cl_2].H_2O and [Co(Phen)(ATET)Cl_2].H_2O complexes were prepared identically.

RESULTS AND DISCUSSION

Physico-chemical and analytical studies

Metal complexes having the composition $M(Phen)_2Cl_2$ (Where, M = Co(II), Ni(II) and Cu(II); Phen = 1,10-Phenanthroline) are reacted with ATET resulted in heteroleptic transition metal complexes with molecular formula [M(Phen)(ATET) Cl_2]H_2O. All the complexes are stable at room temperature, non-hygroscopic, insoluble in water, slightly soluble in methanol and ethanol but quickly soluble in DMF and DMSO. Molecular weights of the complexes are arbitrated based on their ESI-mass spectra. ESI-mass spectrum of [Co(Phen)(ATET)Cl_2]. H_2O complex is shown in Figure 1. The physico-chemical data for the complexes are epitomized in Table 1.

Conductivity measurements

Analytical data of conductivity measurements shown the formation of the complexes. For 1:1 electrolyte the molar conductivity values are in the range 65 - 90 Ω^{-1} cm^2 mol^{-1} in dimethylformamide (DMF). The observed values (11 - 19 Ω^{-1} cm^2 mol^{-1}) specified non-electrolytic nature of complexes [20].

Electronic spectra

The electronic spectra of the complexes are recorded in DMF and data are presented in Table 2. A strong and sharp peak is observed in the region of 37,735 – 31,152 cm^{-1} due to $\pi \rightarrow \pi^*$ transition of aromatic chromophore [21]. Medium intensity bands observed in the range of 30,581 - 28,570 cm^{-1} are correlated to M → L charge transfer transition [22]. A weak band in the region of 17,094 - 10,101 cm^{-1} region may be designated to d-d transitions. A typical ESR spectrum of [Cu (Phen) (ATET) Cl_2].H_2O recorded at LNT is shown in Figure 2.

Infrared spectra

Infrared (IR) spectral data of ATET and its metal complexes and assignment of peaks are given in Table 3. A strong band is observed in the IR spectrum of ATET at 1592 cm^{-1} which is assigned to ν (> C = N) group. In all the complexes, this band is shifted to lower frequency representing that azomethine nitrogen atom took part in coordination [23,24]. A medium band is appeared in the spectrum of ATET at 1201 cm^{-1}, which is assigned to ν (C = S) group. This band is shifted to lower wave number suggesting participation thioke to sulphur in chelation. Thiophene ring deformation modes are observed around 719 and 620 cm^{-1}. These bands are shifted to higher wave number indicating coordination of thiophene sulphur to metal. In far IR region, new peaks are observed in 549 - 596 and 449 - 458 cm^{-1} regions which are assigned to υM-N and υM-S vibrations [25, 26] subsequently.

ESR spectra

ESR spectra of copper complexes were recorded in DMF solution at ambient temperature and at liquid nitrogen temperature. ESR spectra of [Cu(Phen)(ATET)Cl_2]. H_2O recorded at LNT are shown in Figure 3. The spin Hamiltonian, orbital reduction and bonding parameters of complexes are given in Table 4. The $g_{||}$ and $g_{\perp}$ are computed from the spectra using tetracyanoethylene (TCNE) free radical as g marker. The observed g|| values for complexes are less than or equal to 2.3 suggesting significant covalent character of metal ligand bond in agreement with observation of Kivelson and Neiman [27]. The $g_{||}$ and $g_{\perp}$ were more than 2, corresponding to an axial

symmetry. The trend $g_{||} > g_{\perp} > g_e$ (2.0023) observed for these complexes suggests that the unpaired electron is localized in the $d_{x^2-y^2}$ orbital of the copper ion. The axial symmetry parameter G is defined as:

$$G = \frac{[g_{\parallel} - 2.0023]}{[g_{\perp} - 2.0023]} \quad (1)$$

$$\alpha^2 = -A^{\parallel}/p + (g^{||} - 2.0023) + 3/7(g_{\perp} - 2.0023) + 0.04 \quad (2)$$

The calculated G values for these complexes are less than 4.0, indicating the presence of small exchange coupling and molecular axis misalignment. The orbital reduction parameters (K, K), the bonding parameter (2), and the energies of the d-d transitions are calculated using the g||, g, A, A of complexes and the energies of the d-d transitions. The factor 2 is commonly used as a measure of covalence and is calculated as follows Hathaway pointed out that for pure σ bonding $K_{\parallel} \approx K_{\perp} \approx 0.77$, for in-plane π-bonding $K_{\parallel} < K_{\perp}$, while out of plane π-bonding $K_{\parallel} > K_{\perp}$ the following simplified expressions were used to calculate $K_{\parallel}$ and $K_{\perp}$:

$$K^{\parallel 2}(g^{||} - 2.0023)/8x\ \lambda_0\ X \text{ d-d transition}$$

$$K^{\perp 2} = (g_{\perp} - 2.0023)/8x\ \lambda_0\ X \text{ d-d transition} \quad (3)$$

The observed $K_{\parallel} < K_{\perp}$ relation for $Cu(phen)_2Cl_2$ complex reveals the importance in plane π - bonding and $K_{\parallel} > K_{\perp}$ relation for [Cu(Phen)(ATET) Cl_2]. H_2O complex reveals the out of plane π-bonding.

Cyclic voltammetry

Electrochemical properties of complexes are investigated by using cyclic voltammetry, Voltammograms of complexes are recorded in DMF using 0.1 M tetrabutylammonium hexafluorophosphate as supporting electrolyte. The cyclic voltammogram of [Ni(Phen)(ATET)Cl_2]. H_2O complex is given in Figure 4 and the electrochemical data of complexes are stated in Table 5.Stability constants of complexes are calculated using ΔEp values. Stability of complexes may be approximated based on standard free energy change (ΔG) values. The ΔG values are higher for mixed ligand complexes. Data reveals that mixed ligand complexes are more stable than the parent [M(Phen)Cl_2] complexes.Repeated scans at completely different scan rates counsel that the presence of stable chemical reaction species in resolution. it's been ascertained that electrode (Ipc) and anodal (Ipa) peak currents weren't equal. The calculated E1/2 values for $Cu(Phen)2Cl_2$ and [Cu(Phen)(ATET)]Cl2].H_2O are 0.113 and 0.198 V severally. it should be terminated that everyone the divalent metal complexes endure one lepton reduction to their differential M(I) complexes. The non-equivalent currents in electrode and anodal peaks illustrate quasi-reversible behaviour [28]. The divergence, ΔEp for all the complexes is best than the Nerstian demand 59/n mV (n = range of electrons concerned in chemical reaction reduction). The ΔEp values indicate quasi-reversible character of lepton transfer [29].

The complexes show giant separation between anodal and electrode peaks indicating quasi-reversible character. supported analytical, chemical science and spectral knowledge, a general structure (Figure 5) is projected for the complexes and DNA binding studies The binding interaction of the complexes with DNA was monitored by differentiating their absorption spectra with and without CT-DNA. Absorption spectra of [Cu(Phen)(ATET) Cl_2] H_2O complex in the absence and in the presence of CT-DNA are shown in Figure 6. It has been observed (Table 6) that molar absorptivity of complexes decreases (hypochromism, Δε, +20.79 % to +28.01; and absorption maximum is shifted towards higher wavelength (bathochromic shift $\Delta\lambda = 0.5$ - 1.5) upon each addition of CT-DNA. Table 6 shows the intrinsic binding constants of complexes. The hyperchromic and hypochromic effects of DNA are unique properties of its double helix structure. Hypochromism is caused by the contraction of DNA in the helix axis as well as a change in DNA conformation, whereas hyperchromism is caused by damage to the double helix structure. Hypochromism and bathochromic shift indicate that these complexes bind DNA via intercalation, which involves a firmly -assembled interactivity between the aromatic chromophore and DNA base pairs [30].

Antibacterial activity

The diameters of reticence of zones were measured with Verniercallipers in mm and its values are depicted in the Table 7. Antibacterial activity of synthesized complexes is compared (Figure 7) with the reference compound, ciprofloxacin. The reference compound shows higher activity even in the presence of its smaller quantities (5 μg/μL). However, all the complexes show remarkable antibacterial activity with its larger quantities (100 - 300 μg/μL). The zone of inhibition data obtained, proved that the [Cu(Phen)(ATET) Cl_2].H_2O complex inhibits bacteria more strongly than any other complex. Further, the mix Table 6 shows the intrinsic binding constants of complexes. The hyperchromic and hypochromic effects of DNA are noteworthy feautures of its double helix structure. Hypochromism is caused by the contraction of DNA in the helix axis as well as a change in DNA formation, whereas hyperchromism is caused by damage to the double helix structure. Hypochromism and bathochromic shift indicate that these complexes bind DNA via intercalation, which involves a firmly -assembled interactivity between the aromatized chromophore and DNA base pairs [30].ed ligand complex, for example [Cu (Phen)(ATET) Cl_2] .H_2O complex shows enhanced activity than the parent complex Cu $(Phen)_2Cl_2$ possibly due to the symbiotic effect of Phen and ATET ligands against bacteria.

CONCLUSIONS

In conclusion, here in we report that ESI mass spectra, molar conductivity, infrared, and electronic spectroscopy are used to characterize mixed ligand transition metal complexes with Phen and ATET. Cyclic voltammetry is used to study the electrochemical properties of complexes. For the M(II)/M(I) couple, the complexes exhibit quasi-reversible cyclic voltammetric responses. Absorption spectrophotometry is used to investigate the binding properties of these complexes with calf-thymus DNA. Terinary complexes show high binding affinity towards DNA. Metal complexes are examined for their antibacterial activity by using agar well diffusion method against pathogenic microbial strains. Despite antibacterial man oeuvre of present complexes is less than the reference compound (Ciprofloxacin), the mixed ligand compounds show consequential activity when taken in excessive amounts (100 - 300 μg/μL).

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REFERENCES

1. Campbell MJM, *Coord. Chem. Rev*., 1975: 15:279-319. https://doi.org/10.1016/S0010-8545(00)80276-3
2. Padhye S and Kauffman GB, Transition metal complexes of semicarbazones and thiosemicarbazones. *Coord. Chem. Rev*. 1985; 63, 127-60. https://doi.org/10.1016/0010-8545%2885%2980022-9
3. Reddy KH and Reddy DV, Analytical data comparison of analytical potentialities of thiosemicarbazones and semicarbazones. *Quaterly Chem. Rev.,* 1985: 1, 47-98. https://doi.org/10.1080/10408349108051640
4. Krishna PM and Reddy KH, Synthesis, single crystal structure and DNA cleavage studies on first ^{4}N-ethyl substitutedthree coordinate copper(I) complex with thiosemicarbazone. *Inorg. Chim. Acta* 2009: 362: 4185-4190. https://doi.org/10.1016/j.ica.2009.06.022
5. Haribabu PR and Reddy KH, DNA binding and cleavage activity of cationic dinuclear copper(II) and nickel(II) complexes with novel oxime-thiosemicarbazones. *Indian J. Chem.,* 2011: 50A: 996-1001.
6. Prashanthi Y, Kiranmai K and Sathishkumar K. Spectroscopic characterization anbiological activity of mixed ligand complexes of Ni(II) with 1,10-phenanthroline and heterocyclic Schiff bases. *Bioinorg. Chem. Appl.,* 2012: 2012: 948534. https://doi.org/10.1155/2012/948534
7. Mahalakshmi R and Raman N, A therapeutic journey of mixed ligand complexes containing 1,10-phenanthroline derivatives: A review. *Int. J. Curr. Pharm. Res.,* 2016: 8: 1-6.

8. Sugiyarto KH, Kusumawardani C, Wigati H **and** Sutrisno H, Structural study of the powder complex of Cu(II)-1,10-phenanthroline- trifluoroacetate. *Orient. J. Chem.,* 2019: 35: 325-331. http://dx.doi.org/10.13005/ojc/350139
9. Oveisikeikha A and Saravani H, Synthesis and characterization of new binuclear chromium, cadmium complexes based on terpyridine and phenanthroline. *Orient. J. Chem.,* 2018: 34:1323-7. http://dx.doi.org/10.13005/ojc/340317
10. Al-shemary RKR, Karem LKA and Ghanim FH, Structure, and in vitro antimicrobial and antifungal evaluation of some amino benzoic acids, derived ligand Schiff base and their mixed complexes with Cu(II), Hg(II), Mn(II), Ni(II) and Co(II). *Orient. J. Chem.,* 2018: 34: 1105-1113. http://dx.doi.org/10.13005/ojc/340263
11. Prasad. 2001 NBL, Spectrophotometric determination of Cu(II) and Ni(II) in edible oils using oxime-thiosemi-carbazones. Master Thesis. Sri Krishnadevaraya University, India.
12. Lima GMD, Neto JL, Beraldo H, Siebald HGL and Duncalf DJ, Structural and spectral studies of thiosemicarbazones derived from 2-acetylthiophene. *J. Mol. Struct.,* 2002: 604: 287-291. https:// doi:10.1016/S0022-2860(01)00664-0
13. SayajiRao M, Prasad NBL and Reddy KH, Spectrophotometric determination of copper(II) in alloys and edible oils using 2-acetylthiophene thiosemicarbazone. *Indian J. Chem.* 2006; 45A, 1659-62.
14. Shivankar VS, Vaidya RB, Dharwadkar SR and Thakkar NV, Synthesis, characterization and biological activity of mixed ligand Co(II) complexes of 8-hydroxyquinoline and some amino acids. *Synth. React. Inorg. Met. Org. Chem.* 2003: 33: 1597-1622. https://doi.org/10.1081/SIM-120025443
15. Adkhis A, Djebbar S, Benali-Baïtich O, Kadir A, Khan MA and Bouet G, Synthesis, characterization, and electrochemical behavior of mixed-ligand complexes of cobalt(III) with dimethylglyoxime and some amino acids. *Synth. React. Inorg. Met. Org. Chem.,* 2003: 33: 35-50. https://doi.org/10.1081/SIM-120016871
16. Reddy KH, *Bioinorganic chemistry*. New Age International Publishers, New Delhi, India, 2020.
17. Dornala D, Katreddi HR , Kandukuri A , Kummara S and Balaramaiah NY, Synthesis, characterization, deoxyribonucleic acid binding and antimicrobial activity of bivalent metal complexes with 2-acetylpyridine isonicotinoylhydrazone. *J. Appl. Pharm. Sci*., 2021:11: 84-92. http://dx.doi.org/10.7324/JAPS.2021.110211a
18. Nagamani YB, Reddy KH, Srinivasulu K, Dhanalakshmi D and Anuja K, Antibacterial activity and DNA binding properties of bivalent metal complexes with cuminaldehyde benzoylhydrazone (CABH). *Int. J. Pharm. Res.,* 2021: 12:188-195. https://doi.org/10.13040/ijpsr.0975-8232.12(1).188-95
19. Dhanalakshmi D, Reddy KH, Srinivasulu K, Anuja K, Nagamani YB and Shravya P, DNA binding and antibacterial activity studies on transition metal complexes with 2-formylpyridine isonicotinoyl hydrazone. *Res. J. Chem. Environ.,* 2020: 24: 130-40.
20. Geary WJ, The use of conductivity measurements in organic solvents for the characterization of coordination compounds. *Coord. Chem. Rev*., 1971: 7: 81-122. http://dx.doi.org/10.1016/S0010-8545(00)80009-0
21. Tu C, Wu X, Liu Q, Wang X, Xu Q and Guo Z. Crystal structure, DNA-binding ability and cytotoxic activity of platinum (II) 2,2'dipyridylamine complexes. *Inorg. Chim. Acta.,* 2004: 357: 95-102.
22. Szlyk E, Surdykowski A, Barwiołek M and Larsen E, Spectroscopy and stereochemistry of the optically active copper(II), cobalt(II) and nickel(II) complexes with Schiff bases N,N′-(1R,2R)-(−)-1,2-cyclohexylenebis(3-methylbenzylideneiminato) and N,N′-(1R,2R)-(−)-1,2-cyclohexylenebis(5-methylbenzylideneiminato). *Polyhedron.,* 2002: 21: 2711-7. http://dx.doi.org/10.1016/S0277-5387(02)01273-1
23. Mitu L, Natarajan R, Kriza A, Stanica N and Dianu M, Synthesis, characterization and antimicrobial activity of Cu(II), Ni(II), Co(II), Zn(II) complexes with isonicotinoylhydrazone-4-benzyloxy- benzaldehyde. *Asian J. Chem.,* 2009: 21: 5749-5756.
24. Chattopadhyay P and Sinha C, Synthesis and characterization of uranyl complexes and their peroxo derivatives with some thiosschiff bases. *Indian J. Chem.* 1996; 35, 523-6.
25. Dede B, Ozmen I and Karipcin F. Synthesis, characterization, catalase functions and DNA cleavage studies of new homo and heteronuclear Schiff base copper(II) complexes. *Polyhedron.,* 2009: 28: 3967-74. http://dx.doi.org/10.4067/S0717-97072014000300003
26. Peizhi XD and Boz Y, Transition metal complexes of isonicotinoyl-hydrazone-4-diphenylamino benzaldehyde: Synthesis, characterization and antimicrobial studies. *Asian J. Chem.* 2005: 17: 969.
27. Kivelson D and Neiman R, Line shapes in glasses of copper complexes. *J. Chem. Phys.* 1961:35: 156-161. https://doi.org/10.1063/1.1731881

28. Procter IM, Hathaway N and Nicholis P, The electronic properties and stereochemistry of the copper(II) ion. Part V. The tetra-ammine complexes. *J. Chem. Soc. A* 1969:. https://doi.org/10.1039/J 19690000065.
29. Usha S and Palaniandavar M. Influence of chelate-ring size and number of sulfur-donor atoms on spectra and redox behaviour of copper(II) bis(benzimidazolyl) tetra- and penta- thioether complexes. *J. Chem. Soc. Dalton Trans*., 1994: 15: 2277-83. https://doi.org/10.1039/DT9940002277
30. Sirajuddin M, Ali S and Badshah A, Drug-DNA interactions and their study by UV-Visible, fluorescence spectroscopies and cyclic voltammetry. *J. Photochem. Photobiol. B.,* 2013: 124: 1-19. https://doi.org/10.1016/j.jphotobiol.2013.03.013

Table 1: Physicochemical and Analytical data of Cu(II) Ni(II) and Co(II) complexes.

