



## Linear Programming Approach for Workers Scheduling in Textile Industry

G. B. Sumathy and V. Amirthalingam\*

Department of Mathematics, Vinayaka Mission's Kirupanadha Variyar Engineering College, Vinayaka Mission's Research Foundation (Deemed to be University), Salem, Tamilnadu, India.

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### \*Address for Correspondence

#### V.Amirthalingam

Asst. Professor,

Department of Mathematics,

Vinayaka Mission's Kirupanadha Variyar Engineering College,

Vinayaka Mission's Research Foundation (Deemed to be University),

Salem-636 308, Tamil Nadu, India.

E.mail : amirvbm14@gmail.com



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### ABSTRACT

The Worker scheduling problem of this paper is to study and analyze the scheduling process in practice and propose models and heuristics to improve both the process and the quality of the resulting schedule. This paper illustrates how linear programming solves the workers scheduling problem and how it has been effectively used in workers scheduling at a garment industry in textile city Karur.

**Keywords:** Workers scheduling; Linear Programming; Simplex Method.

### INTRODUCTION

Operations Research is a science that aims to provide quantitative tools to decision-makers in order to improve their efficiency. The study of optimal resource distribution is represented by the term "operations research." The purpose of Operations Research is to offer rational bases for decision making by attempting to understand and structure complicated situations, and to use this understanding and structuring to forecast system behavior and enhance system performance as a result of this endeavor. When it comes to difficulties of how to execute and coordinate operations within businesses, the Operations Research approach is applied. The nature of the organization is generally unimportant, and Operations Research has been applied extensively in a wide range of fields, including manufacturing, transportation, telecommunications, public services, health care, and the military, among others. The scheduling of employees is extremely important in any business since a lack of access to or a lack of a source of employees results in a loss of both time and money for the organization. The proper scheduling of employees will improve the overall performance of the organization. It has been decided to consider the scheduling of workers for a garment (Home Textile) company in a textile city in this research paper. The techniques of Linear Programming have

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been used to develop a mathematical model of the world. The approach has been used to determine the bare minimum of workers required for a single day of labor. The real-world problem has been mathematically stated and addressed analytically using Linear Programming techniques in order to arrive at the ideal answer in this case. Given an increase in the number of variables, the problem gets more difficult, necessitating the use of the computational technique Online Calculator. The limitations, in this case, are obtained by calculating the maximum number of workers required for each day, and the goal is to reduce the number of workers allocated to each day to the bare minimum. Both the constraints and the goal function are assumed to be linear in the mathematical sense.

A common problem in the service industry is employee scheduling, which includes the scheduling of nurses in hospitals [3-6], scheduling of staff in hotels, check encoders in banks[7], airlines[8, 9], and hotel reception personnel, telephone operators, patrol officers, the scheduling of waiters in restaurants, and the scheduling of labours in construction companies, among others.

Workforce scheduling is an extremely vital duty in a service firm, and it must be done efficiently and effectively. Serving the needs of the client while maintaining a reasonable price involves accurate forecasting of labor requirements. At any one time, having an insufficient number of employees or personnel who lack the requisite job skills can result in substandard customer service, agitated and overworked employees, a delay in project completion, and a loss of sales. If an excessive number of employees is employed, the labor costs are likely to rise. If additional hours are scheduled, the operating margins are likely to be reduced. From the employee's perspective, having fewer working hours than desired will lower morale and job satisfaction because the available work is distributed thinly among a large number of employees. In this way, labor or workforce scheduling can be defined as the process of reconciling consumer demand with employee work requests while maintaining or improving profitability.

Many studies have been conducted on the various features of staff scheduling and rostering [1, 10], and the results have been published. Since the field has grown in importance as businesses have become more service-oriented and cost-conscious in a global setting, it has become increasingly vital. As a result, there is a wealth of information available on personnel scheduling [11-13], general workforce planning plans, and other topics. A significant increase has been observed in the application of staff scheduling and rostering methods to transportation systems such as airlines [8, 9, 16-18], and railways [19], emergency services such as police [20], ambulance, and fire brigade, call centers [21, 22], and many other service organizations such as hotels, restaurants, and retail stores [23]. Thus, focused studies and surveys on scheduling objective models [24], constraints [25], and methodology for each application area were conducted, which laid the groundwork for future study on the detailed model and algorithm development in future research. The creation of numerous computer software programs for staff scheduling has taken place throughout the years, ranging from spreadsheet adaptations of manual processes to mathematical models based on efficient optimum or heuristic algorithms [26] and everything in between. The key difficulties associated with staff scheduling in various application areas have been identified and examined in order to arrive at an appropriate solution. It is revealed in this sector through research [27] that there are three types of scheduling issues to contend with: day-off scheduling, shift scheduling, and tour schedule. Scheduling days off is connected to the assignment of workers to work and non-work days throughout the course of a whole planning horizon. The challenge of shift scheduling, on the other hand, is concerned with determining the actual shifts within a planning horizon. Tour scheduling challenges, on the other hand, combine elements of both days off and shift scheduling. It should be emphasized that, in most cases, work shifts are assigned to ensure that the daily demand for each shift is met and that each employee's day off plan is met as necessary. Many major studies in this area have been published in the research literature, and many of these papers have investigated the integration of project work scheduling and personnel scheduling and attempted to propose answers. The goal of those articles was to determine the number of employees required on each day, based on the right scheduling of tasks, with the goal of reducing the overall cost to the lowest possible level. As a result, there have been sufficient studies conducted on various aspects of staff scheduling in various service industries to warrant the investigation on which this thesis is based.





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**METHODOLOGY**

Linear Programming is a mathematical technique for generating and selecting the optimal or the best solution for a given objective function. It may be defined as a method of optimizing (Maximizing or Minimizing) a linear function for a number of constraints stated in the form of linear equations.

The General Linear Programming Problem can be stated below.

Objective Function

$$(Max \text{ or } Min)Z = C_1 X_1 + C_2 X_2 + \dots + C_n X_n .$$

Subject to the constraints

$$A_{11} X_1 + A_{12} X_2 + \dots + A_{1n} X_n (\leq, =, \geq) B_1$$

$$A_{21} X_1 + A_{22} X_2 + \dots + A_{2n} X_n (\leq, =, \geq) B_2$$

.....

.....

$$A_{m1} X_1 + A_{m2} X_2 + \dots + A_{mn} X_n (\leq, =, \geq) B_m$$

Non negativity constraints

$$X_1, X_2, \dots, X_n \geq 0.$$

In this paper,

$$\text{Min } Z = \sum_{i=1}^7 X_i \text{ subject to } X_i \geq B_i, \text{ where } i = 1,2,\dots,7.$$

where  $X_i$  is the number of workers assigned to day1, day2, etc, and  $B_i$  is the demand of workers for day1, day2, etc.

**PROBLEM DEFINITION**

Consider a textile industry that is open seven days a week. Based on past experience, the number of workers needed on a particular day is given in Table No. 1. Every worker works six consecutive days and then takes one day off, repeating this pattern indefinitely. How can we minimize the number of workers that staff the company?

Model

$X_1$  = Number of workers starting duty on Sunday (Sun-Fri)

$X_2$  = Number of workers starting duty on Monday (Mon-Sat)

$X_3$  = Number of workers starting duty on Tuesday (Tues-Sun)

$X_4$  = Number of workers starting duty on Wednesday (Wed-Mon)

$X_5$  = Number of workers starting duty on Thursday (Thu-Tue)

$X_6$  = Number of workers starting duty on Friday (Fri-Wed)

$X_7$  = Number of workers starting duty on Saturday (Sat-Thu)

The demand for the number of workers in the company on Sunday (100), Monday (220),

Tuesday (170), Wednesday (200), Thursday (250), Friday (80), and Saturday (300) respectively.

Let  $X_i$  be the number of workers who begin their six-day shift on the day  $i$ . Our objective is

$$X_1 + X_2 + X_3 + X_4 + X_5 + X_6 + X_7$$

Consider the constraint for Sunday staffing level of 100. Those who start their shift on Sunday( $X_1$ ), will work for continuously 6 days, that is Sunday to Friday. Those who start their shift on Monday ( $X_2$ ), will work for continuously 6 days, that is Monday to Saturday. Similarly, Those who start their shift on Tuesday( $X_3$ ), will work for continuously 6 days, that is Tuesday to Sunday and so on. The details are shown in Table No. 2





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Similar arguments give a total formulation of

$$\text{Minimize } Z = \sum_{i=1}^7 X_i$$

Subject to the constraints

$$X_1 + X_3 + X_4 + X_5 + X_6 + X_7 \geq 100$$

$$X_1 + X_2 + X_4 + X_5 + X_6 + X_7 \geq 220$$

$$X_1 + X_2 + X_3 + X_5 + X_6 + X_7 \geq 170$$

$$X_1 + X_2 + X_3 + X_4 + X_6 + X_7 \geq 200$$

$$X_1 + X_2 + X_3 + X_4 + X_5 + X_7 \geq 250$$

$$X_1 + X_2 + X_3 + X_4 + X_5 + X_6 \geq 80$$

$$X_2 + X_3 + X_4 + X_5 + X_6 + X_7 \geq 300$$

$$X_1, X_2, X_3, X_4, X_5, X_6, X_7 \geq 0; / X_i \geq 0 \text{ for all } i.$$

### SOLVING THE MODEL

Since this model has 7 variables, we cannot solve the problem manually. Hence we can solve the LPP by using a solver. If we solve the LPP, we will get the feasible solution  $\text{Min } Z = 300$ .

### CONCLUSION

Workers shifting allotment is a well-established area. It is important to note that our model only includes one type of shift; however, the model may simply be expanded to include additional types of shifts with varying shift prices. The resulting schedule comprises schedules that are balanced in terms of the distribution of shift tasks, fairness in terms of the number of consecutive night duties, and the preferences of the employees, among other things. This is an improvement over the typical manual approach, which is both time-consuming and inefficient in terms of establishing a suitable timetable, as well as labor-intensive. A complex scheduling challenge that impacts industry personnel on a daily basis all around the world, workers rostering is a complex scheduling problem. For a variety of reasons, there is a genuine demand for high-quality software applications. In particular, it is critical to maximize the use of time and effort, to evenly distribute the task among employees, and to make every effort to accommodate individual preferences in the workplace. In addition to increasing employee satisfaction, a high-quality roster can also increase employee productivity. This research article can be applied to all other professions of work that use shift arrangements as well as to the military. However, while the problem in this work has been addressed using Linear Programming, the identical problem can also be solved using Integer Programming.

### REFERENCES

1. Pinedo, M., Zacharias, C. and Zhu, N.(2015) Scheduling in the Service Industries: An Overview. Journal of Systems Science and Systems Engineering, 24, 1 – 48. <https://doi.org/10.1007/s11518-015-5266-0>
2. Semra Agrali, Z., Taskin, C. and Unal, A.T.(2016) Employee Scheduling in Service Industries with Flexible Employee Availability and Demand. Omega,66,159 -169. <http://doi.org/10.1016/j.omega.2016.03.001>
3. Satheesh Kumar, B, Nagalakshmi, G, and Kumaraguru, S.(2014) A Shift Sequence for Nurse Scheduling Using Linear Programming Problem, IOSR Journal of Nursing and Health Science, 3, 24 – 28. <https://doi.org/10.9790/1959-03612428>
4. De Grano, M.L., Medeiros, D.G. and Eitel, D. (2009) Accommodating individual preferences in Nurse Scheduling via Auctions and Optimization. Health Care Management Science,12, 228 – 242. <https://doi.org/10.1007/s10729-008-9087-2>
5. Moz, M. and Pato, M.V. (2007) A Genetic Algorithm Approach to a Nurse Rostering Problem. Computers and Operations Research, 34,667- 691. <https://doi.org/10.1016/j.cor.2005.03.019>
6. Maenhout, B. and Vanhoucke, M.(2013) An Integrated Nurse Staffing and Scheduling Analysis for Longer-Term Nursing Staff Allocation Problems. Omega, 41, 485 – 499. <https://doi.org/10.1016/j.omega.2012.01.002>



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7. Bagatourova, o.and Mallya, S.K. (2004) Coupled Heuristic and Simulation Scheduling in a Highly Variable Environment. Proceedings of the 2004 Winter Simulation Conference, Washington DC, 5- 8 December 2004,1856-1860. <https://doi.org/10.1109/WSC.2004.1371540>
8. Brusco, M., Jacobs, L., Bongiorno, R., Lyons, D. and Tang, B. (1995) Improving Personnel Scheduling at Airline Stations. European Journal of Operational Research, 43, 728 - 905. <https://doi.org/10.1287/opre.43.5.741>
9. Chu, S.C.K.(2007) Generating, Scheduling and Rostering of Shift Crew – Duties: Applications at the Hong Kong International Airport. European Journal of Operational Research, 177, 1764 - 1778. <https://doi.org/10.1016/j.ejor.2005.10.008>
10. Ernst, A.T., Jiang, H., Krishnamoorthy, M. and Sier, D. (2004) Staff Scheduling and Rostering: A Review of Applications, Methods, and Models. European Journal of Operational Research, 153, 3-27. [https://doi.org/10.1016/S0377-2217\(03\)00095-X](https://doi.org/10.1016/S0377-2217(03)00095-X)
11. Van den Bergh, J., Belien, J., De Bruecker, P., Demeulemeester, E. and De Boeck, L. (2013) Personnel Scheduling : A Literature Review. European Journal of Operational Research, 226, 367 - 385. <https://doi.org/10.1016/j.ejor.2012.11.029>.
12. Brucker, P., Qu, R. and Burke, E. (2011) Personnel Scheduling: Models and Complexity. European Journal of Operational Research, 210, 467 -473. <https://doi.org/10.1016/j.ejor.2010.11.017>
13. Pawar, U.S., and Hanchate, D.B. (2013) Literature Review on Personnel Scheduling. International Journal of Computer Engineering and Technology,5.
14. Casado , S., Laguna , M. and Pacheco,J. (2005) Heuristical Labour Scheduling to Optimize AirportPassenger Flows. Journal of the Operational Research Society, 56, 649- 658. <https://doi.org/10.1057/palgrave.jors.2601859>
15. De Bruecker, P., Van den Bergh,J., Belien,J. and Demeulemeester, E. (2015). A Two- Stage Mixed Integer Programming Approach for Optimizing Skill Mix and Training Schedules for Aircraft Maintenance. Social Science Research Network. <https://doi.org/10.2139/ssrn.2697491>.
16. Thiel, M.P. (2008) Team – Oriented Airline Crew Rostering for Cockpit Personnel.In:Hickman, M., Mirchandani, P. and Vos, S., Eds., Computer – Aided Systems in Public Transport. Lecture Notes in Economics and Mathematical Systems, Springer, Berlin, Heidelberg, 600, 91 – 114. [https://doi.org/10.1007/978-3-540-73312-6\\_6](https://doi.org/10.1007/978-3-540-73312-6_6)
17. Hoong, C.L., Zhi, Y., and Aldy, G. (2016) Patrol Scheduling in Urban Rail Network. Annals of Operations Research, 239, 317 – 342. <https://doi.org/10.1007/s10479-014-1648-9>.
18. Kolesar, P.J., Rider, K.L., Crabill, T.B. and Walker, W.E. (1975) A Queuing – Linear Programming Approach to Scheduling Police Patrol Cars. Operations Research, 23, 10451062.
19. Chanpanit, T. and Udomsakdigool, A. (2015) workforce planning for Single Call Center with Service Level Agreement. In: Kachitvichyanukul, V., Sethanan, K.and Golinska- Dawson, P., Eds., Toward Sustainable Operations of Supply Chain and Logistics Systems. Eco Production (Environmental Issues in Logistics and Manufacturing), Springer, Cham, 521- 533. [https://doi.org/10.1007/978-3-319-19006-8\\_36](https://doi.org/10.1007/978-3-319-19006-8_36).
20. Dietz, D.C. (2011) Practical Scheduling for Call Center Operations. Omega, 39, 550-557. <https://doi.org/10.1016/j.omega.2010.12.001>
21. Parisio, A., and Jones, C.N. (2015) A Two-Stage Stochastic Programming Approach to Employee Scheduling in Retail Outlets with Uncertain Demand. Omega, 53, 97 – 103.
22. Henao, C.A., Munoz, J.C. and Ferrer, J.C.(2015) The Impact of Multi – Skilling on Personnel Scheduling in the Service Sector: A Retail Industry Case. Journal of the Operational Research Society, 66, 1949 – 1959. <https://doi.org/10.1057/jors.2015.9>
23. Thomas, P.I. (2013) Scheduling Algorithm with Optimization of Employee Satisfaction.
24. Jaumard, B., Semet, F. and Vovor, T. (1998) A Generalized Linear Programming Model for Nurse Scheduling. European Journal of Operational Research,107,1-18.
25. Cheang, B., Li, H., Lim, A. and Rorigues, B.(2003) Nurse Rostering Problems: A Bibliographic Survey. European Journal of Operational Research,151,447 – 460.
26. Hewitt, M., Chacosky, A., Grasman, S. and Thoms, B. (2015) Integer Programming Techniques for Solving Non – Linear Workforce Planning Models with Learning. European Journal of Operations Research,42,942 – 950.





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27. Baker, K.R. (1976) Workforce Allocation in Cyclical Scheduling Problems: A Survey. Journal of the Operational Research Society, 27, 155 – 167.

**Table No. 1 No. of workers needed on a particular day of the week**

	X <sub>2</sub>	X <sub>3</sub>	X <sub>4</sub>	X <sub>5</sub>	X <sub>6</sub>	X <sub>7</sub>	X <sub>1</sub>
Day	Mon	Tue	Wed	Thu	Fri	Sat	Sun
No.of Workers	220	170	200	250	80	300	100

**Table 2: The formulated model of days and workers**

Day	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>	X <sub>4</sub>	X <sub>5</sub>	X <sub>6</sub>	X <sub>7</sub>
Sunday	*	*	*	*	*	*	-
Monday	-	*	*	*	*	*	*
Tuesday	*	-	*	*	*	*	*
Wednesday	*	*	-	*	*	*	*
Thursday	*	*	*	-	*	*	*
Friday	*	*	*	*	-	*	*
Saturday	*	*	*	*	*	-	*





## A Study on Recent Developments for Ensuring Safety on Highway Projects in India

Saswati Moharana<sup>1\*</sup> and Pradyumna Sagar Sahoo<sup>2</sup>

<sup>1</sup>Student of Civil Engineering Department, Centurion University of Technology and Management, Odisha, India.

<sup>2</sup>M.Tech Transportation Engineering, Confiance Infratech Management Pvt. Ltd, Bhubaneswar, Odisha, India.

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### \*Address for Correspondence

#### Saswati Moharana

Student of Civil Engineering Department,  
Centurion University of Technology and Management,  
Odisha, India.

Email Id: saswatimoharana98@gmail.com



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### ABSTRACT

Rapid increases in vehicle ownership, especially in two wheelers in India and have placed considerable pressure on the road networks, their traffic and control devices, and on users of road facilities. The number of people killed and injured in traffic crashes has been steadily increasing. Unfortunately, in the rush to develop and expand road networks, problems can sometimes arise in new construction and especially in rehabilitation schemes if insufficient attention is given to road safety impacts that can be associated with road infrastructure projects. The higher speeds that become possible on improved roads can lead to an increase in road safety risk for communities along such routes and for vulnerable road users. There is an increasingly concerned about the growing road safety problems. The rate of accidents increases during construction of various road projects as operating environment is different during construction, dust during construction, deep excavations made during construction of CD structures etc. Similarly, rate of accidents increases during nights driving due to poor visibility etc. It is also observe that road user such as pedestrians and animals are largely involved during road accidents due to inadequate project facilities etc. Thus there is an urgent need to develop various strategies to ensure safety of road users and workers. This study identify such strategies. This study presents strategies for safety during construction, strategies for safety during nights and also strategies for safety of vulnerable road users. Relevant Indian standards and specifications are also studied to design and plan such strategies. A case study is also made during this study to illustrate the applications of these identified strategies on a four laning road projects i.e. Lebad – Jaora road project in the state of Madhya Pradesh. It is expected that the strategies identified in this study will be useful to reduce safety problems on Indian roads.

**Keywords:** Strategies for safety, CD structure, Fourlaning road, Lebad- Jaora road.





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## INTRODUCTION

### General

The Safety requirements aim at reduction in injuries, loss of life and damage to property resulting from accidents on the Project Highway, irrespective of the persons at fault. Users of the Project Highway include motorized and non-motorized vehicles as well as pedestrians and animals involved in, or associated with accidents. Vulnerable Road Users (VRU) include pedestrians as well as riders of motorized two-wheelers, bicycles and other vehicles which do not provide adequate occupant protection. Safety requirements apply to all phases of development, construction, operation and maintenance with emphasis of identification of factors associated with accidents, consideration of the same, and implementation of appropriate remedial measures. Safety requirements include measures associated with traffic management and regulation such as road signs, pavement marking, traffic control devices, roadside furniture, highway design elements, enforcement and emergency response.

### Need of Study

- Number of people killed and injured in road accidents has been steadily increasing. Fatalities in road accidents surpass the deaths by disease or epidemic which results in grief and agony to many.
- Accident results in huge economic losses and accidents are a major socio economic cost that can typically amount to 1-2% of annual GDP in each country. Accidents are inhibiting the economic and social development of the region and adding to the poverty and hardship of the poor.
- Higher speeds that become possible on improved road (New construction or rehabilitation projects) can lead to an increase in road safety risk for communities along such routes and for vulnerable road users. Unfortunately in the rush to develop and expand road infrastructure, if insufficient attention is given to road safety impacts, safety problems can arise in new construction and especially in rehabilitation/ widening projects
- Increasing traffic volume, the rapid growth in two wheelers, and the higher speeds made possible by construction improvement and rehabilitation of road can all add to the safety problem.
- The increase speed along the improved road may result in an increase in accidents at sections with poor alignment, particularly at junctions and where the road passes through or is adjacent to small communities. Such accidents occur, not because the rehabilitation work is deficient work, but because the operating environment has not been given adequate attention. It is therefore necessary that adequate attention is paid to the safety needs of road users who are likely to use that road.
- Thus there is need to make every effort to ensure that development of road infrastructure projects do not further worsen the road safety problem.

### Objective and Scope of Study

The main objective of this study is to Development of Some Strategies to Enhance Road Safety. To achieve the above objective, the scope of this study are identified as follows:

- To carry out literature review on road safety in India and problems related with road safety.
- To develop Strategies to Enhance Road Safety.
- To conduct a case study to illustrate the applications of strategies to enhance road safety as above.



**Saswati Moharana and Pradyumna Sagar Sahoo****LITERATURE REVIEW****General**

The main objective of this study is to develop some strategies to enhance road safety. To meet the above objective a comprehensive literature review was carried out to identify various safety related problems on Indian roads. Literature review was also made to identify remedial measures to reduce these problems. This chapter presents the important safety related problems observed during literature survey. This chapter also presents a review on National Road Safety Policy.

**Literature Review on Road Safety Problems**

The Government of India has initiated an ambitious National Highways Development Programme (NHDP) that relies substantially on Public Private Partnerships (PPPs) as a means of attracting private capital, improving efficiencies and reducing costs. Several states are pursuing similar programmes based on PPPs in state highways, while a few others are in different stages of the process. For awarding PPP projects within a competitive, efficient and economic framework based on international best practices, Government of India has adopted a Model Concession Agreement (MCA) for PPPs in National Highways. On similar lines, the Planning Commission has published an MCA for State Highways. These MCAs follow the Design, Build, Finance and Operate (DBFO) approach that requires the Concessionaire to bear the responsibility for detailed design. However, the accountability for providing safe and reliable roads ultimately rests with the Government and the MCA, therefore, mandates a Manual of Standards and Specifications that the concessionaire must conform to. As per Article 18 of MCA, the Authority shall appoint a safety Team to review safety requirements for the project in accordance with schedule-L of MCA. Model Concession Agreement identified scope of safety Team for various stages of highway project. Also MCA identified obligation of Concessionaire for ensuring safety of highway in design, construction and operation period.

**Scope of Safety Team**

Scope of Safety Team It is stipulated in the Concession Agreements of the PPP Projects that the Concessionaire shall develop, implement and administer a surveillance and safety programme for providing a safe environment on or about the Project Highways and shall comply with the safety requirements set forth in Schedule 'L' of the Model Concession Agreement (MCA). It is also stipulated in the Concession Agreement that NHAI shall appoint an experienced and qualified firm or organization as a "Safety Team" for carrying out safety audit of the Project Highway in accordance with the safety requirements set forth in Schedule-L.

Scope of Safety Team according to Schedule-L of Model Concession Agreement is in following three stages-

- For Development Period.
- For Construction Period.
- For Operation Period.

**Scope of Safety Team- for Development Period****Collection and Analyzing of Preceding Two Years Accident Data**

- The road safety team under the guidance of Team Leader will collect the accident data for last 2 years from concerning police stations lying on project highway. The data will be collected on all fatal crashes and other road accidents by obtaining copies of the relevant First Information Report (FIR) and other relevant record etc. from the Police Station having jurisdiction on the project road. The team will seek the help of MPRDC in this matter to expedite the work. Further senior police officers will also be contacted to get the above information.





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- The information collected above will be tabulated in road accident reporting form (Form-A-1) as issued by IRC ( I.R.C.-53-1982 ). Each accident will be posted in different 22 tables which are sub- parts of Form A-1, in view to analyze the accident from different angles.
- The above accident data will be analyzed for the type of victim killed or injured, impacting vehicles, location of accidents and other relevant factors on yearly basis.
- The accidents data will be analyzed in 19 different ways as per road accident Form-4 as issued by IRC ( I.R.C.-53-1982 ) in view to analyze the accident from different angles. A sample of road accident form-4 is provided in Appendix B.
- The results of the above analysis will be summarized and presented in tabular forms in 19 different tables which are sub- part of road accident Form-4 as issued by IRC ( I.R.C.-53-1982 ) This tabulation will be further transformed into a graphical representation to demarcate the black- spots along with causes and type.

### Review of Design Details and Relevant Drawings

- Beside collecting and analyzing the accident data, the road- safety consultancy team will also review the design details and the other relevant matters indicating the safety measures to be adopted for ensuring the safety of workers, road users etc. The safety team will review the feasibility report, D.P.R., project drawings, safety plan etc as provided by the concessionaire for this purpose.
- The design and drawings will be referred from safety perspective for horizontal and vertical alignment , sight-lines, layout of intersection and interchanges, road cross -section, all structures such as Bridges and Culverts, drains, parking places, lane provision for slow –moving vehicles and pedestrians and bay lanes for truck and buses etc. to ensure their consistency with the design standards.

### Safety Audit of the Project Road

- The road safety audit for the existing 2 lane highway will then be conducted in a period of three months to identify the black spots and hazardous objects. In this audit the help of traffic police and other stake- holders will be sought. The dangerous points will be identified and will be categorizes as high, medium and low risk.

### Submit Safety Report

- Safety report will be prepared on the basis of analysis of accident data, review of design details and safety audit.
- All the recommendations and observations regarding design, drawings and safety Audit of existing 2 lanes will be forwarded to M.P.R.D.C. for consideration and their enforcement in accordance with contractual provisions. The recommendations will be discussed with Concessionaire and Independent Engineer while assisting the client to ensure their effective implementation.

### Scope of Safety Team - for Construction Period

- To suggest and review the arrangement made during the construction period for the safety of Workers and road users.
- To conduct Road Safety Audit to assess adequacy of safety measures.
- To prepare the safety report and to recommend additional road safety measures, if any

### Scope of Safety Team- for Operation Period

- To suggest and review the arrangement made during the operation and maintenance period for the safety of road users.
- To conduct Road Safety Audit to assess adequacy of safety measures during operation period.
- To prepare the safety report and to recommend additional road safety measures, if any.





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## **Road Safety Audit**

Road safety audit (RSA) is a formal procedure for assessing accident potential and safety performance in the provision of new road schemes, the improvement and the rehabilitation of existing roads, and in the maintenance of existing roads. It should form an integral part of highway planning, design, construction and maintenance, and it requires an objective approach to the assessment of accident risk. The principal method of ensuring this objectivity is through the independent assessment of schemes by persons who are independent of the original design team. RSA can be applied to all kinds of road projects- new road construction as well as widening of existing road. It can be applied to small and large project and can be used on rural as well as urban roads.

## **METHODOLOGY**

### **Development of Strategies**

#### **General**

The main objective of this study is to developed some strategies to enhance road safety. To meet the above objective a comprehensive literature review was carried out to identify various safety related problems on Indian roads. This chapter is mainly focused on develop strategies of safety during construction of highway, safety during night and safety of vulnerable road users/pedestrian and animals and

### **Development of Strategies to Enhance Road Safety Strategies for Safety During Construction**

#### **Overview**

Construction zones are an integral part of any road system. This is more so in a developing country like India where most of the road construction has been designed as a 'stage construction' process. This method of design provides for the improvement of the road, both in terms of the geometry (for example, widening to increase the number of lanes, widening to create a divided carriageway or changes to the vertical or horizontal alignment) and structurally (for example, to increase the pavement thickness to withstand the axle load of traffic), during the life of the road. In addition, the general maintenance activities on roads give rise to "construction zones" on the roads. Traffic on our roads has increased many times and will continue to do so, as many roads are either operating or expected to operate at their maximum capacity in the near future. Under these circumstances the existing methods of maintenance and construction compromise safety and cause delays and inconvenience which are no longer acceptable. A change in design and implementation practices is, therefore, necessary to overcome the problems.

Road construction and maintenance work is hazardous for both the site operatives and the road user. At work sites in rural areas, traffic is never more than 15 metres away. In addition, speeding vehicles create a whirlwind of dust around the work place and noise from the traffic and maintenance equipment often masks the sound of an impending accident. Under the present system, the traffic operations and safety provisions during improvement/maintenance works depend entirely upon the expertise of the engineer. This has been found to be unsafe and inefficient. Besides, non-uniformity in the methods of traffic control and placement of signs at various locations increases confusion for road users. In our country, where the travel distances extend upto 300 km or more and where the majority of heavy vehicle drivers are, at best, only semi-literate, there is a need for adopting uniform traffic control methods and devices at construction zones to ensure the safety of both the road users as well as the construction workers.





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### Problems

- Excavation is very important activity of road construction. During construction, road side excavation for widening of road and structure excavation of construction of CD work is very initial process. However, this excavation is very dangerous for safe traffic operation during the construction of road and CD structure, if adequate arrangements are not provided. The vehicle may fall down suddenly on excavated site, if proper guidance to the traffic is not provided.
- Dust is created during movement of traffic, which is very dangerous for safe movement of traffic. Because dust reduced visibility and it is very dangerous for high speed traffic.
- During the construction, it is required to divert the traffic at several places like during construction of CD structures, during strengthening at existing carriageway etc. through diversion. Inadequate diversions (very poor geometric) is very dangerous for safe operation of traffic specially at night. Also proper and adequate arrangement of barricading, direction sign board, flags, delineators required for safe movement of traffic during day as well as during night also.
- During the construction, it is require to proper monitoring of safety devices/safety measure at construction site for proper needful arrangement of safety measures at construction site.

### Strategies for Safety During Night

#### Overview

Road accident occurs higher during night in respect of day time. Some design problems or inadequate construction are creates hazardous to the high speed traffic during night. Most of the reflective sheeting seems dull before there warranty time which creates problem in visibility to the traffic during night. All the situations are seems opposite for safe movement of the traffic during night and increase risk of accident to high speed traffic.

#### Problems

- Poor visibility is affected to safe operation of traffic.
- Naturally night time is identify for rest time for our body, so loss of attention is very major problem during night driving.
- It is observed that, the traffic sign can't sustain their retro-reflectivity property up to sometime like 4-5 years i.e. they may be useless after 4-5 years. At this condition they will not working as the sing boards.
- It is observed that the road marking seems to be dull during rainy season or within 1-2 years. However, this is very important for safe operation of traffic during night time especially.
- All the road side structure like median, structure Parapet wall etc. may be dangerous during night for safe operation of traffic.

### Strategies for Safety of Vulnerable Road User

#### Overview

All over India, flows of modern motor vehicles are mixed with animal-drawn carts and bicycle. As well as reducing capacity substantially, the presence of slow-moving vehicles on the same carriageway creates hazardous conditions. Other vehicles may be forced to slow down rapidly, or be tempted to overtake in dangerous circumstances. Non-motorized vehicles are amongst the most vulnerable group of road users and like pedestrians, are unprotected.. Consequently, any impact, even a small one, can results in serious injury to riders and passengers.



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The mix of traffic in India can lead to particularly hazardous situations because of the wide range of vehicle performance characteristics especially in the speed differentials. Segregating the slower moving traffic from the motorized flow can offer safety benefits and an improvement in overall efficiency of the network. Although it is rarely practical to build completely separate networks in existing cities, it may be possible to provide a safer network by providing additional facilities along certain routes and linking these by short, segregated sections. In new developments, segregated road and path systems can be included at the planning stage, often at little cost. Pedestrian and slow moving vehicles can share facilities. The provision of shoulders along rural roads could provide one such facility.

Also Lay-byes and bus stops allow vehicles to stop safely and with the minimum of adverse effects on other traffic. This is best done with a segregated area joined to the main road pavement only at an entry point and an exit point. Vehicles can then stop off the main carriageway without interfering with other traffic and with less risk to passengers getting on or off.

**Problems**

In India, despite the large numbers of cyclists and other human powered Vehicle (e.g. cycle-rickshaws, hand-carts), very few special facilities are available. In India, these vehicles have to compete for road space against cars, trucks and other motorized traffic using the roads. For example, buses stopped adjacent to the kerb can obstruct cyclists and other slow moving vehicles.

Other kinds of slow moving vehicles can be even more problematic, especially if they are physically large - at are many animal drawn carts. Slow moving animal drawn traffic can be particularly hazardous in rural areas and at night. Often unlit, they obstruct (aster moving traffic in an unpredictable way. Unsuspecting drivers run serious risks of collision, either with the car or with other vehicles if they attempt to overtake. It must be recognised however, that in India, animal drawn carts are still fundamental to the local and rural economy and so cannot just be banned. Further, vehicles stopping at a lay-byes or bus stop constitute a temporary obstruction which may obstruct visibility of an important feature. If the lay-byes is close to the road, there is also the risk of collision between parked vehicles and those inadvertently straying from the road.

The slower speeds of vehicles entering and leaving a lay-byes could cause a hazard to faster moving through traffic, although this is often less hazardous and disruptive than buses slopping directly on the carriageway. On heavily trafficked roads, bus drivers may not wish to enter a lay-byes because of the difficulties of merging with the main road flow again. A bus lay-byes which has been produced at the expense of the adjacent footway may not be used fully at peak periods because of pedestrian queues and movements. At existing commercial sites, there may be insufficient room for the construction of a service road, although there may be too many accesses to the compatible with safety.

**CASE STUDY****General**

The main objective of this study is to identified some safety measures for a highway project Lebad –Jaora (SH-31) by case study of the project. This chapter is mainly focused on safety during construction of highway, safety during night and safety of vulnerable road users/pedestrian and animals. This chapter is prepared with a basic aim to recommend a package of additional road safety measures and other project facilities that are required, for reducing accidents hazards on Lebad- Jaora road project. This chapter is based on the road safety audit of the project highway. These chapter were reviewed to identify the accident prone locations, requirements of project as per concessionaire agreements etc. and project facilities required. Road safety audit was conducted for the entire project highway during day time as well as during night time also to identify the problems and potential hazardous locations. This chapter mainly presents some safer practices for safe movement of traffic during the day as well as during night also.



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The main purpose of this chapter is to succinctly address the aspects of the proposed scheme that may contribute to creating unsafe conditions.

**Project Highway**

The Lebad–Ratlam–Jaora Road, Project Highway is a segment of 500 km long Ajmer- Naisirabad-Nimach-Mandsaur – Indore. It provides the shortest route of transportation from northern part of India to the southern part and vice-versa. It takes off from Ajmer in Rajasthan and traverses southwards in the state of Rajasthan connecting the towns Bhilwara and Chittaurgarh. Thereafter, it enters Madhya Pradesh in Nayagaon and continues traversing in the southerly direction upto Lebad, where it joins NH-59 (Ahmedabad-Godhra-Dhar-Indore) and thereafter runs in the easterly direction up to Indore, where it joins NH-3. It commences from Lebad at intersection of NH-59 and traverses in the northern direction through the districts of Dhar, Ratlam. Indore the biggest industrial hub in Madhya Pradesh is just 47 km. from Lebad and Bhopal the capital city is 234 km. The project corridor traverses through the towns of Sadalpur, Nagda, Badnawar, Satrunda, Ratlam, Jaora. The road project plan location is shown in Figure 1.

**Strategies for Safety during Construction**

Road construction work is hazardous for both workers and road users. Construction zone creates an environment where traffic is forced with a series of unusual hazards like dust due to construction, deep excavation at CD structures, substandard alignment, construction equipments, and diversion to other routes. Roads with construction sites have higher accidents rate, when compared with similar section of roads without construction site. Therefore there is a need to reduce the increased risk to road users and site workers during construction also by adopting suitable safety measures.

Some of the important measures recommended improving safety of road users and workers during construction of Lebad-Jaora road project are summarized as follows. These recommendations were suggested on the basis of deficiencies observed during regular monitoring and review of safety measures of the project highway and on the basis of the analysis of accidents occurred on project highway. Relevant photographs showing safety measures during construction are also presented.

- Poor surface condition (presence of big potholes, depressions, crack etc.) of existing carriageway may be dangerous for safe and smooth traffic operation. Photograph-4.1 presents the poor surface condition which may be dangerous for safe traffic operation. Therefore, It is suggested that existing carriageway should be adequately maintained by filling all potholes, depressions, crack repair etc. Photograph-4.2 shows the maintenance on the project highway. Similarly shoulder also needs to be maintained for smooth traffic operation. Basically this is required to ensure that adequate carriageway width will be made available for safe and smooth traffic operation.
- During construction it is observed that dust is created due to movement of traffic and construction vehicles affecting visibility Photo-4.3 shows that visibility is poor due to dust. This is dangerous situation and may lead to accidents. Therefore efforts must be made to reduce dust by adopting various measures like sprinkling water etc. Photo-4.4 shows the sprinkling of water to reduce dust on project highway.
- Diversion needs to be constructed for construction of CD structures/Bridges etc. to divert the traffic. Adequate information and guidance is required so that traffic from the existing carriageway can be diverted safely. Photo 4.7 and photo 4.8 illustrate this point. It is observed that adequate safety measures are not adopted at these diversions and they became hazardous location. It is observed that these measures are not very effective due to improper arrows, placement of boards at improper location .stones put as delineators are not effectively white washed. Further team observed that the above measures are not effective during night and more retro reflective sign boards are required to improve safety during night.
- Due to construction activities, existing carriageway width available may be inadequate for the traffic plying on project highway. Therefore it is suggested that no overtaking sign, go slow etc. may be installed at all such critical locations to guide the traffic and to avoid accidents that may take place during overtaking operation. More information to road users regarding construction work is in progress, go slow, no overtaking, speed limit,



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deep excavation, narrow bridge etc. by installing appropriate sign boards at regular interval ( specially more effective during night also) are required to enhance safety on project highway.

**Strategies for Safety during Night**

- Driving during night is more dangerous due to various construction activities going on project highway. Therefore more precautions is necessary during nights. It is suggested that adequate lighting arrangements at all deep excavations should be made. Further all sign boards and other safety measures should be visible during night also. More information to road users regarding construction work is in progress, go slow, no overtaking, speed limit, deep excavation, narrow bridge etc. by installing appropriate sign boards (specially retro reflective sign boards) are required to enhance safety on project highway during nights
- Provide other road safety devices such as traffic impact attenuators (like assembly of old tires or steel drums etc) to prevent errant vehicles from impacting fix objects, road delineators to provide visual assistance to drivers about the road ahead specially at night, hazard and object markers to indicate hazards and obstruction within the vehicle flow path.

**Strategies for Safety of Vulnerable Road Users**

- Provide various facilities for the safety of pedestrians and animals at habitation, built-up areas, junctions etc. the guard rails shall be provided to enhance safety. Pedestrian guardrail needs to be designed to control and guard pedestrian and road crossing movements' safety.
- Locations of significant pedestrian and animal cross traffic have been identified, based on which the needful facility is proposed for the safety of pedestrians and animals and to ensure smooth flow of vehicular traffic. The tentative location for the pedestrian sub-ways and animal crossing
- Construction of bus lay byes of suitable design at the required locations is desirable to avoid any serious effect on the capacity of the road due to buses standing indiscriminately on the carriageway to drop or pick up passengers. The governing consideration for locating bus stops is the overall safety and minimum interference to the through traffic. The bus stops should be sited away from bridges and other important structures, from embankment section, which are more than four meters high as from horizontal curves and from top of vertical curve summit.
- The provision of truck parking shall be based on the site requirement and as per the MORTH Guidelines (RW/34032/5/880-DOII dated 22nd August, 1988). Parking shall be designed in the form of rectangular/ trapezoidal area/ parallel to the road and separated from the carriageway by a separator.

**CONCLUSIONS AND RECOMMENDATION FOR FUTURE STUDY****CONCLUSION**

The basic objective of this study was to develop some strategies to enhance safety on Indian roads. Some of the important conclusions drawn from the present study are as follows:

- Some of the safety related problems on Indian roads as follows:
- Adequate arrangement not provided for various activities during construction like :
  - Site for deep excavation.
  - Removal of dusty environment.
  - During maintenance work.
  - At Diversions.
  - Poor monitoring of safety measures during construction.





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- Traffic movement during night time is dangerous due to
  - Poor visibility.
  - Loss of driver attention.
- Safety of Vulnerable road users
  - large numbers of cyclists and other human powered Vehicle (e.g. cycle-rickshaws, hand-carts)
  - buses and other good vehicle stopped adjacent to the kerb which creates dangerous to safe movement of traffic and slow moving vehicles also.
- Some of the strategies identifies to enhance safety are as follows:
  - Strategies to ensure safety during construction are identified as follows.
  - Strategies to ensure safety during Night are identified as follows.
  - Strategies to ensure safety for vulnerable road users are identified as follows.

Case study illustrate the applications of these identified strategies on a four lanning road projects i.e. Lebad – Jaora road project in the state of Madhya Pradesh. It is expected that the strategies identified in this study will be useful to reduce safety problems on Indian roads.

**RECOMMENDATION FOR FUTURE STUDY**

This study has identified some strategies to enhance safety on Indian roads. More detailed studies are needed to develop more comprehensive strategies to enhance road safety. It is also recommended to conduct more studies on different type of roads to identify effectiveness of such strategies in reducing accidents on Indian roads.

**REFERENCES**

1. IRC-2, "Route Marker Sign for National Highways (1st Revision)", India Road Congress, New Delhi, 1968.
2. IRC-35, "Code for practice for road markings with Paints (1st revision)", India Road Congress, New Delhi, 1997.
3. Sarin, S.M., & A.C. Sarna (1981) "Pedestrian, The Neglected Road Users", Indian Highways, August.
4. Sarin, S.M., & Mrs. Nishi Mittal & Sh. BasantLal (1995) "Hoarding- A Threat To Road Traffic Safety And Environment", Central Road Research Institute.
5. Dr. Geetam Tiwari (1992)," Accident Recording System: Design of A System For India" International Workshop on Prevention And Control Accidents & Injuries, I.I.T., New Delhi.
6. IRC-38, "Guidelines for design of horizontal curves for highways and design /tables (1st revision)", India Road Congress, New Delhi, 1988.
7. IRC-53, " Road accident forms A-1 &4 (1st revision)" India Road Congress, New Delhi, 1982
8. IRC-73, " Geometric design standard for rural non-urban highways "India Road Congress, New Delhi, 1980.
9. IRC-75," Guidelines for the Design for Highway Embankments" India Road Congress, New Delhi, 1979
10. IRC-103," Guidelines for pedestrians facilities" India Road Congress, New Delhi, 1988.
11. IRC-SP-27," Report containing Recommendation of IRC Regional Workshops on Highway Safety" India Road Congress, New Delhi, 1984.
12. IRC-SP-31." New traffic signs "India Road Congress, New Delhi, 1992.





## Comparative Study of Cost Minimization of Antiepileptic Drugs in Pregnant Women in a Tertiary Care Hospital

Anjali Prasad, A. Ashok Kumar, V. Annamalai, R. Antony Praveen, R. Kothai, B. Arul\*

Department of Pharmacy Practice, Vinayaka Mission's College of Pharmacy, Vinayaka Mission's Research Foundation (Deemed to be University) Salem – 636008, Tamil Nadu, India.

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### \*Address for Correspondence

#### B.Arul

Department of Pharmacy Practice,  
Vinayaka Mission's College of Pharmacy,  
Vinayaka Mission's Research Foundation (Deemed to be University)  
Salem – 636008, Tamil Nadu, India.  
E.mail : arul1971@yahoo.com



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### ABSTRACT

The risk of problems during pregnancy and delivery is higher for pregnant women who have epilepsy. Hospital-based research may be able to provide valuable information about current management and results in this population of patients. The purpose of this study was to discover whether or not pregnant women's seizure frequency, as well as pregnancy and birth outcomes, changed over time. Before and during the pregnancy, information about seizure frequency was gathered; concurrent medications, pregnancy problems, and neonatal outcomes were also recorded. Patient's medical data from 115 different pregnancies were evaluated by the researchers. Patients were treated with antiepileptic drugs throughout their pregnancies, both as monotherapy and as part of a polytherapy regimen. It is necessary to compare drug prices with the prices of resources used in the management of adverse events, as well as prices associated with treatment change, in order to determine the relative quantity of those agents. . We calculated the cost-minimization methodology for the economic evaluation of the information, which was backed by the fact that randomized studies demonstrated that various anti-epileptic medications are aware of comparable effectiveness in treating epilepsy. The analysis was carried out with the goal of treating the patient with direct medical costs of prescribed branded drugs with alternates were taken into consideration. The cost minimization observed in this category of drugs was up to ten times that of prescribed brands.

**Keywords:** Cost minimization; Epilepsy; Anti-epileptic drugs; Pregnant women.



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## INTRODUCTION

Since health care is currently characterized by a high level of cost sensitivity, it has become necessary to conduct economic analyses of medications in order to find those that provide the greatest value for money while treating patients. For those illnesses in which there are numerous competing medications available, economic analyses evaluating the costs and results of therapy with various drugs are becoming more common practice. Similarly, the introduction of new antiepileptic drugs (AEDs) has prompted economic assessments to compare the costs and effects of utilizing either new or established AEDs in the treatment of epilepsy. To determine whether higher charges are justified in cases when enhanced efficacy cannot be demonstrated, economic analyses must analyze the resource implications of different adverse-event profiles' [1]. Patients with epilepsy incur a significant share of the entire costs associated with their treatment, which includes the procurement of AEDs. New AEDs could have a significant influence on the resources available to treat epilepsy in developing countries, particularly if savings from improved clinical results do not fully offset the cost of the medications.

Epilepsy is a broad term that refers to a variety of distinct forms of seizures that might present themselves as a change in sensory perception or in subjective/objective behavior, a disturbance in consciousness, or tonic-clonic convulsions, among other things. It is estimated that approximately 5-7 people in every 1,000 in Europe suffer from epilepsy, making it the most common neurologic disorder. Epilepsy is a central nervous system (CNS) disorder in which brain activity becomes abnormal, resulting in seizures or episodes of abnormal behavior, feelings, and, in some cases, perception loss. Epilepsy can affect anyone at any time. Epilepsy affects men, women, and children of all ages and races, as well as people from all ethnic origins. The symptoms of a seizure can be extremely variable. While having an epileptic seizure, some people with epilepsy simply sit there and stare blankly for a few seconds, while others shiver their arms or legs incessantly. A single seizure does not necessarily imply that a person has epilepsy [2]. The use of pharmaceuticals is the cornerstone of epilepsy treatment. Monotherapy is favored since it can treat up to 80 percent of patients with a single medication; however, the use of other medications in conjunction with monotherapy may enhance seizure control in some patients. The type of seizure experienced by the patient can define the appropriate treatment [3].

In most cases, at least two light seizures are required to establish an epilepsy diagnosis. Medications and, in rare cases, surgical intervention can be used to manage seizures in the vast majority of people who have epilepsy. Some patients require lifetime treatment to manage their seizures, however, for the majority of people, the seizures gradually subside or disappear. Some children who suffer from seizures may outgrow their condition as they get older [4]. Seizures during pregnancy are dangerous to both the mother and the unborn child, and certain anti-epileptic drugs can raise the risk of birth abnormalities in the mother and the unborn child. Pregnancy should be closely watched throughout, and drugs may need to be modified as a result of this [5-6]. Unfortunately, no studies have been conducted to determine the significance of contributing factors such as variances between AEDs, levels of in utero exposure, or epilepsy type. According to recent research, the influence of maternal AED exposure on fetal cognitive and behavioral development has arisen as a source of concern [7].

The Government of India has addressed the issue of differential pricing of medicines, at least to a certain extent, through the periodic notification of the Drug Price Control Order (DPCO), which fixes the prices of certain drugs that are essential and makes them affordable for those who cannot afford them. The National Pharmaceutical Pricing Authority (NPPA) is in charge of putting this policy into effect [8]. This study was undertaken to analyze the cost difference of various anti-epileptic drugs available in the Indian market, as well as to highlight the cost variation among different branded and generic preparations available, in order to prescribe a more cost-effective medicine whenever possible in order to improve patient compliance and to reduce the medicine cost to both the patients and to the healthcare system [9].



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## METHODOLOGY

A retrospective study was conducted at the VMKVMCH in Salem, Tamil Nadu, for a period of six months from October 2020 to March 2021, with a sample size of 115 patients. The patients were chosen based on the inclusion criteria (patients with epilepsy, patients in the gynecological department between the ages of 20 and 50, and patients with other medical conditions) and exclusion criteria (Brainstem stroke, History of peroneal nerve injury). All of the referred individuals were evaluated for the possibility of receiving an anti-epileptic medication. The following information was obtained from either the patient or his or her attendant using a data extraction form: (a) a brief description/referral note of the current illness, (b) presenting complaints, (c) investigations performed previously, (d) interventions performed previously, and (e) the name of the city and hospital where the referring facility or doctor is located. Anti-epileptic medicine prices were obtained from several sources, including the CIMS (Current Index of Medical Specialties) (July-October 2020), Drug Today (July-October 2020), and the internet ([www.1mg.com](http://www.1mg.com)). It was noted in all of the above-mentioned sources [10] that the minimum and maximum costs in rupees (INR) of an anti-epileptic medicine manufactured by different pharmaceutical businesses in the same dose strength were variable. The cost of ten tablets/capsules, one bottle of syrup/drops, and one ampoule/vial were all estimated, and the results were presented. The cost of anti-epileptic-based oral formulations was determined for a period of three days in accordance with WHO regulations [11].

It is defined as the ratio of a drug's maximum cost to its lowest cost, where maximum cost equals minimum cost. It was estimated for all of the anti-epileptic medications were included. This implies an increase in the price of the prescribed drug when the chemical composition is the same but the commercial brands are different [12]. The cost ratio is a way of expressing the cost of pharmaceuticals in relation to the most expensive and least expensive brand of the drug available on the market at the time. It was also necessary to calculate the percentage of cost variation, which was supplied by the following equation:

Percentage of cost variation= (maximum cost-minimum cost / minimum cost) X 100

## RESULTS AND DISCUSSION

According to our findings, carbamazepine and phenytoin were the anti-epileptic drugs that were most commonly prescribed. With the exception of phenobarbitone, a similar pattern of older AED use has been observed in India. When compared to the usage of carbamazepine, phenytoin, or lamotrigine, the use of valproic acid has been linked to a higher risk of malformation [13]. We continue to see valproic acid prescribed often among our patients, and it would be interesting to see if there is a trend in prescription among these patients in the future, given the current public awareness of the effects of valproic acid [14].

Additionally, more than half of the pregnancies among our patients were associated with increased seizure frequency. The reported rates of patients experiencing changes in seizure frequency during pregnancy are extremely diverse, which is most likely due to variances in sample size and methodological approach. According to a separate patient, factors that may impact variations in seizure frequency during pregnancy include changed disposition of antiepileptic drugs (AEDs), poor compliance with treatment (including psychological stressors), and physiological and hormonal changes [15]. Postpartum bleeding has been recognized as a possible cause of maternal death, and anemia has been identified as one of the contributing variables. The use of AEDs during pregnancy has been linked to an increased likelihood of postpartum bleeding, and as a result, anemia must be evaluated and treated appropriately during pregnancy [16]. Women in completely different age groups, such as 20-30, 31-40, and 41-50, were assigned to the age-wise distribution, which included the number of instances 40, 60, and 15, for a total percentage of 34.78 percent, 52.17 percent, and 13.04 percent, respectively. The proportion of women in each age



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group and the number of women in each age group show that the majority of women (52.17 %) are between the ages of 31 and 40, with a very small percentage (13.04 %) of women between the ages of 41 and 50. A total of 115 cases were gathered during the study period, and the cases were divided according to whether or not they were obese. Only 35 (30.4 %) of the women are obese, compared to approximately 80 (69.6 %) of the women who are not obese.

The cohort of pregnant epileptic women consisted of 115 women who had been exposed to AED in the months leading up to their pregnancy. In this study, the majority of the women (55 out of 100) received AED monotherapy during pregnancy; 25 (21.7 %) received polytherapy during pregnancy, and 35 (30.4 %) were not exposed to AED at any point during pregnancy. The study population consists of the risk factors, the number of instances, and the overall proportion of participants. Eclampsia was reported in 10 cases (43.5 %), and head injuries were reported in 3 cases (13.0 %). Dementia 8 (34.8 %), stress 15 (65.2 %), hormone changes 6 (26.1 %), water and sodium retention 11 (44.8 %), Cerebral vein thrombosis 2 (08.7 %), stroke 4 (17.4 %), hypoglycemia 7 (30.4 %). The distribution of the drug is based on cost minimization, as shown in Table No.1. It specifies the prescribed brand as well as another brand of the same medicine that is available at a low cost. Table No. 2 illustrates the percentage variance in cost between the prescribed brand and the alternate brand, with a minimum of 26.67 % and a maximum of 1030.77 % for the difference between the prescribed brand and the alternate brand. Almost a tenfold reduction in the cost of the prescription drug was demonstrated in the study.

As a result, a patient may pay more than 10 times the price of an expensive brand of a drug compared to an alternate brand of the same drug. Especially concerning in the context of India, where patients are still responsible for 50-90 % of the cost of pharmaceuticals and other medical supplies. It has been shown that the high cost of acquiring medications is a significant factor in low compliance [17]. False beliefs held by clinicians about the efficacy or superiority of branded medications over generic medications frequently result in the prescription of expensive medications when less expensive alternatives are readily available [18]. In many cases, this results in patients not complying or only partially complying with their treatment regimen. A measure of medication compliance is how closely prescribed medication is followed in terms of time, dosage, frequency, and duration of use in accordance with treatment guidelines or recommendations. it can be defined as Also known as the degree to which a patient correctly follows medical guidance. Some other reasons that may contribute to patient noncompliance include drug formulation, improvement in symptoms, frequent dosing, drug-induced side effects, and others [19]. Hence, the prescription of high-quality generic pharmaceuticals that are as effective as branded drugs would result in increased patient compliance, which would in turn result in improved outcomes [20].

## CONCLUSION

In accordance with the findings of this study, therapy with various antiepileptic drugs under base-case assumptions resulted in cost savings when taking an alternate brand of antiepileptic medication rather than taking the prescribed brand. The cost difference between using a specific brand prescribed by physicians and utilizing a generic brand is raising the expense of treatment, which places an additional strain on the economically disadvantaged sector of the population already burdened.

## REFERENCES

1. Heaney DC, Shorvon SD, Sander JW, Boon P, Komarek V, Marusic P, Dravet C, Perucca E, Majkowski J, Lima JL, Arroyo S. Cost minimization analysis of antiepileptic drugs in newly diagnosed epilepsy in 12 European countries. *Epilepsia*. 2000 May;41(S5):S37-44.
2. Filippakis GM, Zografos G. Contraindications of sentinel lymph node biopsy: are there any really?. *World journal of surgical oncology*. 2007 Dec;5(1):1-1.
3. Lipton RB, Brennan A, Palmer S, Hatswell AJ, Porter JK, Sapra S, Villa G, Shah N, Tepper S, Dodick D. Estimating the clinical effectiveness and value-based price range of erenumab for the prevention of migraine in





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- patients with prior treatment failures: a US societal perspective. *Journal of medical economics*. 2018 Jul 3;21(7):666-75.
4. Fisher RS, Cross JH, D'souza C, French JA, Haut SR, Higurashi N, Hirsch E, Jansen FE, Lagae L, Moshé SL, Peltola J. Instruction manual for the ILAE 2017 operational classification of seizure types. *Epilepsia*. 2017 Apr;58(4):531-42.
  5. Shorvon SD, Farmer PJ. Epilepsy in developing countries: a review of epidemiological, sociocultural, and treatment aspects. *Epilepsia*. 1988 Apr;29:S36-54.
  6. Moshé SL, Perucca E, Ryvlin P, Tomson T. Epilepsy: new advances. *The Lancet*. 2015 Mar 7;385(9971):884-98.
  7. Citraro R, Aiello R, Franco V, De Sarro G, Russo E. Targeting  $\alpha$ -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate receptors in epilepsy. *Expert opinion on therapeutic targets*. 2014 Mar 1;18(3):319-34.
  8. Katzung BG, Trevor AJ. Basic & clinical pharmacology. In: 13<sup>th</sup>Edn.. vol. 52. New York: McGraw-Hill Education; 2015. p. 886–98.
  9. National Pharmaceutical Pricing Authority, Government of India. Available from: <http://www.nppaindia.nic.in>.
  10. Evans RW, Wilberger JE, Bhatia S. Traumatic disorders. *Textbook of Clinical Neurology*, 3rd ed. Philadelphia: Saunders. 2007 Jan 1.
  11. Mamtamaharajan, Anita pal, "Risk and management of pregnancy in women with epilepsy", April 2018.
  12. M.W.Lammers, Y.A.Hekster, W.O.Renier, "Monotherapy or Polytherapy for epilepsy, a quantitative assessment", 1995.
  13. Noni Richards, David Reith, Alesha Smith, "Antiepileptic drug exposure in pregnancy and pregnancy outcomes from national drug usage data", 2018.
  14. Mervyn J. Eadie, "Antiepileptic drug safety in pregnancy: possible dangers for the pregnant woman and her foetus", Jan 2016.
  15. Evan Gedzelman, Kimford J. Meador, "Antiepileptic drug in women with epilepsy during pregnancy", April 2012.
  16. Eric. K. St. Louis, "Truly 'Rational' Polytherapy: Maximizing Efficacy and Minimizing Drug Interactions, Drug Load, and Adverse Effects", 2009.
  17. Kasper DL. *Harrison's principles of internal medicine*. In: 19th Edn.. vol. 248. New York: McGraw Hill Education; 2015. p. 1368–84.
  18. Kumar PJ, Clark M. *Kumar & Clark's clinical medicine*. In: and others, editor. 8th Edn.. vol. 4. Edinburgh: Saunders, Elsevier; 2012. p. 143–6.
  19. Prasad SN, Vedavathi H. Pharmacoeconomic study of antipsychotic drugs in India. *Int J Basic Clinical Pharmacology*. 2017; 6 (2): 377 – 82. doi: 10.18203 / 2319 - 2003. ijbc20170333.
  20. Walker BR, Colledge NR, Ralston S, Penman ID, Britton R. *Davidson's principles and practice of medicine*. In: 22nd Edn.. vol. 13. New York: Churchill Livingstone/Elsevier; 2014. p. 353–8.

**Table No. 1. The distribution of the various oral anti-epileptic formulation**

S.No	Drug	Formulation	Strength (mg)	No.of Units	Cost Per Unit of Prescribed Brands	Cost Per Unit of Alternate Brand
1	Carbamazepine	Tablets	200	10	2.35	1.3
2	Oxcarbazepine	Tablets	600	10	20.4	10.6
3	Lamotrigine	Tablets	100	10	25.4	10.7
4	Levetiracetam	Tablets	750	10	74.8	16.2
5	Phenytoin Sodium	Tablets	100	10	14.7	1.3
6	Valproate Sodium	Tablets	200	10	4.6	2.25
7	Topiramate	Tablets	100	10	14.5	10.8





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8	Zonisamide	Tablets	100	10	26	16
9	Gabapentin	Tablets	100	10	5.7	4.5
10	Clonazepam	Tablets	2	10	6.5	3.8

**Table No. 2. Percentage of cost variation of various anti-epileptic formulations**

S.No	Drug	Strength (mg)	Percentage of Cost Variation
1	Carbamazepine	200	70.00%
2	Oxcarbazepine	600	92.45%
3	Lamotrigine	100	137.38%
4	Levetiracetam	750	361.72%
5	Phenytoin Sodium	100	1030.77%
6	Valproate Sodium	200	104.44%
7	Topiramate	100	34.26%
8	Zonisamide	100	62.50%
9	Gabapentin	100	26.67%
10	Clonazepam	2	71.05%





## Mathematical Modeling of Rosette Formation

R. Sivaraman\*

Associate Professor, Department of Mathematics, Dwaraka Doss Goverdhan Doss Vaishnav College, Chennai, Tamil Nadu, India.

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### \*Address for Correspondence

**R. Sivaraman**

Associate Professor,  
Department of Mathematics,  
D. G. Vaishnav College,  
Chennai, Tamil Nadu, India.  
Email: rsivaraman1729@yahoo.co.in



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### ABSTRACT

Assuming that the joint area for lymphocyte – RBC adhesion as  $a$  and mean number of available receptors is  $\lambda = \rho a$  in this paper, I had proved that the probability of  $s$  receptors is actually a Poisson distribution and had also obtained the probability of red blood cells bounding to the cell. It turns out that in this case we get the Binomial distribution.

**Keywords:** Lymphocyte – RBC adhesion, Infinitesimal areas, Poisson distribution, Combination coefficient, Binomial distribution.

## INTRODUCTION

When small populations are involved, large fluctuations in any measured quantity is the norm, and probabilistic considerations become important. Rosette formation is an assay for the density  $\rho$  of antibody receptors on the surface of lymphocytes, macrophages, etc. The probes are the relatively small red blood cells (RBCs) whose surfaces are coated with the antibody in question, the Fc (leg) portion of which can bind, for example, to lymphocyte receptors. A number of RBCs attached to a lymphocyte, when viewed in profile, have the appearance of a rosette. The mathematical models involved in studying Rosette formation involve probability at two different levels. This paper discuss the mathematical aspects of the Rosette formation.

### Creating and Solving the Model

In creating the suitable model, let us first suppose that the joint area for lymphocyte-RBC adhesion is  $a$ , so that the mean number of available receptors is  $\lambda = \rho a$ . In this paper, I will determine the probability of  $s$  receptors actually being present in that area. To do this, first let us divide  $a$  into  $n$  infinitesimal areas of size  $\Delta$ , so that  $a = n\Delta$  (2.1).





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The probability that a certain  $s$  of the  $\Delta$  's will be occupied,  $n - s$  unoccupied, is clearly  $(\rho\Delta)^s (1 - \rho\Delta)^{n-s}$  (2.2). Each of the  $s\Delta$  's can be any of the  $n$  possible ones, and all  $s!$  permutations of the order of choice give the same

configuration, so the desired probability is given by  $p_s = \frac{n^s (\rho\Delta)^s (1 - \rho\Delta)^{n-s}}{s!}$  (2.3)

From (2.1),  $n = \frac{a}{\Delta}$  and allowing the limit as  $\Delta \rightarrow 0$ , and making use of Euler's limit, (2.3) becomes

$$p_s = \lim_{\Delta \rightarrow 0} \frac{(\rho a)^s (1 - \rho\Delta)^{\frac{a}{\Delta} - s}}{s!} = \frac{(\rho a)^s e^{-\rho a}}{s!} \quad (2.4)$$

Equation (2.4) is a Poisson distribution with parameter  $\rho a$ .

Now, if it takes  $N$  bound receptors for the RBC to adhere, the probability of a given RBC adhering is simply

$$p = \sum_{s=N}^{\infty} \frac{(\rho a)^s e^{-\rho a}}{s!} \quad (2.5)$$

**Probability of RBCs bounding to the cell**

In this section, I will determine the probability of  $m$  RBCs being bound to the cell in discussion. If the surface area of the cell is  $S$ , we can imagine, tentatively, that there are  $M = \frac{S}{a}$  possible binding areas. The argument can be repeated, but we need to take care, since  $a$  is not infinitesimal here. Now the number of ways that  $m$  areas out of  $M$

can be chosen to bind RBCs is just the combination coefficient  $\binom{M}{m} = \frac{M!}{(M - m)! \times m!}$

Hence the associated probability is  $P_m = \binom{M}{m} p^m (1 - p)^{M-m}$  (3.1)

We see that (3.1) is a Binomial distribution with parameters  $M$  and  $p$ .

Finally, assuming that three or more bound RBCs are identified as a rosette, the relative frequency of lymphocytes appearing as rosettes should be

$$f = \sum_{m=3}^{\infty} P_m = 1 - P_0 - P_1 - P_2 \quad (3.2)$$

**CONCLUSION**

In this short paper, I had proved that the probability of  $s$  receptors by dividing  $a$  in to  $n$  infinitesimal areas of size  $\Delta$  is a Poisson distribution with parameter  $\rho a$  through equation (2.4) in section 2. Knowing that the events occurring in Poisson distribution is rare, we can conclude that the event of occurrence of  $s$  receptors by dividing lymphocyte-RBC adhesion in to infinitesimal areas is quite rare. Moreover for such an event, the mean will be equal to its variance being  $\rho a$

In section 3, I had proved that the probability of  $m$  Red Blood Cells being bound to the cell turns to be a Binomial distribution with parameters  $M$  and  $p$ . Through equation (3.2), given  $f$ , one can work back numerically to find  $q$  so that the assay strategy will be complete. These results reveal the pattern and behavior of Rosette formation in cells.





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## REFERENCES

1. Glass, L., and Kauffmann, S. A. Co-operative components, spatial localization and oscillatory cellular dynamics. *J. Theor. Bio.* 34(2): 219–237, 1973.
2. Perelson, A. S., Mirmirani, M., and Oster, G. F. Optimal strategies in immunology. I. B-cell differentiation and proliferation. *J. Math. Biol.* 3(3-4): 325–367, 1976.
3. R. Sivaraman, On Metric and Ultrametric Trees, *European Journal of Molecular and Clinical Medicine*, Volume 7, Issue 8, 2020, 2611 – 2615.
4. R. Sivaraman, Mathematical Modeling of Cell Packings, *Annals of the Romanian Society for Cell Biology*, Vol. 24, Issue 2, 2020, pp. 149 – 152.
5. R. Sivaraman, Mathematical Modeling of Recovery Curves, *African Journal of Mathematics and Statistics Studies*, Volume 3, Issue 5, 2020, pp. 38 – 41.
6. R. Sivaraman, Markov Process and Decision Analysis, *Journal of Mechanics of Continua and Mathematical Sciences*, Volume 15, No. 7, July 2020, pp. 9 – 16.
7. Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K., and Watson, J. D. *Molecular biology of the cell*. Garland, New York, 1989.





## Molecular Characterization of Bacterial Microbiome Isolated From Wheat Rhizosphere of Agro Climatic Zones of India

Ashish K. Pandey<sup>1</sup>, Arun Kumar Patel<sup>2</sup>, Umakant Banjare<sup>1</sup> and Ramesh K. Singh<sup>1\*</sup>

<sup>1</sup>Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, BHU, Varanasi, Uttar Pradesh, India,

<sup>2</sup>Department of Botany, Institute of Sciences, BHU, Varanasi, Uttar Pradesh, India.

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### \*Address for Correspondence

**Ramesh K. Singh**

Department of genetics and plant breeding,  
Institute of Agricultural Sciences,  
BHU, Varanasi, India.

Email: rksbhu@yahoo.com



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### ABSTRACT

Present investigation revealed the bacterial microbiome study of wheat rhizosphere between two different agro-climatic zones of country viz. Research farm, Institute of Agricultural Sciences, BHU and IARI Regional Research Centre, Wellington, Tamilnadu. Rhizosphere soil samples from different locations in the agro-climatic zones were taken for the isolation, characterization, identification of bacterial communities. After identification, based on biochemical and molecular characterization, their phylogeny was studied. The representative isolates were screened on the basis of multiple growth promoting activities shown by them. Based on 16S rRNA gene sequencing, the representative isolates were identified as *Gibbsiella and quercinecans* IASBHU1, *Bacillus subtilis* IASBHU2, *Agrobacterium salinitolerans* IASBHU3, *Agrobacterium salinitolerans* IASBHU4, *Pseudomonas* sp. IASBHU5, *Bacillus subtilis* IASBHU6, *Bacillus subtilis* IASBHU7, *Arthrobacter* sp. IASBHU8, *Arthrobacter* sp. IASBHU9, *Nitrobacter winogradskyi* IASBHU10, *Sphingomonas echinoides* IASBHU11 and *Pseudomonas* sp. IASBHU12. On the basis of nucleotide sequences obtained after DNA sequencing a phylogenetic tree was constructed that describes the evolutionary relationship of bacterial strains in four distinct OTUs.

**Keywords:** 16S rRNA gene, Bacterial community, Agro-climatic zone, OTUs.

### INTRODUCTION

The plant-microorganisms interaction and association can prove usefulness in this respect. These beneficial associations are very complex and useful. Therefore, more intensive research and studies are required in this field. Plant-microbe associations are most pronounced in the root-soil contact area, called "rhizosphere." Specifically, soil

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particles and various microorganisms adhere to this area often extending a few mm from the root surface is much richer in bacteria than the surrounding bulk soil [9]. The rhizosphere is a hot spot for microbial growth and major microbial activities. The microbial communities inhabiting the rhizosphere region imparts a significant role in the plant growth promotion directly or indirectly. Different prokaryotic and eukaryotic microbial assemblages constitute the microbiome in this hot-spot soil ecosystem. Microbiome diversity is greatly affected in soil ecosystem. The functional microbial communities such as bacteria, fungi, actinomycetes, fermitutes, algae are deviated both in rhizosphere and non-rhizosphere soil. The only alternative to replace chemical fertilizer is the application of beneficial microbes. Consequently, this is a great challenge to search for sustainable strategies to alleviate harmful effects of intensive farming practices based on chemical fertilizers. Plant growth promoting rhizobacteria (PGPR) are soil-borne bacteria that have ability to colonize the rhizosphere or plant roots or both aggressively, leads to increase in plant growth and yields [2, 11]. The diversity of microorganisms in agroecosystems is critical to the maintenance of good soil health because they are involved in many important soil processes [6]. Moreover, species diversity can give rise to ecosystem stability through the ability of species or functional groups it contains to respond differentially and in a compensatory fashion to perturbations in the soil environment [26]. The bacterial community diversity or structure can be used as an indicator of these perturbations or disturbances in the agro ecosystems. Disturbances could be caused by the presence of a plant changes in agronomic practices such as type of amendment [29, 12,18], reduced or no-tillage [10], irrigation system, monocropping, or crop rotation [16]. The traditional method for the determination of microbial diversity consisted identification and characterization of culturable microorganisms in a soil system to species level and use the taxonomic differences to measure diversity [1]. Studies based on molecular techniques have estimated more than 4,000 microbial species per gram of soil.

However, the rhizospheric microbiota of mature wheat plants growing under field conditions remain poorly characterized. The role of bacteria in growth promotion of wheat genotypes and its interactions in the field environment to the resident soil microbiota remain to be more elaborated and elucidated. Although, the effect of different agroclimatic conditions, how to affect the soil microbial diversity around the root zone of the plants in a cultivated field but a very little information is available particularly in Indian agro-climatic condition. This investigation inculcates the characterization of microbiota across a genetically diverse genotype of wheat at their flowering time in two agricultural field environments was studied. The goal of this study is to improve our knowledge about plant-microbe interaction, its impact to environment. Study also aims to provide idea about suitable plant genotype conserved more diverse and identification of beneficial bacteria that can increase productivity without disturbing environment health.

## MATERIALS AND METHODS

### Soil Sampling and Physiochemical Analysis

Soil sampling was done from two different agro-climatic zones of country viz. Research farm, Institute of Agricultural Sciences, BHU and IARI Regional Research Centre, Wellington, Tamilnadu. Soil sampling was performed by random sampling method from different locations in the agro-climatic zones. The wheat genotypes PBW-343, HUW-234, HD-2967, K-307 and Lok-1 (procured from, Department of genetics and Plant breeding, Institute of Agriculture Sciences, Banaras Hindu University, Varanasi) were used for the experiment.

### Isolation of Rhizobacteria

Plant samples were uprooted at the growth stage of. 25-30 days. Plant roots along with good amount of non-rhizosphere soil were taken immediately to the laboratory in polythene bags and samples are air dried within 2 hours, were collected along with bulk rhizospheric soil. For the isolation of bacteria from rhizosphere soil, samples were randomly collected from farm plot's of BHU and wellington field. Isolation was performed using serial dilution technique up to  $10^{-5}$  dilution. From  $10^{-2}$ ,  $10^{-3}$  and  $10^{-4}$  dilution, 10  $\mu$ l of inoculums was spread plated on YEMA media (Appendix), NA, LB, KB and Yeast extract agar media. The plates were incubated at  $30 \pm 2^\circ\text{C}$  in BOD incubator for 24-



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72 hours. From all the crowded plates, morphologically distinct colonies were picked up and streaked on fresh plates for further purification. From this purified culture, the inoculums were built up in broth and were used for rest of the experiment. The slant culture were prepared on respective media and stored at 4°C in a refrigerator. Sub-culturing was done after every three month interval.

**Morphological Characteristics and Gram Staining**

Morphological characteristics *viz.*, colony morphology (colour, chromogenesis, shape, margin, elevation and surface) along with cell morphology (shape and arrangement) of different bacterial isolates were observed macroscopically and microscopically. For numerous isolates obtained, Gram staining was done in which a loopful fresh grown culture is taken on the surface of clean microscopic slide. The suspension was smeared and heat fixed under laminar air flow chamber. The heat fixed smear was flooded with crystal violet to cover the smear and left for 60 seconds. The excess stain was washed off with distilled water. Afterwards, slide was flooded with Gram's Iodine and left for 60 seconds and washed with water. Smear was flooded with decolourizing agent (95% ethyl alcohol) for 30 seconds, or until no more purple dye runs off. Immediately wash the slides with distilled water to remove excess of decolourizing agent. The slide is flooded with counter stain (safranin) and for 30 seconds followed by washing with tap water. The slide is air dried or with blotting paper and examined under microscope (De winter) at 40X and 100X (oil immersion) magnification. Screening based on multiple PGP attributes was further subjected to biochemical characterization and different physiological characterisation. Total twelve final bacterial strains were selected for further studies.

**Screening Based On Multiple PGP (Plant Growth Promoting) Attributes****Estimation of Indole Acetic Acid (IAA) Production by Rhizospheric Bacterial Strains**

IAA production will be estimated by growing the bacterial strains in broth as described by Okon *et al.*[20]. Tubes will be incubated at 28°C for 48 hours with continuous shaking. Cultures will be centrifuged at 10,000 rpm for 15 min. at 4°C. IAA produced ml<sup>-1</sup> culture will be estimated by mixing 4 ml of with culture supernatant followed. After incubation, IAA productions by the different isolates were determined. The culture broth was centrifuged at 10000 rpm for 15 minutes. The supernatant was used for the qualitative detection of IAA production by isolates. To one ml of the supernatant 4 ml of the Salkowsky reagent was added and mixed thoroughly. The tubes were incubated for 30 minutes to allow the colour to develop. Development of pink colour indicated IAA production by the isolate. Intensity of colour was read at 530 nm. by measuring absorbance at 530 nm after 30 min [8]. Quantification of IAA production was done by preparing curve with standard indole acetic acid concentration against OD. Viable cell count was taken after serial dilution of culture and the result were expressed in terms of µg IAA/ml culture.

**Siderophore Production Test**

Suitable media will be inoculated with rhizospheric bacteria and incubated at their optimum growth temperature with Chrome Azurol Sulphonate (CAS) assay and CAS agar plate test will be done [25].

**Phosphate Solubilization Activity**

Double layered plates will be prepared with a bottom layer of suitable medium containing 0.5% (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> as nitrogen source. Mannitol from the medium will be replaced with same amount (1%) of glucose, as sole source of carbon. Plates will be over laid with of 0.4% of CaHPO<sub>4</sub> mixed in 1.5% water agar. To detect phosphate solubilization, plates will be spotted with of exponentially grown isolates ( $\pm 10^9$  CFU ml<sup>-1</sup>). These plates will be incubated at 30°C for 7 days. The radial area of the solubilization zone including colony will be measured and phosphate solubilization activity will be expressed in mm radial area solubilized h<sup>-1</sup>. The solubilization efficiency and the solubilization index on the basis of diameter of clearing halo zones were measured.

Solubilizing Efficiency (% S.E) = Solubilization Diameter/Growth Diameter\*100

Solubilization Index (S.I) = Colony Diameter + Halo zone Diameter/Colony Diameter



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### Molecular Characterization for Identification

The isolates obtained from rhizosphere region of different plant varieties were subjected to DNA extraction, amplification of 16S rRNA gene, purification of amplified product and further Di-deoxy chain termination sequencing. Obtained nucleotide sequences are taken to see the similarity with already available nucleotide sequences in NCBI database using NCBI BLAST search.

### Genomic DNA Extraction

The genomic DNA extraction method was as described by Pospiech and Neumann [22]. Inoculated broth of pure cultures were centrifuged, supernatant discarded and the pelleted cells were washed twice with TE buffer and resuspended in 0.5 ml in SET buffer (75mM NaCl, 25mM EDTA and 20 mMTris). The cell suspension was incubated with 10 µL (10 mg/mL) of lysozyme solution and kept at 37°C for 1hr. The following reagents were then added: 10 µL of sodium dodecyl sulfate (10%), and 10 µL of proteinase K (10 mg/mL). The reaction mixture was incubated at 55°C for 2 hr in water bath to lyse the cells. To the mixture, 150 µL NaCl (5M), equal volume of water saturated phenol and Chloroform: Isoamyl alcohol (24:1) was added and centrifuged at 10,000 rpm for 10 min. The aqueous layer was transferred to fresh tube. To the aqueous layer 0.1 volumes of 3M Sodium acetate and 2 volumes of chilled 95% ethanol was added and kept at 4 °C for 30 minutes. The precipitated DNA was pelleted by centrifugation at 12,000 rpm for 10 min. The DNA pellet was washed with 70% ethanol and the pellet was dried by keeping at 37 °C for 10 min. The DNA was then dissolved in 50µL of milli Q water and stored at 4 °C for further analysis.

### 16S rRNA gene Amplification, Gel Electrophoresis and Sequence Analysis

For the amplification of 16S rDNA universal primers as described by Lane *et al.*[15] were used to obtain partial gene sequence of 16S rRNA gene. The 100µL PCR reaction mixture contains 50-90ng DNA template, 1X Taq buffer, 0.2 mM each of deoxyribonucleotide triphosphate (dNTP) mixture, 10 pmol of each primers, 1.5 mM MgCl<sub>2</sub>, and 2 U of Taq DNA polymerase (Bangalore Genei, India). The PCR reaction was performed in a thermocycler (Bio-Rad) using the following conditions: initial denaturation of 5 min at 94°C, followed by 40 cycles consisting of 40 s at 94°C (denaturation), 40 s at 50°C (annealing) and 1 min 30 s at 72°C (Primer extension) and a final extension period of 7 min at 72°C. The PCR reaction mixture and the amplification conditions were same as described above. After amplification the PCR product was resolved by electrophoresis in 1.2% agarose gel stained with ethidium bromide and visualized on a gel documentation system (Alpha-Imager) and gel images were digitalized. PCR products were sequenced commercially using dye terminator chemistry. All sequenced in order to determine the identity of the isolated cultures was done by comparing the sequences with the database. The sequences obtained were subjected to nucleotide BLAST (Basic Local Alignment Search tool) (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) at the server of the National Centre for Biotechnology information (NCBI) to determine the similarity with the already submitted gene sequences and identity was affirmed by highest percentage of similarity match. All sequences are submitted to NCBI GenBank to obtain NCBI GenBank accession number. The NCBI accession numbers obtained were from MW599239 to MW599250.

### Identification and Phylogeny of Rhizobacteria

The partial 16S rRNA gene sequences of the isolated strains were compared with those available in the databases. Identification to the species level was determined as a 16S rRNA gene sequence similarity of ≥97% with that of a prototype strain sequence in the GenBank. Sequence alignment and comparison was performed using the program CLUSTAL W2 with default parameters. The sequences are multiple aligned using CLUSTAL W algorithms with a set of sequences of representatives of the most closely related genera identified. Based on this alignment a phylogenetic tree is constructed by neighbour joining method. The phylogenetic tree was constructed on the aligned datasets using the neighbour-joining method [23] implemented in the program MEGA 4.0.2 [27]. Bootstrap analysis was performed as described by Felsenstein[7] on 500 random samples taken from the multiple alignments.





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## RESULT AND DISCUSSION

### Isolation of Bacterial Isolates from Wheat Rhizosphere

Bacterial isolates were obtained after isolation through serial dilution, spreading and streaking on different culture media plates with different morphotypes. A total 137 rhizobacterial strains were isolated from soil samples taken from different locations of at Research Farm, Institute of agriculture Sciences, Banaras Hindu University, Varanasi, U.P. in rabi season 2014. Out of 137 isolates 50 were observed on YEMA media, 33 on nutrient agar, 30 on LB media, 19 on KB media and 5 colonies on BAZ. Whereas in year 2015 total 132 bacterial colonies were observed, out of these 49 were observed on YEA media, 40 on nutrient agar, 27 on LB media, 15 on KB media and 1 colony on BAZ. Total 140 isolates were observed sample taken from IARI regional research centre, Wellington, Tamilnadu. Out of these isolates, 65 were observed on YEMA media, 44 on nutrient agar media, 22 on LB media, 16 on KB media and 1 bacterial colony on BAZ. Next year 2016, a total 134 bacterial colonies were observed out of these isolates 54 were observed on YEMA media, 30 on nutrient agar media, 29 on LB media, 19 on KB media and 2 bacterial colonies on BAZ.

### Screening Based on Multiple PGP (Plant Growth Promoting) Attributes

On the basis of multiple plant growth promoting attributes such as phosphate solubilization, IAA production and siderophore production, screening of various isolates obtained from rhizosphere region of different wheat varieties was done. In addition, a total 12 bacterial isolates were obtained with significant and better plant growth promoting activities. These screened bacterial isolates were further selected to observe their physiological and biochemical characteristics. The screening result is described in the table 2. The phosphate solubilization was observed according to the zone of clearance obtained around the bacterial colonies to respective media. Moreover, Indol acetic acid production (IAA) was observed through spectrophotometer analysis in µg/ml as mentioned above in table 2.

### Molecular Characterization, Identification and Phylogeny of Rhizobacteria

There were different screened bacterial strains obtained from the rhizosphere region of five different varieties of wheat plant. Further, DNA fragments were observed on gel electrophoresis. The amplified gene products (PCR products) were also observed on agarose gel electrophoresis in the form of clear brightened DNA band.

This was quite obvious and observed that 16S rRNA gene sequence analysis revealed the similarity search on bioinformatics databases with different bacterial species. Apart from variation in the species, as compared with plant variety, this was also observed that there was the abundance of Rhizobium sp. on the basis of nucleotide sequence similarity search. Identification of the bacterial strains was done on the basis of similarity of sequences more than or equals to 97% with the bacterial 16S rRNA gene sequences present in public database. This nucleic acid based tool was used to further study the diversity of rhizobacteria from different wheat varieties at species level and thus 16S rRNA gene was amplified from the isolates. Based on 16S rRNA gene sequencing, the representative isolates were identified as *Gibbsiella quercinecans* IASBHU1, *Bacillus subtilis* IASBHU2, *Agrobacterium salinitolerans* IASBHU3, *Agrobacterium salinitolerans* IASBHU4, *Pseudomonas* sp. IASBHU5, *Bacillus subtilis* IASBHU6, *Bacillus subtilis* IASBHU7, *Arthrobacter* sp. IASBHU8, *Arthrobacter* sp. IASBHU9, *Nitrobacter winogradskyi* IASBHU10, *Sphingomonas echinoides* IASBHU11 and *Pseudomonas* sp. IASBHU12. The % similarity obtained were based on closest match are mentioned into the table3.

Further, nucleotide sequences obtained after DNA sequencing and their accession numbers were utilised for the construction of phylogenetic tree (Fig 2). In the neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, strains have the lineage comprising to the type strains at a bootstrap resampling value of 50 to 99%. The prevalent genera were found to be *Bacillus* and *Pseudomonas* from rhizosphere of most of the wheat genotypes



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16S rRNA gene sequencing indicated that the strains were most closely related to different rhizobacterial strains. From the phylogenetic tree, this is quite obvious that *Bacillus subtilis* strains obtained from wheat rhizosphere are abundantly present. In the tree, they form a cluster different from other bacterial strains. After looking at the phylogenetic tree, four distinct major OTUs (Operational Taxonomic Units) are obtained. In OTU I, all *Bacillus* strains and *Arthrobacter* strains are grouped. In OTU II, Gram negative *Pseudomonas* strains were clustered. OTU III represents another cluster of Gram negative rhizobacterial strains like *Nitrobacter* and *Sphingomonas*. OTU IV represents unique group of bacteria such as *Gibbsiella quercinecans* and *Serratia* sp.

*Bacillus subtilis* isolated from wheat rhizosphere are reported in several studies to enhance the plant growth by direct or indirect biocontrol mechanism by suppressing plant pathogens. *Bacillus* genera are reported to produce plant hormones, secondary metabolites and several enzymes to protect plants in unfavourable conditions too. In a very recent study that was based on multi-omics approaches, described the structure and functions of plant associated microbes. Phosphate solubilizing *Pseudomonas* sp. isolated from wheat rhizosphere was also reported to enhance the plant growth effectively. The isolates obtained in this study validate the abundance of isolates as compared to earlier investigations [17]. One of the earlier studies explained that bacterial strains of *Arthrobacter* sp. help in the plant growth promotion and are reported as salt tolerant PGPR. They enhance the percentage nitrogen (N), potassium (K) and phosphorus (P) in leaves of wheat significantly [28]. These strains are reported in the current studies also, which gives a clue to exploit these bacterial strains in future. This isolate was further identified as *Bacillus subtilis* IASBHU2 after DNA sequencing (molecular characterization). In several earlier studies related to wheat rhizosphere, this was clearly found that *Bacillus* was predominant genera in the rhizosphere. As, this is quite obvious that the operational taxonomic unit (OTU) is the latest unit of classification, there were total four OTUs were obtained in the phylogenetic study, showing the diversity of bacterial communities obtained from wheat rhizosphere. The identification and characterization of screened, representative isolates based on Sanger DNA sequencing validate the result of identification at species level.

The breakthrough in evolutionary formulation was reached by Carl Woese during the 1970's who, by comparison of ribosomal RNA sequences, established a molecular sequence-based phylogenetic tree that would be used to relate all organisms and reconstruct the history of Life [21]. Because of the likely antiquity of the protein-synthesizing process, ribosomal RNA turned out to be an excellent evolutionary chronometer. Ribosomal RNA is an ancient molecule, functionally constant, universally distributed and moderately well conserved across broad phylogenetic distances [3]. Moreover, there is no evidence of lateral gene transfer of rRNA genes between different species and therefore rRNA genes can bring true information regarding evolutionary relationships [21]. The entry of natural phylogeny into microbial systematic based on nucleic acid sequence data enabled the classification of microorganisms on evolutionary terms as well as the clarification of the phylogenetic lineages between them [21]. Consequently, unique phylogenetic groups could be determined at each taxonomic level, i.e., species, genus, family, etc., based on comparative analysis via DNA sequence databases [19, 14, 13].

Boivin-Jahnset al. [4] have compared bacteria using on one hand the classical phenotypic characterization including morphology, Gram staining, enzyme activities and the utilization of different organic substrates as carbon and energy sources as well as molecular analyses. They found that the phylogenetic analysis of small subunit rRNA gene sequences is more efficient for the identification of bacterial strains because misidentification of bacteria was less with the molecular methods. Application of genotypic and molecular analysis has advanced microbial identification and it has led to the discovery of a number of possibly new species [5,24].

## REFERENCES

1. Alexander, M. (1977) Soil Microbiology. Second Edition, John Wiley and Sons, Inc. Canada. pp. 457.





**Ashish Pandey et al.**

2. Ashrafuzzaman, M., Hossen, F.A., Ismail, M.R., Hoque, M.A., Islam, M.Z., Shahidullah, S.M., Meon, S. (2009) Efficiency of plant growth-promoting rhizobacteria (PGPR) for the enhancement of rice growth. *Afr. J. Biotechnol.* 8: 1247-1252.
3. Asanovich KM, Bakken JS, Madigan JE, Aguero-Rosenfeld M, Wormser GP, Dumler JS. (1997) Antigenic diversity of granulocytic Ehrlichia isolates from humans in Wisconsin and New York and a horse in California. *J Infect Dis.* 176(4): 1029-34.
4. Boivin-Jahns V, Bianchi A, Ruimy R, Garcin J, Daumas S, Christen R. (1995) Comparison of phenotypical and molecular methods for the identification of bacterial strains isolated from a deep subsurface environment. *Appl Environ Microbiol.* 61(9): 3400-6.
5. Bowman JP, McCammon SA, Brown JL, Nichols PD, McMeekin TA. (1997) *Psychroserpensburtonensis* gen. nov., sp. nov., and *Gelidibacteralgens* gen. nov., sp. nov., psychrophilic bacteria isolated from antarctic lacustrine and sea ice habitats. *Int J Syst Bacteriol.* 47(3):670-7.
6. Borneman J, Triplett EW. (1997) Molecular microbial diversity in soil from Eastern Amazonia: evidence for unusual microorganisms and microbial population shifts associated with deforestation. *Appl. Environ. Microbiol.* 63: 2647-53.
7. Felsenstein J. (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. *J. Mol. Evol.* 17: 368–376.
8. Gordon, S. A., and Weber, R. P. (1951) Colorimetric estimation of indoleacetic acid. *Plant Physiol.* 26:192–195.
9. Hiltner L. (1904) Über neuere Erfahrungen und Probleme auf dem Gebiete der Bodenbakteriologie unter besonderer Berücksichtigung und Brache. *Arb. Dtsch. Landwirtschaft. Gesellschaft.* 98:59-78
10. Ibekwe AM, and Kennedy AC. (1998) Phospholipid fatty acid profile and carbon utilization pattern for analysis of microbial community structure under field and greenhouse condition. *FEMS Microbiology Ecology* 26: 151-163
11. Kaymak, H.C. (2011) Potential of PGPR in agricultural innovations. In: Maheshwari, DK (Ed.), *Plant Growth and Health Promoting Bacteria. Microbiology Monographs*, 18. Springer, Berlin, pp.45-79.
12. Kennedy, I.R., Choudhury, A.T.M.A. and Kecskes, M.L. (2004) Non-symbiotic bacterial diazotrophs in crop-farming systems: can their potential for plant growth promotion be better exploited? *Soil Biol. Biochem.* 36: 1229-1244
13. Kumar M, Saxena R, Parihar SS, Rai PK, Tomar RS (2018) Molecular characterization and phylogeny of some cyanobacterial strains isolated from soil and freshwater ecosystem. *Journal of Pure and Applied Microbiology.* 12(2): 897-904
14. Kumar M, Yadav AN, Saxena R, Paul D, Tomar RS (2021) Biodiversity of pesticides degrading microbial communities and their environmental impact. *Biocatalysis and Agricultural Biotechnology.* 31: 101883.
15. Lane D.J. (1991) 16S/23S rRNA sequencing. In: Stackebrandt, E.; Goodfellow, M., (eds.). *Nucleic acid techniques in bacterial systematics.* New York: John Wiley and Sons, p. 115-175.
16. Larkin, R.P. (2003) Characterization of soil microbial communities under different potato cropping systems by microbial population dynamics, substrate utilization, and fatty acid profiles. *Soil Biol. Biochem.* 35: 1451-1466.
17. Liu, X., Jiang, X., He, X. et al. (2019) Phosphate-Solubilizing *Pseudomonas* sp. Strain P34-L Promotes Wheat Growth by Colonizing the Wheat Rhizosphere and Improving the Wheat Root System and Soil Phosphorus Nutritional Status. *J Plant Growth Regul* 38: 1314–1324 <https://doi.org/10.1007/s00344-019-09935-8>
18. Marschner, P., Kandeler, E. and Marschner, B. (2003) Structure and function of the soil microbial community in a long-term fertilizer experiment. *Soil Biol. Biochem.* 35: 453-461.
19. Meena KK, Kumar M, Mishra Snehashish, Ojha SK, Sarkar Biplab. (2015) Phylogenetic Study of Methanol Oxidizers from Chilika-Lake Sediments Using Genomic and Metagenomic Approaches. *Indian Journal of Microbiology.* 55(2):151-62,
20. Okon, Y., Albercht, S. L., and Burris, R. H. (1977) Methods for growing *Spirillum lipoferum* and for counting it in pure culture and in association with plants. *Appl. Environ. Microbiol.* 22: 85-88.





**Ashish Pandey et al.**

21. Pace NR. (1997) A molecular view of microbial diversity and the biosphere. *Science*. 276 (5313):734-40. doi: 10.1126/science.276.5313.734. PMID: 9115194.
22. Pospiech A, Neumann B. (1995) A versatile quick-prep of genomic DNA from gram positive bacteria. *Trends Genet*. 11: 217-218.
23. Saitou N. & Nei M. (1987) The neighbour joining method a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol*. 4: 406-425.
24. Sheridan PP, Miteva VI, Brenchley JE. (2003) Phylogenetic analysis of anaerobic psychrophilic enrichment cultures obtained from a greenland glacier ice core. *Appl Environ Microbiol*. 69(4):2153-60.
25. Schwyn B, Neilands JB. (1987) Universal chemical assay for the detection and determination of siderophores. *Anal Biochem*. 160:47–56. doi: 10.1016/0003-2697(87) 90612-9.
26. Sturz, A.V. and Christie, B.R. (2003) Beneficial microbial allelpathies in the root zone: the management of soil quality and plant disease with rhizobacteria. *Soil Till. Res*. 72: 107-123.
27. Tamura K., Dudley J., Nei M. & Kumar S. (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0.2. *Mol. Biol. Evol*. 24: 1596-1599.
28. Upadhyay SK and Singh DP. (2015) Effect of salt-tolerant plant growth-promoting rhizobacteria on wheat plants and soil health in a saline environment. *Plant Biol (Stuttg)*. 17(1): 288-93
29. Workneh, F. and van Bruggen, A.H.C. (1994) Microbial density, composition, and diversity in organically and conventionally managed rhizosphere soil in relation to suppression of corky root of tomatoes. *Applied Soil Ecology* 1: 219-230.

**Table1: Description of Forward and Reverse Primers Used In the Molecular Characterization**

S.No (Corresponding to template)	Primer to be used (Universal – M13/T7/T3/SP6/Custom)	Tm of Primer	Annealing Temp. used by scientist	Conc. Of Primer (pm/μl)	Volume of medium	Qty of Primer	Is Primer Purified	Length of Primer with sequence
27F 5' TO 3'	Universal primer	57.30	56 C	396.30	100pm/ul	244.00 microgram	HPSF	20bp AGAGTTTGATCCTGGC TCAG
1429r 5' TO 3'	Universal primer	56.53	56 C	354.20	100pm/ul	237.00 microgram	HPSF	22bp TACGGTTACCTTGTTAC GACTT

**Table2. Plant Growth Promoting Activities of Screened Bacterial Isolates**

S.no.	Isolates	Multiple PGP attributes		
		Phosphate solubilization	IAA Production (μg/ml)	Siderophore production
1	HURB1	++	59.12	++
2	HURB2	++	68.34	++
3	HURB3	++	84.01	+++
4	HURB4	+++	55.34	+++
5	HURB5	++	45.56	++
6	HURB6	++	80.56	++
7	HURB7	+++	45.21	++





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8	HURB8	+++	35.56	+++
9	HURB9	+++	81.33	+++
10	HURB10	++	98.23	++
11	HURB11	++	67.43	++
12	HURB12	++	77.52	++

**Table 3: Identification Details for Representative Isolates**

S. No.	Representative isolates	Identification after molecular characterisation	NCBI Accession number	% Similarity in public database	Source Location-Year	Source Variety
1	HURB1	<i>Gibbsiella quercinecans</i> IASBHU1	MW599239	99.09	Location I-2014	PBW-343
2	HURB2	<i>Bacillus subtilis</i> IASBHU2	MW599240	99.80	Location I-2014	HUW-234
3	HURB3	<i>Agrobacterium salinitolerans</i> IASBHU3	MW599241	99.87	Location I-2015	Lok-1
4	HURB4	<i>Agrobacterium salinitolerans</i> IASBHU4	MW599242	99.38	Location II-2015	K-307
5	HURB5	<i>Pseudomonas sp.</i> IASBHU5	MW599243	99.2	Location I-2014	HD-2967
6	HURB6	<i>Bacillus subtilis</i> IASBHU6	MW599244	97.80	Location II-2014	PBW-343
7	HURB7	<i>Bacillus subtilis</i> IASBHU7	MW599245	97.40	Location I-2015	Huw-234
8	HURB8	<i>Arthrobacter sp.</i> IASBHU8	MW599246	99.0	Location I-2015	K-307
9	HURB9	<i>Arthrobacter sp.</i> IASBHU9	MW599247	98.90	Location II-2015	HD-2967
10	HURB10	<i>Nitrobacter winogradskyi</i> IASBHU10	MW599248	99.2	Location II-2014	HUW-234
11	HURB11	<i>Sphingomonas echinoides</i> IASBHU11	MW599249	98.69	Location II-2015	PBW-343
12	HURB12	<i>Pseudomonas sp.</i> IASBHU12	MW599250	97.20	Location I-2014	K-307





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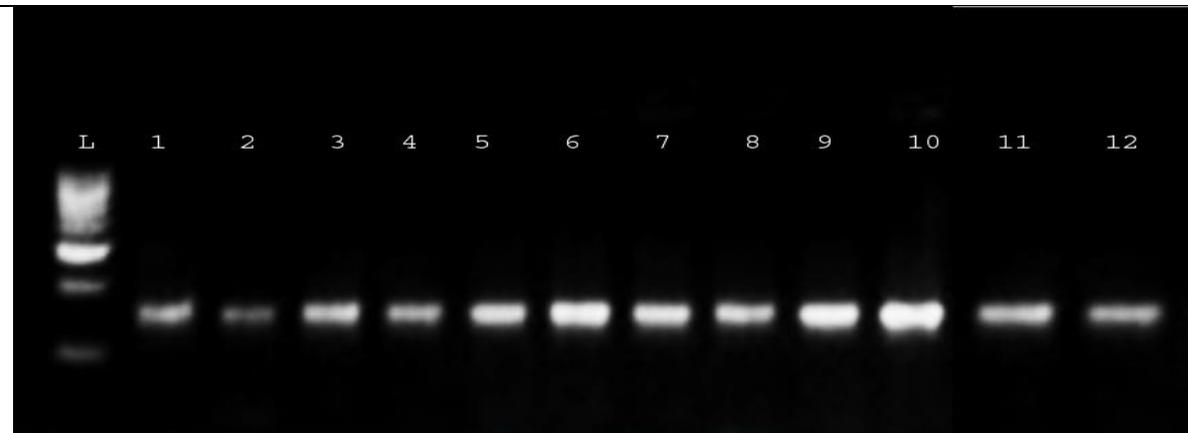


Fig1. 16S rRNA gene amplification using universal gene primers (HURB1 to HURB10).

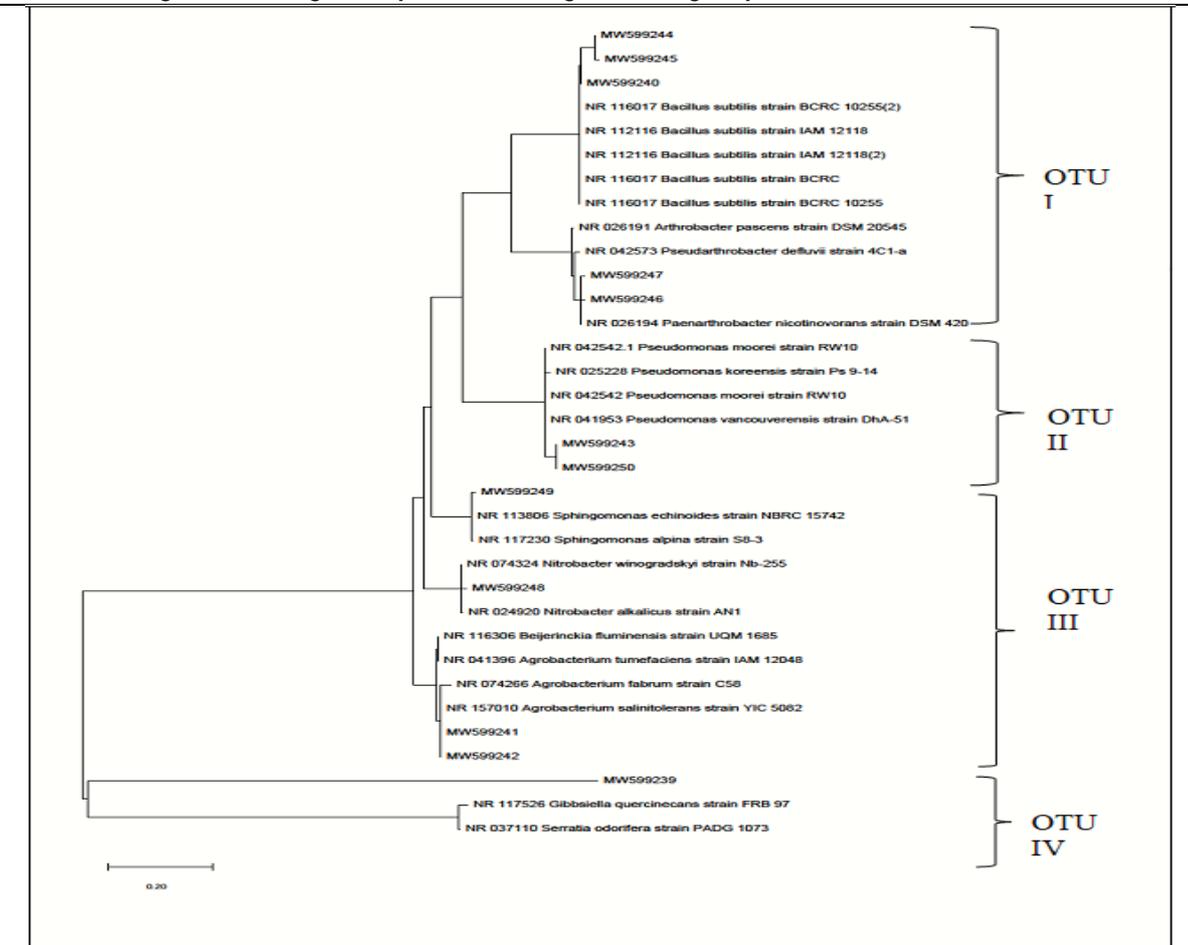


Fig 2. The evolutionary tree constructed based on nucleotide sequences gives the relatedness among representative isolates identified (accession numbers from MW599239 to MW599250 in the tree). Bar below tree represents the nucleotide substitution per time with a probability value 0.20.





## Hepatoprotective Activity of Ethanolic Extract of Leaves of *Sterculia foetida* Linn. Against CCl<sub>4</sub> Induced Hepatotoxicity in Rats.

Manivannan Ekambaram<sup>1\*</sup>, Arul Balasubramanian<sup>2</sup>, Kothai Ramalingam<sup>2</sup> and Vijayarangan Sivasamy<sup>3</sup>

<sup>1</sup>Department of Pharmacology, Vinayaka Mission's Kirupanandha Variyar Medical College and Hospitals, Vinayaka Mission's Research Foundation (Deemed to be University), Seeragapadi, Salem, Tamilnadu, India.