Complex	ESI-MS (F.W)	Melting Point (°C)	Colour (Yield %)	Molar Conductivity*
$[Cu(Phen)_2Cl_2]$	498 (496.0)	Above 300	Light Green (73)	18
$[Cu(Phen)(ATET)Cl_2]\ H_2O$	517.0 (517.9)	246-247	Brown (77)	11
$[Ni(Phen)_2Cl_2]$	491.6 (491. 0)	Above 300	Light Blue (75)	19
$[Ni(Phen)(ATET)Cl_2]\ H_2O$	549.0 (549.7)	256-257	Light Brown (89)	18
$[Co(Phen)_2Cl_2]$	492.0 (491.8)	Above 300	Light Blue (74)	17
$[Co(Phen)(ATET)\ Cl_2\ ]\ H_2O$	552.9 (554.7)	269-270	Brown (89)	17

Units: $(\Omega^{-1}cm^2mol^{-1}$

Table 2: Electronic Spectral Data for Cu(II), Ni(II) and Co(II) Complexes

Complex	Wavelength λ max (nm)	Frequency (cm^{-1})	Assignment
$[Cu(Phen)_2Cl_2]$	295	33,898	π-⟶π* transition
	350	28,570	M→LCT
	755	13,245	d-d transition
$[Cu(Phen)(ATET)Cl_2]\ H_2O$	273	36,630	π-⟶π* transition
	346	28,818	M→LCT
	585	17,094	d-d transition
$[Ni(Phen)_2Cl_2]$	327	30,581	M→LCT
	345	28,985	d-d transition
	627	15,948	d-d transition
	990	10,101	d-d transition
$[Ni(Phen)(ATET)Cl_2]\ H_2O$	272	36,764	π-⟶π* transition
	347	28,818	M→LCT
$[Co(Phen)_2Cl_2]$	290	34,482	π-⟶π* transition
	351	28,490	d-d transition
	608	16,447	d-d transition

Table 3: IR spectral data (cm^{-1}) of Cu(II), Ni(II) and Co(II) Complexes

ATET	[Cu(Phen)(ATET) Cl_2] H_2O	[Ni(Phen)(ATET) Cl_2] H_2O	[Co(Phen)(ATET) Cl_2] H_2O	Assignment
1592	1546	1563	1565	υC=N (Azomethine)
	1497 1234	1497 1333	1480 1345	υC-C (thiophene)
1201	1161	1145	1142	υC=S (thione)
	719	725	728	Thiophene
	620	623	637	Thiophene
	596	549	566	υM-N
	457	449	458	υM-S

Table 4: ESR spectral data of copper complexes in DMF solvent

Parameter	$Cu(Phen)_2Cl_2$		[Cu(Phen)(ATET) Cl_2] H_2O	
	LNT	RT	LNT	RT
$g\|\|$	2.26	2.23	2.15	2.08
$g\perp$,	2.05	2.16	2.02	2.03
gavg	2.12	2.21	2.06	2.05
G	0.51	0.69	7.53	2.20
$A^{\parallel} \times 10^{-5}$	0.00104	-	0.00136	-
$A^{\perp} \times 10^{-5}$	-	-	0.00126	-
$K^{\parallel}$	0.991	-	1.07	-
$K^{\perp}$	1.061	-	0.992	-
λ	432	-	335	-
α	0.366	-	0.157	-

Table 5: CV data of Cu (II), Ni(II) and Co(II) complexes with Phen and ATET

Complex	Ep_c	Ep_a	ΔEp (mV)	$E_{1/2}$	-ic/ia	$LogKc^a$	$-\Delta G^{b}$
$Cu(Phen)_2Cl_2$	-0.086	0.140	226	0.113	1.461	0.0148	854
[Cu(Phen)(ATET) Cl_2] H_2O	-0.092	0.135	227	0.198	1.714	0.0147	853
$Ni(Phen)_2Cl_2$	-1.83	- 0.680	115	-1.25	1.317	0.0292	168
[Ni(Phen)(ATET) Cl_2] H_2O	-1.102	- 0.652	450	-0.877	1.612	0.0746	232
$Co(Phen)_2Cl_2$	-1.21	-0.55	660	-0.880	1.261	0.0508	293
[Co(Phen)(ATET) Cl_2] H_2O	-1.073	- 0.607	466	-0.840	1.813	0.0720	416

[a]log $K_C = 0.434ZF/RT\Delta E_p$; [b]$\Delta G^o = -2.303RT\log K_C$

Table 6: Electronic Absorption Data upon addition of CT –DNA to the Complex

Complexes	λ max(nm)		Δ λ	H%	Kb [M^{-1}]
	Free	Bound			
$Cu(Phen)_2Cl_2$	269	270	1	20.79	1.44 $X10^5$
[Cu(Phen)(ATET) Cl_2] H_2O	335	336	1	23.75	4.09 $X10^5$
$Ni(Phen)_2Cl_2$	270	270.5	0.5	15.83	1.26$X10^5$
[Ni(Phen)(ATET) Cl_2] H_2O	324	325	1	28.01	1.58$X10^5$
$Co(Phen)_2Cl_2$	269.5	270.5	1	12.19	1.73 $X10^5$
[Co(Phen)(ATET) Cl_2] H_2O	280	281.5	1.5	12.74	6.73 $X10^5$

Table 7: Antibacterial activity of different Metal Complexes against pathogenic bacterial strains

Sample	Treatment (Concentration)	*E. coli* (Mean±SE)	K.pneumoniae (Mean±SE)	*S. aureus* (Mean±SE)	B.cereus (Mean±SE)
S-Ciprofloxacin	(5µg/µL)	10.5±0.02	8.98 ±0.09	10.03±0.03	12.16±0.05
$Cu(Phen)_2Cl_2$	100µg/µL	3.98±0.14	2.17±0.17	2.87±0.18	1.14±0.25
	200µg/µL	4.37±0.47	3.47±0.47	3.8±0.32	2.45±0.75
	300µg/µL	5.12±0.8	4.93±0.3	4.97±0.06	3.87±0.36
$[Cu(Phen)(ATET)Cl_2]H_2O$	100µg/µL	4.1±0.02	4.25±0.42	4.5±0.24	4.15±0.06
	200µg/µL	5.6±0.11	5.67±0.69	5.7±0.02	5.5±0.78
	300µg/µL	6.55±0.47	6.12±0.39	6.65±0.05	6.2±0.23
$Ni(Phen)_2Cl_2$	100µg/µL	2.4±0.11	2.4±0.17	1.5±0.28	2.87±0.16
	200µg/µL	2.67±0.09	3.67±0.17	2.34±0.15	1.93±0.26
	300µg/µL	1.98±0.06	5.33±0.17	3.5±0.63	1.4±0.32
$[Ni(Phen)(ATET)Cl_2]H_2O$	100µg/µL	2.5±0.02	4.5±0.03	2.5±0.04	5.7±0.02
	200µg/µL	2.1±0.05	3.5±0.78	4.6±0.36	6.8±0.47
	300µg/µL	3.1±0.86	4.5±0.85	3.2±0.98	3.4±0.98
$Co(Phen)_2Cl_2$	100µg/µL	1.3±0.15	1.2±0.18	1.53±0.3	1.83±0.01
	200µg/µL	2.83±0.17	1.93±0.13	1.87±0.87	2.01±0.34
	300µg/µL	1.5±0.29	2.01±0.15	1.69±0.23	1.57±0.36
$Co(Phen)(ATET)Cl_2]H_2O$	100µg/µL	0.5±0.25	1.9±0.05	2.68±0.14	2.5±0.15
	200µg/µL	1.6±0.36	2.68±0.08	2.64±0.78	3.6±0.68
	300µg/µL	3.5±0.21	3.65±0.09	3.5±0.64	4.64±0.12

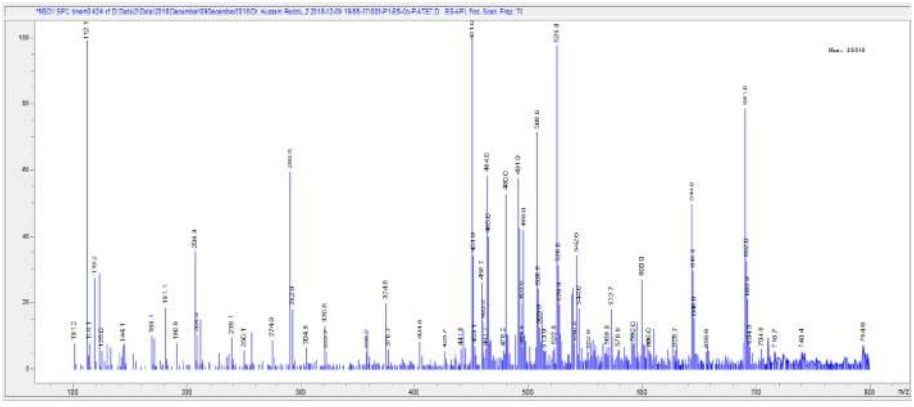


Figure 1: ESI-Mass spectrum of $[Co(Phen)(ATET)Cl_2]H_2O$ Complex

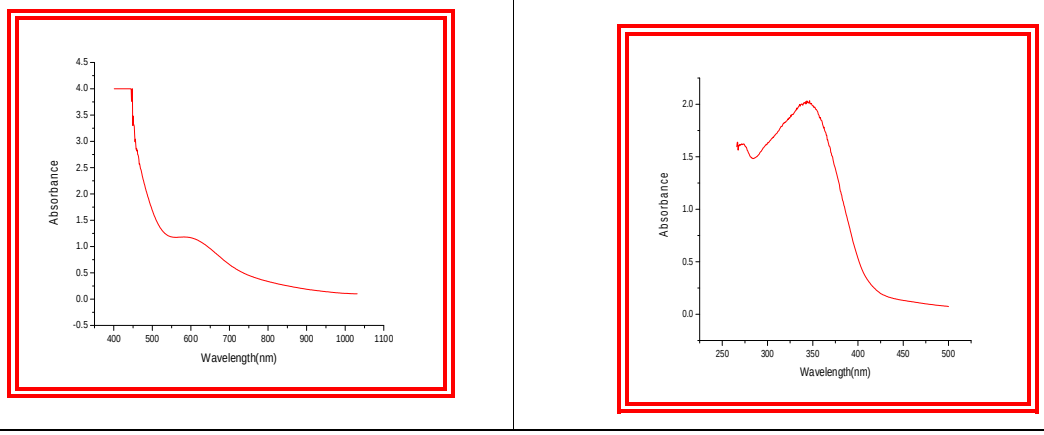
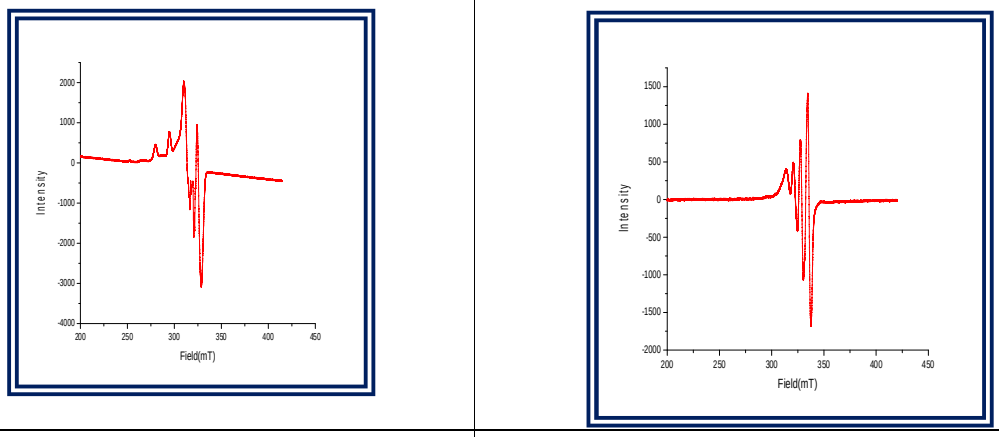


Figure 2: Electronic Spectra of [Cu (Phen)(ATET)Cl_2] H_2O Complex (A) at higher concentration and (b) at lower concentration.



(A) At LNT (B) At RT

Figure 3: ESR Spectrum of [Cu (Phen) (ATET) Cl_2].H_2O Complex.

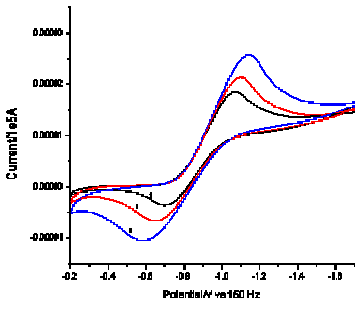


Figure 4: Cyclic voltammogram of [Ni (Phen)(ATET) Cl_2]. H_2O At different scan rates (1) 0.05 (2) 0.1 (3) 0.2 mVs^{-1}

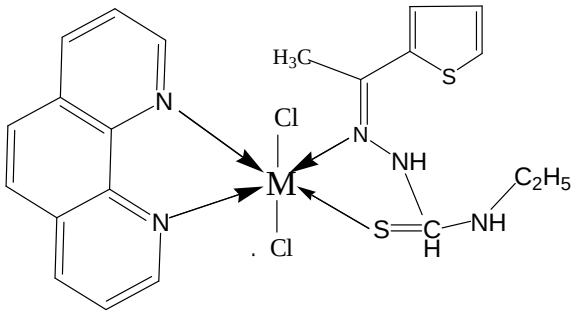
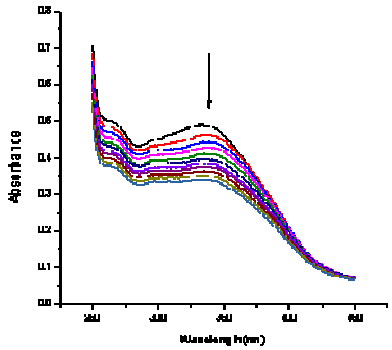
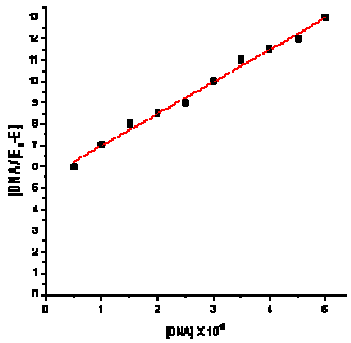


Figure 5: Structure of [M (phen) (ATET) Cl_2]. H_2O Complex. [M= Cu (II), Ni (II) and Co (II)]



(A)



(B)

Figure 6: (A) Absorption Spectra of [Cu(Phen)(ATET) Cl_2] H_2O In the absence and in the presence of increasing concentration of CT-DNA; [The Top most spectrum is recorded in the absence of CT-DNA and below spectra on addition 20µL DNA each time.] (b) A plot [DNA]/ (E_a-E_f) vs [DNA] X 10^{-6} is shown.

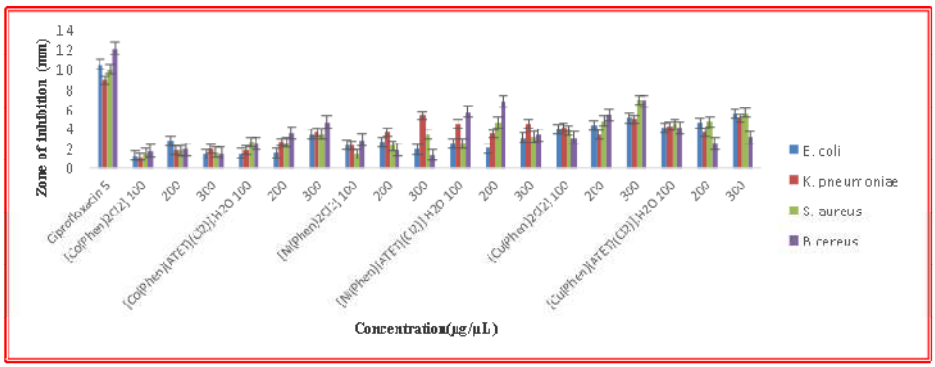


Figure 7: Graphical representation of antibacterial activity of Metal Complexes against pathogenic bacterial strains

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RESEARCH ARTICLE

Diversity of Wild Edible Plants Traditionally used by the Local Inhabitants of South Indian State of Tamil Nadu

T. S. Shynin Brintha and S. Jeeva*

Assistant Professor, Department of Botany, Scott Christian College (Affiliated to Manonmaniam Sundaranar University, Tirunelveli), Nagercoil, Tamil Nadu, India.

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***Address for Correspondence**
S. Jeeva
Assistant Professor,
Department of Botany,
Scott Christian College
(Affiliated to Manonmaniam Sundaranar University, Tirunelveli),
Nagercoil, Tamil Nadu, India.

ABSTRACT

Indigenous communities in different parts of the world use wild edibles plants as supplementary food to increase dietary diversity. In South India, wild edible plants have been in use since time immemorial and they were the important source as a food supplement. Therefore, it is necessary to document indigenous knowledge of the local populace regarding the wealth of wild edible plants, ensuring their long lasting existence. Thus, the present study was carried out among the inhabitants of the Keeriparai village of Kanniyakumari district, Tamil Nadu, south India, in order to make an inventory of the utilization of wild edible plants as dietary supplements. A total of 60 plant species belonging to 33 families were recorded. Enumeration of a list of species, plant part used, method of consumption and the harvesting time is presented. Euphorbiaceae with 6 species is the most utilized family followed by Apocynaceae (5 species), Fabaceae and Solanaceae (4 species each), Amaranthaceae and Annonaceae with at least three species in each. Trees with 23 species were found to be the most dominant growth form followed by herbs with 17 species. Based on parts used fruits with 37 species were recorded to be the most used plant parts followed by aerial parts.

Keywords: Ethnobotany; Tamil Nadu; Wild edible plants

INTRODUCTION

Global food security and economic growth now depends on a declining number of plant species. In human history, 40- 100,000 plant species have been regularly used for food, fibers, shelter, industrial, cultural and medicinal purposes (Magbagbeola et al., 2010). However, only a small number of plants are widely used. The remaining plant

diversity is underutilized (Jaenicke and Hoschele-Zeledon, 2006). Underutilized plants contribute immensely to family food security and serve as means of survival during times of drought, famine, shocks and risks (Assefa and Abebe, 2011). They can also supplement nutritional requirements due to their better nutritional value (van Andel, 2006; Hunde et al., 2011). With alarming increase in human population and depletion of natural resources, it has been felt necessary to explore the possibility of use of new plant resources having potential for food, fodder, energy and industrial uses. Many neglected and underutilized species are nutritionally rich and adapted to low input agriculture. The erosion of these species can have immediate consequences on the nutritional status and food security of the poor (Dansi et al., 2012). The use of wild plant resources has been an integral part of cultural, religious and health aspect of numerous indigenous and rural communities across the globe (Sawian et al., 2007; Jeeva, 2009; Gajurel and Doni, 2020). Out of about 422000 recorded plant taxa globally, nearly 20000 species are reported to be wild edible and more than 85% of world population depends on less than 20 plant taxa for their daily caloric need (Rashid et al., 2015). In Indian subcontinent alone, about 9500 wild plant are utilized for food, medicine and other purposes of by indigenous communities (Jain and Tiwari, 2012). Today the knowledge regarding these wild edibles and their use remains restrained to elderly people of the community. Perusal of literature reveals that several studies have been carried out on wild edible plants across the Tamil Nadu state (Ramachandran, 2007; Arinathan et al., 2007; Rasingam, 2012; Ramachandran and Udhayavani, 2015; Sarvalingam et al., 2015), but in particular, none of the workers have investigated the diversity and use of wild edible plants of Kanniyakumari district, Tamil Nadu. Assuming the importance, the present study was undertaken with the aim of documenting the indigenous knowledge on wild edible plants among the rural inhabitants of Keeriparai and its environs of Kanniyakumari district, Tamil Nadu, India.

MATERIALS AND METHODS

Study area

The present study was conducted at Keeriparai and its vicinity of Kanyakumari district (77^{0}15'E, 8^{0}29'N) (Figure 1). Keeriparai is a small Village/hamlet in Thovalai Block in Kanniyakumari District of Tamil Nadu State, India. It comes under Thadikkarankonam Panchayath. It is located 29 KM towards North from District head quarters Nagercoil. The climate of the area is favorable warm and humid. The summer starts from March to May followed by southwest monsoon from June to September. The mean annual rainfall was 167.64 mm and varied from 70 mm (minimum during February) to 442 mm (maximum - October). The mean monthly temperature varied from a maximum of 32.6^{0}C in the month of May to a minimum of 22.5^{0}C in December. Rice (*Oryza sativa*) is the staple food of the rich and poor, alike in the area. Agriculture is the main occupation of the people of the area, and some of them are involved in rubber tapping. They follow the age-old culture and tradition to utilize the wild resources for food and medicinal requirements. Besides, the wild edible plants also play a vital role in revenue generation as they are being sold regularly in local markets.

Field study

For ethnobotanical data collection, various surveys were conducted from 2021 to 2022 (mostly from April to February) in the study area. During this period, the diet of the people was examined in detail. The information on wild edible plants was collected through semi-structured and open-ended interviews with 70 inhabitants-men (42.85%) and women (57.14%). including teachers, students, shopkeepers, workers, seasonal nomads, and housewives. According to the respondent necessity and response, interviews were conducted into the local common language (Tamil). Most of the people interviewed were 40 years old. We were accompanied by locals during the survey who toured various habitats of plants and obtained data were noted down in the field notebook. All collected wild edible plants (in alphabetical order) and concerning data like scientific names, families, voucher numbers, local names, English names, local names in Tamil, used part(s), mode of consumption, life form, season and use value is summarized in Table 1.

Plant collection and identification

For identification, each mature plant (during flowering and fruiting stages) was collected in the months of mid-April to mid- February from 2021 to 2022. In the second phase collected wild edible plants were pressed, dried, poisoned, and mounted on standard herbarium sheets. Plant specimens were identified by Dr. T.S. Shynin Brintha at the Department of Botany, Scott Christian College (Autonomous), Nagercoil, Tamil Nadu, India using the ''Flora of the Presidency of Madras" (Gamble, 1957) and were confirmed by matching with Herbarium specimens. Finally, each species name was conformed from "The Plant List Database''. If a species was recognized in database, it was classified as taxonomically valid (i.e., "accepted"). Based on the information presented above, the status of synonyms was determined, and verified synonyms were eliminated. After identification and assigning voucher numbers of plants, specimens were deposited at the Herbarium of Scott Christian College, Nagercoil, Tamil Nadu, India.

RESULTS

In the present study, a total of 60 wild food plants belonging to 50 genera and 33 families (31 Angiosperm families; Pteridophytes and Gymnosperms were monospecific) were reported (Table 1). The family Euphorbiaceae (6 species) contributed the highest number of species followed by Apocynaceae (5), Fabaceae and Solanaceae (4 species each), Amaranthaceae and Annonaceae (3 species each), Arecaceae, Convolvulaceae, Moraceae, Myrtaceae, Passifloraceae, Rhamnaceae and Sapindaceae (2 species each) remaining 9 families such as Apiaceae, Asparagaceae, Cactaceae, Caesalpiniaceae, Cornaceae, Cucurbitaceae, Cycadaceae, Erythroxylaceae, Lythraceae, Marsileaceae, Nelumbonaceae, Nyctaginaceae, Primulaceae, Rubiaceae, Rutaceae, Salicaceae, Talinaceae, Verbenaceae and Vitaceae shared with one species each (Table 2; Figure 2). Habit-wise distribution of wild edible plants shows that 38% of the species found were trees (23 species), followed by herbs (28%; 17 species), climbers (22%; 13 species) and shrubs (12%; 7 species) (Figure 3). All reported edible plants are used by locals as a fresh in season or some of them were preserved in a dried form to overcome food crises throughout the year. The fruits of most plants (37 species) were used in food followed by aerial parts (15 species), stem (3 species), tubers (3 species) and roots (1 species) (Figure 4). Wild plants were used in three stages viz., young parts, and mature parts, mature and young.

Most vegetables (except herbal tea plants, condiments, and fruits) were used young stages because most leafy vegetables become hard at the maturity stage and even lose their nutritional value as well. In the study area, the availability of wild edible plants was 8 species in April, 15 species in May, 30 species in June, 30 species in July, 32 species in August, 18 species in September, 9 species in October, 6 species in November, and two species in December. Although most recorded species were available in other months, the period listed in Table 2 was regarded as the most favorable for collecting, consuming, and storing wild food plants (April to December). However, the availability of most plants for consumption and storage was optimum from June to August. The majority of leafy vegetable and fruits are consumed in fresh form through seasonal preference and selection. Thus, it can be stated that the consumption of wild edible plants is a common livelihood option of the people of the study area to fulfill the food and nutritional requirements for all the age groups from the children to the elderly people. The high usage of wild edible plant indicates availability and ease of accessibility of various wild resources coupled with vast associated traditional knowledge on its utility. This indicates the huge gene pool diversity of wild edible plants in the region, which further provides scope for suitable agro-horticultural research interventions for improving economic and livelihood security of the people of the study area.

DISCUSSION

From time immemorial the wild edible plants have been a source of 'hidden harvest' which had supplemented the community with food and income (Heywood, 1999; Grivetti and Ogle, 2000). Tamil Nadu historically has been an agricultural state of India cherishes rich ethnobotanical knowledge about medicinal and edible plants since ancient times. The plant wealth of the state is fully utilized by the local inhabitants as food and medicine. They consume the wild edible plants either eaten raw or cooked by boiling in water. In view of the

food security of local populace, the present study was conducted, and provides detailed information on the diversity and use of wild edible plants used by the local community of Keeriparai village of Kanniyakumari district. The present study confirmed the uses of 60 plant species that are being used for various purposes in the form of wild edible. Fruits were the most utilized plant part (37 species) followed by aerial parts (15 species), stem (3 species), tubers (3 species) and roots. The majority of edible plants are consumed in fresh form through seasonal preference and selection. Thus, the present study reflects the vast traditional knowledge and preference and dependency of the people on wild edible plants to meet their sustenance. Dependency on wild plants for day to day activities and consumption of many wild plants for various dietary requirements by the indigenous people of Tamil Nadu state have already been highlighted in different studies (Ramachandran, 2007; Arinathan et al., 2007; Ramachandran and Udhayavani, 2015). The high usage of wild edible plants indicates availability and ease of accessibility of various wild resources coupled with vast associated traditional knowledge on its utility (Doni and Gajurel, 2020). The potential contribution of wild edible plants towards meeting the daily nutritional requirement of the rural population has also been highlighted previously (Lockett et al., 2000; Agrahar-Murugkar and Subbulakshmi, 2005). Rigorous empirical documentation of this knowledge is imperative as it may be lost forever (Sukumaran et al., 2021).

CONCLUSION

The present study indicates the huge gene pool diversity of wild edible plants of the area, which further provides scope for suitable agro-horticultural research interventions for improving economic and livelihood security of the local communities of the area. Moreover, integrating wild-plant-related knowledge in the school and university curriculum would familiarize the youths with these important wild species and their associated indigenous knowledge.

Authors' contributions

TSSB designed the objectives and plan of work. SJ carried out the field work, analysed the data and wrote the manuscript. TSSB helped in data analysis, interpretation of results and finalization of the manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflict of interest.
Ethical issues: None.

REFERENCES

1. Agrahar-Murugkar D, Subbulakshmi G. Nutritive values of wild edible fruits, berries, nuts, roots and spices consumed by the Khasi tribes of India. Ecol Food Nutr. 2005; 44:207–23.
2. Arinathan V, Mohan VR, Britto A, Murugan C. Wild edibles used by Palliyars of the western Ghats, Tamil Nadu. Indian Journal of Traditional Knowledge, 2007; 6(1), 163–168.
3. Assefa A, Abebe T. Wild edible trees and shrubs in the semi-arid lowlands of southern Ethiopia. J Sci Dev. 2011;1(1):5-19.
4. Dansi A, Vodouh`e R, Azokpota P, Yedomonhan H, Assogba P, Adjatin A et al. Diversity of the Neglected and Underutilized Crop Species of Importance in Benin. The Scientific World Journal, 2012, 1-19.
5. Gajurel PR, Doni T. Diversity of wild edible plants traditionally used by the galo tribe of Indian Eastern Himalayan State of Arunachal Pradesh. Plant Science Today 2020; 7(4):523-533.
6. Gamble JS, Fischer CE. Flora of the Presidency of Madras, Part III, Botanical Survey of India, 1956.
7. Grivetti LE, Ogle BM. Value of traditional foods in meeting macro-and micronutrient needs: the wild plant connection. Nutrition research reviews. 2000 Jun;13(1):31-46.
8. Heywood VH. Use and potential of wild plants in farm households. Food & Agriculture Org.; 1999.

9. Hunde D, Njoka J, Zemede A, Nyangito M. Wild edible fruits of importance for human nutrition in semiarid parts of east shewa zone, Ethiopia: associated indigenous knowledge and implications to food security. Pakistan Journal of Nutrition. 2011; 10:40–50.
10. Jaenicke H, Hoschele-Zeledon I. Strategic framework for underutilized plant species research and development, with special reference to Asia and the Pacific, and to Sub-Saharan Africa. Rome, Italy: International Centre for Underutilized Crops, Colombo, Sri Lanka and Global Facilitation Unit for Underutilized Species; 2006.
11. Jain AK, Tiwari P. Nutritional value of some traditional edible plants used by tribal communities during emergency with reference to central India. Indian Journal of Traditional Knowledge. 2012;11(1):51–57.
12. Jeeva S. Horticultural potential of wild edible fruits used by the Khasi tribes of Meghalaya. Journal of Horticulture and Forestry. 2009 Nov 30;1(9):182-92.
13. Lockett CT, Calvert CC, Grivetti LE. Energy and micronutrient composition of dietary and medicinal wild plants consumed during drought. Study of rural Fulani, North-eastern Nigeria. Int J Food Sci Nutr. 2000;51:195-208.
14. Magbagbeola, J.A., Adetoso, J.A. and Owolabi, O.A. 2010. Neglected and underutilized species (NUS): panacea for community focused development to poverty alleviation/poverty reduction in Nigeria. Journal of Economics and International Finance, 2: 208–211.
15. Ramachandran VS, Udhayavani C. Knowledge and uses of wild edible plants by Paniyas and Kurumbas of Western Nilgiris, Tamil Nadu. Indian Journal of Natural Products and Resources, 2015; 4(4), 412–418.
16. Ramachandran VS. Wild edible plants of the Anamalais, Coimbatore district, western Ghats, Tamil Nadu. Indian Journal of Traditional Knowledge, 2007; 6(1), 173–176.
17. Rashid S, Ahmad M, Zafar M, Sultana S, Ayub M, Khan MA, et al., Ethnobotanical survey of medicinally important shrubs and trees of Himalayan region of Azad Jammu and Kashmir, Pakistan. J. Ethnopharmacol. 2015; 166:340–51.
18. Rasingam L. Ethnobotanical studies on the wild edible plants of Irula tribes of Pillur Valley, Coimbatore district, Tamil Nadu, India. Asian Pacific Journal of Tropical Biomedicine, 2012; 2(3):S1493-1497.
19. Sarvalingam, A., Rajendran, A., and Sivalingam, R. Wild edible plant resources used by the Irulas of the Maruthamalai Hills, Southern Western Ghats, Coimbatore, Tamil Nadu. *Indian J. Nat. Prod. Resour.*, 2015; 5, 198–201.
20. Sawian JT, Jeeva S, Lyndem FG, Mishra BP, Laloo RC. Wild edible plants of Meghalaya, North-East India, Nat. Product. Radiance, 6 (2007) 410–426.
21. Sukumaran S, Sujin RM, Geetha VS, Jeeva S. Ethnobotanical study of medicinal plants used by the Kani tribes of Pechiparai Hills, Western Ghats, India. Acta Ecologica Sinica, 2021; 41(5):365-76.
22. Van Andel T. Non-timber forest products the value of wild plants. Wageningen: Agrodok 39, Agromisa Foundation and CTA, 2006.

Table 1. Preference of wild edible plants by the local people of Keeriparai and its environs

Sl. No	Botanical Name	Family	Common Name	Local Name	Habit	Part Harvested	Purpose of Harvest	Season of Harvest
1	*Aegle marmelos* (L.) Corr	Rutaceae	Wood Apple	Vilvam	Tree	Fruit	Household	March to June
2	*Amaranthus viridis* L.	Amaranthaceae	Pigweed	Kuppaikeerai	Herb	Leaves	Household & Economic	Throughout the year
3	*Annona squamosa* L.	Annonaceae	Custard apple	Munthiri	Tree	Fruit	Household & Economic	August-November
4	*Annona reticulata* L.	Annonaceae	Netted custard apple	Ramachita	Tree	Fruit	Household	September-January
5	*Annona muricata* L.	Annonaceae	Prickly custard apple	Mullu-sitha-pazham	Tree	Fruit	Household & Economic	April-October
6	*Artocarpus heterophyllus* Lam.	Moraceae	Jack Fruit	Palapalam	Tree	Fruit	Household & Economic	June-August
7	*Artocarpus hirsutus* Lam.	Moraceae	Wild jack	Aaeni	Tree	Fruit	Household & Economic	December-March
8	*Borassus flabellifer* L.	Arecaceae	Palmyra palm	Panampazham	Tree	Fruit	Household	March-September
9	*Mangifera indica* L.	Anacardiaceae	Mango	Mampazham	Tree	Fruit	Household & Economic	January-May

10	*Phyllanthus emblica* L.	Euphorbiaceae	Indian Gooseberry	Kattu Nelli	Tree	Fruit	Household & Economic	June-September
11	*Physalis minima* L.	Solanaceae	Little Gooseberry	Sodakku thakkaali	Herb	Fruit	Household	Throughout the year
12	*Psidium guajava* L.	Myrtaceae	Common guava	Koyya	Tree	Fruit	Household & Economic	Throughout the year
13	*Syzygium cumini* (L.) Skeels	Myrtaceae	Indian Blackberry	Naaval	Tree	Fruit	Household & Economic	June-July
14	*Solanum torvum* Sw.	Solanaceae	Prickly Nightshade	Sundakkai	Herb	Fruit	Household & Economic	July-March
15	*Solanum americanum* Mill.	Solanaceae	American Nightshade	Manathakkali	Herb	Leaves	Household & Economic	March-November
16	*Tamarindus indica* L.	Caesalpiniaceae	Tamarind	Puli	Tree	Fruit	Household & Economic	October-December
17	*Phyllanthus acidus* (L.) Skeels	Euphorbiaceae	Malay Gooseberry	Cheema Nelli	Tree	Fruit	Household & Economic	June-August
18	*Carissa spinarum* L.	Apocynaceae	Conkerberry	Sirukila	Shrub	Fruit	Household & Economic	February-April
19	*Ziziphus jujuba* Mill.	Rhamnaceae	Jujube	Elanthai	Tree	Fruit	Household & Economic	April to May
20	*Ziziphus oenopolia* (L.) Mill.	Rhamnaceae	Jackal Jujube	Elanthai	Shrub	Fruit	Household	July-November
21	*Coccinia grandis* (L.) Voigt	Cucurbitaceae	Ivy gourd	Kovakkai	Climber	Fruit	Household & Economic	December-April
22	*Hemidesmus indicus* (L.) R. Br. ex Schult.	Apocynaceae	Indian Sarsaparilla	Nannari	Climber	Root	Household & Economic	November-February
23	*Cissus quadrangularis* L.	Vitaceae	Edible vine	Pirandai	Climber	Stem	Household	June-January
24	*Alternanthera sessilis* (L.) R.Br. ex DC.	Amaranthaceae	Sessile joyweed	Ponnankannikeerai	Herb	Leaves	Household & Economic	Throughout the year
25	*Amaranthus viridis* L.	Amaranthaceae	Wild Amaranth	Kuppaikeerai	Herb	Leaves	Household & Economic	Throughout the year
26	*Cardiospermum halicacabum* L.	Sapinadaceae	Balloon vine	Mudakattan	Climber	Leaves	Household & Economic	June-November
27	*Opuntia dillenii* (Ker Gawl.) Haw.	Cactaceae	Indian fig	Kalli Pazham	Shrub	Fruit	Household	November-February
28	*Solanum trilobatum* L.	Solanaceae	Thai Nightshade	Thoothuvalai	Climber	Leaves	Household	January-August
29	*Passiflora foetida* L.	Passifloraceae	Stinking Passion flower	Kurangu Pazham	Climber	Fruit	Household	October
30	*Cycas circinalis* L.	Cycadaceae	Sago-palm	Chalankai	Tree	Fruit	Household	December-February
31	*Pithecellobium dulce* (Roxb.)Benth.	Fabaceae	Madras thorn	Kodukka puli	Tree	Fruit	Household & Economic	June
32	*Anacardium occidentale* L.	Anacardiaceae	Cashew nut	Kola mavu	Tree	Fruit	Household & Economic	November-April
33	*Rivea hypocrateriformis* Choisy	Convolvulaceae	Common Night Glory	Musuttai Kodi	Climber	Leaves	Household	December-February
34	*Vigna trilobata* (L.) Verdc.	Fabaceae	Wild Gram	Kattupayar	Herb	Fruit	Household	July-December
35	*Clitoria ternatea* L.	Fabaceae	Butterfly Bean	Kakkattan	Climber	Flower	Household	Throughout the year
36	*Erythroxylum monogynum* Roxb.	Erythroxylaceae	Red cedar	Sembulichan	Shrub	Fruit	Household	Throughout the year
37	*Embelia ribes* Burm.f.	Primulaceae	Vidanga	Vayuvidangan	Climber	Fruit	Household	March-August
38	*Flacourtia indica* (Burm.f.) Merr.	Salicaceae	Madagascar plum	Katukalai	Tree	Fruit	Household	November-March
39	*Breynia retusa* (Dennst.) Alston	Euphorbiaceae	Cup Saucer Plant	Aattacherukola	Shrub	Fruit	Household	February-September
40	*Flueggea leucopyrus* Willd.	Euphorbiaceae	Spinous fluggea	Vellaipoola	Shrub	Fruit	Household	June-September
41	*Canavalia ensiformis* (L.) DC.	Fabaceae	Jack bean	Chattavarai	Climber	Fruit	Household	September - October