<sup>2</sup>Department of Pharmacy Practice, Vinayaka Mission's College of Pharmacy, Vinayaka Mission's Research Foundation (Deemed to be University), Salem-636 008, Tamilnadu, India.

<sup>3</sup>Department of Pharmacology, Trichy SRM Medical College Hospital and Research Centre, Tiruchirappalli, Tamil Nadu, India.

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### \*Address for Correspondence

#### Manivannan Ekambaram

Department of Pharmacology,  
Vinayaka Mission's Kirupanandha Variyar Medical College and Hospitals,  
Vinayaka Mission's Research Foundation (Deemed to be University),  
Seeragapadi Salem-636 308, Tamilnadu, India  
E.mail : manipoo73@gmail.com



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### ABSTRACT

Liver disorders are common all over the world, and they have traditionally been treated with medicinal plants, particularly in rural areas where they are readily available and affordable. The majority of traditional plants had yet to be scientifically validated. The aim of this investigation was to see if an ethanolic extract of the shade dried leaves of *Sterculia foetida* Linn. could protect Wistar rats from CCl<sub>4</sub>-induced hepatotoxicity. Biochemical parameters such as serum aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), total bilirubin, and gamma glutamate transpeptidase were used to assess the activity (GGTP). In the carbon tetrachloride-induced hepatotoxicity model, the extract displayed considerable dose-dependent hepatoprotective action at 250, 500, and 750 mg/kg, p.o. body weight, which was comparable to the control and activity exhibited by the reference standard Silymarin. The histology findings corroborated these observations.

**Keywords:** *Sterculia foetida*; Hepatoprotective; CCl<sub>4</sub> induced; Silymarin.



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## INTRODUCTION

The liver is the most essential vital organ in vertebrates, and it serves a variety of functions [1]. When it comes to xenobiotics, it plays an important role in the metabolism and excretion of these substances from the body. Every day, the risk of liver damage increases due to the increased exposure to harmful chemicals. This is especially true for children. Liver disorders are extremely serious health issues. Conventional medications are ineffective in the treatment of liver problems, according to research. Herbs are quite beneficial in the treatment of liver problems. As a result, the development of liver-protective herbal medications is extremely vital, and the need for such treatments is extremely urgent [2]. As of yet, there has been no scientific validation of the use of medicinal herbs in the treatment of liver ailments. There are a plethora of medicinal plants available in traditional medicine, and they are also used to treat various disorders, but there is no scientific proof to support this.

The soft woody tree *Sterculia foetida* Linn., which belongs to the family Sterculiaceae, can grow up to 35 meters in height. Originating in East Africa and North Australia, it now flourishes throughout the western peninsula, as well as in Burma and Ceylon. Known as Wild Indian almond, it has edible seeds that can be consumed after being roasted. The leaves of *Sterculia foetida* Linn. have traditionally been used for a variety of therapeutic purposes, including laxative, carminative, astringent, anti-inflammatory (3), antifungal (4), analgesic, anti-ulcer, liver disease, nausea, and vertigo. They are also used to treat a variety of other ailments. The present study focused to evaluate the hepatoprotective activity of ethanolic extract of leaves of *S. foetida* Linn (ESF) against CCl<sub>4</sub> induced hepatotoxicity in rats.

## MATERIALS AND METHODS

### Plant Material

Plant parts were collected from the foothills of Yercaud in Salem in the month of August 2020 and cleaned to remove any debris before being used. Dr. A. Marimuthu, a botanist, was able to identify and certify the plant that had been collected. Our museum has preserved a voucher specimen (SFM-1) for future use as a source of information. The plant parts were dried at room temperature for 10 days and then coarsely powdered with the aid of a hand-grinding mill and passing through sieve no. 60 to obtain the final product.

### Preparation of the Extract

*S. foetida* leaves powder was extracted separately by a continuous hot extraction process using a soxhlet apparatus with different solvents in increasing order of polarity from petroleum ether to chloroform, acetone, alcohol, and finally a mixture of chloroform and water [5] in a soxhlet apparatus. Following extraction, the extracts were concentrated in a tared vessel with reduced pressure after being filtered. The marc of crude drug powder was then subjected to a second round of sequential extraction with different solvents, with the extractive values computed in relation to the airdried drug as a reference. The dried extracts were subjected to a variety of chemical assays in order to determine the presence of various phytoconstituents in the plant.

### Test Animals

Wister rats of either sex and around the same age, weighing 150-175 g, were utilized in the study. They were housed in polypropylene cages and fed a regular chow diet, as well as unlimited access to freshwater. The animals were subjected to 12 h cycles of darkness and light, which were alternated each day. The animals have fasted for a minimum of 12 h before each experiment. Acute toxicity experiments were conducted on male mice weighing around 20-25 g each. In order to ensure that the experimental protocols were safe, they were reviewed and approved by the Institutional Animal Ethics Committee.



**Manivannan Ekambaram et al.****Hepatoprotective Study**

For the purpose of assessing hepatoprotective activity, the animals were divided into six groups, each of which had six animals. For the usual control group, I received 1 mL of propylene glycol orally once a day for seven consecutive days. Group II was served as a positive control and received 1 mL of propylene glycol after receiving CCl<sub>4</sub>. Groups III, IV, and V were given ethanolic extracts of *S. foetida*(EESF) at concentrations of 250, 500, and 750 mg/kg, p.o., for a total of seven days consecutively. Group VI received the reference chemical Silymarin 200 mg/kg, p.o., for a total of seven consecutive days. On the seventh day, 2 ml/kg, p.o. of CCl<sub>4</sub> [6] was delivered to all of the rats, with the exception of those in group I, 30 min after the last dose. After 36 h, blood samples were taken from all groups of rats using a cardiac puncture technique on non-anesthetized rats. According to previous reports, biochemical parameters such as ALT [7,] [8], AST [9], ALP, total bilirubin [10], total protein [11, 12], and GGTP were estimated. Following standard microtechnique [14], a small part of the liver was removed from each group of animals and stored in neutral buffered formalin before being processed for embedding in paraffin. 5 μ sections of the livers were stained with alum haemotoxylin and eosin and examined for signs of degeneration and necrosis.

**Statistical Analysis**

All results were reported as the mean ± the standard error of the mean. Newman Keul's multiple range test was used to statistically assess the data after the one-way ANOVA was performed on the data. Significant differences were defined as those with a p-value less than 0.05.

**RESULTS AND DISCUSSION**

The plant *S. foetida* was taken from the foothills of Yercaud, Salem, air-dried, and extracted using a continuous hot extraction process utilizing the Soxhlet apparatus. In this study, it was discovered that the average percentage yield of the ethanolic extract of *S. foetida* was 3.2 % w/w. A single intraperitoneal injection of the ethanolic extract did not cause any harmful effects in mice when supplied at a concentration of 5000 mg/kg. As a result, the LD<sub>50</sub> for the ethanolic extract of *S. foetida* was fixed as 5000 mg/kg.

When the biochemical parameters were examined, it was discovered that the enzyme levels in the CCl<sub>4</sub>-treated group had increased, indicating that CCl<sub>4</sub> has caused liver damage. Patients with acute and chronic hepatic disorders had more liver tissue that was high in both transaminase and aminotransferase. The AST level, which is slightly raised by cardiac necrosis, is a more specific sign of liver illness than the other markers (15). The levels of AST, ALT, ALP, GGTP, total bilirubin and total protein in the animals treated with the ethanolic extract of *S. foetida* were shown to be significantly lower than in the control animals. The enzyme levels were practically back to normal after the treatment. As a result, the animals treated with the ethanolic extract of *S. foetida* showed statistically significant (P<0.001) protection against CCl<sub>4</sub>-induced hepatotoxicity in rats, which was comparable to the reference chemical Silymarin in terms of protection. The outcomes of the biochemical studies are supported by histological studies. By the 8th day, the hepatotoxicity caused by CCl<sub>4</sub> had presented itself, with the liver exhibiting extensive degeneration engulfing the less obvious necrotic patches, as opposed to the normal liver condition. Compared to the liver sections from group IV rats, the liver sections from group IV rats were treated with the ethanolic extract revealed microvesicular alterations, including mild congestion and expansion of the sinusoids. There were no signs of necrosis present.

Carbon tetrachloride is one of the most commonly utilized hepatotoxins in the experimental study of liver disease, and it is also one of the most toxic. In part, the hepatotoxic effects of CCl<sub>4</sub> are attributable to the presence of its active metabolite, the trichloromethyl radical (15, 16). These activated radicals bond covalently to macromolecules and cause the peroxidative breakdown of membrane lipids of the endoplasmic reticulum, which are rich in polyunsaturated fatty acids, in the presence of oxygen. Lipid peroxides are formed as a result of this reaction. This lipid peroxidative breakdown of biomembranes is one of the primary reasons for CCl<sub>4</sub>'s hepatotoxic effects on the



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liver (17). This is demonstrated by an increase in the levels of serum marker enzymes such as AST, ALT, ALP, GGTP, total bilirubin, and total protein, among others. Estimation of serum transaminase levels provides a reasonably accurate picture of the liver's functional state in a functional investigation. An effective hepatoprotective drug is one that has the ability to either reduce the harmful effect of a hepatotoxin or restore normal hepatic function after it has been disrupted by a hepatotoxin. In groups III and IV, the extracts lowered CCl<sub>4</sub>-induced higher levels of the enzymes. This suggests a role for the extracts in preserving the structural integrity of the hepatocytic cell membrane or in the regeneration of damaged liver cells.

The liver sections of the rats that had been exposed to the toxicant had extensive centrilobular necrosis and vasculisation, as revealed by histopathological testing. The rats treated with extracts alone and then exposed to toxicants showed significant signals of protection against the toxicants, as evidenced by the creation of normal hepatic cords and the lack of necrosis and vacuoles in the livers of the animals. The fact that serum bilirubin decreased after treatment with extract in liver injury showed that the extracts were efficient in restoring the liver to its normal functional state.

**CONCLUSION**

As a result, the findings of the current study indicate that the ethanolic extract of *S. foetida* has a significant hepatoprotective effect. Further work is required to characterize the active hepatoprotective principle as well as the mechanism of action of this principle.

**REFERENCES**

1. Jesika R, Rajesh J, Bakel RL: Liver diseases and herbal drugs: A review. Journal of innovations in pharmaceutical and biological sciences 2016; 3(2):24-36.
2. Subramoniam A, Pushpagadhan P: Development of phytomedicines for liverdiseases. Indian Journal of pharmacology 1999; 31:166-75.
3. Naik DG, Mujumdar AM, Waghole RJ, Misar AV, Bligh SW, Bashall A, Crowder J. PlantaMedica. 2004; 70(1): 68-9.
4. Schmid KM, Patterson GW. Lipids 1988; 23(3): 248-52.
5. C.K. Kokate, Practical Pharmacognosy, 3<sup>rd</sup>Edn., VallabhPrakashan, New Delhi. pp. 107109,1994.
6. S. Mohideen, R. Ilavarasan, E.Sasikala and R. Thirumalaikumaran, Hepatoprotective activityof *Nigella sativa* Linn. Ind. J.Pharm. Sci., vol. 65, no.5, pp. 550551,2003.
7. S. Reitman and S. Frankel. Acolorimetric method for thedetermination of serum glutamateoxaloacetic acid and glutamicpyruvate transaminases. Am. J. Cl.Path., vol. 28, no.4, pp. 56-63,1957.
8. Armilage and G. Berry, Statisticalmethods of Medical Research,2ndEdn. Blackwell Scientific Publications, Oxford. Pp.186-192,1985.
9. P.R.N. Kind and E.J. King.Determination of serum alkalinephosphatase, Clin. Path., vol. 7,pp.322-326, 1954.
10. C.A. Burtis and E.R. Ashwood.Tietz Fundamentals of ClinicalChemistry, WB Saunders andcompany, Philadelphia. Pp.539552,1996.
11. J.G. Reinhold.StandardmethodsofClinicalchemistry. M. Reiner,1 StEdn, Academic Press, New York. pp.88-96, 1953.
12. R.J. Henry, D.C. Cannon and J.W.Winkelman. Clinical Chemistry2ndEdn. Harper and Row, New York. pp. 881-890, 1974.
13. G. Szasz. A kinetic photometricmethod for serum gammaglutamyltranspeptidase,Clin. Chem., vol. 15, no.2, pp. 124-36, 1969.
14. A.E. Galigher, E.N. Kozloff. Essential Practical Microtechnique, 2<sup>nd</sup> Edn., Lea and Febiger,Philadelphia. pp. 197-210, 1971.





**Manivannan Ekambaram et al.**

15. Sherlock S. Biochemical Assessment of Liver Function, Blackwell Scientific Publications, Oxford. 1981, 14-21.
16. Slater TF, Necrogenic action of CCl<sub>4</sub> in the rat: A speculative mechanism based on activation. Nature. 1966; 209 (18): 36-40.
17. Kaplowitz N, Aw TY, Simon FR, Stolz A. Drug induced hepatotoxicity, Ann. Int. Med., 1986; 104 (3): 826-39

**Table 1: Effect of ethanolic extract of *S.foetida* on CCl<sub>4</sub> induced hepatotoxicity in rats**

Treatment	Dose mg/kg,p.o.	AST U/L	ALT U/L	ALP U/L	GGTP U/L	TotalProtein mg/dl	Totalbilirubin mg/dl
Normal	1ml	112.17±4.49	40.17±1.88	174.33±6.53	112.67±4.41	5.93±0.06	0.47±0.003
Control(CCl <sub>4</sub> )	1.25ml/kg	185.50±4.98	110.67±3.27	268.67±5.22	240.50±3.51	2.77±0.10	0.98±0.02
EESF	250	153.33±5.37*	75.50±2.72*	209.83±8.11*	142.50±7.49*	3.81±0.14*	0.78±0.003*
EESF	500	128.50±6.72*	52.50±3.37*	196.33±7.49*	132.30±7.11*	3.95±0.16*	0.73±0.002*
EESF	750	130.83±5.49*	51.50±3.11*	193.83±8.12*	130.50±7.33*	4.08±0.17*	0.71±0.003*
Silymarin	200	121.67±4.90*	45.33±1.79*	182.50±8.41*	124.33±5.23*	4.62±0.03*	0.67±0.003*

\*P<0.001whencomparedwithcontrol.Numberof individuals used=6in each group .Days of drug treatment =7. Values are expressed as mean ± SEM.





## Graphene Based Polymer Nanocomposites as Packaging Materials: A Review

Niladri Sarkar<sup>1\*</sup>, Gyanaranjan Sahoo<sup>2</sup> and Anupam Sahoo<sup>1</sup>

<sup>1</sup>Department of Chemistry, Centurion University of Technology and Management, Odisha, India.

<sup>2</sup>Department of Basic Science and Humanities, Majhighariani Institute of Technology and Science, Bhubabala-765017, Odisha, India.

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### \*Address for Correspondence

**Niladri Sarkar**

Department of Chemistry,  
Centurion University of Technology and Management,  
Odisha, India.

E.Mail: niladri.sarkar@cutm.ac.in



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### ABSTRACT

Among different nanostructures, graphene analogues (graphene oxides, reduce graphene oxide and graphene) are in the limelight for their unique layer dimension with high aspect ratio, easy processability, ease of chemical modification, high mechanical, and thermal properties. Present review article summarizes some polymer nanocomposite materials, particularly their synthesis and structural analysis along with their applicability towards active packaging materials. Incorporation of graphene oxide (GO) to the polymer core via *in situ* polymerization approach and thereafter its conversion to reduced graphene oxide (r-GO) is generally performed to achieve uniform distribution of r-GO sheets. Graphene oxide also plays an important role in anchoring *in situ* formed metal nanoparticles on its extended surface with inhibiting of stacking behaviors. Therefore, the fabrication of graphene-based hybrid nanostructures has opened a new dimension in making of potent filler for polymer nanocomposites.

**Keywords:** Graphene oxide, nanocomposite, supercapacitor, dielectric properties, synergistic effect.

### INTRODUCTION

Packaging technology is a major part of the industry which takes care of our daily needs by controlling the safety storage and hygienic handling of the packed materials. With the progression of time, this technology has undergone in continuous modification to maintain the consumer's need. Among different packaging application, food packaging has its own importance as it deals with the essential needs of the human being. The two essential requirements for the food packaging are the safe maintenance of foods at packed condition from oxidation and microbial spoilage. The plastic materials like polyethylene, poly (tetrafluoro ethylene) which are well-known as

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“Teflon” materials have been widely used to preserve food for a longer period of time. These plastics are practically non-biodegradable and produce environmental pollution after disposal. Therefore, researchers are annoyed to use plastic materials. Moreover, they are trying to modify the plastics with bio-based materials for making them biodegradable. The use of natural biopolymers also introduces the biocompatibility to the materials. But biopolymers are poor in mechanical and gas barrier properties. Hence, these are not competent to the synthetic polymers in their neat form. Therefore, physical, or chemical modification of the biopolymer is required. Another issue of mechanical stability of the packaging materials is also addressed by the researcher to produce effective packaging materials. In connection to this, polymeric materials are used as composites with proper reinforcement. As compared to the micron sized reinforcement, nanostructure materials are offered unique physical and chemical properties to the composites, and it is because of their high aspect ratio [1]. Among different nanostructure-reinforcements, graphene and its derivatives are considered as the top priorities due to their high aspect ratio and electron mobility. Uniform distribution of graphene in polymer matrix is a challenging issue because of their strong Vander Waals interactions, operating between sheet structures. Therefore, researchers are using graphene oxide (GO) for preparation of different types of polymer nanocomposites due to existence of various oxygenated functional groups [2].

Graphene oxide is also used for fabrication of different multicomponent hybrid material due to its mechanical, optical, thermal, biological, and electronic properties [3, 4]. Graphene oxide is well studied in sensing [5, 6], electronics [7], catalysts [8], water cleaning [9] and biomedical applications [10]. Because of the ultra-thin layered nanostructure, graphene oxide (GO) is known to create efficient barrier against almost all gas molecules and can give a tough competition to other layered nanomaterials like clay [11], MoS<sub>2</sub> [12], h-BN [13] etc. Being the quasi-two-dimensional lattice with oxygen functionality (-OH, -O-, >C=O and -COOH), graphene oxide is well dispersible in aqueous medium and undergoes in physical interactions (H-bonding, hydrophobic interactions) with polymer matrix to achieve uniformly dispersed state. Currently, Pal et al. [14] has incorporated the reduced graphene oxide (r-GO) in poly lactic acid to achieve high anti-bacterial properties. Liu et al. [15] prepared the nacre-mimetic fibers of PAN-g-GO to achieve high mechanical strength. Graphene oxides are used for fabrication of several electronic devices. Reduced graphene oxide (R-GO) is used as chemical sensor and biosensors. Electrode materials in batteries and double-layered capacitors GO and r-GO have been used. GO is also used as fuel cells and solar cells. GO is used in the biomedical field, particularly in drug-delivery systems. GO is used to many other anticancer drugs because it does not target healthy cells, only tumours, and has a low toxicity. Huang et al. [16] took the advantages of layered structure and numerous oxygen containing functionalities (-OH, -COOH, -O-, etc) of graphene oxides to prepare nanocomposite films with polyvinyl alcohol (PVA). Due to the extensive chemical interactions (H-bonding) between PVA and GO, GO layers were observed to be exfoliated in PVA matrix and showed a declination of 98 % and 68 % to the oxygen and water permeability, respectively with incorporation of only 0.72 vol % of graphene oxide nano sheets.

Pinto et al. [17] fabricated the graphene oxide and graphene nanoplatelets incorporated poly(lactic acid) (PLA) nanocomposites through simple solution casting approach and observed a decrease in the gas permeability of PLA based nanocomposites with varying the graphene oxide (GO) and graphene nanoplatelet (GNP) contents. The normal oxygen permeability of PLA ( $3.76 \times 10^{-18} \text{ m}^2\text{s}^{-1}\text{Pa}^{-1}$ ) was effectively reduced to  $1.23 \times 10^{-18} \text{ m}^2\text{s}^{-1}\text{Pa}^{-1}$  and  $1.20 \times 10^{-18} \text{ m}^2\text{s}^{-1}\text{Pa}^{-1}$  with loading of only 0.4 wt % of GO and GNP, respectively. Above 0.4 wt %, oxygen permeability was slightly increased in PLA nanocomposites and the fact was attributed as the agglomeration of graphene-based fillers at higher content (Figure 1). The same pattern was also observed for mechanical performances of PLA nanocomposites. With 0.4 wt % of GNPs, the young modulus and yield strength were observed to be increased by 156 % and 129%, respectively. On the other hand, the young modulus and yield strength for 0.3 wt % of GO was increased to 115% and 95 %, respectively. In connection to this, PLA/GO and PLA/GNP, both the nanocomposites were stated to be good packaging material.

Poly(lactic acid) is also reported to form nanocomposite with incorporation of nanoclay by Chang et al. [18] where an intense two-fold decrease in the oxygen permeability was observed with 10 wt % of nanoclay loading. Hence,



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graphene-based filler showed better performance for the improvement of the oxygen barrier properties in the same polymeric matrix. Swain et al. [19] synthesized the expanded graphite (EG) reinforced polymethyl methacrylate (PMMA) nanocomposites via emulsifier free *in situ* emulsion polymerization techniques. The effective chemical interaction was studied by FTIR spectroscopy. A red shifting of the pure PMMA peaks ( $1745\text{ cm}^{-1}$  C=O and  $2956\text{ cm}^{-1}$  C-H) was observed in the PMMA/EG nanocomposites (Figure 2-a) which was accounted due to the strong chemical interaction between the polar functionalities of PMMA and EG. Transmission electron micrographs (Figure 2-b) showed the presence of flake like EG in the morphology of PMMA/EG nanocomposites. Due to such flaky morphology of PMMA/EG nanocomposites, it showed the 10 times reduction in the value of oxygen permeability with incorporation of 5 wt % of EG.

Ionita et al. [20] fabricated the graphene oxide (GO) incorporated sodium alginate (SA/GO) nanocomposite films. Simple solution casting approach was adopted to prepare SA/GO nanocomposite from their blend suspension. The graphene oxide content was varied as 0 to 6 wt %. Effective chemical interactions between sodium alginate and graphene oxide via intermolecular hydrogen bonding was confirmed by FTIR Spectroscopy, while XRD studies revealed the formation of intercalated nanocomposite structures. As graphene oxides are comparatively stable as compared to the sodium alginate, thermal and mechanical properties were observed to increase with incorporation of graphene oxide. The tensile strength and young modulus of SA/GO nanocomposite films with 6 wt% GO were increased from 71 MPa to 113 MPa and 0.85 GPa to 4.18 GPa, respectively (Figure 3).

Lim et al. [21] prepared the graphene oxide incorporated chitosan nanocomposites for packaging application. Simplified and modified Hummer's method was adopted to obtain large area ( $7000\text{ }\mu\text{m}^2$ ) and small area ( $50\text{ }\mu\text{m}^2$ ) graphene oxides which were subsequently reduced by the treatment of sodium hydroxides to obtain reduced graphene oxide (r-GO). Both type of r-GO was introduced in the chitosan matrix to prepare nanocomposite film and investigated for thermal, mechanical and antibacterial properties. The chitosan nanocomposite film with large area reduced graphene showed the better thermal and mechanical properties as compared to small area reduced graphene oxide. The better performance of high area reduced graphene oxide was anticipated as the restrictor of mobility of polymeric chains. Chitosan nanocomposite films with both type of reduced graphene oxide showed excellent antibacterial performance over *Pseudomonas aeruginosa*.

In another study, Yadav et al. [22] prepared the nanocomposite film with incorporation graphene oxide in the blend of two widely used biopolymers, named sodium alginate and carboxymethyl cellulose (CMC). The blend of these two biopolymers was prepared via means of simple solution mixing, containing 0.75 g of SA and 0.25 g of CMC. The nano graphene oxide was varied from 0.01–0.04 g. The mechanical properties of the SA/CMC/GO nanocomposite films were found to be improved, and the presence of GO augmented both the strength and stiffness of the hybrid nanocomposite films. The complete exfoliation of GO in SA/CMC matrix was observed upto 1 wt % of the GO content and the fact was supported by the complementary analysis of X-ray diffraction pattern and scanning electron micrographs. With incorporation of 1 wt % GO to the binary blend of sodium alginate and carboxymethyl cellulose, the mechanical behavior of the prepared SA/CMC/GO nanocomposites was significantly increased to 40 % for tensile strength and 1128 % for young modulus. The improved mechanical properties of the bionanocomposites were explained as the effective hydrogen bonding between graphene oxide, sodium alginate and carboxymethyl cellulose and the fact was confirmed by FTIR analysis.

Graphene analogues are effectively used to increase the gas barrier performance of polymer nanocomposites because of their layer dimensions. Depending on the nature of polymeric matrix and the distribution of graphene analogues within the polymer matrix, gas permeability was observed to vary significantly in different polymer nanocomposites. Some examples are summarized in Table 1. The recent literature on graphene-based polymer nanocomposites shows that graphene oxide and reduced graphene oxides are widely used improve the thermal, mechanical and barrier properties.



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## CONCLUSION

Graphene based nanostructures like graphene oxide, graphene itself and reduced graphene oxide are well-known as nano-reinforcement to the polymer matrix to improve the mechanical, thermal, gas-barrier and anti-bacterial properties of the pristine polymer. It possesses a unique layer dimension with high aspect ratio, easy processability, and ease of chemical modification. Incorporation of nanostructure intermediate like graphene oxide (GO) to the polymer core via *in situ* polymerization approach and thereafter its conversion to reduced graphene oxide (r-GO) is generally performed to achieve uniform distribution of r-GO sheets. Uniform distribution of graphene sheets help to minimize the porous morphology of neat polymer and therefore, increase the gas barrier behaviour which is an essential criterion for packaging application. Present review illustrates a short collection of graphene-based various polymer nanocomposites for their applications towards active packaging materials.

## REFERENCES

1. D. Pierleoni, Z.Y. Xia, M. Christian, S. Ligi, M. Minelli, V. Morandi, V. Palermo, Graphene-based coatings on polymer films for gas barrier applications. *Carbon* 96 (2016) 503-512.
2. Y. Wang, Z. Li, J. Wang, J. Li, Y. Lin, Graphene, and graphene oxide: biofunctionalization and applications in biotechnology. *Trends Biotechnol.* 29 (2011) 205-212.
3. C. Xu, J. Gao, H. Xiu, X. Li, J. Zhang, F. Luo, Q. Zhang, F. Chen, Q. Fu, Can in situ thermal reduction be a green and efficient way in the fabrication of electrically conductive polymer/reduced graphene oxide nanocomposites?. *Composites Part A: Appl. Sci. Manufact.* 53 (2013) 24-33.
4. J.S. Shayeh, A. Ehsani, M.R. Ganjali, P. Norouzi, B. Jaleh, Conductive polymer/reduced graphene oxide/Au nano particles as efficient composite materials in electrochemical supercapacitors, *Appl. Surf. Sci.* 353 (2015) 594-599.
5. Y. Chen, P. Pötschke, J. Pionteck, B. Voit, H. Qi, Smart cellulose/graphene composites fabricated by in situ chemical reduction of graphene oxide for multiple sensing applications. *J. Mater. Chem. A* 6 (2018) 7777-7785.
6. B. Zhang, J. Liu, X. Cui, Y. Wang, Y. Gao, P. Sun, G. Lu, Enhanced gas sensing properties to acetone vapor achieved by  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> particles ameliorated with reduced graphene oxide sheets. *Sens. Actuators B Chem.* 241 (2017) 904-914.
7. P. Chandrasekhar, Graphene applications in electronics, electrical conductors, and related uses. in *conducting polymers, fundamentals and applications*, (2018) 141-146. Springer, Cham.
8. A.A. Jeffery, S.R. Rao, M. Rajamathi, Preparation of MoS<sub>2</sub>-reduced graphene oxide (rGO) hybrid paper for catalytic applications by simple exfoliation-costacking. *Carbon* 112 (2017) 8-16.
9. L. Chen, Y. Li, Q. Du, Z. Wang, Y. Xia, E. Yedinak, L. Ci, High performance agar/graphene oxide composite aerogel for methylene blue removal. *Carbohydr. Polym.* 155 (2017) 345-353.
10. Z. Zang, X. Zeng, M. Wang, W. Hu, C. Liu, X. Tang, Tunable photoluminescence of water-soluble AgInZnS-graphene oxide (GO) nanocomposites and their application in-vivo bioimaging. *Sens. Actuators B Chem.* 252 (2017) 1179-1186.
11. S. Pandey, K.K. Jana, V.K. Aswal, D. Rana, P. Maiti, Effect of nanoparticle on the mechanical and gas barrier properties of thermoplastic polyurethane, *Appl. Clay Sci.* 146 (2017) 468-474.
12. W. Cai, J. Zhan, X. Feng, B. Yuan, J. Liu, W. Hu, Y. Hu, Facile construction of flame-retardant-wrapped molybdenum disulfide nanosheets for properties enhancement of thermoplastic polyurethane. *Ind. Eng. Chem. Res.* 56 (2017) 7229-7238.
13. T.Y. Kim, E.H. Song, B.H. Kang, S.J. Kim, Y.H. Lee, B.K. Ju, Hydrolyzed hexagonal boron nitride/polymer nanocomposites for transparent gas barrier film. *Nanotechnology* 28 (2017) 12LT01.
14. N. Pal, P. Dubey, P. Gopinath, K. Pal, Combined effect of cellulose nanocrystal and reduced graphene oxide into poly-lactic acid matrix nanocomposite as a scaffold and its anti-bacterial activity. *Int. J. Biol. Macromol.* 95 (2017) 94-105.





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15. Z. Liu, Z. Xu, X. Hu, C. Gao, Lyotropic liquid crystal of polyacrylonitrile-grafted graphene oxide and its assembled continuous strong nacre-mimetic fibers, *Macromolecules* 46 (2013) 6931-6941.
16. H.D. Huang, P.G. Ren, J. Chen, W.Q. Zhang, X. Ji, Z.M. Li, High barrier graphene oxide nanosheet/poly (vinyl alcohol) nanocomposite films. *J. Membrane Sci.* 409 (2012) 156-163.
17. A.M. Pinto, J. Cabral, D.A.P. Tanaka, A.M. Mendes, F.D. Magalhães, Effect of incorporation of graphene oxide and graphene nanoplatelets on mechanical and gas permeability properties of poly (lactic acid) films. *Polym. Int.* 62 (2013) 33-40.
18. J.H. Chang, Y.U. An., G.S. Sur, Poly (lactic acid) nanocomposites with various organoclays. I. Thermomechanical properties, morphology, and gas permeability. *J. Polym. Sci. Part B: Polym. Phys.* 41 (2004) 94-103.
19. S.K. Swain, G. Prusty, A.S. Ray, L. Behera, Dispersion of nanoplatelets of graphite on PMMA matrix by in situ polymerisation technique. *J. Exp. Nanosci.* 9 (2014) 240-248.
20. M. Ionita, M.A. Pandele, H. Iovu, Sodium alginate/graphene oxide composite films with enhanced thermal and mechanical properties. *Carbohydr. Polym.* 94 (2013), 339-344.
21. H.N. Lim, N.M. Huang, C.H. Loo, (2012). Facile preparation of graphene-based chitosan films: Enhanced thermal, mechanical and antibacterial properties. *J. Non-Crystalline Solids* 358 (2012) 525-530.
22. M. Yadav, K.Y. Rhee, S.J. Park, Synthesis and characterization of graphene oxide/carboxymethylcellulose/alginate composite blend films. *Carbohydr. Polym.* 110 (2014) 18-25.
23. S. Mahmoudian, M.U. Wahit, M. Imran, A.F. Ismail, H. Balakrishnan, A facile approach to prepare regenerated cellulose/graphene nanoplatelets nanocomposite using room-temperature ionic liquid. *J. Nanosci. Nanotechnol.* 12 (2012) 5233-5239.
24. K.C. Chang, W.F. Ji, M.C. Lai, Y.R. Hsiao, C.H. Hsu, T.L. Chuang, W.R. Liu, Synergistic effects of hydrophobicity and gas barrier properties on the anticorrosion property of PMMA nanocomposite coatings embedded with graphene nanosheets. *Polym. Chem.* 5 (2013) 1049-1056.
25. K. Fukushima, H. Kalaitzidou, L.T. Drzal, Multifunctional polypropylene composites produced by incorporation of exfoliated graphite nanoplatelets. *Carbon* 45 (2007) 1446-1452.
26. G. Prusty, S.K. Swain, Synthesis, and characterization of conducting gas barrier polyacrylonitrile/graphite nanocomposites. *Polym. Compos.* 32 (2011) 1336-1342.
27. J. Zhu, J. Lim, C.H. Lee, H.I. Joh, H.C. Kim, B. Park, S. Lee, Multifunctional polyimide/graphene oxide composites via in situ polymerization. *J. Appl. Polym. Sci.* 131 (2014).

**Table 1 Oxygen permeability reduction of graphene-based polymer nanocomposites**

Name of Composites	Filler	Methods of Preparation	Reduction in Oxygen permeability	References
Cellulose/GNPS	5 wt%	Solution casting	□27	[23]
PMMA/Graphene	0.5wt%	<i>In situ</i> polymerization	□70	[24]
PP/EGNPs	3 vol%	Melt mixing	□20	[25]
PAN/EG	4 wt%	<i>In situ</i> polymerization	□92	[26]
PI/r-GO	30 wt%	<i>In situ</i> polymerization	□93	[27]





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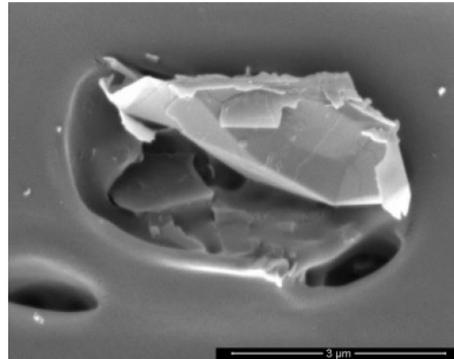


Figure 1. SEM image of GNP after dispersion in PLA matrix [17].

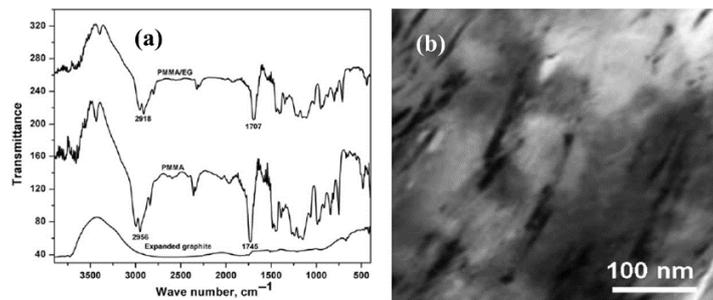


Figure 2. (a) FTIR spectra of EG, PMMA and PMMA/EG nanocomposites (5 wt%) and (b) TEM micrograph of PMMA/EG nanocomposites (5 wt%) [19].

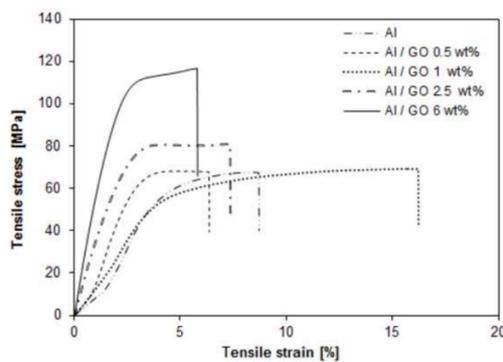


Figure 3. Stress-strain curves for SA and SA / GO films as a function of GO content. [SA in this image is represented as Al] [20].





## Evaluating the Performance of Signalized Intersection and the Associated Economic Impact of Congestion: (A Case Study on Cuttack Road to Ravi Talkies Road, Bhubaneswar)

Haraprasad Behere<sup>1</sup>, Biswajit Mohanty<sup>2</sup>, Biswajit Jena<sup>3</sup> and Sagarika Panda<sup>3</sup>

<sup>1</sup>Student of Civil Engineering Department, Centurion University of Technology and Management, Odisha, India.

<sup>2</sup>M.Tech Transportation Engineering, Centurion University of Technology and Management, Odisha, India.

<sup>3</sup>Asst. Prof. of Civil Engineering Department, Driems, Cuttack, Odisha, India.

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### \*Address for Correspondence

#### Haraprasad Behere

Student of Civil Engineering Department,  
Centurion University of Technology and Management,  
Odisha, India.

Email: haraprasadbehera6@gmail.com



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### ABSTRACT

Now days, traffic congestion is becoming a serious issue in developing countries in general and in Bhubaneswar in particular. Its major impact on the day-to-day activities of road users is not limited to delay in travel time but also it affects the consumption of fuel and the surrounding environment in terms of greenhouse gas emissions. In Bhubaneswar, the issue is becoming a great concern to its residents, transport managers and planners. In light of the interest in testing the performance and optimization of Signalized Intersection timing plan on major route corridor of Bhubaneswar, this study was designed and conducted on Cuttack road to Ravi Talkies square between the JHARPADA & KALPANA, as it is the most signalized route corridor in the city and serves the city's major traffic population. Based on Highway Capacity Manual (HCM), the current performances of signal operation on the route corridor were assessed along with the cost of congestion results from Travel Time Lost, Extra Fuel Consumption and Environmental Pollution costs. In addition, using Synchro 9 Simulation Software, the Intersection signal timing plan was optimized with respect to network cycle length, offset and splits. The result of performance evaluation indicated that all the Signalized Intersections under this study serve the traffic demand with poor level of service (LOS F) resulting in costs to the Country amounted to ETB:1,191,442.76 per hour due to extra fuel consumption and travel time loss and also affected by pollutions with emission rate of 31,668.64 g/h of NO<sub>x</sub>;206,796.22 g/h of HC; 2,874,467.45g/h of CO;3, 736, 807.68g/h of PM<sub>2.5</sub> and 134,375, 604.21g/hofCO<sub>2</sub> attributed by the on roads traffic on the study segment. The simulation analysis



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for optimization shows that service quality at intersections can be improved by changing the current operating cycle length of 188sec to 270sec for Ravi Talkies Intersection and 230sec for all other Intersections in the network, together with phase sequences and splits. As a result, more than 550 vehicles will be relieved from stopping at intersections and also extra 654 liters of fuel consumed by the vehicles per hour will also be saved owing to improvement of signal timing.

**Keywords:** Level of Service, HCM Manual, Synchro Software, Congestion, Optimization, Signalized Intersection

## INTRODUCTION

### General

The general objective of the study was to examine the service quality of junctions' especially signalized intersections and evaluate the impact of congestion in the city of Bhubaneswar. For the accomplishment of stated objectives, all necessary data for signal evaluation, for congestion cost estimation were identified.

### Need of Study

- Currently, the increase in traffic volume of roadways has led transport expertise to explore different means of control mechanisms. Among the different control mechanisms, traffic signals are the most preferable in urban areas where there is right of way limitation and highly accident prone roadway junctions. However, the inappropriate traffic forecasting and/or installation of traffic signals can actually result in an extended long queue, traffic crash, congestion and delays on the road ways. In this connection, the day to day activities of the urban residents has been negatively affected and this effect has also direct impact on the nation's economy.
- In the city of Bhubaneswar, the effect of traffic congestion with regard to limited mobility of road uses, fuel consumption and productivity loses due to travel time delay are significant especially, at the city's major intersection. Consequently, the sustainable development and satisfaction of road users of the city's population has been significantly affected.
- This being the case, to utilize effective and efficient flow of traffic and to overcome the associated economic impact, there is a need to assess the possible causes of traffic jam, updating the traffic signal timing plan.

### Objective and Scope of Study

The specific objectives of this study were to evaluate the performance of signals, optimizing timing plans and its economic impact in the city of Bhubaneswar; by focusing on a case study on Cuttack road To Ravi Talkies square, in relation to constraint analysis of congestion, fuel consumption, level of service and outline a framework on the performance of the route. Particularly, the study has the following specific- objectives:

- To evaluate the performance of each isolated signalized intersection on Cuttack road to Ravi Talkies square;
- To determine the cost of congestion due to jammed traffic; and
- To optimize the road network (i.e. Cuttack road to Ravi Talkies square) signal timing.
- The scope of the study covers performance analysis of the Cuttack road to Ravi Talkies square of Bhubaneswar, considering all physical and timing parameters, mainly focused on the existing travel flow patterns. An evaluation framework was presented for performance analysis based on HCM, 2010 methodologies. Performance analysis was manipulated using Microsoft excel. Synchronization of traffic signals has been done using SYNCHRO version-9 micro simulation software which is the most preferable and recommended software by FHWA of U.S Department of Transportation. Further, Costs relating to traffic congestions were formulated from field data and software output.
- Nevertheless, the existing traffic flow pattern, driver behavior in relation to perception reaction time and red





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signal time enforcement, economic development of area and land use pattern can vary from corridor to corridor in the city and it would be difficult to transfer to other routes.

- The collection of data was a challenging task as the research area involves mixed traffic population. In the end, conclusions were presented and recommendations were formulated based on the major findings of the research.

## Literature Review

### General

The report consists of five chapters. Following this chapter of Introduction, chapter two deals with Literature Review revising the works that have been done in the area of traffic signal coordination with respect to selection of best simulation model, signal timing plan evaluation and performance parameters considered during analysis, design and road way congestion impacts and costs. Chapter three focuses on research approach and plan to assess the performance evaluation. The framework includes the data collection methods together with selection of appropriate simulation model. In addition, Chapter four focuses on results and discussions with respect to the Signal Timing performance evaluations, Signal Timing Optimizations and congestion cost estimations. The last chapter, Chapter five summarizes the major conclusions of the research and identifies the areas of future research. At the end future research areas, as found relevant have been proposed.

## Literature Review

### Signalized Intersection

Intersections in urban roadways form bottlenecks. Accordingly, to accommodate the traffic and to eliminate the queue formed on that limited space, different junction types are considered to be provided in accordance with warrants in the Manual on Uniform Traffic Control Devices (P.Roess,2004). From those junctions control forms signalized intersection is the ultimate control method for at grade intersection. Signals can increase the traffic capacity of the intersection, reduce frequency and severity of crashes, particularly right-angle crashes, and interrupt heavy traffic flow to permit other motor vehicles, pedestrians and bicycles to cross the to Ravi Talkies square. Signalized intersection is an operational strategy which facilitates the movement of traffic on the intersections by reducing crossing accidents and giving pedestrian safe feeling.

Unwarranted or poorly timed traffic signals can have negative impacts, including excessive delay to vehicular and pedestrian traffic, disrespect for traffic control devices in general, increased "cut through" traffic on inappropriate routes, and increased frequency of crashes (MassDOT, 2012). At signalized intersection, signal time is mounted to reduce, eliminate and segregate the potentially conflicting movements. The signal design incorporates cycle and phase parameters. The cycle is a time required for one complete sequence of signal indicators and a phase is a portion of cycle allocated to any combination of traffic movement (Tarekegn Kumala, 2016). The two most important features considered during evaluation of signalized intersection are capacity and delay. Capacity is the maximum rate of flow where the vehicle is expected to traverse a road way under prevailing traffic, control and road way condition whereas; delay is the additional time experienced by the driver beyond the time required to traverse a road way (Bhosale, 2016). Delay and level of service are an indication of the potential capacity and performance measure of an intersection and it is found that the main contributory factors for moderate traffic congestion in the same area is the allocation of long cycle time and inappropriate phase plan at the intersections (Tarekegn Kumala, 2016).

In order to conduct an operational analysis of signalized intersection geometric, traffic and signalization data are required. The geometric conditions includes area type, number of lanes, average lane width, grade, existence of exclusive left turn or right turn lanes, length of stoppage bay left turn or right turn lanes and parking. Traffic conditions also includes demand volume by movement, Base saturation flow rate, Peak-hour factor, Percent heavy vehicles, Approach pedestrian flow rate, Local buses stopping at intersection, Parking activity, Arrival type, Proportion of vehicles arriving on green and Approach speed. In addition, the input parameters of Signalization conditions includes Cycle length, Green time, Yellow-plus-all-red change-and- clearance interval, Actuated or pre-timed operation, Pedestrian push-button, Minimum pedestrian green, Phase plan and Analysis period (HCM,2010).





### Level of service

Level of Service (LOS) is somewhat a subjective indication of the general acceptability of delay to drivers and directly related with average control delay and volume to capacity ratios. When delay levels are unacceptable but v/c ratios are relatively low, the cycle length and/ or the phase plan may be inefficient (Koonce, 2008). It should be noted, however, that when signals are part of a coordinated system, the cycle length at individual intersections is determined by system considerations, and alterations at isolated locations may not be practical. When both delay levels and v/c ratios are unacceptable, the situation is critical. Delay is already high, and demand is near or over capacity. In such situations, the delay may increase rapidly with small changes in demand. The full range of potential geometric and signal design improvements should be considered in the search for improvements (HCM, 2010). LOS is average control delay per vehicle in a lane group. The average control delay of the intersection is estimated by aggregating average delay weighted by adjusted flow of intersection. The adjusted flow of intersection ( $v_p$ ) is the maximum hourly flow rate based on the full hourly volume.

Where,

$v_p$ = Adjusted flow of intersections

$V$ = Traffic Volume

PHF= Peak Hour Factor

### Delay

Traffic delays and queues are principal performance measures of intersection LOS, in the evaluation of the adequacy of lanes, and in the estimation of fuel consumption and emissions. The delay measures include the Control Delay, the Queue Delay and the Total Delay. The Control Delay is uniform delay plus the impacts of coordination and incremental delays. Queue Delays are the additional delay caused by reduction in capacity due to spillback and starvation. Control delay mainly affected by cycle time, ideal saturation flow, inters green time, number of phasing, number of lane, and left turn on red (LTOR) (Abojaradeh,2014). Total delay includes delay introduced by the presence of the signal (control delay) and other delay due to various roadway and traffic characteristics. Control delay includes deceleration as the vehicle approaches a queue or a red signal indication, acceleration away from the signal to get back to free flow speed, and the time spent stopped at a red indication (Avdiu,2013). Control delay of signalized intersection is the weighted average of delay of each approach where the approach delay is also the weighted average of delay of each lane group. In addition, the average control delay per vehicle is determined by HCM Methodology for Signalized Intersection (HCM,2010).

$$d = d_1(PF) + d_2 + d_3$$

where,

$d$  = control delay per vehicle;

$d_1$ = uniform control delay assuming uniform arrivals (s/veh);

$c$ = cycle length (s); cycle length used in pre-timed signal control, or average cycle length for actuated control

$g$  = effective green time for lane group (s)

$x$  = v/c ratio or degree of saturation for lane group

PF = uniform delay progression adjustment factor

$P$  = proportion of vehicles arriving on green

$f_{PA}$  = supplemental adjustment factor for platoon arriving during green

$d_2$ = incremental delay

$T$  = duration of analysis period (h)

$k$  = incremental delay factor that is dependent on controller settings

$I$  = upstream filtering/metering adjustment factor

$C$  = lane group capacity (veh/h)

$x$  = lane group v/c ratio or degree of saturation

$d_3$ = initial queue delay

$Q_b$  = initial queue at the start of period  $T$  (veh)





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c = adjustment lane group capacity (veh/h)

T = duration of analysis period (h)

t = duration of unmet demand in T (h)

u = delay parameter

The study also used the above Highway Capacity Manual (HCM) methodology in order to evaluate the signal timing.

### Essentials of signal coordination

Providing effective real time traffic signal control for a large complex network is an extremely challenging task. Hence, in order to get efficient movement of traffic on to Ravi Talkies squares and highways, optimization of traffic signals is required (Shamshirband, 2008). Using integration microscopic traffic simulation and assignment model the traffic signal re-timing by coordinating isolated signals is essential in order to reduce the variance of the speed, total delay, stopped delay, acceleration and deceleration delay and average crash risk (H. Rakha, 2016).

### Overview of signal Coordination Design

Even if traffic signals are one of the most effective and flexible active traffic control mechanisms that require simple geometric design, it causes large stopped delays, and complexity in the design and implementation. The three parameters that determine the coordination of signalized network are cycle length, offset, and split. Optimization of the network signal timing plan (cycle, splits, and offsets) can be handled by Synchro software. However, when the computer model is not available, the following procedures are suggested by FHWA (Koonce, 2008). Synchro 9 software is also preferred for this study.

a) Calculate cycle length for each primary intersection using Webster equation;

Optimum Cycle Length =  $(1.5 * L + 5) / (1.0 - Y)$

b) Determine the minimum pedestrian cycle length: Pedestrian Minimum Cycle =  $LT + 14 + Wm / SP + Wc / SP + Y$

c) Using the average distance between intersections, calculate the potential resonant cycles; Cycle =  $2 * \text{Distance} / \text{Speed}$ ; Cycle =  $4 * \text{Distance} / \text{Speed}$ ; Cycle =  $26 * \text{Distance} / \text{Speed}$ ; Cycle =  $8 * \text{Distance} / \text{Speed}$  and then select the shortest resonant cycle. The cycle time should be max (optimum Cycle Length, Pedestrian Minimum Cycle and shortest resonant cycle)

d) Calculate the offset using Kell method

e) Split is calculated for the primary intersection using the Critical Movement Method.

Phase difference plan is a better tool in order to coordinate traffic signals for closely spaced intersection having 1000m center to center distance by adopting uniform cycle length. On this study, two different phase plans are designed depends on average speed of vehicle, distance, geometric properties of link and assigned to all consecutive intersections (Bhosale, 2016).

### Traffic Congestion

Traffic congestion becomes a serious problem at road intersections. The costs due to unproductive time loss, extra fuel consumption, accidents, environmental pollution are the major problems accounted for in relation to traffic congestion. On rush hours, the intersections cause long queue which imposes economic impacts on the urban dwellers in many aspects (Agajie, 2015). Mekonnen, 2012, mentioned traffic congestion is the most annoying feature which substantially consumes travel time, creates difficulty for scheduling and on time delivery and occurs at times of high travel demand. Sugiyanto, 2010, on the study on estimation of Congestion Cost of Private Passenger Car Users in Malioboro, Yogyakarta, has developed a model for estimating congestion cost considering that congestion cost is a generalized cost (i.e. vehicle operating cost, travel time cost and pollution cost) in actual condition (8 km/h) less in perceived condition (30 km/h). However, the study is limited to Private Passenger Car. Side effects of traffic congestion give rise to various resource costs that can be expressed in monetary terms: time costs of delays, health costs caused by air pollution, productivity losses due to lives lost in traffic accidents, abatement costs due to climate impacts of transport, etc. In this study, the two major delay related costs; cost of travel time and Cost of Extra Fuel





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Consumption will be estimated (Institute,2017). Mahmud, 2012, used different approach to estimate the congestion cost. He gave estimates of congestion cost for different vehicle and carriage way type considering fuel and travel time cost as a major congestion related cost.

### **Congestion Cost**

#### **Cost of Travel Time Lost**

The value of travel time is a critical factor in evaluating the benefit of transportation infrastructure investment and saving of it mainly depend on the condition of the trip (US Department of Transportation, 2011). Travel time savings are major benefits arising from transport infrastructure and its estimation in a monetary value is important in order to forecast level of traffic which is attracted and to include so value of savings in cost benefit analysis (Bank, 2016). The value of time lost is an opportunity cost caused by waiting at congested road intersection. The lost time may be either working time or nonworking time. The lost time is determined from the hourly income of individuals, average vehicle occupancy, the number of vehicles delayed and average waiting time as developed below (Agajie,2015).

$$C_T = L_d * W_d * t_a * V_p$$

Where:  $C_T$  = Cost of time lost per hour, ETB;

$L_d$  = Difference in number of vehicles waiting in the system;

$W_d$  = Difference in average waiting time per vehicle;

$t_a$  = Average value of time, sec; and  $V_p$  = Vehicle occupancy, pass/veh

#### **Cost of Extra Fuel Consumption Due to Waiting at Signalized Intersections**

Most drivers are less aware of fuel their vehicles consume while traversing a short segment of road but it is important to measure the effectiveness of signalized intersection. The impact of extra fuel consumption on the city economy is significant if it is estimated over multiple vehicles and forecasted to a given service time. The amount of fuel consumption is estimated by the PC- travel software based on the velocity and acceleration of vehicle (Meyer, 2010). Abajie (2015), stated that the cost of fuel lost is rapidly increasing if vehicles are waiting longer time. According to him, the following are considered to estimate the cost of extra fuel consumed by the vehicles: determination of differences in waiting time, number of vehicles waiting, average fuel consumption of single vehicle and the unit cost of fuel are required. In addition, he illustrated in his research that about ETB: 560,000 is wasted due to consumption of extra fuel while vehicles are delayed at junctions for about 6 minutes. In order to determine this cost used the following mathematical equation (Abajie, 2015).

$$ECEFC = C_u * F_c * w_d * L_d$$

Where;

ECEFC= Expected Cost of Extra Fuel Consumed per hour;  $C_u$  = Unit cost of fuel, ETB;

$F_c$ = Extra fuel consumed for one vehicle per hour;

$w_d$  = Difference in average waiting time at the road Junction; and

$L_d$  = The difference in number of vehicles waiting at the road junction.

#### **Emission from in road Vehicles**

There are no emissions standard that vehicles for both new and used vehicles. For this reason, even newer vehicles emit severe exhaust smoke pollution because they enter the country without the modern Emissions Control Technology (ECT) installed. In line with the vehicle usage in Bhubaneswar, many vehicles are not maintained and are actually overloaded, thus these conditions worsen vehicle exhaust smoke emissions (Shankute,2015). The average age of vehicle fleet is 15-20 years. Vehicles are considered to be second- hand ten years after their production date, compared to the global norm of four years after production. The rate of growth of used vehicles registration is greater than that of brand new ones. Excessive import of used vehicles would by far contribute to CO<sub>2</sub> emission, as used vehicles consume more fuel than brand new (Dr. Thomas Schiller, 2017). There are two types of GHG emission estimation method. One is Fuel consumption base (IPCC,USA, Japan), and the other is Vehicle traveling kilometer base (Australia). But most countries have used methods developed on the basis of the IPCC Guideline. For this study the IPCC guideline were used (Jiun-Horng Tsai, 2010).



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## MATERIALS AND RESEARCH METHODOLOGY

### Research Methodology

This chapter will present the materials and research methodology used in this research. This provides framework in which this research was carried out and presented. The knowledge gained from the literature review and the HCM, 2010 methodology in the area of Signalized Intersection and past research carried out in the field of traffic flow problem on multi-lane intersection (Economic analysis), form the main building block for this evaluation framework. This chapter focuses on creating that understanding to formulate a framework for the performance evaluation together with cost estimation of the signalized intersection. In addition, modeling and evaluation using Synchro software has been discussed.

### Study area description

The high economic growth and growth of residential, business and settlements is leading the city to Ravi Talkies squares to overcrowding by traffic jams. The cuttack road to Ravi Talkies square of Bhubaneswar is the Principal Arterial Road with two lanes in each flow direction and is one of the main corridors of the city which serves the east-west traffic of the city in a jammed situation. The Ravi Talkies square is one of the route corridors in the city with high traffic density along the East to West corridor. This study covers the Cuttack road to Ravi Talkies square of Bhubaneswar. The corridor includes three major signal controlled intersections. In addition, because of its great significance during optimization, the Kalpana Signalized intersection has also been included in this study.

### Method of data collection

#### Traffic Data

Traffic flow count is categorized into six classes by visual assessment of the vehicle sizes, Corresponding passenger car equivalent values were considered as depicted in Table below. Table below depicts the vehicle types and passenger Car unit (PCU) considered in this study (Andrew, 2003).

From the collected traffic data, it was noted that the route corridor serves a peak flow during 10/10/2017 and 11/10/2017; which, for Cuttack road and Jharpada Intersection flow 10/10/2017 during morning peak period and for Jharpada Square and KALPANA Intersections Night is a peak period of traffic flow on 11/10/2017.

### Travel time and fuel consumption data

The conditions of travel time together with fuel consumption at congested intersections were analyzed from questionnaires distributed to road users that pass the Cuttack road to Ravi Talkies square. The questionnaire sheet include: job, income, purpose of trip, number of trips per day, travel speed, travel time lost and characteristics of respondents. In addition to the above, amount of fuel consumed on the study route and users understanding on source of congestion were also included in questionnaires. The respondents were selected using cluster sampling strategies because it is better to organize the collected data into clusters like passengers, drivers and private car owners. In connection, the sample size was determined based on National Education Association formula as presented by Krejcie (2012) on his paper on determining sample size for research activities as follows; Accordingly, about 200 copies of the questionnaire were prepared and distributed to road users, of which 100 for passengers and 100 for drivers and private car owners. From the distributed questionnaires, 60 from drivers and private car owners and 59 from passengers were returned.

### Emission

Inroad vehicles emission can be measured directly by directing the vehicles the road side test locations and also using measuring instrument that measure component of atmosphere. Using vehicles emission control technologies (ECT) fixed with the vehicles and equipment like wayer smoke opacity of vehicles can be estimated (Shankute, 2015). Determination of number vehicles is not sufficient to estimate vehicle emissions. Depending on the fuel type used in





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vehicles amount of pollutants vary. The number of gasoline vehicles registered in Ethiopia becomes constant from 2002-2009 with a share of 27.7% and 28.1% in the first and last four years of the aforementioned year respectively from the total registered vehicles. The proportion of vehicles estimated by AAiT should also be used in this study (AAiT, 2012). In addition to the above, the other important parameter while determining amount of the pollutants is emission factor. Emission factors depend on type and compound of the fuel and ignition system. Taking into considerations of those factors Hamit SOLMAZ have determined emission factors depending on types of vehicle for gasoline and diesel fuel (SOLMAZ, 2012). Neway (2016), in his study on Investigation on Pollution Caused by Gasoline and Diesel fuelled Vehicles determined emission factors for Ethiopian conditions. In the study, lots of parameters were taken into account such as, fuel consumption, age of vehicles, average vehicles kilometers traveled in a year and accordingly CO, NOx, CO<sub>2</sub>, PM and HC pollutants emission factors were determined. According to his study, the highest level of NOx emissions were estimated at 3.44 g/km, HC emissions at 6.53 g/km, CO at 13.9 g/km, PM<sub>2.5</sub> at 1.3 g/km and 35.96 g/km of CO<sub>2</sub>. As the research was relatively current the estimated emission factors were used in this study. Having this data the emission from vehicles is estimated by multiplying average vehicles kilometers traveled by emission factor.

## RESULTS AND DISCUSSIONS

### Cost of extra fuel consumption

The total cost of travel time lost from Cuttack road to Ravi Talkies section was estimated to be **ETB: 1,163,916.56** per hour. To calculate extra fuel consumption due to waiting at congested section, the following parameters were required as inputs;

### Average fuel consumptions of single vehicle per hour

Traffic congestion leads to excess fuel usage due to time spent idling in jam and the start-and- stop nature of travel in congested conditions, as travel at a lower speed consumes more fuel. Almost all vehicles operate with fuel efficiency when traveling between 50 and 80km/hr. Otherwise, vehicles consume increasingly more fuel the faster or the slower they travel. In this regard, the following chart was developed by Ardina (2006) to estimate the amount of fuel burned by vehicle with respect to vehicle speed. Ardina (2006) mentioned that for every 10 minutes of idling time, the car consumes around 100ml of petrol. In this study the same was adopted.

### Cost of fuel per liter

Market value of fuel per liter was obtained from gas stations. For the Bhubaneswar condition, the cost of gasoline is ETB: 16.20.

The parameters used to calculate cost of travel time lost were described above. Accordingly, the same formula was used to determine the values in this case.

### Cost of extra fuel consumption (Cf)=Vn\*Vt\*Fv\*Cfl

Where;

Cf=Cost of extra fuel consumption;

Vn=Number of vehicles waiting in the system;

Vt =Average waiting time per vehicle (sec);

Fv=Average fuel consumptions of single vehicle per hour; and

Cfl=Cost of fuel per liter.

The cost of extra fuel consumption from Cuttack road to Ravi Talkies road section was estimated to be **ETB: 27,526.20** per hour.

### Emission

For the entire road segment has been covered by vehicles in peak one hour period. This parameter was directly taken from Synchro software. Having this data emission is calculated by;

Emission=EF\*VKT





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Where;

EF= Emission factors; and

VKT = vehicle kilometer travel

Therefore, from Cuttack Road to Ravi Talkies road section on road vehicles emit 31,668.64 g/h of NO<sub>x</sub>, 206,796.22 g/h of HC, 2,874,467.45 g/h of CO, 3,736,807.68 g/h of PM 2.5 and 134,375,604.21g/h of CO<sub>2</sub>. Hence, the overall costs of congestion were estimated by adding **Cost of travel time lost (C<sub>tt</sub>)** and **Cost of extra fuel consumption (C<sub>f</sub>)**. Based on this specific study, region it was estimated that the country lost **ETB: 1,191,442.76** per hour from extra fuel consumption and travel time lost. In addition, it is also affected by pollution with emission rate of 31,668.64 g/h of NO<sub>x</sub>, 206,796.22 g/h of HC, 2,874,467.45 g/h of CO, 3,736,807.68 g/h of PM<sub>2.5</sub> and 134,375,604.21g/h of CO<sub>2</sub>.

## CONCLUSIONS

This research focused on analyzing the impacts of improperly allocating traffic signal timing of Signalized Intersections on the flow of traffic and the effect of traffic congestion on road vehicles. The case study was the Cuttack road to Ravi Talkies square in Bhubaneswar. For performance evaluation, the Highway Capacity Manual (HCM) was used and also for optimization of signal timing of Signalized Intersections, the Synchro software was used. In addition, the effect of congestion was estimated in monetary values with respect to the following parameters;

- Cost of extra fuel consumption; and
- Cost of travel time lost.

Further, the amount of emission from inroad vehicles with respect to NO<sub>x</sub>, HC, CO, PM<sub>2.5</sub> and CO<sub>2</sub> were also estimated.

Accordingly, the following results were obtained:

- The traffic flow at all intersections of the route corridor was served by poor Level of Service (LOS F).
- The results obtained using the HCM methodologies and Synchro software with respect to performance evaluation were almost the same.
- The saturation flow rate of the route corridor was high as compared to HCM default value which is mainly arising from aggressive nature of drivers.
- Due to the effect of congestion on extra fuel consumption and travel time lost, the country loses ETB:1,191,442.76 per hour due to traffic jam on the route corridor.
- Due to the effect of congestion, the country is subjected to pollution with emission rate of: 31,668.64 g/h of NO<sub>x</sub>, 206,796.22 g/h of HC, 2,874,467.45 g/h of CO, 3,736,807.68 g/h of PM<sub>2.5</sub> and 134,375,604.21 g/h of CO<sub>2</sub>.
- The service quality of the Intersections can be improved by changing the cycle length from 188sec to 270sec for KALPANA Inter section and 230sec for all other intersections and phase sequences together with splits. More than 550 vehicles will be relieved from stopping at the intersections and also extra 654 liters of fuel will be saved from improvement of signal performance.
- Though, effectiveness parameters were improved due to optimization of cycle length, offsets and splits, the level of service of the network is not changed from LOS F to better service. This is happen due to the reason that the arrival rates are too much to be served by the intersections.

## RECOMMENDATIONS

- In order to improve the service quality of the corridor, only signal timing optimization is not enough by itself. Hence, another traffic management mechanism should be applied like overpass and introducing additional lanes to existing network.
- Using HCM default values leads inappropriate judgment, so calibration is required. For example, the value of saturation flow rate in our condition is higher than the HCM default value.





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### Recommendations for Future Research Areas

There are several potential areas which remain to be examined and can have significant influence for improvement of traffic signal operation. Some of the areas can be;

- Calibration of Level of Service thresholds for local conditions;
- Effect of driving behavior on saturation headway estimation;
- Formulation of other improvement methods other than signal re-timing to improve the level of service of roadway;
- Further investigation should also be conducted to determine other types of congestion costs like noise cost;

### REFERENCES

1. (AAiT), A. A. (2012). *Final Report on Pilot Global Fuel Economy Initiative Study in Ethiopia*.
2. Abajie, T. M. (2015). Dynamical Systems and Network Flows: Traffic Flow Problem on Multi- lane Intersections (Economic Analysis). *Science Publishing Group*.
3. Abojaradeh, M. (2014). Evaluation and Improvement of Signalized Intersections in Amman City in Jordan. *Journal of Environment and Earth Science*.
4. Agajie, T. M. (2015). Dynamical Systems and Network Flows: Traffic Flow Problem on Multi- lane Intersections (Economic Analysis). *Science Publishing Group*.
5. Andrew. (2003). *Traffic Data Collection and Analysis*. Gaborone: Botswana, Ministry of Works and Transport .
6. Ardina. (2006). [www.team-bhp.com/forum/technical-stuff/6250-fuel-consumption-while-standing-still.html](http://www.team-bhp.com/forum/technical-stuff/6250-fuel-consumption-while-standing-still.html).
7. Avdiu, P. S. (2013). Optimization of Work of Traffic Signals in Order to Reduce the Length of the Waiting Queue and Delays in Isolated Intersection: A Case Study. *International Journal of Mechanical Engineering and Technology (IJMET)*, ISSN 0976.
8. Bank, W. (2016). *The Cost of Air Pollution Strengthening the Economic Case for Action*. Washington.
9. Bhosale, N. (2016). Analysis of Delay and Travel Time for Pre-timed Traffic Signal Coordination on Close Continuous intersection. *Global Research and Development Journal for Engineering | Volume 1 | Issue 4* .
10. Chunhui Yu, W. (2017). Optimization of vehicle and pedestrian signals at isolated intersecion. *Transportation Research*.
11. DOT, M. (2012). *A Guide on Traffic Analysis Tools*. Boston, Massachusetts: MassDOT Highway Division.
12. Dr. Thomas Schiller, K. P. (2017). *Deloitte Africa Automotive Insights Navigating the African Automotive Sector: Ethiopia, Kenya and Nigeria* .
13. Dr. Tom V, M. (2014). *pedistrian studies*. Transportation systems Engineering.
14. Errampalli, M. (2015). Effect of congestion on fuel cost and travel time cost on multi-lane highways in India. *International Journal for Traffic and Transport Engineering*.
15. Gauff Ingenieure GmbH & Co. KG -JBG. (2017). *Consultancy Service to Design Road Traffic Flow Improvement of Bhubaneswar City*. Bhubaneswar.
16. H. Rakha, A. M. (2016). Coordination of Traffic Signals across Jurisdictional Boundaries: Field and Modeling Results.
17. Harriet, p. &. (2013). Assessment of Traffic congestion and its effect on productivity in urban Ghana. *sociology and social work*, Vol.4 No.3.
18. HCM. (2010). *Volume 3: Interrupted Flow, Chapter 18, Signalized Intersections*. WASHINGTON, DC: TRANSPORTATION RESEARCH BOARD.
19. HDR for the Office of Economic and Strategic Analysis, U.S. Department of. (February 2009). *Assessing the Full Costs of Congestion on Surface Transportation Systems and Reducing Them through Pricing*. Department of Transportation, USA.
20. Henry. (2005). *Signal Timing on a Shoestring*. Washington: U.S. Department of Transportation, Federal Highway Administration.
21. Institute, V. T. (2017). *Transportation Cost and Benefit Analysis II – Congestion Costs*. Victoria Transport Policy Institute.





**Haraprasad Behere et al.,**

22. Jiun-Horng Tsai, Y.-C. Y.-H. (2010). *Greenhouse Gases Emissions from On-road Vehicle A Case Study in Taiwa.*
23. Kang, Y.-S. (2000). Delay, Stop and Queue Estimation for uniform and random traffic arrivals at fixed-time Signalized Intersections.
24. Koonce, P. (2008). *Traffic Signal Timing Manual.* Georgetown: U.S. Department of Transportation, Federal Highway Administration.
25. Mahmud, G. (2012). Possible causes & solutions of Traffic Jam and Their impact on the Economy of Dhaka City. *Business administration, Vol.2, No.2.*
26. Mass DOT. (2012). *A Guide on Traffic Analysis Tools.* Boston, Massachusetts: MassDOT Highway Division.
27. Mathew, P. T. (2017). Lecture Notes in Transportation Systems Engineering, queueing Analysis. Mekonnen, F. (2015). Evaluation of Traffic Congestion and Level of Service at Major Intersections in Adama City.
28. NCDOT. (2015). *Congestion Management Capacity Analysis Guidelines.* North California. NDOT. (2002). *Comparative Evaluation of Simulation Software for Traffic Operation.* Nevada: Nevada Department of Transportation.
29. Neway, S. (2016). Investigation on Pollution Caused by Gasoline and Diesel fuelled Vehicles.
30. *International Journal of Engineering Trends and Technology (IJETT)* , – Volume 36 Number 7- June 2016.
31. P.Roess, R. (2004). *Traffic Engineering, Third Edition.* Upper Saddle River, New Jersey: Pearson Education, Inc.
32. Paper, W. (2007). *Estimating urban traffic and congestion cost trends for Australian cities.* Bureau of Transport and Regional Economics Department of Transport and Regional Services Canberra, Australia.
33. Sen, A. K. (2010). Estimating marginal external costs of transport in Delhi. *Transport Policy.* Shamshirband, H. S. (2008). Coordination between Traffic Signals Based on Cooperative. *World Applied Sciences Journal 5 (5): 525-530.*
34. Shankute, D. (2015). *The Effect of Diesel-Fueled Vehicle Exhaust Smoke Emissions on Air Pollution.* Bhubaneswar: V-SAFE.
36. Smith, D. (2002). *Handbook of simplified practice for traffic studies.* Ames, Iowa: center for transportation research and education Iowa state university.
37. SOLMAZ, H. (2012). Estimation of Amount of Pollutants Generated by Vehicles in Turkey Until 2030 .*Gazi University Journal of Science, 25(2):495-503(2012)* .
38. Sugiyanto, G. (2010). Estimation of Congestion Cost of Private Passenger Car Users in Malioboro, Yogyakarta. *Civil Engineering Dimension, Vol. 12, No. 2, 92-97.*
39. Tadele, k. (2017). Analysis of traffic congestion and its economic cost in Bhubaneswar a case study of meskel square to kaliti interchange.
40. Tarekegn Kumala, P. E. (2016). Effect of Cycle Time and Signal Phase On Average Time Delay, Congestion And Level of Service: A Case Study At Hager Astedader Signalized Intersection in Addis Abeba. *International Journal of Scientific & Engineering Research.*
41. Trafficware. (2014). *Traffic Signal Optimization and Simulation Modeling Software.* U.S: Trafficware.
42. Unit, T. P. (2009). *Traffic Count Guidelines.*

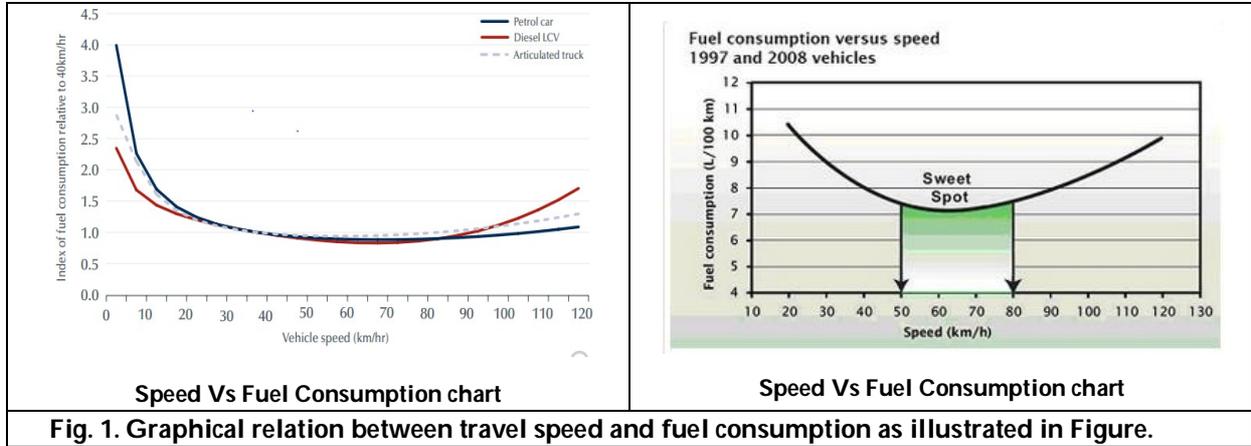
### Traffic Data

SI.No.	Vehicle Type	PCU
1.	Cycle	0.2
2.	M/ cycle	0.5
3.	Car (includes Passengers Cars, Station wagons, pick-up, M/bus)	1
4.	S/Truck includes Small buss 18-48 passenger occupancy and small Truck	2
5.	M/Truck includes Medium Trucks and Large Bus 48-62 passenger Occupancy	2.5
6.	H/Truck includes double cargo city busses and Truck and Trailer	3





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**Fig. 1. Graphical relation between travel speed and fuel consumption as illustrated in Figure.**





## Fuzzy Pseudo $q, a, p$ -Ideals in $d$ -Algebras

N Nagamani<sup>1\*</sup>, P. Ganesan<sup>1</sup> and N. Kandaraj<sup>2</sup>

<sup>1</sup>Research Scholar, PG and Research Department of Mathematics, Saiva Bhanu Kshatriya College, Aruppukottai, Tamil Nadu, India.

<sup>2</sup>Associate Professor, PG and Research Department of Mathematics, Saiva Bhanu Kshatriya College, Aruppukottai, Tamil Nadu, India.

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### \*Address for Correspondence

#### N Nagamani

Research Scholar,  
PG and Research Department of Mathematics,  
Saiva Bhanu Kshatriya College,  
Aruppukottai, Tamil Nadu, India.  
Email: nagamanin28@gmail.com



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### ABSTRACT

In this paper, we introduce characterizations of fuzzy ideals of a pseudo  $d$ - algebra are established. we define fuzzy pseudo  $q$  ideals and fuzzy pseudo  $a$  ideals in pseudo -  $d$ - ideals. We give several characterizations and the extensive theorems about fuzzy pseudo  $q$  ideals and fuzzy pseudo  $a$  ideals.

**Keywords:** Pseudo  $d$ -ideals ; pseudo  $a, p, q$  ideals in  $d$  algebras .

## INTRODUCTION

Y. Imai and K. Iseki introduced two classes of abstract algebras  $BCK$ - algebras and  $BCI$ -algebras [3, 10, 11, ?] It is known that the class of  $BCK$ -algebras is a proper subclass of the class of  $BCI$ - algebras. In Q.P. Hu and X. Li introduced a wider class of abstract algebras  $BCH$ -algebras. They have shown that the class of  $BCI$ -algebras is a proper subclass of the class of  $BCH$ -algebras. J. Neggers and H.S. Kim Introduced On  $d$ -algebras [13]. K. H. Dar introduced the notions of left and right mapping on  $BCK$ -algebras in .Further the authors discussed notions of endomorphisms on  $BCH$ -algebras. Left and Right mapping over  $BCI$ -algebras have been discussed . In this paper, we study the connection between fuzzy and  $d$ -algebras.

### 2 Preliminaries

In this section we site the fundamental definitions that will be used in the sequel.

#### Definition 2.1

Let  $X$  be a set with a binary operation  $*$  and a constant  $0$ . Then  $(X, *, 0)$  is called a  $BCH$ -algebra if it satisfies the following conditions:





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1.  $x * x = 0$
2.  $(x * y) * z = (x * z) * y$
3.  $x * y = 0$  and  $y * x = 0 \Rightarrow x = y \forall x, y, z \in X$ .

**Example 2.2.** Let  $X = \{0, a, b, c\}$  be a set with the following cayley table.

*	0	a	b	c
0	0	0	0	0
E	a	0	a	0
F	b	b	0	0
G	c	c	c	0

**Definition 2.3.** [2, 3, 5] Let  $X$  be a set with a binary operation  $*$  and a constant  $0$ . Then  $(X, *, 0)$  is called a *BCK*-algebras if it satisfies the following axioms.

1.  $((x * y) * (x * z)) * (z * y) = 0$
2.  $(x * (x * y)) * y = 0$
3.  $x * x = 0$
4.  $x * y = 0$  and  $y * x = 0 \Rightarrow x = y \forall x, y, z \in X$
5.  $0 * x = 0$ .

**Definition 2.4.** [12] A *d*-algebra is a non empty set  $X$  with a constant  $0$  and binary operation  $*$  and satisfying the following axioms.

1.  $x * x = 0$
2.  $0 * x = 0$
3.  $x * y = 0$  and  $y * x = 0 \Rightarrow x = y \forall x, y \in X$ .

**Example 2.5.** Let  $(X = \{0,1,2,3\}, *, 0)$  be a set with the following cayley table.

*	0	1	2	3
0	0	0	0	0
1	1	0	1	0
2	2	2	0	0
3	3	3	1	0

Then  $(X, *, 0)$  is a *d*-algebra.

**Definition 2.6.** [1] Let  $X$  be a *BCH*-algebra. If  $X$  is satisfies the condition,  $(x * y) * z = (x * y) * (y * z), \forall x, y, z \in X$ . Then  $X$  is called positive implicative *BCH*-algebra.

**Definition 2.7.[14]** Let  $X$  be a *d*-algebra and  $I$  be a subset of  $X$ . Then  $I$  is called a *d*-ideal of  $X$  if it satisfies the following conditions:

1.  $0 \in I$
2.  $x * y \in I$  and  $y \in I \Rightarrow x \in I$
3.  $x \in I$  and  $y \in X \Rightarrow x * y \in I$  (i.e.  $I \times X \subseteq I$ )

**Definition 2.8.[13]** Let  $\mu$  be a fuzzy set in a *d*-algebra  $X$ . Then  $\mu$  is called a fuzzy *d*-ideal of  $X$  if it satisfies the following inequalities:

1.  $\mu(0) \geq \mu(x)$
2.  $\mu(x) \geq \min \{ \mu(x * y), \mu(y) \}$
3.  $\mu(x * y) \geq \min \{ \mu(x), \mu(y) \} \forall x, y \in X$





**3 Fuzzy Pseudo q, a, p -ideals**

**Definition: 3.1** A fuzzy set  $\mu$  is called a fuzzy pseudo  $q$ -ideal in  $X$ . If

$$(Q1) \mu(x \circ z) \geq \min\{\mu(x * (y \circ z)), \mu(y)\}, \forall x, y, z \in X.$$

$$(Q2) \mu(a * c) \geq \min\{\mu(a \circ (b * c)), \mu(b)\}, \forall x, y, z \in X.$$

**Definition: 3.2** A fuzzy set  $\mu$  is a fuzzy pseudo subalgebra of  $X$ . If

$$(i) \mu(x \circ y) \geq \min\{\mu(x), \mu(y)\} \forall x, y \in X.$$

$$(ii) \mu(a * b) \geq \min\{\mu(a), \mu(b)\} \forall a, b \in X.$$

**Definition: 3.3** A fuzzy set  $\mu$  is called a fuzzy pseudo closed ideal in  $X$ . If

$$(C1) \mu(0 \circ x) \geq \mu(x), \mu(0 * a) \geq \mu(a), x, a \in X \text{ and}$$

$$(C2) \mu(x) \geq \mu(x \circ y) \wedge \mu(y), \mu(a) \geq \mu(a * b) \wedge \mu(b), \forall a, b, x, y \in X.$$

**Theorem: 3.4** Every fuzzy pseudo  $q$ -ideal of  $X$  is a Fuzzy pseudo subalgebra of  $X$ .

**Proof.** If  $\mu$  be a fuzzy pseudo  $q$ -ideal, then putting  $z = y$  in (Q1) and  $c = b$  in (Q2), we have  $\mu(x \circ y) \geq \min\{\mu(x), \mu(y)\}, \mu(a * b) \geq \min\{\mu(a), \mu(b)\}$ .

This completes the proof.

**Theorem: 3.5** Let  $\mu$  be a fuzzy pseudo ideal of  $X$ . Then the following are equivalent.

(i)  $\mu$  is a fuzzy pseudo  $q$ -ideal of  $X$ .

$$(ii) \mu((a \circ b) * c) \geq \mu((a \circ b) * (0 \circ c)) \text{ and } \mu((x * y) \circ z) \geq \mu((x * y) \circ (0 * z))$$

$$(iii) \mu(x \circ y) \geq \mu(x \circ (0 * y)) \text{ and } \mu(a * b) \geq \mu(a * (0 \circ b))$$

**Proof.** (i)  $\Rightarrow$  (ii) Since  $\mu$  is a fuzzy pseudo  $q$ -ideal of  $X$ , we have

$$\mu((a \circ b) * c) \geq \min\{\mu((a \circ b) * (0 \circ c)), \mu(0) = \mu((a \circ b) * (0 \circ c))\}$$

Similarly, Since  $\mu$  is a fuzzy pseudo  $q$ -ideal, we have we

$$\mu((x * y) \circ z) \geq \min\{\mu((x * y) \circ (0 * z)), \mu(0)\}. \mu((x * y) \circ (0 * z))$$

(ii)  $\Rightarrow$  (iii) Letting  $y = 0$  and  $z = y$  in (Q1) and  $b = 0$  and  $c = b$  in (Q2), we get the required implication.

(iii)  $\Rightarrow$  (i) We have  $(x \circ (0 * y)) * (x \circ (z * y)) \leq (z * y) \circ (0 * y) \leq z$ . and

$$(a * (0 \circ b)) \circ (a * (c \circ b)) \leq (c \circ b) * (0 \circ b) \leq c.$$

Then we have  $\mu(x \circ (0 * y)) \geq \min\{\mu(x \circ (z * y)), \mu(z)\}$  and

$$\mu(a * (0 \circ b)) \geq \min\{\mu(a * (c \circ b)), \mu(c)\}. \text{ Therefore by hypothesis}$$

$$\mu(x \circ y) \geq (x \circ (0 * y)) \geq \min\{\mu(x \circ (z * y)), \mu(z)\}$$

and  $\mu(a * b) \geq \mu(a * (0 \circ b)) \geq \min\{\mu(a * (c \circ b)), \mu(c)\}$ . Hence  $\mu$  is a fuzzy pseudo  $q$ -ideal of  $X$ .

**Theorem: 3.6** Let  $\mu$  and  $\nu$  be fuzzy pseudo ideals of  $X$ , such that  $\mu \subseteq \nu$  and

$$\mu(0) = \nu(0). \text{ If } \mu \text{ is a fuzzy pseudo } q\text{-ideals of } X. \text{ then so } \nu.$$

**Proof.** We want to show that by theorem for any  $x, y, a, b \in X. \nu(x \circ y) \geq \nu(x \circ (0 * y))$  and  $\nu(a * b) \geq \nu(a * (0 \circ b))$  putting,  $s = x \circ (0 * y)$ , then  $(x * s) \circ (0 * y) = 0$ , hence  $\mu((x * s) \circ (0 * y)) = \mu(0) = \nu(0)$ . Since  $\mu$  is a fuzzy pseudo- $q$ -ideal of  $X$  and using  $\mu((x * s) \circ y) \geq \mu((x * s) \circ (0 * y)) = \nu(0)$ . Thus  $\nu((x * s) \circ y) \geq \nu(0) \geq \nu(s)$ . Since  $\nu$  is a fuzzy pseudo ideal we have

$$\nu(x \circ y) \geq \min\{\nu((x \circ y) * s), \nu(s)\} = \nu(s) = \nu(x \circ (0 * y)) \text{-----(1)}$$

putting  $t = a * (0 \circ b)$  then  $(a \circ t) * (0 \circ b) = 0$ .

Hence,  $\mu((a \circ t) * (0 \circ b)) = \mu(0) = \nu(0)$ . Since  $\mu$  is a fuzzy pseudo  $q$ -ideal of  $X$  and using  $\mu((a \circ t) * b) \geq \mu((a \circ t) * (0 \circ b)) = \nu(0)$ .

Thus  $\nu((a \circ t) * b) \geq \mu((a \circ t) * b) \geq \nu(0) \geq \nu(t)$  Since  $\nu$  is a fuzzy pseudo ideal we have  $\nu(a * b) \geq \min\{\nu((a * b) \circ t), \nu(t)\} = \nu(t) = \nu(a * (0 \circ b)) \text{-----(2)}$ .





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From (1) and (2)  $v$  is a fuzzy pseudo  $q$ -ideal of  $X$ . This completes the proof.

**Definition: 3.7** A fuzzy set  $\mu$  is called a fuzzy pseudo  $a$ -ideal of  $X$  if,

$$(A1) \mu(y \circ x) \geq \min\{\mu((x * z) \circ (0 * y)), \mu(z)\} \quad \forall x, y, z \in X \text{ and}$$

$$(A2) \mu(b * a) \geq \min\{\mu((a \circ c) * (0 \circ b)), \mu(c)\} \quad \forall a, b, c \in X$$

**Theorem: 3.8** Let  $\mu$  be a fuzzy pseudo ideal of  $X$ . Then the following are equivalent.

(i)  $\mu$  is a fuzzy pseudo  $a$ -ideal of  $X$ .

(ii)  $\mu(b * (a \circ c) \geq \mu((a \circ c) * (0 \circ b))$  and  $\mu(y \circ (x * z)) \geq \mu((x * z) \circ (0 * y))$

(iii)  $\mu(y \circ x) \geq \mu(x \circ (0 * y))$  and  $\mu(b * a) \geq \mu(a * (0 \circ b))$

**Proof.** (i)  $\Rightarrow$  (ii) suppose that  $\mu$  is a fuzzy pseudo  $a$ -ideal of  $X$ , we have

$$\mu(y \circ (x * z)) \geq \min\{\mu((x * z) \circ (0 * y)), \mu(s)\} \text{ and}$$

$$\mu(b * (a \circ c)) \geq \min\{\mu((a \circ c) \circ t) * (0 \circ b), \mu(t)\} \text{ we write } s = (x * z) \circ (0 * y)$$

and  $t = (a \circ c) * (0 \circ b)$ . Since  $((x * z) \circ (0 * y)) * s = 0$  and  $((a \circ c) \circ t) * (0 \circ b) = ((a \circ c) * (0 \circ b)) \circ b = 0$ .

Then we have  $\mu(y \circ (x * z)) \geq \min\{\mu(0), \mu(s)\} = \mu(s) = \mu((x * z) \circ (0 * y))$  and

$$\mu(b * (a \circ c)) \geq \min\{\mu(0), \mu(t)\} = \mu(t) = \mu((a \circ c) * (0 \circ b)).$$

(ii)  $\Rightarrow$  (iii) Letting  $z = 0$  and  $c = 0$  in (ii) we obtain  $\mu(y \circ x) \geq \mu(x \circ (0 * y))$  and  $\mu(b * a) \geq \mu(a * (0 \circ b))$ .

(iii)  $\Rightarrow$  (i) Note that  $(x \circ (0 * y)) \circ ((x * z) \circ (0 * y)) \leq x \circ (x * z) \leq z$  and  $(a * (0 \circ b)) * ((a \circ c) * (0 \circ b)) \leq a * (a \circ c) \leq c$

$$\text{we have } \mu(x \circ (0 * y)) \geq \min\{\mu((x * z) \circ (0 * y)), \mu(z)\}$$

and  $\mu(a * (0 \circ b)) \geq \min\{\mu((a \circ c) * (0 \circ b)), \mu(0)\}$  From condition (iii) we have

$$\mu(y \circ x) \geq \mu(x \circ (0 * y)) \geq \min\{\mu((x * z) \circ (0 * y)), \mu(z)\} \text{ and}$$

$$\mu(b * a) \geq \mu(a * (0 \circ b)) \geq \min\{\mu((a \circ c) * (0 \circ b)), \mu(c)\}.$$

Hence  $\mu$  is a fuzzy pseudo  $a$ -ideal of  $X$ .

**Theorem: 3.9** Let  $\mu$  be a fuzzy pseudo ideal of  $X$ , then  $\mu$  is a fuzzy pseudo  $a$ -ideal of  $X$  if and only if it satisfies the following conditions.

$$(i) \mu(y) \geq \mu(0 \circ (0 * y)) \text{ and } \mu(b) \geq \mu(0 * (0 \circ b)) \quad \forall y, b \in X$$

$$(ii) \mu(x \circ y) \geq \mu(x \circ (0 * y)) \text{ and } \mu(a * b) \geq \mu(a * (0 \circ b)) \quad \forall x, y, a, b \in X.$$

**Proof.** Assume that  $\mu$  is a fuzzy pseudo  $a$ -ideal of  $X$ . Setting  $x = 0$  and  $a = 0$  in the above theorem(iii), we get  $\mu(y) \geq \mu(0 \circ (0 * y))$  and  $\mu(b) \geq \mu(0 * (0 \circ b))$  Note that

$$\begin{aligned} (0 * (0 \circ (y \circ (0 * x)))) * (x \circ (0 * y)) &= (0 * (0 * y) * (0 \circ (0 * x))) * (x \circ (0 * y)) = ((0 \circ (0 * y)) \circ (0 * (0 \circ (0 * x)))) * \\ (x \circ (0 * y)) &= ((0 \circ (0 * y)) \circ (0 * x)) * (x \circ (0 * y)) \\ &= ((0 \circ (0 * y)) * (x \circ (0 * y))) \circ (0 * x) \end{aligned}$$

$$= (0 * x) \circ (0 * x) = 0 * (x * x) = 0.$$

Then  $\mu(0 * (0 \circ (y \circ (0 * x)))) \geq \min\{\mu(x \circ (0 * y)), \mu(0)\} = \mu(x \circ (0 * y))$ ,

Hence  $\mu(y \circ (0 * x)) \geq \mu(0 * (0 \circ (y \circ (0 * x)))) \geq \mu(x \circ (0 * y))$ . Applying above theorem (iii),

we have  $\mu(x \circ y) \geq \mu(y \circ (0 * x)) \geq \mu(x \circ (0 * y))$ . Similarly, we have

$$\begin{aligned} (0 \circ (0 * (b * (0 \circ a)))) \circ (a * (0 \circ b)) &= (0 \circ (0 \circ b) \circ (0 * (0 \circ a))) \circ (a * (0 \circ b)) \\ &= (0 * (0 \circ b) * (0 \circ (0 * (0 \circ a)))) \circ (a * (0 \circ b)) = ((0 * (0 \circ b)) * (0 \circ a)) * (a * (0 \circ b)) \\ &= ((0 * (0 \circ b)) \circ (a * (0 \circ b))) * (0 \circ a) \leq (0 * a) * (0 \circ a) \\ &= 0 \circ (a \circ a) = 0. \end{aligned}$$

We have  $\mu(0 \circ (0 * (b * (0 \circ a)))) \geq \min\{\mu(a * (0 \circ b)), \mu(0)\} = \mu(a * (0 \circ b))$ ,

Hence  $\mu(b * (0 \circ a)) \geq \mu(0 \circ (0 * (b * (0 \circ a)))) \geq \mu(a * (0 \circ b))$  applying above theorem (iii), we have  $\mu(a * b) \geq \mu(b * (0 \circ a)) \geq \mu(a * (0 \circ b))$ .

Conversely, suppose  $\mu$  is a fuzzy pseudo ideal satisfying (i) and (ii) In order to prove that  $\mu$  is a fuzzy pseudo  $a$ -ideal of  $X$ , from above theorem, we show that  $\mu(y \circ x) \geq \mu(x \circ (0 * y))$  and  $\mu(b * a) \geq \mu(a * (0 \circ b)) \quad \forall a, b, x, y \in X$ . By (ii),





we have  $\mu(x \circ y) \geq \mu(x \circ (0 * y))$ . Since  $0 * (y \circ x) \leq x \circ y$ , by above theorem , we get  $\mu(0 * (y \circ x)) \geq \mu(x \circ y) \geq \mu(x \circ (0 * y))$ , thus

$$\mu(0 * (0 * (y \circ x))) \geq \mu(0 * (y \circ x)) \geq \mu(x \circ (0 * y))$$

Applying (i), we get  
 $\mu(y \circ x) \geq \mu(0 * (0 * (y \circ x))) \geq \mu(x \circ (0 * y))$ .

Similarly, we can get  $\mu(b * a) \geq \mu(a * (0 \circ b))$ . Therefore  $\mu$  is a fuzzy pseudo  $a$ -ideal of  $X$ . This completes the proof.

**Definition: 3.10** A fuzzy set  $\mu$  is called a fuzzy pseudo  $p$ -ideal in  $X$ . If

$$(PI1) \mu(x) \geq \min\{\mu((x * z) \circ (y * z)), \mu(y)\}, \forall x, y, z \in X \text{ and}$$

$$(PI2) \mu(a) \geq \min\{\mu((a \circ c) * (b \circ c)), \mu(b)\} \forall a, b, c \in X$$

**Theorem: 3.11** Every fuzzy pseudo  $p$ -ideal is a fuzzy pseudo ideal.

**Proof.** Let  $\mu$  be a fuzzy pseudo  $p$ -ideal of  $X$  and let  $t \in Im(\mu)$ . For every  $y \in U(\mu, t)$ , Let  $a \in *(y, U(\mu, t))$  and  $b \in \circ(y, U(\mu, t))$ . Then  $(b * 0) \circ (y * 0) = b \circ y \in U(\mu, t)$  and

$(a \circ 0) * (y \circ 0) = a * y \in U(\mu, t)$ . Since  $y \in U(\mu, t)$  and  $\mu$  is a pseudo  $p$ -ideal we have  $\mu(a) \geq \min\{\mu((a \circ 0) * (y \circ 0)), \mu(y)\} \geq t$  and

$\mu(b) \geq \min\{\mu((b * 0) \circ (y * 0)), \mu(y)\} \geq t$ . So that  $a, b \in U(\mu, t)$ . This show that  $*(y, U(\mu, t)) \subseteq U(\mu, t)$  and  $\circ(y, U(\mu, t)) \subseteq U(\mu, t)$ . Hence  $U(\mu, t)$  is a pseudo ideal of  $X$  and so  $\mu$  is a fuzzy pseudo ideal of  $X$ .

**Lemma: 3.12** A fuzzy pseudo ideal of  $X$  is a fuzzy pseudo  $p$ -ideal if and only if  $\mu(x) \geq \mu(0 \circ (0 * x))$  and  $\mu(a) \geq \mu(0 * (0 \circ a))$ ,  $\forall x, a \in X$ .

**Proof.** Let  $\mu$  be fuzzy pseudo  $p$ -ideal then  $\forall x, y, z, a, b, c \in X$  we have

$\mu(x) \geq \mu((x * z) \circ (y * z)) \wedge \mu(y)$  ,  $\mu(a) \geq \mu((a \circ c) * (b \circ c)) \wedge \mu(b)$  setting  $x = z, y = 0$  and  $a = c, b = 0$ , we have  $\mu(x) \geq \mu(0 \circ (0 * x))$  and  $\mu(a) \geq \mu(0 * (0 \circ a))$ ,  $\forall x, a \in X$ .

Conversely suppose  $\mu$  satisfies  $\mu(x) \geq \mu(0 \circ (0 * x))$  and  $\mu(a) \geq \mu(0 * (0 \circ a))$ ,  $\forall x, a \in X$ . We have  $(0 * (0 \circ a)) * ((a \circ c) * (b \circ c)) = (((b \circ c) * (b \circ c)) * (0 * a)) * ((a \circ c) * (b \circ c))$

$$= (((b \circ c) * (0 \circ a)) * (b \circ c)) * ((a \circ c) * (b \circ c))$$

$$\leq ((b \circ c) * (0 \circ a)) * (a \circ c)$$

$$= ((b \circ c) * (a \circ c)) * (0 \circ a) \leq (b \circ a) * (0 \circ a) \leq b$$

we have

$$(0 \circ (0 * x)) \circ ((x * z) \circ (y * z)) = (((y * z) \circ (y * z) \circ (0 * x)) \circ ((x * z) \circ (y * z)))$$

$$= ((y * z) \circ (0 * x) \circ (y * z)) \circ ((x * z) \circ (y * z))$$

$$\leq ((y * z) \circ (0 * x)) \circ (x * z) = ((y * z) \circ (x * z)) \circ (0 * x) \leq (y * x) \circ (0 * x) \leq y$$

Then we have  $\mu(0 \circ (0 * x)) \geq \mu(((x * z) \circ (y * z)) \wedge \mu(y)$

and  $\mu(0 * (0 \circ a)) \geq \mu((a \circ c) * (b \circ c)) \wedge \mu(b)$ . Then  $\mu(x) \geq \mu((x * z) \circ (y * z)) \wedge \mu(y)$  and  $\mu(a) \geq \mu((a \circ c) * (b \circ c)) \wedge \mu(b)$ . Hence  $\mu$  is a fuzzy pseudo  $p$ -ideal of  $X$ .

**Theorem: 3.13** Any fuzzy pseudo  $a$ -ideal is a fuzzy pseudo  $p$ -ideal.

**Proof.** Let  $\mu$  be a fuzzy pseudo  $a$ -ideal of  $X$ . Then  $\mu$  is a fuzzy pseudo ideal. setting  $x = z = 0$  and  $a = c = 0$  in theorem 3.8(ii), then we have  $\mu(y) \geq \mu(0 \circ (0 * y))$  and  $\mu(b) \geq \mu(0 * (0 \circ b))$ . From above lemma  $\mu$  is a fuzzy pseudo  $p$ -ideal.

**Theorem: 3.14** Any fuzzy pseudo  $a$ -ideal is a fuzzy pseudo  $q$ -ideal .

**Proof.** Let  $\mu$  be a fuzzy pseudo  $a$ -ideal of  $X$ . Then  $\mu$  is a fuzzy pseudo ideal. In order to prove that  $\mu$  is a fuzzy pseudo  $q$ -ideal, from Theorem 3.5(iii), it is sufficient to show that  $\mu(x \circ y) \geq \mu(x \circ (0 * y))$  and  $\mu(a * b) \geq \mu(a * (0 \circ b))$ , we have

$$(0 * (0 \circ (y \circ (0 * x)))) * (x \circ (0 * y)) = (0 * ((0 * y) * (0 \circ (0 * x)))) * (x \circ (0 * y))$$

$$= (0 \circ ((0 * y) \circ (0 * (0 \circ (0 * x)))) * (x \circ (0 * y)) = ((0 \circ (0 * y)) \circ (0 * x)) * (x \circ (0 * y))$$

$$= ((0 \circ (0 * y)) * (x \circ (0 * y))) \circ (0 * x) \leq (0 * x) \circ (0 * x) = 0 * (x * x) = 0$$

Hence  $\mu(0 * (0 \circ (y \circ (0 * x)))) \geq \mu(x \circ (0 * y))$ ——(6) and we have





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$(0 \circ (0 * (b * (0 \circ a)))) \circ (a * (0 \circ b)) = (0 \circ (0 \circ b)) \circ (0 * (0 \circ a)) \circ (a * (0 \circ b)) = ((0 * (0 \circ b)) * (0 \circ (0 * (0 \circ a)))) \circ (a * (0 \circ b)) = ((0 * (0 \circ b)) * (0 \circ a)) * (a * (0 \circ b)) = ((0 * (0 \circ b)) \circ (a * (0 \circ b))) * (0 \circ a) \leq (0 * a) * (0 \circ a) = 0 \circ (a \circ a) = 0$ . Then  $\mu(0 \circ (0 * (b * (0 \circ a)))) \geq \mu(a * (0 \circ b))$ —(7) Since,  $\mu$  is a fuzzy pseudo  $p$ -ideal by lemma 3.13 we have  $\mu(y \circ (0 * x)) \geq \mu(0 \circ (0 * (y \circ (0 * x))))$  and

$\mu(b * (0 \circ a)) \geq \mu(0 * (0 \circ (b * (0 \circ a))))$  by theorem 3.8 (iii)

$$\mu(x \circ y) \geq \mu(y \circ (0 * x)) \text{—(8)}$$

$$\mu(a * b) \geq \mu(b * (0 \circ a)) \text{—(9)}$$

From (6),(7) and (8), (9), we have

$$\mu(x \circ y) \geq \mu(y \circ (0 * x)) \geq \mu(0 \circ (0 * (y \circ (0 * x)))) \geq \mu(x \circ (0 * y))$$

$$\text{and } \mu(a * b) \geq \mu(b * (0 \circ b)) \geq (\mu(0 * (0 * (b * (0 \circ a)))) \geq \mu(a * (0 \circ b)).$$

Therefore  $\mu$  is a fuzzy pseudo  $q$ -ideal of  $X$ .

**Theorem: 3.15** Let  $\mu$  be a fuzzy pseudo ideal of  $X$ ,  $\mu$  is a fuzzy pseudo  $a$ -ideal if and only if it is both a fuzzy pseudo  $p$ -ideal and a fuzzy pseudo  $q$ -ideal.

**Proof.** If  $\mu$  is a fuzzy pseudo  $a$ -ideal, then  $\mu$  is a fuzzy pseudo  $p$ -ideal and a fuzzy pseudo  $q$ -ideal by theorem 3.14 and theorem 3.15. Conversely, let  $\mu$  be both a fuzzy  $p$ -ideal and a  $q$ -ideal we want to show

$$\mu(y \circ x) \geq \mu(x \circ (0 * y)) \text{ and } \mu(b * a) \geq \mu(a * (0 \circ b)). \text{ By theorem 3.8(iii)}$$

$$\mu(x \circ y) \geq \mu(x \circ (0 * y)) \text{ and } \mu(a * b) \geq \mu(a * (0 \circ b)). \text{ Hence}$$

$\mu(0 \circ (y \circ x)) \geq \mu(x \circ y) \geq \mu(x \circ (0 * y))$  and  $\mu(0 * (b * a)) \geq \mu(a * b) \geq \mu(a * (0 \circ b))$ . Since  $\mu$  is a fuzzy pseudo  $p$ -ideal by lemma 3.12  $\mu(y \circ x) \geq \mu(0 \circ (0 * (y \circ x)))$  and  $\mu(b * a) \geq \mu(0 * (0 \circ (b * a)))$ . Also  $\mu$  is a fuzzy pseudo  $q$ -ideal, therefore  $\mu(0 \circ (y \circ x)) \leq \mu(0 \circ (0 * (y \circ x)))$  and  $\mu(0 * (b * a)) \leq \mu(0 * (0 \circ (b * a)))$  we have  $\mu(y \circ x) \geq \mu(0 \circ (0 * (y \circ x))) \geq \mu(0 \circ (y \circ x)) \geq \mu(x \circ y) \geq \mu(x \circ (0 * y))$  and  $\mu(b * a) \geq \mu(0 * (0 \circ (b * a))) \geq \mu(0 * (b * a)) \geq \mu(a * b) \geq \mu(a * (0 \circ b))$ .

Thus  $\mu$  is a fuzzy pseudo  $a$ -ideal, by theorem 3.8(iii) completing the proof.

## REFERENCES

1. Hu. Q. P and Lix: "On BCH-algebras", Math. Seminar Notes, kobe Univ., 11(1983), (313-320)
2. Hu. Q. P and Lix: "On proper BCH-algebras", Math. Japan 30(1985) (659-669)
3. Imai. Y and Iseki. K: " On axiom systems of propositional calculi, XIV, Proc.Japan. Acad. Ser A,Math Sci., 42(1966), 19-22.
4. Iseki K: " An algebra related with a propositional calculi, Proc. Japan Acad. Ser A. Math.Sci,42(1966),26-29.
5. Iseki K and Tanaka S: " An introduction to theory of BCK-algebras ", Math.Japo., 23(1978), 1-26.
6. Iseki K: "On BCI-algebras, Math. Seminar Notes., 11(1980), 313-320
7. Jun Y.B and Roh E.H and Kim H.s, " On BH-algebras", Sciential Mathematical (1988), 347-354
8. Hu. Q. P and Lix: "On BCH-algebras", Math. Seminar Notes, kobe Univ., 11(1983), (313-320)
9. Hu. Q. P and Lix: "On proper BCH-algebras", Math. Japan 30(1985) (659-669)
10. Imai. Y and Iseki. K: " On axiom systems of propositional calculi, XIV, Proc.Japan. Acad. Ser A, Math Sci., 42(1966), 19-22.
11. Iseki K: " An algebra related with a propositional calculi, Proc. Japan Acad. Ser A. Math.Sci, 42(1966),26-29.
12. Iseki K and Tanaka S: " An introduction to theory of BCK-algebras ", Math. Japo.,23(1978), 1-26.
13. Iseki K: "On BCI-algebras, Math. Seminar Notes., 11(1980), 313-320.
14. Neggers J. and Kim H.S: " On d-algebras", Math. slovac, co., 49 (1999),19-26.





## Clinical Review on Myasthenia Gravis – An Update

T. Gowtham Pradeep, B. Arul and R. Kothai\*

Department of Pharmacology, Vinayaka Mission's Research Foundation (Deemed to be University), Salem-636308, Tamilnadu, India.

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### \*Address for Correspondence

**R.Kothai**

Department of Pharmacology,  
Vinayaka Mission's College of Pharmacy,  
Vinayaka Mission's Research Foundation (Deemed to be University),  
Salem-636008, Tamilnadu, India.  
E.mail : kothaiarul@yahoo.co.in



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### ABSTRACT

Myasthenia gravis is an autoimmune disorder characterized by the weakness and fatigability of the voluntary muscles that are caused by autoantibodies against the nicotinic Acetylcholine Receptor (AChR) on the postsynaptic membrane at the neuromuscular junction which decreases the capacity of neuromuscular end-plate to transmit the nerve signal. A wide variety of conditions can precipitate Myasthenia gravis like as infections, immunization, surgeries, and drugs. According to the presence of antibodies, symptoms, age at onset and thymus pathology, the patients will be grouped. An annual incidence rate of myasthenia gravis is believed to be between 0.25 and 20 per 1,000,000 populations. Women's are additional often affected and concerning 10-15% of cases is associated with thymoma. Treatment of myasthenia gravis be based on subgroup and includes symptomatic treatment using acetyl cholinesterase inhibitors, thymectomy and immunotherapy. The present review aims to focuses on the various aspects of myasthenia gravis like origin, pathophysiology, diagnosis and its pharmacotherapy.

**Keywords:** Acetylcholine, Neurotransmitters, Antibodies, Autoimmune, Immunosuppressant

### INTRODUCTION

Myasthenia gravis (MG) is the most common disorder in neuromuscular junction and characterized clinically by fluctuating painless muscle weakness. It is usually caused by antibodies to postsynaptic proteins of which three namely, Acetylcholine receptor (AChR), Muscle-specific tyrosine kinase (Musk), and Low-density lipoprotein receptor-related protein 4 (LRP4) have been identified[1]. The autoimmune attack is caused by an autoantibody against the acetylcholine postsynaptic receptor at the synapse of skeletal muscles [2]. However, other antigen targets that are components of the synapse have also been implicated within the pathogenesis of MG. The current standard of the case is immunosuppressive therapy; however, many existing therapeutic options have not been validated to be

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used in MG large randomized controlled trials [3]. The most common initial symptoms are vision defect due to ocular muscle weakness, and patients have difficulties in standing up, and possibly even swallowing and or chewing [4].

**Epidemiology**

Myasthenia gravis is an organ-specific disease characterized by weakness and fatigue of voluntary muscles and also the presence of autoantibodies to acetylcholine receptor of the postsynaptic muscle membrane [5]. An annual incidence rate of MG is believed to be between 0.25 and 20 per 1,000,000 populations. Epidemiological studies of MG have indicated a trend of skyrocketing MG prevalence with relatively stable incidence [6]. This reflects the impact of effective treatment, improved diagnostic methods, and prolonged survival [7]. The foremost common age of MG onset is between 20 and 40 years. MG is related to other autoimmune diseases in about 30% of cases. Gender and age appear to influence the occurrence of disease of the neuromuscular junction was given below, [8]

1. Below 40 year old
2. The feminine and male ratio is about (3:2) However, between 40 and 50 years likewise as during puberty, it's roughly equal.
3. Over 50 years, it occurs more commonly in males. Childhood MG is rare in Europe and North America, comprising 10% to 15% of MG cases [9]. Approximately 10% of patients with MG have a thymoma, and it is implicated within the production of auto antibodies [10].

**Etiology**

Myasthenia gravis is an autoimmune disease mediated by an organ-specific antibody [11]. These antibodies are present at the synapse and directed against the nicotinic acetylcholine receptor (AChR) on the postsynaptic muscle membrane in 80-90% of patients. It is mainly caused by auto antibodies and thymus gland. Nearly, 3-7% of the auto antibodies are directed to a different NMJ protein, Muscle-specific tyrosine kinase (Musk) [12].

**Antibodies**

Nerves communicate with muscles by releasing chemicals (neurotransmitters) that fit precisely into receptor sites on the muscle cells at the nerve-muscular junction. [13]. In myasthenia gravis, immune system produces antibodies that block or destroy many of muscles' receptor sites for a neurotransmitter called acetylcholine. With fewer receptor sites available, muscles receive fewer nerve signals, leading in weakness. Antibodies may block the function of a protein called a muscle-specific receptor tyrosine kinase [14]. This protein is involved in forming the nerve-muscular junction.

**Thymus gland**

The thymus gland may be a part of immune system situated within the upper chest beneath breastbone. It was believed that the thymus gland triggers or maintains the production of the antibodies that block Acetylcholine [15]. The thymus gland is large in infancy, and small in healthy adults. In some adults with myasthenia gravis, however, the thymus gland is abnormally large. Some people with myasthenia gravis even have tumors of the thymus gland (thymomas) [16]. Usually, thymomas aren't cancerous (malignant), but they can become cancerous.

**Other causes**

Some people have myasthenia that won't be caused by antibodies blocking acetylcholine or the muscle-specific receptor tyrosine kinase [17]. This kind of disease of the neuromuscular junction is named antibody-negative disease of the neuromuscular junction. Antibodies against another protein, called lipoprotein-related protein, can play a component within the development of this condition [18]. Rarely, mothers with myasthenia have children who are born with myasthenia (neonatal myasthenia gravis). If treated promptly, children generally recover within two months after birth. Some children are born with a rare, hereditary variety of myasthenia, called congenital myasthenia syndrome [19]. Factors which will worsen myasthenia are as follows:  
Fatigue





### Illness and Stress

Some medications like beta-blockers, guanidine gluconate,

Pregnancy

Menstrual periods

Myasthenia gravis is also classified based on age of onset, presence or absence of Anti-AchR antibodies severity, and also the etiology of the disease.

Early-onset MG: Age at onset < 50 years, thymic hyperplasia usually females.

Late-onset MG: Age at onset > 50 years, thymic atrophy, mainly males.

Thymoma-associated MG (10-15%) and MG with anti-MuSK antibodies,

### Pathophysiology Of Myasthenia Gravis

Myasthenia gravis caused by antibodies against proteins in the neuromuscular junction postsynaptic membrane antibodies against Acetylcholine Receptor (AChR) are generalized MG cases the Anti-AchR antibodies are pathogenic because they reduced the number of AChR at the neuromuscular junction [20]. The major abnormalities of the neuromuscular junction in myasthenia gravis include

- Reduced number of the AChRs leading to reduced length of the postsynaptic membrane,
- Shortening of the synaptic folds due to destruction of the terminal expansions, and
- Widening of the synaptic clefts caused by the shortening of the junctional folds.

These changes are caused by autoimmune attack on the postsynaptic membrane. It is worth noting that the abnormalities in myasthenia gravis are postsynaptic in location (in contrast to presynaptic abnormality in Lambert-Eaton syndrome)[21].

The consequence of those abnormalities may be a reduced factor of safety. As previously discussed, reduction in ratio as well as a standard “synaptic rundown” ends up in progressive reduction in amplitude of the EPP. This leads to myasthenia weakness characterized by fatigue on exertion[22].

### Role of AChR antibodies

It is now well established that myasthenia gravis is an antibody-mediated disorder of the neuromuscular junction. There are several lines of evidence supporting the role of antibodies in the pathogenesis of myasthenia gravis [23].

1. AChR antibodies are found in nearly 80%–90% of patients with generalised disease.
2. Circulating anti-AChR antibodies are found in cases of transient neonatal myasthenia gravis and the titre of the antibody declines as the patient recovers.
3. Plasmapheresis lowers the levels of AChR resulting in the improvement in myasthenia.

The serum concentration of AChR antibodies in different patients does not correlate with the clinical severity of myasthenia gravis. It has been suggested that the severity of weakness in myasthenia gravis depends on the functional activities of the antibodies[24].

### Role of the Thymus

The association of disease of the neuromuscular junction and thymomas was noted quite 200 years ago found in nearly 75% of patients with disease of the neuromuscular junction of those germinal hyperplasia is noted in 85% and thymic tumors in 15%[25]. Anti-striated muscle antibodies are found in 90% of patients with myasthenia and a thymoma muscle cell-like cell (Myoid cells) are found within the thymus that express surface AChRs. These cells are surrounded by the helper T- cell and also the antibodies presenting cells.

### Signs and Symptoms

The initial, main symptom in MG could be a painless weakness of specific muscle, not fatigue; the muscle weakness becomes progressively worse during periods of physical activity and improves after periods of rest [26]. The fatigable weakness of skeletal muscle in which smaller muscle responsible for fine movements (e.g. Eye muscle) end to be affected first, which larger muscles become affected later on.



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Eye muscle weakness: Most common initial symptom (Potosi's Diplopia)

1. Bulbar muscle weakness: Slurred speech, Difficulty chewing and/or swallowing.
2. Proximal limb weakness: Rising from a chair, Climbing, Brushing, Deep tendon reflexes are not affected.
3. Head and Neck: Due to weakness of the muscle of facial expression and muscle of mastication, facial weakness may manifest as the inability to hold the mouth closed, with drooping eyelids, facial weakness may make the individual appear sleepy or sad [27].

**Diagnosis of Myasthenia Gravis**

MG can be difficult to diagnose as the symptoms can be subtle and hard to distinguish from both normal variant and other neurological disorders. In patients with a character's history, it may be easy to make a diagnosis on clinical grounds alone; however, it is important to confirm the diagnosis of myasthenia gravis before committing patients to long-term treatment [28]. It was reported that the diagnosis of myasthenia gravis is mostly based on the result of the test for the antibody against AchR and the neurophysiologic tests. The Tensilon test should only be used where the diagnosis is required urgently and the facilities for full resuscitation are available [29].

**Edrophonium (Tensile) test**

Edrophonium is an AchR inhibitor that works within some seconds (30 seconds) and therefore the effect lasts for some minutes (about five minutes). It is desirable to use a placebo injection (for example, normal saline) before the edrophonium injection[30]. Therefore, this test should only be carried out where the diagnosis of myasthenia is required urgently and there are facilities for a full resuscitation. The test is reported as positive if there is a definitive improvement within the weakness[31].

**Ice pack test**

This test can be employed when ptosis is present. The application of an ice pack to lids of the affected eyes improved ptosis due to myasthenia gravis in 80% of cases but it did not improve in ptosis due to other etiologies [32]. Response is explained based on improvement in the safety factor of the neuromuscular junction with local cooling presumably caused by slowing the kinetics of AchRs. The response is not entirely caused by rest. This test is much simpler than the edrophonium test and does not require cardiac monitoring [33].

**Anti- AchR antibody test**

Vincent and Newsom-Davis developed a radioimmunoassay test to detect the antibodies that bind to AchRs. The development of this test has remarkably changed the diagnostic evaluation of myasthenia gravis and is now considered a diagnostic "gold standard"[34]. These antibodies are found in nearly 80%–85% of patients with generalized myasthenia gravis and 50%–60% cases of ocular myasthenia gravis [35].

**Anti- Musk antibodies**

It is well known that about 10%–20% of patients do not have anti-AChR antibodies in their sera (seronegative myasthenia gravis). However, targets for antibody attacks other than the AchR were not known until recently [36]. It was collaboration between Vincent in the UK and a German scientist (Hoch) that led to the recognition of a new target for antibody attack in myasthenia gravis. This region in the neuromuscular junction is a protein called muscle-specific protein kinase or MuSK.

**Physical examination**

During a physical examination to check for MG a Doctor might ask one to look at a fixed point for 30sec and to relax the muscles of the forehead because with MG and ptosis of the eyes might be involuntarily using the forehead muscle to compensate for the weakness in the eyelids. The clinical examiner might also try to elicit the curtain sign in a person by holding one of the person's eyes open [37].





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### Blood test

If the diagnosis is suspected, serology can be performed. One test is for antibodies against the acetylcholine receptor, the test has a reasonable sensitivity of 80-96% but in ocular Myasthenia, the sensitivity falls to 50%. A proportion of the people without antibodies against the acetylcholine receptor have antibodies against the Musk protein. In a specific situation, testing is performed for Lambert-Eat'sson syndrome [38].

### Pharmacotherapy of Myasthenia Gravis

The treatment of myasthenia gravis can be considered to involve three steps: initial treatment usually involves the use of acetylcholinesterase inhibitors. However, these drugs are usually not adequate to control disease on their own and additional therapy is mostly needed. Often an immune directed treatment is added, beginning with either thymectomy or high dose corticosteroids [39]. In the long term, steroid-sparing medications are usually added to facilitate the tapering phase. Short term therapies that are, intravenous immunoglobulin or plasmapheresis may be effective in the early stages of treatment, before thymectomy, or later during an exacerbation [40]. The following treatment modalities are available.

- Acetyl cholinesterase inhibitors. eg. Neostigmine, Pyridostigmine
- Immunosuppressant's eg. Azathioprine, Cyclosporine
- Corticosteroids.
- Plasmapheresis.
- Thymectomy.

### Acetyl cholinesterase inhibitors

These drugs act by inhibiting acetyl cholinesterase and thus increase the availability of acetylcholine to act on the AchRs. They are usually the initial drugs used in the treatment of myasthenia gravis and may be the only drug required to treat mild disease [41]. Neostigmine may be a medication used to treat myasthenia, Ogilvie syndrome, and urinary retention without the presence of a blockage. It is also used together with atropine to finish the consequences of neuromuscular blocking medication of the non-depolarizing type. Onset of action is within 30 min in injection, and 4 hrs in oral intake [42]. Pyridostigmine bromide is the most frequently employed drug in this class, it is often started at a dose of 30 mg 3 times a day and can be gradually increased to 60–90 mg four times a day based on the response and also the tolerability. The main advantage of the drug is its rapid onset of effect (within 15–30 minutes) [43].

### Immuno suppressants

Immunosuppressant medications work to lower the immune system response in order to prevent the immune attack on the NMJ, thereby limiting muscle fatigue.

### Common Medications Used In Myasthenia Gravis Include

Cyclophosphamide is very toxic and mycophenolate is currently expensive [44]. Azathioprine has been in use for a long time and its relatively favorable safety profile makes it the primary choice in this class of medicine. Its effectiveness has been shown in a randomized trial. It is started at a low dose of 50 mg/day and if tolerated, the dose is gradually increased depending on the response. Up to 10% of the patients develop troublesome side effects such as idiosyncratic flu-like illness, bone marrow suppression, and liver toxicity [45].

### Corticosteroids

Corticosteroids are needed to treat myasthenia gravis of moderate or greater severity and sometimes during a mild disease that fails to respond fully to acetyl cholinesterase inhibitors. Because the long-term use of steroids is related to a major associated with a significant risk of potentially serious side effects, it is imperative to discuss them fully with the patient before starting treatment [46]. They could be started at a low dose (for example, prednisolone 10–20 mg/day) to avoid the early worsening noted in nearly 48% of patients on high dose regimens.



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The dose can be gradually increased by 5 mg every third day up to 60 mg/day. They can be used at initial high doses when symptoms are worsening rapidly, though this should be done in the hospital with close monitoring of forced diagnostic assay[47].

**Plasmapheresis**

Plasmapheresis is based on the antibody-mediated pathogenesis of myasthenia gravis and has been used in its treatment for more than 30 years. It produces rapid but temporary improvement by reducing the amount of AChR antibodies [48]. Various methods of plasmapheresis including double filtration plasmapheresis, immune-adsorption plasmapheresis, and plasma exchange have been used. The major indications are Immediate treatment in patients with serious myasthenia gravis or myasthenia crisis, Preparation of patients with severe myasthenia gravis before thymectomy, In the early postoperative period, In cases of symptom worsening during tapering or initiation of immunosuppressive therapy. The improvement rarely persists for more than 4–10 weeks and, therefore, immunosuppressive therapy has to be continued [49].

**Thymectomy**

There are two different aspects of thymectomy in myasthenia gravis:

Thymectomy for thymic tumors associated with around 10% of patients with myasthenia gravis

Thymectomy for the treatment of myasthenia gravis. As regards the first indication, thymectomy should always be done, as thymic tumors are potentially locally invasive.

Thymectomy as a treatment of myasthenia gravis (in the absence of thymoma) has been practice for several years. The young age and the absence of thymoma have been shown to predict a better response in some studies, though age did not have any effect on the response in a recent study[50].

**Future Therapeutic Approaches**

Myasthenia gravis is an autoimmune disease in which autoantibodies to different antigens of the neuromuscular junction cause the typical weakness and fatigability. Treatment includes anticholinesterase drugs, immune suppression, and thymectomy. The autoimmune response is maintained under control by corticosteroids frequently associated with immunosuppressive drugs, with improvement in the majority of patients. In case of acute exacerbations with bulbar symptoms or repeated relapses, modulation of autoantibody activity by plasmapheresis or intravenous immunoglobulins provides rapid improvement. Recently, techniques removing only circulating immunoglobulins have been developed for the chronic management of treatment-resistant patients. In a recent study, increased diagnostic sensitivity was obtained using anti-AchR  $\epsilon$  subunit-specific antibodies compared with the conventional AchR antibody testing. In addition to this, all current therapies for MG are non-specific and focus on the activity of T- and B-cells [51].

However, novel immunotherapy is in clinical development, including a variety of T-cell directed monoclonal antibodies that block the intracellular cascade associated with T-cell activation, monoclonal antibodies directed against key B-cell molecules, and inhibitors of complement, cytokines, and transmigration molecules [52]. Other potential approaches aim to focus on the anti-AchR autoimmune response and re-establish immune tolerance to the AchR. Possible monoclonal approaches include administration of AchR or a portion of its sequence in a manner known to induce tolerance (e.g. oral or nasal)[53].

**Prognosis**

The prognosis of people with Myasthenia Gravis is generally good as is the quality of life, give very good treatment. Monitoring of a person with MG is very important as at least 20% of people diagnosed with it will experience a myasthenia crisis with two years of their diagnosis, requiring rapid medical intervention. Generally, the most disabling period of MG might be years after the initial diagnosis. In the early 1900s, 70% of detected cases died from lung problems now that number is estimated to be around 3-5% which is attributed to increased awareness and medication to manage symptoms [54].





## CONCLUSION

Myasthenia gravis is an autoimmune disorder characterized by the weakness and fatigability of the voluntary muscles that are caused by autoantibodies against the nicotinic Acetylcholine Receptor (AChR) on the postsynaptic membrane at the neuromuscular junction. It's nearly always possible to ascertain the diagnosis of myasthenia with these tests. Modern treatment is very successful and mortality of treated disease is practically zero. Hence it was concluded from the study that there is a need to educate and improve disease awareness among the patients and it is also important to perform the early diagnosis that helps in the management of this neurological disorder.

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## REFERENCES

1. CV J. Massey MJ. Myasthenia gravis. *Orphanet J Rare Dis.* 2007; (2): 14-23.
2. Hughes T. The early history of myasthenia gravis. *Neuromuscular Disorders.* 2005 Dec 1; 15(12):878-86.
3. Jacob S. Myasthenia Gravis—A Review of Current Therapeutic Options.
4. Martins F, Sykietis GP, Maillard M, Fraga M, Ribi C, Kuntzer T, Michielin O, Peters S, Coukos G, Spertini F, Thompson JA. New therapeutic perspectives to manage refractory immune checkpoint-related toxicities. *The Lancet Oncology.* 2019 Jan 1;20(1):e54-64.
5. Kennedy WR, Alter M, Sung JH. Progressive proximal spinal and bulbar muscular atrophy of late onset: a sex-linked recessive trait. *Neurology.* 1998 Mar 1;50(3):58-63.
6. Thanvi BR, Lo TC. Update on myasthenia gravis. *Postgraduate medical journal.* 2004 Dec 1; 80 (950):690-700.
7. Hughes BW, Kusner LL, Kaminski HJ. Molecular architecture of the neuromuscular junction. *Muscle & Nerve: Official Journal of the American Association of Electrodiagnostic Medicine.* 2006 Apr;33(4):445-61.
8. Hepple RT, Rice CL. Innervation and neuromuscular control in ageing skeletal muscle. *The Journal of physiology.* 2016 Apr 15;594(8):1965-78.
9. Katz B, Miledi R. The effect of calcium on acetylcholine release from motor nerve terminals. *Proceedings of the Royal Society of London. Series B. Biological Sciences.* 1965 Feb 16;161(985):496-503.
10. Dabi A, Solieman N, Kurukumbi M, Kalyanam J. Myasthenia gravis: a review. *Autoimmune Diseases.* 2012 Oct 31; 874-680.
11. Loh KH, Stawski PS, Draycott AS, Udeshi ND, Lehrman EK, Wilton DK, Svinkina T, Deerinck TJ, Ellisman MH, Stevens B, Carr SA. Proteomic analysis of unbounded cellular compartments: synaptic clefts. *Cell.* 2016 Aug 25;166(5):1295-307.
12. Marques MJ, Conchello JA, Lichtman JW. From plaque to pretzel: fold formation and acetylcholine receptor loss at the developing neuromuscular junction. *Journal of Neuroscience.* 2000 May 15;20(10):3663-75.
13. Purslow PP. The extracellular matrix of skeletal and cardiac muscle. In *Collagen 2008 Springer, Boston, MA.* 325-357.
14. Mclachlanem. The statistics of transmitter release at chemical synapses. *Int rev physiol.* 1978 Jan 1; 17:49-117.
15. Borodin sky LN, Spitzer NC. Activity-dependent neurotransmitter-receptor matching at the neuromuscular junction. *Proceedings of the National Academy of Sciences.* 2007 Jan 2;104 (1):335-40.
16. Emerson RW. Retching, Vomiting, Feeding Intolerance. *Caring for Children Who Have Severe Neurological Impairment: A Life with Grace.* 2013 Aug 1:131.
17. Raja M. Clozapine safety, 35 years later. *Current drug safety.* 2011 Jul 1;6(3):164-84.



**Gowtham Pradeep et al.**

18. Boers M, Verhoeven AC, Markusse HM, van de Laar MA, Westhovens R, van Denderen JC, van Zeben. Randomised comparison of combined step-down prednisolone, methotrexate and sulphasalazine with sulphasalazine alone in early rheumatoid arthritis. *The Lancet*. 1997 Aug 2;350(9074):309-18.
19. Van Sonderen A, Wirtz PW, Verschuuren JJ, Titulaer MJ. Paraneoplastic syndromes of the neuromuscular junction: therapeutic options in myasthenia gravis, lambert-eatonmyasthenic syndrome, and neuromyotonia. *Current treatment options in neurology*. 2013 Apr 1;15(2):224-39.
20. Huda R. New approaches to targeting B cells for myasthenia gravis therapy. *Frontiers in immunology*. 2020;11:24-70.
21. Donohoe KM. Nursing care of the patient with myasthenia gravis. *Neurologic clinics*. 1994 May 1;12(2):369-86.
22. Batlevi CL, Matsuki E, Brentjens RJ, Younes A. Novel immunotherapies in lymphoid malignancies. *Nature reviews Clinical oncology*. 2016 Jan;13(1):26-85.
23. Sax, T. W., & Rosenbaum, R. B. Neuromuscular disorders in pregnancy. *Muscle & Nerve: Official Journal of the American Association of Electrodiagnostic Medicine*. 2006; 34(5): 55-57.
24. Blattner WA. HIV epidemiology: past, present, and future. *The FASEB journal*. 1991 Jul;5(10):20-8.
25. Tanovska N, Novotni G, Sazdova-Burneska S, Kuzmanovski I, Boshkovski B, Kondov G, Jovanovski-Srceva M, Kokareva A, Isjanovska R. Myasthenia gravis and associated diseases. *Open access Macedonian journal of medical sciences*. 2018 Mar 15;6(3):472.
26. Grob D. Natural history of myasthenia. *Contemporary Neurology Series*. Jun.9 ;(6):131-45.
27. Rezende RM, Weiner HL. Cellular components and mechanisms of oral tolerance induction. *Critical Reviews™ in Immunology*. 2018;38(3).
28. Edmondson HA, Steiner PE. Primary carcinoma of the liver. A study of 100 cases among 48,900 necropsies. *Cancer*. 1954 May;7(3):462-503.
29. Morgenthalert, brown Jr, colbytv, harper jr cm, colesdt. Thymoma. *Inmayo clinic proceedings* 1993 Nov 1 vol. 68, no. 11, pp. 11-112.
30. Hoch W, McConville J, Helms S, Newsom-Davis J, Melms A, Vincent A. Auto-antibodies to the receptor tyrosine kinase MuSK in patients with myasthenia gravis without acetylcholine receptor antibodies. *Nature medicine*. 2001 Mar;7(3):365-8.
31. Berrih-Aknin S. Myasthenia Gravis: paradox versus paradigm in autoimmunity. *Journal of autoimmunity*. 2014 Aug 1;52:1-28.
32. Conti-Fine BM, Milani M, Kaminski HJ. Myasthenia gravis: past, present, and future. *The Journal of clinical investigation*. 2006 Nov 1;116(11):2843-54.
33. Patten BM. Myasthenia gravis: review of diagnosis and management. *Muscle & Nerve: Official Journal of the American Association of Electrodiagnostic Medicine*. 1978; 1(3):190-205.
34. Rea P. *Essential clinical anatomy of the nervous system*. Academic Press; 2015 Jan 5(2):28-26.
35. Gilhus NE, Skeie GO, Romi F, Lazaridis K, Zisimopoulou P, Tzartos S. Myasthenia gravis—autoantibody characteristics and their implications for therapy. *Nature reviews neurology*. 2016 May;12(5):259-68.
36. Goldstein G, Mackay IR. *The human thymus*. Elsevier; 2016 Oct 27. (2) 190-205.
37. Hoch W, McConville J, Helms S, Newsom-Davis J, Melms A, Vincent A. Auto-antibodies to the receptor tyrosine kinase MuSK in patients with myasthenia gravis without acetylcholine receptor antibodies. *Nature medicine*. 2001 Mar; 7(3): 36-58.
38. Greenstein B. *Rapid revision in clinical pharmacology*. CRC Press; 2017 Jul 12. (2):28-26.
39. Sher E, Gotti C, Clementi F. A (Etylcholine Nicotinic Receptor: a key molecule the pathogenesis Myastilenia, *Ann. I st. Super. Sanità*. 1988; 24 (1):103-16.
40. Ciafaloni E. Myasthenia gravis and congenital myasthenic syndromes. *CONTINUUM: Lifelong Learning in Neurology*. 2019 Dec 1; 25 (6):1767-84.
41. Trinh VB. *Therapeutic peptide mimics of the acetylcholine receptor main immunogenic region for treating myasthenia gravis*. University of California, Davis; 2013. 26 (2): 187-265.
42. Maggi L, Mantegazza R. Treatment of myasthenia gravis. *Clinical drug investigation*. 2011; 31(10):691-701.





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43. Koenig SM. Pulmonary complications of obesity. *The American journal of the medical sciences*. 2001 Apr 1; 321 (4):249-79.
44. Vrolix K, Fraussen J, Molenaar PC, Losen M, Somers V, Stinissen P, De Baets MH, Martínez-Martínez P. The auto-antigen repertoire in myasthenia gravis. *Autoimmunity*. 2010 Aug 1;43(5-6):380-400.
45. Statland JM, Barohn RJ. Muscle channelopathies: the nondystrophic myotonias and periodic paralyses. *Continuum: Lifelong Learning in Neurology*. 2013 Dec;19 (2):15-29.
46. Hillel AD, Miller R. Bulbar amyotrophic lateral sclerosis: patterns of progression and clinical management. *Head & neck*. 1989 Jan;11(1):5-19.
47. Scheife RT, Hills JR, Munsat TL. Myasthenia gravis: signs, symptoms, diagnosis, immunology, and current therapy. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*. 1981 Jul 8; 1(1):39-54.
48. Brodsky MC. Complex ocular motor disorders in children. *In Pediatric neuro-ophthalmology* 2010 Sep 3; 30-38.
49. Wolff AS, Kärner J, Owe JF, Oftedal BE, Gilhus NE. Clinical and serologic parallels to APS-I in patients with thymomas and autoantigen transcripts in their tumors. *The Journal of Immunology*. 2014 Oct 15;193 (8):38-90..
50. Leite MI, Jacob S, Viegas S, Cossins J, Clover L, Morgan BP, Beeson D, Willcox N, Vincent A. IgG1 antibodies to acetylcholine receptors in 'seronegative' myasthenia gravis. *Brain*. 2008 Jul 1;131 (7):1940-52.
51. Verma P, Oger J. Treatment of acquired autoimmune myasthenia gravis: a topic review. *Canadian journal of neurological sciences*. 1992 Aug;19 (3):36-75.
52. Feist E, Doerner T, Sörensen H, Burmester GR. Longlasting remissions after treatment of autoimmune myositis. *The Journal of rheumatology*. Jun 1; 35(6):12-30.
53. Meriggioli MN. Myasthenia gravis with anti-acetylcholine receptor antibodies. *Immune-Mediated Neuromuscular Diseases*. 2009; 26:94-108.
54. Jacob S, Viegas S, Lashley D, Hilton-Jones D. Myasthenia gravis and other neuromuscular junction disorders. *Practical neurology*. 2009 Dec 1;9(6):364-71.





## Ethical Perspectives of Advertising among Dental Healthcare Providers and Healthcare Consumers

Priya Thomas<sup>1\*</sup> and R Sujatha<sup>2</sup>

<sup>1</sup>Reader, Department of Oral Pathology, Annoor Dental College, Muvattupuzha, Kerala, India.

<sup>2</sup>Assistant Professor, Department of Biochemistry, PSG Institute of Medical Sciences and Research, Coimbatore, India.

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### \*Address for Correspondence

**Priya Thomas**

Department of Oral and Maxillofacial Pathology,  
Annoor Dental College, Muvattupuzha,  
Ernakulam Dist, Kerala, India  
Email: priyathomask@gmail.com



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### ABSTRACT

Ban on advertisements by dental professionals in India can be traced back to the 1950's with the implementation of the Dentist Act. As the practice of dentistry is mounting towards complexities, an ethical dilemma persists as to whether dentists should advertise their services. This study is set to evaluate the ethical perspectives of advertising among dental health care professionals and dental healthcare consumers. A questionnaire-based survey was conducted among the healthcare providers (dentists), and health care consumers (patients/public). The participants for the study were drawn from different dental colleges and clinics across Kerala. Data was collected by means of a validated closed ended questionnaire format and scored on a five-point Likert scale. Two questionnaires were formatted for each study group and evaluated by three subject experts. 26.8% of the healthcare providers considered advertising by dentists unethical, 52.4% disagreed that advertising can benefit their dental practice. 32.5% of the consumers disagreed that advertising can help them select the right dentist for their dental needs and 43.3% perceived that there is no requirement to advertise their services. Our survey indicated that healthcare providers still adhered to the code of ethics. This survey also projected that the consumers do not depend on the advertisements to select their dentists. A doctor patient relation is built on many virtues of ethics. Unethical advertising is not cherished by neither the public nor the providers.

**Keywords:** Advertising, ethics, dental health care providers, dental healthcare consumers.





## INTRODUCTION

Dentistry as a health care profession has ushered into an era of globalization. This profession has witnessed dramatic changes incorporating new concepts and latest technologies while flaking outdated canons and methodologies [1]. The dentist Act was established in 1948 under the Government of India (Ministry of Law and justice) to supervise professional ethics and to improve the service of dental health care. The Dental Council of India (DCI) with the sanction of the Central Government laid down the Dentists (Code of Ethics) Regulations in 1976 and later revised it in 2014. Advertising prevails in every field and is considered as one of the key ways for marketing and business enhancement. It has not only captured all domains, but also extended itself into field of healthcare services [2]. "Advertisement" by definition (Allied Health Professions of South Africa) refers to any written, illustrated, visual or other descriptive material or verbal statement or reference that appears on the Internet, newspaper, magazine etc that distributed amongst members of the public and which is meant to promote a specific practice or a specific practitioner's technique or to make known a specific practitioner's professional proficiency or knowledge [3]. The Code of Ethics (1976) by the Dental Council of India, brought advertising under the unethical practices and stated that any advertising either directly or indirectly for one's own professional advantage or for the purpose of obtaining patients or projecting the practitioner's skill, knowledge, service or qualifications were unethical [4].

The issue on Ethics of Advertisement by Dental/Medical professionals has drastically changed over the last few decades and revised the regulations (Chapter 6 – DCI Regulations 2014) so as to that a Dentist or a group of Dentists may advertise provided that they maintain decorum, keeping in mind the high moral obligations and the value that society advocates on them [5]. With the evolution of medicine from guilds into professional societies in 19<sup>th</sup> and early 20<sup>th</sup> century, many countries had imposed a ban on advertisement of professional services in-order to maintain professionalism [6]. In 1975, American Medical Association's ban on advertising was challenged in the U.S. Supreme Court by physicians. This led to the revision of its statutes with permission to advertise as long as they do not contain any false or deceptive information and is free to project ones-self through any form of commercial or public communication [6]. The other councils that permit advertisements by physicians are British Medical Council, the Australian Medical Association and the Canadian Medical Association [6]. In India, the scenario is still unchanged. Era of globalization, advancing technologies and unemployment on the rise with surplus entrants of new dentists into the field has posed major challenges to the dentists. An ethical dilemma persists whether dentists should advertise their services taking into consideration, patient's autonomy and right to information, though it an important issue with significant ethical and professional implications. Considering the above facts, the present study was designed to evaluate the ethical perceptions of advertising by dental healthcare service providers (Dentists) and to assess the awareness on dental health care and evaluate the perceptions among Dental Healthcare Consumers (public) on dental health care advertising.

## MATERIALS AND METHODS

The present Cross-sectional survey was done, after Institutional Human Ethical Committee approval on 799 participants drawn from different Dental Colleges and Dental Clinics across Kerala after obtaining informed consent. The survey population consisted of two groups: Dental Healthcare Providers (Dentists - Dental Clinic practitioners / consultants, Teaching Faculties in dental colleges) and Dental Healthcare consumers (Patients/public). Participants were drawn on the basis of convenience sampling. Dental practitioners, academicians of age groups above 22 years of age and health care consumers those visiting the dental Out Patient Department (OPD) of dental colleges/dental clinics for treatment and who were willing to participate were included into the study. Undergraduates and interns in dental colleges were excluded. The data was collected by means of a validated closed ended questionnaire format for each group respectively. The validation of the questionnaire was done by 3 subject experts and were also handed to dental practitioners and the public prior to the conduction of the study. The questionnaires were distributed either in print form or through SurveyMonkey.com. The data was collected through a printed Questionnaire either in





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English or local language for the consumer group/public where questions were formatted to assess awareness on dental health care and ethicality of advertising. All the responses obtained were scored on a five point Likert scale.

## RESULTS

**Comparative analysis of demographic data:** Out of 399 respondents of the dental healthcare provider group, majority who responded belonged to age group between 23-32 years (157/399). Majority among the provider group disagreed to the statement that there is a need to advertise one's practice irrespective of the age group (Fig: 1). The gender variation consisted of 230 males and 169 females. The dentists who participated had either qualification as Bachelor of Dental Surgery (BDS) or Master of Dental Surgery (MDS): 31.6 % (126/399) BDS and 68.4 % (273/399) MDS. (Table 1). Majority of MDS qualified dentists 77(28%) disagreed that advertising is unethical. In the consumer group, 25% of the participants belonged to the age group 23 - 32 years, 20% between 33-42, 17% to 43-52, 13% (53-62 years) and the remaining 9% were 63 years and above. 55.5% were females and 43.8% were males among the public who participated in the study.

### Advertising ethics and code of ethics (Table 2 & Table 3)

A majority (63.2%) of the dentists hadn't read the Code of Ethics (revised) by DCI on advertising. 71.3% of the public participants were not aware that the healthcare providers / dentists are not supposed to advertise their services. 26.8% of the dentists considered advertising to be unethical, 25.3% respondents as ethical or remained neutral in their response. Majority (45.6%) stated that there is no requirement for advertising of their practices. 52.4% disagreed that advertising will benefit their dental practice. In comparison, 32.5% of the consumer group conflicted that advertising will help them to find the right dentist for their dental needs and only 23.0% agreed that dentists should advertise their services while 69.5% disagreed to the above fact. (Fig 2). These responses were almost identical for the two groups. 41.1% of the dentists disagreed that advertising of services can help the public to take better decisions on dental healthcare. 55.9% opposed that it is not right to advertise a new treatment modality that has been introduced into the practice. 31.8% of consumers stressed that advertising will mislead due to its false claims. 38.3% of dentists abided by the fact that the dental practitioner who does not advertise is considered lacking in competence by the public and this view was contradicted by 51.9% of public. 38.8% of the dentists disagreed that dental practitioners who do not advertise are at disadvantage when compared to a corporate dental hospital. In response to the question, "would advertising project dental healthcare as a commercial activity rather than a service?", only 26.15% of dentists agreed on the statement. A majority of the public (30.5%) also abided by the similar opinion. 32.6% of dentists approved that advertising can create a negative reputation to dentistry in general.

**Advertising ethics and modes of advertising:** The mainstream (40.6%) of the dentists considered that conducting camps, surrogate endorsement on articles (45.4%), and websites with factual information (55.8%) were different forms of advertisements. 29.4% of dentists and 34.5% of consumers disagreed on the verity that surrogate advertisements (televised talk shows with scollers) are not forms of advertisement while the remaining participants projected a dissimilar opinion.

**Advertising and dental tourism:** Global advertising has also increased over the years and dentistry has been expanding its horizons into worldwide tourism. Of the healthcare respondents, 52.4% opposed dental tourism and only 36.1% wanted to advertise their practice globally as a component of dental tourism.

**Amendment of code of ethics on advertising:** 32.6% of dentists agreed upon a right to enact a law to ban advertising in dental practice. 35.3% were not in favour of amendment in the existing code of ethics and 35.1 % remained neutral in respect to the same view.

**Preferred mode of Advertising:** The mode of ethical advertising preferred by dentists, if allowed, was internet 29.2%, followed by print media (12.94%) and 11.86% all forms of media (internet, visual, print and radio).





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### General awareness of dentistry and selection of dentists among consumers

Other questions to the consumer groups were to assess their general awareness on dentistry. About 42% of the consumer had single visits to the dentist and 40.4% have not visited any dentist over the last two years. 80.4% of the respondents did not have a family dentist and 44.7% selected their dentist through word of mouth. 32.3% selected their doctors through researched selection (internet) and 51.9% based on the degree affixed on the name board. Nevertheless 63.9% are not aware of different specialties existed in dentistry and those who had knowledge (27.1%) were through their friends. Only 10.6% had visited the dentist following an advertisement, out of which 28.4% received quality treatment that matched the expectations created by advertisements. When allowed for ethical dissemination of information through advertisements, 24.6% of the consumers preferred the type of treatment available to be publicized while 13% stressed on knowledge of dentist's degree, treatment type, different specialties and treatment charges.

## DISCUSSION

Medical advertising can be unprofessional because of the intrinsic and unmanageable risk of misleading the public [7]. Dentistry is a field with emerging trends, lots of new entrants into field and unemployment on the rise. This has led to lots of unhealthy competition among the dental professionals leading to the dilution of professional ethics [9]. This may divert the platform for the dentist to be more of businessman rather than a service provider but with the interest of the consumers at its foremost. The biphasic role of a doctor or a dentist as a health care provider and a business person is the heart of concern for advertising. The study results depicted the lack of awareness and knowledge about the code of Ethics among dentists and the dental healthcare consumers (public) were not aware that dentists are not supposed to advertise their services and such that the healthcare professionals are bounded by a Code of Ethics. It's a high necessity that the undergraduates and postgraduate students are to be taught on Ethics and Code of Conduct and such subjects to be introduced into their curriculum. The results between age and the need for advertising did not show much variability between different age groups. Majority either disagreed or strongly disagreed on the need of advertising. This could be based on the viewpoint that healthcare is more of a service to society rather than business. Only a minority (25%) between the age group 53-62 years agreed on the need for advertising. The results showed a decline, as the number of participants under this category was minimal (n=8) Our survey results were also in accordance with the results of Dable et al that 53.19% of the respondents confirmed that advertising does not have any effect on a dental practice<sup>8</sup>. Advertising the dental practice was not considered to be a concern and even competitive when compared to a corporate dental hospital [8].

As per our study both the groups agreed that advertising will not prove to be beneficial in providing information for a better health care and the public believed that if the claims made in the advertisements are not factual and thereby can mislead them. Advertisement of a new treatment modality that has been introduced into the practice was not considered favourable. The above views were contrary to the survey results of Dable et al that advertising and marketing could serve the community by ensuring better quality services [8]. This difference could be related to the fact the public did not stress on the importance of advertising and selection of dentist, the quality of treatment opted was not based on advertisements. As per the Code of ethics (DCI), use of dentist's name on designate commercial articles, surrogate advertisements in the garb for educating the public through TV programs or other media with dissemination of information like address/email id, telephone number etc., of the Dental Surgeon or the clinic, on-screen 'scrollers' with address of the dentists in television programmes are considered as unethical acts [5]. The study also reflects on the lack of awareness of oral hygiene and the importance of routine regular visits to dentists among consumers. This proves that public awareness on the oral hygiene has to be introduced as a part of advertisement campaigns. The dentists were selected by word of mouth and opinions from the general public and this study also established the reality that word of mouth can be one of the best forms of advertising. These thoughts were also reflected by Pandya that "Word of mouth" can also act as one of the ways to advertise one's practice but it has its disadvantage when it comes to court of law. This kind of dissemination of information provides the doctor with the ambiguity that he was misunderstood and the exaggerated claims of efficacy through word-of-mouth



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dissemination cannot be litigated easily [4]. Under the code of ethics, any formal advertisements by dentists should be limited to the announcement of the opening / change / resumption of a dental clinic or practice, change of address & ownership, temporary absence from duty [5]. Advertisement could be made about the availability of new equipment or services without boastful claims of being the 'best' or 'first' especially if such services are already available in other facilities, insertion in Telephone directories/Yellow pages or on the internet is permissible. Maintenance of websites about dentists or dental clinics where all information is factual and could carry details of treatment facilities available and the fees for the same could be done. This will in fact help patients to make informed choices through a transparent system [6].

Though these codes exist, advertising by health care professionals in India has drastically increased during the past decade. The expanding dentist population and competition in the industry has allowed commercialization to enter dentistry, undermining the ethical status of the profession and has led to the violation of the Code of Ethics [9,10]. Though there is a rise in propaganda of new technologies and media, the ethical issues continue to be the same. Dable RA et al stated "Business by itself is not unethical, and the reality is this: if the practice fails as a business, the practitioner fails as a medical professional [8]." He also affirmed that many dentists still abide by the conventional view that dentistry is a health profession not a business and that the ethical reflections of a service provide should be paramount. The ethics behind a dentist as a service provider and a business entrepreneur are different and both play different roles and display different responsibilities [8]. As per the opinion if allowed to advertise, internet is preferred over other forms of social media. This attributed to the fact that the traditional referral system (print form) was less effective, as most people rely on net, to find a dentist. This depicts the usage of latest technologies available. Our results were also in accordance with statement of Dable et al that the internet and social networks have also changed the milieu enormously [8]. Nowadays both dentists and consumers across the nation use online testimonials and recommendations. This was well proved by our results that the consumer groups select their dentists through researched selection. In contradiction, Sabrinath (2011) projected the disadvantage that use of internet lacks control and the advertisements pertaining to the health services provided may be misleading. He also brought out fact on nonexistence of a squad or board to have a check on this [10].

Advertisements have its disadvantages too. They often fail to cite complications and rarely present treatment alternatives not offered by the physician. The fleeting medium of advertisement (billboards, short radio segments, fliers, rapid television commercials) is not appropriate for instigating the informed decision-making process that underlie all health interventions [11]. Medical health care service has been considered as the quintessence of professions since many eras. The current scenario has changed and advertisement has eroded professionalism. People consider it to be more of a business than service. Yet the distinction of a profession from a trade can be adverted to the codified ethics that is integrated to the former. The doctor/patient relationship is at stake, as doctors continue to display the self-interested decoy of advertisement [11]. Unethical advertising though not appreciated, the demand for relaxation in the restrictions, would be beneficial in helping emerging dentists to establish themselves in a competitive industry has been a suggestion put forward by the providers which is in accordance with the suggestion of Dable et al [8]. Advertising can be for used for the good, but at the same it could also destabilize the health care role of the profession [8].

**CONCLUSION**

The present survey proves that majority of the dental professionals are not in favor of advertising. Approximately equal proportion had for and against view on the ethicality of advertising by healthcare professionals. This depicts that the professionalism and professional integrity still exists among professional and the dentists still adhered to the code of ethics and worked towards the maintaining the integrity of the profession. This survey also projected that the consumers do not depend on the advertisements to select their dentists. A doctor patient relation is built on many other virtues of ethics like compassion, communication skills, trust etc. The societal expectations of a healer attributes to altruistic service, morality, integrity, transparency and accountability of the healthcare providers. Lack of





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knowledge and awareness still exist among the consumer group. Education of the public is a prime requisite. Unethical advertising is not cherished by the neither the public nor the providers. There are a minority of the dentists group preferred advertising to build up their services among a crowd of well-established dentists, as the new entrants into the field are on the rise. New technologies pose a major challenge to the upcoming dentists. A transparent system is a must for dissemination of factual information to the public in the best interest of the patient and ethical advertising could benefit the society and patients. Training of dentists towards Ethical advertising should be considered specially for the upcoming healthcare professionals.

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## CONFLICT OF INTEREST

The authors declare that there are no existing competing interests.

## REFERENCES

1. Soben P. Essentials of Preventive and Community Dentistry. 3<sup>rd</sup> ed. India: Arya (Medi) Publishing House; 2006
2. Oliveira FT, Sales Peres A, Sales Peres SHC, Yarid SD, Silva RHA. Odontological ethics: knowledge of the students and dentists on the ethical aspects of the profession. Rev Odontol UNESP. 2008 Jan-Mar; 37(1):33-9.
3. www.gpwnline.co.za (Code of Ethics: Allied Health Professions of South Africa)
4. <https://www.apstatedentalcouncil.com/Documents/CODE%20OF%20ETHICS>
5. DCI code of Regulations 2014: www.dciindia.org.in
6. Mamdani B, Mamdani M. Ethics of professional advertising. Issues in medical ethics. 2001 Jan; 9(1):18.
7. Jones, James W., and Laurence B. McCullough. "Is medical advertising always unethical, or does it just seem to be?" Surgical Ethics Challenges. 2015: 1635-1636.
8. Dable RA, Prasanth M, Singh SB, Nazirkar GS. Is advertising ethical for dentists? An insight into the Indian scenario. Drug Healthc Patient Saf. 2011; 3:93-98.
9. Tandon S. Challenges to the oral health workforce in India. J Dent Educ 2004; 68:28-33.
10. Sabarinath B, Sivapathasundharam B. Ethics in dentistry. Journal of Education and Ethics in Dentistry. 2011 Jan 1; 1(1):24.
11. Tomycz ND. A profession selling out: lamenting the paradigm shift in physician advertising. Journal of medical ethics. 2006 Jan 1; 32(1):26-8

<b>Do you consider advertising by dentists unethical?</b>						
<b>Qualification</b>	<b>Number of participants</b>	<b>Strongly disagree</b>	<b>Disagree</b>	<b>Neutral</b>	<b>Agree</b>	<b>Strongly Agree</b>
BDS	126 (31.5%)	15(11.9%)	29 (23%)	35(27.7%)	33 (26%)	14(11%)
MDS	273 (68.4%)	28 (10%)	77(28%)	66(24%)	68(24.9%)	34(12.4%)





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**Table 2: Comparative analysis between qualification and ethicality of advertising**

	Questionnaire	Strongly Agree	Agree	Disagree	Strongly Disagree	
1	Do you consider advertising by dentists unethical?	42(10.5)	107(26.8)	101(25.3)	48 (12)	
2	In your opinion, is there a need to advertise Your dental practice?	21(5.3)	62(15.5)	182(45.6)	59(14.8)	
3	Do you think that advertising can benefit Your dental practice?	9(2.3)	31(7.8)	209(52.4)	89(22.3)	
4	In your opinion, will dental tourism benefit your practice?	4(1)	25(6.3)	209(52.4)	97(24.3)	
5	Would you prefer to advertise your practice abroad as a component of tourism package?	24(6)	78(19.5)	144(36.1)	68(17)	
6	Which do you consider as forms of advertisements					
7a	Conducting dental camps	46(11.5)	162(40.6)	76(19)	26(6.52)	
7b	Surrogate advertisements	38(9.5)	100(25.1)	117(29.4)	59(14.8)	
7c	Surrogate advertisements dental surgeon’s endorsement on commercial articals	102(25.6)	181(45.4)	44(11.0)	20(5.0)	
7d	Websites with factual information	103(25.8)	223(55.8)	30(7.5)	7(1.7)	
8	Is it right to advertise a new treatment modality that has been introduced into your practice?	7(1.8)	30(7.5)	223(55.9)	103(25.8)	
9	Do you think that dental practitioners who do not advertise are at disadvantage when compared to a corporate dental hospital? <sup>8</sup>	18(4.5)	63(15.8)	155(38.8)	110(27.6)	
10	In your view, are dental practitioners who do not advertise considered as lacking in competence by the public? <sup>8</sup>	53(13.3)	153(38.3)	81(20.3)	37(9.3)	
11	Can advertising help the public in making better dental health care decisions?	22(5.5)	70(17.5)	164(41.1)	61(15.3)	
12	In your outlook, would advertising create a negative reputation to dentistry in general?	40(10)	130(32.6)	85(21.3)	27(6.8)	
13	Would advertising project dentistry as a commercial activity rather than a health care service?	36(9)	104(26.1)	108(27.1)	55(13.8)	
14	Do you think it is right to enact a law to ban advertising in dental practice?	60(15)	130(32.6)	90(22.6)	32(8.0)	
15	Are you in favour of amendment in the Code of Dental ethics on Advertising?	17(4.3)	46(11.5)	141(35.3)	51(12.8)	
16	If in favour of advertising which means or mode of advertising would you prefer	<b>Internet</b>	<b>Print form</b>	<b>Radio</b>	<b>Visual</b>	<b>All</b>
		111(29.9)	48(12.9)	8(2.1)	36(9.7)	44(11.8)

**Table 3: Advertising Ethics and perspectives of Consumers [n (%)]**

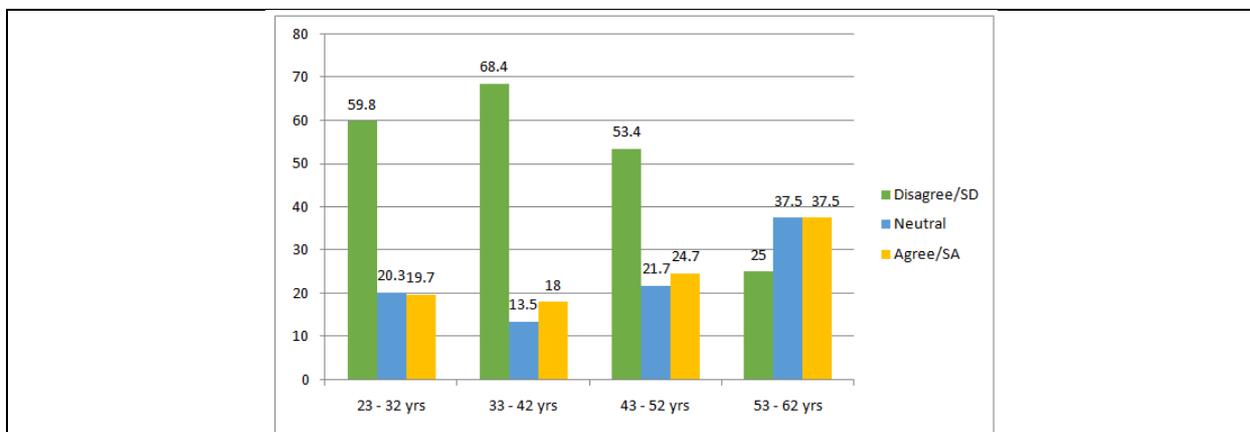
Questionnaire	Strongly Agree	Agree	Disagree	Strongly Disagree
Will advertising help you to choose the correct dentist for your dental needs	22(5)	98(22.1)	144(32.5)	75(16.9)
Do you agree that radio/ televised talk shows by dentists (with their names and phone	49(11.1)	122(27.5)	152(34.3)	32(7.2)



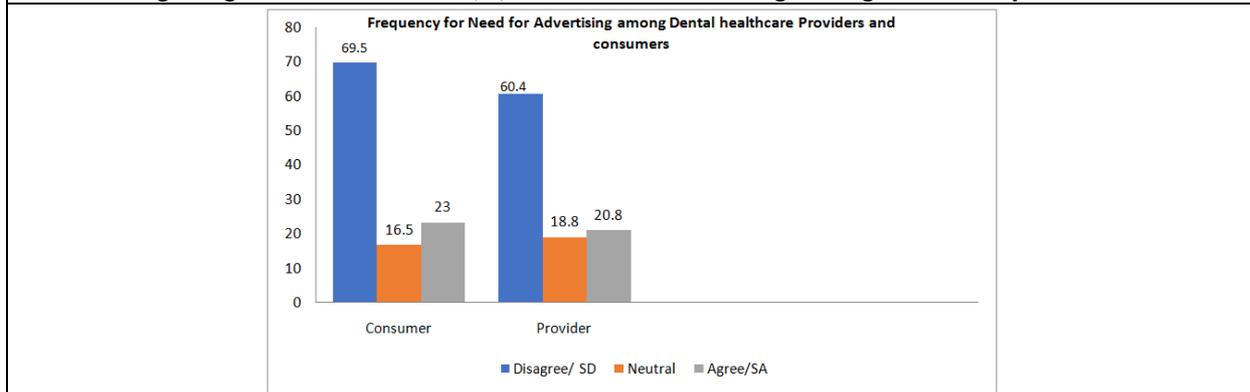


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numbers) are different forms of advertising				
Do you think advertising will mislead because of its false claims?	87(19.6)	141(31.8)	109(24.6)	26(5.9)
Do you feel advertising will project dentistry as a trade rather than a service?	84(19)	128(28.9)	135(30.5)	35(7.9)
Do you think a dentist who does not advertise is lacking in competence	11(2.5)	27(6.1)	230(51.9)	139(31.4)
In your opinion, do you think that dentists should advertise their services?	17(3.8)	75(16.9)	192(43.3)	86(19.4)
	Degree	Specialty	Treatment	Cost
If allowed for ethical dissemination of information, what would you prefer in the advertisement	26(5.9)	20(4.5)	109(24.6)	9(2.0)
				All



**Fig 1: Age wise distribution (%) of need for advertising among healthcare providers**



**Fig 2: Frequency for need for advertising among healthcare providers and consumers**





## Biogenic Synthesis of Silver Nanoparticles and their Applications: A Review

Ayushi Chaturvedi, Rajesh Singh Tomar and Asha Singh\*

Amity Institute of Biotechnology, Amity University Madhya Pradesh, Gwalior (M.P.) India.

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### \*Address for Correspondence

#### Asha Singh

Amity Institute of Biotechnology,  
Amity University Madhya Pradesh,  
Gwalior (M.P.) India.

Email: asingh@gwa.amity.edu



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### ABSTRACT

On a molecular basis, nanotechnology investigates a number of promising methods in the material sciences discipline, with silver nanoparticles (AgNPs) currently receiving the most coverage. There has been use of silver and silver salts since the dawn of humanity, but silver nanoparticles (AgNPs) have recently been discovered. They've been cast-off in agriculture and medicine as antibacterial, antifungal, and antioxidant agents. Several attempts have been made in the last decade to establish green synthesis methods that do not comprise harmful by-products. AgNPs have been suggested to generate free radicals, which cause apoptosis and cell death. A crucial phase of nanotechnology is the development of operative and eco-friendly procedures for nanoparticle synthesis. Bio fabrication of silver nanoparticles (AgNPs) using medicinal plants extract is a novel approach for the reduction of nanoparticles. Their characterization was performed by UV-Visible analysis, FT-IR, TEM, SEM and Zeta analyses. The findings of the analysis showed that biogenic AgNPs showed potential on a biomolecular scale and could afford remarkable protection against hepatic and microbial alteration.

**Keywords:** Silver Nanoparticles, Biogenic Synthesis, Drug Delivery System

### INTRODUCTION

Nanotechnology is the study of science, engineering, and technology at the nanoscale (between 1 and 100 nanometers). A billionth of a metre, or  $10^{-9}$  of a metre, is one nanometre. Nanotechnology is referred to the term for manufacture, portrayal, manipulation, and application of structures by controlling shape and size at nanoscale [1]. Supramolecular scaffolds are formed when the molecules come together, which are in the limits of nanoscale dimension.etc. Richard Feynman, a Nobel laureate in Physics informed the scientific community about the field of future, "Nanotechnology", in his famous lecture "There's plenty of room at the bottom". Nanotechnology provides



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the ability to engineer the properties of materials by controlling their size, and this has prompted analysis towards a multitude of nanomaterials future applications. Nanoparticles have a grain-like structure with a diameter varying from 1–100 nm, according to study; they're used for a range of things, including drug targeting and as a drug carrier in therapeutics. As a result of their optical properties, colloidal suspension of metal nanomaterials is transparent, thus they are useful in cosmetics, coatings, and packaging [2]. A wide variety of materials can be used to make these nanoparticles for example metal oxide ceramics and silicates, magnetic materials, liposomes, dendrimers, emulsion. The major thrust of Nano-biotechnology is the production of new and enhanced nano-materials. They are used in a variety of industries, including health, cosmetics, pharmaceuticals, and automobiles, because of their special properties such as mechanical, electrical, optical, and magnetic properties. Nanoparticles (NPs) fall into three categories: carbon-based, organic-inorganic-based, and composite-based materials. Carbon nanofibers, polymers, dendrimers, carbon nanotubes and metallic oxides such as Au, Ag, Cu, TiO<sub>2</sub>, ZnO, and others are some examples. Among metal nanoparticles, silver nanoparticles have application in medicine due to its antibacterial, antifungal, larvicidal and anti-parasitic properties [3].

**Fabrications of Nanoparticles**

In general, two methods are used to make nanoparticles: top-down and bottom-up. In the top-down approach, bulk material is broken down into nanoscale particles using various lithographic methods for example grinding, milling, and so on, while in the bottom-up approach, atoms self-accumulate to new nuclei, which then transform into a nanoscale component. Nanoparticles can be created using both traditional physical and chemical methods and modern green synthesis. Ion sputtering, solvothermal synthesis, reduction, and the sol-gel technique are examples of traditional methods.

**Biogenic Approach of Nanoparticles**

The development of biogenic nanoparticle synthesis has gained popularity recently around the world, and the usage of metals has already been made for medicinal purposes since ancient times. These metallic nanoparticles can now be used in wound dressing,[4] medical imaging,[5] antimicrobial activity,[6]anti-cancer drug delivery system,[7,8]catalysis sensors for water purification [9]and other applications. These herbal plant formulations, such as polymeric nanoparticles, nano capsules, nano emulsions, phytosomes, liposomes, microspheres, and transfersomes,, were made from bioactive plant extract to deliver drugs in systems that are specifically targeted [10]. It is essential to evaluate the drug's behaviour in a particular tissue *in vitro* or in a model *in vivo*. The commonly used nanoparticles are a) Three-dimensional (3D Confinement) structure, such as nanoparticles, quantum dots, and particle groups Nano wires, quantum wires (semiconductor material), Nano poles, Nano tubes, and Nano strands are examples of one-dimensional structures (2D confinement). Thin films, planar quantum wells, and super lattices are examples of two-dimensional structures (1D confinement). With circular, rounded, and intermittent shapes, they may exist as a single, melded, collected, or agglomerated structure. Nanotubes, quantum dots, and fullerenes are examples of popular nanomaterials. Nanomaterials differ from ordinary bulk materials in terms of their physical and synthetic properties. A plant mediated synthesis of M/MO NPs especially Silver, Gold, Palladium NPs and Zinc, Copper, Titanium, Zirconium oxide NPs has been progressing, because of its simple approach, long-term stability, instant synthesis and environmentally eco-friendly [11].

**Metallic Nanoparticles**

Metal nanoparticles, among the various nanoparticles, have piqued the interest of scientists because of their peculiar properties and efficient synthesis processes [12]. Researchers have attempted to focus on the controlled size and shape of metal nanoparticles, which affects their physicochemical and optical properties [13]. Until now, comprehensive research on the synthesis of various metallic nanoparticles (gold, silver, copper, zinc, titanium, and so on) has been published in the literature for their novel properties and applications. The nanoparticles have a wide range of applications, including catalysts [14],active food packaging materials [15], drug-delivery systems, enhancing contrast agents,[16] antibiotics, antiseptics, disinfectants[17]and components for the construction of nano biosensors, optoelectronics, and biomedical sciences. Phytochemicals found in plant biodiversity are responsible for the



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formation of metal oxide nanoparticles. Metal precursors are initially reduced by the inclusion of phytochemicals in plant extract. At some stage, ambient oxygen or a decaying phytochemical binds to the metallic oxide. As a consequence, metal oxide ions form, and the metal oxide ions clump together due to electrostatic attraction, resulting in the desired nanoparticles. Some of the phytochemicals in the extract then stabilise the nanoparticles, preventing particle aggregation.

### Silver Nanoparticles

The use of silver nanoparticles has gotten a lot of attention due to antimicrobial properties in pharmaceuticals, cosmetics and textiles. They play a crucial role in the field of biology and medicine due to their attractive physicochemical properties. Silver nanoparticles are also stated to be nontoxic to humans and to be most effective against bacteria, viruses, and other eukaryotic microorganisms at extremely low concentrations with no side effects. Silver has long been thought to have potent inhibitory properties as well as a wide range of antimicrobial activities, and it has been used to prevent and cure a variety of diseases [18]. They possess anti-fungal,[19,20] anti-inflammatory,[21] anti-viral, anti-angiogenesis[22] and antiplatelet activity properties [23]. Silver nanoparticles (AgNPs) in suspension release silver ions, which are harmful to microorganisms. The way a substance dissolves in AgNPs is influenced by their structure, dimension, distance, concentration, ionic strength, coating, temperature, and time. This surface-coated material can also deliver therapeutic components like medications, antibodies, and pharmaceuticals. The increasing interest in using nanotechnology to treat cancer is due to its specific advantages in drug delivery, diagnosis and imaging, synthetic vaccine production, and miniature hospital devices, as well as therapeutic applications of nanomaterials. Biologically active form of silver is controllable using various ligand coatings, preventing silver ions from releasing and increasing half-life in systemic circulation for passive targeting. Furthermore, surface functionalization on AgNPs can be achieved by decorating them with various ligands such as sugars, proteins, peptides, and genetic materials for active targeting, which will require more research into their therapeutic efficacy. Biosynthesis of AgNPs by plant extracts such as *Azadirachta indica*,[24] *Chenopodium album*,[25] *Allium cepa*,[26] *Eucalyptus hybrida*,[27] *Cycas*,[28] *Tribulus terrestris* [29]etc have been reported.

### Nanoparticles and their Applications

The use of nanotechnologies in medicine is known as nanomedicine. The use of nanotechnologies in medicine is known as nanomedicine. The use of nanoparticles to improve the behaviour of drug substances is one of the several benefits that have been found. Patients with ovarian and breast cancer, liver cancer, renal disease, fungal infections, elevated cholesterol, menopausal symptoms, multiple sclerosis, chronic pain, asthma, and emphysema, among other disorders, are now being administered with nanomedicines at a global scale. Nanomedicines are being used in order to defeat the challenges that traditional medical therapies have in delivering assistance from the drug molecules used. When medications have a low solubility in water, the human body tries to consume as much as possible to cure the underlying disease [30]. Regardless of the fact that the drug molecule is well digested, the medication is taken out of the human body before it can offer any value. Drugs have the potential to cause negative consequences due to poor delivery at the original location of disease. For example, cancer medicines must be kept away from healthy tissues and organs, or they have the potential to cause damage. Nanomedicines, as a result, play a significant role in verifying whether sufficient drug enters the body, is it retained in the body for large duration or is it targeted mainly to the portions that are in need of treatment [31]. Nanomedicine is one of nanotechnology's fastest-growing fields, with the potential to change healthcare and medicine by the use of novel diagnostic techniques and therapeutic methods. Not only the innovation of new antibiotics and chemical modifications of existing antimicrobial drugs address the issue of microbial resistance, but it will also necessitate the use of long-term efficient metallic nanotechnology in medicine to combat infectious diseases.

### Medicinal Plant: A New Paradigm for Nanoparticles Fabrication

Medicinal plants have been used in India for centuries to treat a number of illnesses, including cancer. [32] Approximately 6000 medicinal plants are expected to be used in folk and herbal medicine in India. Only 3000 medicinal plants have been reported as having medicinal value, while the rest are unknown [33]. These medicinal





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properties are mainly due to bioactive compounds produced as by-products by the plants [34,35]. Around 80% of the world's population uses medicinal plants for their healthcare needs [36]. When compared to 1-3 percent of modern medications, about one-third of all conventional medicines are used to treat skin diseases. Traditional Chinese, Ayurvedic, Unani, and Biomedicine medicines are effective in the treatment of various ailments, particularly in rural areas. Plants contain phytochemicals, which can also be found naturally in plants. The two major types of metabolites are primary and secondary. The method of constructing and preserving living cells involves primary metabolites. Chlorophyll, amino acids, and protein sugars are among the most well-known components. Secondary metabolites are substances that aren't primary metabolites. Secondary metabolites serve as barriers against microorganisms and insects. The secondary constituents include alkaloids, flavonoids, steroids, phenols, glycosides, coumarins, tannins, saponins and terpenoids etc. Many plants are known to contain phytochemicals, but merely have been studied for their pharmacological activity [36].

### **Fabrication of Silver Nanoparticles through Plants**

The first approach to using floras as a source of synthesis of metallic NPs was achieved with Alfalfa sprouts, according to Gardea-Torresdey et al. (2003). This was the first explanation of the synthesis of Ag-NPs by making use of a living plant type [37]. Alfalfa roots can absorb Ag from agar medium and transport it to plant shoots in the same oxidation state. Velmurugan et al. (2015) demonstrated that Ag-NPs can be made from peanut shell extract and compared to commercial Ag-NPs in terms of characteristics and antifungal activity [38]. UV-Vis spectra, XRD peaks, FTIR values revealed that synthesised and industrial NPs are identical, and HRTEM results confirmed that NPs were mainly spherical and oval. Since pathogenic bacteria can contaminate nanoparticles, plant extracts were found to be more effective than microbiological processes for nanoparticle synthesis. Because of their reducing properties, it is one of the most successful approaches for producing nanoparticles because it uses plant extract as a capping and reducing agent (Table 1). *Ficus religiosa*, *Gymnema sylvestre*, *Embilica Officinalis*, *Moringa oleifera*, *Phyllanthus emblica*, *Melia azedarach*, *Annona squamosa*, *Andrographis paniculata*, *Cinnamon zeylanicum*, *Ficus religiosa*, *Gymnema sylvestre*, *Embilica Officinalis*, *Moringa oleif* plants secondary metabolites act as reducing (provide) agents. Since plants have a high potential for storing and detoxifying heavy metals, they play dynamic role in production of nanoparticles as compared to microbial synthesis. Plant products like proteins, enzymes, polysaccharides and amino acids act as reducing agents. According to the requirement stem, root twigs, leaves, flowers and seeds can be used for extraction of these reducing agents. In addition to other plants, medicinal plants leave researchers with plenty of materials for selection and investigation as reducing agents [39].

### **Applications of Biogenic Nanoparticles**

Manufactured silver nanoparticles have appeared in a variety of products in recent years, including drug delivery, imaging, and cancer therapeutics, to name a few. Energy-efficient materials and lightweight armour used for protection. Hydrogen storage, increased performance, catalysis, increased crop yields, safe packaging, chemical/biological detection in agriculture, filtration of water, reduction of air pollution, and remediation of the environment are all examples.

### **Antimicrobial Activity**

Silver has been used as an antimicrobial for a long time to prevent microbial infection. Silver has been used as a biocide to cover milk bottles since the Phoenician civilization. Silver is a widely recognised antimicrobial agent that can destroy over 650 different microorganisms, including gram-negative and gram-positive bacteria, fungi, and viruses. In 1884, it was common practise to give silver nitrate in aqueous form to new-borns' eyes during childbirth to avoid *Neisseria gonorrhoea* transmission from infected mothers. Of all the metals with antimicrobial properties, silver has been discovered to have the most potent antibacterial activity and to be the least toxic to animal cells. . The exact mechanisms of silver nanoparticles' antimicrobial or toxicity activities continue to be researched and are a hotly debated subject. The positive charge on Ag ions is thought to be essential for antimicrobial properties. Silver must be in its ionised state in order to have some antimicrobial properties. Silver is non-reactive in its ionised form, however,



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when it comes into contact with moisture, it releases silver ions [76]. Ions of silver may form complexes with nucleic acids, preferring to interact with nucleosides over phosphate groups. As a consequence, all forms of antimicrobial silver or silver-containing compounds are sources of silver ions ( $Ag^+$ ); these ions of silver can be absorbed into the substance and released gradually over time as in the case of silver sulfadiazine, or they are able to ionise from the surface of a substantial silver piece as in the case of silver nanoparticles.[77,78]The scientific literature has recorded the electrostatic attraction between positively charged nanoparticles and negatively charged bacterial cells.[79] They have been proposed as the most effective bactericidal agent.[80,81]It's been shown that these nanoparticles build up within membranes and then penetrate into cells, causing damage to cell walls and membranes. Silver atoms bind to thiol groups (ASH) of enzymes, forming stable SA-Ag bonds with thiol-containing compounds, deactivating enzymes involved in transmembrane vitality creation and ion transport in the cell membrane. The  $Ag(I)$  ion enters the cell and interpolates between purine and pyrimidine base pairs, disrupting hydrogen bonding between the two anti-parallel strands and denaturing the DNA molecule, according to one theory. One reason for its antibacterial properties is bacterial cell lysis. A bacterial peptide's phosphotyrosine profile was modulated by nanoparticles, which affected signal transduction and inhibited microbial development. There is a dose-dependent antibacterial function which is unaffected because of the growth of antibiotic resistance in bacteria. The accumulation of silver nanoparticles in the bacterial membrane was discovered in *E. coli* bacteria treated with silver nanoparticles, resulting in an increase in permeability and cell death.

#### **Antioxidant Activity**

Antioxidant activity has been observed in AgNPs having nearly spherical morphology. Free radicals such as DPPH,  $H_2O_2$ , hydroxyl, and superoxide radicals are effectively scavenged by biosynthesized AgNPs. When compared to AgNPs synthesised using their respective fruit extract, plant-based AgNPs demonstrated higher antioxidant and antibacterial activity [82]. AgNPs' antioxidant properties are due to their surface-coated bioactive compounds, which are extracted from the biological extract used in their synthesis. With the increasing AgNPs concentrations, the antioxidant properties improve, resulting in a higher percentage of DPPH radical scavenging abilities. The scavenging activity often follows a dose-dependent trend, increasing as the AgNPs dose is increased.

#### **Anticancer Activity**

The use of polymeric nanoparticles as a drug carrier in a targeted delivery system may be a brilliant way to treat cancer. Biodegradability, biocompatibility, non-toxicity, sustained circulation, and a broad payload range of a therapeutic agent are all important features. Other distinguishing characteristics include their distinct size and shape properties for active and passive tissue penetration, complex cellular/subcellular trafficking pathways, and simple cargo release control through sophisticated material engineering. NPs with a diameter of 10 to 100 nm are commonly used in cancer therapy because they can deliver drugs efficiently and have an enhanced permeability and retention (EPR) effect. Smaller particles (less than 1–2 nm) can easily leak from normal vasculature and be filtered by kidneys (less than 10 nm in diameter),[83] while particles larger than 100 nm are more likely to be cleared from circulation by phagocytes.[84] For example, NPs coated with hydrophilic materials such as polyethylene glycol (PEG) reduce opsonization and thus avoid immune system clearance [85]. The use of liposomes in the treatment of breast and prostate cancer [86] is becoming more popular [87,88]. The co-polymerization of glycolic acid and lactic acid results in poly-lactic-co-glycolic acid (PLGA), a common polymeric NP. PLGA is a popular drug delivery carrier [89,90]. Furthermore, dendrimers are a type of polymer that has been used in nanomedicine. They are defined as flexible and biocompatible macromolecules with a three-dimensional branch structure [91]. On the surface, their various functional groups improve the ability to load and distribute therapeutic agents. In addition, polymeric micelles, which are characterized by polymer self-assembly into nano-aggregates as they are composed of amphiphilic copolymers, constitute another kind of widely investigated polymer NPs [92]. The hydrophobic core allows insoluble anticancer drugs to be absorbed and distributed smoothly, while the hydrophilic section improves stability, reducing drug absorption by the reticuloendothelial system and extending drug circulation time [93].



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NPs are unique in biosensors because they can be detected using a variety of methods, including optic absorption, fluorescence, and electric conductivity. The Ag-NPs achieve very high sensitivity and measurements by using SPR [94]. The novel physicochemical properties of metals at the nanoscale have benefited nano biosensors for disease detection, monitoring malady pathogenesis or therapy monitoring, cell tracking, and nanoprobe in vivo detection/imaging [95].

**Wastewater Treatment**

Silver nanoparticles pose a significant threat to wastewater treatment plants and biological systems. Silver nanoparticles inhibit the nitrifying bacteria, which may be harmful to wastewater treatment microorganisms. The risk of silver nanoparticles to the environment was recently investigated by measuring the amount of silver that was released from commercial clothing. Silver nanoparticles with a diameter of 10–500 nm were found in the sock material and waste water.

**Drug Delivery**

The therapeutic efficacy of pharmacologically beneficial drugs prescribed for disease treatment is significantly reduced as they encounter physicochemical and physiological obstacles at disease sites. As a result, one of the major challenges of modern medicine is enhancing the physicochemical properties of medicaments and overcoming physiological obstacles to drug delivery into the disease site for effective therapeutic action [96,97]. The following physicochemical properties are linked to the limited effectiveness of conventional dosage forms: (a) poor water solubility of drugs, (b) ionisation lipophilicity of drugs, (c) large molecular weight of drugs, (d) lower permeability of drugs, (e) difficulty in controlling drug release kinetics, and (f) lack of spatial and progressive release of therapeutic moieties at the diseased site [98]. These restrictions and shortcomings can be overcome by a controlled drug delivery system where the drug is delivered directly to the site of action, capacitated to surpass the effects of physicochemical and physiological barriers, thereby, improving the efficiency of drug delivery.

**Catalytic Activity**

Nano catalysis is a quickly expanding field in which nanomaterials are used as catalysts in a number of catalytic processes. It has the potential to be a promising field of science due to its high activity, selectivity, and productivity. The first papers on poly (vinyl) alcohol-protected palladium and platinum nanoparticle hydrogenation catalysts prepared by reduction of metal salts by H<sub>2</sub> were published in 1941 [99]. Nano catalysts have special properties due to their high surface-to-volume ratio. The borohydride reduction of many organic dyes is catalysed by the nanoparticles. The effective particle-mediated electron transfer from the reducing agent to the dyes is responsible for catalysis. Nanoparticles' catalytic activity can be regulated by their size, which is proportional to their redox potential. By using sodium borohydride to reduce nitro compounds, such as 4-nitrophenol and 2-nitrophenol, to amino compounds, the synthesised metal nanoparticles are used as catalysts [100].

**Human Health Benefits**

Nanoparticles have a variety of health effects when compared to the bulk content from which they are made [101]. Nanoparticles are small enough to enter the body via the skin, lungs, or brain [102]. When metal-containing nanoparticles were exposed to human lung epithelial cells, reactive oxygen species were formed, causing oxidative stress and cell damage [103,104]. Because of its rapid development and transparent body structure, a report on the toxic effects of silver nanoparticles was conducted using Zebrafish as a model. The biocompatibility and toxicity of silver nanoparticles were demonstrated in embryos at different stages of development. The types of abnormalities in Zebrafish were highly influenced by the amount of silver nanoparticles provided to them [105]. Silver nanoparticles have been suggested as an antibiotic and a preservative, meaning they can come into contact with humans. As a result, it's important to look at the impact of silver nanoparticles on human health.



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## CONCLUSION

Nature has produced the most versatile miniaturized functional materials in elegant and ingenious ways. The desire to develop environmentally sustainable techniques has been fuelled by knowledge of green chemistry and the use of green routes for metal nanoparticle synthesis. The benefit of using plant extracts to make silver nanoparticles is that it is cost-effective, energy-efficient, and provides healthy work environments and ecosystems, as well as safeguarding human health and the environment resulting in less waste and safer goods. Plants may be preferable to other biological entities for nanoparticle synthesis because they can avoid the time-consuming process of employing microbes and preserving their culture, which can lead to the loss of their capacity for nanoparticle synthesis. As a result, the use of plant extract for synthesis may have a huge effect in the coming decades. Since cancer is one of the most deadly diseases, nanotechnology's contribution to precise care while preventing life-threatening side effects has the ability to lead to a positive shift in clinical practise toward a more life-saving approach.

## CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

## REFERENCES

1. Sarsar V, Selwal KK, Selwal MK. Green synthesis of silver nanoparticles using leaf extract of *Mangifera indica* and evaluation of their antimicrobial activity. *J Microbiol Biotech Res*. 2013;3(5):27-32.
2. Castro L, Blázquez ML, González FG, Ballester A. Mechanism and applications of metal nanoparticles prepared by bio-mediated process. *Reviews in Advanced Sciences and Engineering*. 2014 Sep 1;3(3):199-216.
3. Tran QH, Le AT. Silver nanoparticles: synthesis, properties, toxicology, applications and perspectives. *Advances in Natural Sciences: Nanoscience and Nanotechnology*. 2013 May 14;4(3):033001.
4. Leaper DJ. Silver dressings: their role in wound management. *International wound journal*. 2006 Dec;3(4):282-94.
5. Muthu MS, Wilson B. Multifunctional radio nanomedicine: a novel nanoplatform for cancer imaging and therapy. *Nanomedicine*. 2010 Feb;5(2):169-71.
6. Sathishkumar M, Sneha K, Won SW, Cho CW, Kim S, Yun YS. Cinnamon zeylanicum bark extract and powder mediated green synthesis of nano-crystalline silver particles and its bactericidal activity. *Colloids and Surfaces B: Biointerfaces*. 2009 Oct 15;73(2):332-8.
7. Brown SD, Nativo P, Smith JA, Stirling D, Edwards PR, Venugopal B, Flint DJ, Plumb JA, Graham D, Wheate NJ. Gold nanoparticles for the improved anticancer drug delivery of the active component of oxaliplatin. *Journal of the American Chemical Society*. 2010 Apr 7;132(13):4678-84.
8. Hanlon PR, Webber DM, Barnes DM. Aqueous extract from Spanish black radish (*Raphanus sativus* L. Var. *niger*) induces detoxification enzymes in the HepG2 human hepatoma cell line. *Journal of agricultural and food chemistry*. 2007 Aug 8;55(16):6439-46.
9. Pradeep T. Noble metal nanoparticles for water purification: a critical review. *Thin solid films*. 2009 Oct 30;517(24):6441-78.
10. Saraf S. Applications of novel drug delivery system for herbal formulations. *Fitoterapia*. 2010 Oct 1;81(7):680-9.
11. Shamaila S, Sajjad AK, Farooqi SA, Jabeen N, Majeed S, Farooq I. Advancements in nanoparticle fabrication by hazard free eco-friendly green routes. *Applied Materials Today*. 2016 Dec 1;5:150-99.
12. Sutradhar KB, Amin M. Nanotechnology in cancer drug delivery and selective targeting. *International Scholarly Research Notices*. 2014;2014.
13. Bar H, Bhui DK, Sahoo GP, Sarkar P, De SP, Misra A. Green synthesis of silver nanoparticles using latex of *Jatropha curcas*. *Colloids and surfaces A: Physicochemical and engineering aspects*. 2009 May 1;339(1-3):134-9.
14. Narayanan R, El-Sayed MA. Catalysis with transition metal nanoparticles in colloidal solution: nanoparticle shape dependence and stability. *The Journal of Physical Chemistry B*. 2005 Jul 7;109(26):12663-76.



**Ayushi Chaturvedi et al.,**

15. Llorens A, Lloret E, Picouet PA, Trbojevich R, Fernandez A. Metallic-based micro and nanocomposites in food contact materials and active food packaging. *Trends in Food Science & Technology*. 2012 Mar 1;24(1):19-29.
16. Liao H, Nehl CL, Hafner JH. Biomedical applications of plasmon resonant metal nanoparticles.
17. Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. *Biotechnology advances*. 2009 Jan 1;27(1):76-83.
18. Shankar SS, Rai A, Ahmad A, Sastry M. Rapid synthesis of Au, Ag, and bimetallic Au core–Ag shell nanoparticles using Neem (*Azadirachta indica*) leaf broth. *Journal of colloid and interface science*. 2004 Jul 15;275(2):496-502.
19. Wiley BJ, Im SH, Li ZY, McLellan J, Siekkinen A, Xia Y. Maneuvering the surface plasmon resonance of silver nanostructures through shape-controlled synthesis.
20. Medina-Ramirez I, Bashir S, Luo Z, Liu JL. Green synthesis and characterization of polymer-stabilized silver nanoparticles. *Colloids and Surfaces B: Biointerfaces*. 2009 Oct 15;73(2):185-91.
21. Panáček A, Kolář M, Večeřová R, Pucek R, Soukupova J, Kryštof V, Hamal P, Zbořil R, Kvítek L. Antifungal activity of silver nanoparticles against *Candida* spp. *Biomaterials*. 2009 Oct 1;30(31):6333-40.
22. Gurunathan S, Lee KJ, Kalishwaralal K, Sheikpranbabu S, Vaidyanathan R, Eom SH. Antiangiogenic properties of silver nanoparticles. *Biomaterials*. 2009 Oct 1;30(31):6341-50.
23. Rogers JV, Parkinson CV, Choi YW, Speshock JL, Hussain SM. A preliminary assessment of silver nanoparticle inhibition of monkeypox virus plaque formation. *Nanoscale Research Letters*. 2008 Apr;3(4):129-33.
24. Tripathy A, Raichur AM, Chandrasekaran N, Prathna TC, Mukherjee A. Process variables in biomimetic synthesis of silver nanoparticles by aqueous extract of *Azadirachta indica* (Neem) leaves. *Journal of Nanoparticle Research*. 2010 Jan;12(1):237-46.
25. Dwivedi AD, Gopal K. Biosynthesis of silver and gold nanoparticles using *Chenopodium album* leaf extract. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*. 2010 Oct 20;369(1-3):27-33.
26. Saxena A, Tripathi RM, Singh RP. Biological synthesis of silver nanoparticles by using onion (*Allium cepa*) extract and their antibacterial activity. *Dig J Nanomater Bios*. 2010 Jun 1;5(2):427-32.
27. Dubey M, Bhadauria S, Kushwah BS. Green synthesis of nanosilver particles from extract of *Eucalyptus hybrida* (safeda) leaf. *Dig J Nanomater Biostruct*. 2009 Sep 1;4(3):537-43.
28. Jha AK, Prasad K. Green synthesis of silver nanoparticles using *Cycas* leaf. *International Journal of Green Nanotechnology: Physics and Chemistry*. 2010 May 20;1(2):P110-7.
29. Gopinath V, MubarakAli D, Priyadarshini S, Priyadharsshini NM, Thajuddin N, Velusamy P. Biosynthesis of silver nanoparticles from *Tribulus terrestris* and its antimicrobial activity: a novel biological approach. *Colloids and Surfaces B: Biointerfaces*. 2012 Aug 1;96:69-74.
30. Moghimi SM, Hunter AC, Murray JC. Nanomedicine: current status and future prospects. *The FASEB journal*. 2005 Mar;19(3):311-30.
31. Vinogradov S, Wei X. Cancer stem cells and drug resistance: the potential of nanomedicine. *Nanomedicine*. 2012 Apr;7(4):597-615.
32. Mamidala E, Gujjeti RP. Phytochemical and antimicrobial activity of *Acmella paniculata* plant extracts. *J. Bio Innov*. 2013;1:17-22.
33. Jagatheeswari D, Deepa J, Ali HS, Ranganathan P. *Acalypha indica* L-An important medicinal plant: A review of its traditional uses and pharmacological properties. *International Journal of Research in Botany*. 2013;3(1):19-22.
34. Prabhu S, Poulouse EK. Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. *International nano letters*. 2012 Dec;2(1):1-0.
35. Ayyanar M, Ignacimuthu S. Medicinal plants used by the tribals of Tirunelveli hills, Tamil Nadu to treat poisonous bites and skin diseases.
36. Nyamai DW, Arika W, Ogola PE, Njagi EN, Ngugi MP. Medicinally important phytochemicals: an untapped research avenue. *Journal of Pharmacognosy and phytochemistry*. 2016 Mar;4(4):2321-6182.
37. Gardea-Torresdey JL, Gomez E, Peralta-Videa JR, Parsons JG, Troiani H, Jose-Yacaman M. Alfalfa sprouts: a natural source for the synthesis of silver nanoparticles. *Langmuir*. 2003 Feb 18;19(4):1357-61.



**Ayushi Chaturvedi et al.,**

38. Velmurugan P, Sivakumar S, Young-Chae S, Seong-Ho J, Pyoung-In Y, Jeong-Min S, Sung-Chul H. Synthesis and characterization comparison of peanut shell extract silver nanoparticles with commercial silver nanoparticles and their antifungal activity. *Journal of Industrial and Engineering Chemistry*. 2015 Nov 25;31:51-4.
39. Ahmad N, Sharma S, Alam MK, Singh VN, Shamsi SF, Mehta BR, Fatma A. Rapid synthesis of silver nanoparticles using dried medicinal plant of basil. *Colloids and Surfaces B: Biointerfaces*. 2010 Nov 1;81(1):81-6.
40. Liu H, Zheng S, Xiong H, Alwahibi MS, Niu X. Biosynthesis of copperoxide nanoparticles using *Abies spectabilis* plant extract and analyzing its antinociceptive and anti-inflammatory potency in various mice models. *Arabian Journal of Chemistry*. 2020 Sep 1;13(9):6995-7006.
41. Ahamed M, Khan MM, Siddiqui MK, AlSalhi MS, Alrokayan SA. Green synthesis, characterization and evaluation of biocompatibility of silver nanoparticles. *Physica E: Low-dimensional Systems and Nanostructures*. 2011 Apr 1;43(6):1266-71.
42. Armendariz V, Herrera I, Jose-yacaman M, Troiani H, Santiago P, Gardea-Torresdey JL. Size controlled gold nanoparticle formation by *Avena sativa* biomass: use of plants in nanobiotechnology. *Journal of nanoparticle research*. 2004 Aug;6(4):377-82.
43. Tripathy A, Raichur AM, Chandrasekaran N, Prathna TC, Mukherjee A. Process variables in biomimetic synthesis of silver nanoparticles by aqueous extract of *Azadirachta indica* (Neem) leaves. *Journal of Nanoparticle Research*. 2010 Jan;12(1):237-46.
44. Kora AJ, Sashidhar RB, Arunachalam J. Aqueous extract of gum olibanum (*Boswellia serrata*): a reductant and stabilizer for the biosynthesis of antibacterial silver nanoparticles. *Process Biochemistry*. 2012 Oct 1;47(10):1516-20.
45. Gangula A, Podila R, Karanam L, Janardhana C, Rao AM. Catalytic reduction of 4-nitrophenol using biogenic gold and silver nanoparticles derived from *Breynia rhamnoides*. *Langmuir*. 2011 Dec 20;27(24):15268-74.
46. Zhao H, Su H, Ahmeda A, Sun Y, Li Z, Zangeneh MM, Nowrozi M, Zangeneh A, Moradi R. Biosynthesis of copper nanoparticles using *Allium eriophyllum* Boiss leaf aqueous extract; characterization and analysis of their antimicrobial and cutaneous wound-healing potentials. *Applied Organometallic Chemistry*. 2020 Feb 27:e5587.
47. Selvan SM, Anand KV, Govindaraju K, Tamilselvan S, Kumar VG, Subramanian KS, Kannan M, Raja K. Green synthesis of copper oxide nanoparticles and mosquito larvicidal activity against dengue, zika and chikungunya causing vector *Aedes aegypti*. *IET nanobiotechnology*. 2018 Jul 27;12(8):1042-6.
48. Rehana D, Mahendiran D, Kumar RS, Rahiman AK. Evaluation of antioxidant and anticancer activity of copper oxide nanoparticles synthesized using medicinally important plant extracts. *Biomedicine & Pharmacotherapy*. 2017 May 1;89:1067-77.
49. Tshireletso P, Ateba CN, Fayemi OE. Spectroscopic and Antibacterial Properties of CuONPs from Orange, Lemon and Tangerine Peel Extracts: Potential for Combating Bacterial Resistance. *Molecules*. 2021 Jan;26(3):586.
50. Sackey J, Nwanya A, Bashir AK, Matinise N, Ngilirabanga JB, Ameh AE, Coetsee E, Maaza M. Electrochemical properties of *Euphorbia pulcherrima* mediated copper oxide nanoparticles. *Materials Chemistry and Physics*. 2020 Apr 1;244:122714.
51. Baghizadeh A, Ranjbar S, Gupta VK, Asif M, Pourseyedi S, Karimi MJ, Mohammadinejad R. Green synthesis of silver nanoparticles using seed extract of *Calendula officinalis* in liquid phase. *Journal of molecular liquids*. 2015 Jul 1;207:159-63.
52. Jamuna KS, Banu S, Brindha P, Kurian GA. Nano-scale preparation of titanium dioxide by *Desmodium gangeticum* root aqueous extract. *Ceramics International*. 2014 Sep 1;40(8):11933-40.
53. Li S, Shen Y, Xie A, Yu X, Qiu L, Zhang L, Zhang Q. Green synthesis of silver nanoparticles using *Capsicum annuum* L. extract. *Green Chemistry*. 2007;9(8):852-8.
54. Gopinath V, MubarakAli D, Priyadarshini S, Priyadharsshini NM, Thajuddin N, Velusamy P. Biosynthesis of silver nanoparticles from *Tribulus terrestris* and its antimicrobial activity: a novel biological approach. *Colloids and Surfaces B: Biointerfaces*. 2012 Aug 1;96:69-74.
55. Armendariz V, José Yacamán M, Duarte Moller A, Peralta Videa JR, Troiani HE, Herrera I, Gardea Torres JL. HRTEM characterization of gold nanoparticles produced by wheat biomass.



**Ayushi Chaturvedi et al.,**

56. Zargar M, Shameli K, Najafi GR, Farahani F. Plant mediated green biosynthesis of silver nanoparticles using *Vitex negundo* L. extract. *Journal of Industrial and Engineering Chemistry*. 2014 Nov 25;20(6):4169-75.
57. Umashankari J, Inbakandan D, Ajithkumar TT, Balasubramanian T. Mangrove plant, *Rhizophora mucronata* (Lamk, 1804) mediated one pot green synthesis of silver nanoparticles and its antibacterial activity against aquatic pathogens. *Aquatic biosystems*. 2012 Dec;8(1):1-7.
58. Mittal, A. K., Kaler, A., & Banerjee, U. C. Free Radical Scavenging and Antioxidant Activity of Silver Nanoparticles Synthesized from Flower Extract of *Rhododendron dauricum*. *Nano Biomedicine & Engineering*, 2012;4(3).
59. Das J, Das MP, Velusamy P. *Sesbania grandiflora* leaf extract mediated green synthesis of antibacterial silver nanoparticles against selected human pathogens. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*. 2013 Mar 1;104:265-70.
60. Umadevi M, Bindhu MR, Sathe V. A novel synthesis of malic acid capped silver nanoparticles using *Solanum lycopersicum* fruit extract. *Journal of Materials Science & Technology*. 2013 Apr 1;29(4):317-22.
61. Ankamwar B, Damle C, Ahmad A, Sastry M. Biosynthesis of gold and silver nanoparticles using *Emblca officinalis* fruit extract, their phase transfer and transmetallation in an organic solution. *Journal of nanoscience and nanotechnology*. 2005 Oct 1;5(10):1665-71.
62. Dubey SP, Lahtinen M, Sillanpää M. Tansy fruit mediated greener synthesis of silver and gold nanoparticles. *Process Biochemistry*. 2010 Jul 1;45(7):1065-71.
63. Reddy NJ, Vali DN, Rani M, Rani SS. Evaluation of antioxidant, antibacterial and cytotoxic effects of green synthesized silver nanoparticles by *Piper longum* fruit. *Materials Science and Engineering: C*. 2014 Jan 1;34:115-22.
64. Anuradha J, Abbasi T, Abbasi SA. An eco-friendly method of synthesizing gold nanoparticles using an otherwise worthless weed *pistia* (*Pistia stratiotes* L.). *Journal of advanced research*. 2015 Sep 1;6(5):711-20.
65. Bindhu MR, Umadevi M, Esmail GA, Al-Dhabi NA, Arasu MV. Green synthesis and characterization of silver nanoparticles from *Moringa oleifera* flower and assessment of antimicrobial and sensing properties. *Journal of Photochemistry and Photobiology B: Biology*. 2020 Apr 1;205:111836.
66. Ibrahim HM. Green synthesis and characterization of silver nanoparticles using banana peel extract and their antimicrobial activity against representative microorganisms. *Journal of Radiation Research and Applied Sciences*. 2015 Jul 1;8(3):265-75.
67. Vijayaraghavan K, Nalini SK, Prakash NU, Madhankumar D. One step green synthesis of silver nano/microparticles using extracts of *Trachyspermum ammi* and *Papaver somniferum*. *Colloids and Surfaces B: Biointerfaces*. 2012 Jun 1;94:114-7.
68. Sathyavathi R, Krishna MB, Rao SV, Saritha R, Rao DN. Biosynthesis of silver nanoparticles using *Coriandrum sativum* leaf extract and their application in nonlinear optics. *Advanced science letters*. 2010 Jun 1;3(2):138-43.
69. Halder KM, Halder B, Chandra G. Fabrication, characterization and mosquito larvicidal bioassay of silver nanoparticles synthesized from aqueous fruit extract of *putranjiva*, *Drypetes roxburghii* (Wall.). *Parasitology research*. 2013 Apr;112(4):1451-9.
70. Von White G, Kerscher P, Brown RM, Morella JD, McAllister W, Dean D, Kitchens CL. Green synthesis of robust, biocompatible silver nanoparticles using garlic extract. *Journal of Nanomaterials*. 2012 Jan 1;2012.
71. Lukman AI, Gong B, Marjo CE, Roessner U, Harris AT. Facile synthesis, stabilization, and anti-bacterial performance of discrete Ag nanoparticles using *Medicago sativa* seed exudates. *Journal of colloid and interface science*. 2011 Jan 15;353(2):433-44.
72. Dhand V, Soumya L, Bharadwaj S, Chakra S, Bhatt D, Sreedhar B. Green synthesis of silver nanoparticles using *Coffea arabica* seed extract and its antibacterial activity. *Materials Science and Engineering: C*. 2016 Jan 1;58:36-43.
73. Chandran SP, Chaudhary M, Pasricha R, Ahmad A, Sastry M. Synthesis of gold nanotriangles and silver nanoparticles using *Aloevera* plant extract. *Biotechnology progress*. 2006;22(2):577-83.
74. Swamy VS, Prasad R. Green synthesis of silver nanoparticles from the leaf extract of *Santalum album* and its antimicrobial activity. *J Optoelectron Biomed Mater*. 2012 Jul;4(3):53-9.



**Ayushi Chaturvedi et al.,**

75. Sharneli K, Ahmad MB, Shabanzadeh P, Al-Mulla EA, Zamanian A, Abdollahi Y, Jazayeri SD, Eili M, Jalilian FA, Haroun RZ. Effect of Curcuma longa tuber powder extract on size of silver nanoparticles prepared by green method. *Research on Chemical Intermediates*. 2014 Mar;40(3):1313-25.
76. Klueh U, Wagner V, Kelly S, Johnson A, Bryers JD. Efficacy of silver-coated fabric to prevent bacterial colonization and subsequent device-based biofilm formation. *Journal of Biomedical Materials Research: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials*. 2000;53(6):621-31.
77. Yakabe Y, Sano T, Ushio H, Yasunaga T. Kinetic studies of the interaction between silver ion and deoxyribonucleic acid. *Chemistry Letters*. 1980 Apr 5;9(4):373-6.
78. Sondi I, Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. *Journal of colloid and interface science*. 2004 Jul 1;275(1):177-82.
79. Cao Y, Jin R, Mirkin CA. DNA-modified core-shell Ag/Au nanoparticles. *Journal of the American Chemical Society*. 2001 Aug 15;123(32):7961-2.
80. Wright JB, Lam K, Hansen D, Burrell RE. Efficacy of topical silver against fungal burn wound pathogens. *American journal of infection control*. 1999 Aug 1;27(4):344-50.
81. Eby DM, Schaeublin NM, Farrington KE, Hussain SM, Johnson GR. Lysozyme catalyzes the formation of antimicrobial silver nanoparticles. *ACS nano*. 2009 Apr 28;3(4):984-94.
82. Ge L, Li Q, Wang M, Ouyang J, Li X, Xing MM. Nanosilver particles in medical applications: synthesis, performance, and toxicity. *International journal of nanomedicine*. 2014;9:2399.
83. Venturoli D, Rippe B. Ficoll and dextran vs. globular proteins as probes for testing glomerular permselectivity: effects of molecular size, shape, charge, and deformability. *American Journal of Physiology-Renal Physiology*. 2005 Apr;288(4):F605-13.
84. Decuzzi P, Pasqualini R, Arap W, Ferrari M. Intravascular delivery of particulate systems: does geometry really matter?. *Pharmaceutical research*. 2009 Jan;26(1):235-43.
85. Yang Q, Jones SW, Parker CL, Zamboni WC, Bear JE, Lai SK. Evading immune cell uptake and clearance requires PEG grafting at densities substantially exceeding the minimum for brush conformation. *Molecular pharmaceutics*. 2014 Apr 7;11(4):1250-8.
86. Yari H, Nkepeng G, Awasthi V. Surface modification of liposomes by a lipopolymer targeting prostate specific membrane antigen for theranostic delivery in prostate cancer. *Materials*. 2019 Jan;12(5):756.
87. Satsangi A, Roy SS, Satsangi RK, Tolcher AW, Vadlamudi RK, Goins B, Ong JL. Synthesis of a novel, sequentially active-targeted drug delivery nanoplatforM for breast cancer therapy. *Biomaterials*. 2015 Aug 1;59:88-101.
88. Tang B, Peng Y, Yue Q, Pu Y, Li R, Zhao Y, Hai L, Guo L, Wu Y. Design, preparation and evaluation of different branched biotin modified liposomes for targeting breast cancer. *European journal of medicinal chemistry*. 2020 May 1;193:112204.
89. Acharya S, Sahoo SK. PLGA nanoparticles containing various anticancer agents and tumour delivery by EPR effect. *Advanced drug delivery reviews*. 2011 Mar 18;63(3):170-83.
90. Nanjwade BK, Bechra HM, Derkar GK, Manvi FV, Nanjwade VK. Dendrimers: emerging polymers for drug-delivery systems. *European Journal of Pharmaceutical Sciences*. 2009 Oct 8;38(3):185-96.
91. Sherje AP, Jadhav M, Dravyakar BR, Kadam D. Dendrimers: A versatile nanocarrier for drug delivery and targeting. *International journal of pharmaceutics*. 2018 Sep 5;548(1):707-20.
92. Zhou Q, Zhang L, Yang T, Wu H. Stimuli-responsive polymeric micelles for drug delivery and cancer therapy. *International journal of nanomedicine*. 2018;13:2921.
93. Cagel M, Tesan FC, Bernabeu E, Salgueiro MJ, Zubillaga MB, Moretton MA, Chiappetta DA. Polymeric mixed micelles as nanomedicines: Achievements and perspectives. *European Journal of Pharmaceutics and Biopharmaceutics*. 2017 Apr 1;113:211-28.
94. Doria G, Conde J, Veigas B, Giestas L, Almeida C, Assunção M, Rosa J, Baptista PV. Noble metal nanoparticles for biosensing applications. *Sensors*. 2012 Feb;12(2):1657-87.
95. Marchiol L. Synthesis of metal nanoparticles in living plants. *Italian Journal of Agronomy*. 2012 Aug 6:e37-.





**Ayushi Chaturvedi et al.,**

96. De Jong WH, Borm PJ. Drug delivery and nanoparticles: applications and hazards. *International journal of nanomedicine*. 2008 Jun;3(2):133.
97. Safari J, Zarnegar Z. Advanced drug delivery systems: Nanotechnology of health design A review. *Journal of Saudi Chemical Society*. 2014 Apr 1;18(2):85-99.
98. Gupta S, Kesarla R, Omri A. Formulation strategies to improve the bioavailability of poorly absorbed drugs with special emphasis on self-emulsifying systems. *International Scholarly Research Notices*. 2013;2013.
99. Rampino LD, Nord FF. Preparation of palladium and platinum synthetic high polymer catalysts and the relationship between particle size and rate of hydrogenation. *Journal of the American Chemical Society*. 1941 Oct;63(10):2745-9.
100. Pradhan N, Pal A, Pal T. Silver nanoparticle catalyzed reduction of aromatic nitro compounds. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*. 2002 Jan 15;196(2-3):247-57.
101. Albrecht MA, Evans CW, Raston CL. Green chemistry and the health implications of nanoparticles. *Green chemistry*. 2006;8(5):417-32
102. Kozziara JM, Lockman PR, Allen DD, Mumper RJ. In situ blood–brain barrier transport of nanoparticles. *Pharmaceutical research*. 2003 Nov;20(11):1772-8.
103. Limbach LK, Wick P, Manser P, Grass RN, Bruinink A, Stark WJ. Exposure of engineered nanoparticles to human lung epithelial cells: influence of chemical composition and catalytic activity on oxidative stress. *Environmental science & technology*. 2007 Jun 1;41(11):4158-63.
104. Xia T, Kovochich M, Brant J, Hotze M, Sempf J, Oberley T, Sioutas C, Yeh JI, Wiesner MR, Nel AE. Comparison of the abilities of ambient and manufactured nanoparticles to induce cellular toxicity according to an oxidative stress paradigm. *Nano letters*. 2006 Aug 9;6(8):1794-807.
105. Asharani PV, Wu YL, Gong Z, Valiyaveetil S. Toxicity of silver nanoparticles in zebrafish models. *Nanotechnology*. 2008 May 14;19(25):255102.