42	*Adenia hondala* (Gaertn.) W.J.de Wilde	Passifloraceae	Hondala	karimutukk	Climber	Leaves	Household	September to January
43	*Alangium salviifolium* (L.f.) Wangerin	Cornaceae	Sage leaved alangium	Azhinjil	Tree	Leaves	Household	March-May
44	*Marsilea quadrifolia* L.	Marsileaceae	Four Leaf Clover	aaraikkeerai	Herb	Leaves	Household	Throughout the year
45	*Phyllanthus indofischeri* Bennet	Euphorbiaceae	Indian Gooseberry	Nelli	Tree	Fruit	Household & Economic	October-January
46	*Asparagus racemosus* Willd.	Asparagaceae	Indian Asparagus	Sathavari	Climber	Tuber	Household & Economic	August-September
47	*Ceropegia spiralis* Wight	Apocynaceae	Spiral Ceropegia	Parai Pandam	Herb	Tuber	Household	August-December
48	*Caralluma umbellata* Haw.	Apocynaceae	Umbelled Caralluma	Kallimulaiyaam	Herb	Stem	Household	March-April
49	*Boerhavia diffusa* L.	Nyctaginaceae	Common Hogweed	Mookarattai	Herb	Leaves	Household	August-December
50	*Caralluma adscendens* (Roxb.) R.Br.	Apocynaceae	Antiobesity plant	Muyal kombu chedi	Herb	Stem	Household	March-April
51	*Ipomoea aquatica* Forssk.	Convolvulaceae	Water Morning Glory	Vallai-k-kirai	Climber	Leaves	Household	November-March
52	*Trapa natans* var. b*ispinosa* (Roxb.) Makino	Lythraceae	Water chestnut	Karimbolam	Herb	Leaves	Household	September-May
53	*Nelumbo nucifera* Gaertn.	Nelumbonaceae	Lotus	Tamarai	Herb	Tuber	Household	July-December
54	*Canthium coromandelicum* (Burm.f.) Alston	Rubiaceae	Wild jessamine	Karay Chedi	Shrub	Fruit	Household	April-June
55	*Lantana camara* L.	Verbenaceae	Lantana weed	Unni Chedi	Herb	Fruit	Household	Throughout the year
56	*Phoenix sylvestris* (L.) Roxb.	Arecaceae	Silvester Palm	Inthupaanai	Tree	Fruit	Household	April-December
57	*Baccaurea courtallensis* (Wight) Müll.Arg.	Euphorbiaceae	Mootapalam	Mootilpazham	Tree	Fruit	Household & Economic	January-June
58	*Schleichera oleosa* (Lour.) Merr.	Sapindaceae	Ceylon Oak	Poovanam	Tree	Fruit	Household	March-June
59	*Talinum paniculatum* (Jacq.) Gaertn.	Talinaceae	Ceylon Spinach	Pasalai Keera	Herb	Leaves	Household	October-January
60	*Centella asiatica* (L.) Urb.	Apiaceae	Indian Pennywort	Vallarai	Herb	Leaves	Household	Throughout the year

Table 2. Family-wise distribution of wild edible plants of the study area

Sl. No.	Family	Genus	Species
1	Euphorbiaceae	4	6
2	Apocynaceae	4	5
3	Fabaceae	4	4
4	Solanaceae	2	4
5	Amaranthaceae	2	3
6	Annonaceae	1	3
7	Amaranthaceae	2	2
8	Arecaceae	2	2
9	Convolvulaceae	2	2
10	Moraceae	1	2
11	Myrtaceae	2	2
12	Passifloraceae	2	2

13	Rhamnaceae	1	2
14	Sapinadaceae	2	2
15	Apiaceae	1	1
16	Asparagaceae	1	1
17	Cactaceae	1	1
18	Caesalpiniaceae	1	1
19	Cornaceae	1	1
20	Cucurbitaceae	1	1
21	Cycadaceae	1	1
22	Erythroxylaceae	1	1
23	Lythraceae	1	1
24	Marsileaceae	1	1
25	Nelumbonaceae	1	1
26	Nyctaginaceae	1	1
27	Primulaceae	1	1
28	Rubiaceae	1	1
29	Rutaceae	1	1
30	Salicaceae	1	1
31	Talinaceae	1	1
32	Verbenaceae	1	1
33	Vitaceae	1	1

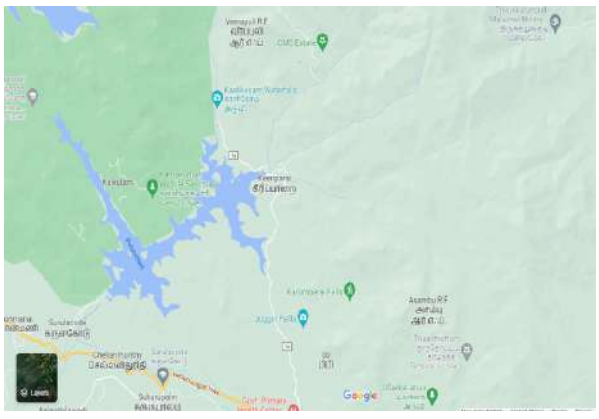

Figure 1. Map of the study area.

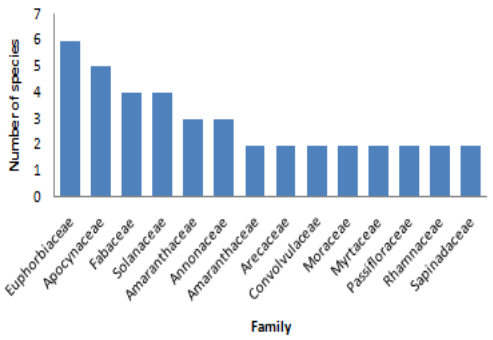

Figure 2. Dominant families of wild edible plant species

Figure 3. Percentage of different growth forms/ habit of recorded wild edible plants species.

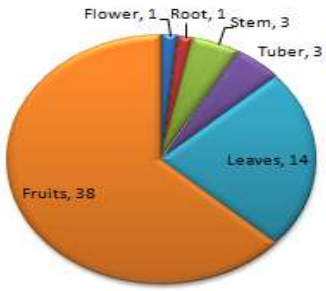

Figure 4. Percentage of parts used.

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RESEARCH ARTICLE

Assessment of Maximum Ramus Breadth in Gender Determination: An Orthopantomographic Study

G. Preethi[1], Reshma Poothakulath Krishnan[2]* and Abirami Arthanari[3]

[1]Undergraduate Student, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai, India
[2]Senior Lecturer, Department of Oral and Maxillofacial Pathology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai, India
[3]Lecturer, Department of Forensic Odontology, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai, India

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***Address for Correspondence**
Reshma Poothakulath Krishnan
Senior Lecturer,
Department of Oral and Maxillofacial Pathology,
Saveetha Dental College and Hospitals,
Saveetha Institute of Medical and Technical Sciences,
Chennai, India
Email: reshmapk.sdc@saveetha.com

ABSTRACT

Forensic science's discipline involves the methodologies ranging from DNA analysis to chemical composition to pattern recognition. Mandibular ramus can be used as an indicator for gender determination as it is found intact and it is very durable and shows resistance to the disintegration process. The aim of this study is to determine the gender using maximum ramus breadth with orthopantomogram. The present study was conducted at Saveetha Dental College and Hospital, Tamilnadu. The study sample consisted of 16 OPG of male and female between the age 30 to 35 years. Maximum ramus breadth was measured for both male and female OPG using a computerized software. The obtained data and results were analyzed using SPSS software. In the present study, it was noted that the mean of maximum ramus breadth was more among males (mean value - 46.11) than females (mean value - 41.10). p value was found to be 0.033 and is statistically significant ($p < 0.05$). The mandibular ramus can be considered as a valuable tool in gender determination with the help of OPG among the studied population.

Keywords: Maximum ramus breadth, forensic science, gender, orthopantomogram, innovative technology, novel method

INTRODUCTION

Forensic science is the application of science to criminal and civil laws. Forensic science's discipline involves the methodologies ranging from DNA analysis to chemical composition to pattern recognition [1]. In the fields of anthropology and forensic sciences, gender estimation is a critical component of research. It is used to establish further interpretations and analyses of bone in gender determination. The sex of a bone is usually determined through morphological and metric investigations [2]. Data from the morphology and metric aspects of the skull and mandible, dental records, soft tissues, and DNA analysis of teeth can be used to determine the gender of an unknown individual [3]. The mandible is very important in determining gender. The mandible is considered as the skull's biggest, dimorphic, and strongest bone. There is a deep layer of compact bone present, which makes it exceptionally tough and allows it to last longer than many other bones [4]. Mandibular development growth rates are usually dissimilar in male and female. Also, masticatory forces are different for each sex which influences the mandibular ramus shape. The form and size of the mandible show dimorphism. Bones in males are typically larger and more stronger than those in females [5]. Previous studies have shown that minimum ramus breadth and maximum ramus breadth are most accurate in predicting gender on OPG while projective height was least reliable [6]. Mandibular ramus can be used as an indicator for gender determination as it is found intact and it is very durable and shows resistance to the disintegration process[7].The efficacy of orthopantomogram has been proved in the determination of morphological dimensions in mandibles. Antemortem OPG is known to have great value in the identification of human remains. Extensive coverage, reduced patient radiation dosage, and quick image gathering are just a few of the benefits of digital panoramic imaging. Other advantages of overlaid image interference are not experienced. In addition, picture contrast and brightness augmentation, as well as image enlargement, give an accurate and repeatable means of measuring the selected locations. The presence of various radiographs provides a wide opportunity to study the sexual dimorphism in a population. Thus the mandibular ramus can be considered another valuable tool in gender determination with the help of OPG [8]. Our team has extensive knowledge and research experience that has translate into high quality publications [9], [10], [11], [12], [13][14], [15], [16], [17], [18], [19], [20], [21], [22], [23],[24] ,[25] ,[26] ,[27] ,[28]. The aim of this study is to determine the gender using maximum ramus breadth with orthopantomogram.

MATERIALS AND METHODS

The present study was conducted at Saveetha Dental College and Hospital, Chennai. The study sample consisted of 16 orthopantomogram of male and female between the age 30 to 35 years. The informed and written consent was taken from the ethical committee clearance of Saveetha Dental College. The inclusion criteria were, radiographs taken with proper positioning and without any magnificent errors and no developmental anomalies. The exclusion criteria includes mandibles with fractures, developmental disturbances and poorly visualized radiographs . The maximum ramus breadth was measured using a computerized software. Maximum ramus breadth was measured by measuring the distance between the most anterior point on the mandibular ramus and a line connecting the most posterior point on the condyle and the angle of jaw (Figure 1). The morphometric analysis of mandibular Ramus was done and the mean was calculated. The obtained data and results were analysed using SPSS software. A p -value less than 0.05 (typically ≤ 0.05) was considered statistically significant.

RESULTS

The total number of orthopantomogram included in the present study were 16. Out of 16, 8 were male and 8 were female. The maximum ramus breadth was calculated using computed software. It was noted that the maximum ramus breadth was comparatively more among males than females. On applying an independent t-test for both genders, the p value was noted to be 0.033 which is statistically significant (p value < 0.05). The mean value for male was 46.11 ± 2 and the mean value for female was 41.10 ± 2 where 2 represents the standard deviation (Figure 2).

DISCUSSION

One of the crucial elements of forensics is to determine the gender from fragmented dentition and jaws. Morphological marks which are used for sex determination is subjective and are mostly inaccurate, but measurements and morphology based methods are accurate and can be a viable tool in sex determination. The precision of orthopantomogram for providing the anatomic measurements has been accepted to be reliable. However orthopantomogram is being used by clinicians to diagnose various oral diseases. But there are limitations like magnification and geometric distortion, the vertical dimensions in contrast to the horizontal dimensions may get altered. In the present study, mean maximum ramus breadth was more among males (mean value - 46.11) than females (mean value - 41.10) with a statistically significant p value of 0.033 was found. A similar result was observed in a study where the maximum ramus breadth was observed to be more among the males than the females. Another study demonstrates that the mean maximum ramus width in females and males was 31.0275 and 30.5625, respectively, which was statistically insignificant (p value = 0.524) [2]. Maximum ramus breadth has been proved to be a valuable tool for sex determination in various studies. A study proved that total ramal height is a reliable method for sex determination using panoramic radiograph [29]. In another study, mandibular ramus breadth was found to be the most effective component in their study, with a 75.8% accuracy [30]. A similar study was conducted where 78.4% of males and 76.8% females were successfully identified by mandibular ramus analysis on OPG with overall accuracy of 77.6% [31]. The mandibular ramus can be contemplated as a valuable tool in sex estimation since it takes over a resistance to damage and disintegration processes. Given the methodological approach and strategy used, as well as the substantial results obtained, it can be stated that orthopantomograph measurements of the mandibular ramus can be used as an authentic parameter for gender assessment. The present study was done on a small sample size and only on one parameter. Further studies should be done on larger samples and other parameters should be included to provide better results.

CONCLUSION

From the present study it can be concluded that maximum ramus breath can be used for gender determination. The mandibular ramus breadth can be considered a useful tool in determining gender because it provides resistance to damage and disintegration processes, and the orthopantomogram can be used as an authentic and accurate tool to record the various measurements needed to determine the gender of a specified mandible. Further population-specific studies with larger samples are needed in the future to substantiate the usefulness of this study.

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Conflict of Interest: Authors have no conflict of interest to declare.

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REFERENCES

1. Bell S, Sah S, Albright TD, Gates SJ Jr, Denton MB, Casadevall A. A call for more science in forensic science. Proc Natl Acad Sci U S A. 2018 May 1;115(18):4541–4.
2. Damera A, Mohanalakhsmi J, Yellarthi P, Rezwana B. Radiographic evaluation of mandibular ramus for gender estimation: Retrospective study [Internet]. Vol. 8, Journal of Forensic Dental Sciences. 2016. p. 74. Available from: http://dx.doi.org/10.4103/0975-1475.186369
3. Datta A, Siddappa SC, Gowda VK, Channabasappa SR, Shivalingappa SB, Dey D, et al. A study of sex determination from human mandible using various morphometrical parameters. Indian Journal of Forensic and Community Medicine. 2015;2(3):158–66.
4. Yadav R, Yadav A, Dhond P. Anatomy of Mandible [Internet]. Fracture Mandible. 2012. p. 12–12. Available from: http://dx.doi.org/10.5005/jp/books/11598_3
5. Muskaan A, Sarkar S. Research Article Mandible-An Indicator for age and sex determination using digital Orthopantomogram. Available from: http://saspjournals.com/wp-content/uploads/2015/01/SJDS-2182-95.pdf
6. Mehta H, Bhuvaneshwari S, Singh MP, Nahar P, Mehta K, Sharma T. Gender determination using mandibular ramus and gonial angle on OPG. Journal of Indian Academy of Oral Medicine and Radiology. 2020 Apr 1;32(2):154.
7. Tejavathi Nagaraj LJ, Gogula S, Ghouse N, Nigam H, Sumana CK. Sex determination by using mandibular ramus: A digital radiographic study. Journal of Medicine, Radiology, Pathology and Surgery. 2017;4:5–8.
8. Saloni, Verma P, Mahajan P, Puri A, Kaur S, Mehta S. Gender determination by morphometric analysis of mandibular ramus in sriganganagar population: A digital panoramic study. Indian J Dent Res. 2020 May;31(3):444–8.
9. Princeton B, Santhakumar P, Prathap L. Awareness on Preventive Measures taken by Health Care Professionals Attending COVID-19 Patients among Dental Students. Eur J Dent. 2020 Dec;14(S 01):S105–9.
10. Mathew MG, Samuel SR, Soni AJ, Roopa KB. Evaluation of adhesion of Streptococcus mutans, plaque accumulation on zirconia and stainless steel crowns, and surrounding gingival inflammation in primary molars: randomized controlled trial. Clin Oral Investig. 2020 Sep;24(9):3275–80.
11. Sridharan G, Ramani P, Patankar S, Vijayaraghavan R. Evaluation of salivary metabolomics in oral leukoplakia and oral squamous cell carcinoma. J Oral Pathol Med. 2019 Apr;48(4):299–306.
12. R H, Hannah R, Ramani P, Ramanathan A, Jancy MR, Gheena S, et al. CYP2 C9 polymorphism among patients with oral squamous cell carcinoma and its role in altering the metabolism of benzo[a]pyrene [Internet]. Vol. 130, Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology. 2020. p. 306–12. Available from: http://dx.doi.org/10.1016/j.oooo.2020.06.021
13. Antony JVM, Ramani P, Ramasubramanian A, Sukumaran G. Particle size penetration rate and effects of smoke and smokeless tobacco products - An invitro analysis. Heliyon. 2021 Mar 1;7(3):e06455.
14. Sarode SC, Gondivkar S, Sarode GS, Gadbail A, Yuwanati M. Hybrid oral potentially malignant disorder: A neglected fact in oral submucous fibrosis. Oral Oncol. 2021 Jun 16;105390.
15. Hannah R, Ramani P, WM Tilakaratne, Sukumaran G, Ramasubramanian A, Krishnan RP. Author response for "Critical appraisal of different triggering pathways for the pathobiology of pemphigus vulgaris—A review" [Internet]. Wiley; 2021. Available from: https://publons.com/publon/47643844
16. Chandrasekar R, Chandrasekhar S, Sundari KKS, Ravi P. Development and validation of a formula for objective assessment of cervical vertebral bone age. Prog Orthod. 2020 Oct 12;21(1):38.
17. Subramanyam D, Gurunathan D, Gaayathri R, Vishnu Priya V. Comparative evaluation of salivary malondialdehyde levels as a marker of lipid peroxidation in early childhood caries. Eur J Dent. 2018 Jan;12(1):67–70.
18. Jeevanandan G, Thomas E. Volumetric analysis of hand, reciprocating and rotary instrumentation techniques in primary molars using spiral computed tomography: An in vitro comparative study. Eur J Dent. 2018 Jan;12(1):21–6.
19. Ponnulakshmi R, Shyamaladevi B, Vijayalakshmi P, Selvaraj J. In silico and in vivo analysis to identify the antidiabetic activity of beta sitosterol in adipose tissue of high fat diet and sucrose induced type-2 diabetic experimental rats. Toxicol Mech Methods. 2019 May;29(4):276–90.