**Table 1. Biogenic Synthesis of Nanoparticles Using Different Parts of Plants**

Name of plant	Parts of plant	Metallic nanoparticles	Size and shape	Citation
<i>Abies spectabilis</i>	Leaves	CuSO <sub>4</sub>	50; spherical	[40]
<i>Allium sativum</i>	Bud	Ag	4-22 nm; spherical	[41]
<i>Avena sativa</i>	leaf	Au	Various shaped	[42]
<i>Azadirachta indica</i>	leaf	Au	20 nm; nearly spherical shape	[43]
<i>Boswellia serrata</i>	Gum	Ag	7.5 ± 3.8 nm; Spherical	[44]
<i>Breynia rhamnoides</i>	stem	Ag	64 nm	[45]
<i>Allium eriophyllum Boiss</i>	Leaf	Cu	30–35 Spherical	[46]
<i>Tridax procumbens (Tridax daisy)</i>	leaves	Cu	16 spherical	[47]
<i>Tamarindus indica</i>	leaves	Cu	12spherical	[48]
<i>Citrus (Orange, lemon)</i>	Peel of fruit	Cu	48–76 Globular	[49]
<i>Euphorbia pulcherrima</i>	Floral	Cu	16-153cubical	[50]
<i>Calendula officinalis</i>	Seed	Ag	5–10 nm; spherical	[51]
<i>Desmodium</i>	Leaf	Ti	100nm/Spherical	[52]
<i>Capsicum annum</i>	leaf	Ag	10-12	[53]
<i>Tribulus terrestris</i>	Fruit	Ag	16-28 spherical	[54]
<i>Triticum aestivum</i>	Biomass	Au	10-30 nm	[55]
<i>Vitex negundo</i>	Leaf	Ag	10-30: fcc	[56]
<i>Rhizophora mucronata</i>	Leaf bud	Ag	4-26: spherical	[57]
<i>Rhododendron dauricam</i>	Flower	Ag	25-40: spherical	[58]
<i>Sesbania grandiflora</i>	Leaf	Ag	10-25: spherical	[59]





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<i>Solanum lycopersicum</i>	Fruit	Ag	10nm spherical	[60]
<i>Tamarindus indica</i>	Leaf	Au	Triangular	[61]
<i>Tanacetum vulgare</i>	Fruit	Ag	16nmtriangular	[62]
<i>Piper longum</i>	Fruit	Ag	15-200nm	[63]
<i>Pistia stratiotes (weed)</i>	Aerial and submerged parts	Au	2-155nm Hexagonal pentagonal	[64]
<i>Moringa oleifera</i>	Flower	Ag	14nm spherical	[65]
<i>Musa paradisiaca</i>	Peel	Ag	20nm cluster	[66]
<i>Papaver somniferum</i>	Seed	Ag	3-7nm spherical	[67]
<i>Coriandrum sativum</i>	Leaf	Ag	26nm spherical	[68]
<i>Drypetes roxburghii</i>	Fruit	Ag	10-35nm	[69]
<i>Garlic</i>	Leaf	Ag	4-7nm	[70]
<i>Medicago sativa</i>	Leaf	Ag	20-40nm	[71]
<i>Coffea arabica</i>	Seed	Ag	2-30nm	[72]
<i>Aloe vera</i>	Leaf	Ag	15nm	[73]
<i>Santalum album</i>	Leaf	Ag	80-200nm	[74]
<i>Curcuma longa</i>	Tuber	Ag	10 nm	[75]





## Effect of Shifting Cultivation on Soil health and Microbial Diversity in Bhor Taluka of Pune District

V. Patturajan<sup>1\*</sup>, R. G. Jaybhaye<sup>2</sup> and Y. Shouche<sup>3</sup>

<sup>1</sup>Dr. D. Y. Patil Arts, Commerce, Science College, Pimpri, Pune-411018, India.

<sup>2</sup>Department of Geography, Savitribai Phule Pune University, Pune-411007, India.

<sup>3</sup>National Centre for Cell Science, Pune University Campus, Pune-411007, India.

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### \*Address for Correspondence

**V. Patturajan**

Dr. D. Y. Patil Arts, Commerce,  
Science College, Pimpri,  
Pune-411018, India.



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### ABSTRACT

Shifting cultivation is also referred to as Swidden cultivation, slash and burn cultivation, bush-fallow agriculture. Its name varies in different parts of the world. The physico-chemical characters of the soil are affected by the crop type and agricultural practices. Microbial biomass of soil and its number also influence the soil health. The present study has attempted to find out the impact of shifting cultivation on soil bacterial communities and soil quality in the western ghat region of Pune district. 16 soil samples, from 5 villages of Bhor tehsil were collected. The soil samples included the fallow land and crop (Varai, Sawa, Nachani and Hulga) harvested soils. Eleven bacterial genus were isolated from all soils in varying number. It was observed that the TVC of the crop harvest soil significantly decreased as compared to the fallow soil. Different soil parameters were also found to be improved in the fallow period, pointing out the importance of the length of the fallowing. Few remedial measures are also suggested to improve the soil health.

**Keywords:** Shifting cultivation, Physico-chemical parameters, Microbial parameters, fallow period.

### INTRODUCTION

The historical background of shifting cultivation is old as the history of agriculture itself [1]. The "Shifting Cultivation", its name varies in different parts of the world and generally known as "Slash and Burn" and "bush fallow" agriculture. It is known as "Jhum" in the hilly states of north-east India, Podu in Orissa, Kumari in western Ghats, watra in Rajasthan, Penda, Bewar or Dahia in Bastar district of Madhya Pradesh [2]. The shifting cultivation is characterized by rotation of fields rather than rotation of crops, absence of manuring, use of human labour, employment of dibbled stick or hoe, and short period occupancy alternating with fallow period. After two or three



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years the fields are abandoned, the cultivators shift to another clearing, leaving the old one to natural recuperation. This explains the term, "Shifting Cultivation" [3]. The clearance of jungles is the prerequisite of shifting cultivation. The felling of trees and clearing of bushes, however, accelerates soil erosion and accentuate variability of rainfall which may lead either to draughts or floods. The overall impact is the decline in soil fertility. The ecosystems lose their salient characteristics. The dependent community faces shortage of food, fuel, wood, and fodder. Consequently the nutritional standard goes down. This process culminates into social poverty and ecological imbalance [4]. As the cycle of shifting cultivation becomes shorter which is 4-5 years, the biomass that depends on the humus of the soil, declines, and the biodiversity is considerably reduced [5]. In addition, shifting cultivation is associated with poor crop yields and rapid soil degradation [6].

Shifting cultivation in India is very common in the North Eastern hilly region and Orissa, besides the hilly Eastern Ghats region of Andhra Pradesh, Madhya Pradesh, where it is moderately practiced, and parts of Western Ghats, where it exists in a modified form. Shifting cultivation was widely practiced in the Western Ghats, extending further north along its forested heights till the south west extension of Aravalis in Dungarpur district. Millets is common term used for a number of small seeded annual grass grain crops. They are produced in the fields with less fertile soil and low rainfall conditions. Nutritionally, Millets are superior to rice and wheat, hence considered as a cheaper source of proteins, minerals and vitamins for all the poor. Also, since they are practically deprived of grain storage pests, they have indefinite storage life [7]. In India, 4.5 per cent of the cultivated area is occupied by Millets. They are restricted to large areas of drylands and hilly tracks. Millet can adapt to a wide variety of soil type from very less fertile to highly fertile soil. They are able to tolerate some level of alkalinity [8].

The appearance, fertility, and chemical characteristics of the soil vary with respect to the minerals and plant materials which form them and keep transforming. Most the reactions involve water, mineral and biological factors. The soil quality is influenced by these physicochemical properties of it [9]. Several properties of the soil work hand in hand to maintain equilibrium in the soil environment. This balance is very much necessary for the sustainability of all of all forms of life. Microorganisms are highly responsible for the increase in the biomass production and maintaining the ecosystem through their decomposition activity thereby affecting the soil fertility. They performances lead to various vital ecosystem processes like nutrient cycling and availability, productivity, plant growth, suppression of root diseases and soil structure [10]. This naturally occurring microbial community inhabit the plant rhizosphere and effectively promote the plant growth through various interactions [11]. This microbial population is influenced by varied factors that lead to the shift in their communities. One of them is shifting cultivation method, which leads to decrease in the microbial population and diversity.

Unfortunately these parameters and their effects on shifting cultivation with respect to accumulation of forest biomass, nutrient cycling and role of microbes that varies with fallow length are still poorly understood. Therefore the need of hour is to search for sustainable ways of cultivation that can transform shifting cultivation system and have less environmental consequences. Understanding the plant-soil-microbe interactions thus can help finding a solution for transforming shifting cultivation system. The main objective of this research was to study the effect of shifting cultivation system on the microbial diversity and thus in return on the soil fertility.

## MATERIALS AND METHODS

### Soil sample collection

16 soil samples from 5 villages of the Bhor tehsil were collected between February, 2014 and April, 2014 after harvesting the cultivated crop and also from fallow land. The sample was collected by laying 5 quadrants of 1m by 1m, within the 2 to 4 acres of the shifting cultivation plots identified in the villages. 200g soil per quadrant was collected from 1cm to 9cm depth, and was pooled together in a 5kg sterile polythene bags and transported at room temperature to the laboratory within 24hours. On arrival at the lab the soil samples were stored at 4°C, the soil



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samples were processed within 3 to 4 days. To determine all the parameters for each soil sample, portion of soil was air-dried and sieved through a 2 mm sieve and then was diluted in water.

**Analysis of Physico-Chemical Properties of Soil**

The pH was measured with the help of a pH meter. An Electrical Conductivity meter was used to measure Electrical Conductivity (EC) [12]. The amount of soil organic carbon, available phosphorus and available potash were determined as described by [12]. The soil  $\text{CaCO}_3$  was mixed with 0.5N HCl, whereby  $\text{CO}_3$  was released, and the excess HCl is neutralized by 0.5N NaOH titration and the amount of  $\text{CaCO}_3$  is estimated from this [13]. Total cation (Ca + Mg) was estimated according to [14], using Na EDTA and complexometric titration. The soil available sodium is estimated by first extracting it with solution of sodium or ammonium acetate and then estimating the soil sodium by flame photometer [15]. The soil moisture was estimated by Gravimetric method or drying method [16]. The soil texture was determined by International Pipette method as described by [17]. Water Holding Capacity was measured by Keen-Raczkowaki-Box Method [18].

**Isolation and Identification of bacteria**

The microbial community analysis was done by total viable count of soil bacteria using serial dilution of the soil. 1gm of soil was mixed in 100ml saline, and serially diluted this up to 10<sup>-10</sup> and then 0.1ml of each dilution was spread on sterile nutrient agar plate in triplicates. These plates were incubated at room temperature for 24 to 72 hours. The subsequent bacterial colonies developing on the plates were counted and the colony forming units (cfu) per ml of soil sample was estimated. The isolated bacterial colonies growing on these plates were visually identified and picked up daily, the colony morphology recorded and the bacterial colonies were subject to Gram staining, Spore staining, capsule staining and biochemical identification was done up to genus level as per the Bergey's manual of Determinative Bacteriology, 9<sup>th</sup> edition.

**Statistical Analysis**

Statistical analyses were performed using SPSS software version 21. The correlation between all the soil parameters tested in this study was generated. Using R software version 3.6.1, Heat maps were developed for this correlation analysis data generated from SPSS. On the basis of the data obtained richness, Simpson's diversity and evenness indexes were calculated. The results were submitted to principal component analysis (PCA) in order to determine the common relationships between parameters.

**RESULTS AND DISCUSSION**

The study area covers the mountainous region of the western ghats of Pune district covering the Bhor tehsil. Bhor is situated south of Pune district, having latitude 18.16 N and longitude 73.85 E.

**Physico-chemical analysis**

16 soil samples of the shifting cultivation plots from 5 villages were collected and the physico-chemical analysis was performed for each sample. Average and standard deviation of each parameter was statistically calculated and was considered for further analysis. The results obtained are compiled and the average data of each parameter including the fallow period is presented below in the table. These results were then utilized to interpret the effect of shifting cultivation on the fertility of the soil. Elevation of the village location was also considered as one of the parameter that may affect the soil fertility with respect to shifting cultivation. Also the microorganisms were isolated from each soil sample to study the same effect and in turn the soil fertility.

**Microbial analysis**

All samples, which were analysed, contained various bacteria in varying population number and thus are dominant in the soil. In the soil samples studied here, they seem to be unevenly spread. All soils shows presence of bacteria but their population number varies and is dependent on the soil texture and organic substrate in the soil. Bacterial



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population is highly influenced by pH, temperature, humidity, agriculture practice, fertilizers, pesticide, and the addition of organic matter [19]. A TVC of  $1218 \times 10^6$ cfu/ml was determined from 16 samples collected from 5 villages of Bhor. Total 11 different bacterial genus isolated from all the 16 soil samples were *Frankia*, *Brocothrix*, *Staphylococcus*, *Acetobacter*, *Kurthia*, *Pseudomonas*, *Streptomyces*, *Nitrobacter*, *Azotobacter*, *Nocardia* and *Bacillus* in the increasing order of the number of each genus isolated from the soil (Figure 1).

**Correlation between soil parameters tested**

The soil analysis of Bhor tehsil shows that the pH of this soil is strongly positively related to available phosphates and strongly negatively related to clay whereas neutral to the moisture. Electric conductivity was shown to be positively related to carbon, nitrogen, potash and calcium. Carbon was strongly positively related to the nitrogen, calcium and magnesium and neutral to apparent and specific density; similarly nitrogen was positively related to Ca and Mg. Available phosphorus was neutral to the Ca and Mg. Also, potash was neutral to  $\text{CaCO}_3$  and WH. In the same way, calcium was strongly positively related to Mg and negatively related to Na. Magnesium showed neutral relation with moisture, clay and specific density whereas  $\text{CaCO}_3$  was neutral to specific density and porosity. Sodium had a neutral relation with clay. It was also seen that sand was strongly negatively related to silt and water holding capacity but positively related to apparent density. Clay was seen to strongly negatively related to apparent density and silt was positively related to WH. Moisture was neutral to porosity. Apparent density was neutral to porosity but specific density was positively related to porosity. Lastly it was observed that water holding capacity was strongly positively related to apparent density and neutral to porosity. The bacterial count of this soil showed a strong positive correlation with electric conductivity, calcium and specific density whereas was strongly negatively related to the porosity and had a neutral relation with the water holding capacity of the soil. This correlation can be seen in Figure 2.

**Simpson's Diversity Index**

It was observed that the SDI was close to 1 which states that the diversity is high in all the soil studied here (Table 1).

**Richness**

The number of species per sample is a measure of richness. The more species present in a sample, the 'richer' the sample. Accordingly the soil of Bhor showed the presence of 12 different isolates (Figure 1).

**Evenness**

Evenness is a measure of the relative abundance of the different species making up the richness of an area. If a community dominated by one or two species, it is considered to be less diverse than the one in which several different species have a similar abundance. Thus, all samples have unevenness because the total numbers of individuals in the sample are quite unevenly distributed between their respective isolates, dominated by *Bacillus* genus. As species richness and evenness increase, diversity also increases. Simpson's Diversity Index takes into account both richness and evenness to measure the diversity. This compliment (1-D) represents the probability that two individuals randomly selected from a sample will belong to different species. Thus high diversity is observed in all the soils.

Few parameters soils of fallow lands were significantly high as compared to the crop harvested soil. The other parameters of fallow lands did not show increase in the values than the crop harvested soil and the reason could be the length of the fallow period. Clay also increased in all the fallow lands but the sand level decreased. Same is observed from the table 2. Thus fallowing such lands for more than 7 to 8 years would bring the soil parameters back to normal. In case of Kondhari village, the fallow soil parameters and the TVC showed some deviation in its results. The TVC of fallow soil was less than crop harvested soil. The reason could be the fallowing period of the land which might be less than two years. Dhamandeo and Salungan villages showed maximum presence of all the isolates in the soils collected after harvesting nachani, while Hirdoshi village soil collected after harvesting hulga and Kondhari



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after harvesting varai, exhibited maximum presence of all isolates as seen in Table 3. This shows that the crop cultivation did not influence the number and diversity of the bacteria in that soil.

**Correlation Analysis**

A Pearson correlation coefficient was computed to assess the relationship between the various soil parameters and bacterial genus isolated from these soils. Many isolates were strongly positively correlated to electric conductivity (EC), organic carbon (OC) and Nitrogen. Strong positive correlation was also observed for Ca, Mg, Potash and CaCO<sub>3</sub> by few isolates. A strong negative correlation was observed for specific density and porosity. Few isolates also showed a strong negative correlation with Na and apparent density. There was a neutral correlation observed for silt, water holding capacity and moisture (Figure 3). During this period the dominant soil bacteria identified were *Bacillus*, *Nocardia*, *Azotobacter*, *Pseudomonas*, *Staphylococcus*, *Brocothrix*. *Kurthia* was isolated from almost all samples. *Kurthia gibsonii* Strains was reported from meat, mince beef, hen faeces on conveyer and cow faeces [20]. Thus, pointing towards the cattle faeces contamination in the soil.

In the present study soil samples from five villages of Bhor tehsil were studied for their microbial content. Eleven different bacterial genuses were isolated from these soil samples. They are *Frankia*, *Brocothrix*, *Staphylococcus*, *Acetobacter*, *Kurthia*, *Pseudomonas*, *Streptomyces*, *Nitrobacter*, *Azotobacter*, *Nocardia* and *Bacillus*. In a different study [21] which used the soil sample from Bhor and Velhetakulas but from the rice fields reported the presence of Bacteria like *Alcaligenes*, *Halomonas*, *Rhizobacterm*. This difference could be attributed to the land use and the crop cultivated in that land. The study area in current research work was the hilly areas of these and other talukas and was under shifting cultivation process. The land in the study area was used to grow millets like varai, nachani, sawa and hulga. Fallow land soil samples were also included in the study.

Similar study could also isolate actinomycetes from the rhizosphere samples of the four varieties of foxtail millet planted into red soil [22]. Among the plant-associated microbes, *Pseudomonads* are prominent bacteria that fight and save plants against several plant pathogens [23], but in the study area this organism was not found in high number. Actinomycetes are considered to be plentiful in the soil but surprisingly only six genus varieties were isolated from the entire study region, may be due to acid pH and low soil nutrient. Most actinomycetes are not tolerant to soil acidity. Another reason would be the depth of soil sample collection. Similar study [19] has reported that higher number of actinomycetes in the total population of microbes in the deeper soil. The actinomycetes identified in this study belonged to *Streptomyces*, *Nocardia*, *Acetobacter*, *Frankia* and *Nocardia*. *Azotobacter* and *Pseudomonas* associated with the rhizosphere of pearl millet (*P. glaucum*); grown in nutritionally poor soils of semi-arid regions were isolated by another group of researches [24]. The abundance of *Nocardia* spp. isolates from soil is affected by pH, soil type, climate, temperature [25]. In current study its presence was noted in all soils mainly due to the temperature and the dry climate in these regions.

Agriculture is the major factor influencing the soil environment and its microbiome[26]. But the farmers in these regions did not use any fertilizer though they practiced shifting cultivation. Thus, to an extent this aspect could not explain the variability of the recorded results. Few of them are pH, soil texture and metal content. Even temperature, precipitation and other climatic events may influence the soil microorganism community[27]. In this research the soils were collected from the lands where only rainfed crops were cultivated were exposed to similar weather conditions. Therefore, the climatic conditions cannot explain the differences between the examined soils. The maintenance of ecosystem functions even under stressful conditions is enabled by large microbial diversity [28]. On the contrary, in soils with low microbial diversity, even a little loss of species may expose the environment to functional changes [29]. In the discussed soils, differences in the activity and structure of the microbes inhabiting them may be important during fallow period.



**Patturajan et al.****Principal Component Analysis**

Agricultural data from different villages were subjected to principal component analysis (PCA) to find having similar overall patterns in the reduced multivariate data space without loss of information due to dimensionality reduction. Varimax rotated first three PCs were used to make a three dimensional scatter plot. Variables that will tend to cluster together in the 2D scatter plot will have similarity in the discriminatory power of the variables included in the study. All the statistical analysis was done in SPSS version 21. Potash, EC shows dis-similarity pattern in the village of Bhor whereas N and OC shown perfectly similar pattern in the component 1 (0.902) of the principal component analysis. Also found similar pattern for TVC (0.299) and Clay (0.276). Whereas in component 2 it can be seen that Moisture and Silt has dissimilarity pattern compared to other variables in the study (Figure 4).

**Remedial measures**

Although the fallow cycle has shown to increase soil erosion and disturb the watershed area, a usual practice of *Alnus nepalensis* plantation along with crops in *jhum* would serve to complete the need for nitrogen (N) [30]. The use of mixed cropping method in this cultivation system not only increases the soil fertility but also protects the crops from insect and pest attacks [31]. Thus deliberate plantation of trees like *Alnus nepalensis* and mixed cropping could help in improving the soil health.

The present work proposes certain remedial solutions for sustainability of this traditional cultivation system.

- Knowledge of ill effects of shifting cultivation should be conveyed in an understandable way to the cultivators. They have to understand the importance of fallow period length. Surely, if they are convinced about the degradation of soil and ecology, they will definitely follow the alternatives prescribed to save the environment as they are more close to the nature.
- They can be made to understand the importance of biodiversity, their knowledge of the plant species and their different uses can be streamlined to maintain and avoid extinction of valuable plants.
- They can be taught to use the forest resources without harming the forest in any way. These can serve as additional income source for them.
- Government should take initiatives to provide them with marketing facilities.
- Initiatives and schemes for helping the shifting cultivators transform to settled cultivation. They can be provided with lands on lease, seeds of exotic or in-demand fruits and vegetables, fertilizers and manure to increase their crop yield.
- Schemes involving secondary income sources like poultry, sericulture etc. can also be beneficial in preventing the long term effects of shifting cultivation.
- They can be also equipped with irrigation facilities to increase their yield.

The results demonstrate significant differences between the soils studied, both regarding physico-chemical and biological parameters. Based on both these parameters it can be concluded that the length of the fallow period influences the soil health. The TVC obtained in the fallow soils was significantly higher than the crop harvested soils. Thus the land management systems should take actions to satisfy the needs of the cultivators by providing various alternatives for their sustainability. Few remedial measures suggested at the end can be considered to improve and protect the environment.

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**Conflicts of Interest**

The authors declare no conflict of interest.





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## REFERENCES

1. Colin C, Haswell M. The Economics of Subsistence Agriculture. 4th ed. London: MacMillan and Co. Ltd; 1970.
2. Mamoria CD. Agricultural Problem of India, KitabMahalPublication, Allahabad; 1953.
3. Borthakur DN, Singh A, Awasthi RP, Rai, RN. Shifting cultivation in the north-eastern region. Proceedings of the National Seminar on Resources, Development and Environment in the Himalayan Region (New Delhi: Department of Science and Technology) 1978; 330-342.
4. Jarosz L. Defining and explaining tropical deforestation: shifting cultivation and population growth in colonial Madagascar. *Econ Geogr* 1993;69(4):366-79.
5. Rasul G, Thapa, G. Shifting cultivation in the mountains of South and Southeast Asia: Regional patterns and factors influencing the change. *Land Degradation & Development* 2003;14:495–508.
6. Christianity L. Shifting cultivation and tropical soils: patterns, problems, and possible improvements. In Marten, G. G. (ed.) *Traditional Agriculture in Southeast Asia*. Boulder and London: Westview Press 1986; 226 – 240.
7. Sampath TV, Razvi SM, Singh DN, Bondale KV. Small Millets in Indian Agriculture in *Small Millets In Global Agriculture*, Proceedings of the First International Small Millets Workshop, Oxford & IBH Publishing Co. Pvt. Ltd; 1986.
8. Joshi PK, Agnihotri AK. Millet Production in India. Problems and prospects. *Agricultural Situation in India* 1984;39:329.
9. Brady NC, Weil RR. The nature and properties of soils. 14th ed. Upper Saddle River, N.J.: Pearson Prentice Hall; 2008.
10. Jesus EDC, Susilawati E, Smith SL, Wang Q, Chai B, Farris R, Tiedje JM. Bacterial communities in the rhizosphere of biofuel crops grown on marginal lands as evaluated by 16S rRNA gene pyrosequences. *Bioenergy Research* 2010;3(1):20-27.
11. Saharan BS, Nehra V. Plant growth promoting rhizobacteria: a critical review life sciences and medicine research. *International Journal of Life Science and Medical Research* 2011; 21:1-30.
12. Jackson ML. Soil chemical analysis. Prentice Hall of India Pvt. Ltd, New Delhi; 1973.
13. Rowell DL. Soil Science: Methods and Applications. Prentice Hall, Harlow; 1994.
14. Tucker BB, Kurtz LT. Calcium and magnesium determinations by EDTA titrations. *Soil Science Society of America Journal* 1961;25:27-29.
15. Havre GN. The flame photometric determination of sodium, potassium and calcium in plant extracts with special reference to interference effects. *Analytica Chimica Acta* 1961;25:557-566.
16. Reynolds SG. The gravimetric method of soil moisture determination. Part I, a study of equipment and methodological problems. *Journal of Hydrology* 1970;11:258-273.
17. Gee GW, Bauder JW. Particle-size analysis. In A. Klute (ed.) *Methods of soil analysis*. Part 1. 2nd Ed. Agronomy Monogram. 9: ASA and SSSA, Madison, WI; 1986:383-411.
18. Keen BA, Raczkowski H. Relation between the clay content and certain physical properties of a soil. *Journal of Agricultural Science* 1921; 11:441-449.
19. Rao NS. Mikroorganism Tanah dan Pertumbuhan Tanaman. Edisikedua. Jakarta: Penerbit Universitas Indonesia; 1994.
20. Shaw S, Keddie RM. A numerical taxonomic study of the genus *Kurthia* with a revised description of *Kurthiazopfi* and a description of *Kurthiagibsonii* sp. nov. *Systemic Applied Microbiology* 1983; 4:253-276.
21. Gaikwad SA, Upadhye AS, Kulkarni DK. Ecology in relation to rice field soils in Bhor and Velhe region of Pune district, Maharashtra state, India. *International Journal of Geology, Earth & Environmental Sciences* 2016;6 (1):12-18.
22. Varalakshmi T, Sekhar K, Rafi M, Charyulu P. Population studies of actinomycetes isolated from the rhizosphere of Foxtail millet. *Current Biotica* 2010; 4(3):305-317.
23. Wang X, Mavrodi DV, Ke L, Mavrodi OV, Yang M, Thomashow LS. et al. Biocontrol and plant growth-promoting activity of rhizobacteria from Chinese fields with contaminated soils. *Microb. Biotechnol* 2015; 8:404-418.





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24. Tiwari M, Paroda S, Dadarwal KR. Associative diazotrophs of pearl millet (*Pennisetum glaucum*) from semi arid region--isolation and characterization. *Indian J Exp Biol* 2003;41(4): 341-345.
25. Arends, JE, Stemerding, AM, Vorst, SP, Neeling, AJ and Weersink, AJ. (First report of a brain abscess caused by *Nocardia veterana*. *Journal of Clinical Microbiology* 2011; 49:4364-4365.
26. Nannipieri P, Ascher J, Ceccherini MT, Landi L, Pietramellara G, Renella G. Microbial diversity and soil functions. *Eur. J. Soil Sci* 2003;54:655-670
27. Li G, Kim S, Park M, Son Y. Short-term effects of experimental warming and precipitation manipulation on soil microbial biomass C and N, community substrate utilization patterns and community composition. *Pedosphere* 2017;27:714-724.
28. Wertz S, Degrange V, Prosser FP, Commeauc C, Guillaumaud N, Le Roux X. Decline of soil microbial diversity does not influence the resistance and resilience of key soil microbial functional groups following a model disturbance. *Environmental Microbiology* 2007;9: 2211-2219.
29. Wagg C, Bender SF, Widmer F, van der Heijden MGA. Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proceedings of the National Academy of Sciences, USA* 2014;111:5266-5270.
30. Rathore S, Karunakaran K, Bhukya P. Alder based farming system a traditional farming practices in Nagaland for amelioration of jhum. *Indian Journal of Traditional Knowledge* 2010;9:677-680.
31. Ramakrishnan PS. The Science Behind Rotational Bush Fallow Agricultural System (Jhum). *Proc. Indian Academy of Science. Plant Science* 1984;93,391-400.

**Table 1: Simpson's diversity index**

Bacterial Isolates	Total number
<i>Frankia</i>	26
<i>Brocothrix</i>	54
<i>Staphylococcus</i>	62
<i>Acetobacter</i>	81
<i>Kurthia</i>	84
<i>Pseudomonas</i>	89
<i>Streptomyces</i>	104
<i>Nitrobacter</i>	114
<i>Azotobacter</i>	118
<i>Nocardia</i>	141
<i>Bacillus</i>	210
Total	1083
Simpson	0.8885734
<b>1/D</b>	<b>1.1254</b>





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**Table 2: Physico-chemical analysis of soil samples**

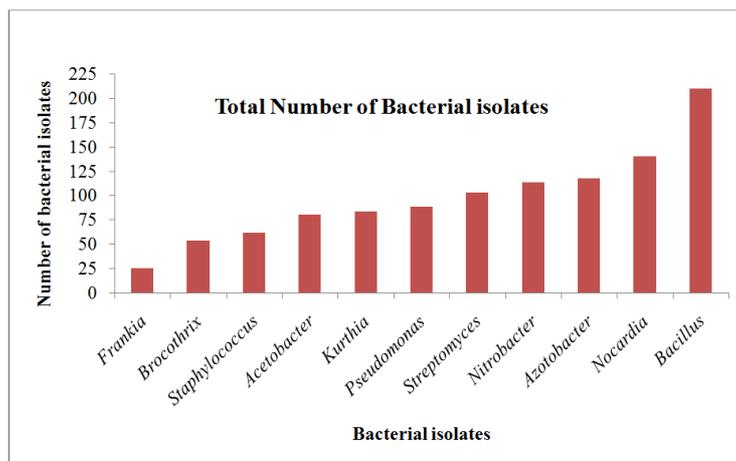
Sample No.	Village	pH	EC	OC	N	Avail . P	Potash	Calci um	Magn esiu m	Sodi um	CaC O <sub>2</sub>	Sand	Clay	Silt	Moist ure	Water Hold. Cap.	Appe rent Den	Spe cific Den	Poros ity	TVC(x 10 <sup>6</sup> cfu/ml)
Fallow	Kondhari	6.76	0.07	0.08	56	13.82	145.75	11.27	5.22	2.69	1.88	3.08	17.97	38.89	5.71	52.21	1.26	2.31	60.5	19
Varai/ sawa	Kondhari	5.51	0.07	0.02	14	6.9	274.6	12.25	4.41	2.29	2.38	21.16	30.02	3.76	6.26	32.59	1.31	1.94	58.17	11
Nachani	Kondhari	5.74	0.07	0.26	182	6.9	267.21	11.76	9.23	3.65	2.13	5.78	30.14	40.5	5.76	66.86	1.17	2.32	60.29	29
Fallow	Hirdoshi	5.82	0.11	0.74	518	7.01	546.03	7.84	5.62	3.81	1.88	5.79	33.89	25.19	5.9	56.26	1.2	2.09	59.63	77
Hulga	Hirdoshi	5.55	0.09	0.66	462	6.9	838.59	12.75	2.01	2.56	2.5	3.31	32.95	31.68	5.45	61.8	1.12	2.01	59.56	58
Sawa	Hirdoshi	5.6	0.12	0.78	546	7.01	688.62	9.8	1.2	2.3	4	28.11	26.02	14.76	5.12	57.31	1.2	2.2	57.96	46
Fallow	Dhamandeo	5.65	0.15	0.5	350	6.9	737.2	15.69	6.42	2.73	2.63	11.95	35.03	26.58	6.14	62.32	1.13	1.2	30.82	302
Nachani	Dhamandeo	5.51	0.08	0.26	182	7.12	356.98	7.35	2.01	5.78	2	8.17	28.31	49.52	5.81	61.79	1.16	2.18	59.61	49
Hulga	Dhamandeo	5.77	0.06	0.17	119	6.9	132.02	6.37	2.41	4.34	2.13	14.48	25.84	40.65	5.49	61.53	1.18	2.09	58.83	36
Fallow	Shilimb	5.68	0.07	0.02	14	7.12	368.6	9.31	4.41	2.64	2	5.32	34.48	44.35	12.18	75.84	1.09	2.22	59.2	56
Nachani	Shilimb	5.41	0.08	0.48	336	6.78	238.69	6.86	1.2	2.28	2.38	0.87	36.68	36.99	6.32	68.16	1.1	2.15	60.56	44
Sawa	Shilimb	5.26	0.05	0.57	399	6.78	383.39	7.84	3.21	3.4	3.13	13.31	38.22	24.73	6.17	65.79	0.99	1.89	57.99	48
Fallow	Salungan	5.78	0.13	1.89	1323	6.67	500.62	27.45	25.27	0.35	2.88	15.27	33.86	13.53	5.81	43.6	1.23	2.18	64.56	185
Nachani	Salungan	5.51	0.08	0.35	245	7.01	217.57	14.71	0.8	0.37	7.75	8.67	33.66	39.75	5.18	68.61	1.04	2.15	60.23	95
Hulga	Salungan	5.39	0.07	0.33	231	7.01	386.55	11.76	5.62	0.33	2	3.45	34.94	47.95	5.88	80.93	1.06	2.24	59.88	88
Varai	Salungan	5.51	0.08	0.3	210	6.56	392.89	10.78	9.23	0.34	2.25	31.86	18.04	19.14	6.12	34.14	1.35	1.97	54.51	75

Note: Fallow soil data is highlighted in yellow

**Table 3. Number of Bacterial genus isolated from each soil sample**

Sample No.	Village	Bacillus	Pseudomonas	Nocardia	Streptomyces	Kurthia	Bronchothrix	Staph	Azotobacter	Acetobacter	Nitrobacter	Frankia	TVC((x10 <sup>6</sup> c fu/ml)
Fallow	Kondhari	4	1	3	2	2	2	1	2	2	1	0	19
Nachani	Kondhari	3	3	1	1	0	0	0	0	0	3	0	11
Varai	Kondhari	8	3	4	2	1	0	2	3	1	3	2	29
Fallow	Hirdoshi	12	8	10	9	6	4	4	9	5	6	3	77
Hulga	Hirdoshi	16	2	13	8	0	0	2	6	3	8	0	58
Sawa	Hirdoshi	12	6	6	7	3	0	5	2	0	5	0	46
Fallow	Dhamandeo	48	8	19	25	10	12	14	21	13	30	6	302
Nachani	Dhamandeo	14	2	6	6	0	0	2	7	4	8	0	49
Hulga	Dhamandeo	13	2	3	2	0	0	0	8	6	2	0	36
Fallow	Shilimb	11	7	14	6	10	2	2	1	1	2	0	56
Nachani	Shilimb	10	5	12	3	4	0	0	6	2	2	0	44
Sawa	Shilimb	9	3	8	9	8	1	0	3	1	4	2	48
Fallow	Salungan	21	13	12	8	9	11	6	13	14	15	6	185
Nachani	Salungan	10	9	13	8	10	12	5	11	9	16	2	95
Hulga	Salungan	9	9	9	8	9	10	6	12	10	6	5	88
Varai	Salungan	10	8	8	0	12	0	13	14	10	3	0	75

Note: Fallow soil data is highlighted in yellow



**Figure 1: Number of each bacterial isolate from all the samples**





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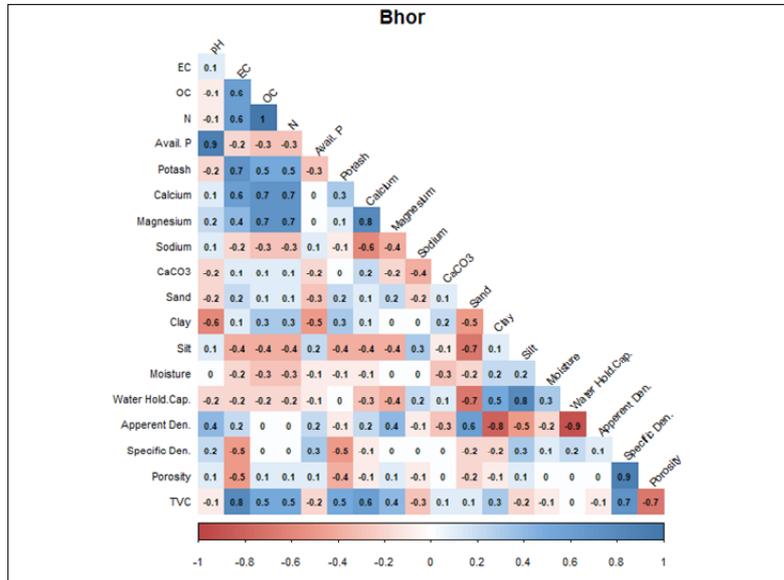


Figure 2: Pearson Correlation coefficient among soil parameters of Bhor Soil; the values indicated in red are a negative correlation, and those in blue are a positive correlation; the number of degrees of freedom (df) = 1, Strong positive correlation (above +0.6), strong negative correlation (above -0.6) and neutral relation (zero).

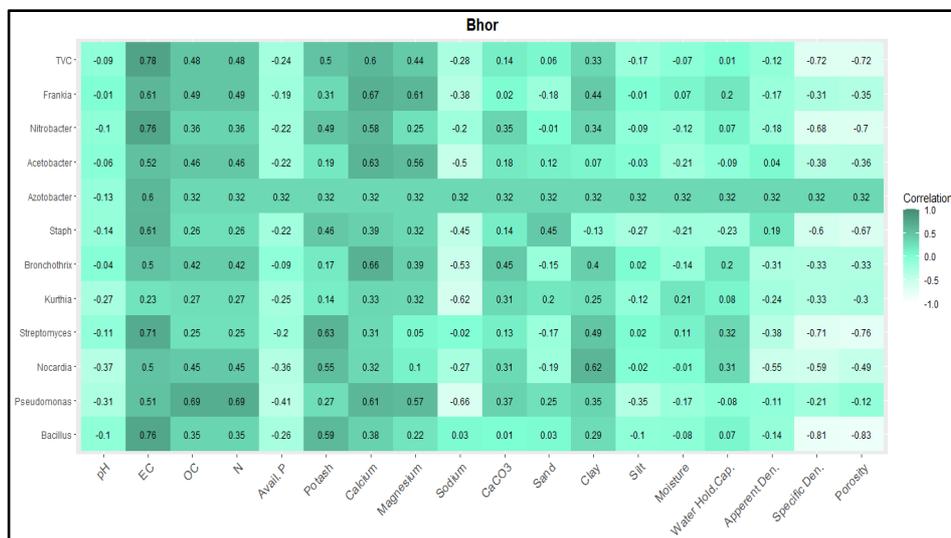


Figure 3: Correlation Analysis of Bhor soil parameters with bacterial isolates





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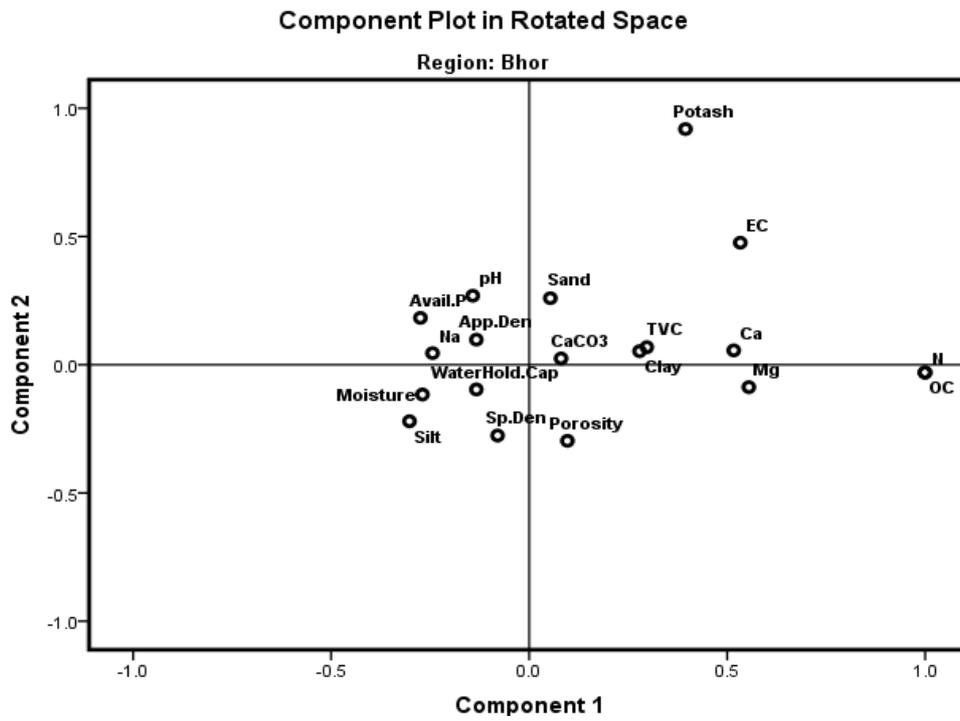


Figure 4: PCA for Bhor Taluka





## Pythagorean Fuzzy Multi- Granulation Soft Rough Set and Its Application

K Akalyadevi\* and C Antony Crispin Sweety

Department of Mathematics, Avinashilingam Institute for Home Science and Higher Education for women, Coimbatore, Tamil Nadu, India.

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### \*Address for Correspondence

**K Akalyadevi**

Department of Mathematics,  
Avinashilingam Institute for Home Science and Higher Education for Women,  
Coimbatore, Tamil Nadu, India.  
E.mail: akalyadevi\_maths@avinuty.ac.in



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### ABSTRACT

Pythagorean Fuzzy Set (PFS) was first initiated by Yager to deal with imprecision and vagueness. As a generalization of intuitionistic fuzzy set, PFS has a greater scope to tackle the ambiguous situations of real physical problems with high precision and accuracy. The theories of soft sets and rough sets, originally proposed by Molodtsov and Pawlak are the mathematical ways to approach with uncertainties. In this work, the above theories are combined to form a new hybrid form of Pythagorean Fuzzy Soft Rough Set (PFSRS) and some of its properties are studied. Then the proposed model is extended to multi-granular environment based on Pythagorean fuzzy soft relation and Pythagorean Fuzzy Multi-granulation Soft Rough Set (PFMGSRs) is introduced. Enduringly, an illustrative example is explored to depict the importance of the proposed model.

**Keywords:** Pythagorean soft relation, Multi- granulation rough set, Optimistic, Pessimistic.

### INTRODUCTION

The idea of fuzzy set theory, proposed by Zadeh (1965), plays a significant role as it handles uncertain or vague information in decision making, characterized by a membership function which assigns a membership value ranging between zero and one. However, in some actual environment, the fuzzy set theory has some limitations when decision maker deals with some uncertain information or vagueness. Intuitionistic fuzzy set (IFS), proposed by Atanassov (1986, 2015) is characterized by a membership and non-membership function satisfies the condition that the sum of membership and non-membership is less than or equal to one. Hence it describes more precisely than fuzzy set. Mendel et al. (2002) discussed a new representation for type-2 fuzzy set. Torra (2010) introduced Hesitant fuzzy sets and some basic operations and also proved that the envelope of the hesitant fuzzy sets is an intuitionistic fuzzy set.



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Pawlak(1982, 1991) proposed the concept of rough set theory, a well-established mechanism. Binary relation is imposed in an approximation space consisting of a universe of discourse. The application domain of rough set model is narrowed by the equivalence relation. Thus, various extensions have been introduced over the past years and widely applied in some branches of artificial intelligence such as automatic classification, feature selection, learning algorithm, inductive reasoning, pattern recognition, knowledge reduction, etc. Yager(2013, 2014) proposed a brand-new extension of fuzzy set called Pythagorean fuzzy set (PFS), which has been successfully applied in many fields for decision making procedures. PFS is characterized by a membership and non-membership function satisfies the condition that the square sum of membership and non-membership is less than of equal to one. It is noted that not all Pythagorean fuzzy set are intuitionistic fuzzy set but an intuitionistic fuzzy set must be a Pythagorean fuzzy set.

Reformat et al (2014) discussed a novel collaboration based recommender system that provides a user with the ability to control a process of constructing a list of suggested items and also proposed a new idea of ranking items rated by multiple users. Chao et al (2016) introduced the models of Pythagorean fuzzy rough set over two universes and Pythagorean fuzzy multigranulation rough set over two universes. Wan et al (2017,2018) developed the Pythagorean fuzzy analytic hierarchy process is proposed to determine the criteria weight of the evaluation criteria and introduced a three-phase method for addressing multi-attribute group decision making (MAGDM) with Pythagorean fuzzy numbers (PFNs) and apply to haze management. The concept of Pythagorean fuzzy sets and some properties of Pythagorean fuzzy set in connection to score and accuracy functions was proposed by Paul (2019). Lazim (2019) introduced a modification fuzzy DEMATEL characterized by Pythagorean fuzzy set for linguistic variables.

On other hand Dubois and Prade (1990) proposed the model of fuzzy rough set on the combination of intuitionistic fuzzy set and classical rough set theory. In the fusion process of rough set theory and intuitionistic fuzzy set, Samanta and Mondal (2001) proposed the intuitionistic fuzzy rough sets and rough intuitionistic fuzzy sets. Greco et al (2002) discussed the new rough set approach is able to approximate this partition by means of dominance relations. Fuzzy rough set over two universes and its applications was proposed by Sun and Ma. (2011).Yang et al.(2013) researched a fuzzy probabilistic rough set model on two universes and its application in medical field. The concept of soft set theory was proposed by Molodtsov(1999 ) to solve imprecise problems in the field of social science, engineering, medical science, environment and economics. Molodtsov (2001, 2004, 2006) applied the soft set theory to several directions such as game theory, measurement theory, smoothness of function, probability and operation research. On the combination of fuzzy set and soft set, the model of fuzzy soft set was proposed by Maji et al.(2003). Multi-granulation rough set theory is beneficial to view a problem through multiple binary relations. Multi-granulation rough set is studied from two types of qualitative combination rules: optimistic and pessimistic by Qian (2010). Liu et al. (2016) discussed the relationship between rule-based systems and granular computing and provides an overview of granular computing concepts in the context of set theory and information granulation. Qi and wei (2018) proposed five types of granules in formal concept analysis with respect to objects and discusses their relationships.

In Section 2, we discuss the basic concepts of intuitionistic fuzzy set, Pythagorean fuzzy set, rough sets over two universes, multi-granulation rough sets over two universes and soft sets. In Section 3, we introduce the model of PFSRS and some properties. In Section 4, two types of PFMGSRS are defined and explored and their properties are studied in detail. Section 5 presents an application of PFMGSRS. In Section 6, we conclude this paper with some remarks.

**BASIC CONCEPTS**

In this section, we review some basic concepts, such as Intuitionistic fuzzy set, Pythagorean fuzzy set, rough sets over two universes, Multi-granulation rough sets over two universes, and so on.





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**Definition 1.** Let  $A$  be an IFS in the universe of discourse  $X$ , shown as follows:

$$A = \{ \langle x, \mu_A(x), \nu_A(x) \rangle \mid x \in X \},$$

Where  $\mu_A(x) : X \rightarrow [0,1]$  and  $\nu_A(x) : X \rightarrow [0,1]$  satisfy  $0 \leq \mu_A(x) + \nu_A(x) \leq 1$  for all  $x \in X$ ,  $\mu_A(x)$  and  $\nu_A(x)$  denote the membership degree and non-membership degree of element  $x$  belonging to the IFS  $A$ , respectively. Moreover,  $\pi_A(x) = 1 - \mu_A(x) - \nu_A(x)$  is called the hesitancy degree of element  $x$  belonging to the IFS  $A$ .

**Definition 2.** Let  $P$  be an PFS in the universe of discourse  $X$ , shown as follows:

$$P = \{ \langle x, \mu_P(x), \nu_P(x) \rangle \mid x \in X \},$$

Where  $\mu_P(x) : X \rightarrow [0,1]$  and  $\nu_P(x) : X \rightarrow [0,1]$  satisfy  $0 \leq (\mu_P(x))^2 + (\nu_P(x))^2 \leq 1$  for all  $x \in X$ ,  $\mu_P(x)$  and  $\nu_P(x)$  denote the membership degree and non-membership degree of element  $x$  belonging to the PFS  $P$ , respectively. Moreover,  $\pi_P(x) = \sqrt{1 - \mu_P^2(x) - \nu_P^2(x)}$  is called the hesitancy degree of element  $x$  belonging to the PFS  $P$ . For convenience, we introduce a Pythagorean fuzzy number denoted by  $\beta = P(\mu_\beta, \nu_\beta)$ , where  $\mu_\beta, \nu_\beta \in [0,1]$  and  $0 \leq (\mu_\beta)^2 + (\nu_\beta)^2 \leq 1$ .

**Definition 3.** Suppose we have three Pythagorean fuzzy numbers  $\beta_1 = P(\mu_{\beta_1}, \nu_{\beta_1})$ ,  $\beta_2 = P(\mu_{\beta_2}, \nu_{\beta_2})$ , and  $\beta = P(\mu_\beta, \nu_\beta)$ . The operations of complement, union, and intersection are defined as follows:

- (1)  $\beta^c = P(\nu_\beta, \mu_\beta)$ ;
- (2)  $\beta_1 \cup \beta_2 = P(\max\{\mu_{\beta_1}, \mu_{\beta_2}\}, \min\{\nu_{\beta_1}, \nu_{\beta_2}\})$ ;
- (3)  $\beta_1 \cap \beta_2 = P(\min\{\mu_{\beta_1}, \mu_{\beta_2}\}, \max\{\nu_{\beta_1}, \nu_{\beta_2}\})$ ;

Moreover, Zhang and Xu further defined some novel operations for PFNs as given below:

- (4)  $\beta_1 \oplus \beta_2 = P\left(\sqrt{\mu_{\beta_1}^2 + \mu_{\beta_2}^2 - \mu_{\beta_1}^2 \mu_{\beta_2}^2}, \nu_{\beta_1} \nu_{\beta_2}\right)$ ;
- (5)  $\beta_1 \otimes \beta_2 = P\left(\mu_{\beta_1} \mu_{\beta_2}, \sqrt{\nu_{\beta_1}^2 + \nu_{\beta_2}^2 - \nu_{\beta_1}^2 \nu_{\beta_2}^2}\right)$ ;
- (6)  $\lambda^\beta = P\left(\sqrt{1 - (1 - (\mu_\beta)^2)^\lambda}, (\nu_\beta)^\lambda\right), \lambda > 0$ ;
- (7)  $\beta^\lambda = P\left((\mu_\beta)^\lambda, \sqrt{1 - (1 - (\nu_\beta)^2)^\lambda}\right), \lambda > 0$ .

**Definition 4.** Suppose we have two Pythagorean fuzzy numbers  $\beta_1 = P(\mu_{\beta_1}, \nu_{\beta_1})$  and  $\beta_2 = P(\mu_{\beta_2}, \nu_{\beta_2})$ . A natural quasi-ordering on the PFNs is defined as follows:

$$\beta_1 \geq \beta_2 \text{ if and only if } \mu_{\beta_1} \geq \mu_{\beta_2} \text{ and } \nu_{\beta_1} \leq \nu_{\beta_2}.$$

**Definition 5.** Let  $\beta = P(\mu_\beta, \nu_\beta)$  be a PFN, the score function of  $\beta$  is defined as follows:

$$s(\beta) = (\mu_\beta)^2 - (\nu_\beta)^2.$$

If we have two PFNs  $\beta_1 = P(\mu_{\beta_1}, \nu_{\beta_1})$  and  $\beta_2 = P(\mu_{\beta_2}, \nu_{\beta_2})$ .  $s(\beta_1)$  and  $s(\beta_2)$  be the scores of  $\beta_1$  and  $\beta_2$ , respectively. If  $s(\beta_1) < s(\beta_2)$ , then  $\beta_1 < \beta_2$ ; if  $s(\beta_1) > s(\beta_2)$ , then  $\beta_1 > \beta_2$ ; if  $s(\beta_1) = s(\beta_2)$ , then  $\beta_1 = \beta_2$ .





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**Definition 6.** Let  $U$  and  $V$  be two universes and  $R$  be a compatibility relation from  $U$  to  $V$ . The mapping  $F : U \rightarrow 2^V$ , for any  $u \in U, v \in V, u \rightarrow \{v \in V | (u, v) \in R\}$  is called a mapping induced by  $R$ . Then, the rough set over two universes is defined as follows:

Let  $U$  and  $V$  be two universes and  $R$  be a compatibility relation from  $U$  to  $V$ . The ordered triple  $(U, V, R)$  is called an approximation space. The lower and upper approximations of  $Y \subseteq V$  are defined as follows:

$$\underline{apr}(Y) = \{x \in U | F(x) \subseteq Y\},$$

$$\overline{apr}(Y) = \{x \in U | F(x) \cap Y \neq \emptyset\}.$$

The pair  $(\underline{apr}(Y), \overline{apr}(Y))$  is called rough sets over two universes.

**Definition 7.** Let  $U, V$  be two nonempty and finite universes of discourse.  $R$  is a family binary compatibility relation from  $U$  to  $V$  induced by binary mapping family  $F_i : U \rightarrow 2^V$ , for any  $u \in U, v \in V, u \rightarrow \{v \in V | (u, v) \in R_i\}, R_i \in R, i = 1, 2, \dots, m$ . The ordered triple set  $(U, V, R)$  is the multi-granular approximation space over two universes.

**Definition 8.** Let  $(U, V, R)$  be multi-granular approximation space over two universes.  $F$  and  $G$  be two binary mappings from universe  $U$  to  $V$ . For any  $X \subseteq V$ , the optimistic lower and upper multi-granular approximations with respect to  $(U, V, R)$  are defined as follows:

$$\underline{apr}_{F+G}^O(X) = \{x \in U | F(x) \subseteq X \vee G(x) \subseteq X\},$$

$$\overline{apr}_{F+G}^O(X) = \overline{apr}_{F+G}^O(X^c)^c.$$

The pair  $(\underline{apr}_{F+G}^O(X), \overline{apr}_{F+G}^O(X))$  is the optimistic multi-granulation rough sets over two universes if  $(\underline{apr}_{F+G}^O(X) \neq \overline{apr}_{F+G}^O(X))$ ; otherwise,  $X$  is definable on  $(U, V, R)$  with respect to  $F$  and  $G$ . Moreover, the boundary region of  $X$  on  $(U, V, R)$  is defined as follows:

$$Bnd_{F+G}^O(X) = \overline{apr}_{F+G}^O(X) - \underline{apr}_{F+G}^O(X)$$

Similarly, the pessimistic lower and upper multi-granular approximations with respect to  $(U, V, R)$  are defined as follows:

$$\underline{apr}_{F+G}^P(X) = \{x \in U | F(x) \subseteq X \vee G(x) \subseteq X\},$$

$$\overline{apr}_{F+G}^P(X) = \overline{apr}_{F+G}^P(X^c)^c.$$

The pair  $(\underline{apr}_{F+G}^P(X), \overline{apr}_{F+G}^P(X))$  is the pessimistic multi-granular rough sets over two universes if  $(\underline{apr}_{F+G}^P(X) \neq \overline{apr}_{F+G}^P(X))$ ; otherwise,  $X$  is definable on  $(U, V, R)$  with respect to  $F$  and  $G$ . Moreover, the boundary region of  $X$  on  $(U, V, R)$  is defined as follows:

$$Bnd_{F+G}^P(X) = \overline{apr}_{F+G}^P(X) - \underline{apr}_{F+G}^P(X)$$

**Definition 9.** Let  $U$  be an initial universe set and let  $E$  be a universe set of parameters. A pair  $(F, E)$  is called a soft set over  $U$  if  $F : E \rightarrow P(U)$ , where  $P(U)$  is the set of all subsets of  $U$ .





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**PYTHAGOREAN FUZZY SOFT ROUGH SETS**

**Definition 10.** Let  $U$  be an initial universe set and let  $E$  be a universe parameter set. A Pythagorean Fuzzy Soft (PFS) relation  $\mathfrak{R}_S$  from  $U$  to  $E$  is defined as follows:

$$\mathfrak{R}_S = \{ \langle (a,b), \mu_{\mathfrak{R}_S}(a,b), \nu_{\mathfrak{R}_S}(a,b) \rangle \mid (a,b) \in U \times E \},$$

where  $\mu_{\mathfrak{R}_S}(a,b)$  and  $\nu_{\mathfrak{R}_S}(a,b)$  are two sets of values in  $[0,1]$ , denoting the membership and non-membership degrees for all  $(a,b) \in U \times E$ , respectively. With the conditions:  $\mu_{\mathfrak{R}_S} : U \times E \rightarrow [0,1]$  and  $\nu_{\mathfrak{R}_S} : U \times E \rightarrow [0,1]$  satisfy  $0 \leq (\mu_{\mathfrak{R}_S}(a,b))^2 + (\nu_{\mathfrak{R}_S}(a,b))^2 \leq 1$  for all  $(a,b) \in U \times E$ . Moreover, in this study, the set of all PFS on  $E$  is denoted by  $PF(E)$  and the set of all Pythagorean fuzzy soft relations on  $U \times E$  is denoted by  $PFSR(U \times E)$ .

**Definition 11.** Let  $U$  be an initial universe and let  $E$  be a universe of parameters. For an arbitrary fuzzy soft relation  $\mathfrak{R}_S$  over  $U \times E$ , The ordered triple set  $(U, E, \mathfrak{R}_S)$  is called Pythagorean fuzzy soft approximation space. For any PFS  $C \in PF(E)$ , the lower and upper approximations of  $C$  with respect to  $(U, E, \mathfrak{R}_S)$  denoted by  $\underline{\mathfrak{R}_S}(C)$  and  $\overline{\mathfrak{R}_S}(C)$ , respectively as follows:

$$\begin{aligned} \underline{\mathfrak{R}_S}(C) &= \{ \langle a, \underline{\mu}_{\mathfrak{R}_S}(C)(a), \underline{\nu}_{\mathfrak{R}_S}(C)(a) \rangle \mid a \in U \}, \\ \overline{\mathfrak{R}_S}(C) &= \{ \langle a, \overline{\mu}_{\mathfrak{R}_S}(C)(a), \overline{\nu}_{\mathfrak{R}_S}(C)(a) \rangle \mid a \in U \}, \end{aligned}$$

where

$$\begin{aligned} \overline{\mu}_{\mathfrak{R}_S}(C)(a) &= \bigvee_{x \in E} [\mu_{\mathfrak{R}_S}(a,b) \wedge \mu_C(b)], \\ \overline{\nu}_{\mathfrak{R}_S}(C)(a) &= \bigwedge_{x \in E} [\nu_{\mathfrak{R}_S}(a,b) \vee \nu_C(b)], \\ \underline{\mu}_{\mathfrak{R}_S}(C)(a) &= \bigwedge_{x \in E} [\nu_{\mathfrak{R}_S}(a,b) \vee \mu_C(b)], \\ \underline{\nu}_{\mathfrak{R}_S}(C)(a) &= \bigvee_{x \in E} [\mu_{\mathfrak{R}_S}(a,b) \wedge \nu_C(b)]. \end{aligned}$$

The pair  $(\underline{\mathfrak{R}_S}(C), \overline{\mathfrak{R}_S}(C))$  is referred to as an Pythagorean fuzzy soft rough set of  $C$  with respect to  $(U, E, \mathfrak{R}_S)$ .

**Example 1.** Let  $U = \{a_1, a_2, a_3\}$  and  $E = \{b_1, b_2, b_3\}$  be universe set and universe parameter set,

$$\begin{aligned} \mathfrak{R}_S = \{ &\langle (a_1, b_1), [0.8, 0.1] \rangle, \langle (a_1, b_2), [0.3, 0.6] \rangle, \langle (a_1, b_3), [0.5, 0.3] \rangle, \\ &\langle (a_2, b_1), [0.5, 0.4] \rangle, \langle (a_2, b_2), [0.9, 0.2] \rangle, \langle (a_2, b_3), [0.6, 0.4] \rangle, \\ &\langle (a_3, b_1), [0.4, 0.5] \rangle, \langle (a_3, b_2), [0.7, 0.3] \rangle, \langle (a_3, b_3), [0.5, 0.1] \rangle \} \end{aligned}$$

$$C = \{ \langle b_1, [0.6, 0.3] \rangle, \langle b_2, [0.7, 0.6] \rangle, \langle b_3, [0.9, 0.2] \rangle \}$$

we have,

$$\begin{aligned} \overline{\mu}_{\mathfrak{R}_S}(C)(a_1) &= \bigvee_{b \in E} [\mu_{\mathfrak{R}_S}(a_1,b) \wedge \mu_C(b)] = [0.8 \wedge 0.6] \vee [0.3 \wedge 0.7] \vee [0.5 \wedge 0.9] = 0.6 \\ \overline{\nu}_{\mathfrak{R}_S}(C)(a_1) &= \bigwedge_{b \in E} [\nu_{\mathfrak{R}_S}(a_1,b) \vee \nu_C(b)] = [0.1 \vee 0.3] \wedge [0.6 \vee 0.6] \wedge [0.3 \vee 0.2] = 0.3 \\ \underline{\mu}_{\mathfrak{R}_S}(C)(a_2) &= \bigvee_{b \in E} [\mu_{\mathfrak{R}_S}(a_2,b) \wedge \mu_C(b)] = [0.5 \wedge 0.6] \vee [0.9 \wedge 0.7] \vee [0.6 \wedge 0.9] = 0.7 \\ \underline{\nu}_{\mathfrak{R}_S}(C)(a_2) &= \bigwedge_{b \in E} [\nu_{\mathfrak{R}_S}(a_2,b) \vee \nu_C(b)] = [0.4 \vee 0.3] \wedge [0.2 \vee 0.6] \wedge [0.4 \vee 0.2] = 0.4 \\ \underline{\mu}_{\mathfrak{R}_S}(C)(a_3) &= \bigvee_{b \in E} [\mu_{\mathfrak{R}_S}(a_3,b) \wedge \mu_C(b)] = [0.4 \wedge 0.6] \vee [0.7 \wedge 0.7] \vee [0.5 \wedge 0.9] = 0.7 \\ \underline{\nu}_{\mathfrak{R}_S}(C)(a_3) &= \bigwedge_{b \in E} [\nu_{\mathfrak{R}_S}(a_3,b) \vee \nu_C(b)] = [0.5 \vee 0.3] \wedge [0.3 \vee 0.6] \wedge [0.1 \vee 0.2] = 0.2 \end{aligned}$$





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Therefore,

$$\begin{aligned} \underline{\mathfrak{R}}_S(C) &= \{ \langle a_1, [0.6, 0.3] \rangle, \langle a_2, [0.7, 0.4] \rangle, \langle a_3, [0.7, 0.2] \rangle \} \\ \mu_{\underline{\mathfrak{R}}_S(C)}(a_1) &= \bigwedge_{b \in E} [v_{\mathfrak{R}_S}(a_1, b) \vee \mu_C(b)] = [0.1 \vee 0.6] \wedge [0.6 \vee 0.7] \wedge [0.3 \vee 0.9] = 0.6 \\ v_{\underline{\mathfrak{R}}_S(C)}(a_1) &= \bigvee_{b \in E} [\mu_{\mathfrak{R}_S}(a_1, b) \wedge v_C(b)] = [0.8 \wedge 0.3] \vee [0.3 \wedge 0.6] \vee [0.5 \wedge 0.2] = 0.3 \\ \mu_{\underline{\mathfrak{R}}_S(C)}(a_2) &= \bigwedge_{b \in E} [v_{\mathfrak{R}_S}(a_2, b) \vee \mu_C(b)] = [0.4 \vee 0.6] \wedge [0.2 \vee 0.7] \wedge [0.4 \vee 0.9] = 0.6 \\ v_{\underline{\mathfrak{R}}_S(C)}(a_2) &= \bigvee_{b \in E} [\mu_{\mathfrak{R}_S}(a_2, b) \wedge v_C(b)] = [0.5 \wedge 0.3] \vee [0.9 \wedge 0.6] \vee [0.6 \wedge 0.2] = 0.6 \\ \mu_{\underline{\mathfrak{R}}_S(C)}(a_3) &= \bigwedge_{b \in E} [v_{\mathfrak{R}_S}(a_3, b) \vee \mu_C(b)] = [0.5 \vee 0.6] \wedge [0.3 \vee 0.7] \wedge [0.1 \vee 0.9] = 0.6 \\ v_{\underline{\mathfrak{R}}_S(C)}(a_3) &= \bigvee_{b \in E} [\mu_{\mathfrak{R}_S}(a_3, b) \wedge v_C(b)] = [0.4 \wedge 0.3] \vee [0.7 \wedge 0.6] \vee [0.9 \wedge 0.2] = 0.6 \end{aligned}$$

Therefore,

$$\underline{\mathfrak{R}}_S(C) = \{ \langle a_1, [0.6, 0.3] \rangle, \langle a_2, [0.6, 0.6] \rangle, \langle a_3, [0.6, 0.6] \rangle \}$$

**Theorem 1.** Suppose  $(U, E, \mathfrak{R}_S)$  is a PFS approximation space. For any  $C, D \in PFS(E)$ , the lower and upper PFS approximation operators in Definition 3.2 satisfy the following properties:

- (1)  $\underline{\mathfrak{R}}_S(C) = \sim \overline{\mathfrak{R}}_S(\sim C)$ ,  
 $\overline{\mathfrak{R}}_S(C) = \sim \underline{\mathfrak{R}}_S(\sim C)$ ;
- (2)  $C \subseteq D \Rightarrow \underline{\mathfrak{R}}_S(C) \subseteq \underline{\mathfrak{R}}_S(D)$ ,  
 $C \subseteq D \Rightarrow \overline{\mathfrak{R}}_S(C) \subseteq \overline{\mathfrak{R}}_S(D)$ ;
- (3)  $\underline{\mathfrak{R}}_S(C \cap D) = \underline{\mathfrak{R}}_S(C) \cap \underline{\mathfrak{R}}_S(D)$ ,  
 $\overline{\mathfrak{R}}_S(C \cup D) = \overline{\mathfrak{R}}_S(C) \cup \overline{\mathfrak{R}}_S(D)$ ;
- (4)  $\underline{\mathfrak{R}}_S(C \cup D) \supseteq \underline{\mathfrak{R}}_S(C) \cup \underline{\mathfrak{R}}_S(D)$ ,  
 $\overline{\mathfrak{R}}_S(C \cap D) \subseteq \overline{\mathfrak{R}}_S(C) \cap \overline{\mathfrak{R}}_S(D)$ .

Proof.

$$\begin{aligned} (1) \overline{\mathfrak{R}}_S(C) &= \{ \langle a, \mu_{\overline{\mathfrak{R}}_S(C)}(a), v_{\overline{\mathfrak{R}}_S(C)}(a) \rangle \mid a \in U \} \\ &= \{ \langle a, \bigvee_{b \in E} [\mu_{\mathfrak{R}_S}(a, b) \wedge \mu_C(b)], \bigwedge_{b \in E} [v_{\mathfrak{R}_S}(a, b) \vee v_C(b)] \rangle \mid a \in U \} \\ &= \{ \langle a, \bigvee_{b \in E} [\mu_{\mathfrak{R}_S}(a, b) \wedge v_{\sim C}(b)], \bigwedge_{x \in E} [v_{\mathfrak{R}_S}(a, b) \vee \mu_{\sim C}(b)] \rangle \mid a \in U \} \\ &= \{ \langle a, v_{\mathfrak{R}_S(\sim C)}(a), \mu_{\mathfrak{R}_S(\sim C)}(a) \rangle \mid a \in U \} \\ &= \sim \underline{\mathfrak{R}}_S(\sim C) \end{aligned}$$

Similarly, it is not difficult to prove  $\underline{\mathfrak{R}}_S(C) = \sim \overline{\mathfrak{R}}_S(\sim C)$ .

(2) For all  $b \in E$ , since  $C \subseteq D$ , according to Definition 2.4., we have  $\mu_C(b) \leq \mu_D(b)$  and  $v_C(b) \geq v_D(b)$ . Then,

$$\begin{aligned} & \{ \langle a, \bigvee_{b \in E} [\mu_{\mathfrak{R}_S}(a, b) \wedge \mu_C(b)], \bigwedge_{b \in E} [v_{\mathfrak{R}_S}(a, b) \vee v_C(b)] \rangle \mid a \in U \} \\ & \leq \{ \langle a, \bigvee_{b \in E} [\mu_{\mathfrak{R}_S}(a, b) \wedge \mu_D(b)], \bigwedge_{b \in E} [v_{\mathfrak{R}_S}(a, b) \vee v_D(b)] \rangle \mid a \in U \} \end{aligned}$$

which means  $\overline{\mathfrak{R}}_S(C) \subseteq \overline{\mathfrak{R}}_S(D)$ .

Similarly, it is not difficult to prove that  $C \subseteq D \Rightarrow \underline{\mathfrak{R}}_S(C) \subseteq \underline{\mathfrak{R}}_S(D)$ .





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(3) For all  $a \in U$ ,

$$\begin{aligned} \underline{\mathfrak{R}}_S(C \cap D) &= \left\{ \langle a, \mu_{\underline{\mathfrak{R}}_S(C \cap D)}(a), \nu_{\underline{\mathfrak{R}}_S(C \cap D)}(a) \rangle \mid a \in U \right\} \\ &= \left\{ \langle a, \wedge_{b \in E} [\nu_{\mathfrak{R}_S}(a, b) \vee \mu_{(C \cap D)}(b)], \vee_{b \in E} [\mu_{\mathfrak{R}_S}(a, b) \wedge \nu_{(C \cap D)}(b)] \rangle \mid a \in U \right\} \\ &= \left\{ \langle a, \wedge_{b \in E} [\nu_{\mathfrak{R}_S}(a, b) \vee (\mu_C(b) \wedge \mu_D(b))], \vee_{b \in E} [\mu_{\mathfrak{R}_S}(a, b) \wedge (\nu_C(b) \wedge \nu_D(b))] \rangle \mid a \in U \right\} \\ &= \left\{ \langle a, \wedge_{b \in E} [(\nu_{\mathfrak{R}_S}(a, b) \vee \mu_C(b)) \wedge (\nu_{\mathfrak{R}_S}(a, b) \vee \mu_D(b))], \right. \\ &\quad \left. \vee_{b \in E} [(\mu_{\mathfrak{R}_S}(a, b) \wedge \nu_C(b)) \wedge (\mu_{\mathfrak{R}_S}(a, b) \wedge \nu_D(b))] \rangle \mid a \in U \right\} \\ &= \left\{ \langle a, \wedge_{b \in E} [\nu_{\mathfrak{R}_S}(a, b) \vee \mu_C(b)], \vee_{b \in E} [\mu_{\mathfrak{R}_S}(a, b) \wedge \nu_C(b)] \rangle \mid a \in U \right\} \wedge \\ &\quad \left\{ \langle a, \wedge_{b \in E} [\nu_{\mathfrak{R}_S}(a, b) \vee \mu_D(b)], \vee_{b \in E} [\mu_{\mathfrak{R}_S}(a, b) \wedge \nu_D(b)] \rangle \mid a \in U \right\} \\ &= \underline{\mathfrak{R}}_S(C) \cap \underline{\mathfrak{R}}_S(D) \end{aligned}$$

Similarly, it is not difficult to prove that  $\overline{\mathfrak{R}}_S(C \cup D) = \overline{\mathfrak{R}}_S(C) \cup \overline{\mathfrak{R}}_S(D)$ .

(4) From the above discussions, it is not difficult to prove that  $\underline{\mathfrak{R}}_S(C \cup D) \supseteq \underline{\mathfrak{R}}_S(C) \cup \underline{\mathfrak{R}}_S(D)$  and  $\overline{\mathfrak{R}}_S(C \cap D) \subseteq \overline{\mathfrak{R}}_S(C) \cap \overline{\mathfrak{R}}_S(D)$ .

**Theorem 2.** Let  $U$  be an initial universe set and let  $E$  be a universe parameter set. Suppose that  $(\mathfrak{R}_S)_1, (\mathfrak{R}_S)_2 \in PFSR(U \times E)$  are two PFS relations. If  $(\mathfrak{R}_S)_1 \subseteq (\mathfrak{R}_S)_2$ , for any  $C \in PFS(E)$ , we have the following properties:

- (1)  $\underline{\mathfrak{R}}_S(C) \supseteq \underline{\mathfrak{R}}_S(C)$ , for all  $C \in PF(E)$
- (2)  $\overline{\mathfrak{R}}_S(C) \subseteq \overline{\mathfrak{R}}_S(C)$ , for all  $C \in PF(E)$

Proof. Since  $(\mathfrak{R}_S)_1 \subseteq (\mathfrak{R}_S)_2$ , for any  $(a, b) \in (U \times E)$ , we have  $\mu_{(\mathfrak{R}_S)_1}(a, b) \leq \mu_{(\mathfrak{R}_S)_2}(a, b)$ ,

$$\begin{aligned} \nu_{(\mathfrak{R}_S)_1}(a, b) &\geq \nu_{(\mathfrak{R}_S)_2}(a, b). \text{ Then,} \\ &\left\{ \langle a, \wedge_{b \in E} [\nu_{(\mathfrak{R}_S)_1}(a, b) \vee \mu_C(b)], \vee_{b \in E} [\mu_{(\mathfrak{R}_S)_1}(a, b) \wedge \nu_C(b)] \rangle \mid a \in U \right\} \\ &\geq \left\{ \langle a, \wedge_{b \in E} [\nu_{(\mathfrak{R}_S)_2}(a, b) \vee \mu_C(b)], \vee_{b \in E} [\mu_{(\mathfrak{R}_S)_2}(a, b) \wedge \nu_C(b)] \rangle \mid a \in U \right\} \end{aligned}$$

Thus, we obtain  $\underline{\mathfrak{R}}_S(C) \supseteq \underline{\mathfrak{R}}_S(C)$ .

Similarly, it is not difficult to prove that  $\overline{\mathfrak{R}}_S(C) \subseteq \overline{\mathfrak{R}}_S(C)$ .

From the above theorem, the lower and upper approximations in Pythagorean fuzzy soft rough sets are monotonic with respect to the monotonic forms of the Pythagorean fuzzy relations.

**Pythagorean Fuzzy Multi-Granulation Rough Soft Set (PFMGRSSs)**

In this section, we extend the PFMGRSSs to PFMGRSSs. In what follows, we provide the definitions and the basic properties of optimistic and pessimistic PFMGRSSs.

**Optimistic PFMGRSS**

**Definition 12.** Let  $U$  be an initial universe set and let  $E$  be a universe parameter set  $(\mathfrak{R}_S)_i \in PFSR(U \times E) (1 \leq i \leq m)$  are  $m$  Pythagorean fuzzy binary relations from  $U$  to  $E$ . For any PFS  $C \in PFS(E)$ , the optimistic lower and upper approximations of  $C$  with respect to  $(U, E, (\mathfrak{R}_S)_i)$  are defined as follows:





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$$\underline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C) = \left\{ \left\langle a, \underline{\mu_{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C)}(a), \underline{\nu_{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C)}(a) \right\rangle \mid a \in U \right\},$$

$$\overline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C) = \left\{ \left\langle a, \overline{\mu_{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C)}(a), \overline{\nu_{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C)}(a) \right\rangle \mid a \in U \right\},$$

where  $\underline{\mu_{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C)}(a) = \bigvee_{i=1}^m \wedge_{b \in E} [\nu_{(\mathfrak{R}_S)_i}(a, b) \vee \mu_C(b)]$ ;  $\underline{\nu_{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C)}(a) = \bigwedge_{i=1}^m \vee_{b \in E} [\mu_{(\mathfrak{R}_S)_i}(a, b) \wedge \nu_C(b)]$ ;

$\overline{\mu_{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C)}(a) = \bigwedge_{i=1}^m \vee_{b \in E} [\mu_{(\mathfrak{R}_S)_i}(a, b) \wedge \mu_C(b)]$ ;  $\overline{\nu_{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C)}(a) = \bigvee_{i=1}^m \wedge_{b \in E} [\nu_{(\mathfrak{R}_S)_i}(a, b) \vee \nu_C(b)]$ .

We call the pair  $\left( \underline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C), \overline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C) \right)$  as an optimistic PFMGSRs of  $C$  with respect to  $(U, E, (\mathfrak{R}_S)_i)$ . If

$\underline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C) = \overline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C)$ , we call  $C$  as optimistic-definable in  $(U, E, (\mathfrak{R}_S)_i)$ ; otherwise,  $C$  is optimistic-indefinable in  $(U, E, (\mathfrak{R}_S)_i)$ . It is also noted that the PFMGSRs will reduce to a PFSRS if  $m=1$ .

**Theorem 3.** Let  $U$  be an initial universe set and let  $E$  be a universe parameter set  $(\mathfrak{R}_S)_i \in PFSR(U \times E) (1 \leq i \leq m)$  are  $m$  Pythagorean fuzzy binary relations from  $U$  to  $E$ . For any PFS  $C, C' \in PFS(E)$ , the optimistic PFMGSRs has the following properties:

- (1)  $\underline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (\sim C) \sim \underline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C)$ ,  $\overline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (\sim C) \sim \overline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C)$ ;
- (2)  $C \subseteq C' \Rightarrow \underline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C) \subseteq \underline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C')$ ,  $C \subseteq C' \Rightarrow \overline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C) \subseteq \overline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C')$ ;
- (3)  $\underline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C \cap C') = \underline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C) \cap \underline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C')$ ,  
 $\overline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C \cup C') = \overline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C) \cup \overline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C')$ ;
- (4)  $\underline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C \cup C') \supseteq \underline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C) \cup \underline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C')$ ,  
 $\overline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C \cap C') \subseteq \overline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C) \cap \overline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C')$ .

Proof.

(1) For any  $a \in U$ , we have

$$\underline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (\sim C) = \left\{ \left\langle a, \bigvee_{i=1}^m \wedge_{b \in E} [\nu_{(\mathfrak{R}_S)_i}(a, b) \vee \mu_{\sim C}(b)], \bigwedge_{i=1}^m \vee_{b \in E} [\mu_{(\mathfrak{R}_S)_i}(a, b) \wedge \nu_{\sim C}(b)] \right\rangle \mid a \in U \right\}$$





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$$\begin{aligned}
 &= \left\langle \left\langle a, \bigvee_{i=1}^m \bigwedge_{b \in E} [v_{(\mathfrak{R}_S)_i}(a,b) \vee v_C(b)], \right. \right. \\
 &\quad \left. \left. \bigwedge_{i=1}^m \bigvee_{b \in E} [\mu_{(\mathfrak{R}_S)_i}(a,b) \wedge \mu_C(b)] \right\rangle \mid a \in U \right\rangle \\
 &= \sim \sum_{i=1}^m (\mathfrak{R}_S)_i \quad (C)
 \end{aligned}$$

Similarly, it is not difficult to prove that  $\sum_{i=1}^m (\mathfrak{R}_S)_i \quad (\sim C) = \sim \sum_{i=1}^m (\mathfrak{R}_S)_i \quad (C)$

(2) For all  $a \in U, C \subseteq C'$ , we have

$$\begin{aligned}
 \sum_{i=1}^m (\mathfrak{R}_S)_i \quad (C) &= \left\langle \left\langle a, \bigvee_{i=1}^m \bigwedge_{b \in E} [v_{(\mathfrak{R}_S)_i}(a,b) \vee \mu_C(b)], \right. \right. \\
 &\quad \left. \left. \bigwedge_{i=1}^m \bigvee_{b \in E} [\mu_{(\mathfrak{R}_S)_i}(a,b) \wedge v_C(b)] \right\rangle \mid a \in U \right\rangle \\
 &\leq \left\langle \left\langle a, \bigvee_{i=1}^m \bigwedge_{b \in E} [v_{(\mathfrak{R}_S)_i}(a,b) \vee \mu_{C'}(b)], \right. \right. \\
 &\quad \left. \left. \bigwedge_{i=1}^m \bigvee_{b \in E} [\mu_{(\mathfrak{R}_S)_i}(a,b) \wedge v_{C'}(b)] \right\rangle \mid a \in U \right\rangle \\
 &= \sum_{i=1}^m (\mathfrak{R}_S)_i \quad (C')
 \end{aligned}$$

Similarly, it is not difficult to prove that  $\sum_{i=1}^m (\mathfrak{R}_S)_i \quad (C) \subseteq \sum_{i=1}^m (\mathfrak{R}_S)_i \quad (C')$ .

$$\begin{aligned}
 (3) \quad \sum_{i=1}^m (\mathfrak{R}_S)_i \quad (C \cap C') &= \left\langle \left\langle a, \mu_{(\mathfrak{R}_S)_i(C \cap C')}(a), v_{(\mathfrak{R}_S)_i(C \cap C')}(a) \right\rangle \mid a \in U \right\rangle \\
 &= \left\langle \left\langle a, \bigvee_{i=1}^m \bigwedge_{b \in E} [v_{(\mathfrak{R}_S)_i}(a,b) \vee \mu_{(C \cap C')}(b)], \bigwedge_{i=1}^m \bigvee_{b \in E} [\mu_{(\mathfrak{R}_S)_i}(a,b) \wedge v_{(C \cap C')}(b)] \right\rangle \mid a \in U \right\rangle \\
 &= \left\langle \left\langle a, \bigvee_{i=1}^m \bigwedge_{b \in E} [v_{(\mathfrak{R}_S)_i}(a,b) \vee (\mu_C(b) \wedge \mu_{C'}(b))], \bigwedge_{i=1}^m \bigvee_{b \in E} [\mu_{(\mathfrak{R}_S)_i}(a,b) \wedge (v_C(b) \wedge v_{C'}(b))] \right\rangle \mid a \in U \right\rangle \\
 &= \left\langle \left\langle a, \bigvee_{i=1}^m \bigwedge_{b \in E} [(v_{(\mathfrak{R}_S)_i}(a,b) \vee \mu_C(b)) \wedge (v_{(\mathfrak{R}_S)_i}(a,b) \vee \mu_{C'}(b))], \right. \right. \\
 &\quad \left. \left. \bigwedge_{i=1}^m \bigvee_{b \in E} [(\mu_{(\mathfrak{R}_S)_i}(a,b) \wedge v_C(b)) \wedge (\mu_{(\mathfrak{R}_S)_i}(a,b) \wedge v_{C'}(b))] \right\rangle \mid a \in U \right\rangle
 \end{aligned}$$





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$$= \left\{ \left\langle a, \bigvee_{i=1}^m \bigwedge_{b \in E} [v_{(\mathfrak{R}_S)_i}(a,b) \vee \mu_C(b)], \bigwedge_{i=1}^m \bigvee_{b \in E} [\mu_{(\mathfrak{R}_S)_i}(a,b) \wedge v_C(b)] \right\rangle \mid a \in U \right\} \wedge$$

$$\left\{ \left\langle a, \bigvee_{i=1}^m \bigwedge_{b \in E} [v_{(\mathfrak{R}_S)_i}(a,b) \vee \mu_{C'}(b)], \bigwedge_{i=1}^m \bigvee_{b \in E} [\mu_{(\mathfrak{R}_S)_i}(a,b) \wedge v_{C'}(b)] \right\rangle \mid a \in U \right\}$$

$$= \underline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C) \cap \underline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C'),$$

Similarly, it is not difficult to prove that  $\underline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C \cup C') = \underline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C) \cup \underline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C')$

(4) From the above discussions, it is not difficult to prove that  $\underline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C \cup C') \supseteq \underline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C) \cup \underline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C')$ ,

$$\underline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C \cap C') \subseteq \underline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C) \cap \underline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C').$$

**Theorem 4.** Let  $U$  be an initial universe set and let  $E$  be a universe parameter set. Suppose that  $(\mathfrak{R}_S)_i, (\mathfrak{R}_S)'_i \in PFSR(U \times E) (1 \leq i \leq m)$  are two  $m$  Pythagorean fuzzy binary relations from  $U$  to  $E$ . If  $(\mathfrak{R}_S)_i \subseteq (\mathfrak{R}_S)'_i$ , for any  $C \in PFS(E)$ , we have the following properties:

- (1)  $\underline{\sum_{i=1}^m (\mathfrak{R}_S)'_i}^O (C) \subseteq \underline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C)$ , for all  $C \in PFS(E)$
- (2)  $\underline{\sum_{i=1}^m (\mathfrak{R}_S)'_i}^O (C) \supseteq \underline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C)$ , for all  $C \in PFS(E)$ .

Proof.

Since  $(\mathfrak{R}_S)_i \subseteq (\mathfrak{R}_S)'_i$ , for any  $(a,b) \in (U \times E)$ , we have  $\mu_{(\mathfrak{R}_S)_i}(a,b) \leq \mu_{(\mathfrak{R}_S)'_i}(a,b)$ ,  $v_{(\mathfrak{R}_S)_i}(a,b) \geq v_{(\mathfrak{R}_S)'_i}(a,b)$ .

Then,

$$\left\{ \left\langle a, \bigvee_{i=1}^m \bigwedge_{b \in E} [v_{(\mathfrak{R}_S)_i}(a,b) \vee \mu_C(b)], \bigwedge_{i=1}^m \bigvee_{b \in E} [\mu_{(\mathfrak{R}_S)_i}(a,b) \wedge v_C(b)] \right\rangle \mid a \in U \right\}$$

$$\geq \left\{ \left\langle a, \bigvee_{i=1}^m \bigwedge_{b \in E} [v_{(\mathfrak{R}_S)'_i}(a,b) \vee \mu_C(b)], \bigwedge_{i=1}^m \bigvee_{b \in E} [\mu_{(\mathfrak{R}_S)'_i}(a,b) \wedge v_C(b)] \right\rangle \mid a \in U \right\}$$

Thus, we obtain  $\underline{\sum_{i=1}^m (\mathfrak{R}_S)'_i}^O (C) \subseteq \underline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C)$ .

Similarly, it is not difficult to prove that  $\underline{\sum_{i=1}^m (\mathfrak{R}_S)'_i}^O (C) \supseteq \underline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^O (C)$ .

From the above theorem, the lower and upper approximations in optimistic Pythagorean fuzzy soft rough sets are monotonic with respect to the monotonic forms of the multiple Pythagorean fuzzy soft relations.

**Pessimistic PFMGSRs**





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**Definition 13.** Let  $U$  be an initial universe set and let  $E$  be a universe parameter set  $\mathfrak{R}_i \in PFSR(U \times E)(1 \leq i \leq m)$  are  $m$  Pythagorean fuzzy binary relations from  $U$  to  $E$ . For any PFS  $C \in PFS(E)$ , the optimistic lower and upper approximations of  $C$  with respect to  $(U, E, \mathfrak{R}_i)$  are defined as follows:

$$\underline{\sum_{i=1}^m \mathfrak{R}_i}^P(C) = \left\{ \left\langle a, \mu_{\underline{\sum_{i=1}^m \mathfrak{R}_i}^P(C)}(a), \nu_{\underline{\sum_{i=1}^m \mathfrak{R}_i}^P(C)}(a) \right\rangle \mid a \in U \right\},$$

$$\overline{\sum_{i=1}^m \mathfrak{R}_i}^P(C) = \left\{ \left\langle a, \mu_{\overline{\sum_{i=1}^m \mathfrak{R}_i}^P(C)}(a), \nu_{\overline{\sum_{i=1}^m \mathfrak{R}_i}^P(C)}(a) \right\rangle \mid a \in U \right\},$$

where  $\mu_{\underline{\sum_{i=1}^m \mathfrak{R}_i}^P(C)}(a) = \bigwedge_{i=1}^m \bigwedge_{b \in E} [\nu_{\mathfrak{R}_i}(a, b) \vee \mu_C(b)]$ ;  $\nu_{\underline{\sum_{i=1}^m \mathfrak{R}_i}^P(C)}(a) = \bigvee_{i=1}^m \bigvee_{b \in E} [\mu_{\mathfrak{R}_i}(a, b) \wedge \nu_C(b)]$ ;

$$\mu_{\overline{\sum_{i=1}^m \mathfrak{R}_i}^P(C)}(a) = \bigvee_{i=1}^m \bigvee_{b \in E} [\mu_{\mathfrak{R}_i}(a, b) \wedge \mu_C(b)]; \quad \nu_{\overline{\sum_{i=1}^m \mathfrak{R}_i}^P(C)}(a) = \bigwedge_{i=1}^m \bigwedge_{b \in E} [\nu_{\mathfrak{R}_i}(a, b) \vee \nu_C(b)].$$

We call the pair  $\left( \underline{\sum_{i=1}^m \mathfrak{R}_i}^P(C), \overline{\sum_{i=1}^m \mathfrak{R}_i}^P(C) \right)$  as a pessimistic PFMGSRs of  $C$  with respect to  $(U, E, \mathfrak{R}_i)$ . If

$\underline{\sum_{i=1}^m \mathfrak{R}_i}^P(C) = \overline{\sum_{i=1}^m \mathfrak{R}_i}^P(C)$ , we call  $C$  as pessimistic-definable in  $(U, E, \mathfrak{R}_i)$ ; otherwise,  $C$  is pessimistic-undefinable in  $(U, E, \mathfrak{R}_i)$ . It is also noted that the PFMGSRs will reduce to a PFSRS if  $m=1$ .

**Theorem 5.** Let  $U$  be an initial universe set and let  $E$  be a universe parameter set  $(\mathfrak{R}_S)_i \in PFSR(U \times E)(1 \leq i \leq m)$  are  $m$  Pythagorean fuzzy binary relations from  $U$  to  $E$ . For any PFS  $C, C' \in PFS(E)$ , the pessimistic PFMGSRs has the following properties:

- (1)  $\underline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^P(\sim C) = \sim \overline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^P(C)$ ,  
 $\overline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^P(\sim C) = \sim \underline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^P(C)$ ;
- (2)  $C \subseteq C' \Rightarrow \underline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^P(C) \subseteq \underline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^P(C')$ ,  
 $C \subseteq C' \Rightarrow \overline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^P(C) \subseteq \overline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^P(C')$ ;
- (3)  $\underline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^P(C \cap C') = \underline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^P(C) \cap \underline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^P(C')$ ,  
 $\overline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^P(C \cup C') = \overline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^P(C) \cup \overline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^P(C')$ ;
- (4)  $\underline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^P(C \cup C') \supseteq \underline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^P(C) \cup \underline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^P(C')$ ,  
 $\overline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^P(C \cap C') \subseteq \overline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^P(C) \cap \overline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^P(C')$ .

Proof.





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(1) For any  $a \in U$ , we have

$$\begin{aligned} \overline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^P (\sim C) &= \left\{ \left\langle a, \bigwedge_{i=1}^m \bigwedge_{b \in E} [v_{(\mathfrak{R}_S)_i}(a, b) \vee \mu_{\sim C}(b)], \right. \right. \\ &\quad \left. \left. \bigvee_{i=1}^m \bigvee_{b \in E} [\mu_{(\mathfrak{R}_S)_i}(a, b) \wedge v_{\sim C}(b)] \right\rangle \mid a \in U \right\} \\ &= \left\{ \left\langle a, \bigwedge_{i=1}^m \bigwedge_{b \in E} [v_{(\mathfrak{R}_S)_i}(a, b) \vee v_C(b)], \right. \right. \\ &\quad \left. \left. \bigvee_{i=1}^m \bigvee_{b \in E} [\mu_{(\mathfrak{R}_S)_i}(a, b) \wedge \mu_C(b)] \right\rangle \mid a \in U \right\} \\ &= \overline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^P (C) \end{aligned}$$

Similarly, it is not difficult to prove that  $\overline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^P (\sim C) = \sim \overline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^P (C)$

(2) For all  $a \in U, C \subseteq C'$ , we have

$$\begin{aligned} \overline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^P (C) &= \left\{ \left\langle a, \bigwedge_{i=1}^m \bigwedge_{b \in E} [v_{(\mathfrak{R}_S)_i}(a, b) \vee \mu_C(b)], \right. \right. \\ &\quad \left. \left. \bigvee_{i=1}^m \bigvee_{b \in E} [\mu_{(\mathfrak{R}_S)_i}(a, b) \wedge v_C(b)] \right\rangle \mid a \in U \right\} \\ &\leq \left\{ \left\langle a, \bigwedge_{i=1}^m \bigwedge_{b \in E} [v_{(\mathfrak{R}_S)_i}(a, b) \vee \mu_{C'}(b)], \right. \right. \\ &\quad \left. \left. \bigvee_{i=1}^m \bigvee_{b \in E} [\mu_{(\mathfrak{R}_S)_i}(a, b) \wedge v_{C'}(b)] \right\rangle \mid a \in U \right\} \\ &= \overline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^P (C') \end{aligned}$$

Similarly, it is not difficult to prove that  $\overline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^P (C) \subseteq \overline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^P (C')$ .

$$\begin{aligned} (3) \quad \overline{\sum_{i=1}^m (\mathfrak{R}_S)_i}^P (C \cap C') &= \left\{ \left\langle a, \mu_{(\mathfrak{R}_S)_i(C \cap C')}(a), v_{(\mathfrak{R}_S)_i(C \cap C')}(a) \right\rangle \mid a \in U \right\} \\ &= \left\{ \left\langle a, \bigwedge_{i=1}^m \bigwedge_{b \in E} [v_{(\mathfrak{R}_S)_i}(a, b) \vee \mu_{(C \cap C')}(b)], \bigvee_{i=1}^m \bigvee_{b \in E} [\mu_{(\mathfrak{R}_S)_i}(a, b) \wedge v_{(C \cap C')}(b)] \right\rangle \mid a \in U \right\} \\ &= \left\{ \left\langle a, \bigwedge_{i=1}^m \bigwedge_{b \in E} [v_{(\mathfrak{R}_S)_i}(a, b) \vee (\mu_C(b) \wedge \mu_{C'}(b))], \bigvee_{i=1}^m \bigvee_{b \in E} [\mu_{(\mathfrak{R}_S)_i}(a, b) \wedge (v_C(b) \wedge v_{C'}(b))] \right\rangle \mid a \in U \right\} \end{aligned}$$





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$$\begin{aligned}
 &= \left\{ \left\langle a, \bigwedge_{i=1}^m \bigwedge_{b \in E} [(v_{(\mathfrak{R}_S)_i}(a,b) \vee \mu_C(b)) \wedge (v_{(\mathfrak{R}_S)_i}(a,b) \vee \mu_{C'}(b))], \right. \right. \\
 &\quad \left. \left. \bigvee_{i=1}^m \bigvee_{b \in E} [(\mu_{(\mathfrak{R}_S)_i}(a,b) \wedge v_C(b)) \wedge (\mu_{(\mathfrak{R}_S)_i}(a,b) \wedge v_{C'}(b))] \mid a \in U \right\rangle \right\} \\
 &= \left\{ \left\langle a, \bigwedge_{i=1}^m \bigwedge_{b \in E} [v_{(\mathfrak{R}_S)_i}(a,b) \vee \mu_C(b)], \bigvee_{i=1}^m \bigvee_{b \in E} [\mu_{(\mathfrak{R}_S)_i}(a,b) \wedge v_C(b)] \mid a \in U \right\rangle \right\} \wedge \\
 &\quad \left\{ \left\langle u, \bigwedge_{i=1}^m \bigwedge_{b \in E} [v_{(\mathfrak{R}_S)_i}(a,b) \vee \mu_{C'}(b)], \bigvee_{i=1}^m \bigvee_{b \in E} [\mu_{(\mathfrak{R}_S)_i}(a,b) \wedge v_{C'}(b)] \mid a \in U \right\rangle \right\} \\
 &= \underbrace{\sum_{i=1}^m (\mathfrak{R}_S)_i}_P(C) \cap \underbrace{\sum_{i=1}^m (\mathfrak{R}_S)_i}_P(C'),
 \end{aligned}$$

Similarly, it is not difficult to prove that  $\overline{\sum_{i=1}^m (\mathfrak{R}_S)_i}_P(C \cup C') = \overline{\sum_{i=1}^m (\mathfrak{R}_S)_i}_P(C) \cup \overline{\sum_{i=1}^m (\mathfrak{R}_S)_i}_P(C')$

(4) From the above discussions, it is not difficult to prove that  $\underbrace{\sum_{i=1}^m (\mathfrak{R}_S)_i}_P(C \cup C') \supseteq \underbrace{\sum_{i=1}^m (\mathfrak{R}_S)_i}_P(C) \cup \underbrace{\sum_{i=1}^m (\mathfrak{R}_S)_i}_P(C')$ ,

$$\underbrace{\sum_{i=1}^m (\mathfrak{R}_S)_i}_P(C \cap C') \subseteq \underbrace{\sum_{i=1}^m (\mathfrak{R}_S)_i}_P(C) \cap \underbrace{\sum_{i=1}^m (\mathfrak{R}_S)_i}_P(C').$$

**Theorem 6.** Let  $U$  be an initial universe set and let  $E$  be a universe parameter set. Suppose that  $(\mathfrak{R}_S)_i, (\mathfrak{R}_S)'_i \in PFSR(U \times E) (1 \leq i \leq m)$  are two  $m$  Pythagorean fuzzy binary relations from  $U$  to  $E$ . If  $(\mathfrak{R}_S)_i \subseteq (\mathfrak{R}_S)'_i$ , for any  $C \in PFS(E)$ , we have the following properties:

- (1)  $\underbrace{\sum_{i=1}^m (\mathfrak{R}_S)'_i}_P(C) \subseteq \underbrace{\sum_{i=1}^m (\mathfrak{R}_S)_i}_P(C)$ , for all  $C \in PFS(E)$
- (2)  $\underbrace{\sum_{i=1}^m (\mathfrak{R}_S)'_i}_P(C) \supseteq \underbrace{\sum_{i=1}^m (\mathfrak{R}_S)_i}_P(C)$ , for all  $C \in PFS(E)$ .

Proof.

Since  $(\mathfrak{R}_S)_i \subseteq (\mathfrak{R}_S)'_i$ , for any  $(a,b) \in (U \times E)$ , we have  $\mu_{(\mathfrak{R}_S)_i}(a,b) \leq \mu_{(\mathfrak{R}_S)'_i}(a,b)$ ,  $v_{(\mathfrak{R}_S)_i}(a,b) \geq v_{(\mathfrak{R}_S)'_i}(a,b)$ . Then,

$$\begin{aligned}
 &\left\{ \left\langle a, \bigwedge_{i=1}^m \bigwedge_{b \in E} [v_{(\mathfrak{R}_S)_i}(a,b) \vee \mu_C(b)], \bigvee_{i=1}^m \bigvee_{b \in E} [\mu_{(\mathfrak{R}_S)_i}(a,b) \wedge v_C(b)] \mid a \in U \right\rangle \right\} \\
 &\geq \left\{ \left\langle a, \bigwedge_{i=1}^m \bigwedge_{b \in E} [v_{(\mathfrak{R}_S)'_i}(a,b) \vee \mu_C(b)], \bigvee_{i=1}^m \bigvee_{b \in E} [\mu_{(\mathfrak{R}_S)'_i}(a,b) \wedge v_C(b)] \mid a \in U \right\rangle \right\}
 \end{aligned}$$

Thus, we obtain  $\underbrace{\sum_{i=1}^m (\mathfrak{R}_S)'_i}_P(C) \subseteq \underbrace{\sum_{i=1}^m (\mathfrak{R}_S)_i}_P(C)$ .

Similarly, it is not difficult to prove that  $\underbrace{\sum_{i=1}^m (\mathfrak{R}_S)'_i}_P(C) \supseteq \underbrace{\sum_{i=1}^m (\mathfrak{R}_S)_i}_P(C)$ .

From the above theorem, the lower and upper approximations in pessimistic Pythagorean fuzzy soft rough sets are monotonic with respect to the monotonic forms of the multiple Pythagorean fuzzy soft relations.





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**The Relation between Optimistic and Pessimistic PFMGSRs**

**Theorem 7.** Let  $U$  be an initial universe set and let  $E$  be a universe parameter set  $(\mathfrak{R}_S)_i \in PFSR(U \times E) (1 \leq i \leq m)$  are  $m$  Pythagorean fuzzy binary relations from  $U$  to  $E$ . For any PFS  $C \in PF(E)$ , the PFMGSRs has the following properties:

$$\frac{\sum_{i=1}^m (\mathfrak{R}_S)_i (C)}{\sum_{i=1}^m (\mathfrak{R}_S)_i (C)} \overset{P}{\subseteq} \frac{\sum_{i=1}^m (\mathfrak{R}_S)_i (C)}{\sum_{i=1}^m (\mathfrak{R}_S)_i (C)} \overset{O}{\subseteq} \frac{\sum_{i=1}^m (\mathfrak{R}_S)_i (C)}{\sum_{i=1}^m (\mathfrak{R}_S)_i (C)}$$

$$\frac{\sum_{i=1}^m (\mathfrak{R}_S)_i (C)}{\sum_{i=1}^m (\mathfrak{R}_S)_i (C)} \overset{P}{\supseteq} \frac{\sum_{i=1}^m (\mathfrak{R}_S)_i (C)}{\sum_{i=1}^m (\mathfrak{R}_S)_i (C)} \overset{O}{\supseteq} \frac{\sum_{i=1}^m (\mathfrak{R}_S)_i (C)}{\sum_{i=1}^m (\mathfrak{R}_S)_i (C)}$$

Proof.

For any  $a \in U$ ,

$$\frac{\sum_{i=1}^m (\mathfrak{R}_S)_i (C)}{\sum_{i=1}^m (\mathfrak{R}_S)_i (C)} \overset{O}{\subseteq} \left\{ \left\langle a, \bigvee_{i=1}^m \bigwedge_{b \in E} [v_{(\mathfrak{R}_S)_i}(a,b) \vee \mu_C(b)], \right. \right.$$

$$\left. \left. \bigwedge_{i=1}^m \bigvee_{b \in E} [\mu_{(\mathfrak{R}_S)_i}(a,b) \wedge v_C(b)] \right\rangle \mid a \in U \right\}$$

$$\geq \left\{ \left\langle a, \bigwedge_{i=1}^m \bigwedge_{b \in E} [v_{(\mathfrak{R}_S)_i}(a,b) \vee \mu_C(b)], \right. \right.$$

$$\left. \left. \bigvee_{i=1}^m \bigvee_{b \in E} [\mu_{(\mathfrak{R}_S)_i}(a,b) \wedge v_C(b)] \right\rangle \mid a \in U \right\}$$

$$= \frac{\sum_{i=1}^m (\mathfrak{R}_S)_i (C)}{\sum_{i=1}^m (\mathfrak{R}_S)_i (C)} \overset{P}{\subseteq} \frac{\sum_{i=1}^m (\mathfrak{R}_S)_i (C)}{\sum_{i=1}^m (\mathfrak{R}_S)_i (C)} \overset{O}{\subseteq} \frac{\sum_{i=1}^m (\mathfrak{R}_S)_i (C)}{\sum_{i=1}^m (\mathfrak{R}_S)_i (C)}$$

Similarly, it is not difficult to prove that  $\frac{\sum_{i=1}^m (\mathfrak{R}_S)_i (C)}{\sum_{i=1}^m (\mathfrak{R}_S)_i (C)} \overset{P}{\supseteq} \frac{\sum_{i=1}^m (\mathfrak{R}_S)_i (C)}{\sum_{i=1}^m (\mathfrak{R}_S)_i (C)} \overset{O}{\supseteq} \frac{\sum_{i=1}^m (\mathfrak{R}_S)_i (C)}{\sum_{i=1}^m (\mathfrak{R}_S)_i (C)}$ .

From the theorem, we have the pessimistic Pythagorean fuzzy multigranulation lower approximation is induced by the optimistic Pythagorean fuzzy multigranulation lower approximation. While the optimistic Pythagorean fuzzy multigranulation upper approximation is induced by the pessimistic Pythagorean fuzzy multigranulation upper approximation.