20. Sundaram R, Nandhakumar E, Haseena Banu H. Hesperidin, a citrus flavonoid ameliorates hyperglycemia by regulating key enzymes of carbohydrate metabolism in streptozotocin-induced diabetic rats. Toxicol Mech Methods. 2019 Nov;29(9):644–53.
21. Alsawalha M, Rao CV, Al-Subaie AM, Haque SKM, Veeraraghavan VP, Surapaneni KM. Novel mathematical modelling of Saudi Arabian natural diatomite clay. Mater Res Express. 2019 Sep 4;6(10):105531.
22. Yu J, Li M, Zhan D, Shi C, Fang L, Ban C, et al. Inhibitory effects of triterpenoid betulin on inflammatory mediators inducible nitric oxide synthase, cyclooxygenase-2, tumor necrosis factor-alpha, interleukin-6, and proliferating cell nuclear antigen in 1, 2-dimethylhydrazine-induced rat colon carcinogenesis. Pharmacogn Mag. 2020;16(72):836.
23. Shree KH, Hema Shree K, Ramani P, Herald Sherlin, Sukumaran G, Jeyaraj G, et al. Saliva as a Diagnostic Tool in Oral Squamous Cell Carcinoma – a Systematic Review with Meta Analysis [Internet]. Vol. 25, Pathology & Oncology Research. 2019. p. 447–53. Available from: http://dx.doi.org/10.1007/s12253-019-00588-2
24. Zafar A, Sherlin HJ, Jayaraj G, Ramani P, Don KR, Santhanam A. Diagnostic utility of touch imprint cytology for intraoperative assessment of surgical margins and sentinel lymph nodes in oral squamous cell carcinoma patients using four different cytological stains. Diagn Cytopathol. 2020 Feb;48(2):101–10.
25. Karunagaran M, Murali P, Palaniappan V, Sivapathasundharam B. Expression and distribution pattern of podoplanin in oral submucous fibrosis with varying degrees of dysplasia – an immunohistochemical study [Internet]. Vol. 42, Journal of Histotechnology. 2019. p. 80–6. Available from: http://dx.doi.org/10.1080/01478885.2019.1594543
26. Sarode SC, Gondivkar S, Gadbail A, Sarode GS, Yuwanati M. Oral submucous fibrosis and heterogeneity in outcome measures: a critical viewpoint. Future Oncol. 2021 Jun;17(17):2123–6.
27. Raj Preeth D, Saravanan S, Shairam M, Selvakumar N, Selestin Raja I, Dhanasekaran A, et al. Bioactive Zinc(II) complex incorporated PCL/gelatin electrospun nanofiber enhanced bone tissue regeneration. Eur J Pharm Sci. 2021 May 1;160:105768.
28. Prithiviraj N, Yang GE, Thangavelu L, Yan J. Anticancer Compounds From Starfish Regenerating Tissues and Their Antioxidant Properties on Human Oral Epidermoid Carcinoma KB Cells. In: PANCREAS. LIPPINCOTT WILLIAMS & WILKINS TWO COMMERCE SQ, 2001 MARKET ST, PHILADELPHIA …; 2020. p. 155–6.
29. Kambylafkas P, Murdock E, Gilda E, Tallents RH, Kyrkanides S. Validity of panoramic radiographs for measuring mandibular asymmetry. Angle Orthod. 2006 May;76(3):388–93.
30. Dayal MR, Spocter MA, Bidmos MA. An assessment of sex using the skull of black South Africans by discriminant function analysis. Homo. 2008 Apr 24;59(3):209–21.
31. Poongodi V, Kanmani R, Anandi MS, Krithika CL, Kannan A, Raghuram PH. Prediction of age and gender using digital radiographic method: A retrospective study. J Pharm Bioallied Sci. 2015 Aug;7(Suppl 2):S504–8.

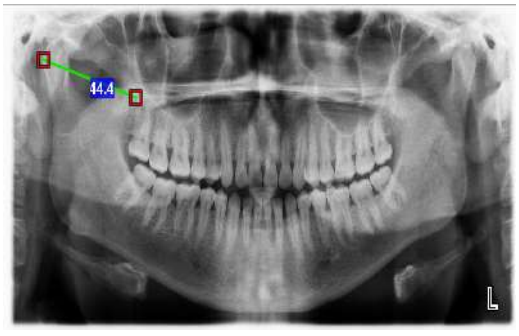

Figure 1: Measurement of maximum ramus breath in an orthopantomograph

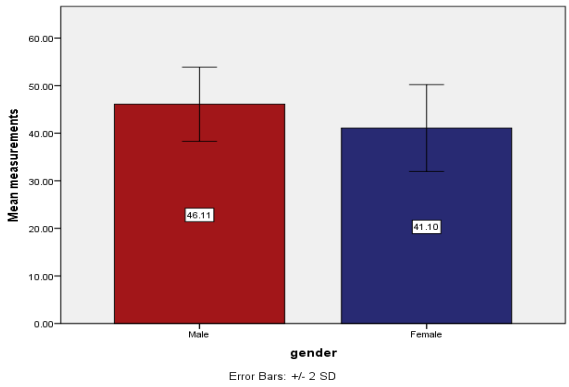

Figure 2 : The graph represents the mean maximum ramus breadth between males and females in gender determination. X axis represents the gender and Y axis represents the maximum ramus breadth. Male is denoted by red colour and female is denoted by blue colour. 46.11 was the mean value for males and 41.10 for females. It was noted that the maximum ramus breadth was comparatively more among males than females. On applying an independent t-test for both genders, the p value was obtained as 0.033 which is statistically significant (p<0.05).

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RESEARCH ARTICLE

Checklist of Marine Algal Collection of BSIS, Industrial Section Indian Museum, Botanical Survey of India

Sudeshna Datta[1], Kangkan Pagag[2]* and Manas Bhaumik[3]

[1]Botanist, Central National Herbarium, Botanical Survey of India, Howrah, West Bengal India
[2]Botanist, Industrial, Section India Museum, Botanical Survey of India, Kolkata, West Bengal, India
[3]Scientist - E , Botanical Survey of India, Kolkata, India.

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***Address for Correspondence**
Kangkan Pagag
Botanist, Industrial,
Section India Museum, Botanical Survey of India,
Kolkata, West Bengal, India
Email: kpagag@gmail.com

ABSTRACT

Marine algae are very rich in diversity in India and they are useful for human consumption or medicinal or commercial purposes. Industrial Section Indian Museum, Botanical Survey of India, Kolkata has vast collections of herbarium specimens of marine algae mostly collected by K.S. Srinivasan and other workers from the period of 1945-1955 in a herbarium which has registered acronym BSIS. K. S. Srinivasan was one of the pioneers of marine algae. The comprehensive checklist of these collections were not done till yet which would be useful for the workers for study and reference. Therefore an attempt has been made to prepare the checklist of these algal collections which would provide a medium to researchers for accessing these data.

Keywords: Database, herbarium, India, taxonomy.

INTRODUCTION

Botanical collections are crucial tool in the field of biodiversity studies, environment impact assessment, genetic as well as taxonomic research. They provide the base for identification as well as evaluation of species conservation status. In India, a study by Krishnamurthy & Joshi (1970) shows a comprehensive checklist of 522 species of Indian marine algae. Srinivasan (1969 & 1973) published Icons of 51 and 53 Indian marine Algae in volumes I & II respectively. Subsequently, Untawale et al. (1983) and Oza & Zaidi (2001) updated the checklist of Indian marine algae. The most recent work by Rao and Gupta (2015) indicates a total of 865 seaweeds from India. CSIR-NIO has recently initiated such efforts to digitize the seaweed collection housed in their reference center under the program entitled bio search: Marine biodiversity data base of India (Kakodkar et al., 2013). The information provided along with the digital herbarium is rudimentary and there is need to integrate more information on the distribution,

ecology, conservation status and available published literature. Thus there is a need for development of herbaria of marine algae of India and providing a source of authentic identification that can be used to further taxonomic studies of algae in India and research information can communicate to the public, government and Industry. BSIS, a herbarium in Industrial Section Indian Museum was established in 1897. The herbarium consists of 4 divisions Dicotyledons, Monocotyledons, Gymnosperms and Algae. In the present study an attempt has been made to prepare the checklist of marine algae herbarium specimens which currently house 5085 marine algae specimens. These marine algae collections were made by Dr. K. S. Srinivasan, curator in Industrial Section Indian Museum, a unit of Botanical Survey of India who was an eminent scholar of Late Prof. M.O.P. Iyengar, a renowned, pioneer algologist in India. Herbarium specimens of Dr. K.S. Srinivasan were collected from the period of 1941 to 1957 and Icons of 51 and 53 Indian marine Algae in volumes I & II by Srinivasan (1969 & 1973) are based on these collections housed at BSIS. Few collections were also made by an anonymous collector during the period of 1914 to 1917 as a part of study of economic plants for British India. There are some deposition of specimens from by Elmer Yale Dawson and A.B. Cribb. Elmer Yale Dawson, an eminent American phycologist, whose collection made during the period 1945-1952. A.B. Cribb is an Australian phycologist and his collections in BSIS were made during the period from 1948-1954. These Herbarium specimens of marine algae of BSIS are of immense importance for all research scholars and scientists. These vast collections of marine algae would serve policymakers or people or researchers who have interest in the marine algae of India and their distribution and documentation of this database could be helpful for the studies of marine algae. Therefore the present work was undertaken to prepare the checklist of marine algae collection of India in BSIS herbarium.

Study area of the conserved herbarium specimens

The collection of marine algae specimen was carried out in different coastel areas of India by Dr. K.S. Srinivasan from the period of 1941 to 1957. These localities are particularly from coastal areas of Goa, Gujarat, Kerala, Karnataka, Tamil Nadu, Maharashtra, Odisha including Andaman & Nicobar Islands and herbarium of these collections are now stored in BSIS herbarium, ISIM, Kolkata which researchers can use for their studies in marine algae.

METHODS

The checklist is based on collections of marine algae specimen of herbarium of Industrial Section Indian Museum, Botanical Survey of India popularly known as Economic Botany Herbarium (BSIS) which holds about 5085 specimens of marine algae. All herbarium specimens were accessed, thoroughly studied. The name, collection number, locality, dates were noted and metadata prepared. The nomenclatures were updated with the help of www.algaebase.org (Guiry & Guiry 2020) and literatures such as Srinivasan (1969 & 1973), Rao and Gupta (2015), Oza & Zaidi (2001). This specimen database consists of the labelled data of all collections. The data includes the updated scientific name, collection date and collection number (where ever present) and locality where collected. Photographs were also taken.

RESULTS

The herbarium of Industrial Section Indian Museum, Botanical Survey of India (BSIS) currently house about 5085 marine algae specimens and out of which 133 species with 3 varieties, 2 forma under 113 genera belonging to 56 families are present (Table 1).

DISCUSSIONS

The specimens are from different coastal areas of India with a large collection from different islands of Andaman and Nicobar archipelago. The largest family is Rhodomelaceae with 12 genera followed by Delesseriaceae with 8 genera and Dictyotaceae with 7 genera. *Sargassum* is the largest genera followed by *Caulerpa*. There is also a minor

representation of marine algae from California by Elmer Yale Dawson and Queensland, Australia by A.B. Cribb. Collections by Elmer Yale Dawson, an eminent American phycologist, made during the period 1945-1952, are represented by about 23 specimens of 14 genera in 6 family, including a paratype of *Herposiphonia hollenbergii* E. Y. Dawson. The genera *Pleonosporium, Tiffaniella, Platythamnion* and *Chondria* from the various genera were collected from California, Mexico are not collected Dr. Srinivasan or present in BSIS from any Indian locality. There is also a specimen of *Sargassum agardhianum* Farlow collected by M. W. Williams from California in 1946. Marine algae collections from Queensland, Australia by A.B. Cribb, an Australian algologist account for 18 specimens, belonging to 17 genera in 10 family. Among these genera, *Pterocladiella, Taenioma, Pseudorhizoclonium, Caloglossa, Martensia, Canistrocarpus, Osmundaria, Amansia* and *Pelotoma* are not found in Indian collections of Dr. Srinivasan. Systematic arrangement of accumulated herbarium data can provide enormous information at a glance without wasting energy and time. An updated nomenclature within the metadata makes an easy access to find out required species information. Scientists and researchers will be highly benefited to get their research material easily through this database, as well as future curatorial maintenance will be easier. The checklist provided here aims to narrows this knowledge gap and should be a key resource in the making of a national repository of algal specimens. In future the aim would be to develop a Marine Algae Virtual Herbarium database for easy access to herbarium specimen and further aggregating and linking information that is presently scattered around India.

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REFERENCES

1. Guiry, M.D., G.M. Guiry. 2020. *Algae Base*. World-wide electronic publication, National University of Ireland, Galway. https://www.algaebase.org; Accessed on 31 December 2020.
2. Kakodkar, A.P., Alornekar, A., Dsouza, R., Thomas, T., Divekar, R., Nath, I. Kavlekar, D.P., Ingole, B.S., Bharathi P.S. 2013. BioSearch: A glimpse into marine biodiversity of Indian coastal waters. Indian Journal of Marine Science. 42: 745–748.
3. Krishnamurthy, V.K., Joshi H.V. 1970. A checklist of Indian marine algae. Central Salt and Marine Chemicals Research Institute: Bhavnagar, India, p 36.
4. Oza, R.M., Zaidi S.H. 2001. A revised checklist of Indian marine algae. Central Salt and Marine Chemicals Research Institute: Bhavnagar, India, p 296.
5. Rao, P.S.N., Gupta R.K. 2015. Algae of India, a checklist of Indian marine algae (Excluding Diatoms & Dinoflagellates). Botanical Survey of India, Kolkata, India, p 93.
6. Srinivasan, K.S. 1969. Phycologia indica (Icons of Indian marine Algae). Vol. 1 N.K. Gossain & Co. Pvt. Ltd.
7. Srinivasan, K.S. 1973. Phycologia indica (Icons of Indian marine Algae). Vol. 2. N.K. Gossain & Co. Pvt. Ltd
8. Untawale, A.G., Dhargalkar, V.K., Agadi V. V. 1983. List of marine algae from India. Technical Report National Institute of Oceanography, Goa, India, p 42.

Table 1: List of marine algae specimens present in BSIS:

Family & Species	Locality	Collection No.	Date
Acinetosporaceae			
Feldmannia duchassaingiana (Grunow) Aisha & M. Shameel	Mahabalipuram	s.n.	20.1.1945
Feldmannia mitchelliae (Harvey) H. -S. Kim.	Mahabalipuram	13/46	20.1.1945
Hincksia thyrsoidea (Børgesen) P. C. Silva	Mahabalipuram	242 MJ45	20.1.1945

Anadyomenaceae			
Anadyomene stellata (Wulfen) C. Agardh.	Krusadai Island, Hare Island	2305, 1444	14.1.1949, 27.6.1949
Astronemataceae			
Asteronema breviarticulatum (J.Agardh) Ouriques & Bouzon	Mahabalipuram	42	5.11.1944
Bonnemaisoniaceae			
Asparagopsis taxiformis (Delile) Trevisan de Saint-Léon	Okha Port	254	5.2.1951
Bryopsidaceae			
Bryopsis hypnoides J. V. Lamouroux	Okha	491	8.2.1951
Bryopsis pennata var. *secunda* (Harvey) Collins & Hervey	Dwarka	628	1.2.1951
Bryopsis plumosa (Hudson) C. Agardh.	Okha	710	8.2.1951
Trichosolen mucronatus (Børgesen) W. R. Taylor	Okha, Dwarka	s.n., 118D	25.2.1955, 1.2.1951
Callithamniaceae			
Spyridia alternans Børgesen	Dwarka	s.n.	3.2.1951
Spyridia fusiformis Børgesen	Krusadai Island	2265	14.1.1949
Spyridia hypnoides (Bory) Papenfuss	Cape Comorin	1718	9.7.1949
Spyridia insignis (J. Agardh) J. Agardh	Cape Comorin	s.n.	March 1944
Caulacanthaceae			
Catenella impudica (Montagne) J. Agardh	Bandra sea face, Bombay	1198	15.2.1952
Caulerpaceae			
Caulerpa chemnitzia (Esper) J.V.Lamouroux	Krusadai Islands, Hare Island	18/46, 2051	October 1941, 26.6.1949
Caulerpa chemnitzia var. *laetevirens* (Montagne) Fernández-García & Riosmena-Rodrigue	Cape Comorin	s.n.	October 1941
Caulerpa corynephora Montagne	Hare Island	1319	27.6.1949
Caulerpa cupressoides (Vahl) C. Agardh	Dwarka, Cape Comorin, Krusadai Island	654, 1772, 2249	2.2.1951, 11.7.1949, 14.1.1949
Caulerpa freycinettii	Krusadai Island	34/46	3.10.1941
Caulerpa lessonii Bory	Krusadai Island	28/46	3.10.1941
Caulerpa mexicana Sonder ex Kützing	Okha, Dwarka, Hare Island	s.n., s.n., 2040	6.2.1951, 3.2.1951, 27.6.1949
Caulerpa racemosa (Forsskål) J.Agardh	Dwarka, Krusadai Island, Hare Island, Okha Port	1006, 2273, 2050, 639-	11.2.55, 14.1.1949, 26.6.1949, 6.2.1951
Caulerpa racemosa frm. *complanata* (J. Agardh) Weber Bosse	Hare Island	2030	27.6.1949
Caulerpa scalpelliformis (R.Brown ex Turner) C.Agardh	Dwarka, Cape comorin, Church Island, Okha, Hare Island	712, 1777 , 2108, 91 D, 2081	31.2.1957, 7.7.1949, 1.7.1949, 8.2.1951, 27.6.1949
Caulerpa sertularioides (S. G. Gmelin) M. Howe	Krusadai Island, Dwarka	2351, 655	15.1.1949, 2.2.1951
Caulerpa taxifolia (M. Vahl.) C. Agardh.	Dwarka, Krusadai Island, Hare Island, Cape Comorin	2268, 2268, 2053, 1768	13.2.55, 14.1.1949, 27.6.1949, 11.7.1949
Ceramiaceae			
Centroceras clavulatum (C. Agardh.) Montagne	Hare Island, Okha	2011, 986	26.6.1949, 8.2.1951

Champiaceae			
Champia indica Børgesen	Dwarka	s.n.	31.1.1951
Champia parvula (C.Agardh) Harvey	Krusadai Island	2297	14.1.1949
Chordariaceae			
Myriogloea sciurus (Harvey) Kuckuck ex Oltmanns	Okha Port, Dwarka, Cape Comorin	1101, 801, 1553	10.2.1957, 31.1.1951, 10.7.1949
Cladophoraceae			
Chaetomorpha antennina (Bory) Kützing	Mahabalipuram, Cape Comorin	17, 1687	10.10.1944, 7.7.1949
Chaetomorpha linum (O.F.Müller) Kützing	Dwarka	1077	12.2.1955
Codiaceae			
Codium dwarkense Børgesen	Okha, Dwarka	s.n.	8.2.1951, 3.2.1951
Codium indicum S.C.Dixit	Dwarka	s.n.	31.1.1951
Codium latum Suringar	Dwarka	s.n.	31.1.1951
Delesseriaceae			
Acrosorium zanardini ex Kützing	Cape Comorin	1823	11.7.1949
Apoglossum spathulatum (Sonder) Womersley & Shepley	Okha Port	s.n.	8 .2.1951
Cryptopleura ramosa (Hudson) L. Newton	Cape Comorin	1819	7.7.1949
Dictyurus purpurascens Bory	Cape Comorin, Hare Island	1980, 1402	7.7.1949, 27.6.1949
Heterosiphonia muelleri (Sonder) de Toni	Okha	242	1.2.1951
Hypoglossum spathulatum (Sonder) Kützing	Okha	s.n.	8.2.1951
Nitophyllum marginale (Kützing) J.Agardh	Krusadai Island	2248	14.1.1949
Dichotomosiphonaceae			
Avrainvillea erecta (Berkeley) A. Gepp & E.S. Gepp	South Point, Port Blair, Andaman Island	2985	9.2.1953
Avrainvillea ridleyi A. Gepp & E.S. Gepp	Car Nicobar, Nicobar Island	2988	15.2.1953
Dictyotaceae			
Dictyota dichotoma (Hudson) J.V.Lamouroux	Dwarka	268	31.1.1951
Dictyota ciliolata Sonder ex Kützing	Hare Island, Krusadai Island	1418, 2247	27.6.1949, 14.1.1949
Dictyopteris australis (Sonder) Askenasy	Dwarka, Muldwarka, Okha	175, 60, 61D	1.2.1951, 19.2.1951, 6.2.1951
Dictyopteris delicatula J. V. Lamouroux	Hare Island, Cape Comorin	2087, 1606	27.6.1949, 9.7.1949
Dictyopteris woodwardii (R. Brown ex Turner) C. Agardh.	Muldwarka, Okha	60D, 433a	14.2.1951, 8.2.1951
Lobophora variegata (J. V. Lamouroux) Womersley ex E. C. Oliveira	Krusadai Island	35/46	3.10. 1941
Padina fraseri (Greville) Greville	Okha	49	5.2.1951
Padina tetrastromatica Hauck	Mahabalipuram	s.n.	20.1.1945
Spathoglossum asperum J.Agardh	Krusadai Island, Okha, Muldwarka	2349, s.n.	s.l, 5.2.1951, 14.2.1951
Stoechospermum polypodioides (J.V.Lamouroux) J.Agardh	Hare Island, Cape Comorin, Okha, Dwarka	1316, s.n., 2041, 706	27.6.1949, March 1944, 6.2.1951, 2.2.1951
Gelidiellaceae			
Gelidiella acerosa (Forsskal) Feldmann & Hamel	Krusadai Island	2314	14.1.1949

Gracilariaceae			
Gracilaria compressa (C. Agardh) Greville	Cape Comorin	1921	9.7.1949
Gracilaria corticata (J. Agardh.) J. Agardh	Mahabalipuram, Cape Comorin, South Point, Port Blair, Andaman Islands, Hare Island	s.n., 911, 3369, s.n.	18.5.1945, 11.7.1949, 9.2.1951, 26.5.1949
Gracilaria debilis (Forskkål) Børgesen	Cape Comorin	1920	9.7.1949
Gracilaria fergusonii J. Agardh	Cape Comorin	1911	9.7.1949
Gracilaria gracilis (Stackhouse) Steentoft, L.M.Irvine & Farnham	South Point, port Blair, Andaman Island	3377	9.2.1953
Gracilariopsis longissima (S. G. Gmelin) Steentoft	Krusadai Island, Chilka Lake, Pigeon Islands	2311, 1459	14.1.1949, 17.4.1950
Hydropuntia edulis (S.G.Gmelin) Gurgel & Fredericq	Cape Comorin	s.n.	October 1942
Halimedaceae			
Halimeda tuna (J.Ellis & Solander) J.V.Lamouroux	Muldwarka, Cape Comorin, Dwarka	s.n., 1849, 1070	14.2.1951, 11.7.1949, 12.2.1955
Halymeniaceae			
Carpopeltis maillardii (Montagne & Millardet) Chiang	Cape Comorin	s.n.	March 1944
Corynomorpha prismatica (J. Agardh.) J. Agardh.	Cape comorin, Okha, Car Nicobar, Nicobar Islands, Nancowri.	s.n., s.n., 2680, 2681	March 1944, 6.2.1951, 16.2.1953, 13.2.1953
Cryptonemia undulata Sonder	Cape Comorin	1778	7.7.1949
Grateloupia filicina (J. V. Lamouroux) J. Agardh.	Chilka lake, Pigeon Island, Cape Comorin	1473, 1909	17.4.1950, October 1941
Grateloupia indica Børgesen	Okha, Muldwarka	s.n.	10.2.1951, 19.1.1951
Grateloupia lithophila Børgesen	Mahabalipuram	s.n.	5.11.1944
Halymenia porphyroides Børgesen	Okha, Dwarka,	s.n.	5.2.1951, 11.2.1955
Halymenia polydactyla Børgesen	s.n.	s.n.	11.2.1955
Halymenia venusta Børgesen	Dwarka	s.n.	12.2.1955
Laminariaceae			
Saccharina latissima (Linnaeus) C. E. Lane	Gujranwala (Imported)	Reg. No. 14266	20.3.1900
Liagoraceae			
Helminthocladia australis Harvey	Okha	s.n.	22.2.1955
Liagora erecta Zeh	Mahabalipuram	6/46	25.1.1946
Liagora ceranoides J. V. Lamouroux	Dwarka	s.n.	31.1.1951
Liagora albicans J. V. Lamouroux	Mahabalipuram	9	10.10.1944
Lithophyllaceae			
Amphioroa anceps (Lamarck) Decaisne	Cape Comorin	s.n.	March 1944
Rhizophyllidaceae			
Portieria hornemannii (Lyngbye) P. C. Silva	Cape Comorin	s.n.	11.7.1949
Rhodomelaceae			
Acanthophora spicifera (M. Vahl) Børgesen	Krusadai Island	27/46	3.10.1941
Enantiocladia prolifera Falkenberg	Cape Comorin	1953, 1959	s.l.
Laurencia papillosa (C. Agardh) Greville	Krusadai Island	17/46	October 1941
Leveillea jungermannioides (Hering & G. Martens)	Cape Comorin, Okha,	s.n., 8d, s.n.	s.l.

Harvey	Dwarka		
Lophocladia lallemandii (Montagne) F. Schmitz	Hare Island	2004	26 .6.1949
Neurymenia fraxinifolia (Mertens ex Turner) J. Agardh	Hare Island, Cape Comorin	2006, 1747	27.6.1949, March 1944
Osmundea pedicularioides (Børgesen) G.Furnari, Serio & Cormaci	Dwarka	s.n.	12.2.1955
Rhodymeniaceae			
Botryocladia leptopoda (J. Agardh)Kylin	Dwarka, Okha, Cape Comorin	122D, 724, 1805	31.1.1951, 10.2.1951, March 1944
Coelarthrum opuntia (J. Ag.) Boergs.	Okha	s.n.	24.2.1955
Rhodymenia disecta Børgesen	Cape Comorin, Dwarka, Okha	1899, 1279, s.n.	11.7.1949, 31.1.1979, 5.2.1951
Sarcodiaceae			
Sarcodia ceylonensis (J. Agardh.) Kylin	Cape Comorin	s.n.	March 1944
Sarcodia dichotoma Børgesen	Dwarka	s.n.	31.1.1951
Sargassaceae			
Sargassum aquifolium (Turner) C. Agardh.	Mahabalipuram, Hare Island	22, 2157	10.10.1944, 26.6.1949
Sargassum biserrula J. Agardh.	Mahabalipuram	s.n.	10.10.1944
Sargassum carpophyllum J. Agardh	Mahabalipuram	5	10.10.1944
Sargassum cinerum var. *beberifolium* Børgesen	Mahabalipuram	16	11.10.1944
Sargassum densifolium Zanardini	Port Blair	36515	27.11.1914
Sargassum dumosum Greville	Port Blair	36519	27.11.1914
Sargassum glaucescens J. Agardh.	Mahabalipuram, Vengurla	10, 36542	10.10.1944, 5.12.1914
Sargassum ilicifolium (Turner) C. Agardh	Port Blair, Andaman	36517	27.11.1914
Sargassum notarisii Zanardini	Port Blair	36524	27.11.1914
Sargassum obovatum Harvey	Port Blair	36514	27.11.1914
Sargassam polycystum J. Agardh.	Mahabalipuram, Port Blair, Vengurla Taluka, Bombay Presidency	17, 36518, 36958	11.10.1944, 27.11.1914, 10.4.1917
Sargassum swartzii C. Agardh	Mahabalipuram	13	4.11.1944
Sargassum tenerrimum J. Agardh.	Mahabalipuram	24	11.10.1944
Sargassum wightii Greville	Port Blair, Mahabalipur, Hare Islandam	36521, 6	27.11.1914, 11.10.1944, 26.6.1949
Sargassum wightii f. *linearis*	Mahabalipuram	3	11.10.1944
Sirophysalis trinodis (Forskkål) Kützing	Church Island, Krusadai Island, Hare Island	2152, 21/46, 2012	1.7.1949, 3.10.1941, 26.6.1949
Turbinaria conoides (J. Agardh) Kützing	Port Blair, Mahabalipuram	s.n., s.n.	27.11.1914, 4.11.1944
Hormophysa cuneiformis (J. F. Gmelin) P. C. Silva	Dwarka	1122	14.2.1955
Scinaiaceae			
Scinaia furcellata (Turner) J. Agardh	Okha	s.n.	20.2.1955
Scinaia halliae (Setchell) Huisman	Okha	s.n.	20.2.1955

Scinaia moniliformis J. Agardh	Okha	s.n.	20.2.1955
Scytosiphonaceae			
Chnoospora minima (Hering) Papenfuss	Cape comorin	1711	9.7.1949
Colpomenia sinuosa (Mertens ex Roth) Derbes & Solier	Okha	159	6.2.1951
Hydrolathrus clathratus (C. Agardh.) M. Howe	Krusadai Island, Okha	2292, 1259	14.1.1949, 6.2.1951
Rosenvingea intricata (J. Agardh.) Børgesen	Okha, Dwarka	s.n.	8.2.1951, 2.2.1951
Sebdeniaceae			
Sebdenia polydactyla (Børgesen) M. Balakrishnan	Dwarka	s.n.	31.1.1951
Siphonocladaceae			
Boergesenia forbesii (Harvey) Feldmann	Krusadai Island, Hare Island	2346, 1389	14.2.1949, 27.6.1949
Chamaedoris auriculata Børgesen	Muldwarka, Dwarka	655a, 649b	14.2.1951, 30.1.1951
Dictyosphaeria cavernosa (Forskkål) Børgesen	South Point, Port Blair, Andaman Islands, Krusadai Island	3384, 2281	9.2.1953, 14.1.1949
Solieriaceae			
Solieria robusta (Greville) Kylin	Okha	s.n.	10.2.1951
Sphacelariaceae			
Sphacelaria furcigera Kutzing	Mahabalipuram	16/46	s.l.
Sphacelaria tribuloides Meneghini	Mahabalipuram	14/46	20.1.1945
Udoteaceae			
Tydemania expeditionis Weber Bosse	Nancowri, Nicobar island,	3092	13.2.1953
Udotea flabellum (J. Ellis & Solander) M. Howe	Hare Island	1453	27.6.1949
Udotea indica A. Gepp & E. S. Gepp	Okha, Dwarka	635, 634	5.2.1951, 3.2.1951
Ulotrichaceae			
Acrosiphonia orientalis (J. Agardh.) P. C. Silva	Mahabalipuram	s.n.	April 1945
Ulvaceae			
Ulva compressa L.	Mahabalipuram, Okha	s.n.	05.11.1944, 6.2.1951
Ulva fasciculata A. P. de Candolle	Shirde Taluq, Ratnagari Rough-Sathkartham through Director of Agric. Pune.	Reg. 36588	9.1.1915
Ulva lactuca L.	Cape comorin, Mahabalipuram, Krusadai Island, Quilon, Dwarka	1539, s.n., s.n., 2095, 653	10.7.1949, 4.11.1944, s.l., 18.7.1949, 3.2.1951
Ulva reticulata Forskkål	Krusadai Islands, Dwarka, Church Island	2378, s.n., 2126	12.1.1949, 31.1.1951, 1.7.1949
Valoniaceae			
Valonia utricularis (Roth) C. Agardh	Car nicobar, nicobar Island	2706	15.2.1953
Valoniopsis pachynema (G. Martens) Børgesen	Cape Comorin, Muldwarka	1871, s.n.	10.7.1949, 14.2.1951
Wrangeliaceae			
Halurus flosculosus (J.Ellis) Maggs & Hommersand	Cape Comorin	s.n.	March 1944
Griffithsia flabellata Montagne	Okha	s.n.	8.2.1951

Figure 1: Map shows area of collection of different algal specimens deposited at BSIS (map source- www.mapsofworld.com)

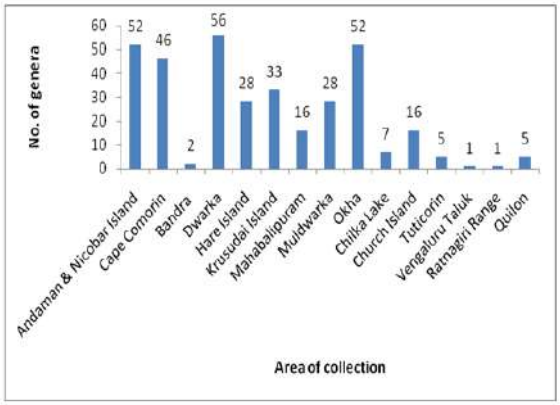

Figure 2: Genera diversity in different collection zone

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RESEARCH ARTICLE

A Study to Assess the Knowledge on Hereditary Breast Cancer and Practice on Screening Methods of Breast Cancer among Women

K.Sivakala[1]* and G.Ambujam[2]

[1]Associate Professor, Vinayaka Mission's College of Nursing, Karaikal, VMRF-DU, Salem, Tamil Nadu, India.
[2]Dean and Professor of Surgery, Vinayaka Missions Medical College, Karaikal, VMRF-DU, Salem, Tamil Nadu, India.

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***Address for Correspondence**
K.Sivakala,
Associate Professor,
Vinayaka Mission's College of Nursing,
Karaikal, VMRF-DU, Salem, Tamil Nadu, India.
Email: sivakalasahithiyan@gmail.com

ABSTRACT

Breast cancer is the most common cancer in women globally and is the leading cause of cancer mortality in women in 103 countries worldwide. Over the past decade, the incidence of breast cancer in low-income and middle-income countries (LMICs) has increased significantly, and by 2020, it is estimated that 1.7million new cases will occur in such countries. To assess the knowledge on hereditary breast cancer among women, to assess the practice of screening methods of breast cancer and To find the association between knowledge and practice of women with their selected demographic variables. This is a descriptive cross-sectional study. Data were obtained by self structured questionnaire. Out of 200 women, 185 (92.5%) agreed to participate in the study. The global percentage of correct answers was not associated with age (p=0.173) or degree/specialization (p=0.815). Questions were classified into two categories. In categories involving knowledge on hereditary breast cancer, the rate of correct answers was 42.4%, respectively. On the practice of screening methods 70% of those interviewed were not sure about the screening methods and Practice of educational actions regarding this subject was reported by 30% of those interviewed. This study reinforces the need to develop qualifying actions for women, so that strategies to control breast cancer become effective in their health care practice.

Keywords: Knowledge; Women, Screening Methods, Breast Cancer.

INTRODUCTION

Cancer is the leading cause of death due to non transmitted diseases worldwide and thus an important public health problem both in developed countries and in underdeveloped or developing countries. Breast cancer is the most frequent type of cancer in women and the second cause of death in this population group worldwide. Cancer is the leading cause of death due to non transmitted diseases worldwide and thus an important public health problem both in developed countries and in underdeveloped or developing countries. Breast cancer is the most frequent type of cancer in women and the second cause of death in this population group worldwide. Breast cancer is a multi-factorial disease in which genetic and environmental factors contribute to its occurrence. In a small percentage of cases, a germ line mutation in a high-penetrance cancer-predisposition gene is present, which can be a major determinant of the occurrence of the disease. Sporadic breast cancer, which is not primarily caused by an inherited high-penetrance mutation, represents more than 90% of breast cancer cases throughout the world. It is estimated that, on average, women who live until the age of 85 will have a chance of 1 in 9 of developing breast cancer. Established risk factors for breast cancer include reproductive factors (early menarche, nulliparity, age at first pregnancy over 30 years, use of high-dose hormonal contraceptives, late menopause and hormone replacement therapy, increasing age, high breast tissue density and family history of cancer, especially breast cancer).

Additional factors that modulate breast cancer risk include nutritional factors, physical activity, history and duration of breast feeding, obesity in post menopause, smoking, alcohol consumption, exposure to ionizing radiation and socio-economic level. Hereditary breast cancer corresponds to approximately 10-15% of all malignant breast tumors. Among these are the tumors caused by highly penetrant germ line mutations in the BRCA1 and BRCA2 genes. Women with mutations in one of these genes present a cumulative risk of between 55% and 85% of developing breast cancer until the age of 70 and a 15% to 65% risk of developing ovarian cancer, depending of the type and location of the mutation(8). Features of the family history that suggest hereditary predisposition to breast cancer include, among others, early age at diagnosis, multiple synchronic or meta chronic primary tumors, male breast cancer and association with other cancer such as ovarian and prostate cancers. Knowledge and identification of risk factors for sporadic breast cancer and focus on risk assessment for the genetic aspects of hereditary breast cancers are key challenges for health promotion and cancer prevention in the community.

Statement of the problem

A study to assess the Knowledge on hereditary breast cancer and practice of screening methods of breast cancer among women

Objectives

- To assess the level of knowledge on hereditary breast cancer among women
- To assess the practice of screening methods of breast cancer among women
- To find the association between knowledge and practice of women with their selected demographic variables

METHODS AND MATERIALS

For the current study quantitative approach, Non – experimental, descriptive design was selected for this study**.** The study was carried out in selected community areas, Karaikal**.** The study target population comprised of women (20-50 years) .The sample size comprised of 200 women and available during the data collection period**.** The Simple random sampling technique - by using lottery method was used to select the sample for the present study. The collected data was analyzed and interpreted by using both descriptive and inferential statistics.