**APPLICATION OF PFMGSRs**

Let  $U = \{a_1, a_2, a_3\}$  be the set of three houses under consideration of Mr.A to purchase. Let  $E$  be universe of parameter, where  $E = \{b_1, b_2, b_3\} = \{\text{expensive, location, environment}\}$ . Mr.A wants to buy the house which is most attractive and feasible with the parameters of  $E$  to the utmost extend from houses in  $U$  by constructing a Pythagorean fuzzy soft rough which is a Pythagorean fuzzy soft relation  $\mathfrak{R}_S$  from  $U$  to  $E$ .

$$\mathfrak{R}_S = \{ \langle (a_1, b_1), [0.5, 0.4] \rangle, \langle (a_1, b_2), [0.9, 0.2] \rangle, \langle (a_1, b_3), [0.6, 0.4] \rangle, \langle (a_2, b_1), [0.4, 0.5] \rangle, \langle (a_2, b_2), [0.7, 0.3] \rangle, \langle (a_2, b_3), [0.9, 0.1] \rangle, \langle (a_3, b_1), [0.3, 0.6] \rangle, \langle (a_3, b_2), [0.8, 0.1] \rangle, \langle (a_3, b_3), [0.5, 0.3] \rangle \}$$

Now suppose that Mr.A gives the optimum normal decision object  $C$  which is an Pythagorean Fuzzy Soft defined as follows:

$$C = \{ \langle b_1, [0.7, 0.6] \rangle, \langle b_2, [0.9, 0.2] \rangle, \langle b_3, [0.6, 0.3] \rangle \}$$

To determine the lower and upper approximations of relation  $\mathfrak{R}_S$ ,

$$\underline{\mathfrak{R}_S}(C) = \{ \langle a_1, [0.6, 0.5] \rangle, \langle a_2, [0.6, 0.4] \rangle, \langle a_3, [0.6, 0.3] \rangle \};$$





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$$\overline{\mathfrak{R}_S}(C) = \{ \langle a_1, [0.9, 0.2] \rangle, \langle a_2, [0.7, 0.3] \rangle, \langle a_3, [0.8, 0.2] \rangle \};$$

We compute the optimistic and pessimistic PFMGSRs, respectively.

$$\underline{\mathfrak{R}_S}^O(C) = \{ \langle a_1, [0.6, 0.3] \rangle, \langle a_2, [0.7, 0.5] \rangle, \langle a_3, [0.7, 0.5] \rangle \};$$

$$\overline{\mathfrak{R}_S}^O(C) = \{ \langle a_1, [0.7, 0.3] \rangle, \langle a_2, [0.6, 0.5] \rangle, \langle a_3, [0.7, 0.7] \rangle \};$$

$$\underline{\mathfrak{R}_S}^P(C) = \{ \langle a_1, [0.6, 0.5] \rangle, \langle a_2, [0.7, 0.5] \rangle, \langle a_3, [0.7, 0.5] \rangle \};$$

$$\overline{\mathfrak{R}_S}^P(C) = \{ \langle a_1, [0.7, 0.3] \rangle, \langle a_2, [0.6, 0.5] \rangle, \langle a_3, [0.7, 0.7] \rangle \};$$

By Definition 2.3, we can compute the following  $\underline{\mathfrak{R}_S}^O(C) \oplus \overline{\mathfrak{R}_S}^O(C)$  and  $\underline{\mathfrak{R}_S}^P(C) \oplus \overline{\mathfrak{R}_S}^P(C)$

$$\underline{\mathfrak{R}_S}^O(C) \oplus \overline{\mathfrak{R}_S}^O(C) = \{ \langle a_1, [0.48, 0.09] \rangle, \langle a_2, [0.48, 0.25] \rangle, \langle a_3, [0.86, 0.35] \rangle \};$$

$$\underline{\mathfrak{R}_S}^P(C) \oplus \overline{\mathfrak{R}_S}^P(C) = \{ \langle a_1, [0.48, 0.15] \rangle, \langle a_2, [0.48, 0.25] \rangle, \langle a_3, [0.86, 0.35] \rangle \};$$

The optimal solution is to choose  $a_3$  ie., Mr.A will select the house  $a_3$  for buying according to his choice parameters in  $E$ .

## CONCLUSIONS

In this paper, we have proposed a new model of PFMGSRs from PFMGRs over two universes. In this study, we have given the definition and some basic properties of PFSRS. Further, we have developed the Pythagorean fuzzy soft rough set into the multi-granulation context. The definition and some properties of optimistic and pessimistic PFMGSRs have been studied. There are still many interesting issues to be explored in this framework of PFMGSRs. We can discuss various attribute reduction approaches and uncertainty measures in future.

## REFERENCES

1. K.T. Atanassov, "Intuitionistic fuzzy sets", Fuzzy Sets Syst, Vol.20, No.1, pp.87–96,1986.
2. K.T. Atanassov, "Intuitionistic fuzzy logics as tools for evaluation of data mining processes", Knowl-Based Syst, Vol.80, pp.122–130, 2015.
3. Cengiz Kahraman, "A Special issue on extensions of fuzzy sets in decision-making", Vol.22, No.15, pp.4251-4853, 2018.
4. Chao Zhang, Deyu Li and RuiRen, "Pythagorean fuzzy multigranulation rough set over two universes and its applications in merger and acquisition", Int J IntellSyst, pp.1-23, 2016.
5. D. Dubois and H. Prade, "Rough fuzzy sets and fuzzy rough sets", Int J Gen Syst, Vol.17, pp.191–209, 1990.
6. S. Greco, B. Matarazzo and R. Slowinski, "Rough approximation by dominance relations", Int J IntellSyst, Vol.17, Issue.2, pp.153–171, 2002.
7. Lazim Abdullah and Pinxin Goh, "Decision making method based on Pythagorean fuzzy sets and its application to solid waste management", Complex & intelligent systems, Vol.5, pp.185-198, 2019.
8. H. Liu, A. Gegov and M. Cocea, "Rule-based systems: a granular computing perspective", Granular Computing, Vol.1, Issue.4, pp.259–274, 2016.
9. P.K. Maji, R. Biswas and A.R. Roy, "Soft set Theory", Computers and Mathematics with Applications. Vol.45, Issue 4-5, pp.555-562, 2003.
10. J.M. Mendel and R.I.B. John, "Type-2 fuzzy sets made simple", IEEE Trans Fuzzy Syst, Vol.10, Issue.2, pp.117–127, 2002.
11. D.A. Molodtsov, "Soft set theory-first result", Computers and Mathematics with applications, Vol.37, pp.19-31, 1999.



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12. D.A. Molodtsov, "The description of a dependence with the help of soft sets", Journal of Computer and Systems Sciences International, Vol.40, Issue.6, pp. 977-984, 2001.
13. D.A. Molodtsov, "The Theory of Soft Sets", URSS Publishers, Moscow (in Russian), 2004.
14. D.A. Molodtsov, V. Y. Leonov and D.V. Kovkov, "Soft sets technique and its application", Nechetkie Sistemy i Myagkie Vychisleniya, Vol.1, Issue.1, pp.8-39, 2006.
15. Paul Augustine Ejegwa, "Pythagorean fuzzy set and its application in career placements based on academic performance using max-min-max composition". Complex & intelligent systems Vol.5, pp.165-175, 2019.
16. Z. Pawlak, "Rough sets", Int J Comput Inform Sci, Vol.11, Issue.5, pp.341-356, 1982.
17. Y. Qian, J. Liang, W. Pedrycz and C. Dang, "Positive approximation: an accelerator for attribute reduction in rough set theory", ArtifIntell, Vol.174, Issue.9, pp. 597-618, 2010.
18. Y. Qian, J. Liang, Y. Yao and C. Dang, "MGRS: A multi-granulation rough set", Inform Sci, Vol.180, Issue.6, pp.949-970, 2010.
19. J. Qi, L. Wei and Q. Wan, "Multi-level granularity in formal concept analysis", Granular Computing, 2018.
20. M.Z. Reformat and R.R. Yager, "Suggesting recommendations using pythagorean fuzzy sets illustrated using Netflix movie data", In: Information processing and management of uncertainty in knowledge-based systems; Berlin: Springer, pp.546-556, 2014.
21. S. Samanta and T. Mondal, "Intuitionistic fuzzy rough sets and rough intuitionistic fuzzy sets", J Fuzzy Math, Vol.9, Issue.3, pp.561-582, 2001.
22. B. Sun and W. Ma, "Fuzzy rough set model on two different universes and its application", Appl Math Model, Vol.35, Issue.4, pp.1798-1809, 2011.
23. V. Torra, "Hesitant fuzzy sets", Int J IntellSyst, Vol.25, Issue.6, pp.529-539, 2010.
24. W.R. Wan Mohd and L. Abdullah, "Pythagorean fuzzy analytic hierarchy process to multi-criteria decision making", In: AIP Conference proceedings, Vol.1905, Issue.1, 2017.
25. S.P. Wan, S.Q. Li and J.Y. Dong, "A three-phase method for Pythagorean fuzzy multi-attribute group decision making and application to haze management", ComputIndEng, Vol.123, pp.348-363, 2018.
26. W. Wei, J. Liang and Y. Qian, "A comparative study of rough sets for hybrid data", Inform Sci, Vol.190, pp.1-16, 2012.
27. R.R. Yager, "On the theory of bags", Int J Gen Syst, Vol.13, Issue.1, pp.23-37, 1986.
28. R.R. Yager, "Pythagorean fuzzy subsets", In: Joint IFSAWorld Congress and NAFIPS Annual Meeting (IFSA/NAFIPS), Edmonton: Canada; June 24-28, pp.57-61, 2013.
29. H. Yang, X. Liao, S. Wang and J. Wang, "Fuzzy probabilistic rough set model on two universes and its applications", Int J Approx Reason, Vol.54, Issue.9, pp.1410-1420, 2013.
30. R.R. Yager and A.M. Abbasov, "Pythagorean membership grades, complex numbers, and decision making", Int J IntellSyst, Vol.28, Issue.5, pp.436-452, 2013.
31. R.R. Yager, "Pythagorean membership grades in multi-criteria decision making", IEEE Trans Fuzzy Syst, Vol.22, Issue.4, pp.958-965, 2014.
32. D.S. Yeung, D. Chen, E.C. Tsang, J.W. Lee and W. Xizhao, "On the generalization of fuzzy rough sets", IEEE Trans Fuzzy Syst, Vol.13, Issue.3, pp.343-361, 2005.
33. L.A. Zadeh, "Fuzzy sets". Inform control Vol.8, Issue.3, pp.338-353, 1965.
34. L.A. Zadeh, "The concept of a linguistic variable and its application to approximate reasoning—I", Inform Sci, Vol.8, Issue.3, pp.199-249, 1975.
35. X. Zhang and Z. Xu, "Extension of TOPSIS to multiple criteria decision making with Pythagorean fuzzy sets", Int J IntellSyst, Vol.29, Issue.12, pp.1061-1078, 2014.





## Equitable Detour Global Domination Number of a Graph

A. Punitha Tharani<sup>1</sup> and A. Ferdina<sup>2\*</sup>

<sup>1</sup>Associate Professor, Department of Mathematics, St. Mary's College (Autonomous), Thoothukudi, Tamil Nadu, India, Affiliated to Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli, Tamil Nadu, India.

<sup>2</sup>Research Scholar (Register Number: 19122212092006), Department of Mathematics, St. Mary's College (Autonomous), Thoothukudi, Tamil Nadu, India, Affiliated to Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli, Tamil Nadu, India.

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### \*Address for Correspondence

#### A. Ferdina

Research Scholar (Register Number: 19122212092006),  
Department of Mathematics, St. Mary's College (Autonomous),  
Thoothukudi, Tamil Nadu, India,  
Affiliated to Manonmaniam Sundaranar University,  
Abishekapatti, Tirunelveli, Tamil Nadu, India.  
E.Mail: aferdinafdo@gmail.com



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### ABSTRACT

In this paper, we introduce a new domination parameter, called equitable detour global domination number of a graph. A subset  $D$  of  $V(G)$  is a detour global dominating set if for every vertex of  $G$  is contained in a longest path between any pair of vertices in  $D$  and global dominating set. The minimum number of vertices taken over all detour global dominating sets of  $G$  is called the detour global domination number of  $G$  and is denoted by  $\gamma_{dng}(G)$ . A detour global dominating set of cardinality  $\gamma_{dng}(G)$  is called a  $\gamma_{dng}$ -set of  $G$ . A detour global dominating set  $D$  of  $V(G)$  is called an equitable detour global dominating set if for every vertex  $a \in V$  not in  $D$ , there exists a vertex  $b \in D$  such that  $ab$  is an edge of  $G$  and  $|deg(a) - deg(b)| \leq 1$ . The minimum number of vertices taken over all equitable detour global dominating sets of  $G$  is called the equitable detour global domination number of  $G$  and is denoted by  $\gamma_{dng}^e(G)$ . We determine  $\gamma_{dng}^e$  for some standard class of graphs and characterize the detour global domination and equitable detour global domination parameters are equal.

**Keywords:** Detour set, detour global dominating set. equitable detour global dominating set  
Mathematical subject classification 05C12, 05C70.





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**INTRODUCTION**

By a graph  $G = (V, E)$ , we consider a finite undirected connected graph without loops and multiple edges. The order and size of  $G$  are denoted by  $p, q$  respectively. The concept of Detour Global Dominating graphs was introduced in [3]. For underlying definition and results, see references.

**Preliminaries**

**Theorem 1.** Every detour global dominating set of  $G$  contains all the pendant vertices of  $G$ .

**Theorem 2.** For any connected graph  $G$  of order  $p \geq 2$ . Then,  $2 \leq dn(G) \leq \gamma_{dng}(G) \leq p$ .

**Results**

1. For the path graph  $G = P_p$  of  $p$  vertices,  $\gamma_{dng}(G) = \left\lceil \frac{p+2}{3} \right\rceil, p \geq 4$ .

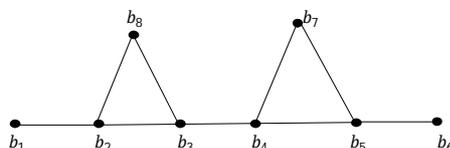
2. For the Cycle graph  $G = C_p$  of  $p$  vertices,  $\gamma_{dng}(G) = \left\lceil \frac{p}{3} \right\rceil, p \geq 6$ .

**Equitable Detour Global Domination Number of a Graph**

**Definition**

A detour global dominating set  $D$  of  $V(G)$  is called an equitable detour global dominating set if for each vertex  $a \in V$  not in  $D$  there exists a vertex  $b \in D$  such that  $ab$  is an edge of  $G$  and  $|deg(a) - deg(b)| \leq 1$ . The minimum number of vertices taken over all equitable detour global dominating sets of  $G$  is called the equitable detour global domination number of  $G$  and is denoted by  $\gamma_{dng}^e(G)$ . An equitable detour global dominating set of cardinality  $\gamma_{dng}^e(G)$  is called a  $\gamma_{dng}^e$ -set of  $G$ .

For illustration, we consider the graph  $H$  as shown in Figure 1



**Figure 1. graph H**

In Figure 1, the detour global dominating sets have minimum number of vertices of  $H$  are  $D_1 = \{b_1, b_6, b_7, b_8\}, D_2 = \{b_1, b_2, b_4, b_6\}, D_3 = \{b_1, b_2, b_5, b_6\}, D_4 = \{b_1, b_3, b_4, b_6\}, D_5 = \{b_1, b_3, b_5, b_6\}$  Also, the sets  $D_1, D_2, D_3, D_4, D_5$  are equitable detour global dominating sets with minimum cardinality of  $H$ . Therefore,  $\gamma_{dng}^e(H) = |D_1| = |D_2| = |D_3| = |D_4| = |D_5| = 4$ .

**Remark**

(i) Every detour global dominating set is obviously equitable global dominating set but converse is not true.

(ii) Every equitable global dominating set is also an equitable dominating set but converse is not true.

In the following proposition, the equitable detour global domination number of some standard graphs are determined.

**Proposition**

1. For the path  $P_p (p \geq 4), \gamma_{dng}^e(P_p) = \left\lceil \frac{p+2}{3} \right\rceil$ .

2. For the cycle  $C_p (p \geq 6), \gamma_{dng}^e(C_p) = \left\lceil \frac{p}{3} \right\rceil$ .





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3. For the wheel  $W_p$  of order  $\gamma_{dng}^e(W_p) = \begin{cases} 4 & p = 4, 6 \\ \lfloor \frac{p-1}{3} \rfloor + 1 & p = 5 \text{ and } p \geq 7 \end{cases}$ .

4. For the complete graph  $K_p (p \geq 2)$ ,  $\gamma_{dng}^e(K_p) = p$ .

5. For the complete bipartite graph  $K_{m,n}$ , we have

$$\gamma_{dng}^e(K_{m,n}) = \begin{cases} 2 & \text{if } |m - n| \leq 1 \quad \text{other than } K_{1,2} \\ m + n & \text{if } |m - n| \geq 2, \quad \text{where } m, n \geq 2 \end{cases}$$

**Proof**

1. Let  $D$  be a  $\gamma_{dng}$  – set of  $P_p$ . For every vertex of  $P_p$  has degree either one or two. Then every vertex in  $D$  is equitable dominates with some vertices in  $V - D$ . Therefore,  $D$  is itself an equitable. Hence  $\gamma_{dng}^e(P_p) = |D| = \gamma_{dng}(P_p)$ . By Result 2.3,  $\gamma_{dng}^e(P_p) = \lfloor \frac{p+2}{3} \rfloor$ .

2. Let  $D_1$  be a  $\gamma_{dng}$  – set of  $C_p$ . Since every vertex of  $C_p$  has same degree. Then  $D_1$  is equitable detour global dominating set with minimum number of vertices and so

$$\gamma_{dng}^e(C_p) = |D_1| = \gamma_{dng}(C_p) = \lfloor \frac{p}{3} \rfloor, \text{ by Result 2.3.}$$

3. Let  $W_p (p \geq 4)$  be a wheel graph is obtained by connecting a single universal vertex to all the vertices of the cycle of order  $p - 1$ . Let  $\{b, b_1, b_2, \dots, b_{p-1}\}$  be the vertex set of  $W_p$  where  $b$  is the universal vertex and  $\{b_1, b_2, \dots, b_{p-1}\}$  are rim vertices. Then degree of  $b_i$  is three, for all  $i, 1 \leq i \leq p - 1$  and degree of the universal vertex  $b$  is  $p - 1$ . We consider the following cases.

**Case (i)**

When  $p = 4$ , the set  $D = \{b, b_1, b_2, b_3\}$  is a  $\gamma_{dng}$  – set and so  $\gamma_{dng}(W_4) = 4$ . Then  $\deg(b) = 3$  and  $\deg(b_i) = 3; 1 \leq i \leq 3$

3. Therefore  $W_4$  is a regular graph. Hence,  $D$  forms an equitable detour global dominating set and so  $\gamma_{dng}^e(W_4) = 4$ .

**Case (ii)**

When  $p = 6$ , any two adjacent rim vertices along with universal vertex forms a  $\gamma_{dng}$  – set but it is not equitable. Then  $D = \{b, b_1, b_2, b_4\}$  forms an equitable detour global dominating set. Therefore,  $\gamma_{dng}^e(W_6) = 4$ .

**Case (iii)**

When  $p \geq 7$ , degree of universal vertex  $b$  is greater than or equal to 6 and degree of all rim vertices  $b_1, b_2, \dots, b_{p-1}$  is three. Then there exists a  $\gamma_{dng}$  – set say

$D_1 = \{b, b_i, b_{i+1} / 1 \leq i \leq p - 2\}$  but it is not equitable.

$$\text{We consider, } D = \begin{cases} \{b, b_1, b_4, \dots, b_{3r-2}\}, & \text{if } p - 1 = 3r; \\ \{b, b_1, b_4, \dots, b_{3r-2}, b_{3r-1}\}, & \text{if } p - 1 = 3r + 1; \\ \{b, b_1, b_4, \dots, b_{3r-2}, b_{3r+1}\}, & \text{if } p - 1 = 3r + 2 \end{cases}$$

Then for every  $b_i \in V - D$  is degree equitable dominates with some  $b_j \in D$ .

Therefore,  $D$  is an equitable detour global dominating set of  $W_p$ . Now,  $D' = D - \{b\}$  is an equitable detour global dominating set of  $C_{p-1}$ . Then by proposition 3.3(2),  $|D'| = \gamma_{dng}^e(C_{p-1}) = \lfloor \frac{p-1}{3} \rfloor$ . Therefore,  $|D| \geq |D'| + 1$ . Hence,

$$|D| = \gamma_{dng}^e(W_p) = \lfloor \frac{p-1}{3} \rfloor + 1$$

4. Any one of the vertices of the complete graph  $K_p$  is an equitable dominating set of  $K_p$  but  $\gamma_{dng}(K_p) = p$ . Therefore,  $\gamma_{dng}^e(K_p) = p$ .





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5. Let  $A, B$  be the two partition sets of a complete graph  $K_{m,n}$ . Let  $\{a_1, a_2, \dots, a_m\}$  be the vertex set of  $A$  with cardinality  $m$  and  $\{b_1, b_2, \dots, b_n\}$  be the vertex set of  $B$  with cardinality  $n$ . Then degree of any vertex  $a \in A$  is  $n$  and degree of any vertex  $b \in B$  is  $m$ . We consider the following two cases

**Case (i):**  $|m - n| \leq 1$ , Then  $D = \{a, b / a \in A, b \in B\}$  is a  $\gamma_{dng}$  - set which is equitable. Therefore,  $\gamma_{dng}^e(K_{m,n}) \leq 2$ . Since  $m, n \geq 2$ , There is no full degree vertex in  $K_{m,n}$ . Therefore,  $\gamma_{dng}^e(K_{m,n}) \geq 2$  and so  $\gamma_{dng}^e(K_{m,n}) = 2$ .

**Case (ii):**  $|m - n| \geq 2$ . Let  $D$  be a minimum equitable detour global dominating set of  $K_{m,n}$ . Suppose  $|D| < |V(K_{m,n})|$ . Then for every vertex  $a \in V - D$  there exists  $b \in D$  such that  $ab$  is an edge of  $K_{m,n}$  and  $|deg(a) - deg(b)| = |n - m| = |m - n| \geq 2$ . Which is a contradiction to equitable dominating set. Hence,  $|D| = \gamma_{dng}^e(K_{m,n}) = m + n$ .

**Theorem 3.** For any connected graph  $G$  with  $p$  vertices, we have  $2 \leq \gamma_{dng}^e(G) \leq p$  and the bounds are sharp.

**Proof:** The lower bound follows from Theorem 2.2 and the upper bound is obvious. For  $C_6$  the lower bound is attained and for  $K_4$  the upper bound is attained.

**Theorem 4.** For any connected graph  $G$ ,  $\gamma_{dng}(G) \leq \gamma_{dng}^e(G)$ .

**Observation 1.** For a connected graph  $G$ ,  $2 \leq \max\{\gamma_{dng}(G), \gamma^e(G)\} \leq \gamma_{dng}^e(G)$ .

**Observation 2.** For any complete graph of order  $p$ ,  $\gamma^e(G) < \gamma_{dng}(G) \leq \gamma_{dng}^e(G)$ .

**Theorem 5.** For any regular or semiregular bipartite graph  $G$ , then  $\gamma_{dng}(G) = \gamma_{dng}^e(G)$ .

**Proof:** Let  $G$  be a regular graph and  $V(G) = \{b_1, b_2, \dots, b_p\}$ . Then  $deg(b_i) = l$ ,  $1 \leq i \leq p$  and  $l$  is any natural number. Let  $D$  be a  $\gamma_{dng}$  - set of  $G$ . Let  $b_i \in V$  not in  $D$ . Since  $D$  is a dominating set, there exists a vertex  $b_j \in D$  such that  $b_i b_j$  is an edge of  $G$ . Also, degree of the vertices  $b_i$  and  $b_j$  are same. Therefore  $|deg(b_i) - deg(b_j)| = 0 \leq 1$ . Also,  $D$  is an equitable so that  $\gamma_{dng}^e(G) \leq |D| = \gamma_{dng}(G)$ . By theorem 3.5,  $\gamma_{dng}(G) \leq \gamma_{dng}^e(G)$ . Therefore  $\gamma_{dng}(G) = \gamma_{dng}^e(G)$ . Let  $G$  be a semiregular bipartite graph and  $A$  and  $B$  be the two bipartite sets of  $G$ . Then every vertex  $a \in A$  has the same degree say  $l$  and every vertex  $b \in B$  has the same degree say  $l+1$  where  $l$  is any natural number. Let  $D$  be a  $\gamma_{dng}$  - set of  $G$ . We can choose a vertex  $a \in V$  not in  $D$ . Then there exists  $b \in D$  such that  $ab$  is an edge of  $G$  and so  $|deg(b) - deg(a)| = |l + 1 - l| = 1$ . Therefore,  $D$  is itself an equitable and so  $\gamma_{dng}^e(G) \leq |D| = \gamma_{dng}(G)$ . But  $\gamma_{dng}(G) \leq \gamma_{dng}^e(G)$ . Hence detour global domination number and equitable detour global domination number are same.

**Definition** An equitable detour global dominating set  $D_1$  is called a minimal equitable detour global dominating set if there is no proper subset of  $D_1$  is a  $\gamma_{dng}^e$  - set.

**Definition** A vertex  $b \in V(G)$  such that  $|deg(b) - deg(a)| \geq 2$  for all  $a \in N(b)$ , then  $b$  is called an equitable isolate vertex.

**Theorem 6.** For any equitable detour global dominating set of a connected graph  $G$  is minimal if and only if  $\forall b \in D$ , satisfies one of the following conditions.

(i) Either a vertex  $b$  which is not adjacent to any vertex in  $D$  or there is no degree equitable vertex  $c$  which is adjacent to  $b \in D$ .

(ii) There exists a vertex  $c \in V - D$  such that  $c$  is adjacent with only one vertex  $b$  in  $D$  and a vertex  $c$  is degree equitable with  $b$ .





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**Proof:** Let  $D$  be a minimal equitable detour global dominating set of  $G$ . Suppose  $D$  does not satisfy (i) and (ii). Then there exists  $c \in D$  which is adjacent to some  $b \in D$  such that  $b$  is degree equitable with  $c$  and for all  $c \in V - D$ , either  $c$  is adjacent with more than one vertex in  $D$  or  $c$  is not degree equitable with  $b$  or both. Therefore  $D - \{b\}$  is a  $\gamma_{dng}^e$ -set which is a contradiction to minimality of  $D$ . Hence  $D$  satisfies (i) or (ii).

Conversely, assume that for every  $b \in D$  satisfies the condition (i) or (ii). Suppose  $D$  is not minimal. Then there exists an equitable detour global dominating set  $D_1 = D - \{b\}$  where  $b \in D$ . Therefore, there exists  $c \in D_1$  such that  $c$  equitably dominates  $b$ . That is  $bc \in E(G)$  and  $|\deg(b) - \deg(c)| \leq 1$ . Hence a vertex  $b$  does not satisfy (i) and so must satisfy (ii). Then there exists a vertex  $c \in V - D$  such that  $c$  is adjacent with only one vertex  $b$  in  $D$  and a vertex  $c$  is degree equitable with  $b$ . Since  $D_1$  is an equitable detour global dominating set, there exists  $d \in D_1$  such that  $d$  is adjacent with  $c$  and  $|\deg(d) - \deg(c)| \leq 1$ . Therefore, vertex  $c$  is adjacent with more than one vertex say  $b, d$ . Which is a contradiction. Hence  $D$  is a minimum equitable detour global dominating set.

**Theorem 7.** For any connected graph  $G$  of order  $p$ . Then,

(a) Every equitable detour global dominating set of  $G$  contains its pendant vertices, full vertices and equitable isolates

(b) If the set  $D$  contains only pendant vertices, full vertices and equitable isolates is a  $\gamma_{dng}^e$ -set of  $G$ . then  $D$  is the unique minimum  $\gamma_{dng}^e$ -set of  $G$ .

**Proof:** (a) Clearly every equitable detour global dominating set of  $G$  contains its pendant vertices, full vertices. Let  $D$  be an equitable detour global dominating set of a connected graph  $G$ . Let  $c \in V$  be an equitable isolate vertex of  $G$ . Suppose that  $c \notin D$ . Since  $D$  is a dominating set, there exists  $b \in D$  such that  $bc$  is an edge of  $G$ . Then  $|\deg(b) - \deg(c)| \geq 2$ . Hence  $D$  is not an equitable dominating set of  $G$ , It follows that  $D$  is not equitable detour global dominating set, which is a contradiction. Hence every equitable detour global dominating set of  $G$  contains all equitable isolate vertices.

(b) Follows directly from (a)

### Conflicts of Interest

The authors declare that they have no conflicts of interest.

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### REFERENCES

1. Chartrand, G., Haynes, T.W., Henning, M.A., and Zhang, P.(2004). Detour Domination in Graphs, *Ars Combinatoria*, 149-160.
2. Haynes, T.W., Hedetniemi, S.T. and Slater, P.J. (1998) *Fundamentals of Domination in Graphs*, Marcel Dekker, Inc., NY.
3. PunithaTharani.A, Ferdina. A, Detour Global Domination Number of Some Standard And Special Graphs, *International Journal of Advanced Science and Technology*, vol.29.pp:185-189.
4. Punitha Tharani. A, Ferdina. A, Detour Global Domination Number of Corona Product Graphs, Communicated to "Sarajevo Journal of Mathematics ANUBIH"
5. Punitha Tharani. A, Ferdina. A, Edge Detour Global Domination Number of a Graph, *MuktShabd Journal*, Vol IX, Issue IX, 137-143(2020).
6. Punitha Tharani. A, Ferdina. A, Connected edge detour global domination number of a graph, *Malaya Journal of Matematik*, Vol 8, Issue 4,1580 – 1582  
Sampathkumar.E, Equitable Dominations, *Kragujevac Journal of Mathematics*, 35(1) (2011) 191-197.





## A Fuzzy Model for Deteriorating Products using Preservation Technology

S.K. Indrajitsingha<sup>1\*</sup>, Padmini Raula<sup>2</sup>, P.N. Samanta<sup>3</sup> and U.K. Misra<sup>4</sup>

<sup>1</sup>Department of Mathematics, Saraswati Degree Vidya Mandir, Neelakantha Nagar, Berhampur-760 002, Odisha, India.

<sup>2</sup>Department of Mathematics, Chikiti Mahavidyalaya, Chikiti, Odisha, India.

<sup>3</sup>Department of Mathematics, Berhampur University, Berhampur-760 001, Odisha, India.

<sup>4</sup>Department of Mathematics, National Institute of Science and Technology, Golanthara-761 008, Odisha, India.

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### \*Address for Correspondence

**S.K. Indrajitsingha**

Department of Mathematics,  
Saraswati Degree Vidya Mandir,  
Neelakantha Nagar, Berhampur-760 002, Odisha, India.  
Email: susantamath86@gmail.com



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### ABSTRACT

Parameters like demand, holding cost, deterioration cost, lost sale cost, shortage cost etc play important role in inventory control. Further, as these parameters in realistic situations are uncertain in nature, in the present article a fuzzy economic order quantity (EOQ) inventory model with partial backlogging is developed. In order to slow down the deterioration rate preservation technology is applied. Parameters are taken as triangular fuzzy numbers. The model is developed to find fuzzy total average cost function which is defuzzified by using the method of graded mean integration representation (GMIR), A numerical example has been given in crisp as well as in fuzzy approach to demonstrate and validate the proposed model. Finally, the effects of different parameters have been rigorously studied through sensitivity analysis.

**Keywords:** Inventory, deterioration, triangular fuzzy number, Graded mean integration representation method.

**AMS Classification No:** 90B05

### INTRODUCTION

Inventory is a physical stock that a business-person keeps on mind in order to promote the smooth and efficient running in its affairs. During this world pandemic, developing countries like India, Pakistan, Sri Lanka and





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Bangladesh which strictly depend upon their own farming face critical situations on harvesting the food products. Supplying of deteriorating products is horrible and much expensive in such times, consequently there is a great economic loss. It is observed that small cities particular of East and South India like Odisha, West Bengal, Jharkhand, Tamil Nadu, Kerala, Karnataka vegetable items are sometimes available in the market with very low price and sometimes not at all due to lack of cold storages. At the harvesting time in Odisha, the vegetables like tomato, onion, cauliflower, the price is as low that the farmers sometimes left the product in the field itself for destroying. As such they face financial crises which urge them to suicide. However the prices of these vegetables are very high in other unseasonal months of the year. In accounting these difficulties, we developed a model of deteriorating products using preservation technology cost. Here we considered that, the above items can be collected from local farmers, stored them using preservation technology cost and sold them at their peak value. Finally, basic price of the product to be given to the farmers from which they came out from loans. In this model we use fuzzy technology since the economic parameters like holding cost, demand, deterioration cost, lost sale cost, shortage cost probably will have some little fluctuations or vague in nature. So in these practical situations, we treat these parameters as triangular fuzzy variables which will be more realistic. Hence fuzzy set theory is necessary for the formulation of such inventory models.

In 1965, Zadeh the first person introduced the concept of fuzziness. Later, Zadeh and Bellman (1970) applied fuzzy set theory on the development of an inventory model. Thus, this new development of fuzzy concept gives more accurate result as compared to classical probability theory. Many researchers attracted towards the development of inventory models applying fuzzy concept. It is found that fuzzy inventory models have been developed by Roy and Maiti (1998), Maiti and Maiti (2006), Rong, Mahapatra and Maiti (2008), Roy et al. (2009), Yadav et al. (2012), Kumar et al. (2013), Singh and Anuradha (2014), Raula et al. (2018), Indrajitsingha et al. (2018, 2019, 2020) and many others. Most of them consider the fuzzy variables as triangular fuzzy number and for defuzzification used graded mean integration representation method. Deterioration plays an important role while developing inventory models of seasonal products like potatoes, onions, food-grains etc. To slow down the deterioration, preservation technology cost may be used for the preservation of these above products, so that one can get more profit as compared to previous models. During the last decade of the present century, use of preservation technology cost while developing inventory model, in various times, had been considered by different scientist such as Hsu et al. (2010), Dye and Hsieh (2012), Dye (2013), Yang et al. (2015), Singh et al. (2016), Saha et al. (2017), pal et al. (2018), Mishra et al. (2018), Mishra et al. (2019), Das et al. (2020). In the present paper, we develop an inventory framework of deteriorating items applying preservation technologies to minimize the deterioration rate in which the preservation technology investment parameter and critical point are assumed to be the decision variables.

## Model Notation and Assumptions

### Notations

Throughout the paper we use the following notations:

$d(t)$	:	Demand rate.
$\alpha$	:	Initial demand rate.
$\beta$	:	Positive demand coefficient.
$m(\xi)$	:	Deterioration rate, after applying preservation technology.
$m_o$	:	The deterioration rate, before applying preservation technology.
$\delta$	:	Sensitive parameter of investment to the deterioration rate.
$h$	:	Inventory holding cost per unit per unit time.
$s$	:	Shortage cost per unit.
$l$	:	Lost sale cost per unit.
$c$	:	Deterioration cost per unit.
$t_1$	:	Time at which inventory level becomes zero.
$A$	:	Ordering cost per order.





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$Q$	:	Initial inventory level.
$T$	:	The duration of the replenishment cycle .
$k$	:	A constant, $0 < k \leq 1$ .
$\tilde{\alpha}$	:	Fuzzy initial demand rate.
$\tilde{\beta}$	:	Fuzzy positive demand coefficient.
$\tilde{h}$	:	Fuzzy inventory holding cost per unit per unit time.
$\tilde{c}$	:	Fuzzy deterioration cost per unit.
$\tilde{l}$	:	Fuzzy lost sale cost per unit.
$\tilde{s}$	:	Fuzzy shortage cost.
$TAC(\xi, t_1)$	:	Total average cost, $0 \leq t_1 \leq T$ .
$\overline{TAC}(\xi, t_1)$	:	Fuzzy total average cost.
$\overline{TAC}_g(\xi, t_1)$	:	Defuzzified value of $\overline{TAC}(\xi, t_1)$ applying GMIR method, $0 \leq t_1 \leq T$ .

**Assumptions**

The model is established by considering the following assumptions:

- i. Demand rate is assumed to be  $d(t) = \alpha + \beta I_1(t)$
- ii. The inventory system involves only one product.
- iii. The market demand depends only on the availability of stock.
- iv. The lead time is assumed to be negligible.
- v. Shortages are allowed with partially backlogging.
- vi. There is no cost to dispose or store deteriorated products.
- vii. It is assumed that  $m(\xi) = m_0 e^{-\delta \xi}$ .

**Mathematical Model Development**

Suppose the system is considered with initial stock  $Q$  units and at time  $t = t_1$ , the inventory level reduces to zero due to the combined effect of deterioration and demand. After that shortage occurs in the system it is backlogged partially. In the present article a single retailer’s inventory model is considered in which deteriorating rate is considered by using preservation technology. Using the above assumptions, the inventory level pattern is depicted in fig. 1.

Thus, the inventory system can be described by the following differential equations:

$$\frac{dI_1(t)}{dt} + m_0 e^{-\delta \xi} I_1(t) = -d(t), \quad 0 \leq t \leq t_1 \tag{1}$$

$$\frac{dI_2(t)}{dt} = -k\xi, \quad t_1 \leq t \leq T \tag{2}$$

with boundary conditions

$$I_1(0) = Q, \quad I_1(t_1) = 0 \tag{3}$$

The solutions of these equations are given by

$$I_1(t) = \frac{\alpha}{\beta + m_0 e^{-\delta \xi}} \left[ e^{(\beta + m_0 e^{-\delta \xi})(t_1 - t)} - 1 \right] \tag{4}$$

$$I_2(t) = k\xi(t_1 - t) \tag{5}$$

Using equations (5) and (6) the value of the parameters has been calculated:

Economic order quantity

$$Q = \frac{\alpha}{\beta + m_0 e^{-\delta \xi}} \left[ e^{(\beta + m_0 e^{-\delta \xi})t_1} - 1 \right] \tag{6}$$

Holding cost

$$HC = h \left\{ \frac{\alpha}{\beta} (\beta + m_0 e^{-\delta \xi}) \right\} \frac{t_1^2}{2} \tag{7}$$





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Deterioration cost

$$DC = c \left[ \frac{\alpha}{\beta} \left( e^{(\beta+m_o e^{-\delta\xi})} - 1 \right) - \left( \alpha t_1 + \alpha \beta \frac{t_1^2}{2} \right) \right] \tag{8}$$

Ordering cost

$$OC = A \tag{9}$$

Shortage cost

$$SC = ks\xi(T - t_1) \tag{10}$$

Lost sale cost

$$LSC = l\xi(1 - k)(T - t_1) \tag{11}$$

Preservation technology cost

$$PTC = \xi T \tag{12}$$

The average total cost  $TAC(\xi, t_1)$  for the period T

$$TAC(\xi, t_1) = \frac{1}{T} [HC + DC + OC + SC + LSC + PTC] \\ = \frac{1}{T} \left[ h \left\{ \frac{\alpha}{\beta} (\beta + m_o e^{-\delta\xi}) \right\} \frac{t_1^2}{2} + c \left\{ \frac{\alpha}{\beta} \left( e^{(\beta+m_o e^{-\delta\xi})} - 1 \right) - \left( \alpha t_1 + \alpha \beta \frac{t_1^2}{2} \right) \right\} + A \right] \\ + ks\xi(T - t_1) + l\xi(1 - k)(T - t_1) + \xi T \tag{13}$$

To minimize the average total cost function  $TAC(\xi, t_1)$  per unit time, the value of  $t_1$  and  $\xi$  that minimizes average cost can be obtained by solving the equations

$$\frac{\partial ATC(\alpha, t_1)}{\partial \xi} = 0 \quad \text{and} \quad \frac{\partial ATC(\alpha, t_1)}{\partial t_1} = 0 \tag{14}$$

satisfying the conditions

$$\frac{\partial^2 TAC(\xi, t_1)}{\partial \xi^2} > 0, \quad \frac{\partial^2 TAC(\xi, t_1)}{\partial t_1^2} > 0 \quad \text{and} \\ \left( \frac{\partial^2 TAC(\xi, t_1)}{\partial \xi^2} \right) \left( \frac{\partial^2 TAC(\xi, t_1)}{\partial t_1^2} \right) - \left( \frac{\partial^2 TAC(\xi, t_1)}{\partial \xi \partial t_1} \right)^2 > 0 \tag{15}$$

Equations (14) are equivalent to

$$\frac{1}{T} \left[ T - \frac{\alpha}{\beta} \left( m_o c e^{\beta - \delta\xi + m_o e^{-\delta\xi}} \right) - \frac{\alpha h \delta t_1^2 m_o e^{-\delta\xi}}{2\beta} \right] = 0$$

and

$$\frac{1}{T} \left[ \frac{\alpha}{\beta} h t_1 (\beta + m_o e^{-\delta\xi}) - \alpha c (1 + \beta t_1) - \alpha l (1 - k) - \alpha k s \right] = 0$$

**Fuzzy Model**

Due to uncertainty in nature, it is not easy to define all the system parameters exactly. Hence, it is assumed that some of the parameters namely  $\tilde{\alpha}, \tilde{\beta}, \tilde{h}, \tilde{c}, \tilde{l}$  may change within some limits. Suppose  $\tilde{\alpha} = (\alpha_1, \alpha_2, \alpha_3)$ ,  $\tilde{\beta} = (\beta_1, \beta_2, \beta_3)$ ,  $\tilde{h} = (h_1, h_2, h_3)$ ,  $\tilde{c} = (c_1, c_2, c_3)$ ,  $\tilde{s} = (s_1, s_2, s_3)$  and  $\tilde{l} = (l_1, l_2, l_3)$  are considered as triangular fuzzy numbers. Then the fuzzy total average cost  $\widetilde{TAC}(\xi, t_1)$  is given by

$$\widetilde{TAC}(\xi, t_1) = \frac{1}{T} \left[ \tilde{h} \left\{ \frac{\tilde{\alpha}}{\tilde{\beta}} (\tilde{\beta} + m_o e^{-\delta\xi}) \right\} \frac{t_1^2}{2} + \tilde{c} \left\{ \frac{\tilde{\alpha}}{\tilde{\beta}} \left( e^{(\tilde{\beta}+m_o e^{-\delta\xi})} - 1 \right) - \left( \tilde{\alpha} t_1 + \tilde{\alpha} \tilde{\beta} \frac{t_1^2}{2} \right) \right\} + A \right] \\ + k\tilde{s}\xi(T - t_1) + \tilde{l}\xi(1 - k)(T - t_1) + \xi T \tag{16}$$

Defuzzifying the fuzzy total average cost  $\widetilde{TAC}(\xi, t_1)$  by

Graded mean integration representation method:





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$$\overline{TAC}_g(\xi, t_1) = \frac{1}{6} [\overline{TAC}_{g1}(\xi, t_1) + 4\overline{TAC}_{g2}(\xi, t_1) + \overline{TAC}_{g3}(\xi, t_1)] \tag{17}$$

where

$$\overline{TAC}_{gi}(\xi, t_1) = \frac{1}{T} \left[ h_i \left\{ \frac{\alpha_i}{\beta_i} (\beta_i + m_o e^{-\delta \xi}) \right\} \frac{t_1^2}{2} + c_i \left\{ \frac{\alpha_i}{\beta_i} (e^{(\beta_i + m_o e^{-\delta \xi})} - 1) - \left( \alpha_i t_1 + \alpha_i \beta_i \frac{t_1^2}{2} \right) \right\} + A \right] + k s_i \xi (T - t_1) + l_i \xi (1 - k) (T - t_1) + \xi T$$

*for i = 1,2,3*

To minimize the total average cost function  $\overline{TAC}_g(\xi, t_1)$  per unit time, the optimal value of  $t_1$  and  $\xi$  can be obtained by solving the equations

$$\frac{\partial \overline{TAC}_g(\xi, t_1)}{\partial \xi} = 0 \quad \text{and} \quad \frac{\partial \overline{TAC}_g(\xi, t_1)}{\partial t_1} = 0 \tag{18}$$

satisfying the equations

$$\frac{\partial^2 \overline{TAC}_g(\xi, t_1)}{\partial \xi^2} > 0, \quad \frac{\partial^2 \overline{TAC}_g(\xi, t_1)}{\partial t_1^2} > 0 \quad \text{and}$$

$$\left( \frac{\partial^2 \overline{TAC}_g(\xi, t_1)}{\partial \xi^2} \right) \left( \frac{\partial^2 \overline{TAC}_g(\xi, t_1)}{\partial t_1^2} \right) - \left( \frac{\partial^2 \overline{TAC}_g(\xi, t_1)}{\partial \xi \partial t_1} \right)^2 > 0 \tag{19}$$

**Numerical example**

The reduced deterioration rate,  $m(\xi)$ , is a function of preservation technology cost  $\xi$  such that  $(\xi) = m_o e^{-\delta \xi}$ , where  $\delta$  is the simulation coefficient representing the percentage of increase in  $m(\xi)$  per increase in  $\xi$ , which means  $m(\xi)$  is an increasing function bounded above by  $m_o$ .

**Crisp Model**

Consider  $\alpha = 200$ ,  $\beta = 0.05$ ,  $h = 0.5$  ₹/unit,  $c = 4$  ₹/unit,  $l = 8$  ₹/unit,  $T = 50$  days,  $\delta = 0.1$ ,  $k = 0.07$ ,  $s = 6$  ₹/unit,  $c_0 = 100$ ,  $m_o = 0.02$  in appropriate units. The values of different parameters considered here are realistic, though these are not taken from any case study. By using Mathematica 11.1 software, we get the optimum value of critical point  $t_1 = 39.1118$  days, preservation technology cost  $\xi = ₹ 41.2491$  and economic order quantity  $Q = 24473.9$  with optimum average total cost  $TAC(\xi, t_1) = 704.032$ . However, the concavities of the average total cost function with respect to  $t_1$  and  $\alpha$  are shown in the fig. 2 below.

**Fuzzy Model**

Suppose  $\tilde{\alpha} = (180, 200, 220)$ ,  $\tilde{\beta} = (0.04, 0.05, 0.06)$ ,  $\tilde{h} = (0.45, 0.5, 0.55)$ ,  $\tilde{c} = (3, 4, 5)$ ,  $\tilde{s} = (4, 6, 8)$  and  $\tilde{l} = (5, 8, 10)$  which are considered as triangular fuzzy numbers and all other parameters are kept same as in crisp model. Then the fuzzy total average cost obtained by GMIR method is  $\overline{TAC}_g(\xi, t_1) = 652.737$  with the optimum value of critical point  $t_1 = 38.8321$  days, preservation technology cost  $\xi = ₹ 40.7493$  and economic order quantity  $Q = 31279$ .

**Sensitivity analysis**

It is very important in an inventory system for a retailer to know the behavior of the system parameters which impacted upon the average total cost function. Retailer should know when one will get minimum expenditure after increase or decrease of the related parameters. Henceforth to illustrate the applicability of the model and to locate some significant managerial ramifications in the vegetable farm houses, we study sensitivity analysis with the variation of different parameters.





### Managerial insights

1. From the numerical example, we see that fuzzy model gives best optimal solution as compared to crisp model.
2. From table. 1, we observe that when holding cost  $\tilde{h}$  of the vegetable increases keeping all other parameters unaltered, both the preservation technology cost  $\xi$  as well as critical point  $t_1$  decreases with the increase of the minimum expenditure  $\overline{TAC}(\xi, t_1)$ .
3. With increase in deterioration cost parameter  $\tilde{c}$  in table. 2, we see that the value of the preservation technology cost  $\xi$  as well as critical point  $t_1$  increases with decrease in the minimum expenditure  $\overline{TAC}(\xi, t_1)$ .
4. With increase demand parameter  $\tilde{a}$  in table. 3, we see that the value of the preservation technology cost  $\xi$  as well as a critical point  $t_1$  increases with increase in the minimum expenditure  $\overline{TAC}(\xi, t_1)$ .
5. With increase in demand parameter  $\tilde{\beta}$  in table. 4, we see that the value of the preservation technology cost  $\xi$  increases slowly and critical point  $t_1$  increases with decrease in the minimum expenditure  $\overline{TAC}(\xi, t_1)$ .
6. From table. 5, we observe that when lost sale cost  $\tilde{l}$  of the vegetable increases keeping all other parameters unchanged, the value of the preservation technology cost  $\xi$  as well as critical point  $t_1$  increases with increase in the minimum expenditure  $\overline{TAC}(\xi, t_1)$ .
7. From table. 6, we observe that when shortage cost  $\tilde{s}$  of the vegetable increases keeping all other parameters remains unchanged, the value of the preservation technology cost  $\xi$  increases as critical point  $t_1$  increases with the increases of the minimum expenditure  $\overline{TAC}(\xi, t_1)$ . However in this case the increase is very slow.

### CONCLUSION AND SCOPE

The public demand always plays a vital role in case of purchasing vegetables, fruits etc. Keeping this in mind we consider that the demand depends upon the stock level. Further, since deterioration conveys an important role for modeling on vegetables and fruit products, we apply preservation technology cost to reduce the rate of deterioration but shortage is considered which is partially backlogged. Due to uncertainty nature during the pandemic, the price of the product fluctuates depending upon different related parameters. On focusing the above assumptions we developed this model and found that it gives always profit in the sense of economic as well as humanity. In this conclusion we observe that fuzzy model give minimum expenditure as compared to classical crisp model. Moreover this model is highly essential at the time of lockdown and shutdown of the market during the pandemic COVID-19 in the countries like India and Pakistan. For future research, the proposed model can be extended in several ways. For example, one can extend by adding purchasing cost and selling price. Also one can extend this article by adding some other related parameters like credit policy, inflation etc.

### REFERENCES

1. Das, S.C., Zidan, A.M., Manna, A.K., Shaikh, A.A., Bhunia, A.K., 2020. An application of preservation technology in inventory control system with price dependent demand and partial backlogging, Alexandria Engineering Journal, 59(1), 1359-1365.
2. Dye, C.Y., 2013. The effect of preservation technology investment on a non-instantaneous deteriorating inventory model, Omega, 41(5), 872-880
3. Dye, C.Y., Hsieh, T.P., 2012. An optimal replenishment policy for deteriorating items with effective investment in preservation technology, European Journal of Operational Research, 218(1), 106-112.
4. Hsu, P.H., Wee, H.M., Teng, H.M., 2010. Preservation technology investment for deteriorating inventory, Int. J. of Production Economics, 124(1), 388-394.
5. Indrajitsingha, S.K., Samanta, P.N., Misra, U.K. (2018) 'A fuzzy inventory model for deteriorating items with stock dependent demand rate', *Int. J. Logistics Systems and Management*, ISSN(P) 1742-7967, ISSN(O) 1742-7975, Vol-30, Issue-4, pp. 538-555, DOI: 10.1504/IJLSM.2018.10014590





**Indrajitsingha et al.,**

6. Indrajitsingha, S.K., Samanta, P.N., Raju, L.K., Misra, U.K. (2019) 'Two-storage inventory model for deteriorating items with price dependent demand and shortages under partial backlogged in fuzzy approach', *LogForum, e ISSN 1734-459X, p ISSN 1895-2038, Vol-15, Issue-4, pp.487-499*, DOI: 10.17270/J.LOG.2019.344,
7. Indrajitsingha, S.K., Sahoo, A.K., Samanta, P.N., Misra, U.K., Raju, L.K. (2020) 'Fuzy EOQ inventory model for price-dependent-demand of deteriorating items', *Indian J. of Natural Sciences, ISSN 0976-0997, Vol-11, Issue-64, pp.28868-28877*.
8. Kumar, N., Singh, S.R. and Kumari, R. (2013) 'Two-warehouse inventory model of deteriorating items with three-component demand rate and time-proportional backlogging rate in fuzzy environment', *Int. J. of Industrial Engineering Computations*, Vol. 4, No. 4, pp. 587-598.
9. Maiti, M.M. and Maiti, M. (2006) 'Fuzzy inventory model with two-warehouse under possibility constraints', *Fuzzy Sets & Systems*, Vol. 157, No. 1, pp. 52-73.
10. Mishra, U., Aguilera, J.T., Tiwari, S., 2018. Retailer's joint ordering, pricing and preservation technology investment policies for a deteriorating item under permissible delay in payments, *Mathematical Problems in Engineering*, Article ID 696.2417, 1-14, <https://doi.org/10.1155/2018/696217>.
11. Mishra, U., Wu, J.Z., Tseng, M-L., 2019. Effects of a hybrid-price-stock-dependent demand on the optimal solutions of a deteriorating inventory system and trade credit policy on remanufactured product, *Journal of Cleaner Production*, 241(1), 1-15.
12. Pal, H., Bardhan, S., Giri, B.C., 2018. Optimal replenishment policy for non-instantaneously permissible items with preservation technology and random deterioration start time, *Int. J. of Management Science and Engineering Management*, 13:3, 188-199, DOI: 10.1080/17509653.2017.1372228
13. Raula, P., Indrajitsingha, S.K., Samanta, P.N., Raju, L.K., Misra, U.K. (2018) Inventory model of deteriorating items for supermarket: A fuzzy approach, *Asian J. of Mathematics and Computer Research, ISSN(P) 2395-4205, ISSN(O) 2395-4213, Vol-23, Issue-4, pp.231-239*
14. Rong, M., Mahapatra, N.K. and Maiti, M. (2008) 'A two-warehouse inventory model for a deteriorating item with partially/fully backlogged shortage and fuzzy lead time', *European J. of Operational Research*, Vol. 189, No. 1, pp. 59-75.
15. Roy, A., Maity, K., Kar, S. and Maiti, M. (2009) 'A production inventory model with remanufacturing for defective and usable items in fuzzy environment', *Computers & Industrial Engineering*, Vol. 56, No. 1, pp.87-96.
16. Roy, T.K. and Maiti, M. (1998) 'Multi-objective inventory models of deteriorating items with some constraints in a fuzzy environment', *Computers & Operations Research*, Vol. 25, No. 12, pp. 1085-1095.
17. Saha, S., Nielsen, I., Moon, I., 2017. Optimal retailer investments in green operations and preservation technology for deteriorating items, *J. of Cleaner Production*, 140(3), 1514-1527.
18. Singh, S.R. and Anuradha (2014) 'Two-storage inventory model for deteriorating items under fuzzy environment', *Proc. of the 3<sup>rd</sup> Int. Conf. on Soft Computing for Problem Solving*, Vol. 258, pp. 867-879.
19. Singh, S.R., Khurana, D., Tayal, S., 2016. An economic ordered quantity model for deteriorating products having stock dependent demand with trade credit period and preservation technology, *Uncertain Supply Chain Management*, 4(1), 29-42.
20. Yadav, D., Singh, S.R. and Kumari, R. (2012) 'Inventory model of deteriorating items with two-warehouse and stock dependent demand using genetic algorithm in fuzzy environment', *Yugoslav J. of Operations Research*, Vol. 22, No. 1, pp. 51-78.
21. Yang, C.T., Dye, C.Y., Ding, J.F., 2015. Optimal dynamic trade credit and preservation technology allocation for a deteriorating inventory model, *Computers & Industrial Engineering*, 87(1), 356-369.
22. Zadeh, L.A. (1965) 'Fuzzy Set', *Information Control*, Vol. 8, pp. 338-353.
23. Zadeh, L.A. and Bellman, R.E. (1970) 'Decision making in a fuzzy environment', *Management Science*, Vol. 17, pp. 140-164.





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**Table 1: Sensitivity analysis for parameter  $\tilde{h}$**

$\tilde{h}$	$t_1$	$\xi$	$\overline{TAC}(\xi, t_1)$
(0.45,0.46,0.47)	45.2161	43.2899	561.258
(0.46,0.47,0.48)	43.5243	42.7476	600.851
(0.47,0.48,0.49)	41.9505	42.2284	637.596
(0.48,0.49,0.5)	40.4792	41.7305	671.789

**Table 2: Sensitivity analysis for parameter  $\tilde{c}$**

$\tilde{c}$	$t_1$	$\xi$	$\overline{TAC}(\xi, t_1)$
(2,2.5,3)	27.2127	33.9792	1053.45
(2.5,3,3.5)	30.6542	35.9615	955.75
(3,3.5,4)	34.6222	38.7729	839.21

**Table 3: Sensitivity analysis for parameter  $\tilde{\alpha}$**

$\tilde{\alpha}$	$t_1$	$\xi$	$\overline{TAC}(\xi, t_1)$
(200,220,240)	39.1494	42.2074	770.048
(220,240,260)	39.1819	43.0961	835.996
(240,260,280)	39.2093	43.9121	901.873
(260,280,190)	39.2310	44.6090	962.209

**Table 4: Sensitivity analysis for parameter  $\tilde{\beta}$**

$\tilde{\beta}$	$t_1$	$\xi$	$\overline{TAC}(\xi, t_1)$
(0.040,0.042,0.044)	35.3088	40.9723	793.92
(0.042,0.044,0.046)	36.1923	40.9943	773.021
(0.044,0.046,0.048)	37.1224	41.0493	751.094

**Table 5: Sensitivity analysis for parameter  $\tilde{l}$**

$\tilde{l}$	$t_1$	$\xi$	$\overline{TAC}(\xi, t_1)$
(5,6,7)	32.8321	37.7649	597.734
(6,7,8)	35.9749	39.5724	655.751
(7,8,9)	39.1110	41.2279	702.089
(8,9,10)	42.2481	42.7554	736.772
(9,10,11)	45.3684	44.1735	759.815

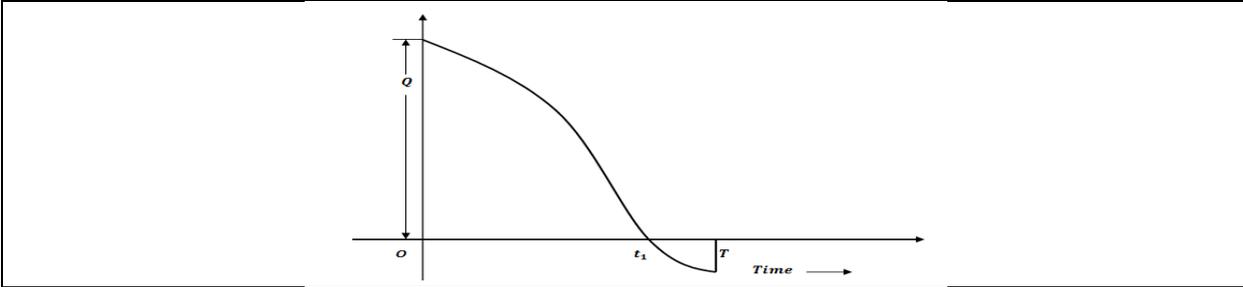
**Table 6: Sensitivity analysis for parameter  $\tilde{s}$**

$\tilde{s}$	$t_1$	$\xi$	$\overline{TAC}(\xi, t_1)$
(4,5,6)	38.876	41.1291	700.939
(5,6,7)	39.1118	41.2489	709.298
(6,7,8)	39.3476	41.368	736.772
(7,8,9)	39.5834	41.48618	759.757

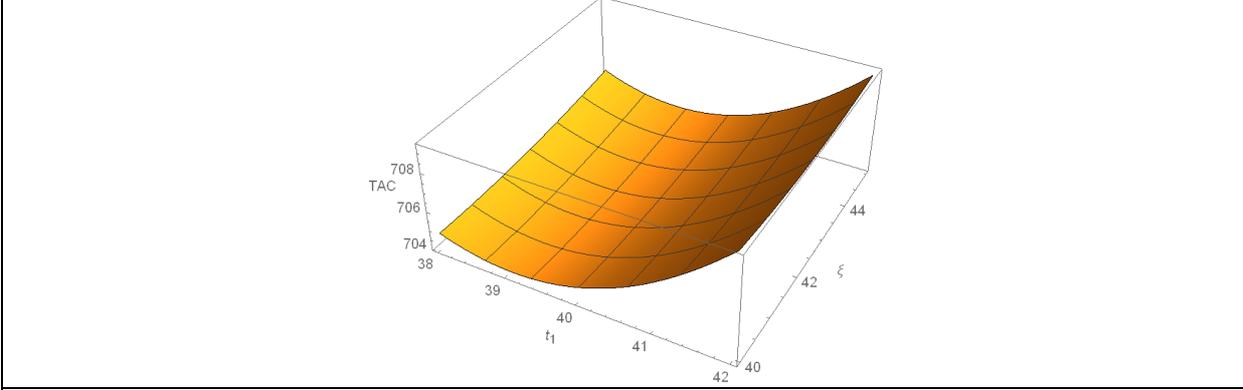




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**Fig. 1 The graphical representation of the inventory system: inventory versus time**



**Fig.2. Crisp Model**





## High Order OSCM for Two Point Neumann Boundary Value Problem

Santosh Kumar Bhal\* and Balaji padhy

Centurion University of Technology and Management, Odisha, India

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### \*Address for Correspondence

**Santosh Kumar Bhal**

Centurion University of Technology and Management,

Odisha, India

E.mail : Santosh.bhal@cutm.ac.in



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### ABSTRACT

In this paper, the Orthogonal spline collocation strategy (OSC) has been utilized for the two-point limit esteem issue. Hermite cubic premise capacities are utilized to estimate the solution for linear and boundary value problems. At last, we have played out a few mathematical investigations and utilizing framework refinement examination, we have registered the request for the intermingling of the mathematical strategy. Near to existing strategies, we show that the Orthogonal spline collocation techniques (OSC) provide the ideal request of assembly for the principal subordinate at the knots.

**Keywords:** Orthogonal cubic spline collocation methods (OCSCM), Two-point boundary value problem, Hermite Cubic basis functions, and Almost block diagonal (ABD) matrix.

## INTRODUCTION

There are numerous mathematical strategies are accessible to track down the surmised arrangements of nonlinear partial differential equations, throughout some undefined time frame symmetrical spline collocation techniques are overwhelmed in view of their prevalence analysed over B-splines as far as security and effectiveness. Contrasted with existing strategies OCSCM is considerably more predominant and proficient.

### Spaces of Piecewise Polynomial Functions

The decision of the subspace  $S_h$  is pivotal in the accomplishment of the Orthogonal Spline Collocation Methods. It is fundamental that  $S_h$  be picked so the collocation estimate can be registered productively and have great guess properties. The subspace  $S_h$  is generally picked to be a space of piecewise polynomial capacities. To characterize such spaces, let  $P_r$  signify the arrangement of polynomials of degree  $\leq r$ , let

$\pi : 0 = x_0 < x_1 < x_2 < \dots < x_N < x_{N+1} = 1$  denote a partition of  $I$ , and set

$$I_j = [x_{j-1}, x_j], \quad j = 1, \dots, N + 1,$$

$h_j = x_j - x_{j-1}$  and  $h = \max_j h_j$ . We define  $M_k^r(\pi) = \{v | v \in C^k(I), v|_{I_j} \in P_r, j = 1, \dots, N + 1\}$





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where  $C^k(I)$  denotes the space of functions which are  $k$  times continuously differentiable on  $I, 0 \leq k \leq r$ , and  $v|_{I_j}$  denote the restriction of the function  $v$  to the interval  $I_j$ . We denote by  $M_k^{r,0}(\pi)$  the space  $M_k^r(\pi) \cap \{v|v(0) = v(1) = 0\}$ . It is to see that  $M_k^r(\pi)$  and  $M_k^{r,0}(\pi)$  are linear spaces of dimensions  $N(r - k) + r + 1$  and  $N(r - k) + r - 1$ , respectively. We now give two examples of commonly used piecewise polynomial spaces.

**The Space of Piecewise Hermite Cubic Function  $M_1^3(\pi)$**

This space has dimension  $2N + 4$ . We express the basis function on  $[x_{i-1}, x_i]$ , with  $x_i = ih$ ,

$$vl_{i-1}(x) = -2 \left[ \frac{x_i - x}{h} \right]^3 + 3 \left[ \frac{x_i - x}{h} \right]^2, vl_i(x) = -2 \left[ \frac{x - x_{i-1}}{h} \right]^3 + 3 \left[ \frac{x - x_{i-1}}{h} \right]^2,$$

and

$$sl_{i-1} = -h \left\{ \left[ \frac{x_i - x}{h} \right]^3 - \left[ \frac{x_i - x}{h} \right]^2 \right\}, sl_i = h \left\{ \left[ \frac{x - x_{i-1}}{h} \right]^3 - \left[ \frac{x - x_{i-1}}{h} \right]^2 \right\}.$$

The function  $vl_i$  and  $sl_i$  are known as the value function and the slope function respectively, associated with the point  $x_i \in \pi$ . If we express the approximate solution as

$$u_h(x) = \sum_{j=0}^{N+1} \{ \alpha_j vl_j(x) + \beta_j sl_j(x) \},$$

then

$$\alpha_j = u_h(x_j), \beta_j = u'_h(x_j), j = 0, \dots, \dots, N + 1.$$

Since

$$vl_j(x_i) = \delta_{ij}, vl'_j(x_i) = 0 \text{ and } sl_j(x_i) = 0, sl'_j(x_i) = \delta_{ij}, i, j = 0, \dots, N + 1,$$

where  $\delta_{ij}$  is the kronecker delta function with  $\delta_{ij} = 1$ , if  $i = j$  and  $\delta_{ij} = 0$ , if  $i \neq j$ . A basis for  $M_1^{3,0}(\pi)$  is obtained by omitting the functions  $vl_0(x)$  and  $vl_{N+1}(x)$ .

**Hermite Cubic Bases**

Assume that, on each subinterval  $[x_{i-1}, x_i], i = 1, 2, \dots, N$ , the collocation approximation  $u_h \in M_1^3(\pi)$  has the form

$$u_h(x) = \sum_{i=0}^n \alpha_i vl_i + \beta_i sl_i,$$

so that

$$u'_h(x) = \sum_{i=0}^n \alpha_i vl'_i + \beta_i sl'_i,$$

and

$$u''_h(x) = \sum_{i=0}^n \alpha_i vl''_i + \beta_i sl''_i.$$

Note that the continuity is inbuilt into the approximate solution. With this representation of  $u_h$ , the collocation equations on the subinterval  $[x_{i-1}, x_i], i = 1, 2, \dots, N$ , are of the form

$$V_i y_i + W_i z_i = q_i,$$

$$\text{where } V_i \text{ and } W_i \text{ are } 2 \times 2 \text{ matrices, } y_i = (y_{i1}, y_{i2})^T, z_i = (z_{i1}, z_{i2})^T.$$

For more details of the above basis functions, we refer to [1,2,4,8,10]

The ubiquity of OCSCM is because of its pertinence. It is to comprehend and simple to apply to take care of an issue. It is a lot of better than B-splines as far as steadiness, proficiency and moulding of the subsequent lattice [6]. Contrasted with finite element methods (FEM), the computation of the coefficients of the mass and firmness networks, deciding the inexact arrangement is quick since no integrals should be assessed or approximated.

Another benefit of this strategy is that it methodically handles a wide range of limit conditions with interface conditions. The semi-discrete framework is settled by RADAU 5 programming library [8-9] which is reasonable for addressing differential mathematical conditions (DAEs)

Orthogonal spline collocation (OSC) technique for straight two-point boundary value problems (BVPs) for ordinary differential equations (ODEs) is portrayed and first investigated in the fundamental paper of deBoor and Swartz





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[3,5,9,11], which established the framework for the detailing and examination of OSC strategies for a wide assortment of issues and the improvement of programming bundles for their answer; see [7]. The constant time OSC technique and the discrete-time Crank–Nicolson OSC strategy for linear parabolic initial–boundary value problems (IBVPs) in one space variable and blueprint utilizations of OSC to different conditions in a single space variable, for example, Schrodinger-type conditions were first planned and examined in [8], and Cerutti and Parter [8] integrated the consequences of [10] and those of Douglas and Dupont. Following the methodology of Douglas and Dupont, Houstis [11] considered OSC for nonlinear second- order hyperbolic problems.

$$u_{xx} + u_x + u = 0, \quad x \in (0,1) \quad \text{--- (1)}$$

Subject to boundary condition

$$u'(0) = \alpha, \quad u'(1) = \beta$$

where  $\alpha$  and  $\beta$  are known constants. Once more, ideal request  $H^1$  and  $H^2$  blunder gauges are determined. For this situation, the OSC straight framework is somewhat confounded and is settled by an immediate strategy which depends on the capacitance grid procedure. On a uniform segment, the absolute expense of the capacitance framework strategy for figuring the OSC arrangement is  $O(N^3)$ . Since the capacitance framework is first shaped unequivocally and afterward addressed by Gauss disposal. Consequences of some mathematical trials are introduced which, specifically, show the fourth-request precision of the approximations and the super intermingling of the subordinate approximations at the nodal focuses.

Coming about framework (1), we utilize symmetrical cubic spline collocation strategy and rough the discrete arrangement utilizing monomial premise capacities or piecewise Hermite cubic basis functions. The semi-discrete framework is then coordinated in time utilizing RADAU 5 [8-9] time integrator.

### Numerical Example

Consider the problem  $u_{xx} + u_x + u = \sin(\pi x), \quad x \in (0,1)$

With boundary conditions  $u'(0) = u'(1) = 0$

Below, we have shown the error in tabular form.

## CONCLUSION

In this paper, we have applied an orthogonal cubic spline collocation methods (OCSCM) to one-dimensional fourth-order linear boundary value problems. One numerical experiment is performed and obtain the fourth order convergence at the grid points.

## REFERENCES

1. P. Danumjaya, A. K. Pani, Orthogonal cubic spline collocation method for extended Fisher-Kolmogorov equation, *J. Compt. Appl. Math.*, 174, 2005, 101-117.
2. P. Danumjaya, A. K. Nandakumaran, Orthogonal cubic spline collocation method for the Cahn-Hilliard equation, *J. Compt. Appl. Math.*, 182, 2006, 1316-1329.
3. P. Danumjaya, Orthogonal cubic spline collocation method for the Fisher-Kolmogorov equation, *Industrial Mathematics*, Narosa Pub., 2006, 87-96.
4. A.V. Manickam, K. M. Moudgalya, A. K. Pani, Second order splitting and orthogonal spline collocation methods for Kuramoto-Sivashinsky equation, *Compt. Math. Appl.*, 35, 1998, 5-25.
5. A.V. Manickam, A. K. Pani, S. K. Chung, A second order splitting combined with orthogonal cubic spline collocation method for the Rosenau equation, *Numer. Methods PDEs*, 14, 1998, 695-716.
6. U. Ascher, S. Pruess, R. D. Russel, On spline basis selection for solving differential equations, *SIAM J. Numer. Anal.*, 20, 1983, 121-142.





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7. G. Fairweather, D. Meade, A survey of spline collocation methods for the numerical solution of differential equations, J.C. Diaz (Ed.), Mathematics for Large Scale Computing, *Lecture Notes in Pure and Applied Mathematics, Marcel Dekker*, New York, 120, 1989, 297–341.
8. E. Hairer, C. Lubich, M. Roche, The Numerical Solution of Differential Algebraic Systems by Runge-Kutta Methods, in Lecture notes in Mathematics, *Springer*, New York, 1409, 1989.
9. E. Hairer, G. Wanner, Solving Ordinary Differential Equations II: Stiff and Differential Algebraic Problems, *Springer*, New York. 1991.
10. P. M. Prenter, Splines and Variational Methods, *John Wiley & Sons*, 1989.
11. C. de Boor, B. Swartz, Collocation at Gauss points, *SIAM J. Numer. Anal.*, 10, 1973, 582–606.

**Table : 1 Numerical example the error in tabular form.**

N	$L^\infty(u)$	order	$L^\infty(u')$	order
10	2.4097e-04		1.9093e-03	
20	3.6074e-06	4.0598e+00	1.9801e-04	3.9165e+00
30	1.3908e-06	4.5834e+00	3.0052e-05	4.0329e+00
40	4.5056e-07	3.7875e+00	1.8081e-05	3.8509e+00
50	1.8021e-07	3.9996e+00	6.9095e-06	4.0015e+00
60	9.0007e-08	3.9085e+00	3.9541e-06	3.9990e+00





## $\beta$ -glucan: An Immunostimulant in Aquaculture Studies

Adyasha Parida<sup>1</sup>, Preetha Bhadra<sup>2\*</sup> and Pradipta Banerjee<sup>3</sup>

<sup>1</sup>Department of Fisheries, Centurion University of Technology and Management, Paralakhemundi, Odisha, India.

<sup>2</sup>Department of Biotechnology, Centurion University of Technology and Management, Paralakhemundi, Odisha, India.

<sup>3</sup>Department of Biochemistry and Plant Physiology, Centurion University of Technology and Management, Paralakhemundi, Odisha, India.

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### \*Address for Correspondence

**Preetha Bhadra**

Department of Biotechnology,  
Centurion University of Technology and Management,  
Paralakhemundi, Odisha, India.  
E.Mail: preetha.bhadra@cutm.ac.in



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### ABSTRACT

The prevalence of a variety of diseases in farming systems is a significant impediment to the aquaculture sector's growth and long-term viability. To combat such illness outbreaks, preventative and control measures are now a top priority. Immunostimulants are thought to be a good way to improve the immunological state of farmed organisms.  $\beta$ -glucan, a homopolysaccharide comprising glucose molecules bonded by a glycoside bond, is one of the most potential immunostimulants utilised in aquaculture. Because of their potential to stimulate the immunological system,  $\beta$ -glucans are characterised as "biological response modifiers."The management of  $\beta$ -glucans via way of means of distinctive means, inclusive of injection, immersion, or dietary inclusion has been located to enhance a number of immunological responses, which includes infection tolerance and stress tolerance. In industrial aquaculture,  $\beta$ -glucan has been demonstrated to be effective at inducing immunity. It is also employed as a food substitute with immunomodulatory properties. This review focuses on the role of  $\beta$ -glucan as an immunostimulant, as well as effective doses and delivery methods, as well as the impact of -glucan in increasing fish and shellfish growth, survivability, and protection against infectious diseases.

**Key words:** immunostimulant, immunomodulator,  $\beta$ -glucans, aquaculture, prebiotic, adjuvant.





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## INTRODUCTION

Aquaculture is increasingly establishing itself as a vital food producing business. A few of the ongoing challenges is health monitoring, as the increasing density of fish creates intense stress, which often leads to the outbreak of fatal diseases or infections. The main obstacle to the development and viability of the aquaculture sectors is the presence of numerous infections in the farming practices. Immune stimulants are thought to be an effective means to improve the immunological state of cultivating organisms.  $\beta$ -glucan is a potential immunostimulant, amongst several immunostimulants utilised in aquaculture practices in which the glycoside bond connects homopolysaccharide comprising glucose molecules. It constitutes the main component of the cell walls of certain plants, bacteria, algae, mushroom and yeast.

A number of studies on fish have shown that  $\beta$ -glucan is a potent, effective and valuable immunostimulants, which can improve the immune state in the fish farming and prevent diseases. The usage of  $\beta$ -glucan in fishes have received a lot of interest from past few years. Various species, like as Atlantic salmon, have become the subject of several research (Paulsen et al. 2001), Rainbow trout (Jorgensen et al. 1993; Djordjevic et al. 2009), Snapper (Cook et al. 2001), African catfish (Yoshida et al. 1995) and Prawn (Hai and Fotedar 2009). These researches shows that  $\beta$ -glucan has an effect on development (Cook et al. 2003), survival, tolerance, and defence against pathogens (Welker et al. 2007), antibody production, immune related gene expression (Zhang et al. 2009), and as adjuvant (Kawakami et al. 1998) in a various fish species.

### Sources, Types and Structure of $\beta$ -Glucan

The term "glucan" refers to a category of polysaccharide polymers that are categorised as either  $\alpha$  or  $\beta$  linked depending on inter chain linkages.  $\beta$ -glucans from various origins have a variety of primary structures and conformations. The type of glycosidic bond and the degree of polymerization and branching are characteristics of the primary structure, where as conformation of the  $\beta$ -glucans also appears in the form of random helix, single or triple helix, and depends on the temperature, intermolecular pressure, primary structure and solvent (Wang et al.; 2017).

$\beta$ -glucans are may be found in a variety of structural forms in algae, bacteria, plants and fungi (Barsantiet al.; 2011). In natural,  $\beta$ -glucans are widely present in the cell walls of many plants (barley, wheat, rye and oat), brewer's yeast (*Saccharomyces* genus) and baker's, and *Echinaceae* species (Tokunaka et al.; 2000). Additional resources of  $\beta$ -glucan comprised of mushrooms of numerous kinds, such as Reishi (*Ganoderma lucidum*), Shiitake (*Lentinus edodes*), Maitake (*Grifola frondosa*) (Weis and Wasser; 1999), seaweed like *Laminaria* sp. (Teas ; 1983).

Their structure comprises of a primary chain of  $\beta$  (1,3) and  $\beta$  (1,4) - d - glucopyranosyl units, with non-repetitive but non-random sequences and side chains of variable lengths. Different  $\beta$ -glucan molecules have different immunomodulatory activity as well as molecules with identical shapes, solution conformations and molecular weights may have significant differences (Rodrigues et al.; 2020). The structure of  $\beta$ -glucan generated from different sources are differs.  $\beta$ -glucans of barley and oats are linear, having (1, 3) and (1, 4) linkages, respectively. Short  $\beta$ (1, 6)-linked branches from the  $\beta$ (1, 3) backbone are found in mushroom  $\beta$ -glucans. Yeast  $\beta$ -glucans has extra  $\beta$  (1, 3) region and  $\beta$  (1, 6) branches. The structural variations might lead to extraction problems and variances on the activity. Larger molecular weight glucans activate leukocytes, stimulating their cytotoxic, phagocytic, antimicrobial as well as the generation of reactive oxygen species (ROS) (Akramiene et al.; 2007). Low-molecular-weight glucan has little effect on cells, while very short-molecular-weight glucan is considered inactive. Insoluble (1, 3/1, 6) compounds have been shown to have higher biological activity than their soluble (1, 3/1, 4) equivalents. (Ooi et al.; 2000).

Fundamentally, the two kinds of  $\beta$ -glucan molecules based on glycosidic bonds found in them are  $\alpha$ -glucan (dextran with 1,6, starch with 1,6 and 1,4 glycosidic linkages) and  $\beta$ -glucan (zymosan with 1,3, cellulose with 1,4, laminarin with 1,3- and 1,6, lichenin with 1,3 and 1,4 glycosidic linkages).  $\beta$ -glucan has a higher capacity to trigger the immune response and function as biological response modifiers due to their complex structure (Miura et al.; 1996). Certain



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properties of  $\beta$ -glucan distinguish it from other immunostimulants, like its capacity to naturally activate the immune system without over-activating it (Chihara G.;1992), its capacity to decrease high cholesterol level (Braaten et al.;1994), and its capacity to lower sugar levels.

**EFFECTS OF  $\beta$ -GLUCANS**

Because  $\beta$ -glucans have the capacity to directly bind and activate macrophages and the other white blood cells (neutrophils and natural killer (NK) cells), they are responsible for the multiple activities such as protecting and stimulating the immunity and providing the best resistance to the potential health threats (Herre;2004). Bacteria, viruses, and pathogenic microbes have all been proven to be prevented by the  $\beta$ -glucans. All the immune activities are enhanced when  $\beta$ -glucan receptors are activated, including phagocytosis (the ability of engulfing foreign particles and cells); production of the cytokines such as IL-6, GM-CSF, IL-1, and interferons; and antigen processing. These are the cytokines which promote the production of the new white blood cell, providing the immunity to  $\beta$ -glucan binding receptor found in all the vertebrates from fishes to humans (Raa;1996).

 **$\beta$ -GLUCANON PROTEIN AND GENE EXPRESSION**

It has been revealed that  $\beta$ -glucan has a function in immune related gene and the protein expressions in a variety of fish species. Macrophages immunostimulated with the LPS and  $\beta$ -glucan from the Atlantic salmon and rainbow trout (*Oncorhynchus mykiss*) head kidney produced high levels of IL, but the induction of C3 transcription in trout was the same as in salmon. The C3 complement subtypes were shown to be differently regulated after in vivo stimulation with the LPS and  $\beta$ -glucans over 48 hours. These results corroborated previous results of C3 deficiency in spotted wolf fish (*Anarhichas minor*) macrophages (Løvoll et al.;2007). Likewise, the oral administration of  $\beta$ -1, 3-glucan for the 5 days promoted the synthesis of  $\alpha$ (TNF-  $\alpha$ ), (interleukin)1 $\beta$ ,  $\alpha$ (TNF-  $\alpha$ ), (interleukin)-12, and (interleukin)-10 cytokines like protein of the tumour necrosis in the fish's plasma. Two separate dosages (1.0 and 0.1  $\mu$ M) of  $\beta$ -glucan were applied to juvenile rainbow trout baths within 45 minutes, four times with a one-week period demonstrated improved expressions of genes with respect to the pro-inflammatory cytokine such as IL-6, TNF- $\alpha$  and IL-1 $\beta$  as well as the anti-inflammatory cytokine such as TGF- $\beta$  and IL-10, at the initial bathing. But, there was no discernible difference in IL-17A transcripts, a pro-inflammatory cytokine, when contrasted to the regulation. After the fourth bathing, expression of genes level in  $\beta$ -glucan in fish that has been medicated revealed no major variations in comparison to the control fish (Zhang et al.; 2009). For 37 days, those same were administered lentinan, found in mushroom  $\beta$ -glucan, and then given an inflammatory drug i.e. bacterial LPS. When lentinan was used instead of bacterial LPS in fish feed, the gene expression involved in acute inflammatory response to inflammatory agents decreased, but there was no difference in the genes expressions linked with the significant immunity responses (Djordjevic et al.;2009).

Grass carps are being treated with  $\beta$ -glucan for the 15 days before being injected with grass carp haemorrhage virus (GCHV) displayed enhanced MX gene expression levels during the early stage of GCHV infection (12 and 36 h) and survival rate was substantially improved to 60%. In comparison to the community that was not pretreated with  $\beta$ -glucan, erythrocytes had enhanced activity of catalase (CAT) and superoxide dismutase (SOD), as well as MX gene expressions, signalling that  $\beta$ -glucan improves antiviral responses (Kim et al.;2009). When fishes are given 5 mg/ml  $\beta$ -glucan, no major changes in TNF- $\alpha$  or IL1 $\beta$  expression were observed. However, there has been evidence of chemokine expressions and IFN- $\gamma$  regulation in the kidneys (Rodríguez et al.;2009).

 **$\beta$ -GLUCANAS IMMUNOSTIMULANT**

$\beta$ -glucan have been researched extensively as an immunostimulant in a variety of vertebrate and invertebrate species. *In vitro* experiment showed the increase of respiratory burst activities (RBA) of the head kidney macrophages was observed after dietary treatment of  $\beta$ -glucan to Atlantic salmon (*Salmo salar*). *In vivo* experiments revealed that confronting them with the infectious pathogen had a devastating impact on amoebic gill



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diseases (AGD), RBA, disease tolerances and serum lysozymes development (Bridle et al.; 2005). According to a variety of studies, in Chinook salmon,  $\beta$ -glucan supplementation in the diet enhances susceptibility to the infections for the brief periods of times (Niki et al.; 1993). The grade of the immunostimulant ( $\beta$ -glucan) provided is important in improving the immune response. Applications of the commercial  $\beta$ -glucan drugs have been shown to have an effect on the immunity responses. In the in vitro analysis, Pink snapper (*Pagrus auratus*) head kidney macrophage pre-incubated with the commercially used  $\beta$ -glucan (EcoActiva) and then introduced with either lipopolysaccharide (LPS) or phorbol myristate acetate (PMA) showed significantly increased respiratory burst activities (RBA) and superoxide anion comparing to macrophage activation with the Eco Activa alone (Cook et al.; 2001). The results of these research show that administration of the  $\beta$ -glucan, leading to improved the detection of LPS contained in the cell walls of gram negative fish microorganisms, lead to increased macrophage preventing ability against these pathogens. Another study found that giving Eco Activa to Pink snappers enhanced  $O_2$  radical in macrophages, particularly in the winter, but had no effect on classical or alternative pathway function, indicating that the best time to feed snapper in order to increase disease resistance is during the winter (Cook et al. ; 2003). In the European sea bass (*Dicentrarchus labrax*), the impact of consuming 1,3/1,6  $\beta$ -glucan on the innate and adaptive immune systems was examined (Bonaldo et al.; 2007).

The immunological responses of the Mediterranean mussels (*Mytilus galloprovincialis*) and clams (*Ruditapes decussatus*) fed  $\beta$ -glucans was strengthened. Nitric oxide synthesis were increased in the both species, but the mussel hemocytes released more phorbol 12-myristate 13-acetate (PMA) and free oxygen radicals. However, combininzymosan and a higher concentration of  $\beta$ -glucans reduced the mussel's respiratory burst activity. *Escherichia coli*, *Vibrio alginolyticus* and *Vibrio splendidus* were all inhibited by the hemolymph treated with the various dosages of  $\beta$ -glucans, but the antibacterial action was only modulated in clams (Marcosta et al. ; 2008).

**GLUCAN AS PREBIOTICS**

Prebiotics are a type of nondigestible diet components which help the consumer by promoting the development as well as activities of the beneficial microorganisms in the gastrointestinal (GI) tract (Gibson and Roberfroid; 1995). Prebiotics have the benefit over probiotics in that they are the natural food constituents, limiting regulatory control over the dietary intake. The use of most antibiotics is prohibited owing to the risk of developing antibiotic resistant microorganisms, the presence of residue in seafood, the destruction of bacterial communities in aquatic medium and inhibition of immune system of aquatic animals. Antibiotics have been demonstrated to impair development and feed efficiency in several animal species by destroying intestinal microorganisms and so limiting amino acid consumption by the consumer (Rawles et al.; 1997). These side effects, as well as the high expense of antibiotics to treat illnesses, prompted researchers to look for alternatives, one of which is the use of prebiotics. Fermacto prebiotic supplementation increased Common carp (*Cyprinus carpio*) fry development and feed efficiency ratio (FER). Although feeding prebiotic xylooligosaccharides to crucian carp (*Carassius auratus*) had no effect on growth or survival, there was a change in amylase and protease activities when compared to the controlled one (Xu et al.; 2008). In Pacific white shrimp (*Litopenaeus vannamei*), feeding short-chain fructooligosaccharide (scFO) aided the formation of the GI tract's microbial flora and improved immunity (Li et al.; 2007). Fructooligosaccharide and mannan oligosaccharide, on the other hand, had a beneficial impact on Atlantic salmon (*Salmo salar*) development (Grisdale-Helland et al.; 2008). In the stomach of coho, (*Rachycentron canadum*) larvae, mannan oligosaccharide improved salt tolerance and the growth of microvilli (Salze et al.; 2008).

**DOSAGE OF  $\beta$ -GLUCAN IN AQUACULTURE**

Immunostimulant doses provided to the marine species plays a vital role in the stimulation of immunological responses. To obtain the optimal effects, it is important to understand the proper immunostimulant dosages and administration routes. Rainbow trout (*Oncorhynchus mykiss*) being fed a diet that included 0.5 g  $\beta$ -1,3/1,6-glucan (Macrogard) /100 g pelleted feed everyday for a week before being immunised with anti-*Yersinia ruckeri* vaccine. As a result, the numbers of specific Ig concentrations in serum and antibody secreting cells (ASC) increased, improving the efficacy of the *Yersinia ruckeri* vaccine in the fishes. However, feeding them 0.1 percent  $\beta$ -glucan for the four



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weeks and then exposing them to approximately 2 hours of transportation stress increased their innate immunological responses (oxidative radicals and phagocytosis production), which helped to avoid the negative effects of stress as well as provided prevention against *Flexibacter columnaris*. The 0.1 percent feeding  $\beta$ -glucan population has the lowest stress resulted in an increase cortisol level in the plasma and hyperglycemia (Jeney et al.; 1997). Various injection with various doses of  $\beta$ -glucans 0, 5, 10, 15 mg/kg of the body weights applied to improve immunological responses and disease resistances against *Edwardsiella tarda* and *Aeromonas hydrophila* in the fingerlings of *Labeo rohita*. The outcomes revealed that injecting glucan 10 mg/kg body weight three times was recommended to improve immunological responses (Misra et al.; 2006). In rohu,  $\beta$ -glucan was recommended at a dose of 250 mg/kg diet for improving immune response, development, as well as resistance to opportunistic infections causing pathogens including *Edwardsiella tarda* and *Aeromonas hydrophila*.

Immunised and non-immunised Nile tilapia being given varied doses of  $\beta$ -glucan (0, 50, 100, 200 mg) with the diet for fourteen weeks. After 10 weeks, survival and growth were remain unaffected by  $\beta$ -glucan inoculation or their combinations. However, fish fed with 100 and 200 mg  $\beta$ -glucans resulted a partial drop in feed efficiency ratios (FER) in comparison to fish given 50 mg glucan. Immunized fishes appeared lower percentage mortalities (PM) and higher relative percentage survivals (RPS) against *S. iniae*; further, their combinations showed no effects over the percentage mortality as well as relative percentage survival (Whittington et al.; 2005).

**ROUTES OF  $\beta$ -GLUCAN ADMINISTRATION**

$\beta$ -glucan could be administered externally or internally in a various routes such injections intravenously, intraperitoneally, or subcutaneously (parenterally); or dietary administration; immersion; or as part of a cream (Volman et al.;2008).The efficacy of various routes of administration (intraperitoneally injection, washing, and dietary administration) has been investigated. In a model that contains *Cyprinus carpio*, fishes were administered  $\beta$ -glucan and LPS to explore survival and immunological responses after being confronted with *Aeromonas hydrophila*.In all  $\beta$ -glucan concentrations, intraperitoneal injection resulted in 100 percent relative percentage survival, whereas the oral administration of increased concentration (1 percent  $\beta$ -glucan + 0.25 percent LPS) resulted in high relative percentage survival, immersion did not result in a rise in the relative percentage of survival level (Selvaraj et al. 2006). In a model of the streptococcus produced by *Streptococcus iniae*, Red tilapia were administered a formalin killed vaccines via immersion, oral and injection immunisations.It is found that intraperitoneal injection was the most effective route in terms of potency, and that adding soluble  $\beta$ -glucan to the vaccine improved its effectiveness even further(Whittington et al.;2005)

**INJECTION**

In diverse species, the preventive role of  $\beta$ -glucans injection has been proven in a dose-dependent responses against a variety of infections(Misra et al.; 2006).Although intraperitoneal injections are an efficient way to administer  $\beta$ -glucans and activate the immunological responses, they may not be the most efficient. As an example, in Rainbow trout, a single dose of  $\beta$ -glucan administered intraperitoneally provided prevention against disease with the microsporidian, *Loma salmonae* similar to the degree of prevention caused by the three-week feeding experiment employing ten times greater  $\beta$ -glucan levels. Interestingly, the effects of a single intraperitoneal dose may be monitored prolonged period in vivo (Guselle et al.; 2010).This notion might clarify the uses of the  $\beta$ -glucan as a vaccination adjuvant. Glucans do not have to be a component of the vaccination in order for it to be effective, it could be a valuable source of dietary, experiment on Gibel carp(*Carassius auratus gibelio*) indicated that immersion effectiveness on inactivated herpesvirus vaccination was significantly improved(Yan et al.;2020).

Because of their immunomodulatory properties,  $\beta$ -glucan has been researched in great detail about the vaccination adjuvants as well as vaccine administration methods (Gregorio et al.;2009). Turbot (*Scophthalmus maximus L.*) were treated before, during, and after the administration of yeast  $\beta$ -glucan in some kind of a model with *Vibrio damsela* vaccine (Figueras et al.;1998).After bacterin injection,  $\beta$ -glucans were administered and the maximum activity was





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reported across all immunological measures. The results of this research show that the order in which  $\beta$ -glucan is administered is essential when using it as a vaccination adjuvant.

#### ORAL

Orally administered  $\beta$ -glucan make their way into the gastrointestinal system, where they must first be absorbed into the bloodstream even before to being carried to the bone marrow. Orally given approach is significantly slower than injectable approaches and is considered to have a less substantial effect. However, because  $\beta$ -glucans may easily be added to feed, this is generally a more practical strategy (Rice et al.;2005). For example, feeding salmon a rich diet in  $\beta$ -glucan and discovered that the rich diet in  $\beta$ -glucan increased adaptive and innate immunological responses by raising the transcription of essential cytokine genes including IL-12 and INF- $\gamma$ , which potentiated the immune response to vaccination (Rodríguez et al.;2016).

Pyro sequencing of the intestinal microbiota in Sea bass given  $\beta$ -glucan for four to eight weeks demonstrated a temporary change in the makeup of the autochthonous microbiota at the family taxonomic level (Carda et al.;2013). Majority of experiments conducted on bass species,  $\beta$ -glucan that are administered orally improved innate immunological measures including phagocytic ability, oxidative burst, lysozyme, and complement activity (Chang et al.;2013). However, it was also resistant to a variety of bacterial diseases, such as *Vibrio alginolyticus* and *Aeromonas hydrophila* (Boshy et al.;2010).

#### IMMERSION

The immersion treatment provides a potentially intriguing alternate use of  $\beta$ -glucan-induced immunostimulant. As an example, a 3-minute bath in  $\beta$ -glucan fertilised egg or gametes of Chum salmon (*Oncorhynchus keta*) were enough to give considerable prevention against *Saprolegnia* spp. infections (Kiseleva et al.;2013). To get the intended effects, it is essential to know the proper immunostimulant doses and routes of administration. Chinook salmon were bath challenged with *Aeromonas salmonicida* after being fed a diet containing 0%, 0.01 percent, 0.1 percent, and 1.0 percent  $\beta$ -glucans for the seven days. Significant protection against *A. salmonicida* was observed in diets including 1.0 percent and 0.1 percent VitaStim-Taito glucan, but not in any of the group bath treated salmon (Nikl et al.;1993). Immersion treatment of the  $\beta$ -glucan as skin or gill mucosal surface modulators might be a promising new research direction, particularly now is that tools for testing mucosal immunity are becoming more widely available (Salinas;2015).

#### NEW PROSPECT OF $\beta$ -GLUCAN IN AQUACULTURE

$\beta$ -glucans appears to have an impact on more than only the immune system. Using proteomic analysis, researchers discovered that Rainbow trout (*Oncorhynchus mykiss*) given a dietary supplementation for the sixty days had different expression of structural muscle proteins (Ghaedi et al.; 2016). Supplementing with glucans could have extra impacts on nutrition, such as amelioration of toxic effects produced by delta-methrin, in addition to direct impacts on improving different immunological response. Latest discoveries in the area of innate immune system suggest that the innate immune response might have long-term impacts, which perhaps could be addressed by the phenomena of trained immunity (Petit et al.; 2016). It is characterized by three parameters and

those are (i) It could be developed after a first primary infection or vaccination and then provide resistance from a secondary infection without the involvement of T and B cells, (ii) Despite being less specific than the adaptive immunological responses, it provides enhanced protection whenever the host is reinfected and innate cell types such as macrophages and neutrophils are involved, (iii) NK-cell plays a significant role in this process, that consists of enhanced pathogen detection as well as a higher inflammatory response (Netea et al.; 2011). Another mechanism of glucan activity is its impact on the neuroendocrine axis. The neuroendocrine and immunological systems are well known for their bidirectional communication. Through a variety of cytokines that serve as auto- or endocrine factors to influence pituitary growth, hormone release, cell proliferation and responses or feedback regulation of the



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hypothalamic-pituitary-adrenal axis. Nonetheless, there is currently a scarcity of knowledge on the effects of glucan in fish.

## CONCLUSION

The growing human population has resulted in escalating food demand, which must be met through novel approaches while keeping human health and environmental sustainability in mind. Despite these concerns,  $\beta$ -glucan has enormous potential for use in the fish farming, treatment of diseases and the development of fishery products via a biotechnological methods.  $\beta$ -glucan, a prophylactic and disease-management agent, confers immunity against a variety of fish pathogens. As per the reports, the  $\beta$ -glucan quality, doses, administering method and administration period, as well as treatment duration, all play a role in improving various Immunity, survivability and growth development parameters. There appears to be a need to concentrate on enhancing the quality and doses of  $\beta$ -glucan utilised in diverse species of fish. It's also worth investigating whether  $\beta$ -glucan may be obtained from other sources, including plants or seaweed. Another emerging area that requires further investigation is the application of the  $\beta$ -glucan as prebiotics in the aquaculture. More study on undiscovered activities of  $\beta$ -glucan should be done in order to utilize it as an effective immunostimulant in the aquaculture

## REFERENCES

1. Paulsen, S. M., Engstad, R. E., & Robertsen, B. (2001). Enhanced lysozyme production in Atlantic salmon (*SALMO salar* L.) MACROPHAGES treated with YEAST B-glucan and bacterial lipopolysaccharide. *Fish & Shellfish Immunology*, 11(1), 23-37. doi:10.1006/fsim.2000.0291
2. Jørgensen, J. B., Sharp, G. J., Secombes, C. J., & Robertsen, B. (1993). Effect of a yeast-cell-wall glucan on the bactericidal activity of rainbow trout macrophages. *Fish & Shellfish Immunology*, 3(4), 267-277. doi:10.1006/fsim.1993.1026
3. Cook, M. T., Hayball, P. J., Hutchinson, W., Nowak, B., & Hayball, J. D. (2001). The efficacy of a COMMERCIAL B-glucan preparation, EcoActiva™, on Stimulating respiratory burst activity of HEAD-KIDNEY MACROPHAGES from Pink snapper (*PAGRUS auratus*), sparidae. *Fish & Shellfish Immunology*, 11(8), 661-672. doi:10.1006/fsim.2001.0343
4. YOSHIDA, T., KRUGER, R., & INGLIS, V. (1995). Augmentation of non-specific protection in african CATFISH, *Clarias GARIEPINUS* (BURCHELL), by the Long-term oral administration of immunostimulants. *Journal of Fish Diseases*, 18(2), 195-198. doi:10.1111/j.1365-2761.1995.tb00278.x
5. Van Hai, N., & Fotedar, R. (2009). Comparison of the effects of the Prebiotics (bio-mos® AND  $\beta$ -1,3-D-glucan) and the customised PROBIOTICS (*pseudomonas synxantha* and *p. aeruginosa*) on the culture of juvenile western king Prawns (*penaeuslatisulcatus* Kishinouye, 1896). *Aquaculture*, 289(3-4), 310-316. doi:10.1016/j.aquaculture.2009.02.001
6. Cook, M. T., Hayball, P. J., Hutchinson, W., Nowak, B. F., & Hayball, J. D. (2003). Administration of a Commercial Immunostimulant preparation, EcoActiva™ as a Feed Supplement ENHANCES MACROPHAGE respiratory burst and the growth rate of snapper (*Pagrus AURATUS*, Sparidae (BLOCH and schneider)) in winter. *Fish & Shellfish Immunology*, 14(4), 333-345. doi:10.1006/fsim.2002.0441
7. Welker, T. L., Lim, C., Yildirim-Aksoy, M., Shelby, R., & Klesius, P. H. (2007). Immune response and resistance to stress And *edwardsiella ICTALURI* challenge in Channel Catfish, *ICTALURUS PUNCTATUS*, fed diets Containing COMMERCIAL WHOLE-CELL yeast or Yeast Subcomponents. *Journal of the World Aquaculture Society*, 38(1), 24-35. doi:10.1111/j.1749-7345.2006.00070.x
8. Zhang, Z., Swain, T., Bøgwald, J., Dalmo, R. A., & Kumari, J. (2009). Bath immunostimulation of rainbow trout (*oncorhynchus mykiss*) fry induces enhancement of inflammatory cytokine transcripts, while repeated bath induce no changes. *Fish & Shellfish Immunology*, 26(5), 677-684. doi:10.1016/j.fsi.2009.02.014
9. Kawakami, H., Shinohara, N., & Sakai, M. (1998). The non-specific IMMUNOSTIMULATION and ADJUVANT effects OF *VIBRIO Anguillarum* BACTERIN, M-glucan, chitin and FREUND'S COMPLETE





**Adyasha Parida et al.**

10. Adjuvant against Pasteurella Piscicida infection In yellowtail. *Fish Pathology*,33(4), 287-292. doi:10.3147/jsfp.33.287
11. Wang, Q., Sheng, X., Shi, A., Hu, H., Yang, Y., Liu, L., . . . Liu, H. (2017). B-Glucans: Relationships between modification, conformation and functional activities. *Molecules*,22(2), 257. doi:10.3390/molecules22020257
12. Barsanti, L., Passarelli, V., Evangelista, V., Frassanito, A. M., &Gualtieri, P. (2011). Chemistry, physico-chemistry and applications linked to biological activities Of  $\beta$ -glucans. *Natural Product Reports*,28(3), 457. doi:10.1039/c0np00018c
13. Tokunaka, K., Ohno, N., Adachi, Y., Tanaka, S., Tamura, H., &Yadomae, T. (2000). Immunopharmacological and IMMUNOTOXICOLOGICAL activities of a water-soluble (1 $\rightarrow$ 3)- $\beta$ -d-glucan, Csbg from CANDIDA SPP. *International Journal of Immunopharmacology*,22(5), 383-394. doi:10.1016/s0192-0561(99)00093-4
14. Teas, J. (1982). The dietary intake of laminaria, a brown seaweed, and breast cancer prevention. *Nutrition and Cancer*,4(3), 217-222. doi:10.1080/01635588209513760
15. Weis, A. L., & Wasser, S. P. (1999). Therapeutic effects of substances occurring in higher basidiomycetes mushrooms: A modern perspective. *Critical Reviews™ in Immunology*,19(1), 32. doi:10.1615/critrevimmunol.v19.i1.30
16. Rodrigues, M. V., Zanuzzo, F. S., Koch, J. F., De Oliveira, C. A., Sima, P., &Vetvicka, V. (2020). Development of fish immunity and the role of  $\beta$ -glucan in immune responses. *Molecules*,25(22), 5378. doi:10.3390/molecules25225378
17. Akramienė, D., Kondrotas, A., Didžiapetrienė, J., &Kėvelaitis, E. (2007). Effects of  $\beta$ -glucans on the immune system. *Medicina*,43(8), 597. doi:10.3390/medicina43080076
18. C.Ooi, V. E., & Liu, F. (2000). Immunomodulation and Anti-Cancer activity of Polysaccharide-Protein Complexes. *Current Medicinal Chemistry*,7(7), 715-729. doi:10.2174/0929867003374705
19. Miura, N. N., Ohno, N., Aketagawa, J., Tamura, H., Tanaka, S., &Yadomae, T. (1996). Blood clearance of (1  $\rightarrow$  3)- $\beta$ -d-glucan IN MRLlpr/lpr mice. *FEMS Immunology & Medical Microbiology*,13(1), 51-57. doi:10.1111/j.1574-695x.1996.tb00215.x
20. Chihara G. (1992). Recent progress in immunopharmacology and therapeutic effects of polysaccharides. *Developments in biological standardization*, 77, 191–197.
21. Braaten, J. T., Wood, P. J., Scott, F. W., Wolynetz, M. S., Lowe, M. K., Bradley-White, P., & Collins, M. W. (1994). Oat beta-glucan reduces blood cholesterol concentration in hypercholesterolemic subjects. *European journal of clinical nutrition*, 48(7), 465–474.
22. Herre, J. (2004). Dectin-1 and its role in the recognition of  $\beta$ -glucans by macrophages. *Molecular Immunology*,40(12), 869-876. doi:10.1016/j.molimm.2003.10.007
23. Raa, J. (1996). The use of immunostimulatory substances in fish and shellfish farming. *Reviews in Fisheries Science*,4(3), 229-288. doi:10.1080/10641269609388587
24. Løvoll, M., Fischer, U., Mathisen, G. S., Bøgwald, J., Ototake, M., &Dalmo, R. A. (2007). The c3 subtypes are differentially regulated after immunostimulation in rainbow trout, but head kidney macrophages do not contribute to c3 transcription. *Veterinary Immunology and Immunopathology*,117(3-4), 284-295. doi:10.1016/j.vetimm.2007.03.005
25. Djordjevic, B., Škugor, S., Jørgensen, S. M., Øverland, M., Mydland, L. T., &Krasnov, A. (2009). Modulation of splenic immune responses to bacterial lipopolysaccharide in rainbow trout (ONCORHYNCHUS mykiss) FED lentinan, a BETA-GLUCAN from Mushroom lentinulaedodes. *Fish & Shellfish Immunology*,26(2), 201-209. doi:10.1016/j.fsi.2008.10.012
26. Kim, Y., Ke, F., & Zhang, Q. (2009). Effect of  $\beta$ -glucan on activity of antioxidant enzymes and mx gene expression in virus infected grass carp. *Fish & Shellfish Immunology*,27(2), 336-340. doi:10.1016/j.fsi.2009.06.006
27. Rodríguez, I., Chamorro, R., Novoa, B., &Figueras, A. (2009). B-Glucan administration enhances disease resistance and some innate immune responses in zebrafish (danio rerio). *Fish & Shellfish Immunology*,27(2), 369-373. doi:10.1016/j.fsi.2009.02.007
28. Bridle, A. R., Carter, C. G., Morrison, R. N., & Nowak, B. F. (2005). The effect of Beta-glucan administration ON MACROPHAGE respiratory burst activity and atlantic salmon, Salmo salar L., challenged With AMOEBIC gill





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- disease - evidence of inherent resistance. *Journal of Fish Diseases*,28(6), 347-356. doi:10.1111/j.1365-2761.2005.00636.x
29. Niki, L., Evelyn, T., & Albright, L. (1993). Trials with an orally and immersion-administered ( $\beta$ -1,3 glucan as AN IMMUNOPROPHYLACTIC against AEROMONAS SALMONICIDA in juvenile Chinook SALMON ONCORHYNCHUS TSHAWYTSCHA. *Diseases of Aquatic Organisms*,17, 191-196. doi:10.3354/dao017191
  30. Cook, M. T., Hayball, P. J., Hutchinson, W., Nowak, B., & Hayball, J. D. (2001). The efficacy of a COMMERCIAL B-glucan preparation, EcoActiva™, on Stimulating respiratory burst activity of HEAD-KIDNEY MACROPHAGES from Pink snapper (PAGRUS auratus), sparidae. *Fish & Shellfish Immunology*,11(8), 661-672. doi:10.1006/fsim.2001.0343
  31. Cook, M. T., Hayball, P. J., Hutchinson, W., Nowak, B. F., & Hayball, J. D. (2003). Administration of a Commercial Immunostimulant preparation, EcoActiva™ as a Feed Supplement ENHANCES MACROPHAGE respiratory burst and the growth rate of snapper (Pagrus AURATUS, Sparidae (BLOCH and schneider)) in winter. *Fish & Shellfish Immunology*,14(4), 333-345. doi:10.1006/fsim.2002.0441
  32. Bonaldo, A., Thompson, K., Manfrin, A., Adams, A., Murano, E., Mordenti, A. L., & Gatta, P. P. (2007). The influence of dietary  $\beta$ -glucans on the adaptive and innate immune responses of European SEA bass (*Dicentrarchus labrax*) vaccinated AGAINST VIBRIOSIS. *Italian Journal of Animal Science*,6(2), 151-164. doi:10.4081/ijas.2007.151
  33. MARCOSTA, M., NOVOA, B., & FIGUERAS, A. (2008). Influence of B-GLUCANS on the immune responses of carpet shell clam (*Ruditapes decussatus*) and Mediterranean mussel (*MYTILUS GALLOPROVINCIALIS*). *Fish & Shellfish Immunology*,24(5), 498-505. doi:10.1016/j.fsi.2007.10.003
  34. Gibson, G. R., & Roberfroid, M. B. (1995). Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. *The Journal of Nutrition*,125(6), 1401-1412. doi:10.1093/jn/125.6.1401
  35. Rawles, S. D., Kocabas, A., Gatlin, D. M., Du, W. X., & Wei, C. I. (1997). Dietary supplementation of terramycin and romet-30 does not enhance growth of channel catfish but does influence tissue residues. *Journal of the World Aquaculture Society*,28(4), 392-401. doi:10.1111/j.1749-7345.1997.tb00286.x
  36. Xu, B., Wang, Y., Li, J., & Lin, Q. (2008). Effect of prebiotic xylooligosaccharides on growth performances and digestive enzyme activities of allogynogenetic crucian carp (*carassius auratus gibelio*). *Fish Physiology and Biochemistry*,35(3), 351-357. doi:10.1007/s10695-008-9248-8
  37. Li, P., Burr, G. S., Gatlin, D. M., Hume, M. E., Patnaik, S., Castille, F. L., & Lawrence, A. L. (2007). Dietary supplementation of Short-Chain fructooligosaccharides Influences GASTROINTESTINAL Microbiota composition and Immunity characteristics of Pacific White Shrimp, *Litopenaeus vannamei*, cultured in a RECIRCULATING SYSTEM. *The Journal of Nutrition*,137(12), 2763-2768. doi:10.1093/jn/137.12.2763
  38. Grisdale-Helland, B., Helland, S. J., & Gatlin, D. M. (2008). The effects of dietary supplementation with Mannan oligosaccharide, FRUCTOOLIGOSACCHARIDE Or Galactooligosaccharide on the growth and Feed utilization of Atlantic salmon (*Salmo salar*). *Aquaculture*,283(1-4), 163-167. doi:10.1016/j.aquaculture.2008.07.012
  39. Salze, G., McLean, E., Schwarz, M., & Craig, S. (2008). Dietary mannan oligosaccharide enhances salinity tolerance and gut development of larval coho. *Aquaculture*,274(1), 148-152. doi:10.1016/j.aquaculture.2007.11.008
  40. Jeney, G., Galeotti, M., Volpatti, D., Jeney, Z., & Anderson, D. P. (1997). Prevention of stress in rainbow trout (*oncorhynchus mykiss*) fed diets containing different doses of glucan. *Aquaculture*,154(1), 1-15. doi:10.1016/s0044-8486(97)00042-2
  41. Misra, C. K., Das, B. K., Mukherjee, S. C., & Pattnaik, P. (2006). Effect of MULTIPLE injections Of B-GLUCAN on NON-SPECIFIC immune response and disease resistance in labeorohita fingerlings. *Fish & Shellfish Immunology*,20(3), 305-319. doi:10.1016/j.fsi.2005.05.007
  42. Whittington, R., Lim, C., & Klesius, P. H. (2005). Effect of dietary  $\beta$ -glucan levels on the growth response and efficacy of *Streptococcus iniae* vaccine in Nile tilapia, *Oreochromis niloticus*. *Aquaculture*,248(1-4), 217-225. doi:10.1016/j.aquaculture.2005.04.013
  43. Volman, J. J., Ramakers, J. D., & Plat, J. (2008). Dietary modulation of immune function by  $\beta$ -glucans. *Physiology & Behavior*,94(2), 276-284. doi:10.1016/j.physbeh.2007.11.045





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44. Selvaraj, V., Sampath, K., & Sekar, V. (2006). Adjuvant and IMMUNOSTIMULATORY effects Of B-GLUCAN administration in combination With Lipopolysaccharide enhances survival and some immune parameters IN CARP challenged with aeromonashydrophila. *Veterinary Immunology and Immunopathology*, 114(1-2), 15-24. doi:10.1016/j.vetimm.2006.06.011
45. Guselle, N. J., Speare, D. J., Markham, R. J., & Patelakis, S. (2010). Efficacy of INTRAPERITONEALLY and orally administered provale, a Yeast B-(1,3)/(1,6)-D-GLUCAN product, in Inhibiting Xenoma formation by the MICROSPORIDIAN LOMA Salmonae on rainbow TROUT GILLS. *North American Journal of Aquaculture*, 72(1), 65-72. doi:10.1577/a09-017.1
46. Yan, Y., Huo, X., Ai, T., & Su, J. (2020). B-Glucan and anisodamine can enhance the Immersion immune efficacy of INACTIVATED cyprinid herpesvirus 2 vaccine In *Carassius auratus Gibelio*. *Fish & Shellfish Immunology*, 98, 285-295. doi:10.1016/j.fsi.2020.01.025
47. De Gregorio, E., D'Oro, U., & Wack, A. (2009). Immunology of TLR-INDEPENDENT vaccine adjuvants. *Current Opinion in Immunology*, 21(3), 339-345. doi:10.1016/j.coi.2009.05.003
48. Figueras, A., M. Santarém, M., & Novoa, B. (1998). Influence of the sequence of administration Of B-GLUCANS and a VIBRIO DAMSELA vaccine on the immune response Of turbot (*Scophthalmus maximus* L.). *Veterinary Immunology and Immunopathology*, 64(1), 59-68. doi:10.1016/s0165-2427(98)00114-7
49. Rice, P. J., Adams, E. L., Ozment-Skelton, T., Gonzalez, A. J., Goldman, M. P., Lockhart, B. E., . . . Williams, D. L. (2005). Oral delivery and gastrointestinal absorption of Soluble Glucans stimulate increased resistance to Infectious Challenge. *Journal of Pharmacology and Experimental Therapeutics*, 314 (3), 1079-1086. doi:10.1124/jpet.105.085415
50. Rodríguez, F. E., Valenzuela, B., Farías, A., Sandino, A. M., & Imarai, M. (2016). B-1,3/1,6-Glucan-Supplemented diets antagonize immune inhibitory effects of hypoxia and enhance the immune response to a model vaccine. *Fish & Shellfish Immunology*, 59, 36-45. doi:10.1016/j.fsi.2016.10.020
51. Carda-Diéguez, M., Mira, A., & Fouz, B. (2013). Pyrosequencing survey of intestinal MICROBIOTA diversity in cultured sea BASS (DICENTRARCHUS labrax) FED functional diets. *FEMS Microbiology Ecology*, 87(2), 451-459. doi:10.1111/1574-6941.12236
52. Chang, C., Huang, S., Chen, S., & Chen, S. (2013). Innate immune responses and efficacy of using MUSHROOM Beta-glucan Mixture (MBG) On orange-spotted grouper, *Epinephelus coioides*, aquaculture. *Fish & Shellfish Immunology*, 35(1), 115-125. doi:10.1016/j.fsi.2013.04.004
53. El-Boshy, M. E., El-Ashram, A. M., AbdelHamid, F. M., & Gadalla, H. A. (2010). Immunomodulatory effect of DIETARY Saccharomyces CEREVISIAE, B-glucan and LAMINARAN IN mercuric chloride treated Nile tilapia (*Oreochromis NILOTICUS*) and experimentally infected with aeromonashydrophila. *Fish & Shellfish Immunology*, 28(5-6), 802-808. doi:10.1016/j.fsi.2010.01.017
54. Kiseleva, M., Balabanova, L., Elyakova, L., Rasskazov, V., & Zvyagintseva, T. (2013). Effect of treatment of chum SALMON *Oncorhynchus KETA* (Walbaum) eggs with 1,3;1,6-β-D-glucans on their development and susceptibility to SAPROLEGNIA INFECTION. *Journal of Fish Diseases*, 37(1), 3-10. doi:10.1111/jfd.12043
55. Salinas, I. (2015). The mucosal immune system of teleost fish. *Biology*, 4(3), 525-539. doi:10.3390/biology4030525
56. Ghaedi, G., Keyvanshokoo, S., Mohammadi Azarm, H., & Akhlaghi, M. (2016). Proteomic analysis of muscle tissue from rainbow trout (*Oncorhynchus mykiss*) fed dietary β-glucan. *Iranian journal of veterinary research*, 17(3), 184–189.
57. Petit, J., & Wiegertjes, G. F. (2016). Long-lived effects of administering β-glucans: Indications for trained immunity in fish. *Developmental & Comparative Immunology*, 64, 93-102. doi:10.1016/j.dci.2016.03.003
58. Netea, M., Quintin, J., & Van der Meer, J. (2011). Trained immunity: A memory for innate host defense. *Cell Host & Microbe*, 9(5), 355-361. doi:10.1016/j.chom.2011.04.006.