DISCUSSION

This study was intended to conclude knowledge on hereditary breast cancer and practice on screening methods of breast cancer among Women .The present study shows that are most of women (52%) are having inadequate knowledge and (28%) women having moderately adequate knowledge. Regarding the practice of screening methods of breast cancer the breast self examination(BSE) the result view that 25% women had practice, 20% women had practice of clinical breast examination and 10% women had mammogram. There will be statistically associated the knowledge and practice with selected demographic variable of educational status (P<0.05). Some of the study supported to that actual results. The findings of the present study supported by A community based cross sectional study was conducted in Mumbai, Maharastra. Total 100 subjects were selected by multistage sampling technique. Structured questionnaire were used to test their knowledge about breast cancer and practice regarding BSE.

RESULTS

Out of 200 women, 58% had knowledge that breast cancer was the most prevalent cancer among women, 52% knew what is breast self-examination and 28% were practicing breast self-examination. A study supported by A cross sectional study was conducted among reproductive age women in Akatsi South district of Volta region of Ghana. The mean age of the women was 24.54±7.19. Only 3.1% of women had no formal education and 58.9% were single. Although 88.3% of the respondents were aware of breast cancer, 64.9% of the respondents had good or sufficient knowledge of breast cancer and only 94 (37.6%) practice BSE. Over 50% of the respondents did not know how to perform BSE. There was a significant association between knowledge on breast cancer and practice of BSE ($\chi 2$ = 36.218 p = 0.000). The higher the age of a participant, the lower practice of breast self-examination and this was significant ($\chi 2 = 11.324$, $p = 0.003$). The Demographic information of women shows highest percentage of the were in the age group 20- 30 years (40%), According to their occupation shows that private sector (31%), According to their residence (57%) of women living in urban area, Regarding the educational status of the women had graduation(46%), 62% of women had marriage, According to their region were Hindus (44%), 67% of women were living in Nuclear family , Highest percentage (64%) of women had age at menarche at earlier , Regarding sources of information (54%) of women through health care professionals.

RECOMMENDATION

1.A similar study can be conducted on a larger sample
2.Effectiveness of Screening programs to be conducted for early detection and prevention of breast cancer among women.
3.A follow up study can be conducted to determine the effectiveness of structure teaching program.
4.A study to assess the effectiveness of risk factor modification in prevention of breast cancer among high risk population.
5.A similar study can be conducted as retrospective study.
This will be continuation of result.

CONCLUSION

The current study showed that the knowledge on hereditary breast cancer and screening practice was poor among women. Among the study participant only small proportions are actually practicing breast cancer early detection methods. Therefore, conducting awareness program on breast cancer screening methods for women to enhance their knowledge and practice is highly recommended.

REFERENCES

1. Ersumo T. Breast cancer in an Ethiopian population, Addis Ababa. East Central Afr J Surg. (2006) 11:81–6. Available online at: https://www.ajol.info/ index.php/ecajs/article/view/136575
2. American Cancer Society. Breast Cancer Facts & Figures 2013 – 2014. Atlanta USA, Inc. (2013). Available online at: http://www.cancer.org/research/ cancer facts statistics/breast-cancer-facts-figures
3. Martha NS, Benford M. Breast cancer knowledge and awareness among university students in Angola. Pan Afr Med J (2012) 11:70.
4. Parkin DM, Whelan SI, Ferlay J, Storm H. Cancer Incidence in Five Continents. Vol. 1–8. Lyon: IARC Press; IARC Cancer Base no 6 (2005)
5. Lamport L, Andre T. AIDS knowledge and responsibility. Youth Soc. (1993) 25:38–62. doi: 10.1177/0044118X93025001003
6. Yip C, Mohd TN, Ibraham M. Epidemiology of breast cancer in Malaysia. Asian Pac J Cancer Prev. (2006) 7:369–74.

Table 1: Knowledge on hereditary breast cancer among women

S. No	Level of knowledge	Percentage (%)
1	Inadequate knowledge	52 %
2	Moderately adequate knowledge	28 %
3	Adequate knowledge	20 %

Table 2: Percentage distribution on Practice of screening methods of breast cancer among women.

S. No	Level of practice	Percentage (%)
1	Breast self examination (BSE)	25 %
2	Clinical breast examination	20 %
3	Mammography	10 %

Table 3: Association between knowledge and practice score with selected demographic variables

S. No	Demographic variables	DF	Table value	X2 value	Level of significance
1	Age	4	9.49	0.3739	Not significant
2	Religion	6	12.59	3.8079	Not significant
3	Residence	6	12.59	2.695	Not significant
4	Marital status	6	12.59	0.485	Not significant
5	Education	2	5.99	5.4256	Significant
6	Occupation	6	4.79	3.943	Not significant
7	Income	4	12.59	0.3739	Not significant
8	Age at menarche	6	12.59	1.335	Not significant
9	Source of information	6	12.59	0.769	Not significant

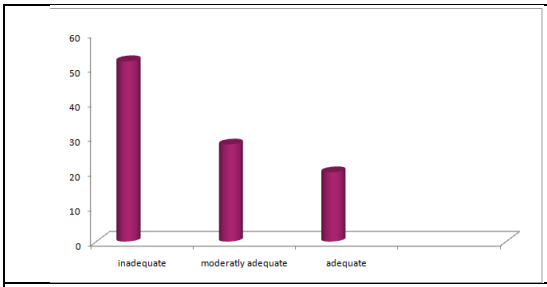

Fig.1: percentage distribution of knowledge on hereditary breast cancer

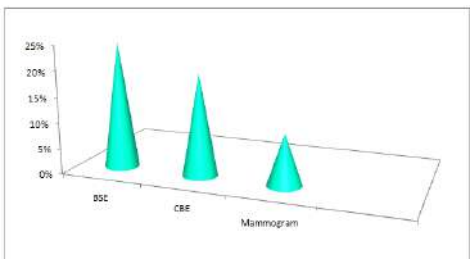

Fig. 2: Percentage distribution on Practice of screening methods of breast cancer

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RESEARCH ARTICLE

Translation and Validation of the Iraqi Anti-Diabetic Medication Adherence Scale in Guajarati

Kaushik K. Patel[1]* and Shraddha J. Diwan[2]

[1]Ph.D. Scholar (Gujarat University, Ahmedabad) and Assistant Professor, SPB Physiotherapy College, Surat, Gujarat, India.
[2]Ph. D. Guide (Gujarat University, Ahmedabad) and Lecturer, SBB Physiotherapy College, V.S. hospital, Ahmedabad, Gujarat, India.

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***Address for Correspondence**
Kaushik K. Patel
Ph.D. Scholar (Gujarat University, Ahmedabad)
and Assistant Professor,
SPB Physiotherapy College,
Surat, Gujarat, India.
Email: kaushik4748@gmail.com

ABSTRACT

Medication adherence is an important determinant of outcomes in patients with type 2 Diabetes Mellitus (T2DM). there is no scale available in Gujarati language for measuring adherence to Medication in Type 2 DM patients, the purpose of our study is to translate The Iraqi anti-diabetic medication adherence scale(IADMAS) in Gujarati language and establish psychometric properties of translated scale. The study was conducted among patients with diabetes mellitus (n=79) and Cross – cultural adaptation guideline was used to translate IADMAS in Gujarati language. Scale was translated in Gujarati language and validity and reliability was established in subjects with DM. Result of the study showed that IADMAS had good test - retest reliability (0.81) and acceptable internal consistency (0.70), Concurrent validity of IADMAS Gujarati showed good correlation (0.68).Result of the study demonstrate The IADMS Gujarati is reliable and valid tool to assess adherence in T2DM.

Keywords: Diabetes mellitus, IADMAS, Cross cultural adaptation, Reliability, Validity

INTRODUCTION

The prevalence of type 2 diabetes in countries has increased dramatically in the last three decades. India has increased the number of people with diabetes from 26 million in 1990 to 65 million in 2016[1]. According to the National Diabetes and Diabetic Retinopathy Survey Report released in 2019 by the Ministry of Health and Family

Welfare, the incidence was 11.8% among people over the age of 50 [2]. The International Federation of Diabetes (IDF) report estimates that India carries nearly 17% of the global burden of diabetes mellitus. It is estimated that US$ 1.7 trillion will be required for diabetes care for the period 2011 to 2030 [3]. Uncontrolled high glucose can increase morbidity and mortality rate in diabetic population, uncontrolled type diabetes has significant negative impacts on the physical and psychological wellbeing and thus affect the quality of life of both people with the disease and their families [4-5]. There are effective approaches available to control high glucose level and to prevent diabetes complication and premature death result from diabetes. These approaches include policies and practice in all population and specific setting like school, home, workplace that contribute to better health regardless of whether they have diabetes or not such as life style modification and self-care activities. Although there are many factors that affect glucose control in patient with diabetes, Medication adherence is an important determinant of outcomes in patients with diabetes malitus [6]. Inadequate adherence compromises safety and the effectiveness of treatment increases the mortality and morbidity with significant direct and indirect costs to the healthcare system [7-8]. For those with diabetes, adherence to medications is associated with better control of risk factors. Improvement in adherence to medication and treatment utility helps to maintain good glucose control in diabetes patient [9-10]. Many tools have been developed and used to evaluate adherence to medication among diabetes patients and unfortunately there is no gold standard measure of medication adherence [11]. Self-reported and semi structured questionnaires have been used for measuring adherence because they are low cost and time saving. Studies have been suggested that the self-reported questionnaires was underestimating non adherence when compared with ill count or biological assay [12-13]. However, subsequent research suggests that the self-report method may provide a reasonably accurate estimate of adherence [14-15]. Further more all these scales are in English language and not specific for Guajarati diabetes mellitus patients. and most commonly used tools for assessing medication adherence (Medication Adherence Questionnaire, The Morisky Medication Adherence Scale, and The Medication Adherence Rating Scale) were originally not designed for assessing adherence among diabetes patients [16-17].The Iraqi Anti-Diabetic Medication Adherence Scale (IADMAS) was developed and validated to assess adherence to antidiabetic medication among Iraqi patient. The IADMAS isonly a reliable and valid tool that can be used to evaluate the adherence to antidiabetic drugs in patients with type 2 DM [18]. Since there is no scale is available in Gujarati language for measuring adherence to Medication in Type 2 DM patients, the purpose of our study is to translate IADMAS in Gujarati language and establish psychometric properties of translated scale.

METHODOLOGY

The Iraqi Anti – Diabetic Medication Adherence Scale(IADMAS) consisted of two parts: part one collects socio-demographic data, part two consist of the eight-item; first three questions are used to assess medication – taking behaviour by giving five responses: (1) always (daily), (2) often, (3) sometimes, (4) rarely and (5) never. The remaining five items are used to measure the determinant of non-adherence by giving a dichotomous response of "Yes" or "No". The first questions identify the extent of unintentional missing of medication doses and others questions are to identify the extend of intentional medication non adherence. 1 and 3 are to identify the extent of non-adherence to the time of medication taking. 2, 6, 7 and 8 are to identify the extent of intentional non-adherence with the prescribed medication dose. Only one item (5) aims to identify the extent of intentional non-adherence through discontinuation of taking DM medication. Scoring of all items ranged from 0 to 1, 0 for non-adherent answer and 1 for total adherence. All items, except item 4, are inversely calculated. The first three items use a 5-point Likert scale that can take one of five values: 1, 0.75, 0.5, 0.25 or 0. The total score of the IADMAS ranged from 0 to 8. Medication adherence in the IADMAS was categorized into three levels of adherence: high adherence (total score=8), medium adherence (6 to <6) and low adherence (<6) [19]. Cross – cultural adaptation guideline was used to translate IADMAS in Gujarati language. Initially scale was translated in Gujarati language. Its validity and reliability was established in subjects with Diabetes mellitus.

Phase 1: Contact with the IADMAS developers

A contact was made with the author of IADMAS in Iraq that originally developed and validated the English version of the scale. The objective was to inform them and ask for their approval then translation and cultural adaptation processes were started.

Phase 2: Initial translations (English to Gujarati)

For the forward translation, Gujarati translators whose mother tongue was Gujarati and fluent in both English and Gujarati language were approached. The IADMAS was given for translation into Gujarati language.

Phase 3: Synthesis of the translations

The original and initial Gujarati questionnaire was synthesized, the process involving comparison and registration of translation differences that reflect potentially ambiguous words. Choices of appropriate terms were identified between the translators and the written report made up of this synthesis process, in which action was taken to resolve the problems that arose.

Phase 4: Backward translations

For the backward translation, The IADMAS Gujarati version was given to English translators who were fluent in both English and Gujarati language. They were blinded to original version.

Phase 5: Expert committee review

For obtaining cross cultural equivalence of translated instrument, the scale was given to expert panel. The expert committee consisted 1 senior Diabetologist, 2senior Physician, and 5senior Physiotherapist with average experience of 14.6 years and who were not the part of the study. Gujarati translated and original version of IADMAS was given to them and a pre- final Gujarati version of IADMAS was developed as per their suggestions and modifications.

Phase 6: Test of pre final version

It was done by conducting a pilot study on a small population of patient who understand Gujarati language and were having diabetes mellitus. The process involved detail Interview of the patients about their understating of the words and translation alternatives. Final version of the scale was developed on the basis of their interview, discussion of expert committee and the findings.

Phase 7: Final version

The final Gujarati version of IADMAS was developed and submitted to the developer of the instrument.

Concurrent validity

Translated Gujarati version of IADMAS was correlated with the Medication Adherence Questionnaire (MAQ) for establishing concurrent validity. No gold-standard tool exists for measuring drug adherence [20]. The MAQ is most acceptable at the stage of care and throughout the population and has been validated in the wide range of diseases and widely used tool for research [21]. The MAQ is four items self- administered questionnaire; that assess medication adherence history. Respondent were asked to answer yes or no and answer will scored as either 0 (Yes) or 1 (No). Total 79 Gujarati person of age group 18 to 65 years with type 2 diabetes mellitus and on antidiabetic medication for at least 3 months were selected. Patients with comorbid conditions, T2DM complications, cognitive impairment and depression and pregnant women were excluded from the study. Table 1 showing base line demographic data. After taking informed consent, participants were asked to complete the MAQ and Gujarati version of IADMAS. Face to face interview was conducted for the participants who were not able to understand questionnaire. Composite score of IADMAS and The MAQ were calculated and spearmen's correlation was found.

Reliability

The selected participants(n = 79) were given Gujarati version the IADMAS scale after 48 hours and combined scores were calculated to find out the reliability of test-retest. An inter-class correlation coefficient was found between the two combined scores and reliability was established.

Statistical analysis

Data input and analysis were done using the SPSS V.17. Spearman's correlation coefficients of the IADMAS and the MAQ was established for the concurrent validity. Internal consistency was measured by Cronbach's alpha and Test retest reliability was determined by Intra- class correlation coefficient (ICC)

RESULT

Total 79 Participant were selected for the study, out of them 46 were male and 33 female with mean age of 54.28 ± 6.2 years participated in the study. Spearman's correlation coefficients was established between IADMS and MAQ for the concurrent validity and it was found to be 0.68 ($p < 0.01$) which indicate good concurrent validity. Correlation coefficient value < 0.25 were considered as small; 0.25 -0.50 as moderate, 0.5 to 75 as good; and > 0.75 considered as excellent [22]. Internal consistency was measured using Cronbach's alpha and the value was 0.70, values indicate acceptable internal consistency. Test retest reliability was determine by intra class coefficient (ICC) and value found was 0.81 which suggested good intra rater reliability.

DISCUSSION

The purpose of this study was to report the reliability and validity of a translated version of the IADMAS among patients with T2DM. It was the first study to systematically translate and validate the IASMAS in Gujarati. The original 8 times IADMAS was developed and tested by Mikhael EM et al[23]. On T2DM patients and it was found that IADMAS was reliable and valid instrument and can be used for assessing antidiabetic medication adherence among patients with type 2 DM. Our study among gujarati population with T2DM showed that IADMAS had good test - retest reliability (0.81) and acceptable internal consistency (0.70), Concurrent validity of IADMAS gujarati was measured by comparing with MAQ and Both the IADMAS Gujarati and the MAQ showed good correlation (0.68).The result of this study was nearly similar to the original IADMAS. Small sample size was our major limitation and this can affect the result, So the IADMS Gujarati need to validate with large sample size, The IADMS is translated and validated only in Gujarati language so it is recommended to translate and validate the IADMS in different language commonly used in India. Furthermore The IADMS Gujarati was validated with only T2DM patients, so further studies are needed to validate in other types of diabetes.

CONCLUSION

The study demonstrate The IADMS Gujarati is reliable and valid tool to assess adherence in T2DM. Hence the IADMS can be used to measure the medicine adherence in Gujarati population.

REFERENCES

1. Tandon N, Anjana RM, Mohan V, Kaur T, Afshin A, Ong K, Mukhopadhyay S, Thomas N, Bhatia E, Krishnan A, Mathur P. The increasing burden of diabetes and variations among the states of India: the Global Burden of Disease Study 1990–2016. The Lancet Global Health. 2018 Dec 1;6(12):e1352-62.
2. Sharma, Neetu Chandra (2019-10-10). "Government survey found 11.8% prevalence of diabetes in India". Livemint. Retrieved 2020-04-29.