## A Comparative Study on the Organoleptic Properties and Antioxidant Activity of The Hot Water Extracts of Dried Flowers of *Aegle marmelos*, A Sri Lankan Traditional Beverage

KDK Peshala Kumari<sup>1\*</sup>, Shiroma M Handunnetti<sup>2</sup>, Kamani Samarasinghe<sup>3</sup> and T Sugandhika Suresh<sup>4</sup>

<sup>1</sup>Department of Basic Sciences, Faculty of Allied Health Sciences, General Sir John Kotelawala Defence University, Sri Lanka.

<sup>2</sup>Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, Sri Lanka.

<sup>3</sup>The Medical Education Development and Research Centre, Faculty of Medicine, University of Colombo, Sri Lanka.

<sup>4</sup>Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka.

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### \*Address for Correspondence

**K.D.K P. Kumari**

Department of Basic Sciences,  
Faculty of Allied Health Sciences,  
General Sir John Kotelawala Defence University,  
Sri Lanka.  
E.Mail: krishanthi.peshala@kdu.ac.lk



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### ABSTRACT

Currently, consumption of herbal beverages gaining popularity among populations globally due to their therapeutic and energizing properties, as they are rich source of natural bioactive compounds. The hot water extract of dried flowers of *Aegle marmelos* is a popular traditional herbal beverage consumed by Sri Lankan folk in rural areas. The health benefits of the beverage are already established in preclinical as well as clinical studies. Therefore the current study was designed to investigate the organoleptic properties of the infusion and the anti-oxidant activity compared to tea, as an effort to popularize it among population. For evaluation of organoleptic properties, 30 healthy individuals were given infusions of dried flowers of *A. marmelos* prepared by different methods and black tea following a questionnaire. Trolox equivalent antioxidant capacity of test extract, black and green tea was measured based on the ABTS decolorization. Between 80-100 % of participants rated the flavor, color and fragrance of TEA and Traditionally prepared infusion of *A. marmelos*, under the categories of excellent, very good or good, but it was only 55 – 85 % for the infusions prepared instantly by powered dried flowers and instant sachets. The Trolox equivalent antioxidant capacity for test extract was 1143.8 ( $\mu\text{mol/g}$ ), while it was 1128.4 ( $\mu\text{mol/g}$ ) and 1115.8 ( $\mu\text{mol/g}$ ) for green tea and black tea respectively. Results of the current study revealed that the traditionally prepared extract has appreciable organoleptic properties and



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antioxidant activity which are comparable to that of black tea. But those properties of the extract prepared by the instant methods were not comparable to that of tea or traditionally prepared infusion. Therefore, if the traditionally prepared infusion of dried flowers of *A. marmelos* is promoted among the population, there is a high possibility to accept it as a herbal beverage which can be consumed routinely. It will be a great advantage for the consumers as the infusion has been proven to possess many bioactivities which leads to a healthy life.

**Key words:** organoleptic properties, *Aegle marmelos*, Tea, antioxidant activity

## INTRODUCTION

Herbal beverages are prepared either as a mixture of parts of different plants or as a single ingredient tea. Currently, consumption of herbal beverages is gaining popularity among people globally due to their therapeutic and energizing properties. The studies done previously reported that the herbal infusions or teas possess various bioactive properties and directly contribute to health benefits for consumers. Some herbal beverages relieve stress by inducing calm and relaxation of mind and providing cleansing properties of the body, while some contribute to boosting energy levels and refreshing the body. Some of them are involved in improving digestive problems, nourishing the nervous system, strengthening the immune system and supporting heart health [1]. Most of the population consumes these infusions as daily beverages in order to maintain a healthy life.

They are considered as a rich source of natural bioactive compounds, such as alkaloids, carotenoids, coumarins, flavonoids, polyacetylenes and terpenoids which are required for a healthy life [2]. Previous studies revealed that these bioactive compounds present in herbal infusions exert a diverse range of biological effects, such as antioxidant, anti-bacterial, anti-inflammatory, anti-allergic, anti-cancer, anti-ageing effects, etc [2]. The most popular herbal beverages are black tea, green tea, chamomile tea, ginger tea, lemon balm, peppermint and rosehip teas. It was reported that the routine consumption of foods and beverages rich in antioxidants is directly associated with reducing the rate of chronic oxidative stress-related diseases in different populations [3]. Therefore, the herbal infusions with potent anti-oxidant activity have gained the attention of consumers.

The antioxidant activity is the process of neutralization of oxidative stress created within cells and it delays or inhibits the oxidative damage to cellular components. During the process of oxidative stress, highly reactive free radicals are formed and they readily react with most of the cellular components which leads to cellular damage. This cellular damage is involved in the pathogenesis of many human diseases including neurodegenerative diseases such as Parkinson's and Alzheimer's diseases, diabetes, atherosclerosis, inflammatory diseases such as asthma as well as cancers. Reactive free radicals are also involved in the human aging process [4].

Antioxidants are the compounds which have the ability to trap free radicals which are created during the process of oxidative stress. Different types of natural chemical substances such as phenolic acids, flavonoids and polyphenols are active against oxidative stress. Plants are reported as rich sources of antioxidants and most of them were used to treat human diseases by traditional medicine practitioners from ancient times [5]. Therefore, the use of herbal infusions rich with natural antioxidants, as nutritional supplements, has already been established. During ancient times, before British colonialism in Sri Lanka (1796- 1948), the people consumed herbal infusions of different medicinal plants to refresh themselves during their laborious work. However, after the British introduced tea as a plantation to Sri Lanka, it became the major export product in Sri Lanka and it also became popular as the main refreshing beverage among the population. Thereafter, the health effects of traditional beverages have been ignored by the Sri Lankans and depend on tea as well as instant artificial beverages imported from western countries. However, currently there is a trend in revising the traditional knowledge on natural products due to the adverse effects exerted



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by artificial products. Therefore, our research group focused the attention on scientific validation of the health effects exerted by traditional herbal beverages, in order to popularize them among Sri Lankans. The hot water infusion of dried flowers of *Aegle marmelos* is a popular traditional herbal beverage consumed by Sri Lankan folk in rural areas to get refresh. Our research group carried out investigations on the health benefits of this beverage for the first time. The *in vitro* studies done on antimicrobial activity showed a promising antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* strains indicating a broad spectrum antibacterial activity in different extracts of flowers [6]. The preclinical study carried out in Wistar rats revealed that flower infusion of the plant exerts a potent hypoglycaemic, anti-inflammatory and ATPase Activity [7,8].

The clinical trials carried out observed that the continuous consumption of the concentrated infusion does not exert any toxic effect in healthy volunteers. It also showed that the consumption of the infusion enhances the ATPase activity in erythrocyte membranes significantly within a short period of time (unpublished data) in healthy volunteers, which may be responsible for the refreshing nature of the beverage. We observed that the dried flower extract exerts a significant hypoglycaemic activity, without showing any adverse effect in alloxan-induced diabetic Wistar rats as well as in diabetic patients on diet control<sup>7</sup>. The investigations done on the possible mechanisms of hypoglycaemic and inflammatory activity in Wistar rats revealed that the dried flower extract stimulates multiple biochemical pathways (unpublished data).

The results of our investigations proved that the herbal infusion prepared by dried flowers of *A. marmelos* is a beverage with many health effects. Therefore the current study was designed to investigate the organoleptic properties of the infusion and the anti-oxidant activity compared to tea, as an effort to popularize it among population.

## MATERIALS AND METHODS

### Ethical approval

Ethical approval was taken from the Ethics Review Committee of University of Sri Jayewardenepura, Sri Lanka. (Ref no: 600/2011). The human study was supervised by a family physician. The clinical trial was registered at Clinical Trials Registry, Sri Lanka (APPL/2012/027).

### Collection and authentication of plant material

Dried flowers were collected from different provinces of the country and pooled together. Required amounts were taken from this pool for the preparation of extracts. The plant material was authenticated by Prof. P. Tissera, Professor of Botany, Department of Botany, Faculty of Applied Sciences, University of Sri Jayewardenepura. A voucher specimen (USJP FMS 6/2010) has been deposited at the herbarium of the Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura.

### Preparation of the infusions

For the sensory evaluation, four infusions were prepared. The traditional infusion was prepared by boiling 5 g of dried flowers in 500 ml. Another infusion was prepared instantly by soaking 1g of finely ground dried flowers in 250ml of hot water for five minutes. The commercially available instant sachet was soaked in 250 ml of hot water for five minutes. The black tea infusion was prepared by soaking 1 g of unbranded black tea powder in 250 ml of hot water for five minutes. Three test extracts were prepared for *in vitro* antioxidant study. The hot water extract of dried flowers of *Aegle marmelos* (WEAM) was prepared by boiling 25 g of dried flowers in 500ml of water and reduced to 50 ml. The extracts of black tea and green tea were also prepared in the same method and all the extracts were freeze dried. The samples were stored in 4 °C until further studies.



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Healthy volunteers were selected by an open advertisement and the inclusion criteria for the selection of individuals for the study were, both males and females between the ages of 20-60, while the exclusion criteria were the pregnant or nursing mothers, patients with any disease, person on any medication.

**Sensory evaluation**

The organoleptic properties of the traditionally prepared extract (TPE) were compared with the extract prepared by the powdered dried flowers (PFE), commercially available instant sachets (CIS) and unbranded black tea (TEA). Thirty healthy individuals (16 females, 14 males) were consumed the different preparations on four separate days and a questionnaire was given to each participant for sensory evaluation. They were asked to provide scores for each factor such as fragrance, colour and taste as well as the overall score for each beverage they consumed. The responses were requested provide in categories according to a Likert scales which have a rank order as mentioned in the questionnaire.

**Assay for total antioxidant capacity**

*In vitro* total antioxidant activity of test extracts was evaluated according to the previously described method by Re et al., (1999) with a few modifications [9]. The total antioxidant capacity of the test extract was compared to that of the green and black tea. To prepare a free radical solution 7 mM ABTS [2, 2'-Azino-bis (3- ethylbenzothiazoline-6-sulphonic acid) di-ammonium salt] from Sigma Aldrich, USA was mixed with the oxidizing agent, potassium persuphate [(2.45 mM) (Himedia, India)] and allowed to react for 12-16 h at room temperature in the dark for free radical formation. This ABTS free radical (ABTS+) stock solution was dark green in colour and was diluted with PBS (pH 7.4) until the absorbance reached  $0.700 \pm 0.02$  at 734nm (Labomed UVD-3200).

Thereafter 0.5 g of the freeze dried samples of test extract, green tea and black tea were dissolved in 10 ml distilled water and 100  $\mu$ l of each sample was added to the ABTS free radical solution which had an initial absorbance of  $0.700 \pm 0.02$  at 734 nm. In each experiment the second absorbance was recorded at 6 min and the trolox equivalent antioxidant capacity (TEAC) for the same reduction in the absorbance was taken from the standard curve drawn for a dilution series of Trolox ( $2.1 \times 10^{-6}$ mol/L -  $12.5 \times 10^{-6}$ mol/L).

**Statistical analysis**

Results were expressed as mean  $\pm$  S.E.M. Significance was evaluated by two tailed t-test by SPSS version 20.0 and the level of statistical significance was set to  $P < 0.05$ .

**RESULTS AND DISCUSSION****Sensory evaluation**

Between 80-100% of participants rated theflavour, color and fragrance of TEA and TPE were under the category of excellent, very good or good, but it was 55 – 85 % for the PFE and CIS. For the CIS, none of participants categorized under excellent (Table 01 and 02). When considering the flavor of the beverages, majority of the participants stated excellent (43.3 %) for TEA, very good for TPE (40.0 %), while good for PFE (40.0 %) and CIS (43.3 %). Regarding the appearance and color, the majority said very good for TEA (43.3 %) and TPE (53.3 %), while good for good for PFE (53.3 %) and CIS (50.0 %). Considering the fragrance or aroma of the beverages, 30.0% of the participants stated excellent for TEA, while 33.3 % of them stated very good. Half of the participants (50.0 %), said very good for the fragrance of TPE, while majority of the participants scored good for PFE (40.0 %) and CIS (43.30%) (Table 01 and 02). The mean overall score for TEA, TPE, PFE and CIS were  $3.3 \pm 0.3$ ,  $3.1 \pm 0.5$ ,  $2.7 \pm 0.7$  and  $2.3 \pm 0.6$  respectively. There was no significant difference between the mean overall scores for TPE and TEA ( $P > 0.05$ ), while there was a significant difference between the mean overall score of TEA and that of PFE and CIS ( $P < 0.001$ ).



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The results of the sensory evaluation suggest that preference of the most of the participants on the taste, color as well as the fragrance of the traditionally prepared extract is almost similar to Tea. But their preference for the taste, fragrance and the color of the beverages prepared instantly with powder of dried flowers or using sachets, was less than Tea.

The chemical analysis done by GCMS showed that the flower extracts of *A. marmelos* extract contain several volatile compounds such as eugenol, 3-hexanol-4-methyl, Oxirane (2-methylbutyl) and Cyclopentanol-1-methyl<sup>[10]</sup>. These volatile compounds may contribute for the pleasant fragrance as well as the color and taste of the beverage. Tea is the most popular herbal beverage globally. The results of the current study suggest that the preference of the participants for the traditionally prepared infusion of dried flowers of *A. marmelos* is more or less similar to Tea. Therefore, if it is promoted among the population, there is a high possibility to accept it as a herbal beverage which can be consume routinely as tea. It will be a great advantage for the consumers as the infusion has been proved to possess many bioactivities which leads to a health life.

The result of the previous studies revealed that the infusion is rich in many bioactive compounds such as tannins, flavonoids, anthocyanin, terpenoids, phenols and coumarins [10]. These bioactive compounds have been reported to exert wide range of biological activities including anti-oxidant, anti-inflammatory, anti-septic, anti-allergic and anti-tumor, etc [11]. Therefore, routine consumption of the infusion of dried flowers of *A. marmelos* may leads to a healthier life of consumers.

The questionnaire also assessed the maintenance of coolness to the body, muscle relaxation, and relieving of stress in participants after consumption of each beverage. The participants note down what they felt within 30 minutes after consumption of each beverage in the questionnaire. Majority of participants (60 %) felt a feeling of cooling throughout 30 min after drinking TPE, while it was 46.7% for TEA. It was only 36.7% and 26.7% for the PFE and CIS respectively. Among participants 33.3% and 46.7% felt a feeling of muscle relaxation within 30 min of consumption of TEA and TPE, while it was only 26.7% and 23.3% for the PFE and CIS respectively. The percentage of participant who had a feeling of relieving of stress throughout 30 min after consumption of TEA and TPE was 43.3% and it was only 23.3% was for the PFE and 16.7% for the CIS (Figure 01).

The results of the current study scientifically proves that the traditionally prepared infusion is highly contribute for the coolness of the body after consumption. It also provide muscle relaxation and stress relieving in consumers. Our research group carried out a study on the effect of the hot water extract of the dried flowers of *A. marmelos* on Na<sup>+</sup>/K<sup>+</sup>ATPase activity in erythrocyte membrane of health humans. We observed Na<sup>+</sup>/K<sup>+</sup> ATPase activity in erythrocyte membrane was significantly enhanced after consumption of the hot water extract of the dried flowers of *A. marmelos* (unpublished data). The test result reflects that the test extract is involved in stimulating Na<sup>+</sup>/K<sup>+</sup> ATPase in the body. This phenomena may be mediated for the refreshing properties and the muscle relaxation of the body after consumption of the beverage. The refreshing nature of the tea and traditionally prepared infusion is more or less similar but the results suggest that the infusion which prepared instantly did not meet the quality of traditionally prepared infusion. Therefore in order to obtain the best quality in fragrance, color, and taste as well as the refreshing properties of the beverage it should be prepared by the traditional method.

#### **Total antioxidant capacity**

The trolox equivalent antioxidant capacity was given as μmol trolox/g of the extract. The Trolox equivalent antioxidant capacity (TEAC) for test extract was 1143.8 (μmol/g), while it was 1128.4 (μmol/g) and 1115.8 (μmol/g) for green tea and black tea respectively. Trolox equivalent antioxidant capacity assay measures total antioxidant capacity based on the ABTS decolorization. The antioxidant capacity of the test sample is given compared to the standard, Trolox. There is no significant difference (P > 0.05) between the total antioxidant capacity of the test extract and that of the black and green tea. This result reflects the antioxidant capacity is more or less similar to the activity of the black and green tea. Thus, the health effects exerted though the antioxidant capacity of the test infusion may



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also similar to that of the consumption of teas. The previous studies done on black and green teas revealed, that they exert the effect against oxidative stress-related chronic diseases have been attributed in part to free-radical scavenging and metal-chelating activity<sup>[12]</sup>. The ingestion studies done on healthy volunteers observed that consumption of tea routinely has a clear impact on the enhancement of plasma total antioxidant activity of consumers [13]. A study done by Kyle *et al.* (2007) reported that the consumption of black tea was associated with significant increases in plasma total antioxidant activity as well as the plasma concentrations of total phenols, catechins, quercetin and kaempferol within 80 minutes of ingestion<sup>[14]</sup>.

Thus, as the hot water extract of dried flowers of *A. marmelos* exerts *in vitro* total antioxidant capacity to a similar extent of the black and green tea, it may also exert similar effect in consumers' body. Therefore, further studies are recommended to study the effect of this infusion on plasma total antioxidant activity as well as the concentrations of bioactive compound in healthy people, in order to confirm the health benefits.

**CONCLUSIONS**

The hot water extract of the dried flowers of *A. marmelos* is one of most popular traditional beverage used by Sri Lankan rural population to get refreshed during their laborious work. Results of the current study revealed that the traditionally prepared extract has appreciable organoleptic properties and antioxidant activity which are comparable to that of black tea. But those properties of the extract prepared by the instant methods were not comparable to those properties of tea or traditionally prepared extract. Therefore, if it is promoted among the population, there is a high possibility to accept it as a herbal beverage which can be consumed routinely as tea. It will be a great advantage for the consumers as the infusion has been proved to possess many bioactivities which leads to a healthy life.

**Conflicts of Interest**

All the authors declared that they have no conflict of interest regarding the content of the paper.

**REFERENCES**

1. Ravikumar C. Review on Herbal Teas. J Pharm Sci & Res 2014; 6(5):236-238.
2. Chandrasekara A, Shahidi F. Herbal beverages: bioactive compounds and their role in disease risk reduction – a review. J Tradit Complement Med 2018;8(4):451-458.
3. Anna RA, Franciszek Ś, Bożena W. Antioxidant properties of tea and herbal infusions – a short report. Pol J Food NutrSci 2010;60(1):33-35.
4. Mahdi-Pour B, Subramanian LJ, Lachimanan YL, Yeng C, Sreenivasan S. Antioxidant activity of methanol extracts of different parts of *Lantana camara*. Asian Pac J Trop Biomed 2012;2(12): 960-965.
5. Wu YY, Li W, Xu Y, Jin EH, Tu YY. Evaluation of the antioxidant effects of four main theaflavin derivatives through chemiluminescence and DNA damage analyses. UnivSci B 2011;12:744-751.
6. Hasanthi KB, Gamage RNN, Kumari KDKP. Anti-microbial activity of different solvent extracts of dried flowers of *Aegle marmelos*. Int J PharmacognPhytochem 2018;7:1827-1830.
7. Kumari KDKP, Samarasinghe K, Suresh TS. Hypoglycaemic effect of the traditional drink, the water extract of dried flowers of *Aegle marmelos* (L.) Correa (bael fruit) in Wistar rats. Indian J Tradit Know 2013;12(3):384-389.
8. Kumari KDKP, Weerakoon TCS, Handunnetti SM, Samarasinghe K, Suresh TS. Anti-inflammatory activity of dried flower extracts of *Aegle marmelos* in Wistar rats. J Ethnopharmacol 2014;151:1202-1208.
9. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 1999;26 (9-10):1231-1237.





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10. Kumari KDKP, Handunnetti SM, Samarasinghe K, Suresh TS. Preliminary Studies on Activity Guided Fractionation of the Ethanolic Extract of Dried Flowers of *Aegle marmelos*. Int J Green Herb Chem 2016;5(2):122-138.
11. Pari L, Rajarajeswari N. Efficacy of coumarin on hepatic key enzymes of glucose metabolism in chemical induced type 2 diabetic rats. Chem Biol Interact 2009;181(3):292-296.
12. Seeram NP, Aviram M, Zhang Y. Comparison of antioxidant potency of commonly consumed polyphenol-rich beverages in the United States. J Agric Food Chem 2008;56:1415–1422.
13. Williamson G, Manach C. Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies. Am J Clin Nutr 2005;81(1):243S–55S.
14. Kyle JA, Morrice PC, McNeill G. Effects of infusion time and addition of milk on content and absorption of polyphenols from black tea. J Agric Food Chem 2007;55(12):4889–4894.
15. Devi KV, Pai RS. Antiretrovirals: Need for an Effective Drug Delivery. Indian J Pharm Sci 2006;68:1-6.

**Table 1: The percentages of scores obtained for Tea and traditionally prepared extract (TPE)**

	Tea				Traditionally prepared extract			
	Flavor	Color	Fragrance	Overall score	Flavor	Color	Fragrance	Overall score
Bad	0	0	0	<b>0</b>	0	0	0	<b>0</b>
Not Satisfactory	0	0	3.3	<b>0</b>	0	0	3.3	<b>0</b>
fair	3.3	0	3.3	<b>0</b>	6.7	6.7	10	<b>6.7</b>
Good	26.7	20	30	<b>13.3</b>	26.7	20	23.3	<b>16.7</b>
Very good	26.7	43.3	33.3	<b>53.3</b>	40	53.3	50	<b>56.7</b>
Excellent	43.3	36.7	30	<b>33.3</b>	26.7	20	13.3	<b>20</b>

Between 80-100 % of participants rated the flavour, color and fragrance of Tea and TPE were under the category of excellent, very good or good.

**Table 2: The percentages of scores obtained for drink prepared instantly using powered flower (PFE) and commercially available sachets (CIS).**

	Drink prepared using powered flower				Drink prepared using commercially available sachets			
	Flavor	Color	Fragrance	Overall score	Flavor	Color	Fragrance	Overall score
Bad	0	0	0	<b>0</b>	3.3	0	0	<b>0</b>
Not Satisfactory	3.3	0	6.7	<b>3.3</b>	13.3	6.7	20	<b>10</b>
fair	40	16.7	26.7	<b>26.7</b>	16.7	10	10	<b>6.7</b>
Good	40	53.3	40	<b>53.3</b>	43.3	50	43.3	<b>50</b>
Very good	10	16.7	16.7	<b>13.3</b>	23.3	33.3	26.7	<b>33.3</b>
Excellent	6.7	13.3	10	<b>3.3</b>	0	0	0	<b>0</b>

Between 55 – 85 % of participants rated the flavour, color and fragrance of PFE and CIS were under the category of excellent, very good or good. For the CIS, none of participants categorized under excellent.





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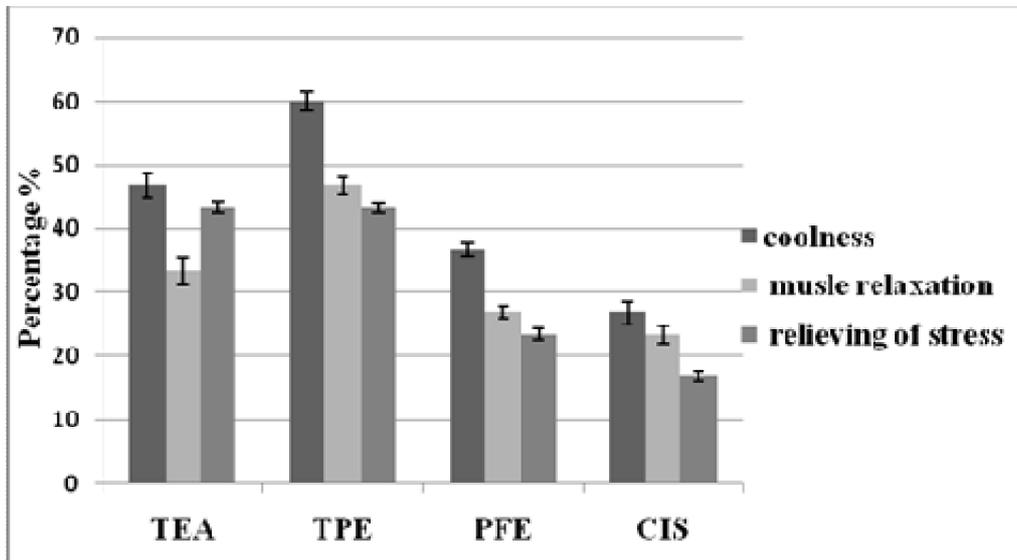


Figure 01: Percentage of the participants felt a feeling of coolness, muscle relaxation and relieving of stress throughout 30 min after drinking traditionally prepared extract (TPE), powdered dried flowers (PFE), commercially available instant sachets (CIS) and unbranded black tea (TEA). The TEA and TPE showed a higher percentage for feeling of coolness, muscle relaxation and relieving of stress throughout 30 min after drinking, while the percentage for PFE and CIS were low.





## Process of Development and Nutritional Value of Gluten Free Burfi with Supplementation of Buckwheat and Muskmelon Seed Flour: An Indian Sweet Delicacy

Akanksha Yadav<sup>1\*</sup>, Bharti Tanwar<sup>2</sup> and Upsana Sarma<sup>2</sup>

<sup>1</sup>Assistant Professor, Department of Nutrition and Dietetics, Faculty of Allied Health Sciences, Shree Guru Gobind Singh Tricentenary University, Gurgaon, Haryana. India.

<sup>2</sup>Department of Nutrition and Dietetics, Faculty of Allied Health Sciences, Shree Guru Gobind Singh Tricentenary University, Gurgaon, Haryana. India

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### \*Address for Correspondence

#### Akanksha Yadav

Assistant Professor,  
Department of Nutrition and Dietetics,  
Faculty of Allied Health Sciences,  
Shree Guru Gobind Singh Tricentenary University,  
Gurgaon, Haryana. India.  
E.Mail: akankshayadav5@gmail.com



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### ABSTRACT

Buckwheat flour and muskmelon seed flour are better supplier of nutrients and are rich in protein, carbohydrate, fiber, vitamins, minerals and antioxidants therefore these are used to develop the gluten free food product. This study aimed to explore the nutrient content of gluten free burfi as a functional food and to evaluate sensory attributes of the product. The gluten free burfi were prepared by using composite blends of buckwheat flour (BWF) and muskmelon seed flour (MSF) in different combination such as 50:0 (BWF) sample code T0, 40:20 (MSF 20%) T1, 30:20 (MSF 20%) T2, 20:30 (MSF 30%) T3, and 10:40 (MSF 40%) T4 respectively. The experiment was replicated five times and Data obtained were then analyzed statistically using ANOVA at 5% level of significance by SPSS software version 0.25. Gluten free burfi were determined according to the AOAC methods, and their sensory attributes by using 9-point Hedonic Scale. Sensory properties of the samples revealed that, T4 had highly acceptable to control sample. A proximate test showed that sample T4 gluten free burfi contained protein (18.3%), carbohydrate (22.3%), and fat (36.9%), iron (7.8 mg) and energy value (493.7 Kcal/100g). The gluten free burfi gained acceptability in terms of sensory attributes hence was concluded to be a potential nutritious product for celiac disease with nutrient deficiency as well as for all vulnerable age group. Overall, the study leads to a development of a protocol and an innovative, nutritious sweet delicious product (gluten free burfi) with a view to popularize among other parts of the globe.

**Keywords:** Nutritional value, Gluten free, Buckwheat flour, Muskmelon seed flour, Sensory properties, Functional ingredients.



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## INTRODUCTION

Food Based approaches are recognized as an essential part of an urgently needed more comprehensive strategy for improving nutrition by increasing the availability, health promoting functional ingredients into traditional recipes and consumption to combat nutrient deficiencies. In recent years, there has been increasing interest in the benefits of buckwheat as a raw base food material owing to its “re-discovered” nutritional quality for healthy life style. Buckwheat is a pseudo-cereal from the family of Polygonaceae used as a functional ingredient in the form of flour [1]. Buckwheat is rich in starch, lipids micronutrients as well as nutritionally valuable protein with a well-balanced amino acid profile. Besides high-quality proteins, buckwheat flour contains bioactive components with healing benefits: flavonoids and flavones, phytosterol and thiamin-binding proteins. These are a source of dietary fiber, antioxidants, along with other health-promoting components such as phenolic compounds and sterols, which have attracted growing attention to buckwheat as a potential functional food [2]. Buckwheat is well known to contain high concentrations of rutin compared with other common plant foods [3]. Buckwheat or buckwheat-incorporated products is associated with a wide range of health benefits, including anticancer, anti-inflammatory, hypoglycemic and hypocholesterolemic effects, although the specific bioactive components responsible for the beneficial effects of buckwheat [4]. Buckwheat has the potential of improving lipid metabolism and could be utilized as a functional food for the obviating of NAFLD and hyperlipidemia [5].

In addition, the absence of gluten makes buckwheat-containing products potential alternatives for patients suffering from celiac disease and as such has become a popular functional ingredient incorporated in diverse recipes/products with particular use in the gluten free market. Due to gluten free, buckwheat or buckwheat enriched ingredients has increased significantly over recent years with many buckwheat-based products appearing globally [6]. Another ingredient for making the base of gluten free burfi was muskmelon seed flour. The seeds of muskmelon are generally considered as agro-waste and are spitted out in spite of having its high nutritional value as well as therapeutic benefits. Muskmelon seeds are a good source of lipids, proteins, dietary fibers and antioxidant properties in addition to their prebiotic [7,8]. Seeds are also rich in minerals such as magnesium, phosphorus, sodium, and potassium [9]. In addition, the presence of flavonoids and phenolic compounds reaffirms that the muskmelon seed flour *Cucumis melo L.* has high antioxidant properties [10]. They contain significant amounts of lipids, mainly polyunsaturated fatty acids, especially linoleic and linolenic as well as considerable amounts of essential amino acids [11]. The seed flour has positive impact on growth; cardio protective effect, anti-diabetic effect, and anti-inflammatory, anti-obesity as well as it can be used as functional ingredients during food formulations.

Burfi is a sweet, square shaped or rectangular Indian specialty prepared with khoa, fruit, pulses, cereals and millets. Once confined to household production, burfi is gaining an international market in recent years owing to its delicious taste, flavour and texture and also as a nutrition dense delicacy for consumers. The most popular varieties of burfi are mawa / khoa, mango, moongdal, coconut, pista, chocolate, cashew, saffron and rava burfi. These ingredients can be used singly or in combination [12]. The present study designed the development of a burfi made from buckwheat flour and muskmelon seed flour, in the form of healthy gluten free sweets, is essential with the aim of producing functional foods. This product then can be used in patients of celiac disease as well as an effort to overcome the problem of nutrition-health, especially in communities in India. The gluten free burfi developed should be acceptable to celiac and all the groups i.e. convenient for everyone to carry and use. Therefore, a powdering process for this food may be a good way to maintain the existing of the product and keep it as ready-to-eat-food products, which contain 18% of protein with high-quality amino acid along with 493kcal/100g as energy dense sweets.

### Aim of the study

The aim of the present study is to develop a gluten free nutritional sweet food product and to study its nutritional value with sensory properties.





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## MATERIALS AND METHODS

### Locale of the study

The study was executed in the research laboratory of Nutrition & Dietetics, Faculty of Allied Health Sciences, SGT University, Gurugram and Haryana.

### Procurement of raw material

The raw materials for preparation of buckwheat flour, musk melon seed flour, sugar powder, almond, ghee were purchased from the local market of Gurugram, Haryana. The raw material was carefully monitored and sorted out for any deformities or weeds present and stored at room temperature in glass containers.

### Design

This research was pre-experimental design. Samples formation was at first started and first step was to divide the ratios. Sample burfi were divided into 5 types (formula) and the different ratios of two flour were taken detailed in (Table 1). The total numbers of trials were 30 in this study.

### Process and development of gluten free burfi

Buckwheat seed and muskmelon seed was purchased from market and then it grinded in laboratory by mixer and grinder and roasted. Similarly almond were also grinded to powder form and roasted. Mixing of roasted ingredients with addition of sugar and ghee. It was cut into a desired shaped and kept in freezing for 10 minutes. The various operations involved in the preparation of burfi are detailed in (Fig. 1). Five types of smples are represented in (Fig.2).

### Sensory evaluation

The sensory evaluation was carried out to find the overall acceptability of burfi. Gluten free burfi were analysed for different sensory characteristics like colour and appearance, body and texture, flavor and taste and overall acceptability and it was performed by a panel of 20semi - trained panelists from Faculty of Allied Health Sciences, SGT University, Gurugram ,Haryana(India). Sensory evaluation was carried out by using 9-point Hedonic rating scale (1 = dislike extremely, 9 = like extremely) was used for evaluating the appearance, body & texture, flavor, taste and overall acceptability of the gluten free burfi [13].

### Nutrition composition of gluten free burfi

#### Protein Content –

##### Micro Kjeldahl Method

The estimation of various gluten free burfi was done by Micro Kjeldahl method [14]. as described in (AOAC 2000).

The following formula was used:

$$N (\%) = 1.4(V_2 - V_1) \text{ Normality of } HCl \times 250 (\text{dilution}) / \text{weight of Sample}$$

Where,

$$(V_2 - V_1) = \text{Volume made of the digest}$$

$$\text{Protein } \% = N\% \times \text{Conversion factor } (6.25)$$

#### Crude fat

The method of fat extraction was followed accordance with AOAC 1995 [15].

Calculations

$$\text{Crude fat } (\%) = \frac{\text{Wt. of ether soluble material}}{\text{Wt. of sample}} \times 100$$

#### Energy value

The Energy value of the product was calculated based on given formula-



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Energy value (Kcal/100 g) = (4 X Protein %) + (9 X Fat %) + (4 X Carbohydrate %)

**Total carbohydrate**

The total Carbohydrate was calculated by the difference method [16].

**Iron**

Iron content of gluten free burfi was determined using atomic absorption spectrophotometer method [17].

**Cost evaluation**

Cost of the developed products was analyzed based on input cost of different ingredients used for the preparation of the gluten free burfi.

**Statistical analysis**

The significant difference of mean value was assessed through analysis of variance ANOVA using a software SPSS version 25.0. Mean and standard deviation was calculated for each sample and each parameter.

**RESULTS AND DISCUSSION****Sensory properties of gluten free burfi**

Sensory analysis is an integral part of the development of products that fulfill consumer expectations. Sensory analysis was performed primarily to identify the gluten free burfi with the highest acceptability and to contribute to one's understanding of consumer's product selection. Gluten free burfi were evaluated for color and appearance, body and texture, flavor and taste, as well as overall acceptability of different sample presented in (Table 2). In this study, it was observed that the colour and appearance, of the muskmelon seed flour enriched gluten free was perceived to be significantly different ( $p < 0.05$ ) from that of the control sample. It was also noticed that gluten free burfi with higher content of muskmelon seed flour (40g/ 100 g gluten free burfi) sample T4 had a stronger appeal than those with considerably lesser amounts. The research showed that the sensory acceptance of the cake developed using melon seed flour as an ingredient [18].

Body and texture of the gluten free burfi were mostly affected by blending of buckwheat flour and muskmelon seed flour used for making the different sample of burfi. It was found that there was an increase in body and texture score in the gluten free burfi sample T4 with increased levels of muskmelon seed flour highly acceptable by panelists. The results showed significantly ( $P \geq 0.05$ ) difference from that of the control sample. The better flavor and taste, the more likely it is for the product to gain the acceptability of consumers. It was observed in the study that sample T4 had highest flavor and taste score. The results observed significantly ( $P \geq 0.05$ ) difference from that of the control sample. All samples had mean scores that ranged from 'like slightly' to 'like very much'. Samples did not fall into the category of dislike or neither like nor dislike. Sample T4 had the highest overall acceptability, which significantly vary ( $p < 0.05$ ) from the control sample. It was found that gluten free burfi sample T4 with mixing of 40 % muskmelon seed flour and 10 % buckwheat flour was the most favorite product by panelists based on sensory analysis which was indicated by the highest value in terms of, color and appearance ( $8.05 \pm 0.76$ ), body and texture ( $7.9 \pm 0.72$ ), flavor and taste ( $7.9 \pm 0.72$ ), so that more accepted by the control sample. Sensory markers can affect the level of preference and quality of food products. The incorporation of health promoting functional ingredients such as buckwheat into traditional recipes are often complemented by the evaluation of appearance, aroma, taste and texture as well as overall quality through standardized procedures involving trained judges or consumer panels [19].

**Nutrition composition of gluten free burfi**

Proximate composition of gluten free burfi made from BWF-MSF blend is shown in (Table 3). The present study, there



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was increase in the protein content of the product with the incorporation of muskmelon seed flour. The control sample was found to have  $11.1 \pm 0.35$ g-protein/100 g sample. It was noticed that the highest protein content,  $18.3 \pm 0.26$  g/100 g of gluten free burfi, was found in the variant containing 40g muskmelon seed flour/ 100 g sample T4. The protein content of the enriched was subject to the quantity of muskmelon seed flour, which helped as an increased protein in the gluten free burfi. Statistically significant difference ( $p \leq 0.05$ ) was observed in the protein content of gluten free burfi. Buckwheat flour and muskmelon seed flour which are good sources of protein, also contributed to its increase in the gluten free burfi. Buckwheat proteins may show a strong supple- mental effect due to the well-balanced amino acid composition [20].

The fat content of the samples ranged from  $23.5 \pm 0.035$ -g/100 g T0 to  $36.9 \pm 0.2826$ -g/100 g T4. It was found that samples of gluten free burfi had high fat content. There was a gradual increase in fat content with increasing composition of muskmelon seed flour and vice versa. It was found that the high levels of fat content from sample T4 containing (40g muskmelon seed flour/ 100 g) with increased levels muskmelon seed flour, which is known as high fat content. The results showed significantly ( $P \geq 0.05$ ) difference was found in fat content among the samples. The carbohydrates content in the gluten free burfi were mostly contributed by sugar, buckwheat flour and muskmelon seed flour. The total carbohydrate content was found to be  $45.0 \pm 0.2$  g/100 g in the control sample containing 50g buckwheat flour/100g, while the lowest carbohydrate content was at  $22.3 \pm 0.18$  g/100 g, found in sample (T4) which was made with 40g muskmelon seed flour/ 100 g sample. It was concluded that there was a significant increase in the total carbohydrate content of the variants enriched with buckwheat flour. There was significant difference ( $P \leq 0.05$ ) observed among the samples. Formulated products like biscuits, chapaties and namakpar using buckwheat flour which have low glycemic index, these developed foods was beneficial for diabetes patients [21].

The iron content of the samples ranged from  $2.69 \pm 0.34$ -mg/100 g T0 to  $7.85 \pm 0.1926$ -mg/100 g T4. The sample T4 had highest iron content from that of the control sample. It was found that with the increase in the muskmelon seed flour proportion in the blends, there was increase in iron content of gluten free burfi. The analysis of variance showed significant differences ( $P \leq 0.05$ ) among the samples. Energy value of control as well as treated sample were calculated and found to be 436.9, 451.1, 464.8, 479.5, and  $493.7 \pm 0.5$  for T0, T1, T2, T3 and T4 respectively. The analysis of variance showed that there was significant difference ( $P \leq 0.05$ ) among the samples. The addition of buckwheat in wheat flour, may not only improve the physico-chemical and functional properties of the blended flour but may also enhance the nutraceutical potential of the product prepared from it [22].

**Cost of the product**

The cost of this gluten free burfi is a crucial parameter for marketing of this product. Hence, it is calculated based on the current market price of raw ingredients used in the development of gluten free burfi. Highest cost of product (89 Rs) was recorded in sample T4 followed by T0 (57 Rs), T1 (65 Rs), T2 (73 Rs) and T3 (81Rs) respectively.

**CONCLUSION**

From our study, it can be concluded that the addition of muskmelon seed flour and buckwheat flour enhances the nutritional composition, in reference to energy, protein, fat and iron as well as functional and sensory properties of gluten free burfi. Nutritional properties were also significantly affected. Buckwheat and muskmelon seed flour flour could be a potential raw material for gluten free burfi with improved bioactivity potential. This product is gluten free hence it can be a good option/ choice for people having celiac disease and under nutrition. Gluten-free food products for celiac sufferers are essential for healthy living. The gluten free burfi can be useful in diseases where macronutrients and micronutrients are needed. Therefore, the newly developed gluten free burfi with balanced nutrition composition is beneficial to society as well as our food industry. This study may provide scope for the utilization of buckwheat flour and muskmelon seed flour in other food products.



**Akanksha Yadav et al.****AVAILABILITY OF DATA AND MATERIAL**

The data mentioned was thoroughly analysed and reported in this work

**FUNDING**

This is a self-funded work by the authors

**AUTHOR'S CONTRIBUTION**

The first author planned and conceived the main part of work. The second author was instrumental in working out experiments in the lab along with first author. The third author helped in editing this manuscript and giving important insights.

**COMPETING INTEREST**

The authors declare there are no competing interests.

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**REFERENCES**

1. Skrabanja, V., Kreft, I., Golob, T., Modic, M., Ikeda, S., Ikeda, K., Kreft, S., Bonafaccia, G., Knapp, M., Kosmelj, K., Nutrient Content in Buckwheat Milling Fractions. *Cereal Chemistry*, 81, (2004). 2172-2176.
2. Krkošková, B and Mrázová, Z, Prophylactic components of buckwheat. *Food Res.Int*, 38,(2005). 561–568. <https://doi.org/10.1016/j.foodres.2004.11.009>
3. Saturni, L.; Ferretti, G.; Bacchetti, T., The gluten-free diet: Safety and nutritional quality. *Nutrients*.2, (2010).16–34.
4. Giménez-Bastida, Juan Antonio, and Henryk Zieliński. "Buckwheat as a Functional Food and Its Effects on Health." *Journal of agricultural and food chemistry* vol. 63,36 (2015): 7896-913. doi:10.1021/acs.jafc.5b02498
5. Zi-Rui Huang.,Jia-Cong Deng.,Qiu-Yi Li.,Ying-Jia Cao.,Yi-Chen Lin.,Wei-Dong Bai.,Bin Liu.,Ping-Fan Rao.,Li Ni ., Xu-Cong Lv ., Protective Mechanism of Common Buckwheat (*Fagopyrum esculentum* Moench.) against Nonalcoholic Fatty Liver Disease Associated with Dyslipidemia in Mice Fed a High-Fat and High-Cholesterol Diet.*J. Agric. Food Chem.* ,68, 24,(2020) 6530–6543.
6. Małgorzata Starowicz, Georgios Koutsidis & Henryk Zieliński., Sensory analysis and aroma compounds of buckwheat containing products—a review, *Critical Reviews in Food Science and Nutrition*, 58:11, (2018).1767-1779, DOI: 10.1080/10408398.2017.1284742
7. Rolim P. M.; Fidelis G. P.; Padilha C. E. A.; Santos E. S.; Rocha H. A. O.; Macedo G. R., Phenolic Profile, Antioxidant Activity from Peel and Seed of Melon (*Cucumis Melo* L. Var. *Reticulatus*) and Its Anti-proliferative Effect in Cancer Cells. *Braz. J. Med. Biol. Res.* ; 51(4): (2018) 1–14.
8. Fundo JF, Miller FA, Garcia E, Santos JR, Silva CLM, Brandão TRS., Physicochemical characteristics, bioactive compounds and antioxidant activity in juice, pulp, peel and seeds of Cantaloupe melon. *Food Meas.* 2018; 12: 292–300
9. Olubunmi IP, Olajumoke AA, Bamidele JA, Omolara OF., Phytochemical Composition and in vitro Anti-oxidant Activity of Golden Melon (*Cucumis melo* L.) Seeds for Functional Food Application. *Int. J. Bio-chem. Res. & Rev.* 25 (2): (2019). 1–13.
10. Bouazzaoui N, Drici W, Bouazzaoui W, Lemerini W, Arrar Z, Bendiabdellah D., Fatty acids and mineral composition of melon (*Cucumis melo* L. *inodorus*) seeds from West Algeria. *Mediterranean J. Chem.* ; 5(1) (2016). 340–346.
11. Oluwabamiwo F, Adegoke G, Denloye S, Akinoso R, Bruno D., Proximate composition and fatty acid profile of Nigerian melon seeds. *Life Sci. Arch. (LSA)*. 1(1) (2015).59–65.





**Akanksha Yadav et al.**

12. Aneja, R.P., Mathur, B.N., Chandan, R.C. and Banerjee, A.K., Technology of Indian milk products, Dairy India Year book, A Dairy India Publication, New Delhi, 74- 96 (2002) 99–101.
13. Amerine M. A., Pangborn R. M., Roessler E. B, Principles of sensory evaluation of food. Elsevier. 2013.
14. A.O.A.C., Official Methods of Analysis, Association of Official Analytical Chemists 18th ed Washington DC(2000)..
15. A.O.A.C., Official Methods of Analysis, Association of Official Analytical Chemists 17th ed Washington DC(1995).
16. FAO. Carbohydrates in Human Nutrition II. Report of a Joint FAO/WHO Expert Consultation (FAO Food and Nutrition Paper 66) Food and Agriculture Organization. Rome1998).
17. Raghuramulu N, Madhavan N, Kalyanasundaram K., A Manual of Laboratory Techniques. National Institute of Nutrition. Indian Council of Medical Research, India.(2003.) pp. 1-38
18. Da Cunha JA, Rolim PM, Damasceno KSFDSC, de Sousa Ju nior FC, Nabas RC, Seabra LMJ., From seed to flour: Sowing sustainability in the use of cantaloupe melon residue (Cucumis melo L. var. reticulatus). PLoS ONE 15(1) (2020) :e0219229.https://doi.org/10.1371/journal.pone.021929
19. Małgorzata Starowicz, Georgios Koutsidis & Henryk Zieliński., Sensory analysis and aroma compounds of buckwheat containing products—a review, Critical Reviews in Food Science and Nutrition, 58:11, (2018).1767-1779, DOI: 10.1080/10408398.2017.1284742
20. Li S., Zhang and Q.H., Advances in the development of functional foods from buckwheat. Critical Reviews in Food Science and Nutrition, 41: (2001). 451–464.
21. Verma M., Nutritional quality evaluation, product formulation, glycemic and lipidemic response of buckwheat: an underutilized plant food of Uttaranchal hills. Ph.D.thesis, G. B. Pant University of Agriculture and Technology, Pantnagar, (2003).65-71.
22. Jan, U., Gani, A., Ahmad, M., Shah, U., Baba, W. N., Masoodi, F. A., Maqsood, S., Gani, A., Wani, I. A., & Wani, S. M., Characterization of cookies made from wheat flour blended with buckwheat flour and effect on antioxidant properties. *Journal of food science and technology*, 52(10), (2015) 6334–6344. https://doi.org/10.1007/s13197-015-1773-8

**Table1: Formulation of gluten free burfi of different sample combinations.**

Ingredient	T0	T1	T2	T3	T4
Buckwheat seed flour	50g	40g	30g	20g	10g
Muskmelon seed flour	-	10g	20g	30g	40g
Ghee	10g	10g	10g	10g	10g
Sugar	20g	20g	20g	20g	20g
Almond	20 g	20g	20g	20g	20g

T= treatments, g= grams

**Table 2: Sensory properties of gluten free burfi**

S. No.	Parameters	T0	T1	T2	T3	T4
1.	Colour & appearance	7.25±0.55	7.75±0.72	7.1±0.55	6.3±1.03	8.05±0.76
2.	Texture	7.15±0.49	7.6±0.68	7.1±0.55	6.4±0.66	7.9±0.72
3.	Flavour & taste	7.1±0.55	7.7±0.66	7.1±0.55	6.4±0.82	7.9±0.72
4.	Overall acceptability	7.2±0.39	7.6±0.64	7.1±0.42	6.38±0.55	7.95±0.66

\*Values are presented Mean ± SD from the samples of gluten free burfi.





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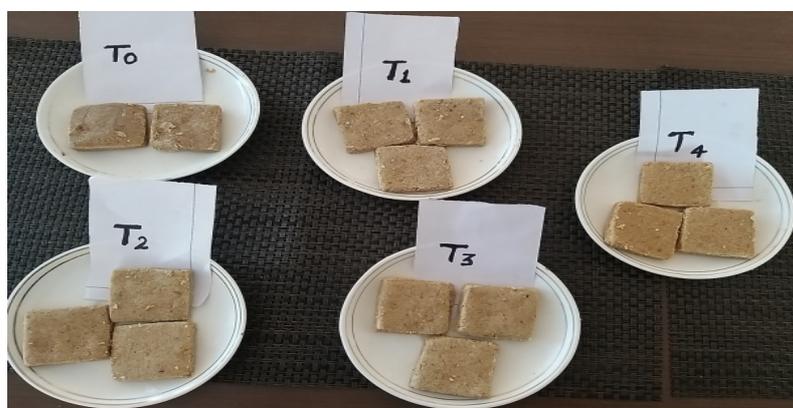
**Table 3: Nutrition composition of gluten free burfi.**

S. No.	Parameters	T0	T1	T2	T3	T4
1	Energy value (kcal/100g)	436.9±0.37	451.1±0.47	464.8±0.31	479.5±0.39	493.7±0.5
2	Total carbohydrate (g)	45.0±0.2	39.3±0.23	33.6±0.23	28.0±0.29	22.3±0.18
3	Protein (g)	11.1±0.35	12.9±0.46	14.7±0.33	16.5±0.26	18.3±0.26
4	Fat (g)	23.5±0.035	26.9±0.29	30.2±0.33	33.6±0.26	36.9±0.28
5.	Iron (mg)	2.69±0.34	3.98±0.28	5.27±0.29	6.56±0.28	7.85±0.19

\*Values are presented Mean ± SD from the samples of gluten free burfi.



**Fig 1: Flow sheet for the process development of gluten free burfi enriched with buckwheat and muskmelon seed flour**



**Fig 2. Formulation of five type samples of gluten free burfi enriched with buckwheat and muskmelon seed flour**





## Some Applications of Semi Group Theory

Mantha Srikanth<sup>1,2\*</sup> and G. Shobhalatha<sup>3</sup>

<sup>1</sup>Research Scholar, Department of Mathematics, Sri Krishnadevaraya University, Ananthapuramu, Andhra Pradesh, India.

<sup>2</sup>Assistant Professor, Department of Mathematics, Malla Reddy Engineering College (Autonomous), Hyderabad, Telangana, India.

<sup>3</sup>Professor, Department of Mathematics, Sri Krishnadevaraya University, Ananthapuramu, Andhra Pradesh, India.

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### \*Address for Correspondence

#### Mantha Srikanth

Assistant Professor, Department of Mathematics,  
Malla Reddy Engineering College (Autonomous),  
Hyderabad, Telangana, India.

E.Mail: manthasrikanth9@gmail.com



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### ABSTRACT

This paper deals with some applications of semi groups in the various fields. Consider such applications Partial differential equations; automate theory, biology, formal language, sociology, network analogy, etc.

**Keywords:** Semigroup, Partial differential equation, Sociology, Kinship system.

## INTRODUCTION

In mathematics, a semigroup is an algebraic structure consisting of a set together with an associative binary operation. The binary operation of a semigroup is most often denoted multiplicatively : ' $a.b$ ', or simply ' $ab$ ', denotes the result of applying the semigroup operation to the ordered pair (a,b). Associativity is formally expressed as that  $(a.b).c = a.(b.c)$  for all  $a,b$  and  $c$  in the semigroup. The theory of finite semigroups has been of particular importance in theoretical computer science since the 1950s due to the natural link between finite semigroups and finite automata via the syntactic monoid. In probability theory, semigroups are associated with Markov processes. In other areas of applied mathematics, semigroups are fundamental models for linear time-invariant systems. In partial differential equations, a semigroup is associated to any equation whose spatial evolution is independent of time. The areas of applications of such as Partial differential equations, formal languages and the software use the language of recent algebra in terms of Boolean logic, semigroups et al..But also parts of other area like biology, biochemistry and sociology.





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The theory of automata has its origins within the work by Turing (Shannon 1984 and Heriken 1994). Turing developed the theoretical concept of what's now called Turingmachines, so as to offer computability a more concrete and precise meaning. Many various parts of pure mathematicians are used as tools like abstract algebra, universal algebra, lattice theory, graph theory and therefore the theory of algorithms. The beginning of the of formal languages can be traced Chomsky, who introduced the concept of context-free language so as to model natural languages in 1957. Semigroups are often utilized in biology to explain certain aspets within the crossing of organisms, in genetics and in consideration of metabolisms. The expansion of plants are often described algebraically in Hermann and Rosenberg (1957). Language theory is employed in cell – development problems, as introduced by Lindenmayes (1968), Hermann and Rosendalg (1975). Sociology includes the study of human interactive behavior in group situations, especially in underlying structures of societies. Such structures are often revealed by mathematical analysis. This means how algebraic techniques could also be introduced into studies of this type.

### Applications of Semi groups

#### Semi groups - partial differential equations

Semigroup theories are often wont to study some problems wthin the field of partial differential equations. Roughly speaking, the semigroup approach is to regard a time-dependent partial differential equation as an ordinary differential equation on a function space.

For example, consider the subsequent initial/boundary value problem for the heat equation on the spatial interval  $(0, 1) \subset \mathbf{R}$  and times  $t \geq 0$ :

$$\begin{cases} \partial_t u(t, x) = \partial_x^2 u(t, x), & x \in (0,1), t > 0; \\ u(t, x) = 0, & x \in \{0,1\}, t > 0; \\ u(t, x) = u_0(x), & x \in (0,1), t = 0. \end{cases}$$

Let  $X = L^2((0,1)R)$  be the  $L^p$  of square-integrable real-valued functions with domain the interval  $(0, 1)$  and let  $A$  be the second-derivative operator with domain  $D(A) = \{u \in H^2((0,1); R) / u(0) = u(1) = 0\}$ ,

Where  $H^2$  is a Sobolev space. Then the above initial/boundary value problems are often interpreted as an initial value problem for an ordinary differential equation on the space  $X$ :

$$\begin{cases} \dot{u}(t) = Au(t); \\ u(0) = u_0. \end{cases}$$

On a heuristic level, the answer to the present problem "ought" to be  $u(t) = e^{(tA)}u_0$ . however, for a rigorous treatment, a meaning must tend to the exponential of  $tA$ . As a function of  $t$ ,  $e^{(tA)}$  is a semigroup of operators from  $X$  to itself, taking the initial state  $u_0$  at time  $t = 0$  to the state  $u(t) = e^{(tA)}u_0$  at time  $t$ . The operator  $A$  is said to be the infinitesimal generator of the semigroup.

#### Semi groups - Biology

Semi groups are often utilized in biology to explain certain aspects in the crossing of organisms, in genetics, and in consideration of metabolisms.

#### Example

In breeding a strain of cattle, which can be white or red monochromatic or spotted, it's known that white is dominant and red receive which monochromatic is dominant over spotted. Thus there are four possible sorts of cattle in this herd.





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x = white monochromatic  
 y = white spotted  
 z = Red monochromatic  
 w = Red spotted.

Due to dominance, in crossing a white spotted one with a red monochromatic one, we expect a white monochromatic one.

This will be symbolized by

$y \circ z = x$ . The operation 'o' can be studied for all possible pairs to get the table.

<i>o</i>	x	y	z	w
x	x	x	x	x
y	x	y	x	y
z	x	x	z	z
w	x	y	z	w

Then  $A = \{x, y, z, w\}$  is a semi group with identity element w.

**Semi groups – Sociology**

Sociology includes the study of human interactive behavior in group situations, especially in underlying structures of societies. Such structures are often revealed by mathematical analysis. This means how algebraic techniques could also be introduced into studies of this type.

$(S(A), *)$  is called the relation semi group on A. The operation 'o' is called the relation product, where A is a monoid. A kinship system may be a semi group  $R = \{X, A\}$  where A is a relation on X, which express equality of kinship relationships.

**Example**

Let the Kinship system  $R = \{X, A\}$  be defined by

$X = \{U = (\text{"is mother of"}), V = (\text{"is son of"})\}$ ,

$R = \{(UU, U), (UV, VU), (VV, V)\}$ . Let x,y,z be the equivalence class of U, V and UV respectively. Now "o" is given by

<i>o</i>	x	y	z
x	x	z	z
y	z	y	z
z	z	z	z

Then  $(R,o)$  is a semi group.

**CONCLUSION**

We've seen different areas of applications of semi groups. We identified some examples in biology, sociology and partial differential equations etc. Further we would like to review some more structures of semi groups which can find applications in several areas.





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## REFERENCES

1. Wikipedia Encyclopedia.
2. Boyd, J. P., Haehl, J. H. and Sailer, L.D. "Kinship Systems and Inverse semigroups", *J.Math.Sociol.*76,(1972), 37-61. (Google Books).
3. Clifford, A. H. and Preston, G. B. "The algebraic theory of semigroups", *Math.Surveys No.7,Amer.math.soc.*, Vol. I, (1967). (Google Books).
4. Clifford, A. H. and Preston, G. B. "The algebraic theory of semigroups", *Math.Surveys No.7,Amer.math.soc.*, Vol. II, (1967). (Google Books).
5. P.Sreenivasulu Reddy & Mulugeta Dawud. "Applications of semigroups", *Global Journal of Science Frontier Research: F Mathematics and Decision Sciences*. Vol.15, Issue 3, version 1.0, year 2015.





## Ecological and Breeding Behaviour of Selected Anurans in India: A Review

Bhagyashree. Panda\* and Siba Prasad Parida

Department of Zoology, School of Applied Science, Centurion University of Technology and Management, Bhubaneswar Campus, Odisha, India.

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### \*Address for Correspondence

**Bhagyashree. Panda**

Department of Zoology,  
School of Applied Science,  
Centurion University of Technology and Management,  
Bhubaneswar Campus, Odisha, India.  
Email: lucky.panda79@gmail.com



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### ABSTRACT

Anurans are mostly wetland species and found near perennial water. Some are found on trees, in tree holes and in house. Foam nest of anurans of Rhacophorids is adhered to twigs above the water level. Anurans of Ranid family are found in permanent ponds rich with hydrophytes or thick vegetation nearby. If we study the nature, anurans of Rhacophorids are mostly arboreal and frequently encountered near the habitation of human. In anurans, vocalizations of the male and tactile cues are important in courtship and mating. Sound production is an important reproductive fraction of male anurans. Species specific advertisement calls attract females for breeding and announce to other males that a given territory is occupied. Most anurans are normally solitary but large breeding aggregations are found during the spring or rainy season. Amplecting pairs of Ranid move and lay eggs in scattered manner which adhered to aquatic plant. Construction of foam nest and parental care is important characters of rhacophorid anurans. In spite of close relationship between the families, difference in ecological niche, habitat and breeding behaviour is prominent.

**Keywords:** foam nest, amplecting pair, Arboreal, parental care, advertisement calls

### INTRODUCTION

Information on systematics, natural history, distribution, description and various other biological data of Indian anurans have been published by Guenther, Ahl, Parker, Rao, Daniel, Pillai and Chanda, Inger and Dutta, Daniel and Sekar [1][2][3][4][5][6][7][8]. Amphibians are grouped into three orders: Caudata (Urodela), Gymnophiona or Apoda (Caecilia) and Anura (Salientia) and they represent 5150 species all over the world, and 209 species in India Among



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amphibians, the order Anura forms the largest living order, which constitutes total of 4120 species under 17 families and 248 genera are known from the world, of which only 180 species under 6 families and 24 genera have been found from Indian region [9]. The amphibian fauna of India constitute 210 species [10][11]. The Ranids are represented by 90 species and the majority of the species are the inhabitants of wetlands and they may be facultative and obligatory aquatic animal. Either temporary or permanent rainwater pools are utilised by these species for breeding. However, variations in the time period of breeding and resource utilization of such species are noticed. In addition, the larval forms of several sympatric species are morphologically similar. As most of the Indian ranids are the inhabitants of wetlands and some of them also live in similar environmental conditions, it is necessary to know more about the variations and similarities in their morphology and biology. Publication by Boulenger [12][13][14][5], Chanda [16], Daniel [17][18][19][20], Rao [21] provide data on the above parameters. Dutta *et al* [22] established size analysis of *Rana crassa*. Rath [23] reported a detailed account on the morphometric analysis of *Polypedates maculatus* for the first time. Sex ratio of Indian anurans was studied by Mohanty- Hejmadi and Dutta [24] who reported the sex ratio of *Rana tigerina*. Kalb and Zug[25] provided data on age estimation of *Bufo americanus*. Twenty nine modes of reproduction were identified among the anurans [26]. Publications of Mc Cann [27], Inger and Greenberg [28][29], Basu[30], Dutta[31] and Platt[32] described reproductive behaviour of asiatic anurans. There could be of two types of reproductive pattern, continuous (acyclic) and potentiality continuous (seasonal) breeder [33]. The acyclic species are abundant to the area where temperature, humidity and rainfall are quite high. The seasonal breeders are confined to areas where there is fluctuation in temperature, humidity and rainfall. Majority of anurans of odisha fall within the limits of the second category. Ratio of clutch size to body weight serves as a useful approximation of reproductive effort [34]. A detailed study on the ontogeny of teeth row structure of Indian anurans was by Dutta and Mohanty-Hejmadi [35]. There are series of papers on life history and development of Indian anurans [36][37][38][39]. Seshadri *et al* [40] observed a new reproductive mode in the white spotted bush frog, *Raorchestes chalazodes* who breeds and lays eggs in live bamboo with narrow openings. The reproductive mode of anurans includes oviposition site, clutch characteristics, developmental mode, stages and type of parental care, if any. Utilisation of similar habitat but different breeding behaviours are observed in between the allied families.

**Body form and size of anurans**

Since the time of Darwin [41] body forms and sizes of organisms have been considered which are determined by the powerful forces of natural selection, and may mirror a wide range of ecological interactions. Size of animals is an evolutionary response to demands of the immediate environment, related to key life history traits, such as fecundity, foraging, locomotion and reduction of predation, desiccation, heating and cooling. Optimal size, according to Phillipson [42] is dependent on competitive abilities and survival probabilities of the various size and age classes. However, large species tend to appear later in a groups evolutionary history and an exception being the Amphibia [43]. Within ecological communities, environmental resources are partitioned according to dimension and differences in comparative sizes of the organism [44][45][46]. Infact such apparent differences were reported much earlier by Hutchinson [47], who found constant differences in the ratio of linear dimension of the trophic (feeding) apparatus among closely related sympatric species.

**Nature of habit and habitat**

Rhacophorids are arboreal frogs with long limbs and enlarged toe pads for holding on to leaves and branches of trees, and some genera have extensive webbing on the hands and feet inhabit in dense forest of the Western Ghats and Eastern Himalayas. Frogs of rhacophorids family have horizontal pupil, free and deeply notched tongue. They possess specialized breeding habits and build foam nests on rocks or vegetation overhanging water bodies. The tadpoles drop into the water for further development at the time of hatching. The Rhacophoridae of India comprises of two subfamilies (Rhacophorinae and Philautinae) and five genera (*Rhacophorus*, *Polypedates*, *Philautus*, *Chirixalus* and *Theloderma*). Two species of the genus *Polypedates*, five species of the genus *Rhacophorus* and six species of the genus *Philautus* from Northeast India have been reported by Sen[48]. Ranids are found in restricted pockets where permanent source of stagnant water is observed. Liem [49] and Taylor [50] described that *Polypedates* live in trees and tree holes or among grass and weeds near water. McCann [51] suggested this species lives near perennial water and



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in house where water is at hand and can be seen all the year round. Anurans found in Odisha are seasonal breeders and observed in areas with temperature and rainfall fluctuation.

**Morphometry**

Lack of data on morphometries of Indian anurans is mainly based on the size analysis from systematic description. Mohanty-Hejmadi and Dutta [24] reported the size correlation of the Indian Bull frog, *Hoplobatrachus tigennus*. Shaffer [52] published the data on the size and scaling in the Indian frogs *Nyctibatrachus* and *Nannobatrachus* (Ranidae). Rath [23] studied on *Polypedates maculatus* and Das [53] reported on *Tomopterna rolandae*. Das [54] has also given the data on size gradation in sympatric frogs in South India.

**Sex ratio**

Sex ratio analysis are rather limited with regard to Indian anurans. For statistical analysis significant number of specimen of both sexes of any species of 3 are required. The first study Mitra and Ray [55] studied on the sex ratio of the Indian anurans and reported on the sex ratio of *Bufo melanostictus*. Mohanty- Hejmadi and Dutta [56] described the sex ratio of *H tigennus*. Abdulali [57][58] reported the sex ratio of the same species from Western India. A report on sex ratio were studied on *H crassus* by Dutta *et al* [22] which is closely related to *H tigennus*. A study on sex ratio of another ranid, *L limnochans* by Mohanty [59] which lives in sympatry with *H tigennus* and *H crassus*. Excluding the above ranid, the study of a burrowing ranid, *T rolandae* has been reported by Das [53] and of a rhacophorid, *P maculatus* has been reported by Rath [23].

**Clutch size**

Reproductive pattern and clutch size of amphibians are well studied for temperate climate species [60][61][62][63][64]. However, the information is available on the annual reproduction of tropical species of anurans from Southeast Asia is, *Bufo melanostictus* in Java [65] and Singapore [66], *Rana cancrivora* in Java [67] *L limnochans* in Singapore [66] *Rana erythraea* in Borneo[68] *Kaloula pulchra*, *Microhyla butlen*, *Microhyla heymonsii* and *Leptobrachium mgrops* in Singapore [66] *Rana blythi*, *Rana macrodon*, *Rana ibanroum*, *Rana hosei* and *Amolops charginensis* in *charginensis* in Borneo[69]. A series of papers on the egg per clutch of *Hyla tigrinus*, *L limnochans*, *Hyla crassus*, *Rana vanegata*, *Bufo melanostictus*, *Polypedates maculatus* and *T rolandae* by Das [53], Dutta [70], Dutta and Mohanty-Hejmadi [37], Dutta *et al*[56][31], Mohanty [59] , Mohanty-Hejmadi and Dutta[71] , Rath [23] are available with regard to Indian anurans.

**Reproductive cycle**

Data on reproduction and breeding of Indian anurans are limited to only few species (*Bufo melanostictus*, *Hoplobatrachus tigrinus*, *Hoplobatrachus crassus*, *Euphlyctis cyanophlyctis*, *Euphlyctis hexadactylus*, *Polypedates maculatus*, *Tomopterna rolandae* and *Microhyla ornata* [72][73][74][75][76][77][78][79][53][37][80][81][33] [82][83][84][85][86][87] [71] [88][89][90][91][92]. Reproductive pattern could be of two types, continuous (acyclic) and potentially continuous (seasonal) breeders [33]. Premating congregation has been observed in both groups of ranids and rhacophorids. Construction of foam nest in rhacophorids has been reported by Ikeda [93] and Bhaduri [94]. The seasonal breeders are confined to areas where there is fluctuation in temperature, humidity and rainfall and the acyclic species are restricted to the areas where the temperature, rainfall and humidity are high. Majority of the anurans of eastern India lie under of first category. A series of papers report the patterns of testicular and ovarian activity of some Indian Anurans [94][95][96][97]. The reproduction in anurans begins when a male clasps a female in a process called amplexus and sheds sperm over the eggs as they are being extruded from the female into the water. Fertilization is almost always external and eggs and larvae are typically aquatic in nature. Oviposition and fertilization are accomplished by synchronized movements by both partners. First the female chooses the oviposition site in an amplexus. The male maintains a close contact with the dorsum of the female in arboreal oviposition and we will find a juxtaposed vents. To avoid predators of larvae female frogs of several species select oviposition sites or conspecific eggs or larvae [97][98][99][100][101]. Some amphibians lay their eggs while others lay eggs and guard them by



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showing parental care. The external fertilization and development of their eggs make them a prime candidate for investigations involving biological and mechanical manipulations

**Anuran communication with respect to breeding behaviour**

In anurans, vocalizations of the male and tactile cues are important in courtship and mating. Sound production is an important reproductive fraction of male anurans. Advertisement calls attract females for breeding and announce to other males that a given territory is occupied. Advertisement calls are species specific, and any one species has a very limited repertoire of calls. Most anurans are normally solitary but they often come together in large breeding aggregations during the spring or rainy season [99][100]. Anurans depend on acoustic communication to attract mates and advertise territory ownership. The hypertrophied muscles of male frogs can be used as sexually selected trait, driven by competition among males for attracting females. Anurans have high calling rates and they possess highly aerobic muscles with high mitochondrial and capillary densities. Most male anurans have advertisement calls to advertise their species identity, sexual receptivity, and spatial location to females and to other males. During close-range courtship interactions males of some species change advertisement calls with females, and in some species, females respond with calls of their own. There is lack of knowledge of both male and female courtship calls and much more common than currently recognized. [104]

**Tadpole characteristics**

The tadpoles are radiated into a variety of microhabitat and modes of life has been accompanied by modification of their ovoidal body form [105][106]. Morphological structures of tadpoles show the differentiation between ranids and rhacophorids and in between two species of ranids. When larval mouth structures are lost, forelimbs emerge and the mouth takes on adult form as the tail begins to shorten [107]. The oral teeth rows and structures are quite variable between tadpole of two different species. The detailed study on the ontogeny of teeth row structures of Indian anurans, *H. tigerinus* is reported by Dutta and Mohanty-Hejmadi [108]. Agarwal and Niazi [109] studied on the development of mouth parts of the same species. There are various publications on the oral structure or teeth row formula of several Indian anurans. Oral morphology of anuran tadpoles differs specifically reflecting adaptive radiations of each species. Basic elements constituting the oral disc are same in tadpoles belonging to different genera and families. Tadpoles are permanently herbivores, they become opportune carnivores occasionally supplementing their carbohydrate rich primary diet with protein by feeding on dead tadpoles and other animals, a bid to hasten development [110].

**CONCLUSION**

If Comparative analysis on the nature of habit, habitat, breeding biology, anatomical peculiarities, and development of anurans of different families will be worked out, the study will add to conservation of the species.

**REFERENCES**

1. Guenther A. Third report on collection of Indian reptiles obtained by the British museum. Proc. Soc. London 1975; 187(5): 567-577.
2. Ahl E. Anura III. Polypedatidae. Das Tierreich, Lief 1931; 55: 1-447.
3. Parker HW. A monograph of frogs of family Microhylidae. British Mus. London 1934; 1-208.
4. Rao CRN. On some new forms of Batrachia from S India. Proc Indian Acad. Sci 1937; 6(6):387-427.
5. Daniel JC. Field guide to the amphibians of Western India. J.Bom. Nat.hist. soc., India 1975; 79:159-168.
6. Pillai RS and Chanda, SK. An overview of amphibian fauna of Garo hills, Meghalaya with description of a new species of Rana. Re. Zool. Surv. India 1981; 79: 159-168.
7. Inger RF and Dutta, SK. An overview of amphibian fauna of India. J.Bom. Nat.Hist.Soc 1986; 83:135-146.




**Bhagyashree. Panda and Siba Prasad Parida**

8. Daniel JC. Notes on some amphibians of the Darjeeling area, West Bengal. J.Bombay Nat.Hist Soc 1962; 56:666-668.
9. Chanda SK. Amphibia: In Faunal Diversity in India. Eds., J. R. B. Alfred, A. K.Das and A. K. Sanyal, Zoological Survey of India, Calcutta 1998; 427-433..
10. Dutta SK. Amphibians of India updated species list with distribution record. Hamdrydad 1992; 17: 1-13.
11. Dutta SK. Amphibians of India and Sri Lanka (checklist and bibliography); 1997.
12. Boulenger GA. Catalogue of the Batrachia Gradients s caudata Batrachia Apoda in the collection of the British Museum. British Mus Publ, London 1882a; 8 -127.
13. Boulenger, GA Catalogue of the Batrachia Salientia s caudata in the collection of the British Museum. London Trustees of the British Mus, London 1882b; 16 -503.
14. Boulenger GA. The Fauna of British India, including Ceylon and Burma, Reptilia and Batrachia. Taylor and Francis 1890; 18 -541.
15. Boulenger GA. A monograph of the South Asian, Papuan, Melanesian and Australian frogs of the genus Rana, Rec. Indian Mus 1920; 20: 1-226.
16. Chanda SK. Memoirs of the Zoological Survey of India. Anuran (Amphibia);1994
17. Daniel JC. Notes on some amphibians of the Darjeeling area, West Bengal. J.Bombay Nat.Hist Soc 1962; 56:666-668.
18. Daniel JC. Field guide to the amphibians of Western India Part-I. J. Bombay. Nat. Hist. Soc 1963; 60(2):415-438.
19. Daniel JC. Field guide to amphibians of Western India, Part-II. J. Bombay. Nat. Hist. Soc 1973; 60:690-702.
20. Daniel JC. Field guide to the amphibians of Western India Part-III J Bombay Nat Hist.Soc. 1975; 72:506-522.
21. Rao, CRN. On some new forms of Batrachia from S India. Proc Indian Acad. Sci. 1937; 6(6):387-427
22. Dutta SK and Mohanty-Hejmadi, P. Size analysis and sex ratio of Jerdon's bull frog Rana crassa Jerdon (Anura Ranidae). J.Bombay Nat His Soc 1992; 88:234-241.
23. Rath S. Biology of Indian tree frog, Polypedates maculates (Anura: Rhacophoridae), Ph.D. Thesis. Utkal university; 1995.
24. Mohanty-Hejmadi P and Dutta SK. Studies on the sex ratio and size correlation of the Indian Bull frog, Rana tigerina. Pranikee 1981; 2: 29-36.
25. Kalb HJ and Jug GR. Age estimates for a population of American toad, Bufo americanus (Silentia: Bufonidae), in Northern Virginia. Brimleyana 1990; 16: 79-86.
26. Duellaman WE. Reproductive modes in anuran amphibians: Phylogenetic significance of adaptive strategies. S. Afr.J.Sci 1985; 81: 174-178.
27. Mc cann C. Notes on Indian Batrachians. J. Bombay Nat. Hist.Soc 1932; 32:152-180.
28. Inger RF and Greenberg B. Morphology and seasonal development of sex characters in two Sympatric African toads. j. Morphol 1956; 99: 549-574.
29. Inger RF and Greenberg, B. The annual reproductive pattern of the frog Rana erythraea in Sarawak. Physiol. Zool 1963; 36(1): 21-33.
30. Basu SL. Effects of testosterone and estrogen on spermatogenesis in Rana hexadactyla. J Expt Zool 1968; 169:133-142.
31. Dutta SK. Biology and effect of chemicals and fertilizers on the eggs, developmental stages, juveniles and adults of Indian Bull frog, Rana tigerina. Ph.D. Thesis, Utkal University, India 1979.
32. Platt DR (1986). Comparative notes on reproduction of ecology of Bufo staticus and Bufo melanisticus in Western Orissa, India. Press, Baltimore and London: Pranikee 1986; 7:17-23.
33. Gopalakrishanan M and Rajasekarasetty MR. The annual reproductive behaviour of the green frog, Rana hexadactyla in and around Mangalore and Mysore city (India). Proc Indian Acad Sci 1978; (6):81-89.
34. Kuramoto, M. Correlations of quantitative parameters of fecundity in amphibians. Evolution 1978; 32(2): 287-296.
35. Dutta SK and Mohanty-Hejmadi P. Ontogeny of teeth row structure in Rana tigenna tadpoles. J Bombay Nat. Hist. Soc 1984; 80(3):517-528.




**Bhagyashree. Panda and Siba Prasad Parida**

36. Bhati DPS. Normal stages in the development of the larvae of *Rana tigerina* and *Bufo andersoni*. Agra univ. J.Res.Sci 1969; 18(1):1-13.
37. Dutta SK and Mohanty-Hejmadi P. Breeding and life history of the Indian bull frog, *Rana tigerina*. Prakruti-Utkal Univ. J.Sci 1976; 13(1-2): 51-59.
38. Agarwal SK and Niazi. Normal table of developmental stages of the Indian bull frog, *Rana tigerina*. Proc. Nat. Acad., Sci., India 1977; 47 (B): 79-92.
39. Sekar AG. Observations on the developmental stages of tadpole of Malabar gliding frog, *Rhacophorus malabaricus* (Anura: Rhacophoridae). J.Bombay Nat. Hist. Soc 1990; 87: 223-226.
40. Seshadri SK, Gururaja KV and Bickford DP (2014). Rare bush frog breeds in bamboo. The Linnean Society of London's *Biological J. Lin. Soc* 2014; 114: 1-11.
41. Darwin C. On the origin of species by means of natural selection. Murray:London; 1959
42. Phillipson J. Bio-energetic options and phylogeny In C R Townsend and P Calow (Eds) Physiological ecology. An evolutionary approach to resource use Blackwell Scientific Publishers. Oxford 1981; 20-45.
43. Peters RH. The ecological implications of body size, Cambridge University Press, Cambridge; 1983.
44. Schoener TW. The evolution of bill size differences among sympatric congeneric species of birds. Evolution 1965; 19: 189-213.
45. Schoener T W. Resource partitioning in ecological communities. Science 1974;185: 27-39.
46. Schoener TW. Resource partitioning. In J Kikkawa and DJ Anderson (Eds) Community Ecology Patterns and Processes Blackwell Scientific Publications, Melbourne 1986; 91-126.
47. Hutchinson GE. Homage to Santa Rosalia, or why are there so many kinds of animals? American Naturalist 1959; 93:145-159.
48. Sen N. Further notes on statewise distribution of the amphibian fauna of Northeast India. Rec. Zool. Surv. India 2004; 102(3-4): 105-112.
49. Liem SS. The morphology, systematics and evolution of the old world tree frogs (Rhacophoridae and Hyperoliidae). Fieldiana Zool, 1970; 57, 1-127.
50. Taylor EH. The amphibian fauna of Thailand. Univ. Kansas Sc. Bull 1962; 43(8), 265-599.
51. McCann C. Reptile and Amphibian miscellany. J. Bonn. Nat.hist. soc.1940; 11: 44-65.
52. Shaffer HB. Size and scaling in the Indian frogs *Nyctibatrachus* and *Nannobatrachus* (Ranidae). Fieldiana Zool (New Series) 1988; 46, 1-10.
53. Das SM. Morphometric, Growth, Breeding and Development of *Tomopterna rolandae* (Anura Ranidae). Ph.D Thesis, Utkal University, India; 1996.
54. Das I. Size gradation in syntopic anurans in South India. Asiatic Herpetol Res 1995; 6 38-44.
55. Mitra B and Ray AK. Further observations on the sex ratio in *Bufo melanostictus*. Proc 58th Indian Set Congr, 1971; 3:543-544. (abstract).
56. Mohanty-Hejmadi P and SK Dutta. Studies on the sex ratio and size correlation of the Indian bull Frog, *Rana tigrina*. Pranikee 1991; 2:29-36.
57. Abdulali H. On the export of frog legs from India. J Bombay Nat Hist. Soc.1985; 82(2): 347-375.
58. Abdulali H. On the export of frog legs from India, Proc First World Conf Trade in Froglegs, vis-a-vis Env Considerations Calcutta 1986; 2:115-149.
59. Mohanty AK. Biology of Indian Paddy field frog, *Rana hmnochans* Ph.D thesis, Utkal University, Orissa; 1994.
60. Brockelman WY. Competition, the fitness of offspring and optimal clutch size. Amer Naturalist 1975; 109:677-699.
61. Bruce RC. Fecundity in primitive plethodontid Salamanders. Evolution 1969; 23:50-54.
62. Crump, ML. Reproductive strategies in a tropical anuran community. Misc Pub! Univ Kansas, Mus Nat Hist 1974; 61:1-68.
63. Salthe SN. Reproductive modes and the number and size of ova in the urodeles. Amer Midi Nature 1969; 81: 467-490.
64. Tilley SG. Size-fecundity relationship and their evolutionary implications in five desmognathus salamanders. Evolution 1968; 22: 806-816.




**Bhagyashree. Panda and Siba Prasad Parida**

65. Church G. Annual and lunar periodicity in the sexual cycle of the Javanese toad, *Bufo melanostictus*, Schneider *Zoologica* 1960a; 44:181-188.
66. Berry PY. The breeding patterns of seven species of Singapore Anura. *J. Amm Ecol* 1964; 33:227-243.
67. Church G. The effect of seasonal and lunar changes on the breeding pattern of the edible Javanese frog, *Rana cancrivora*. *Gravenhorst Treubia* 1960b; 25:215-233.
68. Inger RF and Greenberg B. The annual reproductive pattern of the frog *Rana erythraea* in Sarawak. *Physiol. Zool* 1963; 36(1): 21-33.
69. Inger RF and Bacon JP. Annual reproduction and clutch size in ram forest frogs from Sarawak. *Copeia* 1968; 3:602-606.
70. Dutta SK, Mahapatra P, Mahanty-Hejmadi P. Size analysis and sex ratio of Jerdon's bull frog *Rana crassa* (Anura: Ranidae). *J. Bom. Nat. Hist. Soc* 1992; 88:234-241.
71. Mohanty-Hejmadi P and Dutta SK. Life history of the common Indian tree frog, *Polypedates maculatus* (Anura Rhacophoridae). *J. Bombay Nat. Hist. Soc* 1988; 85(3):512-517.
72. Alcalá AC. Notes on the eggs and egg laying of some amphibians of Negros Island, Philippines. *The Silhman J* 1955; 2(2):103-106.
73. Alcalá AC. Breeding behaviour and early development of frogs of Negros, Philippine Islands. *Copeia* 1962; 679-726.
74. Basu SL. A study of the spermatogenesis of common Indian frog (*Rana tigrina*) treated with testosterone. *Naturwissenschaften* 1962a; 49:188.
75. Basu SL. Seasonal reproductive cycle in the female toad, *Bufo melanostictus*. *Proc Rajasthan Acad Sci* 1962b; 9(1):37-42.
76. Basu SL. Impact of some endo and exogenous factors on the spermatogenesis of common Indian toad, *Bufo melanostictus*. *Folia Biol (Warsaw)* 1962c; 12: 203-210.
77. Basu SL and Mondal A. The annual spermatogenic cycle of the common Indian frog, *Rana tigrina*. *Folia Biol (Warsaw)* 1961a; 9:135-142.
78. Basu SL and Mondal A. Spermatogenesis in normal hypophysectomised toads (*Bufo melanostictus*) following pituitary extract administration. *Naturwissenschaften* 1961b; 48:739-740
79. Basu SL. Effects of testosterone and estrogen on spermatogenesis in *Rana hexadactyla*. *J Expt Zool* 1968; 169:133-142
80. Dutta SK, S Jena, Mohanty-Hejmadi P (1990-1991). Breeding and development of *Ramanella vanegata*. *J Zool Soc India* 1990-1991; 42-43, 55-76.
81. Dutta SK, Mahapatra P and Mohanty-Hejmadi P. Breeding and life history of *Rana crassa* Herpeton effect of predators, competitors, and oviposition sites. *Ecology* 1993; 72: 778-786.
82. Inger RF and Greenberg B. Morphology and seasonal development of sex characters in two Sympatric African toads. *J. Morphol* 1956; 99: 549-574.
83. Kasmathan S and Basu SL (1979). Effect of hormones on spermatogenesis in hypophysectomised *Rana hexadactyla*. (Lesson) *Acta Morphologies Acad Sci, Hung* 1979; 21(3):249-259.
84. Kanamadi RD and Jirankali JS. Testicular activity in the burrowing frog, *Tomopterna breviceps*. *Zool Anz* 1991a; 227:80-92.
85. Kanamadi RD and Jirankali CS. Ovarian activity in the tree frog, *Polypedates maculatus* a qualitative and quantitative study of ovarian cycle and its relation to oviduct and fat body cycle. *Zool! Anz* 1991b; 226:1-14.
86. Kunan T and Saidapur SK. Frequency distribution of cell number in the spermatocytes during the annual testicular cycle in the Indian frog *Rana tigrina*. *J Herpetol* 1983; 17:315-319.
87. Kiyasetuo and Khare MK. Annual breeding cycle and development of *Rhacophorus leucomystax* *Studies in Herpetology*. *Rockez (Ed): Prague* 1986; 417-422.
88. Mohanty-Hejmadi P, Dutta SK and SC Mallick. Life history of Indian frogs II. The marbled balloon frog *Uperodon systoma*. *J Zool Soc* 1979a; 31(1-2) 65-72.
89. Mohanty-Hejmadi P, Dutta SK and Panda S. Life history of the Indian frogs :The burrowing frog. *Rana breviceps*. *J Zool! Soc India* 1979b; 31(1-2) 29-37.



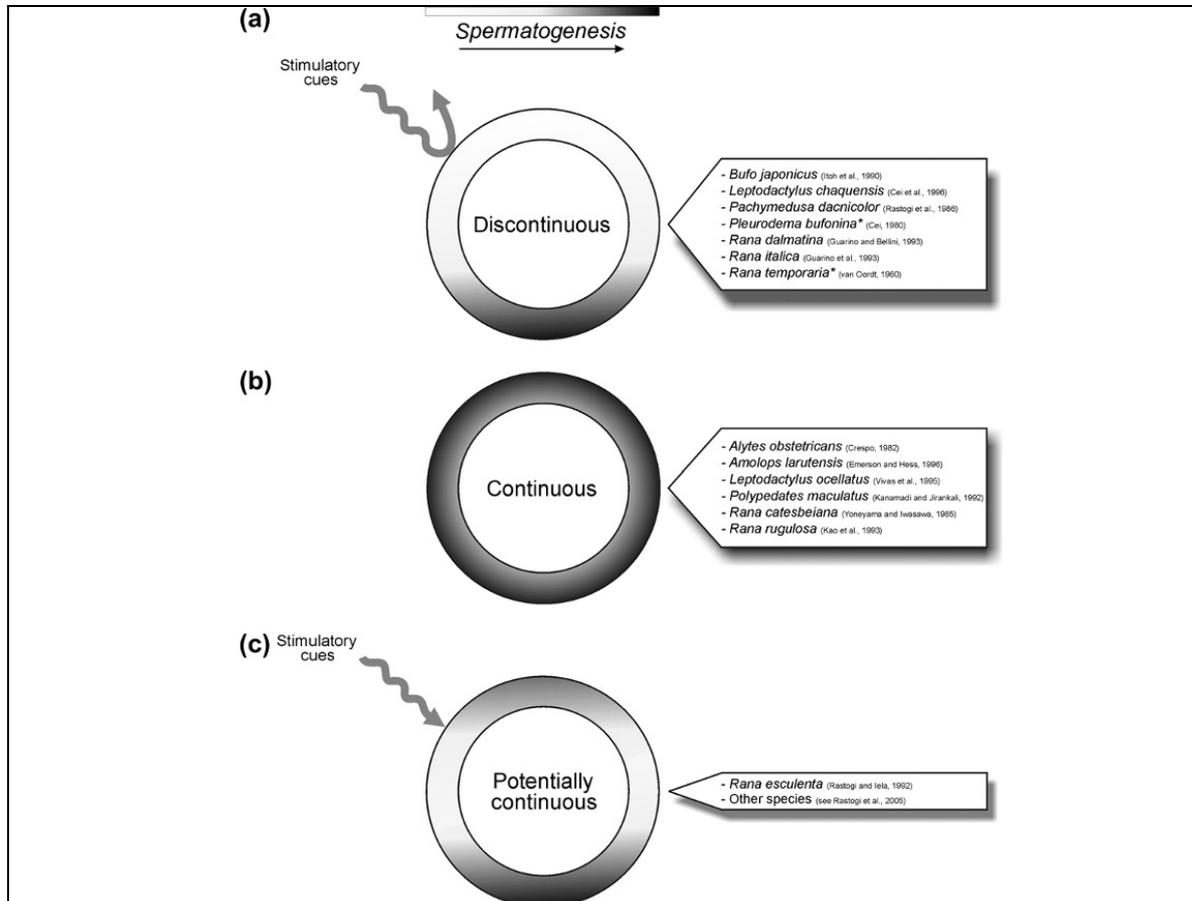
**Bhagyashree. Panda and Siba Prasad Parida**

90. Mohanty-Hejmadi P, Dutta SK and I Khan. Life history of Indian frogs III. The ornate frog, *Microhyla ornata*. J Zool Soc India 1980; 32(1-2) 43-47.
91. Mohanty-Hejmadi P, Nayakand BK and Kanungo J. The reproductive biology of the Indian skipper frog *Rana cyanophlyctis*. Herp Review 1983; 14(1) 11-12.
92. Saidapur SK. Reproductive cycles of amphibians in Reproductive cycles of Indian vertebrates. Saidapur, S K (Ed) .Allied Publ Ltd: New Delhi 1989; 166-224.
93. Ikeda S. Notes on the breeding habit and development of *Rhacophorus schlegeli*. Annot. Zool. Japan 1897; 1: 113:122.
94. Bhaduri JL. Observation on the urinogenital system of the frogs of the genus, *Rhacophorus*, with remarks on their breeding habits. Anat, Anz 1932; 74:336-345.
95. Saidapur SK. Pattern of testicular activity in the Indian Amphibia. Ind Rev Life Sci 1983; 3:157-184.
96. Saidapur SK. Patterns of ovarian activity in Indian amphibians. Indian Rev Life Sci 1986; 6:231-251.
97. Saidapur SK. Reproductive cycles of amphibians in Reproductive cycles of Indian vertebrates. Saidapur, S K (Ed), Allied Publ Ltd: New Delhi 1989; 166-224.
98. Saidapur SK and Nadkarm VB. The occurrence of oocytes in the tests of three amphibians. Karnatak Univ (Science) 1973a; 18:559-564.
99. Resetarits WJ and Wilbur HM. Choice of oviposition site by *Hyla chrysoscelis*: role of predators and competitors. Ecology 1989; 70:220-228.
100. Petranka JW, Hopey ME Jennings BT, Baird SD and Boone SJ. Breeding habitat segregation of wood frogs and American toads: the role of interspecific tadpole predation and adult choice. Copeia 1994: 691-697.
101. Crump ML. Choice of oviposition site and egg load assessment by a tree frog. Herpetologica 1991; 47: 308-315.
102. Spieler M and Linsenmair KE. Choice of optimal oviposition sites by *Hoplobatrachus occipitalis* (Anura: Ranidae) in an unpredictable and patchy environment. Oecologia 1997; 109:184-199.
103. Rastogi RK, Pinelli C, Polese G, Aniello BD and Baccari GC. Hormones and reproductive cycles in anurans Amphibians. Elsevier B.V, Academic Press: 2011; 171-186.
104. Wells KD and Schwartz JJ. The Behavioral Ecology of Anuran Communication. Part of the Springer Handbook of Auditory Research book series SHAR 2007; 28: 45-86.
105. Orton GL. The systematic of vertebrate larvae. Syst Zoo! 1953; 2:63-75.
106. Duellman WE and Trueb L. Biology of Amphibians, McGraw Hill Book Co: New York; 1986.
107. Nodzenszi E and Inger RF. Uncoupling of related structural changes in metamorphosing torrent-dwelling tadpoles. Copeia 1990; 4:1047-1057.
108. Dutta SK and Mohanty-Hejmadi P. Ontogeny of teeth row structure in *Rana tigrina* tadpoles. J Bombay Nat. Hist. Soc 1984; 80(3):517-528.
109. Agarwal SK and Niazi A. Normal table of developmental stages of the Indian bull frog, *Rana tigerina*. Proc. Nat. Acad., Sci. India 1977; 47 (B): 79-92.
110. Altig R and Johnston GF. Guilds of anuran larvae relationships among developmental modes, morphologies and habitats. Herpetol Monogr 1989; 3:81-109.





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**Figure 1: Generalized diagram of the three types of spermatogenic cycle displayed in anuran species [103].**



**Figure 2**



**Figure 3**

**Calling males of some anurans in which vocal communication (Fig.2) *Hyla ebraccata* (Hylidae). (Fig.3) *Hyla microcephala* (Leptodactylidae) has been studied [104].**



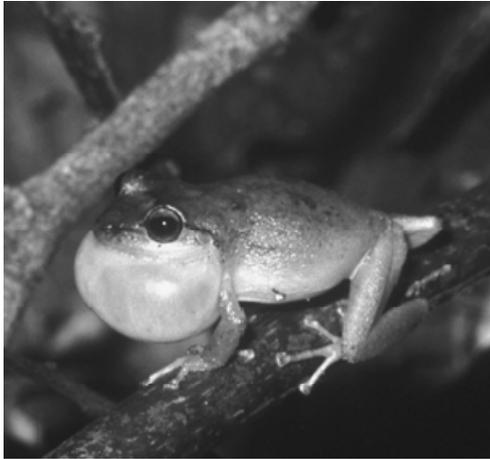


Figure 4

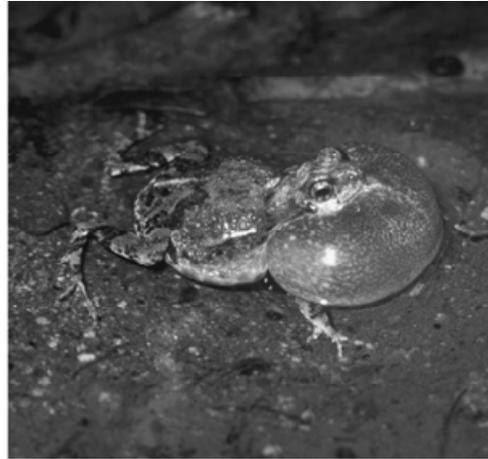


Figure 5

Calling males of *Eleutherodactylus coqui* (Leptodactylidae) (Fig.4) and *Physalaemus pustulosus* (Leptodactylidae) (Fig.5) in which vocal communication has been studied [104].





## A Study on Data Mining Techniques for Cyber Security

V.JeyaKumar\* and S.Deepankumar

Assistant Professor, Department of Computer Science, KG College of Arts and Science, Coimbatore, Tamil Nadu, India.

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### \*Address for Correspondence

**V.JeyaKumar**

Assistant Professor,  
Department of Computer Science,  
KG College of Arts and Science,  
Coimbatore, Tamil Nadu, India.  
E.Mail: jeyakumar28@gmail.com



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### ABSTRACT

Cyber security is that the world that deals with protecting from cyber terrorism. Cyber-attacks include access control violations unauthorized intrusions and denial of service also as insider threat. Security of a data or information system is its vital property especially today when computers are interconnected via internet. Because no system is often absolutely secure the timely and accurate detection of intrusions is important. For this purpose, Intrusion Detection Systems were designed. The IDS together with data processing can provide the safety with next level data processing is that the process of posing queries and extracting patterns, often previously unknown from large quantities of data using pattern matching or other reasoning techniques. This Paper gives the over view of the various data processing techniques which may be utilized in Cyber security for intrusion detection.

**Keywords:** Cyber security, Intrusion Detection System, Data Mining.

### INTRODUCTION

Cyber security cares with protecting computer and network systems from corruption owing to malicious software including Trojan horses and viruses. Data processing for cyber security applications for instance, anomaly detection techniques might be want to detect unusual patterns and behaviours. Data processing or mining is that the process of identifying patterns in large datasets. Data processing techniques are heavily utilized in research project also as in business, mostly to collect statistics and valuable information to improve customer relations and marketing strategies. In this paper, we specialise in data processing application for cyber security. To grasp the mechanism to be adopted so as to safeguard the computers and network, it's imperative to know the kinds of threats that endanger the cyber network.

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**JeyaKumar and Deepankumar****Cyber Security**

Cyber security is about of rules and technologies which are mean to guard our systems, network, and data from unauthorized access, attacks, and unwanted interrupts. they're aim to take care of the confidentiality, integrity, and availability of data and knowledge management systems through various cyber defence systems. To safe the cyber infrastructure against possibly malicious threats, a rising collaborative effort between cyber security professionals and researchers from institutions, private industries, and various agencies has engaged in abusing and designing a spread of cyber defence systems. Cyber security systems are composed of network security systems and host security systems. Each of those has, firewall, antivirus software, and an intrusion detection system (IDS). IDS discover, determine, and identify unauthorized use, duplication, alteration, and destruction of data systems [1].The second line of cyber defence consists of reactive security solutions, like intrusion detection systems (IDSs). IDSs detect intrusions supported the knowledge from log files and network flow, in order that the extent of injury is often determined, hackers are often tracked down, and similar attacks are often prevented within the future. data processing or knowledge discovery (KDD) may be a method want to analyse data from a target source and compose that feedback into useful information. In cyber security data processing techniques are getting used to spot unsure conditions.

**Cyber Terrorism, Threats and Malicious Software**

Now a days internet has allowed for a huge exchange of data. Thus, has created a cyber space during which terrorists can implement attack. Cyber-terrorism, consistent with the O' Leary (2010) is committed through the utilization of cyberspace or computer resources. This use of cyber space leads to there not being simply a physical threat of terrorism. Janczewski, & Colarik (2008) defines cyber terrorism as: "Cyber terrorism means pre-mediated, politically motivated attacks by sub national groups or clandestine agents or individuals against information and computer systems, computer programs, and data that leads to violence against non-combatant targets." Cyber Terrorism is one among the main threat to world now. Over recent decades, it's become apparent that our society is becoming increasingly information technology dependant.

For instance, of banking system. If surprise attack such a system and deplete accounts of funds, then the bank could lose millions or billions of dollars. Crippling the pc system many hours of productivity might be lost, which is ultimately like money loss. Even an easy power outage at work could cause several hours of productivity loss which ends in loss. Therefore, it's imperative that our data system might be secured. Threats can occur from outside or inside a corporation. Malicious software are the codes or procedures or programs which are mean to damage the systems, networks, clients and servers, databases. The most common sorts of this are virus, worms, trojan horses. Intruders try to tap into network and get vital information. It is often a person's or malicious software set by humans.

**Data Mining**

In general, it's a process that involves analysing information, predicting future trends, and making proactive, knowledge-based decisions supported large datasets. It is a process that involves scanning the knowledge, predicting future trends, and making the knowledge-based decisions supported large datasets. Data mining consistent with Silltow (2012) automates the detection of relevant patterns during a database, using defined approaches and algorithms to seem into current and historical data which will then be analysed to predict future trends.

While the term data processing is typically treated as a synonym for Knowledge Discovery in Databases (KDD), it's actually only one of the steps during this process. The main goal of KDD is to get useful and sometimes previously unknown information from large sets of knowledge. Due to the supply of huge amounts of knowledge in cyber infrastructure and increasing number of cyber criminals attempting to realize unauthorized access to the information, there's need of capabilities to address the challenges of cyber security.



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Data mining tools predict future trends and behaviours by reading through database for hidden patterns, learning these behaviours is vital, as they will identify and describe structural patterns which helps to get the knowledge on the idea of that data, and helps the organization to answer the questions that were too time-consuming pervasively. Data mining application for cyber security is that the use of knowledge mining techniques to detect cyber threats. Data mining with the mixture of machine learning is being applied to problems areas like intrusion detection and auditing in cyber security and which is extremely effective technique. In recent years, many IT industry giants like Comodo, Symantec, and Microsoft have started using data processing techniques for malware detection.

**Data Mining Methods for Cyber Security**

This segment labels the different Data Mining methods for cyber security.

**Association Rule**

The association rule mining discovers the relationship among variables in database. Consider an example IF (P AND Q) THEN S. This rule implies that IF P and Q are present, then it is also presence of S. Association rules have metrics that tell how often a given relationship occurs within the data. Association Rule Mining was introduced by Agrawal et al. [2] as how to get interesting co-occurrences in supermarket data. It finds frequent sets of things (i.e., combinations of things that are purchased together in a minimum of N transactions within the database), and from the frequent items sets such as {A, B}, generates association rules of the form:  $A \rightarrow B$  and/or  $A \rightarrow B$ .

**Clustering**

Clustering is employed to assign the similar data object in groups called clusters in order that the objects in one cluster are more almost like one another than objects in other clusters. In simple word this process is employed to spot data items that have similar characteristics. Clustering [4] may be a set of techniques for locating patterns in high-dimensional unlabelled data. The main advantage of clustering for intrusion detection is that it can learn from audit data without requiring the supervisor to supply explicit descriptions of varied attack classes.

**The decision tree technique**

The decision tree may be a tree like structure having leaves which represent the classification and branches which represent the conjunction of features that cause those classifications. Decision tree depends on if-then rules, but it doesn't require parameters and metrics. This simple and interpretable structure allows decision trees to unravel multi-type attribute problems. Decision trees also can manage missing values or noise data. However, they cannot guarantee the optimal accuracy that other machine-learning methods can. [5] The advantages of decision trees are simple implementation.

**The neural network**

Neural Networks are inspired by the brain and composed of organized artificial neurons capable of certain calculations on their inputs [6]. The input data to the first layer activate the neurons of the network whose output is the input to the second layer of neurons in the network. Neural networks re long training times and are therefore more suitable for applications where this is often feasible.

IDS use two kind of Neural Networks, they are

- Multilayered Feedforward NN
- Kohonen's Self-Organizing NN



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These techniques are used to model complex relationships between inputs and outputs and to discover new patterns. The combination of Self organizing map and back propagation neural network supply a very efficient mean for detection of new intrusions.

**Data Mining in Malware Detection**

Data mining is one of the wide method used today for detecting malware. While building a security app, software developers uses the data mining methods to improve the speed and quality of malware detection.

There are three strategies used to detect malwares:

- Anomaly Detection
- Misuse Detection
- Hybrid Detection

Anomaly Detection is the identification of infrequent actions or observations which raise uncertainties by differing significantly from the majority of the data. It contains demonstrating the normal behaviour of a system or network in order to identify deviations from normal usage patterns. Anomaly based detection can also detection the previous unknown attacks and use for defining the signature for misuse detectors. The main problem with anomaly detection is that any deviation from the normal, even if it is a legitimate behaviour, will be reported as an anomaly, thus producing a high rate of false positives.

Misuse Detection is also referred as a signature-based detection, identifies only known attacks based on examples of their signatures. It states to detection of attacks by observing for specific patterns, such as byte sequences in network traffic, or identified malicious instruction sequences used by malware.

Hybrid approach is a combination of anomaly and misuse detection techniques in order to rise the number of detected intrusions while reducing the number of false positives. It does not build any models, but in its place uses information from both harmful and clean programs to create a classifier – a set of rules generated by the data mining algorithm.

**CONCLUSION**

In this paper we have studied the different data mining techniques for cyber security. It is a young interdisciplinary, drawing from areas such as database systems, data warehousing, statistics, machine learning, data visualization, information retrieval, and high-performance computing. Data mining have great potential in place of malware detection tool which allows you to analyse enormous sets of information and extract new knowledge from it. When determining the efficiency of the methods, there is not only one principle but several that need to be taken into account. Depending on a particular Intrusion Detection System some might be more important than other [9]. Another vital aspect data mining for cyber intrusion detection is the importance of the data sets for training and testing the systems. The main advantage of using data mining techniques for detection malicious software is the ability to identify the both known and zero day attacks.

**REFERENCES**

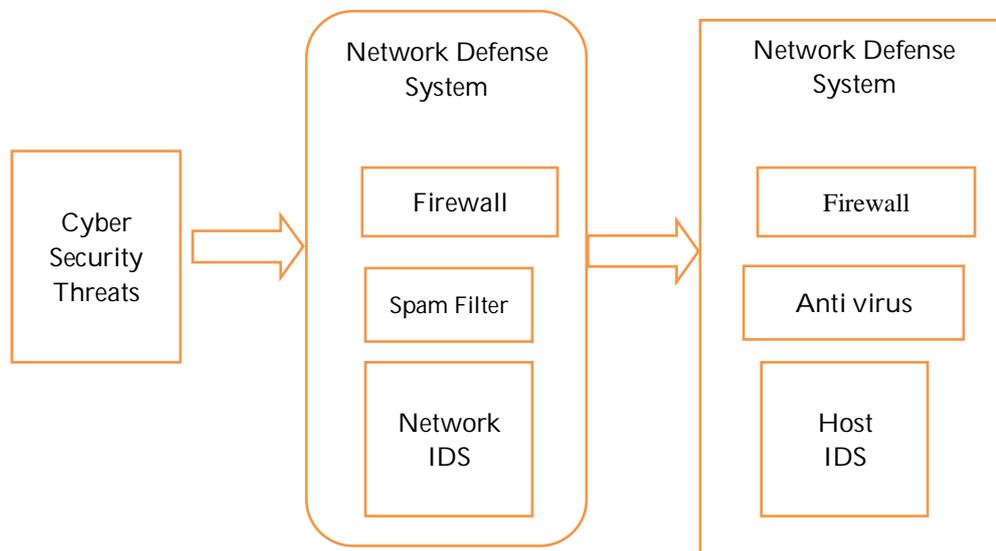
1. A. Mukkamala, A. Sung, and A. Abraham, "Cyber security challenges: Designing efficient intrusion detection systems and antivirus tools," in Enhancing Computer Security with Smart Technology, V. R. Vemuri,Ed. New York, NY, USA: Auerbach, 2005, pp. 125–163.





**JeyaKumar and Deepankumar**

2. R. Agrawal, T. Imielinski, and A. Swami, "Mining association rules between sets of items in large databases," in Proc. Int. Conf. Manage. Data Assoc. Comput. Mach. (ACM), 1993, pp. 207–216.
3. H. Brahmi, B. Imen, and B. Sadok, "OMC-IDS: At the cross-roads of OLAP mining and intrusion detection," in Advances in KnowledgeDiscovery and Data Mining. New York, NY, USA: Springer, 2012, pp. 13–24.
4. K.Jain and R. C. Dubes, Algorithms for Clustering Data. Englewood Cliffs, NJ, USA: Prentice-Hall, 1988.
5. Sumeet Dua and Xian Du "Data Mining and Machine Learning in Cyber security"
6. K. Hornik, M. Stinchcombe, and H. White, "Multilayer feedforward networks are universal approximators," Neural Netw., vol. 2, pp. 359–366, 1989.
7. Bolton, R. and D. Hand, Statistical fraud detection: A review. Statistical Science 17 (3), pp. 235-255, 2002.
8. <https://www.apriorit.com/dev-blog/527-data-mining-cyber-security>



**Fig. 1. Conventional cyber security**





## Functional Food as an Immuno-Modulator

Vadditandra Bhavani<sup>1</sup>, Pradipta Banerjee<sup>2\*</sup> and Preetha Bhadra<sup>3</sup>

<sup>1</sup>Department of Agriculture, Centurion University of Technology and Management, Odisha, India.

<sup>2</sup>Department of Biochemistry and Plant Physiology, Centurion University of Technology and Management, Odisha, India.

<sup>3</sup>Department of Biotechnology, Centurion University of Technology and Management, Odisha, India.

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### \*Address for Correspondence

#### Pradipta Banerjee

Department of Biochemistry and Plant Physiology,  
Centurion University of Technology and Management,  
Odisha, India.

E.Mail: pradipta.banerjee@cutm.ac.in



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### ABSTRACT

Functional foods are the foods that proffer health benefits beyond their nutritional value. Functional foods are known to stimulate the immune system by providing adequate amount of nutrients which will have direct effect on our immune system. Such functional foods or super foods are packed with rich amount of nutrients and antioxidants which can strengthen the immune system. Malnutrition can cause immune dysfunction. Individuals with malnutrition are also prone to severe infection. Therefore; functional food plays an important role in overcoming such malnutrition. Functional foods such as probiotics and prebiotics offer immense immune boosting effects. It is also seen that any effect on the gut microbiota can directly or indirectly can affects the immune system. Probiotics and prebiotics play a very important role in maintaining the gut microbiome ultimately enhancing the immune system. Prebiotic enhances the immunity by increasing the beneficial microbes and reducing the harmful bacteria. Probiotics are also known to modulate the immune system if taken in appropriate amount. Functional food regulates the immune system by enhancing the immune responses and inhibiting the infections. The micronutrients such as vitamin A, C, E and B<sub>6</sub>, folate or B<sub>9</sub>, zinc and iron acts as an immuno-modulator. This review highlights the relationship between the functional food, gut microbiota immunity and their action of mechanism as immuno-boosters.

**Keywords:** Functional foods, mechanism of action, micronutrients, gut microbiota.



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## INTRODUCTION

Functional foods are those fortified, enriched or enhanced foods that will deliver health benefits beyond its nutritional value i.e. essential nutrients (eg: Vitamins and minerals). Nutrients play a major role in the development of strong immune system. Malnutrition impairs the immune system by inhibiting the immune responses, which are essential for the overall health of an individual. Immunity is affected by deficiency of nutrients such as essential amino acids, fatty acids, vitamin A, folic acid, vitamin C, vitamin B<sub>6</sub>, vitamin B<sub>12</sub>, vitamin E, zinc, iron and (Calder & Kew 2002). The intake of micronutrients are essential for the efficient running of the immune system. Micronutrient deficiency affects the innate immune system and adaptive antibody responses (adaptive immune system) resulting in immune dysfunction (Silvia Maggini et al., 2007).

Functions of various nutrients in nervous system (J M Bourre 2006):

1. Vitamin B<sub>1</sub> : It helps in modulating cognitive performance
2. Vitamin B<sub>9</sub>: It helps in preserving brain during aging.
3. Vitamin B<sub>6</sub>: It helps in treatment of premenstrual depression.
4. Vitamin B<sub>12</sub>: It helps in the synthesis of neurotransmitters.
5. Vitamin D: It prevents neuroimmune diseases
6. Vitamin E (alpha toopherol): It helps in protecting nervous membrane.
7. Iron: It helps in producing energy in cerebral parenchyma.

Brain diseases can be cause for immune dysfunction due to deficiency of some micronutrients. Copper and iron plays a major role in immunity and well-being of an individual (Carlos Munoz et al., 2007). The intake of functional foods having immuno modulatory effect packed with various essential minerals and nutrients helps in enhancing the immune system of an individual. The immune system is mainly comprises of innate and adaptive immune system. The adaptive immune system identifies the particular foreign antigen for longer period of time. The T-cells and B-cells (lymphocytes) plays a major part in the adaptive immune system. B-cells will produce the antibodies against the foreign antigen. T-cells helps in controlling immune cells and secretion of cytokines. The innate immune system responds to the pathogen attack by recognizing the specific microbial pattern. NK-cells (Natural killer cells) are the cells responsible for innate immunity, which will eliminate the virus infected cells (Hachimura et al., 2018).

The figure 2 is taken from the review "Hippo Pathway in Mammalian Adaptive Immune system" by Yamauchi and Moroishi published in 2019 which represents the typical mammalian immune system.

### Basic nutrients for immune system

#### Vitamins and minerals

Vitamins are the organic molecule that is essential micronutrients which an organism needs in small amount for proper bodily function and metabolism. It plays a key role in both the immunity i.e. innate and adaptive. Vitamin helps in enhancing immune system by activating the lymphocytes and proliferation of helper cell, production of antibodies and regulation of immune responses. Vitamin A and D acts as an immune-modulator by inhibiting and handling inflammation and autoimmune diseases. Vitamin A improves cytotoxicity and T-cell proliferation and also it can blocks the proliferation of B-cell (Mora et al., 2008). Vitamin A helps in maintaining mucosal surface, production of antibody and in the functioning of T and B lymphocytes. Vitamin A is also very important in maintaining the gut immunity. Vitamin E acts an antioxidant and its reduction can cause a decrease in immune responses (Hachimura et al., 2018). Vitamin B<sub>1</sub> is known to maintain the immune homeostasis (Kunisawa et al., 2015). People with vitamin C deficiency suffered from scurvy which later cured by consuming citrus fruits which are rich in vitamin C. Micronutrients are the essential part of the immune system. Both vitamin C & E together helps in cell membrane protection from free radicals. The higher concentration of the reactive oxygen species can leads to cellular damage, oxidative stress and DNA damage whose concentration can be reduced by action of antioxidants



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(Gombart et al., 2020). Folate mainly decreases the rate of infection, proliferation of T lymphocytes and enhances the immunoglobulin production. Zinc activates the T lymphocytes and iron helps in cell-mediated immune mechanism. Different functions of micronutrients are as follows (Maggini et al., 2018) Table 1.

**Fatty acids**

Fatty acids are carboxylic acids with long aliphatic or straight chain or branched, saturated or unsaturated. The activity of polyunsaturated fatty acids (PUFA) is very much appreciated in enhancing the immune functions. These can influence the inflammatory responses. The intake of omega-3 or omega-6 fatty acid can help in activation of T-cells and neutrophils by improving their functions by incorporating them into membrane phospholipids. Diet rich in monounsaturated fatty acid helps the individual by decreasing the chances or possibility of cardiovascular diseases (Kumar et al., 2019). Fatty acids are known to take part in innate and adaptive immune system by playing a key role. Omega-3 fatty acid is known to regulate the generation of cytokines and chemokines that are essential for the stimulation of macrophages, which plays a major role in innate immune system. It also regulates the inflammation process (Gutiérrez et al., 2019).

**Amino acids**

The human intestine and the gut-associated lymphoid tissue (GALT) play a very important role in the overall mechanism of immune system of an individual. Gut-associated lymphoid tissue helps in the defense of mucosa. Amino acids play an important part in the development of the intestine and GALT. Glutamine helps in maintaining redox homeostasis in lymphocytes and also maintains the mucosal structure in the intestine. L-Arginine is also known to exhibit immuno-modulatory function. It is also observed that amino acids containing sulphur are known to modify immune functions as well. Threonine helps in maintaining the gut barrier function. Therefore, the intake of some amino acids such as glutamine, glutamate, threonine, arginine, methionine and cysteine enhances the immune functions of intestine and GALT immune cells (Ruth and Field 2013).

**Carbohydrates**

These are the biomolecules and compound of carbon, hydrogen and oxygen which occur in the plant food and in some dairy products. Carbohydrates are also known as saccharides because their basic components are sugar. It acts as a reservoir of energy. There are some carbohydrates which are involved in immunity. Polysaccharides are known to activate the immune responses by enhancing the production of cytokine and chemokines and it also helps in proliferation of the cell. They can activate the T-cells and innate immune cells (Ibrahim kankia 2015). Higher intake of carbohydrates can cause hyperglycemia, metabolic syndromes and dysfunction of immune system. Dietary fiber can act as an anti-inflammatory agent and it feeds on gut bacteria, *Bifidobacterium* and *Bacteriodes*. Dietary polysaccharides are known to improve the health in gut microbiome, which will eventually improve the nutrition, stimulates the immune system and resistance to pathogens. Glucose, fructose and sucrose provides energy as well as some essential calories in an individual. Flavonoid glycosides will act as an antioxidant and anti-inflammatory agent (Smith et al., 2015).

**Carotenoid**

Carotenoid is the colorful pigments present in various fruits and vegetables. Vegetables and fruits containing carotenoids such as lycopene, lutein,  $\beta$ -carotene, and zeaxanthin are known to exhibit many health benefits. Food rich in  $\beta$ -carotene are well-known for decreasing the incidence of various types of chronic diseases. Carotenoids in lower rate can cause immunodeficiency in GALT immune system (Toti et al., 2018). Carotenoid are recognized to lower the chances of breast cancer and cervical cancer in women. Lycopene has many properties such as inhibitory agent as antioxidant agent, anti-inflammatory agent, anti-mutagen, anti-carcinogenic & cardio protective. Carotenoid helps in stimulating the immune responses through proliferation of lymphocytes, releasing cytokines and phagocytosis. Curcumin which is a carotenoid, it can act as an Immunomodulatory agent by stimulating the adaptive immunity through T-cells proliferation.  $\beta$ -carotene is known to be very effective in reducing the breast



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cancer. Carotenoids are also known to reduce the various cardiovascular diseases by decreasing the blood pressure, reducing cytokines, reducing inflammation and by stimulating insulin sensitivity in muscles, adipose tissue and liver (Milani et al., 2016).

**Examples of immune boosting functional foods**

A functional food mainly helps in reducing the risk of diseases and also provides health benefits beyond its nutritional value. Functional food can be classified according to the active ingredients present in them i.e. vitamins, minerals, fatty acids, carbohydrates, amino acids and carotenoids or as medicines which can be used in treating microbial diseases such as prebiotics and probiotics (Ashaolu 2020). The criteria for food to be considered as functional food according to Japanese FOSHU (Foods for Specified Health Uses) are as follows (Varela et al., 2002):

1. It should be taken as normal diet on daily basis.
2. It should stimulate the immune system.
3. It should prevent the various microbial diseases.
4. Ageing process should also be slowed down with functional food.
5. It should control both physical and psychological factors.

The components which can be added to food are as follows:

1. Probiotics: These are the live microbial food taken in appropriate quantities which provides health benefits on consumption.
2. Prebiotics: These are the indigestible materials that provide a beneficial physiological effect for the host by enhancing favorable growth of limited number of beneficial indigenous bacteria.
3. Synbiotics: It will act as both prebiotic and probiotic.
4. Nutrients: It includes dietary fibers, vitamins, minerals, carotenoids, fatty acids, amino acids and carbohydrates.

**Examples of immune boosting functional foods****Tomato**

It is a rich source of vitamin B9 commonly called as folic acid which reduces the incidence of microbial infections due to its antimicrobial property and it can also act as an Immunomodulatory agent (Fernández et al., 2019). It also contains vitamins A, B<sub>6</sub> and C and a modest amount of magnesium as well which will act as an anti-inflammatory agent. The bioactive compound present in tomato i.e. lycopene is known to lower the chances of prostate cancer (Clare Hasler 2002). Carotenoids are the potent antioxidant which can help in treating various cardiovascular diseases. It is also seen that increase in the intake of tomatoes is known to lower the possibility of cardiovascular diseases (Volker Böhm 2018). Lycopene is known to exhibit more effective antioxidant property as compared to alpha-tocopherol and  $\beta$ -carotene. It will also act as a cardioprotective agent (Mozos et al., 2018).

**Green leafy vegetables**

Green leafy vegetables are very beneficial for the overall health of an individual as it is rich in vitamins, minerals, carbohydrates and carotenoids. Green vegetables like spinach, broccoli, cauliflower and kale are rich in vitamin C which is very beneficial for the immune system in case of elderly people. Green leafy vegetables are abundant source of vitamin E, which is necessary for maintaining overall health of an individual mainly in elderly people. It will act as a potent antioxidant which provides protection against infection, harmful bacteria and viruses. It also conserves humoral and cell-mediated immunity. Covid-19 is known to mainly affect the individual with lower immunity, so one should restore his/her immunity by consuming such functional foods abundant in vitamins like vitamin C, D&E, minerals like magnesium and zinc and by drinking plenty of water (Arshad et al., 2020). Vitamin E (tocopherol) is one of the greatest effective vitamins well-known to boost the immune system. Vitamin E deficiency affects the cell-mediated immunity and antibody production. It reduces the production of reactive oxygen species (ROS) and oxidation which have direct influence on the functioning of the immune system. Vitamin E is also known to suppress



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respiratory infections such as *Streptococcus pneumonia*, asthma and influenza by boosting the innate immunity (Lewis et al., 2018).

**Garlic and onion**

These are well known to boost the immune system. Garlic is rich in vitamin C and also it is an antimicrobial agent. Garlic is also very effective against various types of infection caused by bacteria, virus and fungi and it exhibit anticancer properties as well (Mirabeau & Samson 2012).It will exhibit anti-inflammatory properties. Garlic maintains the homeostasis in the immune system by enhancing cytokine secretion, increasing phagocytosis, activating macrophage and promoting production of immunoglobulin. Due to presence of fructooligosaccharide in garlic, it exhibits immunomodulatory property (Arreola et al., 2015).Onion maintains the balance of bacteria in gut eventually benefiting the immune system of an individual. Onion is also a strong powerful antioxidant. Red onions are known to decrease the risk of heart diseases, certain cancer including stomach & colorectal and diabetes due to presence of anthocyanins. It will act as an antibacterial agent against harmful bacteria such as *E.coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus cereus* (Sharma et al., 2018).

**Cereals (brown rice, wheat, oat, buckwheat, flaxseed and barley)**

Cereals are rich in vitamins, minerals, antioxidant, proteins and it also provides dietary fibre. Cereals can also be used for the development and growth of probiotic microbes such as lactobacilli and bifid bacteria as fermented substrate (Das et al., 2011).The outer bran layer of cereals is known to be rich in vitamin B and phytonutrients such as flavonoids and indoles. Endosperm is mainly rich in carbohydrates and germ layer is packed with minerals like iron and zinc. Cereals can reduce the risk of cardiovascular diseases and cancers. It can also helps in maintaining gastrointestinal health. There are various phytochemicals present along with antioxidants in the bran layer of brown rice. The main reasons for the less consumption of brown rice over normal rice is its appearance, limited, more cooking time and less awareness regarding its nutritional value (Ravichanthiran et al., 2018).Buckwheat has numerous benefits on the health as it reduces the cholesterol, hypertension and it will also act as an anti-inflammatory as well as an antioxidant agent (Giménez et al., 2015).Flaxseed is the best source of omega-3 fatty acid for vegetarians. It also a rich source of linolenic acid, protein, fibers and phenols and amino acids such as arginine, aspartic and glutamic acid. It will also act as an effective antifungal agent against *Alternaria solani*, *Candida albicans* and *Aspergillus flavus*. Therefore, flaxseed is considered as a functional food due to presence of some bioactive compounds (Kajla et al., 2014).

**Milk and dairy products (Yogurt and cheese)**

Milk is the most abundant source of vitamin A (retinol), vitaminB<sub>12</sub>and minerals. The intake or consumption of dairy products is known to lower the chances of higher blood pressure, stroke and colon cancer. Milk also helps in maintaining immune system in case of babies (Mohammed et al., 2017).Vitamin A and D are necessary for stimulating the mucosal immune system. The consumption of milk known to treat the respiratory tract infection, asthma, hay fever and allergies (Perdijk et al., 2018).It also helps in strengthening both the innate and adaptive immune system as it contains antibodies. Yogurt is also known to stimulate the immune system due to presence of bacteria in it. It enhances the immune responses of gut-associated lymphoid tissue (GALT) and systemic immune system (Meydani & Ha 2000).Cheese can enhance the immune system as well in case of elderly people as it can act as a carrier for probiotic bacteria which is necessary for overall health restoration (Shaikh 2010).

**Pulses and legumes**

Pulses are the superior source of protein, vitamins, minerals, omega-3 fatty acid & dietary fibre. Pulses such as beans and lentils exhibits antioxidant property which helps in reducing the production free radicals which are harmful for the body. Lentil contains a mineral i.e. selenium which helps in decreasing the rate of tumor growth. Legumes are good source of proteins and amino acids such as lysine and methionine as compared to cereals. Saponins are also present in all the legumes which help in preventing cancers, higher cholesterol and high blood glucose (Martin-Cabrejas 2019).



**Vadditandra Bhavani et al.****Citrus fruits**

Citrus fruits are the abundant source of vitamin C (ascorbic acid) which plays a major part in modulating the immune system by encouraging the generation of more white blood cells which helps in preventing infections. Citrus fruits like grapefruit, lime, lemon and oranges are good for health.

**Apples**

Apples are rich source of dietary flavonoids and antioxidants and it helps in preventing chronic diseases. Apples can prevent liver and colon cancer due to antioxidant property. The bioactive compounds present in apple are catechins, epicatechin, procyanidins and phloridzin which are responsible for its antioxidant property (Boyer & Liu 2004).

**Mango**

Mango is rich in vitamins A, B<sub>6</sub>, C and E, minerals, dietary fiber and polyphenolic flavonoids such as  $\beta$ -carotene,  $\alpha$ -carotene and  $\beta$ -cryptoxanthin. It helps in restricting the cardiovascular diseases and cancer. The vitamin C present in mango is mainly responsible for boosting the immune system in an individual (Board 2021).

**Effects of functional food on immune system**

Functional foods helps in maintaining well-being of an individual, preventing certain chronic diseases and providing numerous health benefits contributing in enhancing the immune responses. Vitamins, minerals, dietary fibers, amino acids, fatty acids and carotenoids have a huge effect on the functioning of the immune system (López-Varela et al., 2002; Hachimura et al., 2018). There are several factors that are responsible for weakening of the immune system such as aging, pollutants in the environment, obesity, chronic diseases, malnutrition, irregular sleep pattern and stress which can depress the immune responses of an individual (Nutrition and immunity 2021). Gastrointestinal tract is known to contribute 70% of the immune responses and functional food plays an important role in maintaining the gut microflora via the means of probiotics. Probiotics are the live microbial food such as yogurt, kimchi, cheese and pickles stimulates the immune system by antimicrobial activity, enhancing defense mechanism and activating metabolic enzymes (Shandilya 2017). It exhibits excellent immune boosting ability. Intake of probiotics such *Lactobacillus* and *Bifidobacterium* improved the immune phagocytosis. The consumption of yogurt increases the amount of beneficial bacteria in the gut as the result cytokine production is boosted (López-Varela et al, 2002). Probiotics can also prevent gastrointestinal diseases such as irritable bowel syndrome and diarrhoeas. Prebiotics can also be used as a synergist with probiotics called as synbiotics as it helps in maintaining the balance of beneficial bacteria in the gut eventually enhancing the immune system (Markowiak & Śliżewska 2017).

**Mode of action of functional food as immuno-boosters (Ashaolu 2020)****Action mechanism of probiotics**

1. pH reduction in the intestine by producing organic acids.
2. Probiotic bacteria will compete with other harmful microbes for food and nutrition preventing colonization of pathogenic bacteria as a result harmful bacteria growth is terminated.
3. Production of antimicrobials such as hydrogen peroxide and bacteriocins against harmful pathogenic bacteria both gram-positive and gram-negative by direct interaction with gut microbes. The low molecular weight bacteriocins are antimicrobial in nature. It also leads to the production of acetic and lactic acid which can inhibit *Salmonella* spp.
4. Host-probiotic relationship is mediated via pilli, mucin-binding protein and tool-like receptor ligands.
5. Production and activation of metabolic enzymes and antibodies for enhancing defensive mechanism against pathogens.
6. Epithelial barriers are maintained via secretion of mucin which helps in maintaining acidity in intestine and it also provides protection against gastrointestinal diseases.
7. Immunostimulation by reducing the inflammation and phagocytosis. Probiotics exhibit an important role in innate and adaptive immunity along with monocytes and lymphocytes. Probiotic bacteria can use capsule and fimbriae as driving force for Immunomodulation.



**Vadditandra Bhavani et al.****Action mechanism of prebiotics (Ashaolu 2020)**

The most common prebiotics are fructo-oligosaccharide (FOS), galacto-oligosaccharides (GOS) and xylo-oligosaccharides (XOS) which cannot be digested by mammalian enzymes but it can be used by gut bacteria like lactobacilli and bifidobacteria.

1. Maintain the defense mechanism by increasing the phagocytic and natural killer cell activity resulting in immunomodulation. Therefore, risk of infections is also minimized via production of antimicrobials.
2. Mineral absorption is improved.
3. Immune cell responses are modulated by the action of prebiotics.
4. Improved bowel functions resulting in prevention of gastrointestinal diseases.

**Functional food and gut microbiota**

The gut health is maintained through epithelial barriers in the intestine and gut microbiota which will have direct effect on the immune system. The components of functional foods such as probiotics, prebiotics and synbiotics have beneficial effect on the gut health and overall well-being of an individual (Wan et al., 2018). Functional food components like dietary fiber and polyunsaturated fatty acids can limit the development of pathogenic bacteria like *Helicobacter pylori*. The consumption of functional food maintains the normal metabolism via reducing the bacterial translocation, decreasing inflammation, maintaining intestinal epithelium and helping in regulation of T-cells while the intake of unhealthy fast foods such as fried ones, saturated fats and foods with excessive salt and sugar damages the intestinal epithelium through increased bacterial translocation and inflammation leading to higher chances of colorectal cancer (Mendonça et al., 2018). The maximum number of microbes are present in the distal region of the digestive tract where they help in maintaining the overall health of the host by synthesizing the essential vitamins and amino acids that are essential for stimulating the immune system of an individual. The probiotic microbes like bifidobacteria present in the intestine breakdown the prebiotic food into short chain fatty acids which are important for maintaining intestinal barriers. These short chain fatty acids are directly engaged in the inflammation of the gut. Lactobacilli will also breakdown the prebiotics into short chain fatty acids resulting in anti-inflammation and anti-cancer activities. The plant proteins are preferred more over the animal protein as it increases the number of beneficial bacteria like bifidobacteria and lactobacilli leading to more production of short chain fatty acids thereby decreasing the inflammation and strengthening the gut barrier. The intake of animal proteins increases the number of harmful bacteria like *Bacteriodes spp.*, *Bilophila spp.*, *Ruminococcus spp.* and *Alistipes spp.* resulting in decreased level of short chain fatty acids causing cardiovascular diseases and gastrointestinal diseases like irritable bowel syndrome (Singh et al., 2017).

**CONCLUSION**

The intake functional foods therefore can enhance the immune system as well as preventing various types of diseases if consumed in appropriate amount because excess amount of nutrients can cause over-activation of the immune responses leading to chronic and autoimmune diseases. This review mainly explains how various components of functional food play an important role in managing various kinds of diseases and also providing health benefits to an individual. Functional foods such as probiotics and prebiotics can be used as an alternative to antibiotics in preventing and treating various microbial diseases. The requirement of various nutrients for immune modulation can also be fulfilled with the intake of various types of functional foods like cereals, legumes, dairy products, fruits and beverages. Therefore, diet plays a major key role in affecting the metabolic and cardiovascular health.

**REFERENCES**

1. Calder, P.C., & Kew, S. (2002). The immune system: a target for functional foods? The British journal of nutrition, 88 Suppl 2, S165-S177. <https://doi.org/10.1079/BJN2002682>





**Vadditandra Bhavani et al.**

2. Maggini, S., Wintergerst, E.S., Beveridge, S., & Hornig, D.H. (2007). Selected vitamins and trace elements supports immune function by strengthening epithelial barriers and cellular and humoral immune responses. *The British journal of nutrition*, 98 Suppl 1, S29-S35. <https://doi.org/10.1017/S0007114507832971>
3. Bourre J.M. (2006). Effects of nutrients (in food) on the structure and function of the nervous system: update on dietary requirement for brain. Part 1: micronutrients. *The journal of nutrition, health & aging*, 10(5), 377-385
4. Munoz, C., Rios, E., Olivos, J., Brunser, O., & Olivares, M. (2007). Iron, copper and immunocompetence. *The British journal of nutrition*, 98 Suppl 1, S24-S28. <https://doi.org/10.1017/S0007114507833046>
5. Mora, J.R., Iwata, M., & Von Andrian, U.H. (2008). Vitamin effects on the immune system: Vitamin A and D take centre stage. *Nature Reviews Immunology*, 8(9), 685-698. doi:10.1038/nri2378
6. Hachimura, S., Totsuka, M., & Hosono, A. (2018). Immunomodulation by food: Impact on gut immunity and immune cell function. *Bioscience, Biotechnology, and Biochemistry*, 82(4), 584-599, doi:10.1080/09168451.2018.1433017
7. Kunisawa, J., Sugiura, Y., Wake, T., Nagatake, T., Suzuki, H., Nagasawa, R., Shikata, S., Honda, K., Hashimoto, E., Suzuki, Y., Setou, M., Suematsu, M., & Kiyono, H. (2015). Mode of Bioenergetic Metabolism during B Cell Differentiation in the Intestine Determines the Distinct Requirement for Vitamin B1. *Cell reports*, 13(1), 122-131. <https://doi.org/10.1016/j.celrep.2015.08.063>
8. Gombart, A.F., Pierre, A., & Maggini, S. (2020). A Review of Micronutrients and the immune system-Working in Harmony to Reduce the Risk of Infection. *Nutrients*, 12(1), 236. doi:10.3390/nu12010236
9. Maggini, S., Pierre, A., & Calder, P. (2018). Immune function and micronutrient requirements change over the life course. *Nutrients*, 10(10), 1531. doi:10.3390/nu10101531
10. Kumar, N.G., Contraifer, D., Madurantakam, P., Carbone, S., Price, E.T., Van Tassell, B Wijesinghe, D.S. (2019). Dietary bioactive fatty acids as modulators of immune functions: Implications on human health. *Nutrients*, 11(12), 2974. doi:10.3390/nu11122974
11. Gutiérrez, S., Svahn, S.L., & Johansson, M.E. (2019). Effects of omega-3 fatty acids on immune cells. *International Journal of Molecular Sciences*, 20(20), 5028. doi:10.3390/ijms20205028
12. Ruth, M.R., & Field, C.J. (2013). The immune modifying effects of amino acids on gut-associated lymphoid tissue. *Journal of Animal Science and Biotechnology*, 4(1). doi:10.1186/2049-1891-4-27
13. Kankia, H.I. (2015). Immunomodulatory effects of carbohydrates and advanced glycation end products. *Biotechnology and Molecular Biology Reviews*, 10(1), 1-11. doi:10.5897/bmbr2014-0237
14. Smith, R., Tran, K., Richards, K., & Luo, R. (2015). Dietary carbohydrates that modulate the immune system. *Clinical Immunology, Endocrine & Metabolic drugs*, 2(1), 35-42. doi:10.2174/221270700201151216151927
15. Toti, E., Chen, C.O., Palmery, M., Villano Valencia, D., & Peluso, I. (2018). Non-Provitamin a AND Provitamin a carotenoids as Immunomodulators: Recommended Dietary Allowance, therapeutic index, or PERSONALIZED NUTRITION? *Oxidative Medicine and Cellular longevity*, 2018, 1-20. doi:10.1155/2018/4637861
16. Milani, A., Basirnejad, M., Shahbazi, S., & Bolhassani, A. (2016). Carotenoids: Biochemistry, pharmacology and treatment. *British Journal of Pharmacology*, 174(11), 1290-1324. doi:10.1111/bph.13625
17. Ashaolu, T.J. (2020). Immune boosting functional foods and their mechanisms: A critical evaluation of probiotics and prebiotics. *Biomedicine & Pharmacotherapy*, 130, 110625. doi:10.1016/j.biopha.2020.110625
18. López-Varela, S., González-Gross, M., & Marcos, A. (2002). Functional foods and the immune system: A review. *European Journal of Clinical Nutrition*, 56(S3). doi:10.1038/sj.ejcn.1601481
19. Hasler, C.M. (2002). Functional foods: Benefits, concerns And Challenges-A position paper from the American Council on science and health. *The Journal of Nutrition*, 132(12), 3772-3781. doi:10.1093/jn/132.12.3772
20. Böhm, V. (2018). Lycopene, Tomatoes, and Cardiovascular Diseases. *Lycopene and Tomatoes in Human nutrition and Health*, 51-68. doi:10.1201/9781351110877-4
21. Mozos, I., Stoian, D., Caraba, A., Malainer, C., Horbańczuk, J.O., & Atanasov, A.G. (2018). Lycopene and Vascular health. *Frontiers in Pharmacology*, 9. doi:10.3389/fphar.2018.00521
22. Fernández-Villa, Aguilar, & Rojo. (2019). Folic acid Antagonists: Antimicrobial And Immunomodulating mechanisms and applicatons. *International Journal of Molecular Sciences*, 20(20), 4996. doi:10.3390/ijms20204996





**Vadditandra Bhavani et al.**

23. Arshad,M.S.,Khan,U.,Sadiq,A.,Khalid,W.,Hussain,M.,Yameen,A Rehana,H.(2020).Coronavirus disease(covid-19) and immunity booster green foods: A mini review. Food science & Nutrition,8(8),3971-3976. doi:10.1002/fsn3.1719
24. Lewis,E.D.,Meydani,S.N.,&Wu,D.(2018).Regulatory role of vitamin e in the immune system and inflammation.IUBMB Life,71(4),487-494.doi:10.1002/iub.1976
25. Mirabeau, T., & Samson, E. (n.d.).Effect of allium cepa and allium sativum on some immunological cells in rats. Retrieved May 26, 2021,from <https://www.ajol.info/index.php/ajtcam/article/view/81430>
26. Arreola,R.,Quintero-Fabián,S.,López-Roa,R.I.,Flores-Gutiérrez,E.O.,Reyes-Grajeda,J.P.,Carrera-Quintanar,L.,&Ortuno-Sahagun,D.(2015).Immunomodulation and anti-inflammatory effects of garlic compounds. Journal of Immunology Research,2015,1-13.doi:10.1155/2015/401630
27. Sharma, K., Mahato, N., & Lee, Y.R. (2018).Systematic study on active compounds as antibacterial and antibiofilm agent in aging onions. Journal of Food and Drug Analysis,26(2),518-528.doi:10.1016/j.jfda.2017.06.009
28. Das, A., Raychaudhuri, U., & Chakraborty, R. (2011).Cereal based functional food of Indian SUBCONTINENT: A review. Journal of Food Science and Technology,49(6),665-672.doi:10.1007/s13197-011-0474-1
29. Ravichanthiran,K.,Ma,Z.,Zhang,H.,Cao,Y.,Wang,C.,Muhammad,S .Pan,B.(2018).Phytochemical profile of brown rice and ITS Nutrigenomic Implications.Antioxidants,7(6),71.doi:10.3390/antiox7060071
30. Giménez-Bastida, J.A., & Zieliński, H. (2015).Buckwheat as a functional food and its effects on health. Journal of Agricultural and Food Chemistry,63(36),7896-7913.doi:10.1021/acs.jafc.5b02498
31. Kajla, P., Sharma, A., & Sood, D.R. (2014).Flaxseed-a potential functional food source. Journal of Food Science and Technology,52(4),1857-1871.doi:10.1007/s13197-014-1293-y
32. MAA,G.(2017).Health benefits of milk and functional dairy products.MOJ Food Processing; Technology, 4(4). doi:10.15406/mojfpt.2017.04.00099
33. Perdijk,O., Van Splunter, M.,Savelkoul,H.F.,Brugman,S.,& Van Neerven,R.J.(2018).Cow's milk and immune function in the RESPIRATORY TRACT: Potential mechanisms. Frontiers in Immunology, 9.doi:10.3389/fimmu.2018.00143
34. Meydani, S.N., & Ha, W. (2000).Immunologic effects of yogurt. The American Journal of Clinical Nutrition,71(4),861-872.doi:10.1093/ajcn/71.4.861
35. Shaikh, T. (2010, May 14).Cheese boosts immune system of the elderly. Retrieved May 26, 2021,from [https://edition.cnn.com/2010/HEALTH/diet.fitness/05/14/finland.cheese.immune.system.elderly/index.html#:~:text=London%2C%England%20\(CNN\)%20--,as%20beneficial%20to%20immune%20health](https://edition.cnn.com/2010/HEALTH/diet.fitness/05/14/finland.cheese.immune.system.elderly/index.html#:~:text=London%2C%England%20(CNN)%20--,as%20beneficial%20to%20immune%20health).
36. Health benefits of pulses.(n.d.).Retrieved May 26, 2021,from <https://www.icicilombard.com/blog/health-insurance/hi/health-benefits-of-pulses>
37. Martin-Cabrejas, M.A. (2019).CHAPTER 1.LEGUMES: An overview. Food Chemistry, Function and Analysis,1-18.doi:10.1039/9781788015721-00001
38. Boyer, J., & Liu, R.H. (2004).Apple phytochemicals and their health benefits. Nutrition Journal,3(1).doi:10.1186/1475-2891-3-5
39. Board, M. (2021, April 09).Mango benefits-health benefits of MANGO: National Mango Board. Retrieved May 26, 2021, from <https://www.mango.org/blog-mango-benefits/#:~:text=Combat%20colds%20with%20mangos!,and%20helps%20in%20wound%20healing>.
40. López-Varela, S., González-Gross, M., & Marcos, A. (2002).Functional foods and the immune system: A review. European Journal of Clinical Nutrition,56(S3).doi:10.1038/sj.ejcn.1601481
41. Hachimura,S.,Totsuka,M.,& Hosono,A.(2018).Immunomodulation by food: Impact on gut immunity and immune cell function.Bioscience,Biotechnology, and Biochemistry,82(4),584-599.doi:10.1080/09168451.2018.1433017
42. Nutrition and immunity.(2021,January 27). Retrieved May 27, 2021,from <https://www.hsph.harvard.edu/nutritionsource/nutrition-and-immunity/>
43. Shandilya, U.K. (2017).Functional foods and their benefits: An overview. Journal of Nutritional Health & Food Engineering,7(4).doi:10.15406/jnhfe.2017.07.00247
44. Effects of Probiotics, Prebiotics, and Synbiotics on Human Health. (2017). Nutrients,9 (9), 1021. doi:10.3390/nu9091021





**Vadditandra Bhavani et al.**

45. Wan, M.L., Ling, K.H., El-Nezami, H., & Wang, M.F. (2018). Influence of functional food components on gut health. *Critical Reviews in Food Science and Nutrition*, 59(12), 1927-1936. doi:10.1080/10408398.2018.1433629
46. Mendonça, L.A., Dos Santos Ferreira, R., De Cássia Avellaneda Guimarães, R., De Castro, A.P., Franco, O.L., Matias, R., & Carvalho, C.M. (2018). The complex puzzle of interactions among Functional Food, gut Microbiota, and colorectal cancer. *Frontiers in Oncology*, 8. doi:10.3389/fonc.2018.00325
47. Singh, R.K., Chang, H., Yan, D., Lee, K.M., Ucmak, D., Wong, K., Liao, W. (2017). Influence of diet on the gut microbiome and implications for human health. *Journal of Translational Medicine*, 15(1). doi:10.1186/s12967-017-1175-y
48. Yamauchi, T., & Moroishi, T. (2019). Hippo pathway in mammalian adaptive immune system. *Cells*, 8(5), 398. doi:10.3390/cells8050398

**Different functions of micronutrients are as follows (Maggini et al., 2018).**

Micronutrients	Function
Vitamins (A, C, D, E, B <sub>6</sub> , B <sub>12</sub> & folate) and minerals (iron and zinc)	Contributes to integrity
Vitamins (C & E) and minerals (iron, zinc, copper, selenium and magnesium)	Oxidative burst and self-protection
Vitamins (A, D, C, E, B <sub>6</sub> , B <sub>12</sub> & folate) and minerals like zinc, iron, copper, selenium and magnesium.	Differentiation and proliferation of innate immune-cells
Vitamins (A, D & C) and minerals such as zinc, iron, copper and selenium	Antimicrobial activity
Vitamins (A, D, C, E, B <sub>6</sub> , B <sub>12</sub> & folate) and minerals like zinc, copper, selenium and magnesium	Production of antibodies
Vitamins (A, D, C, E, B <sub>6</sub> & B <sub>12</sub> ) and minerals such as zinc, iron, copper and selenium	Proliferation and Differentiation of T-cells
Vitamins D, E and B <sub>6</sub>	Inhibitory activity
Vitamins (A, D, C, E, B <sub>6</sub> , B <sub>12</sub> & folate) and minerals such as zinc, iron, copper and selenium	Cell-mediated immunity
Vitamins (A, C, E & B <sub>6</sub> ) and minerals (zinc, iron, copper, selenium and magnesium)	Regulation of inflammation

**Table 2: Different sources of food which provides basic nutrients**

Basic nutrients	Sources
Vitamins such as A, D, E, C, B <sub>6</sub> , B <sub>9</sub> or folate and B <sub>12</sub>	Carrot, tomato, papaya, mango, eggs, milk, cod liver oil, green leafy vegetables, citrus fruits, amla, guava, cereals, peanuts, soyabean, meat, banana, fish and spirulina
Minerals (zinc, iron, copper, selenium and magnesium)	Meat, shellfish, fish, legumes, nuts, eggs, milk, yogurt, whole grains, seeds of pumpkin, watermelon and hemp, cereals, spinach, peas, broccoli, dark chocolates, avocados
Fatty acids (omega-3)	Fishes such as salmon, mackerel, shrimp, sardines and tuna, nuts, seeds, seaweed and algae
Amino acids (Glutamine, arginine, threonine, methionine and cysteine)	Pumpkin seeds, sesame seeds, sunflower seeds, nuts, fish, chicken, beef, lamb, soyabean, dairy products
Carbohydrates	Whole grains, fruits, potatoes, dairy products, green leafy vegetables, nuts and seeds
Carotenoid	Tomato, carrots, spinach, broccoli, bell pepper, orange and mango





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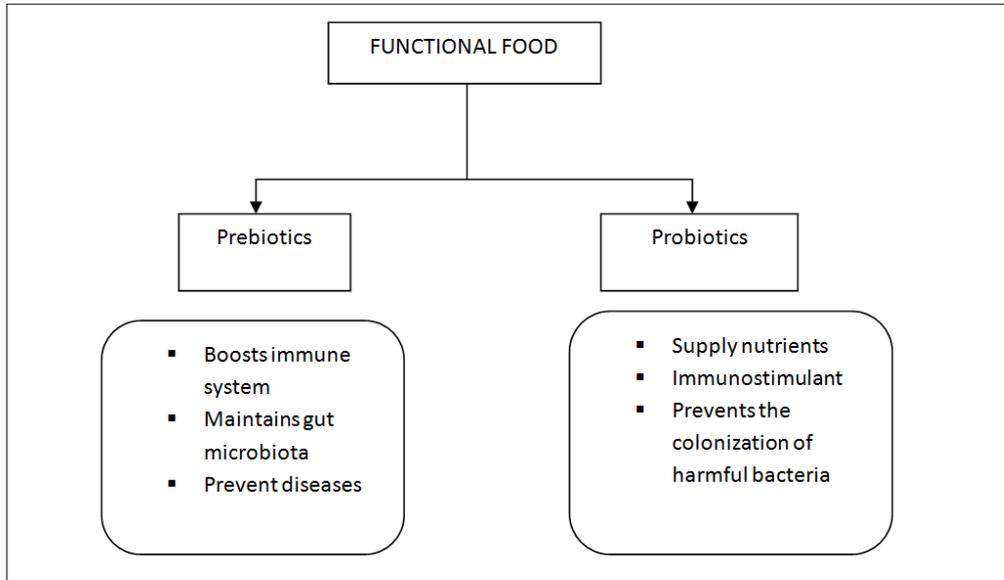


Figure 1: The flowchart describes the classification of functional food and their functions

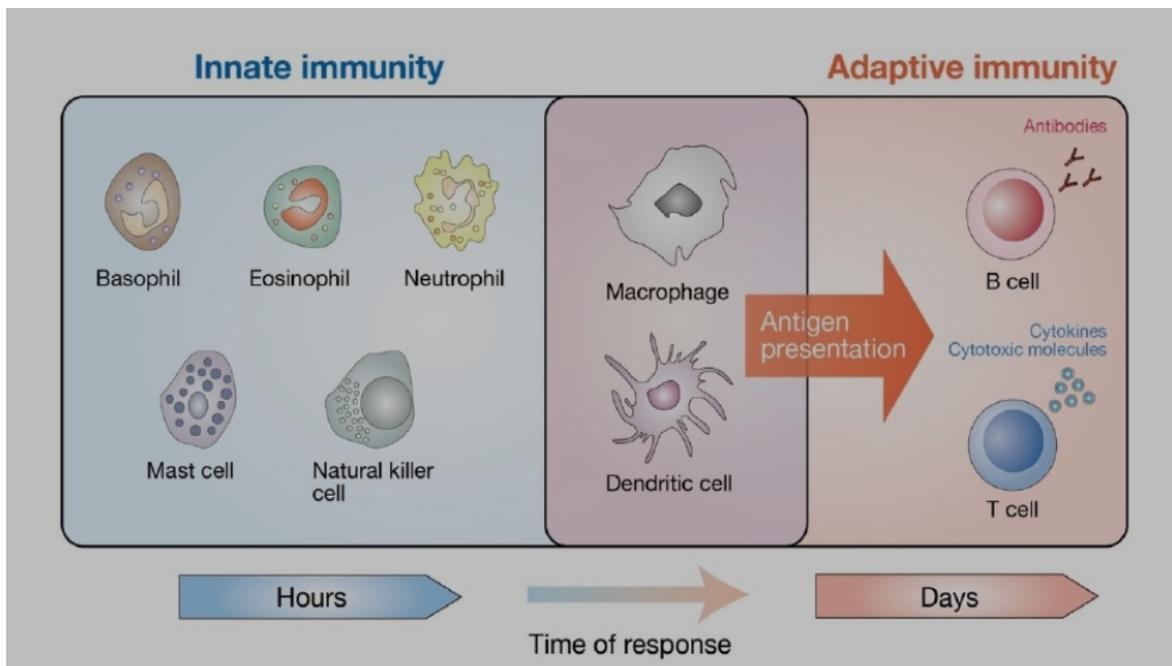


Figure 2. Hippo Pathway in Mammalian Adaptive Immune system





## Review on - "Cumin Wilt and Its Management"

Neekita D. Charan<sup>1</sup>, Kiransinh N. Rajput<sup>2</sup> and Rakeshkumar R. Panchal<sup>2\*</sup>

<sup>1</sup>Research Scholar, Department of Microbiology and Biotechnology, School of Sciences, Gujarat University, Ahmedabad, Gujarat, India.

<sup>2</sup>Associate Professor, Department of Microbiology and Biotechnology, University School of Sciences, Gujarat University, Ahmedabad, Gujarat, India.

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### \*Address for Correspondence

#### Rakeshkumar R. Panchal

Associate Professor,

Department of Microbiology and Biotechnology,

School of Sciences,

Gujarat University,

Ahmedabad, Gujarat, India.

E. Mail: panchalrrce@yahoo.com / rrpanchal@gujaratuniversity.ac.in.



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### ABSTRACT

Cumin (*Cuminum cyminum* L.) is widely grown as an important spice crop in arid and semi-arid regions of the Indian subcontinent. India is the world's largest producer, consumer, and exporter of cumin. The fungal disease wilt, caused by the soil-borne phytopathogens, *Fusarium oxysporum* f. sp. *cumini* (Foc), is one of the major threats to cumin production in India and worldwide. A few chemical compounds and microbes are utilized for the administration of cumin wilt disease. Compound fungicides utilized are Azoxystrobin, Carbendazim, Tricyclazole, Propiconazole, Mancozeb, Carboxin. Powder-based definitions of *Trichoderma viride*, *Aspergillus versicolor*, *T. harzianum*, and *Pseudomonas fluorescens* microbes are utilized. A review of the research findings on symptomatology, ecology, various types of disease prevention strategies, their potential effects on the causative agent, and the only successful way to treat this threat is effective bio control agents, seed dressing with fungicides, and wilt resistant varieties, as well as other traditional approaches such as organic amendment, summer ploughing, crop rotation throughout the year, solarization of soil and time of seed sowing to reduce the pathogen's population below the economic threshold level.

**Keywords:** Cumin, Cuminwilt, Fusarium wilt, Soil borne pathogen.





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## INTRODUCTION

Flavors are a significant horticultural item that has affected human development. It is most established herb normally known as "Jeera" in India. Its dried seeds are used as a flavor. It is a little yearly herbaceous plant that is an individual from the fragrant plant family (Apiaceae). There are three varieties accessible based on their seed colour for example Black, White, yellowish-brown [1][2].

### History and Development

It has been used since ages. It is cultivated in Chile, Iran, Uzbekistan, Tajikistan, Morocco, Turkey, Syria, Mexico, Egypt, Bulgaria, and India. India is the world's chief in the creation of cumin seeds with over 90% productivity, trailed by Syria and Turkey [3]. It is farming monetarily in Gujarat, West Bengal, Punjab, Jharkhand, Himachal Pradesh, Bihar, Assam, and Rajasthan. Uttar Pradesh, Madhya Pradesh, and Tamil Nadu are small-scale cumin cultivar states. In India, the area under cumin improvement is around 8,41,940 hectares with a yearly production of 5,46,750 tones [Table -1] [4].

### Weather and Field Requirement

Cumin develops best on all around depleted sandy topsoil and desirable pH for farming is between 6.6 and 8.3 [5]. This plant cannot withstand high moistness and hefty precipitation. It needs cool and dry climatic conditions for better development and crop required temperatures up to 25-30°C. It is usually filled in dry locales like Gujarat and Rajasthan.

### Disease prevalence in cumin

Wilt, blight, and mildew are significant infections. Cuminvascular wilt is caused by *Fusarium oxysporum* f.sp. *Cumini*, while *Alternaria burnsii* is a pathogen of blight, and infectious agent of mildew is *Erysiphe polygoni*. All these fungi are responsible for a hefty decline in productivity. Shriveling is damaging infection of the crop. From that point forward the infection has likewise been accounted for numerous different nation become a customary issue in limiting the creation of cumin seed yield between 25-40% and sometimes possibly high as up to 80% [6,7].

### Symptoms

The plants are influenced by wilt causing mold at all development stages, however, the seriousness of shriveling increments with the age. Around one month old, the sickness shows up in the field. At the point when they achieve a stature between 2.5 cm to 5.0 cm. Diseased plants show particular indications of dropping of tips and leaves, darkening of roots and leaves, drying of leaves and stems, hindrance in plant development, restraint of root arrangement, and prompting mortality of the whole plant [8]. Seeds whenever framed are dainty, little, and withered. It is regularly defiled during the gathering and the microorganism spreads to more current territories. In halfway withered plants, development used to be captured and the leaves become pinkish yellow in shading.

### Disease Cycle

The growth of mycelium continues to attacking the crops underlying foundations. Roots can be tainted straightforwardly through the root tips, through injuries in the roots, or at the development point of horizontal roots. It is developed through the cortex, xylem and stays in the vessels. The mycelium branches and delivers microconidia, which are conveyed upward inside the vessel at a point when microconidia grow and infiltrate the xylem vessel [9]. The hereditary qualities of biosynthesis of mycotoxins and their method of activity are being accounted for pathogenicity [10]. The enzymes that can degrade the cell wall of a plant are the crucial elements in pathogenicity [11].



**Neekita D. Charan et al.****Management of wilt**

Individuals from the *Fusarium oxysporum* species complex are phylogenetically assorted [12]. It has two sorts of strains viz. pathogenic and non-pathogenic strains. *F. oxysporum* can get by in the soil of the farm through chlamydospores for around 3 years [13]. Plant microbes interactions will in general arise, reappear, and can be endemic [14]. To create climate agreeable and maintainable yield in this advanced logical period. Microbial inoculants containing microorganisms as beneficial plant-organism combinations have an extraordinary potential to arrive at the objective [15]. The pathogen populate and stays stable for more than a half-year time frame and their level used to be higher when added to conducive soils [16]. The impact of mold and bacterial populace, dampness, and temperature on the populace elements of *F. oxysporum* f. sp. *cumini* were concentrated in soils with or without a hostcrop [17]. *Fusarium* wilt develops in warm regions and under drying conditions. This sickness is accounted for as an issue restricting the productivity of cumin [18]. According to a review of Suthar [19], 10 to 45 % loss of cumin creation happened by vascular wither sickness. According to outcome arrangement was adjusted 99.78% to *Fusarium* species at nearby BLAST worker, in FUSARIUM-ID v. 1.0, utilizing ITS1&2 groundwork. Talaviya [20] investigated fungicide for controlling wilt. Soil dousing with 0.1% Carbendazim before one month of planting *Fusarium* populace in the dirt can be decreased. Summer furrowing and utilization of neem mixture and mixture of mustard and pearl millet for a control shrink incident. Powder-based *Aspergillus versicolor* and *Trichoderma viride* used [21]. A few summer furrows diminished wilt frequency [22].

Champawat and Pathak [23, 24, 25] recommended expanded utilization of K<sup>+</sup>, carbofuran, carbendanzim and benomyl, phorate and aldicarb 30:10:10 NPK kg ha<sup>-1</sup> was best in diminishing wilting. Jadeja and Nandoliya. [26] Use of carbendazim granules one month after planting and vapam was used for soil drenching treatment, it is powerful in inhibition of wilt. Aghnoom [27] contemplated the impact of fungicides and found that combination of captan, iprodion and Carbendazim, carboxyl and thiram, diminished mycelial development. Seed treatment and soil dousing with Bavistin and Provax at a period of days from seedling to blooming stage lessening vascular wilt frequency and expanded seed yield [5]. Tawfic & Allam [28] revealed that until this point in time, no wilt-safe assortment has been grown so one just uses physical and organic control of fungi. Mawar and Lodha [29] studied the impact of powder-based bio-seed dresser where *Trichoderma viride* best followed by *Aspergillus versicolor*, *T. harzianum*, and *Pseudomonas fluorescens*. Sharma revealed that [30] *P. fluorescens* and *P. aeruginosa* are isolated from the rhizosphere the outcomes show that *P. fluorescens* gave the most elevated impact in limiting the disease 7.70 and 7.73%. According to Mohamed [31] particular blend of *T. viride* and *P. fluorescens* could have the more prominent adequacy in the limiting development of pathogen in contrasted and individual strains. Double culture of *T. viride* fundamentally diminished 29.0% to 82.2% mycelial development of pathogenic organisms [32].

**CONCLUSION AND FUTURE PERSPECTIVES**

In this survey, an exertion has been made to arrange research discoveries created during recent a very long time on symptomatology, nature, and the board techniques. To deal with this disease certain microorganisms, chemical, and integrated techniques are a successful approach. It will improve the yield cumin productivity

**Authorship**

All authors contributed to the conception of the study, preparation, and design of this review article. All authors made their comments on it. All authors read, revised, and approved the final.

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### Conflicts of Interest

The authors report no conflicts of interest.

### Funding

There is no funding to report.

### Ethical Approvals

This study does not involve experiments on human or animal subjects.

### ABBREVIATION

f.sp. - Formae Speciales

Ha-Hectare

cm – Centimeter

% - Percent

K<sup>+</sup> -Potassium ion

NPK -Nitrogen, Phosphorus, and Potassium

BLAST -Basic Local Alignment Search Tool

ITS -Internal Transcribed Space

### REFERENCES

1. Gangopadhyay S., Gopal R. Evaluation of Trichoderma spp. along with farmyard manure for the management of Fusarium wilt of cumin (*Cuminum cyminum* L.). *Journal of Spices and Aromatic Crops*,2010; Vol. 19 (1 & 2): 57–60.
2. Pruthi, J. S. *Spices and Condiments*, National Book Trust, Delhi, India.2014.
3. Azeez, S. Cumin. In: Parthasarathy, V.A., Champakam, B. and Zachariah, T.J., eds.: *Chemistry of Spices*. CABI International, Wallingford, UK This review article deals with botany, distribution and medicinal properties of cumin, and with the chemistry of cumin. 2008.
4. Indian spices board[Internet]. India: Spices Board (Ministry of Commerce and Industry, Government of India); Available from [Https://www.indianspices.com](https://www.indianspices.com)
5. Weiss E.A. *Spice Crops*. CABI International, Wallingford, UK. Family wise description of spice crops belonging to cruciferae, lauraceae, leguminosae, myristicaceae, myrtaceae umbelliferae and zingiberaceae. All aspects of cultivation processing and industrial applications are discussed 2002, p.299.
6. Khalequzzaman KM. Effect of Fungicides in Controlling Alternaria Blight of Cumin *Asian Journal of Applied Science and Engineering*.2016; 5, 7-14.
7. Gaur M M. Plant protection work in AjmerMerwara in 1948 *Plant Diseases*. *Plant Prot. Bull. Govt. India*. 1949;1: 20–21
8. Bhatnagar K., Tank S.K. , Sharama R.S., majumdar V.L. and Meena R.L. . Management of cumin wilt caused by *Fusarium oxysporum* f. sp. *cumini* through chemical and biological agents. *Indian Phytopath.* 2013,66 (1) : 101-102.
9. Ma LJ, Geiser DM, Proctor RH, Rooney AP, O'Dnnello K, Trail F, Gardiner DM, Manners JM ,Kazan K: *Fusarium pathogenomics*.*Annu. Rev.Microbial* 2013, Vol.67:399-416
10. Nicholson, M. J., Koulman, A., Monahan, B. J., Pritchard, B. L., Payne, G. A., and Scott, B. Identification of two aflatrem biosynthesis gene loci in *Aspergillus flavus* and metabolic engineering of *Penicillium paxilli* to elucidate their function. *Appl. Environ.Microbiol.* 2009; 75, 7469–7481. doi: 10.1128/AEM.02146-08
11. Garcia-Maceira, F.I., A. Di Pietro and M.I.G. Roncero. Cloning and disruption of *pgx4* encoding and in planta expressed exopolysaccharuronase from *Fusarium oxysporum*. *Mol. Plant Microbe. Interaction.* 2000; 13: 359-365.





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12. O'Donnell, K., Sutton, D. A., Fothergill, A., McCarthy, D., Rinaldi, M. G., Brandt, M. E., et al. Molecular phylogenetic diversity, multilocus haplotype nomenclature, and in vitro antifungal resistance within the *Fusarium solani* species complex. *Journal of Clinical Microbiology*. 2008; 46, 2477–2490
13. Haware, M.P., Nene, Y.L., Natarajan, M. Survival of *Fusarium oxysporum* f. sp. *ciceri* in soil in the absence of chickpea. *Phytopath. Mediter*. 1996; 35: 9–12.
14. Miller, S.A., Rowe, R.C. and Riedel, R.M. *Fusarium* and *Verticillium* wilts of tomato, potato, pepper, and eggplant. Fact Sheet. Columbus, Ohio State University. 2011.
15. Bhattacharya, C., Bakshi, U., Mallick, I., Mukherji, S., Bera, B., Ghosh, A. Genome-guided insights into the plant growth promotion capabilities of the physiologically versatile *Bacillus aryabhatai* strain AB211. *Front. Microbiol*. 2017; 8, 411. doi: 10.3389/fmicb.2017.00411
16. Larkin, R.P., Roberts, D.P., & Gracia-Garza, J.A. Biological control of fungal diseases. In *Fungicidal activity. Chemical and biological approaches*. (pp 141-191). New York, NY: Wiley. 1998.
17. Sunil, L and Satish, L. Factors influencing population dynamics of *Fusarium oxysporum* f. sp. *cumini* in the presence and absence of Cumin in crops in arid soil. *Phytopathologia mediterranea*. 2004; 43: 3- 13.
18. Twafik, A.A. and Allam, D.A. Improving cumin production under soil infestation with fusarium wilt pathogen: I-screening of biocontrol agents. *Ass. Univ. Bull. Environ. Res*. 2004; 7 (2):1-11.
19. Suthar R, Bhatt PN, and Bhatt DP. Morphological and molecular identification of *Fusarium* isolated from cumin wilt. *Internat.J.Plant Protec*. 2011; vol.4. (2):359-362.
20. Talaviya, J. R., Kapadiya, I. B., & Shah, K. Fungicidal management of cumin wilt and its effect on soil microbial population. *Journal of Mycopathological Research*. 2018; 56(2), 109-115.
21. Thamburaj, S. and Singh, N. *Vegetables, Tubercrops and Spices* by Directorate of Knowledge Management in Agriculture. ICAR, New Delhi. 2014.
22. Sharma P, Sharma M, Raja M, Shanmugam V. Status of *Trichoderma* research in India: A review. *Indian Phytopathol*. 2014; 14(67): 1-19.
23. Champawat R S & Pathak V N. Role of nitrogen, phosphorus and potassium fertilizers and organic amendments in cumin (*Cuminum cyminum*) wilt incited by *Fusarium oxysporum* f. sp. *cumini*. *Indian J. Agric. Sci*. 1988a; 58: 728–730.
24. Champawat R S & Pathak V N. Soil application of different insecticides and nematicides for the control of wilt (*Fusarium oxysporum* f. sp. *cumini*) of cumin. *Indian J. Plant Prot*. 1988b; 16: 195–196.
25. Champawat R S & Pathak V N. Management of cumin wilt by summer ploughing. *Indian Cocoa Arec. Spices J*. 1990b; 13: 107– 108.
26. K B Jadeja & D M Nandoliya. Integrated management of wilt of cumin (*Cuminumcyminum* L.). *Journal of Spices and Aromatic Crops*. 2008; Volume 17 (3) : 223–229.
27. Aghnoom R, Falahati R M & Jafarpour B. Comparison of chemical and biological control of cumin wilt (*Fusarium oxysporum* f. sp. *cumini*) in laboratory and greenhouse conditions. *Iranian J. Agri. Sci*. 1999; 30: 619-630.
28. Tawfic A A & Allam A D A. Improving cumin production under soil infestation with *Fusarium* wilt pathogen: I-Screening of biocontrol agents. *Assiut Univ. Bulletin Res*. 2004a; 7: 35–44.
29. Mawar R & Lodha S. Brassica amendments and summer irrigation for the control of *Macrophomina phaseolina* and *Fusarium* in hot arid region. *Phytopath. Mediterr*. 2002; 41: 45–54.
30. Sharma yk, Kant K, Solanki RK, Saxena RP. Prevalence of cumin disease on farmer's field: a survey of Rajasthan and Gujarat states. *International J. Sees Spices*. 2013; vol.3. (2) 46-49.
31. Mohamed K. Al-Sman, Kamal A. M. Abo-Elyousr, Amal Eraky & Aida El-Zawahry. Efficiency of *Pseudomonas* spp.-based formulation for controlling root rot disease of black cumin under greenhouse and field conditions. *Archives of Phytopathology and Plant Protection*. 2020; DOI: [10.1080/03235408.2019.1707384](https://doi.org/10.1080/03235408.2019.1707384)
32. Rajeswari, P. Combination of *Trichoderma viride* and *Pseudomonas fluorescens* for the enhanced control of *Fusarium* wilt disease caused by *Fusarium oxysporum* infecting *Arachis hypogaea* L. *Journal of Applied and Natural Science*. 2019; 11(1), 138-143. <https://doi.org/10.31018/jans.v11i1.1985>





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**Table - 1. State-wise area and production of cumin.**

State wise area and production of cumin								
State	2016-17		2017-18		2018-19		2019-20	
	Area	Production	Area	Production	Area	Production	Area	Production
Gujarat	278750	291490	382720	384470	349552	319862	337007	331940
Rajasthan	500140	206940	581360	302930	676240	378654	503230	213070

(Production area in ha and national production in tons)

(Souce:<https://www.indianspices.com/sites/default/files/majorspicestatewise.pdf> )





## Investigation of the Phase Formation Mechanism of SrBi<sub>2</sub>Ta<sub>2</sub>O<sub>9</sub> Ferroelectric Ceramic Prepared through Mixed-oxide Route.

G. K. Sahu<sup>1</sup>, S. Behera<sup>1\*</sup> and S.R. Mishra<sup>2</sup>

<sup>1</sup>Department of Physics, School of Applied Sciences, Centurion University of Technology and Management, Odisha, India.

<sup>2</sup>Department of Chemistry, Gandhi Institute for Education and Technology, Baniatangi, Khorda, Odisha, India.

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### \*Address for Correspondence

**S. Behera**

Department of Physics,  
School of Applied Sciences,  
Centurion University of Technology and Management,  
Odisha, India.

Email: saubhagalaxmi.behera@cutm.ac.in



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### ABSTRACT

A layered perovskite ferroelectric SrBi<sub>2</sub>Ta<sub>2</sub>O<sub>9</sub> ceramic has been synthesized by solid-state route and studied its phase formation mechanism from XRD and FTIR analysis. The precursors are preferred as the oxides and carbonates for more stability and low cost. Mixed oxide precursors are calcined at different temperature ranging from 800-1000°C interval of 50°C for 2 and 4 hrs durations for phase study. At 800°C onwards, XRD shows the formation of SrBi<sub>2</sub>Ta<sub>2</sub>O<sub>9</sub> phase with some intermediate phases like SrBi<sub>2</sub>O<sub>4</sub>, BiTaO<sub>4</sub> and carbonate groups. XRD spectrum at 1000°C confirms the complete phase formations of SrBi<sub>2</sub>Ta<sub>2</sub>O<sub>9</sub> and the temperature has been chosen for the further synthesis process. Using FTIR, the stretching and bending vibrations of the metal-oxide and carbonate bonds have been observed for various temperatures. Vibrations around 530-790 cm<sup>-1</sup>, confirms the vibration of Sr-O, Ta-O and Bi-O bonds.

**Keywords:** Solid state route, Phase formation, Infrared spectroscopy,

### INTRODUCTION

Recently ferroelectric materials have attracted interest for their application in ferroelectric random access memories (FRAM). These memories may replace the silicon-based electrically erasable programmable read-only memories (EEPROM) as they possess low operating voltage, high reading and writing speed, and non-volatility [1-5]. Lead Zirconium Titanate (PZT) has been a promising material for the application of FRAM; however, it suffers severe polarization fatigue on platinum electrodes after long switching cycles. In order to improve the resistance to fatigue, conductive oxide electrodes have been used, but they have lower electrical conductivity than platinum electrodes [4,5]. On the other hand, Aurivillius compounds have been found to exhibit high fatigue resistance and polarization





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retention up to  $10^{12}$  switching cycles on Pt electrodes [6]. These layered-structured perovskites exhibit the characteristics of low leakage current, low operating voltage, stable imprinted characteristics, and high polarization retention up to long switching cycles [JPCS.69.480]. Because of these excellent electrical properties,  $\text{SrBi}_2\text{Ta}_2\text{O}_9$  is an attractive material for FRAM. The increasing interest is also due to fatigue-free behavior and low leakage currents of these compounds [7-8]. The chemical formula of Aurivillius compounds is  $(\text{Bi}_2\text{O}_2)^{2+}(\text{A}_{n-1}\text{B}_n\text{O}_{3n+1})^{2-}$ , where  $n$  indicates the number of perovskite layers ( $\text{BO}_6$ ) between two  $(\text{Bi}_2\text{O}_2)^{2+}$  layers, while A and B represents cations with low and high valences in the structure respectively. The  $\text{BO}_6$  octahedrons construct continuous layers perpendicular to  $c$ -axis direction, but in the  $c$ -axis direction these octahedrons are separated by the  $\text{Bi}_2\text{O}_2$  layers. The presence of the  $\text{Bi}_2\text{O}_2$  layers has been thought to serve as the shock-absorber for enduring the fatigue of polarization [9]. The ceramic form of  $\text{SrBi}_2\text{Ta}_2\text{O}_9$  was first synthesized by Aurivillius, [10] and its dielectric properties and phase transformation were later studied by Smolenskii [11] and Subbarao [12]. The microscopic origin of the ferroelectric instability in  $\text{SrBi}_2\text{Ta}_2\text{O}_9$  was investigated from first-principles calculations by one of the authors [13]. Lattice instabilities along the [110] direction of the tetragonal paraelectric structure were searched by determining the phonon frequencies and eigenvectors of the infrared-active modes polarized perpendicular to the  $c$ -axis. One unstable phonon mode was found which mainly involves movements of the  $\text{Bi}_2\text{O}_2$  planes relative to the  $\text{SrTa}_2\text{O}_7$  perovskite like layers. This result indicated that most of the polarization in  $\text{SrBi}_2\text{Ta}_2\text{O}_9$  comes from the relative displacements of the Sr-Ta-O layers to the Bi-O layers, plus a small additional contribution arising from the movement of Ta relative to its surrounding oxygens. Extensive studies have been carried out examining the formation of  $\text{SrBi}_2\text{Ta}_2\text{O}_9$  films by different processes [14–22]; however, the literature regarding the preparation of  $\text{SrBi}_2\text{Ta}_2\text{O}_9$  powder is very limited [23-25]. Lu and Lee [24] reported a solid-state two-step synthesis method using  $\text{SrCO}_3$  and  $\text{BiTaO}_4$ . However, the solid-state method requires calcination at high temperature, which causes coarsening of the powder. The formation processes of  $\text{SrBi}_2\text{Ta}_2\text{O}_9$  in this process were investigated for adjusting the calcination conditions to produce the pure phase. In order to better control the reaction processes, the formation mechanism and the decomposition reaction of  $\text{SrBi}_2\text{Ta}_2\text{O}_9$  at elevated temperatures were studied with the help of XRD and FTIR.

### Experimental techniques

The precursors were used to prepare the  $\text{SrBi}_2\text{Ta}_2\text{O}_9$  are  $\text{SrCO}_3$  (99% purity, Merck Germany),  $\text{Bi}_2\text{O}_3$  (99.5% Lobachemie, Kolkatta),  $\text{Ta}_2\text{O}_5$  (99.5%, Merck Germany). All the precursors are measured stoichiometrically and mixed in agate mortar long time for the homogenous mixer. After mixing, the mixed precursors are calcined in a programmable furnace at different temperatures in various durations. The calcined powders were characterized the phase formation by XRD, IR spectra of the calcined powders were analyzed by FTIR spectrophotometer. XRD was studied by the powder X-Ray diffractometer (*Phillips PANalytic Instruments*) from 20-60 degree range in  $2\theta$  and matched with JCPDS cards no. 05-0418 for  $\text{SrCO}_3$ , 16-0909 for  $\text{BiTaO}_4$ , 39-1424 for  $\text{SrBi}_2\text{O}_4$  and 46-0906 for  $\text{SrBi}_2\text{Ta}_2\text{O}_9$ . FTIR has studied by the *Perkin Elmer* range from the 400 to  $4000\text{cm}^{-1}$  by taking the medium of KBr in the form of pellet.

Spectroscopic analysis using FTIR was studied on the calcined powders in different temperature and timings. Fig. 1 shows the FTIR results of calcined powders. For complete  $\text{SrBi}_2\text{Ta}_2\text{O}_9$ , vibration starts from the  $950^\circ\text{C}$  onwards. For the calcined powder at  $800^\circ\text{C}$  for 2 and 4 hrs, the peak appears at the around  $1450\text{cm}^{-1}$ , indicating the presence of carbonate groups [26]. Upon calcining at  $850^\circ\text{C}$  for 4hrs, the band intensity of the carbonate group was reduced significantly, but the bands remained visible. All carbonate groups disappeared after the sample was heated at  $900^\circ\text{C}$ , and also the removal of carbonate groups (i.e.,  $\text{SrCO}_3$ ) is confirmed by XRD spectrum, as shown in the Fig. 2. Less intensity of carbonate groups in the spectrum at  $850^\circ\text{C}$  for 2 hrs but not in the spectrum of  $850^\circ\text{C}$  for 4 hrs, this may be due to the fast removal of carbonate groups in long soaking duration. At  $\geq 950^\circ\text{C}$  for 2hrs and  $\geq 900^\circ\text{C}$  for 4hrs, three low frequency bands appeared at  $\sim 550$ ,  $\sim 650$  and  $\sim 780\text{cm}^{-1}$ , indicates that the formation of  $\text{SrBi}_2\text{Ta}_2\text{O}_9$  phase has started at this temperature and it also denotes the vibration of Ta-O, Sr-O and Bi-O bonds respectively [26]. The metal-oxide vibration is appeared at  $700\text{-}730\text{cm}^{-1}$  in all the spectrums above  $800^\circ\text{C}$ , this is may be due to the metal-oxide vibration of  $\text{SrBi}_2\text{O}_4$  and  $\text{BiTaO}_4$  phases. These intermediate phases have confirmed by the XRD spectrum. But the samples calcined at  $900$ ,  $950$  and  $1000^\circ\text{C}$  show the characteristic peak of  $\text{SrBi}_2\text{Ta}_2\text{O}_9$  at  $\sim 550$ ,  $\sim 650$ , and  $\sim 780\text{cm}^{-1}$ ,

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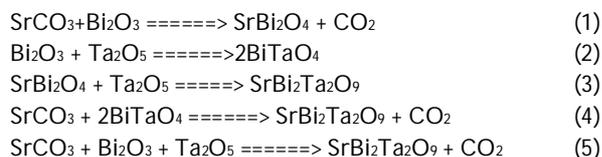


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indicating the complete removal of carbonates from the precursor and the precursors forming the complete compound of SrBi<sub>2</sub>Ta<sub>2</sub>O<sub>9</sub>. The 600–810 cm<sup>-1</sup> TO-LO (Transverse-Optical and Longitudinal-Optical) bands are the main feature of the SrBi<sub>2</sub>Ta<sub>2</sub>O<sub>9</sub> spectrum. The 503cm<sup>-1</sup> band should be the TO frequency of Sr-O with rock salt structure [27].

To understand the fundamental issues of SrBi<sub>2</sub>Ta<sub>2</sub>O<sub>9</sub> formation in the present case, the precursors were heated in air at different temperatures and different durations and then were analyzed by XRD. The XRD patterns for the samples were calcined for 2 and 4hrs at 800-1000°C in the interval of 50°C is shown in Fig.2. Small amount of SrBi<sub>2</sub>Ta<sub>2</sub>O<sub>9</sub> phase with extra phases like SrBi<sub>2</sub>O<sub>4</sub>, BiTaO<sub>4</sub> and carbonate groups were found as intermediate phases after calcination at 800°C. At this temperature, the formation of SrBi<sub>2</sub>Ta<sub>2</sub>O<sub>9</sub> has also started. With an increase in the heating temperature and duration, the amount of SrBi<sub>2</sub>Ta<sub>2</sub>O<sub>9</sub> rapidly increased, accompanied with a corresponding decrement in the amount of extra phases. After calcining at 1000°C for 2 and 4 hrs, the single phase spectrum of well-developed layered structure SrBi<sub>2</sub>Ta<sub>2</sub>O<sub>9</sub> is observed. The formation of SrBi<sub>2</sub>Ta<sub>2</sub>O<sub>9</sub> powders calcined at 1000°C for 2hrs and 4hrs is not observed much variation in the phase pure spectrum (as shown in fig. 5). The relative amounts of extra phases and SrBi<sub>2</sub>Ta<sub>2</sub>O<sub>9</sub> are shown in Fig.3.

XRD quantitative estimation of SrCO<sub>3</sub>, BiTaO<sub>4</sub> and SrBi<sub>2</sub>O<sub>4</sub> on the samples heated at different temperatures for 2 and 4 hours shows that the quantity of SrCO<sub>3</sub>, SrBi<sub>2</sub>O<sub>4</sub> and BiTaO<sub>4</sub> slowly decreases and SrBi<sub>2</sub>Ta<sub>2</sub>O<sub>9</sub> slowly increases up to 950°C. At 950°C, SrCO<sub>3</sub> and BiTaO<sub>4</sub> decomposed and formed SrBi<sub>2</sub>O<sub>4</sub> and SrBi<sub>2</sub>Ta<sub>2</sub>O<sub>9</sub>. Decomposition of SrBi<sub>2</sub>O<sub>4</sub> with BiTaO<sub>4</sub> gives the formation of SrBi<sub>2</sub>Ta<sub>2</sub>O<sub>9</sub> is rapid from 900 to 1000°C. When the temperature reached 1000°C both the phases were entirely consumed, revealing that the formation of SrBi<sub>2</sub>Ta<sub>2</sub>O<sub>9</sub> was complete as well as the crystallinity of the pure SrBi<sub>2</sub>Ta<sub>2</sub>O<sub>9</sub> is increased. According to our XRD spectrum, we are concluding the reactions between the precursors used in the starting material and intermediate phases in the different heating duration as follows,



At 800°C, we found the SrBi<sub>2</sub>O<sub>4</sub>, BiTaO<sub>4</sub> and SrCO<sub>3</sub> compounds in the XRD spectrum. The reason for the formation of SrBi<sub>2</sub>O<sub>4</sub> and BiTaO<sub>4</sub>, may be due to the fast decomposition of Bi<sub>2</sub>O<sub>3</sub> compounds. Because of fast decomposition, Bi atoms easily diffused with the other precursors and forms a new extra phase. According to Chung-Hsin Lu et al [25], Ta<sub>2</sub>O<sub>5</sub> and SrCO<sub>3</sub> precursors are having a high decomposition temperature, so the extra phases like SrTa<sub>2</sub>O<sub>6</sub> would need to form above 1150°C.

Fig.4 shows the indexed spectrum of SrBi<sub>2</sub>Ta<sub>2</sub>O<sub>9</sub> calcined at 1000°C for 2 and 4 hrs. From these spectrums, we calculated the crystalline size, 2θ position and other related parameters are tabulated in Table.1. The crystallite size of the calcined powder was calculated from the broadening at half maximum of the X-ray spectral (115) peak of SrBi<sub>2</sub>Ta<sub>2</sub>O<sub>9</sub> based on Scherrer's formula [29].

$$L = \frac{K\lambda}{\beta \cos\theta} \quad (6)$$

Where L is the crystallite size, K is a constant (0.9), λ is the wavelength of x-rays (1.5406Å), β is the Full Width at Half Maximum (FWHM) and θ is the angle of the diffracted peak. Fig.5 shows the variation of crystallite size and calcination temperature. Crystallite size is increasing gradually with increasing the calcination temperature which corresponding to the higher angle shifts of diffracted peaks. The shift of 2theta position for 4 hrs calcination is almost comparatively parallel to the 2hrs calcination. It is indicating the peak position shift may be linear in long duration soaking hours. May be this is the reason to coincide the crystallite size in higher temperature calcination for 2 and 4 hrs duration.





## CONCLUSION

Bismuth layered ferroelectric  $\text{SrBi}_2\text{Ta}_2\text{O}_9$  had been synthesized by a solid state route using oxide precursors. The removal of carbonate groups has found by the FTIR analysis from the calcination temperature above  $900^\circ\text{C}$  and metal-oxide vibration has observed around  $550\text{-}780\text{cm}^{-1}$ . The  $600\text{-}810\text{ cm}^{-1}$  TO-LO bands are the main feature of the  $\text{SrBi}_2\text{Ta}_2\text{O}_9$  spectrum. The complete phase formation of  $\text{SrBi}_2\text{Ta}_2\text{O}_9$  has confirmed by the XRD at the calcination temperature above  $1000^\circ\text{C}$  and also confirms the  $\text{SrBi}_2\text{Ta}_2\text{O}_9$  compound formation starts at above  $800^\circ\text{C}$ . Calcination temperature and duration dependence of crystallite size and peak shift has been calculated.

## REFERENCES

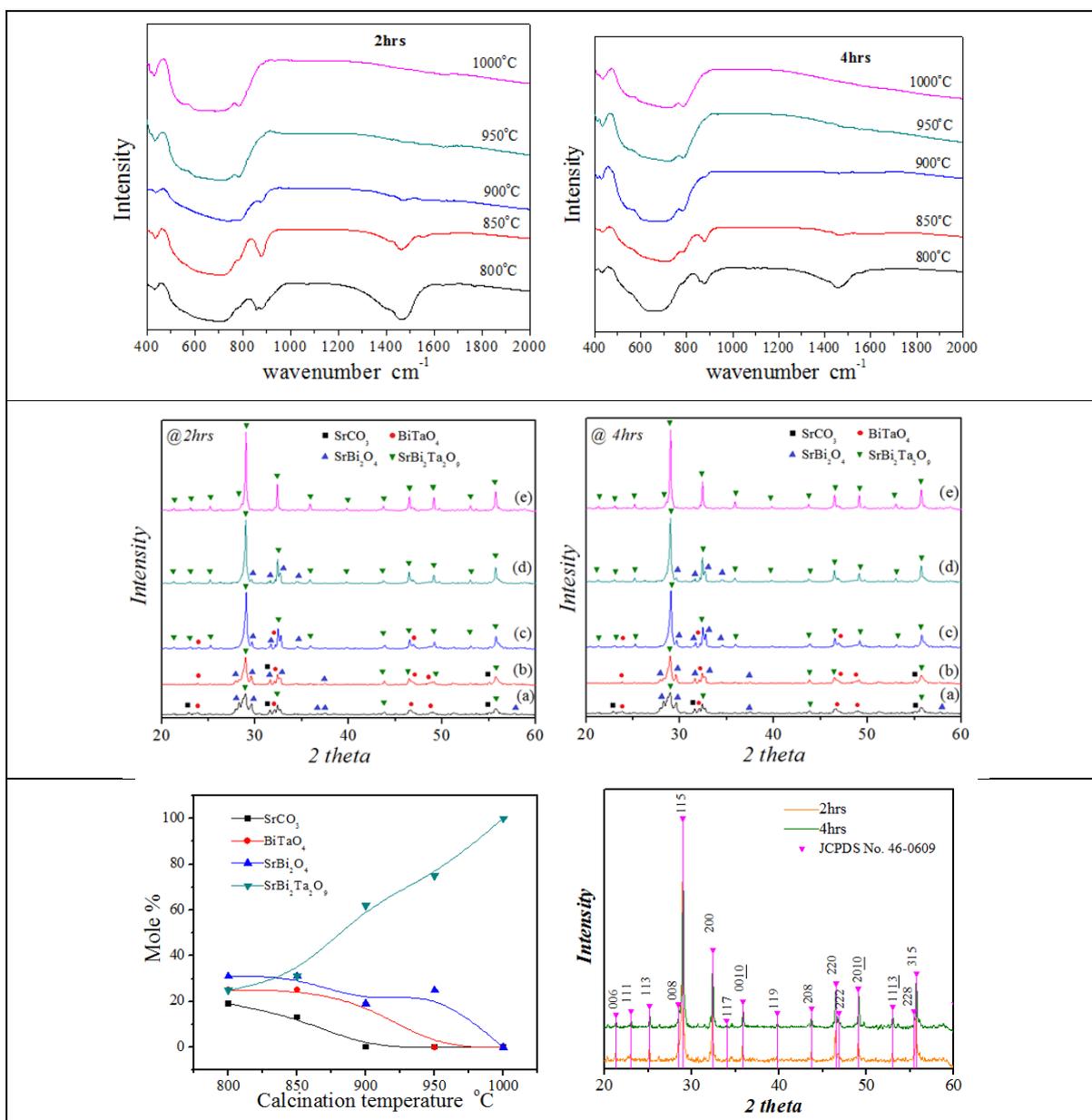
1. Paz de Araujo, C. A., Cuchiario, J. D., McMillan, L. D., Scott, M. C. and Scott, F., Fatigue-free ferroelectric capacitors with platinum electrodes. *Nature*, 1995, 374, 627-629.
2. Jones, R. E. Jr., Maniar, P. D., Moazzami, R., Zurcher, P., Witowski, J. Z., Lii, Y. T., Chu, P. and Gillespie, S. J., Ferroelectric non-volatile memories for low-voltage, low-powder applications. *Thin Solid Films*, 1995, 270(1-2), 584-588.
3. Jones, R. E. Jr. and Desu, S. B., Process integration for nonvolatile ferroelectric memory fabrication. *Mater.Res.Soc. Bull.*, 1996, 21(6), 55-58.
4. Al-Shareef, H. N., Kingon, A. I., Chen, X., Bellur, K. R. and Auciello, O., Contribution of electrodes and microstructures to the electrical properties of  $\text{Pb}(\text{Zr}_{0.53}\text{Ti}_{0.47})\text{O}_3$  thin film capacitors. *J. Mater. Res.*, 1994, 9(11), 2968-2975.
5. Kushida-Abdelghafar, K., Miki, H., Yano, F. and Fuji-saki, Y.,  $\text{IrO}_2/\text{Pb}(\text{Zr}_x\text{Ti}_{1-x})\text{O}_3(\text{PZT})/\text{Pt}$  ferroelectric thin-film capacitors resistant to hydrogen-annealing damage. *Jpn. J. Appl. Phys.*, 1997, 36(8A), 1032-1034.
6. Yoshimori, H., Watanabe, H., Paz de Araujo, C. A., McMillan, L. D., Cuchiario, J. D. and Scott, M. C., US Patent No. 9310021, 1993.
7. Y. Shimakawa, H. Imai, H. Kimura, S. Kimura, Y. Kubo, E. Nishibori, M. Takata, M. Sakata, K. Kato and Z. Hiroi, *Phys. Rev. B* **66** (14) (2002), p. 144110-1-5.
8. D. Nuzhnyy, S. Kamba, P. Kužel, S. Veljko, V. Bovtun, M. Savinov, J. Petzelt, H. Amorín, M.E.V. Costa, A.L. Kholkin and Ph. Boullay, *Phys. Rev. B* **74** (13) (2006), p. 134105-1-7.
9. Paz de Araujo, C. A., Cuchiario, J. D., Scott, M. C. & Mcmillan, L. D., Layered superlattice material applications background of the invention. *Inter. Patent Appl. WO93/12542*. 1993.
10. Aurivillius, B., Mixed bismuth oxides with layer lattices, I. The structure type of  $\text{CaNb}_2\text{Bi}_2\text{O}_9$ . *Arkivjirrkemi*, 54 (1949) 463-80
11. Smolenskii, G. A., Isupov, V. A. & Agranovskaya, A. 1. Ferroelectrics of oxygen-octahedral type with a layer structure. *Fi. Tverdogo Tela*..3 (1961) 895-90 1.
12. Subbarao. E. C. A family of ferroelectric bismuth compounds. *J. Phys. Chem. Solids*, 23 (1962) 665-676
13. M. G. Stachiotti, C. O. Rodriguez, C. Ambrosch-Draxl, and N. E. Christensen, *Phys. Rev. B* 61, 14 434 (2000). [PRB1]
14. S.S. Park, C.H. Yang, S.G. Yoon, J.H. Ahn, H.G. Kim, *J Electrochem Soc* 144 (1997) 2855–2858.
15. H.M. Yang, J.S. Luo, W.T. Lin, *J Mater Res* 12 (1997) 1145–1151.
16. S.B. Desu, D.P. Vijay, X. Zhang, B.P. He, *Appl Phys Lett* 69 (1996) 1719–1721.
17. K. Amanuma, T. Hase, Y. Miyasaka, *Appl Phys Lett* 66 (1995) 221–223.
18. P.Y. Chu, R.E. Jones Jr., P. Zurcher, D.J. Taylor, B. Jiang, S.L. Gillespie, Y.T. Lii, *J Mater Res* 11 (1996) 1065–1069.
19. C.H. Lu, B.K. Fang, *J Mater Res* 12 (1997) 2104–2110.
20. T. Li, Y. Zhu, S.B. Desu, C.H. Peng, M. Nagata, *Appl Phys Lett* 68 (1996) 616–619.
21. T.J. Boyle, C.D. Buchheit, M.A. Rodriguez, H.N. Al-Shareef, B.A. Hernandez, B. Scott, J.W. Ziller, *J Mater Res* 11 (1996) 2274–2281.
22. K. Kato, C. Zheng, J.M. Funder, S.K. Dey, K. Torii, *J Am Ceram Soc* 81 (1998) 1869–1875.





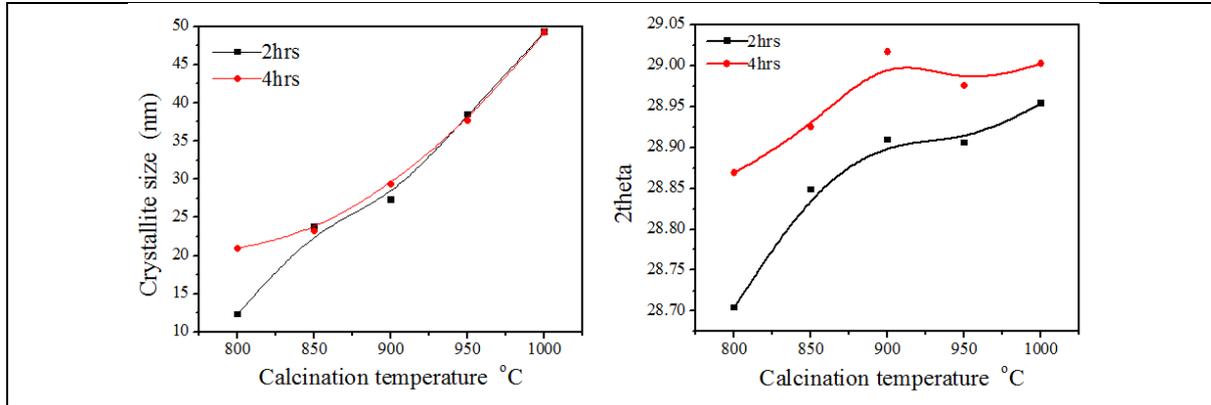
Sahu et al.,

23. E.C. Subbarao, J PhysChem Solids 23 (1962) 665–676.
24. C.H. Lu, J.T. Lee, Ceram Int 24 (1998) 285–291.
25. C.H. Lu, Y.C. Chen, J Euro Ceram Soc 19 (1999) 2909–2915. [SBT kinetics2]
26. C.H. Lu, S.K.Saha, Materials Research Bulletin 35 (2000) 2135–2143 [SBT kinetics3] and [FTIR and TGA important]
27. M.P. Moret, R. Zallen, R.E. Newnham, P.C. Joshi, and S.B. Desu, Phys. Rev. B. 57, 5715 (1998).
28. K.H. Rieder, R. Migoni, and B. Renker, Phys. Rev. B. 12, 3374 (1975). [FTIR and TEM Important]
29. H.P. Klug, L.E. Alexander, X-ray Diffraction Procedures for Polycrystalline and Amorphous Materials, Wiley, New York, 1954, p. 491.
30. D.L. Wood, J. Tauc, Phys. Rev. B 5 (1972) 3144.





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## Impact of Internal Marketing on Employees' Performance in Government Sectors

Odai Falah Mohammad AL-Ghaswyneh<sup>1\*</sup> and Khaled Adnan Oweis<sup>2</sup>

<sup>1</sup>Assistant Professor, Department of Marketing, Faculty of Business Administration, Northern Border University, Box: 1321, Arar, P.O. 91431, Northern Region, Kingdom of Saudi Arabia.

<sup>2</sup>Associate Professor, Department of Accounting, Faculty of Business Administration, Northern Border University, Box: 1321 Arar, P.O. 91431, Northern Region, Kingdom of Saudi Arabia.

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### \*Address for Correspondence

**Odai Falah Mohammad AL-Ghaswyneh**

Assistant Professor,  
Department of Marketing,  
Faculty of Business Administration,  
Northern Border University,  
Box: 1321, Arar, P.O. 91431 Saudi Arabia.  
Email: odai.ghasawneh@hotmail.com



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### ABSTRACT

The study aimed to identify the impact of internal marketing on employees' performance in government sectors. The study sample was selected randomly from the government's employees, where a questionnaire designed to collect data from respondents, and 550 questionnaires distributed to the study sample. The study adopted the descriptive analytical approach to testing hypotheses using the SPSS. Findings showed a statistically significant impact at the level of 0.05 of internal marketing in dimensions of (internal communications, training, courses, leadership, incentives and rewards) on the employees' performance in the government sectors. The dimensions of empowerment and authority have no impact on the employees' performance. The study recommended empowering employees to encourage them to participate in decision making and express their opinions on various issues related to the working environment in government sectors. In addition, the government must pay attention to internal marketing as the cornerstone of the success of external marketing of clients.

**Keywords:** Internal marketing, employees, government sectors, performance.





**Odai Falah Mohammad AL-Ghaswyneh and Khaled Adnan Oweis**

## INTRODUCTION

Recent developments in organizations adopted a new idea in marketing called internal marketing, which is based mainly on the interest of employees within organizations to satisfy their needs and desires. The essence of internal marketing is dealing with the organization's staff as clients and recipients of services. However, suppose governmental organizations attract, retain and understand their mission and objectives as well as the needs and desires of their employees. In that case, the outcome is a constant development of the quality of services provided by these employees, if they were clients from abroad. In addition, it will raise the morale of employees through self-continuous development by receiving training courses. In addition, clarifying their career roles provides a good working environment and makes communication easier between all organizational units. Moreover, to provide incentives and rewards, leading employees willing to provide a high level of services and high quality to domestic residents, then achieve an unprecedented level in governmental performance according to Saudi Vision 2030. Lately, a strategic goal has been set within the national transformation program to increase the productivity of public services. Meanwhile, it is applicable by governing frameworks and regulations of the civil service sector and empowering government activities to create a stimulating working environment. Accordingly, it will raise the efficiency of human capital, thereby enhancing the implication and productivity of the government sector, such as initiatives and objectives that are also suitable for implementation in the private sector that would contribute to creating stimulating work environments for employees within the Kingdom.

### Study problem

The study problem arises through a lack of complete awareness of applying internal marketing to the employees of government organizations. Meanwhile, internal marketing is in the interest of the customers outside the organizations. In addition, the problem arises because of low achievement; satisfying employees' needs to be a central pillar of the organization through applying the concept of internal marketing in all its dimensions. It is applicable based on motivating employees with financial rewards, training, involving them in decision-making and improving their situation. That is all to encourage them to work with all their energies. In addition, providing better services will help them raise their productivity and help achieve the satisfaction of the external customers of the organizations. The problem of study stems from the following question: Do the government sectors apply the dimensions of internal marketing to increase the performance of their employees?

### Study Importance

The study's importance stems from recognizing the reality of applying internal marketing in the Saudi government sectors from employees' perspectives. Meanwhile, expected results appeared to improve organizations' internal marketing implementation to determine the weaknesses and shortcomings and correcting them through the proposals to be presented by the study.

### Study Objectives

Through research and investigation, the study seeks to achieve several objectives, the most important of which are:

- Identify the level of application of internal marketing to government organizations.
- Identify the level of staff satisfaction with internal marketing processes in government organizations.
- Identify the level of performance of employees in government sectors in government organizations.
- We are exploring obstacles to the application of internal marketing.
- Reveal recommendations and make recommendations not decision-makers in government sectors to work under them.



**Odai Falah Mohammad AL-Ghaswyneh and Khaled Adnan Oweis****Study hypotheses**

**The first hypothesis:** There is a statistically significant effect of the dimensions of internal marketing in increasing the employees' performance in the government sectors.

**Sub-hypotheses**

The following sub-hypotheses derived from the first hypothesis:

- **H1:** there is a statistically significant effect of the Dimension of training in increasing the employees' performance in the government sectors.
- **H2:** there is a statistically significant effect of the Dimension of empowerment in increasing the employees' performance in the government sectors.
- **H3:** There is a statistically significant effect of the incentives and rewards in increasing the employees' performance in the government sectors.
- **H4:** There is a statistically significant effect of the Dimension of internal communications in increasing the employees' performance in the government sectors.
- **H5:** There is a statistically significant effect of the distance of the granting of authority in increasing the employees' performance in the government sectors.
- **H6:** There is a statistically significant effect of leadership distance in increasing the employees' performance in the government sectors.

**The second hypothesis:** There are no statistically significant differences from sample perspectives about staff performance in government sectors due to demographic variables (gender, age, income, social status, years of experience).

**Theoretical Framework and Previous studies**

[1] explored the impact of internal marketing practices on the job satisfaction of Saudi universities. The analysis results showed a statistically significant relationship between internal marketing and the emotional intelligence of faculty members in Saudi universities. Recommendations revealed that training courses and workshops urging faculty members to identify emotions, feelings, self-emotions and the ability to control them.[2] explored the compatibility or difference between both internal marketing literature and conducting human resources by following public service laws. They concluded that public service laws are constantly changing to keep pace with developments in human resource management. Public institutions need internal marketing, which is an effective tool that contributes significantly to the success of these institutions, both of which counterpart each other.[3] investigated the role of internal marketing pillars and their impact on the performance of the university's administrative and academic staff and after the analysis showed a positive relationship between the dimensions of internal marketing used by the University of the Northern Border in Saudi Arabia, particularly the training processes of university members to improve their performance of work [1].