3. Bloom DE, Cafiero ET, Jane-Llopis E, Abrahams-Gessel S, Bloom LR, Fathima S, et al. The global economic burden of non-communicable diseases (Working Paper Series). Geneva: Harvard School of Public Health and World Economic Forum; 2011.
4. Verma K, Dadarwal M. Diabetes and quality of life: a theoretical perspective. J Soc Health Diabetes 2017;5:5-8. 4.
5. Vanstone M, Rewegan A, Brundisini F, Dejean D, Giacomini M. Patient perspectives on quality of life with uncontrolled type 1 diabetes mellitus: a systematic review and qualitative metasynthesis. Ont Health Technol Assess Ser 2015;15:1-29
6. Asche C, LaFleur J, Conner C.A review of diabetes treatment adherence and the association with clinical and economic outcomes. ClinTher 2011;**33**:74–109pmid:21397776
7. Liebl A, Neiss A, Spannheimer A, Reitberger U, Wagner T, Gortz A. Costs of type 2 diabetes in Germany. Results of the CODE-2 study. Dtsch Med Wochenschr 2001;18:585–9.
8. Osterberg L, Blaschke T. Adherence to medication. N Engl J Med 2005;353:487–9
9. Rhee MK, Slocum W, Ziemer DC, Culler SD, Cook CB, ElKebbi IM, et al. Patient adherence improves glycemic control. Diabetes Educ 2005;31:240–50.
10. Krapek K, King K, Warren SS, George KG, Caputo DA, Mihelich K, et al. Medication adherence and associated hemoglobin A1C in type 2 diabetes. Ann Pharmacother 2004;38:1357–62
11. Donnan PT, McDonald TM, Morris AD. Adherence to prescribed oral hypoglycemic medication in a population of patients with type 2 diabetes: a retrospective cohort study. Diabet Med 2002;19:279–84
12. Gordis L, Markowitz M, Lilienfeld AM. The inaccuracy in using interviews to estimate patient reliability in taking medications at home. Med Care 1969;7:49–54.
13. Park LC, Lipman RS. A comparison of patient dosage deviation reports with pill counts. Psychopharmacology 1964;6:299–302.
14. Craig HM. Accuracy of indirect measures of medication compliance in hypertension. Res Nurs Health 1985;8:61–6.
15. Grymonpre RE, Didur CD, Montgomery PR, Sitar DS. Pill count, self-report, and pharmacy claims data to measure medication adherence in the elderly. Ann Pharmacother 1998;32:749–54
16. Capoccia K, Odegard PS, Letassy N. Medication adherence with diabetes medication: a systematic review of the literature. Diabetes Educ 2016;42:34–71
17. Nguyen T-M-U, Caze AL, Cottrell N. What are validated self-report adherence scales really measuring?: a systematic review. Br J ClinPharmacol 2014;77:427–45.
18. Mikhael EM, Hussain SA, Shawky N, Hassali MA. Validity and reliability of anti-diabetic medication adherence scale among patients with diabetes in Baghdad, Iraq: a pilot study. BMJ Open Diabetes Research and Care. 2019 Jul 1;7(1):e000658.
19. Morisky DE, Ang A, Krousel-Wood M, et al. Predictive validity of a medication adherence measure in an outpatient setting. J ClinHypertens 2008;10:348–54.
20. Lavsa SM, Holzworth A, Ansani NT. Selection of a validated scale for measuring medication adherence. Journal of the American Pharmacists Association. 2011 Jan 1;51(1):90-4.
21. Culig J., Leppée M. From Morisky to Hill-bone; self-reports scales for measuring adherence to medication. *Collegium Antropologicum.* 2014;38(1):55–62.
22. Portney LG, Watkins MP. Foundations of clinical research: applications to practice. Upper Saddle River, NJ: Pearson/Prentice Hall; 2009.
23. Pedersini R, Vietri J. Comparison of the 4-item and 8-item Morisky Medication Adherence Scale in patients with type 2 diabetes. *Value in Health* 2014;17.

Table 1: Demographic data of participant

Age (Mean ± SD)	54.28 ± 6.2
Gender	
Male	46 (58.23 %)
Female	33 (41.77 %)
Education level	
None or primary	12(15.19%)
Secondary	24(30.38%)
Diploma or college	35(44.3 %)
Post-graduation	08 (10.13%)
Duration of diabetes mellitus (Mean ± SD)	8.46 ± 5.56(3 months – 25 years)
No of prescribed medications (Mean ± SD)	1.85 ± 0.90 (Minimum 1- Maximum 5)
Types of medications	
Oral	43 (54.43%)
Injectable	21 (26.58%)
Combination	16 (20.25%)

Table 2: Concurrent Validity

Spearman's correlation coefficients of the IADMAS and the MAQ	0.68
Level of significance (p)	0.01

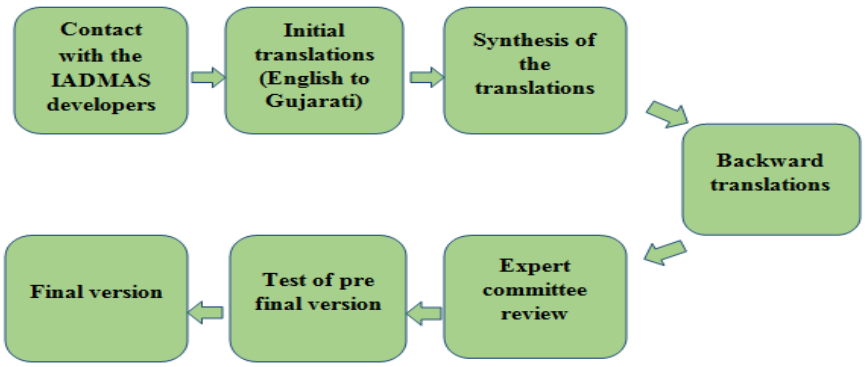

Figure: 1.Steps of Translation Process.

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RESEARCH ARTICLE

Effect of Simplified Kundalini Yoga on Blood Pressure among College Students

C.Viswanathan[1]* and R.Sivakumar[2]

[1]Research Supervisor, WCSC-VISION SKY Research Centre, Temple of Consciousness, Aliyar, Pollachi, Coimbatore, Tamil Nadu, India.
[2]Research Scholar, WCSC-VISION SKY Research Centre, Temple of Consciousness, Aliyar, Pollachi, Coimbatore, Tamil Nadu, India.

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***Address for Correspondence**
C.Viswanathan
Research Supervisor,
WCSC-VISION SKY Research Centre,
Temple of Consciousness,
Aliyar, Pollachi, Coimbatore,
Tamil Nadu, India.

ABSTRACT

There is an increasing prevalence of hypertension amongst young adults in Tamil Nadu. The Objective of the study was to find the effect of Simplified Kundalini Yoga on Blood Pressure among college students. The Practices of Simplified Kundalini Yoga would give a significant difference in Blood Pressure among the college students who are participating in the study as three groups such as experimental group I. experimental group- II than the control group. The experimental study adopted true random group design. Seventy Five Students (age ranging 17 to 23) from K.S.G College of Arts and Science, Coimbatore were selected and assigned randomly to three groups of Twenty Five each. The subjects of the experiments group I and group II underwent the Yoga exercise Practice, Simplified Kundalini Yoga training Program for a period of Sixteen weeks and the Control group did not undergo any practices. The Pre-test and post- test were conducted for three groups. The Blood Pressure was took by SPHYS nonagon meter in variable reading. The data were analyzed using the statistical tool. ANNOVA the test of significance was fixed at 0.05 Level Result and conclusion. The analysis showed a significant difference in Blood Pressure (Systolic and Diastolic) among college students due to the simplified Kundalini Yoga and also a significant reduction in Blood Pressure for the experimental group-I, group –II and control group. It was concluded that simplified Kundalini Yoga Practices can alleviate Blood Pressure among College Students and can be used as a complementary therapy.

Keywords: Blood Pressure, Systolic, Diastolic, Simplified Kundalini Yoga, Yoga

INTRODUCTION

College students are particularly prone to psychological distress caused by interpersonal and social problems, pressures to succeed academically, financial strains, and uncertain futures. For the entire sample in this study, there was a significant improvement in students' mental health. This is the systemized controlled study to show the risk of hypertension in young adults that were associated with changes in psychological distress and coping. A large-scale study undertaken recently in rural Tamil Nadu has confirmed the high prevalence. A study published in the International Journal of Public Health reported 21.4 per cent hypertension prevalence in about 10,500 people, aged 15-64 in 11 villages in the State. 75% percentage of people remaining ignorant of their condition is indeed a major cause for concern. "The ignorance was more in the younger age group" This is one of the major findings of the study.

BLOOD PRESSURE

Blood Pressure are of two types

- Systolic blood Pressure
- Diastolic blood pressure
- In India they measure blood pressure with sphygmomanometer.
- Diastolic less than 80 and systolic between 120-129 has been defined in India

Reason of Blood Pressure

1. Anxiety
2. Stress
3. Emotional
4. Aggression
5. Physical Conditions
6. Physiological conditions
7. Psychological conditions
8. Personal life events
9. Social conditions
10. Location of College

Disease Caused by Blood Pressure

1. Heart Related Problem.
2. Kidney Disorder
3. Stock
4. Paralisis.

Simplified Kundalini Yoga

Simplified Kundalini Yoga was founded by Vethathri Maharishi. He sacrificed his entire life for the service of Society. He Practiced and Preached love and blissfulness. Maharishi felt that the changes towards better living should happen intuitively in the individual. Maharishi's Kundalini yoga is subdivided into

1. Simplified Physical Exercise
2. Kayakalpa Yoga
3. Introspection
4. Meditation.

Purposes of simplified physical exercise

Simplified Physical Exercise Strengthens the body and helps to regularize the Functioning of all systems to eradicate the animal imprints.

Purposes of Kayakalpa Yoga

The Purpose of Kayakalpa is to have

1. Long- Life by postponing death
2. Putting off old age and retaining youth
3. Good health

This Practice strengthens the body and internal organs, which begin to function better. It consists of 1. Ashwini Mudra and 2. Ojus Breath. Through this practice the old age and delay of death are possible for the human.

Introspection

Introspection is the rein to rein the wandering mind. Divine factors like mind, soul, wisdom, magnetism and Divine Force are understood through. "Introspection" with the help of a Guru, One should Practice 'Introspection' until you make your mind a static state. Then you understand that the eternal wisdom is God. These together form Introspection. If there was a mistake made. make a strong auto suggestion not to repeat it, if an action was done well. that should be recollected to imprint it strongly in the mind, to be repeated when possible.

Meditation

Meditation is a stylized mental technique respectively Practiced for the subjective experience that is frequently described as a very useful silent and heightened alertness often characterized as blissful.

Hypothesis

There would be a significant difference in Blood Pressure among College Students due to the Practices of Simplified Kundalini Yoga and there would also be a significant difference in depression among the experimental group I and II than the control group.

Delimitations

1. The study is delimited only in K.S.G College of Arts and Science College Coimbatore Men Students.
2. The data were collected from Seventy Five Men divided into three groups namely Experimental group-I, Experimental group-II and control group. Consisting of Twenty Five each.
3. The ages of the subjects ranged from 17 to 23 years college students only.

Significance of the study

The Study was Significant in assessing the selected physiological and Psychological variable Blood Pressure among college students.

METHODOLOGY

For this study, Seventy Five College Students have been selected and divided into Experimental group-I, Experimental group-II and Control group of Twenty five students for each. Four types of methodology 1. Scientific Method 2.Psychological Method 3. Analysis Method 4. Descriptive Method. Pretest and Post test were conducted with a sphys mongano meter in variable reading before and after the completion of training.

Training schedule

Experimental group –I

- Trains Program for a period of 12 weeks 6 days per week and 1 hour per day.
- Yoga Asanas.

Experimental group - II

- Traing Program for a period of 12 weeks 6 days per week nad I hour per day.
- Simplified Physical exercise.

- Kayakalpa Yoga.
- Introspection
- Meditation

Control group

Control group did not undergo any training.

RESULTS AND DISCUSSIONS

The paired sample 't' was computed on selected dependent variables. The results are presented in the above Table 1. The 't' test value yoga exercise practice group, sky yoga practice group, and control group are 1.76, 5.81 and 0 for Systolic Blood Pressure. The experimental 't' values are significantly higher than the required table value of 2.04 with degrees of freedom 11 at 0.05 level of confidence. The result of the study shows that yoga exercise practice group and sky yoga practice group has significantly improved the performance of Systolic Blood Pressure. The one way analysis of covariance on Systolic Blood Pressure of experimental and control groups has been analyzed and presented in Table 2. Table-.2 shows that the adjusted posttest mean value of Systolic Blood Pressure for yoga exercise practice group, sky yoga practice group, and control group are 122.18, 123.00 and 129.98 respectively. The obtained F-ratio of 12.63 for the adjusted posttest mean is more than the table value of 3.10 for df 2 and 86 required for significance at 0.05 level of confidence. The results of the study indicate that there are significant differences between the experimental groups and control group on Systolic Blood Pressure To determine which of the paired means had a significant difference, Scheffe's test was applied as Post hoc test and the results are presented in Table-3. Table-3 shows that the adjusted post-test mean differences on yoga exercise practice group and control group, sky yoga practice group group and control group are -70.93 , 79.66 and -28.73 respectively and they are greater than the confidence interval value 0 which shows significant differences between the experimental groups and control group at 0.05 level of confidence. The results of the study further have revealed that there were significant differences between the adjusted posttest means of yoga exercise practice group and sky yoga practice group, yoga exercise practice group and control group, sky yoga practice group group and control group in Systolic Blood Pressure. The paired sample 't' was computed on selected dependent variables. The results are presented in the above Table 4.

The 't' test value yoga exercise practice group, sky yoga practice group, and control group are 2.56, 5.81 and 0 for Diastolic Blood Pressure. The experimental 't' values are significantly higher than the required table value of 2.04 with degreesof freedom 11 at 0.05 level of confidence. The result of the study shows that yoga exercise practice group and sky yoga practice group has significantly improved the performance of Diastolic Blood Pressure. The one way analysis of covariance on Diastolic Blood Pressure of experimental and control groups has been analyzed and presented in Table 5. Table-5. shows that the adjusted posttest mean value of Diastolic Blood Pressure for yoga exercise practice group, sky yoga practice group, and control group are 80.28, 79.02 and 83.10 respectively. The obtained F-ratio of 50.89 for the adjusted posttest mean is more than the table value of 3.10 for df 2 and 86 required for significance at 0.05 level of confidence. The results of the study indicate that there are significant differences between the experimental groups and control group on Diastolic Blood Pressure. To determine which of the paired means had a significant difference, Scheffe's test was applied as Post hoc test and the results are presented in Table-6. Table-6. shows that the adjusted post-test mean differences on yoga exercise practice group and control group, sky yoga practice group and control group are 79.02, 83.10 and –4.08 respectively and they are greater than the confidence interval value 0 which shows significant differences between the experimental groups and control group at 0.05 level of confidence. The figure shows a significant difference in diastolic pressure in yoga group when compared to the other two groups. The results of the study further have revealed that there were significant differences between the adjusted posttest means of yoga exercise practice group and sky yoga practice group, yoga exercise practice group and control group, sky yoga practice group group and control group in Diastolic Blood Pressure.

CONCLUSION

- Within the limitations and delimitations of the study.
- Simplified Kundalini Yoga controlled the Blood Pressure among college Students.
- The findings of the study have proved that simplified Kundalini Yoga significantly improved the health condition of college students.

REFERENCES

1. Yogiraj Vethathiri Maharishi (2009) Rejuvenating life force and mind, vethathiri publications, Erode- Tamilnadu.
2. Yogiraj Vethathri Maharishi (2006) Yoga for modern Age, Vethathiri Publications Erode-Tamilnadu.
3. Yogiraj Vethathiri Maharishi (1997) simplified physical Exercises Vethathiri Publications Erode-Tamilnadu.
4. Yogiraj Vethathiri Maharishi (2009)Kayakalpa Yoga vethathiri Publications Erode- Tamilnadu.
5. Yogiraj Vethathiri Maharishi (2013) Sky Yoga for Human Excellence Volume-1, Vethathiri Publications Erode-Tamilnadu.
6. https://www.deccanchronicle.com/nation/current-affairs/140619/rising-hypertension- cases-in-tamil-nadu-causes-concern.html
7. https://www.thehindu.com/sci-tech/health/medicine-and-research/214-pc-prevalence- of-hypertension-in-rural-tamil-nadu-study/article4192172.ece
8. www.health.en.m.wikipedia.org www.healthline.com/bloodpressure/symptoms

Table 1: The summary of mean and dependent 't' test for the pre and post-tests on systolic blood pressure of Experimental and control groups

Mean	YEPG Group – (I)	SKYPG Group – (II)	Control Group – (III)
Pre- test	127.28	129.52	130.80
SD(±)	16.19	18.10	16.05
Post-test	121.20	123.16	130.80
SD(±)	6.08	5.81	16.05
't'-test	+1.76	5.81	0

* Significant at 0.05 level.

(Table value required for significance at 0.05 level for 't'-test with df 11 is 2.04)(Systolic Blood Pressure).

Table 2: Values of analysis of covariance for experimental groups and control group on systolic blood pressure

YEPG	SKYPG	CG	SOV	SS	DF	MS	F-Ratio
122.18	123.00	129.98	B.S	914.35	2	457.17	12.63
			W.S	2569.63	71		

*** Significant at. 0.05 level of confidence**

(The table value required for Significance at 0.05 level with df 2 and 71 is 3.10).

Table 3: The Scheffe's Test For The Differences Between The Adjusted Post-Tests Paired Means On Systolic Blood pressure

SKYPG	CG	SOV	SS	DF	MS	F-Ratio
123.00	129.98	B.S	914.35	2	457.17	12.63
		W.S	2569.63	71	36.19	

*Significant at 0.05 level of confidence

Table 4: The summary of mean and dependent 't' test for the pre and post-tests ondiastolic blood pressur of experimental and control groups

Mean	YEPG Group – (I)	SKYPG Group – (II)	Control Group – (III)
Pre- test	79.56	85.44	83.40
SD(±)	16.53	14.58	15.99
Post-test	78.68	80.32	83.40
SD(±)	3.67	5.81	15.99
't'-test	+2.56	5.81	0

* Significant at 0.05 level.

(Table value required for significance at 0.05 level for 't'-test with df 11 is 2.04) (Diastolic Blood Pressure).

Table 5: Values of analysis of covariance for experimental groups and control group on diastolic blood pressur

YEPG	SKYPG	CG	SOV	SS	Df	MS	F-ratio
80.28	79.02	83.10	**B.S**	218.89	2	109.45	50.89
			W.S	2927.34	71	41.23	

* Significant at. 0.05 level of confidence

(The table value required for Significance at 0.05 level with df 2 and 71 is 3.10).

Table 6: The scheffe's test for the differences between the adjusted post-tests paired means on diastolic blood Pressur

YEPG	SKYPG	CG	MD	CI
80.28	79.02	---	**1.26**	
80.28	---	83.10	**-2.82**	
--	79.02	83.10	**-4.08**	

* Significant at 0.05 level of confidence

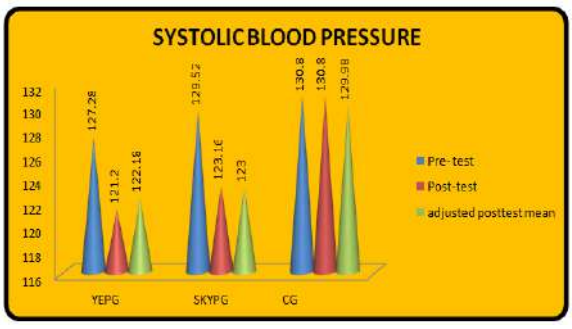

Fig:1 The Pre test, Post Test and adjusted post test mean of Systolic Blood pressure The figure shows the difference in the pre test and post test for the yoga group is better than the other groups

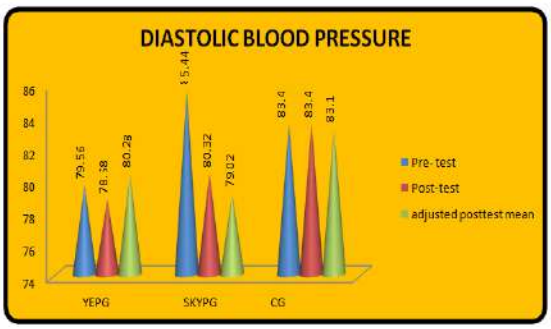

Fig2 The Pre test, Post Test and adjusted post test mean of Diastolic Blood pressure The figure shows a significant difference in diastolic pressure in yoga group when compared to the other two groups.