[4] addressed the dimensions of internal marketing and its impact on achieving satisfaction and increasing the performance of employees in commercial banks in Karak province. Findings showed a statistically significant effect of internal marketing dimensions on job satisfaction and work performance [5]. observed a positive relationship that affected employee motivation and job stability, leading to the provision of high-quality services desired by the customer when banks use internal marketing. [6] found a positive association when the hospital implements the concept of internal marketing, and the positives reflected in dealing with the hospital's reviewers and achieving functional commitment [7]. observed in their study entitled that internal marketing programs significantly impact employees' commitment to commercial banks and a significant impact on increasing the organization's profitability.



**Odai Falah Mohammad AL-Ghaswyneh and Khaled Adnan Oweis****Internal marketing**

Internal marketing is one of the marketing activities that emerged in the 1990s until now. It pushed organizations to take care of their employees and consider them partners who strive to achieve their goals by caring for employees and considering them the first customers[8]. Internal marketing can satisfy employees' needs and desires. It makes them one of the priorities for organizations to make them happy, reflecting positively on their performance, increasing their productivity, and providing high-level services to overseas customers. It gives a distinct impression to the recipients of services and builds a mental image of the organization and its employees[9]. Therefore, internal marketing is ambiguous as the process that is interested in providing products or services to sell them. Also, as a process that cares about working individuals and provides them with services and benefits and works to improve their abilities and performance and achieve satisfaction and retention [10]. Here comes work on the development of workers through training courses, incentive system, nature of supervision, employee empowerment and promotion system. Therefore, when organizations give some importance to their staff, they will improve the capacity and skills of their team and increase their productive efficiency, which all organizations want to achieve. Internal Marketing has been defined by [11] as a planned effort using a marketing-like approach to motivating employees to implement and integrate regulatory strategies towards customers.[12] defined internal marketing as a proactive attempt to use the approach input. He observed the meaning of marketing to overcome regulatory resistance, change, harmonization, motivation and coordination among staff, and their unification to implement career strategies [13].

defined the concept of internal marketing as a management philosophy of meeting many previously separate areas and working methods, such as strategic management, quality management, human resources management, corporate communications and customer relationship marketing based on the idea of employees as internal clients. The concept of internal marketing based on a simple equation: "saturated employees equal to saturated customers", if the organization can attract, maintain and instil in its minds an understanding of its mission and objectives as well as the needs of its customers, the recent result is a permanent development of the quality of service provided by these employees[9]. Internal marketing has been defined as "internally connected marketing and works towards employees within the organization, which is one of the requirements for the success of the organization's work[14]. Internal marketing defined as the way and method by which qualified employees within the business treated as internal clients. Accordingly, with a range of procedures and steps taken by senior management, this definition is attracting efficiently and successfully to achieve the goals of all parties dealing with the organization[15]. Internal marketing defined as recruiting, training and motivating qualified human resources who can provide services to customers well[16].

**Internal marketing objectives**

Internal marketing as an administrative philosophy aims to increase employees' awareness of customers' needs and more motivated to serve them. It aimed at the strategic level to create the appropriate internal environment that supports customer understanding and mental openness among individuals. Its target at the tactical level is to sell services and promote marketing efforts to employees. Internal marketing also sought to create a clear vision of the organization and make the staff attract customers and achieve their ambitions and aspirations [17]. Internal marketing also ensures that employees perceived management concern with their well-being and needs. When internal marketing is applied effectively, employees positively affect their tasks, including job satisfaction. It positively affects job satisfaction in services and affects job satisfaction among employees [18]. Internal marketing aims to determine staff's outcomes they accomplished, interact with customers or other employees, and activate internal communication channels between employees. Internal marketing seeks to create an effective internal environment and increase employees' attitude to escalate efforts to achieve organizations' goals as loyal consumers. In addition, internal marketing focuses on making employees like the clients treat them as a resource of any helpful idea in the organization [19].



**Odai Falah Mohammad AL-Ghaswyneh and Khaled Adnan Oweis****The importance of internal marketing**

Internal marketing is a strategic direction that is concerned with providing a suitable working environment for employees and success in applying internal marketing, which leads to a successful performance. It increases production efficiency and achieves better customer services[20]. Internal marketing also has a significant and clear impact on organizational processes. One of the essential factors that have led to employees' commitment is to treat employees as internal corporate clients, to enhance and improve their commitment and involvement. Moreover, the organizations act with employees as responsible, independent and active entities to realize they are a capital asset rather than a cost to be controlled [21]. Internal marketing is the best activity in attracting and retaining the best talent by providing a healthy working environment that enhances employees' motivation for creativity, efficiency and performance [22]. Internal marketing also raises employee loyalty to the organization, impacting the quality of services and products offered to customers [23].

**Internal marketing properties**

Internal marketing has features and characteristics emanating from the integration of human resources management and marketing management applications, and the essential attributes of internal marketing mentioned as follows:

- Internal marketing is generally not a separate activity. Still, it serves as the essential quality of the customer service program, strategies and tactical work, where the structure of accompanying activities is a preliminary structure for external marketing activity.
- Communication is a critical point for the success of internal marketing and has a crucial role to play in gaining a competitive advantage and reducing conflict within the staff.
- Marketing is successful when the top management and all employees commit, and the open-door style is the prevailing method.
- Internal marketing is also an optional process that enhances results and facilitates creativity[2].
- Internal marketing is a social process that goes beyond the function of satisfying the material needs of employees and works to provide them with job stability and security and increase job satisfaction, which will increase productivity among employees[24].

**Blended marketing**

Recently, a developed model used in the marketing field to present the mixture of internal marketing that combines the most critical activities and procedures that appear to play an essential role in the application of internal marketing in organizations while at the same time mimicking the elements of external marketing. The following is an explanation of the crucial aspects of this model:

- Internal product (job): It expressed by job or job, which is the set of tasks organized and accomplished by a particular person in a specific place.
- Internal price (obligation): Means the psychological cost of adopting new ways of working, i.e. the cost of abandoning the usual methods and methods to implement these new policies or when moving from work in one section to another.
- Distribution (workplace): Distribution means placing the suitable person in the proper position, especially frontline employees (liaison workers), and distribution generally refers to the workplace or working environment.
- Internal promotion: Promotion defined as a marketing communication activity that aims to inform, convince or remind individuals to accept, repurchase, use a particular product, accept the idea and deal with the organization[18].

**Internal marketing components**

Internal marketing has specific components as well as attributes and properties, which could be as follows:

- Training: defined as an administrative and organizational efforts that linked to the state of continuity. In addition, it makes a skilled, cognitive and behavioural change in the individual's current and future



**Odai Falah Mohammad AL-Ghaswyneh and Khaled Adnan Oweis**

characteristics. So, they will meet working requirements or develop a better functional and behavioural performance.

- Empowerment: means an internal state of mind that needs to be adopted. The individual applies this situation to have self-confidence and conviction with the knowledge abilities that help make decisions and test results. It is a liberation from restrictions, motivation, and rewarding for entrepreneurship and creativity[25].
- Motivation and rewards: Incentives and rewards raise the employee's performance and increase commitment to the work. Motivation increases production efficiency, which contributes to achieving the organization's objectives[26].
- Internal communications: Communications defined as activities through which information and ideas exchanged to achieve a perception that reflects positively on conducting administrative process and thus gains the objectives of the organization[20]
- Granting powers: Giving powers to employees is an essential factor in producing and providing the best services to customers, provided that this process linked to the employees' efforts in their best efforts to take responsibility for their work.
- Leadership: leadership is the process of pushing and encouraging individuals towards achieving specific goals. Also, leadership defined as the manager's ability to understand staff behaviour to guide working to achieve the organization's objectives [27].
- Employees' performance: employees' performance is the degree of achievement and completion of the tasks. It is a Job reflection of how the individual accomplished the requirements of the job and the ability to convert the organization's inputs into outputs in goods, processes and services with determined specifications and at the lowest possible cost [9].

**Methodology**

The study relied on the descriptive-analytical approach and divided it into two sections. The first section interested in extrapolating the mentioned results in the theoretical literature and previous studies. Moreover, to select the related subjects of the current research, whether in books or scientific periodicals. Meanwhile, the second section concerned the field side by preparing survey lists as a primary tool for collecting data.

**The study community and sample**

The study community were the employees of government sectors. While the sample of the study was a total of (600) employees of the government sectors randomly selected, where (600) questionnaires distributed among the study sample and (550) questionnaires valid for statistical analysis for 91.6% of the total questionnaires distributed. However, it is an acceptable ratio for scientific research purposes, and the following table shows the distribution of the study sample members according to their demographic variables.

**Study tool**

The study instrument was a questionnaire that authors designed to meet study purposes based on Leckert fifth-scale ranging from "strongly disagree" to "strongly agree" by relative weight (1-5), which included three sections, as follows:

- The first section concerns the personal and functional variables of the sample.
- The second section concerned with the tool related to internal marketing and included (22) paragraphs distributed over (6) domains.
- The third section concerned with the tool related to the employees' performance included (10) paragraphs.





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## RESULTS AND STATISTICAL ANALYSIS

**Results First:** Answering study questions Question 1: What is the impact of internal marketing on increasing the performance of employees in government sectors?

Upon question, arithmetic averages and standard deviations of the independent variable calculated. Table two explains the results.

The table shows that the calculation averages of the internal marketing researchers as a whole came in an average, with an average calculation (3.29) and a standard deviation (0.10). At the dimensions' level, after "leadership", it ranked first, with an average calculation of (0.10). 3.43) With an average approval rating, followed by "internal communications" with an average account of 3.43 and an average approval rating, and in third place came after the "powers" with an average account average of (3.28) and an average approval score. Then, it followed by the fourth place after "training" with an average account average of (3.35) and a medium approval score, and in fifth place after "incentives and rewards" with an average account average of (3.38) and an average approval score, and in the sixth and final place came after the "empowerment service" with an average account average of (2.91) and with medium approval. The following is the presentation of the calculation averages and standard deviations for each Dimension at a limit.

The table shows that the calculations of the researchers' estimates of the variable performance of employees in the government sectors at the macro level came with an average analysis (3.29) and standard deviation (0.17), and at the paragraph level, the paragraph "The training courses I obtain improved the performance of the work" ranked first, with an average calculation of (3.72) and a high approval rating. Meanwhile, the paragraph "My authorities help me complete the work in the right way."

### Prior – Testing hypotheses

Before analyzing the regression test to examining the study hypotheses, some tests were conducted to ensure that the data were appropriate to regression analysis assumptions. Data confirmed that there was no high correlation between independent variables (Multicollinearity). After using the Variance Inflation Factory and Tolerance test (VIF) for each study variable, and taking into account that the (VIF) does not exceed the value (10) and the tolerance test value at (0.05), a Normal Distribution calculated Skewness Factors, taking into account that the data tracked natural distribution. Meanwhile, this is a fact if the value of the twisting factor obtained. See Table 4, which shows the results of these tests. We note that VIF test values for all variables are less than ten and range from (1.369-1.681) and that tolerance test values ranged from (0.596-0.731), which is more significant than (0.05) and this is an indication of the lack of a high correlation between independent variables (Multicollinearity). Then, it confirmed that the data follow the natural distribution by calculating skewness, where values were close to the value (0) meaningless than (1), so it can be said that there is no real problem with the natural distribution of study data. The validity of the form and table five shows the results.

Table 5 shows the study hypothesis test model's validity after calculating F value allowance and the accompanying indication level at the  $\alpha \leq 0.01$ . Internal marketing as overall explains (9.6%). From the variation of dependent variables: staff performance in government sectors confirms the impact of internal marketing on interpreting the disparity in employee performance, so we can test the research hypotheses as follows.



**Odai Falah Mohammad AL-Ghaswyneh and Khaled Adnan Oweis****Testing hypotheses**

The first hypothesis: there is a statistically significant impact at the level of ( $\alpha \leq 0.05$ ) for internal marketing in all its dimensions (empowerment, training, incentives and rewards, internal communications, powers, leadership) in the performance of employees in government sectors. The statistical results in Table 6, beta transaction follow-up and testing (t) show that the following sub-variables (training, incentives and rewards, internal communications, leadership) impact employees' performance in government sectors. Beta transactions for these variables showed an indication of increment in T value. It calculated from their scheduling value at the indicative level of ( $\alpha \leq 0.05$ ). There is a statistically significant impact at the level of ( $\alpha \leq 0.05$ ) for some dimensions of internal marketing (training, incentives and rewards, internal communications, leadership) in the employees' performance of government sectors from the sample's perspectives. To determine the importance of each independent variable to predict the change in the effectiveness of Stepwise Multiple Regression analysis, table 7 shows the results.

Table 7, which shows the order of entry of independent variables into the regression equation, shows that after incentives and equivalents, it followed by contacts after training after leadership. The researcher attributes this result to the fact that internal marketing will help employees in the government sectors achieve and complete the business as desired by the government sectors, increasing production efficiency. Internal marketing achieves the delivery of information to employees in a serial manner that makes employees get the information correctly. This process will lead to proper completion if the information is available in the hands of the staff and will help provide services to the reviewers in a way that achieves satisfaction and satisfies their needs. The training process will work to develop the skills of employees, especially those who need such a type of courses that suit their needs. It is customary that the training process will make employees knowledgeable in the performance of their work and keep employees away from getting into problems, mainly if these courses aim at the needy and understaffed, as well as internal marketing through the application of an effective system in the process of incentives and rewards. A practical leadership approach on employees that strengthens the relationship between presidents and subordinates will benefit employees and the sectors in which they work and achieve the goals properly.

**Sub-study hypotheses**

- **H1:** There is a statistically significant effect at the level of ( $\alpha \leq 0.05$ ) after training in increasing the performance of employees in government sectors. The statistical results in Table 8, beta transaction follow-up and t testing show that the variable (training) impacts increasing the performance of employees in government sectors.
- **H2:** There is a statistically significant effect at the level of ( $\alpha \leq 0.05$ ) for internal communications in increasing the performance of employees in government sectors. Statistical results in Table 9, beta transaction follow-up and t testing show that the variable (internal communications) impacts increasing the performance of employees in government sectors.
- **H3:** There is a statistically significant effect at ( $\alpha \leq 0.05$ ) motivation and rewards in increasing the performance of employees in government sectors. The statistical results in Table 10, beta transaction follow-up and t testing show that the variable (incentives and rewards) has an impact on increasing the performance of employees in government sectors.
- **H4:** There is a statistically significant effect at ( $\alpha \leq 0.05$ ) to enable the increased performance of employees in government sectors. The statistical results in Table 11, beta transaction follow-up and t testing show that the "empowerment" variable does not affect the increased performance of employees in government sectors. Sub-hypothesis 5: There is a statistically significant effect at ( $\alpha \leq 0.05$ ) for leadership in improving the performance of employees in government sectors. The statistical results in Table 12, beta transaction follow-up and t testing show that the (leadership) variable impacts increasing the performance of employees in government sectors.
- **H5:** There is a statistically significant effect at the level of ( $\alpha \leq 0.05$ ) to grant authority in increasing the performance of employees in government sectors. It is clear from the statistical results in table 13, the follow-up of beta transactions and the test (t) that the variable (granting authority) does not affect the increased performance of employees in the government sectors.



**Odai Falah Mohammad AL-Ghaswyneh and Khaled Adnan Oweis****The second hypothesis**

There are no statistically significant differences in the perceptions of staff performance of the study sample members in government sectors due to demographic variables (gender, age, income, social status, years of experience).

Herewith, to test this hypothesis, multiple Anova analysis used, and table 14 shows the results. Data in Table 14 indicate no statistically significant differences at the indicative level ( $\alpha \leq 0.05$ ) in the study sample in terms of estimating the level of performance of employees in government sectors due to variables (sex, age, social status, income, years of experience).

**DISCUSSION**

After examining the study's hypotheses, it turns out that there is a statistically significant effect at the level of ( $\alpha \leq 0.05$ ) of training in increasing the employees' performance in the government sectors. Therefore, the government sectors consider identifying the problems facing employees and working to solve them through employee training, whether central or decentralized. In addition, the selection of trainers who have the capabilities, knowledge, and experience will improve the employees' performance. Also, there is a statistically significant effect at the level ( $\alpha \leq 0.05$ ) of internal communications in increasing the employees' performance in the government sectors from the sample's perspectives. The researcher considered that this result is a fact because government sectors' contribution to the communication process within the sectors has dramatically helped employees obtain information. According to these results, it works to develop performance when data is available. They indicate the nature of the regulations in place that allow the exchange and transmission of information to staff. There is a statistically significant effect at ( $\alpha \leq 0.05$ ) incentives and rewards in increasing the employees' performance in the government sectors from the sample's perspectives. The researchers attribute this finding to the fact that the systems used for incentives and rewards in government sectors have impacted the performance of employees.

Because these sectors use a fair system between employees in distributing rewards and incentives, this factor affected the employees' performance in the desired form and completed the work assigned to them in the best way. There is no statistically significant effect at ( $\alpha \leq 0.05$ ) to increase the employees' performance in the government sectors from the study sample's perspectives. There is also a statistically significant effect at ( $\alpha \leq 0.05$ ) for leadership in increasing the employees' performance in the government sectors from the sample's perspectives. The direction of the sectors in which they work through sound guidance, and when there is no statistically significant effect at the level of ( $\alpha \leq 0.05$ ) to increase the employees' performance in the government sectors from the sample's perspectives. The employees achieve these things will push them to perform their work and accomplish it in high quality. The results of the second hypothesis showed that there were no statistically significant differences at the level of ( $\alpha \leq 0.05$ ) among sample's perspectives in terms of estimating the level of employees' performance in government sectors due to demographic variables (sex, age, social status, income, years of experience).

**CONCLUSION**

It was concluded that internal marketing dimension have impactful direction on employees' performance[3]. Government sectors should take care of internal marketing as the cornerstone of the success of external marketing of customers[5]. In addition, part of the authority must be given to staff, not their concern, and enable them to make decisions about the reviewers' problems[9]. Governments that utilizes from expanding empowering to staff to encourage them to participate in decision-making and express their views on various issues related to their sector has organic growth in assets and productivity[21]. Moreover, government should pay more attention to training employees at all administrative and professional levels because of their importance in improving their performance[17, 27]. It was observed the need to focus on the internal communication process enables employees to obtain accurate and correct information. Also, to activate the channels of official communication in the delivery of information[24].





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**RECOMMENDATIONS**

Based on the findings, the research recommends:

- Government sectors should motivate employees with material, moral, individual and collective incentives without relying on one type of incentive.
- It must pay attention to the human aspect of the staff and the treatment of staff in an atmosphere of civility and kindness.

**Declarations of interest**

None

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**REFERENCES**

1. Al-Kahtani A. Employee emotional intelligence and employee performance in the higher education institutions in Saudi Arabia: A Proposed theoretical framework. *International Journal of Business and Social Science*. 2013;4(9).
2. Hebbaz N, Chergui K. Using Internal Marketing to Extract Value: An Empirical Study on the Algerian Private Health Organizations. *International Journal of Business and Management*. 2019;14(4).
3. AL-Ghaswyneh OFM. Pillars of internal marketing and their impact on staff performance. 2018.
4. AL-Majali MM, Bashabsheh AA. Factors that affect commercial banks customers intention towards electronic payment services in Jordan. *International Business Research*. 2016;9(3):79.
5. Boukis A, Kaminakis K, Siampos A, Kostopoulos I. Linking internal marketing with customer outcomes. *Marketing Intelligence & Planning*. 2015.
6. Tsai Y. Learning organizations, internal marketing, and organizational commitment in hospitals. *BMC health services research*. 2014;14(1):1-8.
7. Zaman K, Javaid N, Arshad A, Bibi S. Impact of internal marketing on market orientation and business performance. *International Journal of Business and Social Science*. 2012;3(12).
8. Rafiq M, Ahmed PK. Advances in the internal marketing concept: definition, synthesis and extension. *Journal of services marketing*. 2000.
9. De Bruin L, Roberts-Lombard M, De Meyer-Heydenrych C. Internal marketing, service quality and perceived customer satisfaction. *Journal of Islamic Marketing*. 2020.
10. Chao P, Dolnicar S, Lazarevski K. Marketing in non-profit organizations: an international perspective. *International marketing review*. 2009.
11. Nointin A, Chui CTB. EFFECTS OF INTERNAL MARKETING ON TURNOVER INTENTION OF FRONTLINE EMPLOYEES IN THE TRAVEL AND TOUR AGENCIES IN SABAH. *The Turkish Online Journal of Design, Art and Communication*. 2018;Special edition:711-20.
12. Al-Borie HM. Impact of internal marketing on job satisfaction and organizational commitment: A study of teaching hospitals in Saudi Arabia. *Business and Management Research*. 2012;1(3):82-94.
13. Sinčić Ćorić D, Pološki Vokić N. The roles of internal communications, human resource management and marketing concepts in determining holistic internal marketing philosophy. *Zagreb International Review of Economics & Business*. 2009;12(2):87-105.
14. Bazazo IK, Alananzeh OA, Alshawagfih KFA. Exploring the effect of marketing mix elements on the quality of medical tourism services in Jordan. *European Journal of Social Sciences*. 2016;51(3):317-26.




**Odai Falah Mohammad AL-Ghaswyneh and Khaled Adnan Oweis**

15. Al-Zyadat MA, AL-Sha'ar IM, Al-Awamreh MA. The Impact of Human Capital in Competitive Strategy for small and medium Enterprises (SMES). Journal of Islamic University for Econmical and Managerial Studies JIUEMS. 2015;23(1):123-54.
16. Kotler P. Marketing Management: The Millenium Edition, Phipe Prentice Hall. Inc; 2010.
17. Al-Dmour HH, Asfour F, Al-Dmour R, Al-Dmour A. The Effect of Marketing Knowledge Management on Bank Performance Through Fintech Innovations: A Survey Study of Jordanian Commercial Banks. Interdisciplinary Journal of Information, Knowledge, and Management. 2020;15:203-25.
18. Yusuf GO, Sukati I, Andenyang I. Internal marketing practices and customer orientation of employees in Nigeria banking sector. International Review of Management and Marketing. 2016;6(4S).
19. Joung H-W, Goh BK, Huffman L, Yuan JJ, Surles J. Investigating relationships between internal marketing practices and employee organizational commitment in the foodservice industry. International Journal of Contemporary Hospitality Management. 2015.
20. Alshura MS, Nusair WKI, Aldaihani FMF. Impact of Internal Marketing Practices on the Organizational Commitment of the employees of the Insurance Companies in Jordan. International Journal of Academic Research in Economics and Management Sciences. 2016;5(4):168-87.
21. Sharifabadi AM, Bideh SS. EFFECT OF MANAGEMENT COMMITMENT TO INTERNAL MARKETING ON EMPLOYEES'SATISFACTION. A CASE STUDY: IMAM JAFAR SADEGH HOSPITAL NURSES. Asian Academy of Management Journal. 2016;21(2):135.
22. Winarja W, Sodikin A, Widodo DS. The effect of Organizational Commitment and Job Pressure to Job Performance through the Job Satisfaction in Employees Directorate transformation Technology Communication and Information Indonesia. International Journal of Business and Applied Social Science (JBASS). 2018;4(2).
23. Abu Hamza SS. Internal marketing in the Palestinian universities and its relationship to achieving their competitive feature. 2017.
24. Balta S. The influence of internal marketing on employee satisfaction in the service industry. Business Management Dynamics. 2018;8(1):12.
25. Melhem Y. Empowerment as a contemporary administrative concept. Cairo: Arab Organization for Administrative Development. 2006.
26. Hassouna F. Human Resources Management. Dar Osama Publishing And Distribution, Amman, Jordan. 2008.
27. Van Wart M. Administrative leadership theory: A reassessment after 10 years. Public Administration. 2013;91(3):521-43.

**Table 1. demographic variables distribution**

Variable	Domain	Frequency	Percent
sex	male	322	58.54%
	female	228	41.45%
	total	550	100%
(Years) age	30-20	113	20.54%
	40-31	155	28.18%
	50-41	172	31.27%
	more than 50	110	20%
	total	550	100%
Social manner	single	121	22%
	married	387	70.36%
	otherwise	42	7.64%
	total	550	100%
(SAR) Income	less than 10000	192	34.90%
	15000-10001	222	40.36%
	20000-15001	97	17.63%





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	20001more than	39	7.11%
	total	550	100%
Experience) Years(	Less than	160	29.10%
	-10May	247	44.90%
	more than 10	143	26%
	total	550	100%

**Table 2. internal marketing dimensions means and importance index II**

Dimensions	mean	Std. Dev	Average - degree	II
Empowerment	2.91	0.41	Medium	6
Training	3.35	0.49	Medium	4
Incentives and Rewards	3.38	0.64	Medium	5
Internal Authorities	3.41	0.63	Medium	2
Authority	3.28	0.34	Medium	3
Leadership	3.43	0.45	Medium	1
Overall	3.29	0.1	Medium	

**Table 3. Internal marketing impact on employees' performance – Major Paragraphs**

Dimensions	mean	Std. Dev	Average - degree	II
Internal communication and quick access to information enabled me to perform the work as required	3.7	0.75	H	2
I feel like I am part of the team that developed my performance	3.47	0.77	M	5
Employees in government sectors are selected according to the specifications and conditions of the job.	3.08	0.34	M	9
The promotion system used to promote employees is fair	3.26	0.69	M	7
The services I need to perform my work are available.	3.27	0.49	M	6
The support I get from the leader has made me complete the work to achieve the sector's goals in which I work.	3.12	1.13	M	8
Doing my job, the way I see it is appropriate enables me to do the job successfully.	3.61	0.54	M	4
The authority which I have to help me get the job done the right way.	3.06	0.64	M	10
The incentive and reward systems used in the sector I work in have made me work efficiently and effectively.	3.64	0.74	H	3
The training courses I get have improved the performance of the work.	3.72	0.44	H	1
Overall	3.29	0.17	M	

**Table 4. VIF, tolerance and Multicollinearity test**

Dimensions	VIF	tolerance	Multicollinearity
Empowerment	1.681	.596	-1.702
Training	1.542	.689	0.328
Incentives and Rewards	1.492	.718	-1.403
Internal Authorities	1.369	.731	-1.673
Authority	1.475	.651	0.358
Leadership	1.584	.687	0.324





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**Table 5. Analysis Of – Regression – Model Variance**

Dependent variable	source	R <sup>2</sup>	Sum-Sqr	Mean-Sqr	F	Correlation F*
Employees' Performance	variance	0.096	0.386	0.386	14.593	0.000*
	error		3.653	0.026		

**Table 6. multiple regression analysis**

Independent variable's Dimension	B	Std-Error	Beta	T-Value	T*
Training	-0.33	0.029	-0.949	-11.466	.000*
Incentives and rewards	-0.109	0.025	-0.416	-4.414	.000*
Internal communication	0.1	0.035	0.371	2.893	.004*
leadership	-0.132	0.029	-0.355	-4.611	.000*

\*: Significance Correlation at the level of ( $\alpha \leq 0.05$ )

**Table 7. Stepwise Multiple Regression**

Ranking dependent variable Pre-prediction	R <sup>2</sup> value	T	T*
	Cumulative Factor	value	
Training	0.612	9.084	0.000*
Incentives and rewards	0.747	7.53	0.000*
Internal communication	0.772	3.614	0.000*
leadership	0.791	3.269	0.000*

\*: Significance Correlation at the level of ( $\alpha \leq 0.05$ ) – variable regression equation excluded (empowerment, authorities)

**Table 8. Excluded Multiple Regression- Training**

Independent variable's Dimension	B	Std-Error	Beta	T-Value	T*
Training	.096	0.034	235	2.835	.005*

\*: Significance Correlation at the level of ( $\alpha \leq 0.05$ )

**Table 9. Multiple Regression- Internal Communication**

Independent variable's Dimension	B	Std-Error	Beta	T-Value	T*
Internal Communication	0.084	0.021	0.318-	3.941	.000*

\*: Significance Correlation at the level of ( $\alpha \leq 0.05$ )

**Table 10. Multiple Regression- Incentives and rewards**

Independent variable's Dimension	B	Std-Error	Beta	T-Value	T*
Incentives and rewards	0.213	0.023	0.612	9.084	.000*

\*: Significance Correlation at the level of ( $\alpha \leq 0.05$ )

**Table 11. Excluded Multiple Regression- Empowerment**

Independent variable's Dimension	B	Std-Error	Beta	T-Value	T*
Empowerment	0.002	0.032	0.004	0.048	.962**

\*: Significance Correlation at the level of ( $\alpha \leq 0.05$ )

**Table 12. Multiple Regression- Leadership**

Independent variable's Dimension	B	Std-Error	Beta	T-Value	T*
Leadership	0.076	0.022	0.28	3.424	.001*

\*: Significance Correlation at the level of ( $\alpha \leq 0.05$ )





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**Table 13. Excluded Multiple Regression- Authorities**

Independent variable's Dimension	B	Std-Error	Beta	T-Value	T*
Authorities	0.052	0.041	0.107	1.268	.207**

\*: Significance Correlation at the level of ( $\alpha \leq 0.05$ )

**Table 14. ANOVA analysis**

Variable	Sum-Sqrt	free-Degree	mean - sqrt	F value	F*
sex	0.016	1	0.016	0.557	.457**
age	0.033	3	0.011	0.397	.755**
social manner	0.001	1	0.001	0.03	.863**
income	0.003	2	0.001	0.048	.954**
experience	0.011	2	0.006	0.201	.818**
Error	3.496	125	0.028		
Total	1527.32	550			

\*: Significance Correlation at the level of ( $\alpha \leq 0.05$ )





## A Study on Measures Taken to Control Air Pollution in Bangalore City

M. Gurusamy<sup>1\*</sup>, K. Deepika Rani<sup>2</sup> and G. Gunaseelan<sup>3</sup>

<sup>1</sup>Professor and Head, PG Department of Commerce and Management Studies, Brindavan College, Bengaluru, Karnataka, India.

<sup>2</sup>Assistant Professor, PG Department of Commerce and Management Studies, Brindavan College, Bengaluru, Karnataka, India.

<sup>3</sup>Associate Professor, PG Department of Commerce and Management Studies, Brindavan College, Bengaluru, Karnataka, India

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### \*Address for Correspondence

**M. Gurusamy**

Professor and Head,

PG Department of Commerce and Management Studies,

Brindavan College, Bengaluru, Karnataka, India.

E.Mail: gurusamyphd@gmail.com



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### ABSTRACT

Air pollution happens when gases, dust particles, smoke are introduced into nature in a way that makes it a problem to people, animals, and plants. Air pollution injurious to the health of humans and other living beings on our planet. It creates smog and acid rain, causes cancer and respiratory diseases, decreases the ozone layer environment, and contributes to global warming. Air pollution is influenced by the presence in the environment that poisonous or causes health effects, it affects every human being and to all sectors: that are animals, cities, forests, ecosystems. In recent years, we though interested in two areas, which hurt many unfavourable consequences of air pollution: nature or environment and human health. The situation in Bangalore is particularly worst because of the cocktail of contamination recorded daily, in which a lot of pollutants exceed the air quality standards at the exact time. If the small particle contamination levels in there ever reached the same levels as Bangalore, this is all about the health care how it is affecting human health and same to measures taken to control the study the air contamination in Bangalore city. Air pollution is a major environmental cause. It can affect human health and life support systems as well, however, fresh air is an essential thing of life.

**Keywords:** Air Pollution, Diseases, Ecosystems, Environment, Human Health.





## INTRODUCTION

Air pollution currently one of the most squeezing natural concerns. While nine out of the ten most dirtied polluted cities in the world lie in India and Pakistan that leaves no room for self-satisfaction amongst other countries somewhat because they also have their issues and because we live in an associated biological system. Air pollution is a multiplex thing as so many components or elements are answerable for the poor quality of our air. Air pollution happens when gases, dust particles, smoke are introduced into nature in a way that makes it a problem to people, animals, and plants. Air pollution injurious to the health of humans and other living beings on our planet. It creates smog and acid rain, causes cancer and respiratory diseases, decreases the ozone layer environment, and contributes to global warming. In this industrial age, air pollution cannot be evicted fully, but steps can be taken to deduct it. The government has developed and continues to develop, guidelines and instructions for air quality and order to restrict outflow to control air pollution. On an individual level, we can reduce our contribution to the pollution threat by using public transportation. furthermore, purchasing energy-efficient light bulbs and machines or otherwise reducing our electricity use will reduce the pollutants released in the production of electricity, which creates most of the industrial air pollution.

Air pollution is the introduction into the atmosphere of chemicals, particulates, or biological materials that cause discomfort, disease, or death to humans, harm other living organisms such as food crops or harm the natural atmosphere or built environment. Fabric in the air that can be disadvantageous to humans and the atmosphere is known as an air pollutant. Pollutants can be in the form of strong particles, fluid beads, or gases. What is more, they might be natural or manmade. Air pollution can be classified into two different categories; visible and invisible, both can guide to an alternation of the elegant balance of our environment and threat to health on a local scale and also the risk to the system globally in types of consumption of the ozone layer leading to weather change, air pollution can arise in two main paths, first pollutants can be discharged straight into the climate are and known as basic pollutants can consolidate to frame optional pollutants and can be found in the arrangements of issues, for example, smog.

### IMPORTANCE OF THE PROBLEM

Air pollution is the most significant one among those causes natural pollution, reducing poisons noticeable all around is significant for human wellbeing and condition, henceforth the investigation of air pollution is significant since the air pollution control is important for better condition, all have to bothered about the reality of the air pollution and battle against for the control, the investigation about the air pollution is designed to expectation the environment counteractive action. because air pollution is one of the best wrongdoings ever committed, even though dirtying the air is deemed satisfactory. It kills many people every year, it's additionally the main thrust behind climate change, we study air pollution so that we can better understand its effects and sources, we use the information to make informed decisions on meaningful ways to prevent and mitigate air pollution, we're still a long way away from having clean air. 90% of the world's total populace breaths messy air the size of wretchedness from birth surrenders, ailment, wellbeing, loss of efficiency is stunning, we cannot settle on information-based choices without information, furthermore, that is the reason we must study air pollution.

Air pollution is one of the significant contaminations among the natural pollution because each living being needs air in each second, none of the living things can make it without air. Also, that air ought to be crisp and clean, since the investigation of air pollution is important, air pollution is likewise prompt's environmental change, the investigation of air pollution is comprehending the impacts and source, so we can take the counteractive action strategy effectively.



**Gurusamy et al.****STATEMENT OF THE PROBLEM**

Air pollution is influenced by the presence in the environment that poisonous or causes health effects, it affects every human being and to all sectors: that are animals, cities, forests, ecosystems. In recent years, we though interested in two areas, which hurt many unfavourable consequences of air pollution: nature or environment and human health.

**THE CONSEQUENCES OF AIR POLLUTION ON THE ENVIRONMENT**

In our environment all people, animals, trees, entire things can suffer facing problems facing from air pollution, that hide shapes and colours, air pollution particles, in the long run, fall back on the earth, air pollution can straightforwardly pollute the surface of bodies of water and soil, this can murder crops or diminish their yield, it can murder youthful trees and different plants. Like people creatures can experience the ill effects of the introduction of air pollution, birth imperfections, infections, and lower conceptive rates have all been attributed to air pollution. The contaminated air, floating on the outside of the earth, is diverted by wind and downpour. Clouds and high temperatures likewise help to scatter contamination to arrive at extremely huge spans from its place of the starting point.

- Air pollution has a great crash on the process of plant evolution by averting photosynthesis in many cases, with genuine results on the filtration of the air we relax or breathe.
- If there is a clean environment only the human beings, animals, plants are also in a clean that is the reason it is harmful to the environment, that is only the thing mentioned in above point.
- Air pollution adds to the arrangement of acid rain, barometrical precipitation as downpours, which are discharged during the burning of petroleum products. by contact with water vapor in the air, acid.

**THE CONSEQUENCES OF AIR POLLUTION ON HUMAN HEALTH**

Air pollution impacts on humans: humans experience a long range of health effects from being exposed to air pollution, effects can be fell into short-term effects and long-term effects. Short-term effects that are temporary this is not permanent, include illness, they also include discomforts such as irritation to the nose, skin, throat, and eyes, this pollution affects humans a lot and it creates headaches. And here long-term impacts of air pollution can keep going for quite a long time for a whole lifetime, they can even prompt a person's passing, long term health effects from air pollution include all disease, lung cancer, a few researchers suspect air pollution cause birth defects, almost 2.5 million individuals die world wide every year from the impacts of open-air or indoor air pollution.

Our continued subjection to these air pollutants is reliable for the decline of human health., Specifically, air pollution can cause cardiovascular issues, sensitivities, asthma assaults, blood issues in the psychological advancement of the youngster, among others. The most helpless are youngsters the older, pregnant ladies, and the sick. Here how the air pollution is a consequence of human health, that is.

- Because of the air pollution, human health is not in a good position, he had to face a lot of problems because of the air pollution.
- Human beings are very sensitives, they can live without pollutants in nature, if there is pollution happens means human beings are in a dangerous situation.

Air pollution might be introduced as the presence of two or more pollutants like dust, smoke, colour in the environment that are affect human beings, forests, and animals. And all these related for the reason of this pollution that how it causes the pollution itself.

**THE CONSEQUENCES OF AIR POLLUTION TO DIFFERENT SOURCES**

Air pollution cause in various ways in all that these different sources it means that it affects the things that harmful to the human beings and also affected by the human beings that gives a lot of strength to the study of this here, that is the reason it will include here itself, Different sources it means I want to tell here about that global warming, global warming is an environmental phenomenon caused by natural and anthropogenic air pollution. It refers to

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raise air and sea temperatures around the globe, this temperature rise is in any event in part brought about by an expansion in the measure of ozone-depleting substances in the air, ozone-depleting substances trap heat vitality in the earth's air, Here in this I will going to tell about the pollution how it is acquiring different sources in all different categories, in that the air pollution is acquiring dust and construction, waste burning, transport, diesel generator, industries, domestic cooking these different things that would be the pollution acquiring things.

**CAUSES OF AIR POLLUTION**

- The burning of fossil fuels: sulfur dioxide released from the burning of fossil fuels like coal, petroleum and another factory inflammable is one the major reason of air pollution, pollution emitting from vehicles including trucks, jeeps, cars, trains, airplanes cause a huge amount of pollution we depend on them to fulfill our daily basic needs of transportation. But their overuse is killing our nature as dangerous gases are polluting the environment or nature. Carbon monoxide caused by improper or incomplete burning and generally discharged from vehicles is another major pollutant along with nitrogen oxides, that are produced from both natural and manmade processes.
- Because of agricultural activities : here because of air pollution affects for the agricultural activities a lot and it is a threat or causes for the agricultural things what we are do in the agricultural things, most farmers center around addressing to difficulties of nuisances and sicknesses and agronomic practices yet a major test could be the impact of air pollution. Crops can be harmed when presented to high groupings of different air poisons, damage ranges from unmistakable markings on crop leaves, to decreased development and yield to unexpected passing, if there should arise an occurrence of creatures, it might prompt pollution because of eating bad feed and water, the development and seriousness of the damage depend not just on the grouping of the specific poison yet in addition on various things, these incorporate the length of introduction to the poison the plant species and its phase of advancement just as the ecological variables helpful for the development of the pollution and to the preconditioning of the plant, which makes it either defenceless or impervious to damage.

**NEED AND RELEVANCE OF THE STUDY**

The techniques introduced to decrease or delete the emission into the nature of substances that can threaten the nature or atmosphere or human health. The control of air contamination is one of the principal areas of contamination control, along with wastewater treatment, solid-waste management, hazardous-waste management. The better path to save air quality is to decrease the release of pollutants by changing to cleaner fuels and processes, pollutants do not remove in this way must be collected or catch by related air-cleaning devices as they are showed and before they can went-off into the environment, these devices are portrayed below. The accentuation of this article is air pollution control innovation as it is intended to expel particulate and vaporious toxins from the outflows of stationary sources, including force plants and mechanical offices.

The situation in Bangalore is particularly worst because of the cocktail of contamination recorded daily, in which a lot of pollutants exceed the air quality standards at the exact time. If the small particle contamination levels in there ever reached the same levels as Bangalore, this is all about the health care how it is affecting human health and same to measures taken to control the study the air contamination in Bangalore city.

**OBJECTIVES OF THE STUDY**

- To create public awareness about air contamination and its impacts to help the community or the society.
- To save the public are from different types of diseases, including tuberculosis, asthma, and other skin diseases.



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## RESEARCH METHODOLOGY

Here in the methodology of the research, I will brief the description which I have collected or received from the respondents, people, and all. In all the places they have said that what is all in the ways they are facing the problem with the pollution that comes from the air and that company and the employees are facing the problems these are all the problems that company employees facing the problems. I visited one of the companies in the Bangalore they are all said that due to the pollution only they have not done works properly and what are all the threats they are taken to come up from that pollution this is also one of the main reasons to that and also the companies want the good atmosphere nature for them and their work. there is now and then seen in the structure of an overview the evident aim to describe the environment of a region by itemized concoction investigation of the greatest number of substances as can be taken care of in the research facility. After-effects of such works, notwithstanding, uncover a drearily comparative character in the air solids in various urban areas all through the nation and such contrasts as are seen are discouraging without real noteworthiness, the most significant one here is that nobody knew for a long time what the reason for brown haze was and all specialized research was committed for an extensive stretch to responding to this inquiry. Generally, for all the companies they want good nature with unpolluted air or nature, the pollution is sometimes it creates by vehicles, factories all those of thinks. This is about the methodology of my research.

### LIMITATIONS FOR THE STUDY

Air pollution has been one of the highest threats that people have been facing for a lot of years, this is not a problem for some specific places, some people across the world need to restrict the issues related to air contamination. The air contamination index has been at every time high for quite some of the time, the effects of air contamination can be serious and very affecting to your overall health.

### SWOC ANALYSIS

#### Strengths

When talking about the strength of this article it is a social subject, that is the reason all people responsible for this thing and they must think about threats which are coming on the earth, human beings, animals, plants and also creatures, and this article's strength is to that what I have collected the data and analysis that is only the strength of my report. Information's which I have got regarding the air pollution it is very useful for this article and also it is very understood to the public, and also make very neat clear picture regarding control the threats of this, And one more thing is what the data I have collected from the air pollution board that is all enough to control the pollution in the city, and also which I have got the information's from the respondents about the topic that also comes under this, finally these are all the strength of this report.

#### Weakness

In this also the weakness of this article is to the information's which I have collected by the people or with the respondents I thought that that is all not the full information's that I have got that is only the reason this is the weakness of the article, no one is ready to follow the rules and regulations which regarding the air pollution, it cause all weakness to this pollution and also in this nation many are not able to understand the problems which arise by the pollution, they have to understand and precautions must be taken from them, and one more thing is many are still doing threat to the society with the help of pollution, and also they must follow the rules and regulations from the government and also they have to think on it and they have to work on it then the only pollution will be controlled in the earth, and then only humans are chance to leave safely in the beautiful environment this is all about the weakness of this report.





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**Opportunities**

There is a lot of measurement want to take from the government and from all the people that who are including in the society that is the reason it is only about the topic of all the setting of the people that would be under the method of this. Here in opportunities also which things are right to have an opportunity means personnel care and measures have to be taken from the people because different kinds of situations decide different things and also different minds have different kinds of ideas or plans also, all the things were very important and matters here telling that because of this pollution measures would be taken and also care will be taken from the people then only we escape from the things which we have face the threats and also in a certain way that we follow the things matters a lot, and another thing is there is a lot of observations would take from the government it is very necessary for that if there is a government will strict and serious only the public also becomes serious, if the government will leave the things which cause the people the public also do the same, that is the reason this is also one of the opportunities to overcome with this.

**Challenges**

Day by day nature and also in the environment will change, every day it is very from one atmosphere to another, here the challenge is to control or limit with the climate change, sometimes it decides how the things will going on and also it is our responsibility to keep the climate in balance that is the reason it is one of the challenge to the report and the people, and another thing is there are no people who come and support for this social activities, there are busy with some of the works, no one is there to come and give stand with anyone, that is the reason it is also one of the challenges to the report, and final thing is that the reducing risks or threats from the toxic air pollutants, this is also one of the challenges to this, these are all the challenges to this report.

**OUTCOME OF THE RESEARCH**

**PESTANALYSIS**

**Political**

In political analysis, there is no understand between the state and also in central government, if there is an understanding and when cooperation between the state and central government only the works should be done perfectly, and if the state government is accepted to do some activities regarding the air pollution at the same time not ready to do the central government for these activities because every party, they have own clashes on the things here. There are very bad politics they are going to make some of the non-sense in their way that is the reason no chances to bring the part of it, and a lot of misunderstanding between the political parties this is about the political things in this analysis.

**Economical**

In this report economically how it affect or impact the study of air pollution here and also economically how the report will be considering these points here, here increasing the economic extension and energy demand will lead to a notable increase in global discharge of air pollutants and the GDP level also decreasing because of this reason and also air pollution is effects on the market because of the air pollution, which include impacts on labor productivity, agricultural crop surrender, and health costs. This is about the economic thing, it is caused only for the set of all the problematic thing of the situation that arises by the way of the economical thing, and it will come under the method of the way of it, economically it is very harmful to the people and to the environment.

**Social**

Here in the social thing the things which are must follow by the people they are not following the rules and regulations properly and also they have done the things that are safe to the society, and also the people they are not aware of the pollution that is the reason they must aware about the pollution and also which are the bad things to the society and all and another thing is the pollution is affects the trees, rivers, soils and also wildlife that is the reason the people follow the precautions that give by the government to protect the environment and also nature, it is the



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social thing that will affect the things in a socialistic way and make some of the things that ever consuming some of the situations also in a proper source of this, air pollution is not good to the society and also that is our responsibility to protect the environment from the pollution. This is the social thing of the research.

**Technology**

In these technological ways also a lot of things must follow in the way and give things to the in a good way of all the systematic problems of this. Nowadays a lot of technologies are implemented in the world which are not affected to the people as well as the environment, here we are using the petroleum vehicles, better to use solar vehicles and battery charging vehicles because it does not give the air that comes from the vehicle, if we are using the petrol vehicles it gives the bad air to the environment that is the reason better to use the solar vehicles and battery charging vehicles behalf of the petrol or diesel vehicles, this is the study of air pollution.

**EXPERIENCES, LEARNINGS, AND CONCLUSION****Experiences**

- When I go to collect the information regarding the air pollution, Peoples or respondents were positive and cooperative.
- It is very eager to know the information's regarding air pollution because of this study.
- It gives the full information regarding how we would be in society and how we are as human beings.
- I understand a lot of things related the air pollution that from which are all the way air pollution happening and affecting society.
- My experience is about this study is that I thought it gave full information to control air pollution.
- This study helps to study more things related to pollution and about the air pollution control board.
- It was an amazing experience, sometimes the respondents they were not reacted properly.

**Learnings**

- In this, I got more knowledge regarding how pollution happening in the city, that when I visited the places relating to the pollution.
- Lots of things I got to know that what I am studied regarding things everything is a new thing to study.
- Another thing that I learned from this is that many things that teach a lot of knowledge regarding this.
- Huge necessary information was collected from the air control board relating to the study which controls air pollution.
- Made plans for how to take measures to control air pollution in Bangalore city.
- In this, I learned that in the future how to control air pollution with measures that are not affected society.
- It gives knowledge in how the air pollution is affecting and how the measures are taken the way these are all the things that I knew from this study and very useful it is for everyone.

**CONCLUSION**

Air pollution creates very bad impacts on the earth, as above all saying that all the precautions that to be taken for the control of the pollution, we must follow that, and also that is our responsibility to keep the good atmosphere in the earth because of the plants, animals, human beings and creatures. Air contamination can be prevented just if people and organizations quit utilizing dangerous substances that reason air contamination, in any case, this would require the end of all non-renewable energy source consuming procedures, from modern assembling to home utilization of forced air systems, this is a far-fetched situation right now, be that as it may, we need to make rules which set most grounded guidelines on modern and power supply assembling and taking care of. The guidelines are to be intended to further decrease hurtful outflows into the earth's climate. Nowadays the air pollution is very problematic to society and the environment, also to public health. Because of the huge dust in the city, the public is



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falling diseases to various complex victims, including asthma, respiratory complications, other skin diseases, tuberculosis, and bronchitis, etc, the only path to brief or expand the accountability of public administration is government should speedily translate is national atmosphere policy, transport policy into action to advantage the people of this nation, otherwise, this nation will be in risk.

The main thing I am going to conclude for this study is to all the peoples who are in the nation they must take care of the environment and they must make it in clean. The system which gives the government to reduce the air pollution we must follow and control the threats which are going to happen in the future, we want to reduce the bad air consuming which comes from the vehicles, and we must precaution that control to the environment, behalf of the petroleum vehicles we can use battery vehicles and solar system vehicles then only we control the air pollution. Air pollution is from different sources and includes vehicles that come waste air, fuels have been shown to have effects of air pollution. In air pollution, we must follow the things which control from the pollution. Air pollution causes health effects on human beings and plants. And we must take measures strictly to take control of air pollution.

Air pollution is a major environmental cause. It can affect human health and life support systems as well, however, fresh air is an essential thing of life. Air contamination has long been a threat in the world and without air, the earth would be not able to leave a beautiful life, effective administration and outside water can be tuff for build articles, groundwater can be reduced by pumping factors and barring methods, environmental impacts including ground settlement and movement of contaminated groundwater. It must be is being done to control, screen, and amend harm done by contaminations, the issues different and some are just being perceived yet it is essential to keep a nearby power over poisons with the goal that we can keep up nature in an adequate condition for who and what is it come. And this air contamination is a major nature-related health problem to children and a tuff thing for both acute and chronic respiratory disease, to control this problem want to action by central or state government and also raise awareness among the public, the conclusion of this report is we have to follow the rules and regulations of all the measures taken to control the pollution, and we take precautions to control the air pollution primarily, it is the conclusion of this article.

**REFERENCES**

1. Avinash G Mulky (2013). Distribution challenges and workable solutions. IIMB ManagementReview, 25(3),179-195.
2. B. Naga Parameswari, V.Yugan Dhar. (2015). The Role of Human ResourceManagement in Organizations. International Journal of Engineering Technology,Management, andApplied Sciences, 3(7),58-63.
3. Bisiaux, Justine &Gidel, Thierry &Huet, Frederic & Millet, Dominique. (2015). The Business Model, A Tool for Transition to Sustainable Innovation. ICED15 Design Society. Milan, Italy. Retrieved from <https://www.researchgate.net/publication/280233851>
4. Jasmine Kaur. (2016). Customer Relationship Management: A Study of CRM Policies of Different Companies. Global Journal of Finance and Management, 8(2), 153-159. Retrieved from [https://www.ripublication.com/gjfm16/gifmv8n2\\_05.pdf](https://www.ripublication.com/gjfm16/gifmv8n2_05.pdf)
5. Leila Saari, Tanja Suomalainen, RajjaKuusela. (2017). A Cookbook for Predicting the FutureIntroduction of Foresight Tools. Helsinki, Finland: DIMECC Publications, Series No.13. Retrieved from [www.dimecc.com](http://www.dimecc.com)
6. Mohammadreza Khorshidi. (2018). Value Proposition as a Main Dimension of Business Model forNationalIranian Oil Company. International Journal of BusinessandManagementInvention,7(5),37-45.
7. SudamsettiNaveen, D.N.M Raju(2014). A Study on Recruitment & Selection Process withReference to Three Industries, Cement Industry, Electronics Industry, Sugar Industry inKrishna Dt AP,India. IOSR Journal of Business and Management, 15(5), 60-67. Retrieved from <http://www.iosrjournals.org>.





## ***Spirulina* as an Immune Booster for Fishes**

Khumbar Debbarma<sup>1</sup>, Pradipta Banerjee<sup>2\*</sup> and Preetha Bhadra<sup>3</sup>

<sup>1</sup>Department of Fisheries, Centurion University of Technology and Management, Odisha, India.

<sup>2</sup>Department of Biochemistry and Plant Physiology, Centurion University of Technology and Management, Odisha, India.

<sup>3</sup>Department of Biotechnology, Centurion University of Technology and Management, Odisha, India.

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### **\*Address for Correspondence**

#### **Pradipta Banerjee**

Department of Biochemistry and Plant Physiology,  
Centurion University of Technology and Management,  
Odisha, India.

E.Mail: pradipta.banerjee@cutm.ac.in



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### **ABSTRACT**

Not only does fish play a significant role in food demand for people, but has also become a crucial model for several scientific studies. There have been various tests in fish with the use of different medications. Bacterial diseases in fish are most common. Antibiotics are often used to manage bacterium-led fish illnesses, although antibiotic resistant forms of bacteria are being developed. Medicinal herbs can serve as immune stimulants, allow the non-specific defensive systems of fish to be activated early and increase a particular immune response. The plant contains various components of the immune system, including as polysaccharides, organic acids, alkaloids, glycosides and volatile oils that might improve immune activities. The immune system means an assembly of cells, chemical production processes that guards our bodies against invading antigens like viruses, bacteria, cancer cells and so on. The immune system's key duties include protecting against bacterial infection organisms pathogenic. Antigens in today's society are increasingly resistant to antibodies and numerous illnesses are caused. We are concentrating in this review on the action of the unicellular alga *spirulina platensis* as an immune stimulator. *Spirulina* is historically used as a dietary supplement that can reinforce the immune system. Furthermore, it is unavoidable that such an immunological booster will remain free from sickness as *Spirulina platensis* is rich in protein, vitamins, and minerals and carotenoid.

**Keywords:** Immune Boosters, *Spirulina*, Immunity, Fish, Health Benefits..



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## INTRODUCTION

Fish is a delicious and protein-rich food for humans. India is on the brink of the Blue Revolution and has made great strides in inland fisheries. In addition to playing an important role in meeting human nutritional needs, fish are also widely used in various biological experiments. In addition to playing an important role in meeting human nutritional needs, fish are also widely used in various biological experiments. In relation to the pharmacokinetic and pharmacodynamic parameters, the antibacterial, ant parasitic and anaesthetic drugs were widely experimented on the fish. A successful fish trial has been conducted on medications such as tetracycline's, penicillin's, macrolides, quinolones, sulfonamides, anti-cancer agents, herbal medicinal medicines, vaccines, etc. Antibiotics are widely used to fight diseases caused by these bacteria. However, there is an increased risk of developing bacterial strains resistant to antibiotics. Medicinal plants can be used as immunostimulants to activate the non-specific defense mechanisms of fish in the early stage and enhance specific immune responses. Medicinal plants have been used as medicines and immune enhancers in humans for thousands of years. In recent years researchers and feed companies have shown increasing interest in the use of plants in animal feed. Plants contain many immune-active ingredients, such as polysaccharides, organic acids, alkaloids, glycosides and essential oils, which can improve immune function. The therapeutic plants have been utilized as medication to treat distinctive fish infections and to control of shrimp, particularly within the nations like China, Mexico, India, Thailand and Japan. The immune stimulating role of some herbs in aquaculture has raised interest. Some blends of Chinese herbs in shrimp and tilapia have increased the non-specific immune system, such as bactericidal activity and leucocyte function.

In contrast to the induction of other inflammatory elements, the non-specific protective mechanism of fish involves neutrophil activation, peroxidase and oxidative radical synthesis. As mentioned below, certain herbs with immunostimulatory effects were used both experimentally and clinically to diagnose and monitor various diseases of fish in various medicinal plants (Govind et al. 2012). Fish are in close interaction with aquatic diseases. Overpopulation and low hydrodynamics and diet lead to increased infection susceptibility. Different drugs are being used for treating and preventing illnesses to avoid significant economic losses related to diseases. The use of antimicrobial medicines in aquaculture will contribute to bacterial pathogens becoming resistant. Over the past few years, alternatives to antibiotics are being pursued and medicinal plants are one of the choices available (Stratev et al. 2017).

### IMMUNOSTIMULATING HERBS IN FISH

By use of red clover in tilapia diet has indeed been observed to enhance feed intake, growth performance, the protein digestibility, and visible protein usage (Yilmaz et al. 2018). By use of immunomodulators for the prevention of fish diseases in fish crops is gaining the interest of many researchers as a solution to medications, chemicals and antibiotics. In this sense, many concentrated on the use of herbs to deter and prevent fish diseases as possible therapeutic steps for the modulation of immune response and, in particular, on the use of herbs. Medicinal plant rehabilitation are profoundly established and intimately related to the preservation of better health and to the cultural history of many people from various cultures and nations. Macrophages, monocytes, granulocytes, Humoral components, including lysozymes or the supplementary system are the principal components of the Innate immune system. Injection, bath or oral should be used for immunostimulants, which are the most feasible in this. The most commonly recognized immune stimulants are bacterial cell membrane components such as lipo polysaccharides and glucans, but they can also stimulate the unspecific immune response of fish by means of synthetic materials, polysaccharides, vitamins and animal and plant extracts. Substances that encourage growth, antimicrobial agent, nutrients and many other applications are currently used in commercial aquaculture. They are also studying their capacity for preventing and controlling fish diseases. A comprehensive scientific research has focused on modulate the immune response using medicinal herbs as a therapeutic potential measure. Medicinal plants form a deep-rooted part of the cultural patrimony and are closely tied, as such, to the preservation of good health of many people from varied cultures and nations (Galina et al. 2009).



**Khumbar Debbarma et al.****HERBS AND VACCINATION**

The use of immunostimulants with fish vaccinations shows potentiality as a way of raising fish's safeguarding capacity and reducing the immunostimulant's vaccine dose to improve the vaccine's strength while decreasing the dose required achieving the same impact. In association with carp vaccine Ganoderma (Ganoderma is a genus of fungi in the family Ganodermataceae commonly known as called Lingzhi, it is one of the most known medicinal species. Recognized as the "marvelous herb". It is widely used in china) and Astragalus (It is a flowering plant in the family Fabaceae. It is one of the herbs used in traditional Mongolian medicine. Commonly combined with other herbs, *it* has been utilized as a dietary supplement in case of several conditions, including the respiratory infections.) noted that the respiratory burst response of the phagocytes as well as increased phagocytosis and lysozyme activities in bloodstream were significantly improved in these fish. There were also high levels of immune reactions, but the vaccinated community did not include herb extracts with herbal extracts and vaccinated fish did not show significant variations. The highest survival rate was seen by fish feeding all herbs vaccine. Herbal products can be applied in the fish culture as a vaccine, particularly as they are easy to produce, not costly and work against a wide range of pathogens. Most herbal products can be administered orally, as it is the most efficient immunostimulation process (Galina et al. 2009).

**SEAWEEDS**

Seaweed is renowned chief constituent hydrocolloids, i.e. agar, alginates and carrageenan. The aquatic organisms are regarded as aquatic macro algae or algae. They can flourish in shallow waters and 180 m deep ocean. The algae are divided into three major groups: brown, red, and green. They are also known as Phaeophyceae, Rhodophyceae, and chlorophyceae. The aquatic algae have several microbial, medicinal and sulfated polysaccharide behaviors. Furthermore, the health effects of nutritional quality are many. It has plenty of molecules that promote fitness, including nutritional fibers, essential fatty acids, amino acids and vitamins A, B, C and E. Eatable seaweeds are rich in bioactive compounds, which function as a food product for disease control and with a variety of diagnostic properties, including anti-obesity, antivirals, antifungals, antibacterial, antidiabetics, anti-hypertensions, antibiotics, anticancer, antioxidants and thyroids (Singh et al. 2020). Oceans, whose top layer is occupied by algae, are two thirds of the earth. This suggests that these photosynthetic microbes are widely available. In recent decades, algae have seen a surge with concomitant breakthroughs and progress in this area. Not only are algae of significant environmental significance, but are also quite economically important (Gomez-Zavaglia et al. 2019). The seaweed had lost its initial or genuine identity by this time and, in its broadest sense, had been understood to be the closest approximation of algae. Seaweeds or algae are a crucial ingredient in coastal marine environment primary biomass production and perform vital ecological functions as a habitat and substratum for invertebrates, fish, mammals and birds (Rebours et al. 2014). Seaweeds provide fascinating growth elements for innovative food and nutraceuticals. A nutraceutical item is a food that does not have a nutritional value but has a favorable impact on human health and might therefore assist to avoid health issues like cancer, arthritis, diabetes, auto-immune disorders, the eye and cardiovascular disease (Cotas et al. 2021). In the development and proliferation of macro algae in direct or indirect ways, seaweed related bacteria play a vital role. Phyla-borne bacterial colonies the most common surfaces of seaweed are proteobacteria and firm cuts. Proteobacteria a consortium comprised of microbes comprising bacteria, fungus, diatoms, protozoa, spores and larva of invertebrates is a very sophisticated, dynamical and complicated microbe that lives on the surface (Singh et al. 2014).

**SEAWEED CLASSIFICATION**

Seaweeds are classified into three major groups according to pigmentation: brown algae (Phaeophyceae), green algae (Chlorophyta), and red algae (Rhodophyta). These are all different in terms of characteristics – morphology and size. Seaweed are divided into two classes according to their size, that is, phytoplankton and seaweed with 6000 various species (Singh et al. 2020).



**Khumbar Debbarma et al.****BROWN ALGAE**

Brown seaweed has historically been exploited for nutritional, functioning and technical uses by coastal people (Reboleira et al. 2019). Algae in cold areas around the continental shores, the class contains around 1,500 species of seaweed in the Chromophyta division. Species colors ranges, depending on the ratio of brown (fucoxanthin) to green pigments, from dark brown to olive green (chlorophyll). Brown algae varies in their shape and size from little epiphytic filament to complicated massive kelps of 1 to 100 meters (Bhutia et al. 2018). This brown color is derived by the supremacy of the component xanthophyll fucoxanthin, which conceal other components, like chlorophyll a and c (there seems to be no chlorophyll b), beta carotene and xanthophyll. The majority of brown algae are haploid and diploid. The haploid thallic isogamous gametes, anisogamous or oogamous, and the diploid thallic, often meiosis, create zoospores (Guiry, 2014). The brown seaweed holds a number of anti-oxidant compounds. These substances are developed to deter bodily damage that can lead to cancer and other disorders. Brown algae chemicals may potentially affect inflammation and the body's immunological system. They are utilized for treating cancer, fibromyalgia, arthritis, stress, weight reduction, cardiovascular disease, high cholesterol, etc. (Wong, 2021).

**GREEN ALGAE**

Green algae are an extensive, diverse category of algae possessing chlorophyll a and b as main photosynthetic activity, xanthophyll, beta carotene, as well as auxiliary pigments. Green algae for the photosynthesis are used by higher species. Other green algae are related to other creatures symbiotically. The member's includes simple, multi-cell, colonial and flagellate. Notable examples include *Spirogyra*, *Ulothrix*, *Volvox*, etc. Green algae. The typical mobile or non-mobile green algal cell has a central vacuolar, plastid pigments, and two-layer cellulose and pectin cell wall. The cell is formed of several kinds (Rimsa, 2007). Green algae have cellular structure which comprises cellulose-like assemblies of polymers, hemicelluloses, arabinogalactan, extension and lignin found in embryophytic walls (Domozych et al. 2012). The majority of green algae reside in fresh water, generally on rocks and woods or as scum in standing water; terrestrial and marine species also exist. Microscopic species that float freely assist aquatic creatures as food and oxygen sources. In the evolutionary plants research, Green algae are also of importance; *Chlamydomonas* with a single cell is seen as the ancestral shape likely to give rise to terrestrial plants (Rimsa, 2007).

**RED ALGAE**

The color red is obtained by the phycoerythrin and phycocyanine pigments, which hide other pigments, chlorophyll a and chlorophyll b absent, beta-carotene and many distinct xanthophylls. Typically Florida starch and floridoside are the primary reservations; there is no real starch like those of higher and green plants. Cellulose and agar walls and carrageenans, both of which are long-chained polysaccharides for commercial usage. A few unicellular agents of varying origin; filaments are made of more sophisticated thallic. Coralline algae, which produce calcium carbonate into their cells, are a particularly significant category of red algae. Some of these corals are interconnected. These corallines were employed in therapy for bone repair. Coral algae were used as insect repellent in ancient times. Therefore, the binomial *Corallina officinalis*. The *Kappaphycus* red algae and *Betaphycus* are currently the leading carrageenic sources, a popular element in foods, yoghurt and chocolate milk and puddings. In the creation of all-important, extensively used, micro-organism agar as a growth medium and in food and biotechnological applications *Gracilaria*, *Gelidium*, *Pterocladia* and other red algae are employed (Guiry, 2014). Red algae have been used as a source of nutrition and to treat diseases for thousands of years. It is rich in vitamins, minerals and antioxidants, and the body can easily use it. The main benefit of red algae is their ability to promote healthy blood circulation. It can regulate blood sugar and lower bad cholesterol in the human body, because it is rich in fiber, a rich source of calcium and magnesium, which can promote bone health; in addition, because it contains antioxidants, it helps to strengthen the immune system. Systematize and nourish your skin (Lawrence, 2014).

**MEDICINAL VALUE OF SEaweEDS**

The nutritional and physiological advantages of seaweed were intensively investigated among the functional impacts. Seaweed is rich in wellness chemicals and components such as nutritional fiber comparison to terrestrial plants and animal-based diets (Rajapakse et al. 2011). In many coastal countries, seaweed is used as a food source,



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industrial use and fertilizer. The biggest food use of these crops is in Asia, especially Japan, South Korea and China, where algae cultivation has become an important industry. In most western countries, the consumption of food and animals is limited, and there is almost no pressure to develop algae growth methods. These are the current and potential uses of algae. , Some excellent biochemical reagents. Today, algae are used as human nourishment, cosmetics, and fertilizers and for industrial gum and chemical production. They are capable of being employed as a source of medical and industrial usage for big or small chain compounds. Marine algal can also be utilized as energy collectors. Fermentation and pyrolysis can extract potentially valuable chemicals (Guiry, 2014).

**ANTIOXIDANT PROPERTIES**

The hunt for natural antioxidants has attracted substantial interest over the past decade and there has almost been a quadruple in the number of publications on antioxidants and oxidative stress. A variety of illnesses such as chronic inflammation, atherosclerosis, cardiovascular and cancer illnesses and ageing processes are crucial for antioxidant chemicals, explaining their great economic potential in medicine, food production and the beauty business. Marine algae are exposed to a mixture of light and oxygen, like other photosynthesis plants, that lead to the development of free radicals and other powerful oxidizing agents. Moreover, the lack of oxidative damage and stability in preservation in the structural components of microalgae indicate that the cells contain antioxidant-protective protection mechanisms. Algae have protective enzymes as well as antioxidant molecules, which are comparable in size to those of vascular plants, such as phlorotannins, ascorbic and carotenoids, phospholipids, chlorophyll, amino acids, polysaccharides, etc. Antioxidant effects of algae extracts have been researched in several geographical zones, but only a few studies on tropical algae have been conducted. Antioxidant activity of tropical macro algae is remarkable as the antioxidant defense system in the tropical environment is predicted to provide a very powerful antioxidant system. Earlier investigations have shown that UV radiation promotes antioxidant protection of macro algae. Many macro algae species have been subject to strong sunlight on the Gulf of Mexico and the Caribbean coast and some have been never examined for antioxidant activity (Shelar et al. 2012).

**ANTIFOULING PROPERTIES**

Marine bio fouling is defined as a binding of microbial slime, macro algae and invertebrates on any evolving surface, such as barnacles, moles and sponges. This phenomenon has several repercussions and many sectors, such as fisheries and the industrial sector, have to face bio fouling implications. For example, the ship hull marine bio fouling increases fuel use, drag and therefore cleanup and painting, resulting in the estimated cost for the US Navy alone of 1 billion US dollars a year. Moreover, fastened hulls and propeller blades boost engine stress, thus expanding carbon dioxide emissions, adding significantly to global warming. Bio fouling in the process known as epibiotic, affects marine organisms themselves. Their surface colonization can lead to the change from plankton to benthic existence and to the transition from motility to the form of the sessile life. Marine organizations have developed fouling mechanisms, including mechanical defense, physical defense or chemical defense. Marine algae, produce a wide variety of active chemical metabolites that can help avoid or minimize access to light and nutrients, and guard themselves from other settling organisms. Active metabolites are reported as antibacterial, anti-antifungal, anti-algal, and anti-macro fouling agents beneficial in preventing the bio fouling of many species of marine and microalgae (Shelar et al. 2012).

**ANTIMICROBIAL PROPERTIES**

Seaweed is a bioactive source since it may create a number of secondary metabolites with a broad diversity of biological activities. While seaweed grows in a hostile environment, throughout metabolism it rarely suffers from major photodynamic damage. This means that marine cells are protected by certain defensive substances and processes. Since seaweed is an excellent source of antibiotic agent, omega-3 fatty acids, antioxidants and other bioactive substances, these items should be used in nutraceuticals and functional meals. Selected methanol extracts of red or brown seaweed have also been discovered as polyphenolic chemicals, such as catechins, flavanols and flavanol glycosides and exhibit antioxidant and antibacterial action. Experts successfully identified brown algae *Sargassum sagamianum*, sargaquinoic acid derivatives with antibacterial activity. The effects of various levels of



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brown algae, *Listeria monocytogenes*, *Salmonella*, *Enterococcus faecalis* and *Pseudomonas aeruginosa*. The *In vitro* antibacterial property against gram positive and gram negative bacteria was demonstrated in the extracts and active ingredients of several algae; selected cases are listed. Antimicrobial production was seen as a sign of the capability of seaweed to produce secondary bioactive compounds. Many microalgae substances, including anti-bacterial, anti-antivirals, anti-tumor, anti-coagulant and anti-fouling, have been reported in this field. Antioxidants are by far the most thoroughly discussed among the various compounds with functional characteristics. Furthermore, there was a demonstration of the crucial role of antioxidants in one's nutrition, thus boosting customer attention in and demand for these products. The marine algae provide for bioactive natural compounds as essential resources. Phenolic substances were found in Brazilian red algae. The nutrients and antioxidants of *Kappaphycus* have a varied antibacterial activity. The thalli are also recognized in several portions (Shelar et al. 2012).

**ANTICANCER ACTIVITY**

Many species have been identified to be isolated from algae to reduce the spread of cancer cells. The bioactive compounds of algae may kill cancer cells through apoptosis by induction. Separated bioactive compounds with an anti-cancer activity by multiple modes of action along with cancer causing inhibitory activity and incursion, and metastatic action. Apoptosis can either be triggered by intrinsic or external paths. Colorectal and breast cancers are caused by Fucoidan isolated from the brown algae fucus species. Anti-proliferative action against cellular cancer was observed by enzyme elimination of algae, *eclonia cava* with polysaccharides and polyphynolic (Singh et al. 2020).

**ANTI-INFLAMMATORY PROPERTY**

A major part of anti-inflammatory compounds has been provided by marine algae in recent years (Abad, 2013). Lung dysfunction, gastrointestinal problem, atherosclerosis and endothelial dysfunction are all influenced by oxidative stress. The two varieties of red algae species identified as having anti-inflammatory characteristics are *Gracilaria verrucosa* and *Gracilaria textorii*. *Gracilaria tenuistitata* contributes to the suppression of viral inflammation (Singh et al. 2020).

**ANTIVIRAL PROPERTY**

A wide range of viruses have demonstrated anti-viral potential with water-soluble compounds from seaweeds. Hong Kong's coastal areas include more than 200 types of algae (Wang et al. 2008). A variety of antiviral action is contained in marine derived polysaccharides and in their lower molecular weight oligosaccharide derivatives. Sulfated polysaccharides help prevent enveloped virus, arena virus, rhabdovirus, orthopox virus etc. from replicating. Carrageenan helps avoid human papillomavirus from being first infected. The prevention of influenza virus caused pulmonary edema in mouse has been well recognized to have low molecular weights of carrageenan and its products. The action against both enclosed and non-enveloped viruses is highly antiviral (Singh et al. 2020).

**BIOACTIVE COMPOUNDS IN SEAWEED  
POLYSACCHARIDES**

Polysaccharides are characterized by all species, whether marine or upper flora as an energy reservoir and structural constituents. Polysaccharides in algae are the largest macro molecule with a weight of about 80%. These stable polysaccharides are called nutritional fibers not to be metabolized in the body; they can ferment to different degrees owing to the enzyme action of bacteria in the gut. In plants like *Codium*, *Gracilaria*, *Ulva* where polysaccharides are greater above wheat bran, edible marine algae have a significant quantity of dietary fiber ranging from 23.5% to 64% DW (dry weight). Algal polysaccharide varies from tropical Polysaccharides; some are methylated, acetylated, pyruvylated or sulfated, and they consist of unusual polyuronides (Ganesan et al. 2019).

**LIPIDS AND FATTY ACIDS**

Seaweed lipids are mostly made up of the long-chain fatty acids with the numerous saturated fatty acids (SFA) and palmitic acid in each species, notably polyunsaturated fatty acids (PUFA) with atoms of 18 and 22 carbohydrates according to species. In Brown Marine Algae, accompanied by Green and Red Algae, also essential fatty acids (EFA)



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and PUFA were identified in abundance. In the normal metabolic profile of cardiac and obese linked questions, PUFA is highly variable across species of seaweed and omega 6-fatty acid ratio and the omega-3-fatty acid ratio. In Ulva species, *Acanthophora*, *Gracilaria* and *Ulva* fatty acids are discovered to be rich. These are widely documented for anti-inflammatory, anti-hypertensive, anti-hyperlipidemic and angiotensin inhibitors I-converting enzyme (Ganesan et al. 2019).

**VITAMINS**

Vitamins are the main micronutrients that initiate a number of metabolic pathways and are prior to the co-factors of enzymes. As higher species do not have the method of synthesizing these cofactors metabolically, they must thus be received from an external entity. It contains practically all crucial and indispensable vitamins. Algae are the finest source of vitamins. Many algae's such as *Porphyra*, *Himantalia* and *Gracilaria* have a high content of vitamin C comparison to land vegetables. Vitamin B12 is often processed by prokaryotes that are closely related to the surface of eukaryotic alga's and further strengthen levels of B12 in maritime algae. The vitamin B12 level of *Chlorella* and *Spirulina* microalgae is much greater than the one of nori macro algae. In algae, such *Codium fragile*, and *Gracilaria chilensis*, vitamin A (carotenoids) is comparatively high than in carrots (Ganesan et al. 2019).

**USES OF SEAWEEDS****AS A FOOD**

Seaweeds are mostly used in the Far East and Australian regions as food. Fresh seaweed is often used as food by folks residing on the shore in these nations. Nori, Kombu and Wakame seem to be the most significant food species in Japan. It is one of the largest edible algae, and the output Amanori and Asacusa-Nori are created from it in Japanese *Porphyria tenera* (Nori). Kelps have been used in China from at least the 5th century. Laminaria is the most common species utilized, but few species are primarily employed in Japan. Plants would be dried and chopped into bands or crushed upon collecting. Kombu is often used in Japan as a vegetable for preparing fish, meat meals, soups and rice. Powder form Kombu may be either used in sauces and soups or used as curry to rice. There are some types used to make tea-like beverage. The Nori is served in sauces, soups and broths, which may be roasted to provide the green color. It's just moistened and consumed occasionally. For cool rice balls, which are a favourite midday meal for Japanese youngsters dry nori sheets are utilized. The dietary content of nori, vitamins, mineral salts and particularly iodine, is its main cause (Madhusudan et al. 2011).

**AS A SOURCE OF AGAR**

The most effective agar is generated from Rhodophyceae *Gelidium*, which is also known as vegetable agar. 95% is produced in the world. Agar is also made out of numerous different marine algae and produced from eleven species *Gelidium*, *Sarconema*, *hypnea*, and *Gracilaria* the output of agar, the settings of temperatures and the gel strength of the result. Japan is the largest producers of agar and sells agar to almost all of the major countries. The agar is handled in several ways. It is used in ice cream, jellies, pastry production etc., fabric size prepping and liquid cleaning. It is also used in lotions, cosmetics and shoe polishing. The agar is continuously used for media production in biological labs (Madhusudan et al. 2011).

**AS A FERTILIZER**

Because of the abundance in seaweed potassium chloride (KCl) is utilized in several nations, like Japan, France, the United States, England and South India, as a fertilizer. Marine algae are employed for diverse soil cultivations in various places of the world as a fertilizer. Retrieved and cast onshore algae are often used as fertilizer for coconut planting in India in Tamil Nadu and Kerala, possibly directly or in the form of compost. Seaweed manure is known to be preferable than manure from the farming field (Madhusudan et al. 2011).

**AS A MEDICINE**

Pharmaceutical companies have begun in recent times in the quest for novel pharmaceuticals of natural ingredients from marine species, including algae (Smit, 2004). The salty flavor of seaweeds shows that the material may disperse



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the phlegm build-up especially since the material creates soft masses, including goiter, the thyroid enlargement indicating serious iodine shortage (Madhusudan et al. 2011).

**SEAWEED AS AN IMMUNOSTIMULANT**

Notable achievement was obtained with application of more ecologically friendly immunostimulants by framework of the management of fish diseases. It's also famous the inborn immune system can be activated in fish .Through numerous antibiotics. However, a few of the immunostimulants cannot be employed due to its huge cost. In contrary, several plants were employed in indigenous remedies for many illnesses prevention and diagnosis. Ergosan consists of 0.002% non-specified plant extract based on algae. It is utilized for its immunomodulation activities in aquaculture. Immunity in the rainbow trout and snakehead showed Improvement after intraperitoneal utilization of Ergosan operation. The antibiotic and antioxidant effects of seaweeds are widely established. They include polysaccharides, alkaloids; fatty amino acids, micro vitamins and nutrients, and they have important traces of them. Various investigations have found that elements of seaweed can improve immunological function (Balaraman et al. 2017).

**SPIRULINA**

*Spirulina* is like a helix of long slender strands underneath *Arthrospira* genus, the phylum Oscillatoriaceae, a photosynthetic and filamentous cyanobacteria. It is named blue-green algae since this cellular structure contains both green and blue pigmentation. The two most commonly Known species that are utilized due to its nutrition purpose, *Spirulina maxima* and *Spirulina plantensis* (Mosh, 2019). *Spirulina* is an algae used for generations because of the excellent potential and the alleged beneficial effects on health. Today the people of support popular *spirulina* lifestyles as a hidden, powerful super-food. It was formerly categorized as a plant due to "its abundance in plant characteristics as well as its capability of photosynthesis," as per an issue of the journal Cardiovascular Therapeutics. *Spirulina* is grown in minute springs, which are usually adhered to and harvested easily. Its hue is strong blue and green yet its flavor is very modest (Cox, 2018). It is manufactured commercially under specific situations in enormous outdoor pools. The information provided on the human clinical possibilities and uses of *spirulina*, the efficacy and impact of *spirulina*, has been summarized. It is largely beneficial to its chemical makeup, including proteins, carbonates, vital amino acids, minerals, vital fats, vitamins and colors. The major reasons for this are its significant health advantages (Reboleira et al. 2019).Two of the most famous genera of algae are *Chlorella* and *Spirulina*. *Chlorella* is single and *spirulina* is a filamentous cyanobacterium, both exist in fresh water (Andrade, 2018).

**SPIRULINA AS AN IMMUNE BOOSTER**

The earliest category of gram negative blue green algae, widespread in nature, is the oxygen photoautotrophic prokaryotes with a broad variety of bioenergy provider in both aquatic environment and terrestrial, from hydrothermal vents to the Arctic and Antarctic areas. They are the precious resource of industrial and pharmaceutical natural assets. *Spirulina* is a blue-green microalga famous due to use by NASA, as it includes 70% of the protein, it also includes vitamins, including B12 and carotenoids. it is a nutritional remedy for astronauts. The *Arthrospira platensis* is a tiny, filamentous, unicellular prokaryote, which is often popularly called *Spirulina platensis* and may be seen in fresh water resources, ponds and sea-water. No such feature was assigned to *Arthrospira*, unlike some of the other cyanobacteria which have shown to be poisonous. Macronutrients and micronutrients are high in *spirulina*. For thousands of years, *spirulina* is being used for nourishment. *Spirulina* has various uses in farming, pharmacies, perfumery, medicine and research. There are several other uses. It aims to improve the immune function as *spirulina* contains chlorophyll and removes bloodstream impurities. *Spirulina* contributes to the improvement of immunity and boosts virus - induced infections resistance. It helps to establish the mucosal and systemic immune response components by activating the innate cells of the immune system. It demonstrates activities like antioxidant, anti-inflammatory activity, and immunosuppressive activities and bio-modulatory qualities more than the high active ingredients of phycocyanine found in *Spirulina* (Praveena et al. 2020).



**Khumbar Debbarma et al.****IMMUNITY**

Immunity is defined as a complicated biological system, capable of recognizing, accepting and rejecting what is unfamiliar to itself (Rodgers, 2011). Immunity is an individual's strength to protect from infections. The immune system defends us against the invasion of pathogens into the body. It is a body-based defensive system for recognizing non-self or external materials. Immune defense or bodily immunity works in two ways that are to some extent interrelated.

- i. Innate immunity.
- ii. Adaptive immunity.

Innate immunity offers instant protection against pathogens in particular. This is not organism-specific and offers initial line of protection from contamination. It is claimed to be non-specific and not unique to a certain disease. It is a fast reaction that takes place in a few minutes; there is no memory and it will not provide the organism long-term immunity. In comparison a particular immune response focused at an invading pathogen is different from an innate immunity which is a non-specific adaptive immunity. There is always an initial EFFECTOR RESPONSE after introduction to a foreign organism that removes or neutralizes a disease. Later re-exposure to the very same external organism generates a faster reaction rate to the MEMORY RESPONSE that removes illness and removes the pathogens. It is only in vertebrates that this reaction found (Praveena et al. 2020).

**HEALTH BENEFITS OF SPIRULINA**

The characteristic of *Spirulina* is distinctive and is an immunological boon. It demonstrates immunomodulative activities and biomodulative qualities in addition to antioxidant, anti-inflammatory action. Many researches have demonstrated the immunological enhancement capabilities of *Spirulina*. As well as in the individuals with different cancers, AIDS, and other viral diseases, this alga boosts immunologic tolerance (Praveena et al. 2020). *Spirulina* has been widely used as a protein and vitamin boost in aquaculture meals and has become more common in the wellness food business. The supplement has often been used by humans because the concentration of macro and micro in shellfish cultivation, it is greatly enhanced in the diet of giant freshwater prawn, independently of the supplementary amount, by 5 - 20 percent in growth, survival and consumption of feeding stuffs. In young Pacific white shrimp, the partial substitution of fishmeal by *Spirulina* was also studied, with positive results. Nutrients are relatively high. In addition, the manufacturing, extraction and preparation need basic technologies (Mosh, 2019). Several *spirulina* antioxidants have actions against inflammation in the body. The contribution of chronic inflammation to cancer and other disorders. Photosynthetic pigments in *spirulina* has been proven to not only lower bodily inflammation but inhibit tumor development and destroy cancer cells, it also causes in destroying cancer cells. Studies have indicated that the *spirulina* protein can diminish the body's cholesterol absorption and decrease cholesterol levels. This helps maintain a clean artery and decreases the stress on your heart which can contribute to cardiovascular disease and blood coagulation (Brennan, 2020). The threat related to *spirulina* supplements may sometimes outweigh the benefits (Greger, 2007).

*Spirulina*'s significant health advantages:-

- Radioprotection.
- Protection of kidneys and liver.
- Improvement of blood quality and prevention of anemia.
- Prevention of liver and renal toxicity.
- Immune protection.
- Removal of heavy metals from the body.
- Relief in allergic reaction.
- Reduction of blood pressure (Praveena et al. 2020).



**Khumbar Debbarma et al.****HOW SPIRULINA INCREASES IMMUNITY IN THE BODY?**

*Spirulina* is filamentous alga that is multicellular and able to reduce inflammation and to display antioxidant properties. In old adults with a history of anemia, it may improve anemia and immunosenescence (Selmi et al. 2011). The extract of *Spirulina platensis* has a wide variety of uses in medicinal practice; and has the qualities of anti-AIDS in vitro activity, anti-inflammatory, anti-microbial, immunomodulatory. Hyperlipidemia, diabetes and cancer are also reduced effectively in people and animals, as are anticancer and antiviral effects (Praveena et al. 2020).

**IN FISHES**

The outcome of the fish test demonstrated a boost in fish development by substituting *Spirulina* with 5 percent FM protein. The *Spirulina*'s growth impact is related to its function in the digestion of nutrients and its great nutritional content, such as vitamins and minerals. Including fish into meals would guard against injury to tissues by inhibiting the generation of reactive oxygen agents per an increase in dietary antioxidant capability of *Spirulina platensis*. It has a function in the digestion of nutrients and a high level of nutrients like vitamins and minerals. Including fish into meals would guard against injury to tissues by inhibiting the generation of reactive oxygen intermediates according to an increase in dietary antioxidant capability of *Spirulina platensis*. It is a possible immunostimulant that increases mucosal and systemic immune systems by activating the non-specific immune response. Its extract has been influenced by increased phagocytic activity and activation of NK immune system cells. The rate of growth of the fish fed with *spirulina* additive also increased considerably and the hematocrit, nitro-blue tetrazolium, and lysozyme activity increased considerably. There were also considerable increases in growth rate. The sulfated polysaccharides in algae are highly likely to prevent viral infections. These algae have been shown to be anti-viral. Since *Spirulina* is called a super-food, it could be used to improve the resistance and management of the disease, as prevention is thought to be better than treatment (Praveena et al. 2020). *Spirulina* is farmed extensively to fulfill market requirements. The open canal ponds with paddles are used to tumble the water. In the United States, Thailand, India, China, and Pakistan, the principal *spirulina* farms are located (Hvizdak, 2014).

**REFERENCES**

1. Saranraj, P. (2014). (PDF) *SPIRULINA PLATENSIS – FOOD FOR FUTURE: A REVIEW*. Retrieved May 25, 2021, from [https://www.researchgate.net/publication/259503619\\_SPIRULINA\\_PLATENSIS\\_-\\_FOOD\\_FOR\\_FUTURE\\_A\\_REVIEW](https://www.researchgate.net/publication/259503619_SPIRULINA_PLATENSIS_-_FOOD_FOR_FUTURE_A_REVIEW)
2. Pandey, G., & Sharma, M. (n.d.). (PDF) Immunostimulant effect of medicinal plants on fish. Retrieved May 25, 2021, from [https://www.researchgate.net/publication/343376550\\_Immunostimulant\\_effect\\_of\\_medicinal\\_plants\\_on\\_fish](https://www.researchgate.net/publication/343376550_Immunostimulant_effect_of_medicinal_plants_on_fish)
3. Balaraman D, Subramanian V (2017) Immunity Enhancement through Seaweed Extracts in Fish. *Ann Aquac Res* 4(2): 1035.
4. Stratev, D., Zhelyazkov, G., Noundou, X. S., & Krause, R. W. (2017). Beneficial effects of medicinal plants in fish diseases. *Aquaculture International*, 26(1), 289-308. doi: 10.1007/s10499-017-0219-x
5. Galina, J., Yin, G., Ardó, L., & Jeney, Z. (2009). The use of immunostimulating herbs in fish. an overview of research. *Fish Physiology and Biochemistry*, 35(4), 669-676. doi:10.1007/s10695-009-9304-z
6. Shelar, P. S., Reddy, V. K., Shelar, G. S., Kumar, G., Reddy, G. S., & Kavita, M. (2012). MEDICINAL VALUE OF SEaweEDS AND ITS APPLICATIONS – A REVIEW. Retrieved May 25, 2021, from [https://www.researchgate.net/publication/280527895\\_MEDICINAL\\_VALUE\\_OF\\_SEaweEDS\\_AND\\_ITS\\_APPLICATIONS\\_-\\_A\\_REVIEW#:~:text=Seaweeds%20are%20one%20of%20the,healing%2C%20antimicrobial%20and%20antioxidative%20properties.](https://www.researchgate.net/publication/280527895_MEDICINAL_VALUE_OF_SEaweEDS_AND_ITS_APPLICATIONS_-_A_REVIEW#:~:text=Seaweeds%20are%20one%20of%20the,healing%2C%20antimicrobial%20and%20antioxidative%20properties.)
7. Singh, H. (2020). Common Marine Organisms: A Novel Source of Medicinal Compounds. *International Journal of Bioresource Science*, 7(2). doi:10.30954/2347-9655.02.2020.1





**Khumbar Debbarma et al.**

8. Guiry, M. D. (2014, January). The Seaweed Site: Information on marine algae. Retrieved May 25, 2021, from <https://www.seaweed.ie/algae/seaweeds.php>
9. Reboleira, J., Freitas, R., Pinteus, S., Silva, J., Alves, C., Pedrosa, R., & Bernardino, S. (2019). Brown seaweeds. *Nonvitamin and Nonmineral Nutritional Supplements*, 171-176. doi:10.1016/b978-0-12-812491-8.00024-2
10. Bhutia, T. K., Pallardy, R., Petruzzello, M., & Rimsa, C. (2018). Brown algae. Retrieved May 27, 2021, from <https://www.britannica.com/science/brown-algae>
11. Wong, C. (2021, January 21). *The health benefits of brown seaweed*. <https://www.verywellhealth.com/the-benefits-of-brown-seaweed-89551>.
12. Rimsa, C. (2007, November 2). Green algae. Retrieved May 27, 2021, from <https://www.britannica.com/science/green-algae>
13. Lawrence, S. (2014, April 23). The many benefits of red Algae (WITH Product picks). Retrieved May 27, 2021, from <https://www.onegreenplanet.org/natural-health/vegan-health/the-many-benefits-of-red-algae-with-product-picks/>
14. Ganesan, A. R., Tiwari, U., & Rajauria, G. (2019). Seaweed nutraceuticals and their therapeutic role in disease prevention. *Food Science and Human Wellness*, 8(3), 252-263. doi:10.1016/j.fshw.2019.08.001
15. Madhusudan, C., Manoj, S., Rahul, K., & Rishi, C. M. (2011). Seaweeds: A diet with NUTRITIONAL, medicinal and industrial value. *Research Journal of Medicinal Plant*, 5(2), 153-157. doi:10.3923/rjmp.2011.153.157
16. Gomez-Zavaglia, A., Prieto Lage, M. A., Jimenez-Lopez, C., Mejuto, J. C., & Simal-Gandara, J. (2019). The Potential of Seaweeds as a Source of Functional Ingredients of Prebiotic and Antioxidant Value. *Antioxidants*, 8(9), 406. doi:10.3390/antiox8090406
17. Rebours, C., Marinho-Soriano, E., Zertuche-González, J., Hayashi, L., Vásquez, J., Kradolfer, P., Robledo, D. (2014, May 03). Seaweeds: An opportunity for wealth and sustainable livelihood for coastal communities. Retrieved May 27, 2021, from <https://link.springer.com/article/10.1007/s10811-014-0304-8>
18. Cotas, J., Pacheco, D., Gonçalves, A., Silva, P., Carvalho, L., & Pereira, L. (2021, March 09). Seaweeds' nutraceutical and BIOMEDICAL potential in Cancer therapy: A concise review. Retrieved May 27, 2021, from <https://jcmjournal.com/article/view/3932>
19. Singh, R., & Reddy, C. (2014, April 01). Seaweed–microbial interactions: Key functions of seaweed-associated bacteria. Retrieved May 27, 2021, from <https://doi.org/10.1111/1574-6941.12297>
20. Hvizdak, J. (2014, June 28). *What is Spirulina and benefits of Spirulina for fish?* Top reasons why to feed your fish Spirulina. <https://en.aqua-fish.net/articles/benefits-spirulina-food-aquarium-fish>.
21. Praveena, B., Yashas, M., Harish, B., Harshavardhana, R., & Yamuna, M. (2020, June 10). A REVIEW ON *SPIRULINA PLATENSIS* AS AN IMMUNITY BOOSTER FOR THE PRESENT SITUATION. Retrieved May 28, 2021.
22. Mosha, D. (2019, April). The Significance of *Spirulina* Meal on Fishmeal Replacement in Aquaculture: A Review. Retrieved May 28, 2021, from [https://www.academia.edu/38221367/The\\_Significance\\_of\\_Spirulina\\_Meal\\_on\\_Fishmeal\\_Replacement\\_in\\_Aquaculture\\_A\\_Review](https://www.academia.edu/38221367/The_Significance_of_Spirulina_Meal_on_Fishmeal_Replacement_in_Aquaculture_A_Review)
23. Cox, L. (2018, February 07). Spirulina: Nutrition Facts & Health Benefits. Retrieved May 28, 2021, from <https://www.livescience.com/48853-spirulina-supplement-facts.html>
24. Brennan, D. (2020, October 06). Spirulina: Are there health benefits? Pros and cons, nutrition, and more. Retrieved May 28, 2021, from <https://www.webmd.com/diet/spirulina-health-benefits#1>
25. Reboleira, J., & Bernardino, S. (2019). *Spirulina* - an overview. Retrieved May 28, 2021, from <https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/spirulina>
26. Greger, M. (2007, August 24). Blue-Green algae & SPIRULINA. Retrieved May 28, 2021, from [https://nutritionfacts.org/video/blue-green-algae-spirulina/?gclid=CjwKCAjwqCkFBhAhEiwAfEr7zU4PAPnUbmS58RA4yvDvmA4pTVLhGMPx6qTy6z3zo98tz1UelgGKzxoC4KYQAvD\\_BwE](https://nutritionfacts.org/video/blue-green-algae-spirulina/?gclid=CjwKCAjwqCkFBhAhEiwAfEr7zU4PAPnUbmS58RA4yvDvmA4pTVLhGMPx6qTy6z3zo98tz1UelgGKzxoC4KYQAvD_BwE)
27. Andrade, L. M. (2018). Chlorella and spirulina MICROALGAE as sources of functional Foods, nutraceuticals, and Food Supplements; an overview. *MOJ Food Processing & Technology*, 6(1). doi:10.15406/mojfpt.2018.06.00144
28. Yilmaz, E., Tasbozan, O., & Erbas, C. (2018, May 5). Potential of medical herbal products to be used in aquaculture. Retrieved May 30, 2021.



**Khumbar Debbarma et al.**

29. Domozych, D. S., Ciancia, M., Fangel, J. U., Mikkelsen, M. D., Ulvskov, P., & Willats, W. G. (2012). The cell walls of green algae: A journey through evolution and diversity. *Frontiers in Plant Science*, 3. doi:10.3389/fpls.2012.00082
30. Rajapakse, N., & Kim, S. (2011). Nutritional and digestive health benefits of seaweed. *Marine Medicinal Foods - Implications and Applications, Macro and Microalgae*, 17-28. doi:10.1016/b978-0-12-387669-0.00002-8
31. Abad, M. (2013). Anti-inflammatory properties of algae. *Functional Ingredients from Algae for Foods and Nutraceuticals*, 338-368. doi:10.1533/9780857098689.2.338
32. Wang, H., Ooi, E., & Ang, P. (2008, December). Antiviral activities of extracts from Hong KONG SEaweeds. Retrieved May 30, 2021, from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2596289/#:~:text=The%20water%2Dsoluble%20extracts%20of,et%20al.%2C%202003>.
33. Smit, A. J. (2004). Medicinal and pharmaceutical uses of seaweed natural products: A review. *Journal of Applied Phycology*, 16(4), 245-262. doi:10.1023/b:japh.0000047783.36600.ef
34. Rodgers, J. (2011). Immunity - an overview. Retrieved May 30, 2021, from <https://www.sciencedirect.com/topics/immunology-and-microbiology/immunity>
35. Selmi, C., Leung, P. S., Fischer, L., German, B., Yang, C., Kenny, T. P., . . . Gershwin, M. E. (2011). The effects of *spirulina* on anemia and immune function in senior citizens. *Cellular & Molecular Immunology*, 8(3), 248-254. doi:10.1038/cmi.2010.76





## Evaluation of Growth, Proximate Composition, Antioxidant and Phytochemical Properties of Marine Diatom *Amphiprora paludosa* (PSBDU-005)

N. Krishnaveni, P. Santhanam\*, S. Dinesh Kumar, A. Gowthami, K. Nanthini Devi and P. Perumal

Marine Planktonology & Aquaculture Lab., Department of Marine Science, School of Marine Sciences, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India

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### \*Address for Correspondence

#### P. Santhanam

Marine Planktonology & Aquaculture Lab.,  
Department of Marine Science,  
School of Marine Sciences, Bharathidasan University,  
Tiruchirappalli, Tamil Nadu, India.  
E-mail: santhanamcopepod@gmail.com



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### ABSTRACT

In recent years, bio resources derived antioxidant and byproducts attracting much attentions due to replace chemical based antioxidants. Among the bioresources, microalgae being prime candidate to convert the biomasses to the valuable byproducts. This study dealing with assessment of growth with reference to media and analyses of proximate compositions, antioxidant, and phytochemical constituents of marine diatom *Amphiprora paludosa*. Among the five media tested, TMRL medium result higher growth and protein being as dominant constitutes in proximate compositions. The DPPH radical, super dioxides radicals, and hydroxyl radicals were higher in 10 mg of *A. paludosa* with the value of 65, 52 and 53 % respectively. In phytochemical constituents analysed methanol reacted in all the constituents and revealed better results. The present findings inferred that the *A. paludosa* biomass and their extracts provided much biological applications for economical and eco-friendly development.

**Keywords:** *Amphiprora paludosa*; diatom; proximate compositions; antioxidant; phytochemicals

### INTRODUCTION

Microalgae are photosynthetic eukaryotes that constitute one of the major components of both marine and freshwater environments as primary producers and they form food source for secondary and tertiary marine organisms. They are also excellent sources of pigments, lipids, carotenoids,  $\omega$ -3 fatty acids and other fine chemicals [1]. As photosynthetic organisms, this group plays an important role in the productivity of marine environs and which constitutes the basis of the marine food chain or food web. Moreover, phytoplankters are responsible for half



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of the oxygen release in the atmosphere [2]. Their long evolutionary and adaptive diversification to a multitude of habitats and extreme conditions (e.g., cold/hot environments, hydrothermal vents) make them good candidates for drug discovery, because they may have evolved compounds for communication, defense and survival that are often unique and such things may not have any terrestrial counterparts [3]. There are around 40 % of global photosynthesis is assumed to be performed by microalgae [4,5] thus, becoming important in the alteration of inorganic nutrients (e.g, C, N, and P) towards the transformation into organic forms [5] and thus contributing to approximately 50 % of the total planetary primary productivity [6].

Microalgae have been considered to be an important source of bioactive compounds since they are the sustainable resources, easy to culture that do not require an arable land and freshwater unlike the terrestrial plants. Some researchers have already reported the interesting and remarkable antioxidant potential of microalgae [7]. As regard their biotechnological applications there is a constant effort actually provided for both finding and exploiting new microalgal resources and developing their putative commercial utilization.

Antioxidants are molecules that inhibit or quench free radical reactions and which delay or inhibit cellular damages [8]. The antioxidant enzymes convert dangerous oxidative product in to hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and then to water, through a multi-step process in the presence of cofactors such as copper, zinc, manganese, and iron. Non-enzymatic antioxidants work by interrupting free radical chain reactions. The free radicals used to attack all the major classes of biomolecules, mainly the polyunsaturated fatty acids (PUFA) of cell membranes, alter the enzyme activity and increase the susceptibility of protein to proteolytic attack. These may lead to diseases like rheumatoid arthritis and cataract through oxidative damage to lens crystalline [9]. Microalgae represent an almost untapped resource of natural antioxidants, as they exhibit enormous biodiversity, much more diverse than higher plants. During the photosynthesis process, they absorb solar light which is converted into chemical energy, later used in the conversion of CO<sub>2</sub> into carbohydrates, and at the same time, generating molecular oxygen, which can reach locally at a high concentration levels. However, not all groups of microalgae can be used as natural sources of antioxidants, due to their widely varied contents of target products and growth rate or yields. Hence, the present research work was focused to examine the biochemical, phytochemicals and antioxidant activity of aquaculture important microalgae. Thus, this research aims to characterize microalgae of interest for aquaculture for their potential biological activities, especially in antioxidant properties.

## MATERIALS AND METHODS

### Indoor Cultivation and Molecular Characterization

The marine diatom *Amphiprora paludosa* were taken from Marine Planktonology & Aquaculture Laboratory, microalgae culture collections and the indoor cultivation of diatom was made according to the method of Perumal et al. [10]. For indoor cultivation F/2 medium were used and the detailed composition of medium were provided in Perumal et al. [10]. Initially the culture media optimization were carried out and the detailed medium composition were given in our previous publication Santhanam et al. [11]. The *A. paludosa* strains were preliminary identified by microscopic analysis, then were further confirmed by 18S rDNA sequence analysis. The cultured cells was harvested in mid-exponential growth phase by centrifugation (10,000g for 10min) and the cell pellets were washed twice using sterile deionized water in a sterile centrifuge tube. Total DNA was extracted using a CTAP method according to the Doyle and Doyle [12]. The DNA extracts were analyzed by electrophoresis on 1.5% agarose gel to confirm the presence and approximate concentration of products, and then used for PCR amplification. Then the amplified sequence further analysed with primers followed by genbank submission of accession number.

### Evaluation of Phytochemical Compounds and Proximate Composition

The extracts were obtained from different solvents based on the polarity (polar: methanol, ethanol and water, Intermediate: chloroform, Non polar: petroleum ether and hexane). 1mg/ml of the microalgal powder was taken for



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secondary metabolites extraction and soaked in different solvents and kept in room temperature for 24 hours. After the extraction, the solvent was filtered through Whatman no.1 filter paper. The crude extracts were stored at 4°C for further analyses. The phytochemical compounds viz; tannins, flavonoids, terpenoids, steroids, saponins and alkaloids were estimated as described [13]. The protein content level estimated according to the method of Lowry et al. [14]. Lipid was estimated by using the chloroform-methanol method as described by Folch et al. [15]. Carbohydrate was estimated according to the method of Dubois et al. [16].

**Evaluation of Antioxidant Activities**

The free radical scavenging activity of the microalgal extracts was evaluated using standard procedures and Ascorbic Acid was used as the reference compound. All analysis were run in triplicates and the average was taken. The potential of marine microalgal extract to scavenge the stable radical DPPH (1, 1-diphenyl-2-picrylhydrazyl) was measured by the method described by Brand-Williams et al. [17] with slight modifications. The hydroxyl radical scavenging activity of different crude extract was determined by the method of Halliwell et al.[18]. The super oxide radical of the sample was determined by adopting the method of Liu et al. [19].

**RESULT AND DISCUSSION**

Figure-1a shows the microscopic view of *A. paludosa*. The genomic DNA from *A. paludosa* isolates were extracted and subjected to the agarose gel. Then the isolates procured genomic DNA with bp of 615. Then the amplified sequences were analyzed using NCBI database and proceed with highest similarity of *Amphiprora paludosa* (MN966871). The obtained NCBI accession and identification were confirming that *Amphiprora paludosa* (Fig. 1b). Figure 2 shows the growth curve of the *A. paludosa* at various culture medium and highest growth were observed at TMRL (0.423 abs) medium followed by Conway's (0.414 abs), Miquel's (0.405 abs), F/2 (0.211 abs), and Schreiber's (0.171 abs) medium. Santhanam et al. [11] stated that the growth and biomass production of microalgae may vary based on nitrogen and phosphorus availability on the medium and present study confirms that the higher growth were observed in TMRL medium followed by Conway's medium. Figure-3 shows the proximate composition of the *A. paludosa*; among the three parameters tested protein (30.6%) was found high followed by carbohydrate (12.15%) and lipid (11.58%).

The fluctuation of salinity in the culture medium may affect the protein accumulation in algal cells opined by Gu et al. [20]. The synthesis of protein getting triggered when the ATP emissions was null while unstapled NaCl concentration occur [21]. The lipid and carbohydrate getting stimulated when nitrogen, light intensity, salinity, and pH was supplied at enough concentration and even the supply of CO<sub>2</sub> was varying [22]. The carbohydrate and starch accumulation might be varried species to species when the salinity and their metabolism changed due to favorable and unfavorable environmental conditions [23]. Borowitzka [24] stated that microalgae considered bank for protein, pigments, fatty acids, and polysaccharides. Due to the presence of different bioactive compounds, microalgae consisting many antioxidant proprieties from their crude extract. The antioxidant activity like DPPH radical, hydroxyl radical, and super dioxide radical has been given in the Figure. 4a-c. The DPPH radical treatment were analyzed in different concentration of crude extracts like 2, 4, 6, 8, and 10 mg. The highest DPPH radical were observed in 65% whereas ascorbic acid (control) result 79%. The same trend has been observed in super dioxide radicals, and hydroxyl radicals were highest was observed in 10 mg of crude extract and percentage of activity were 52 and 63 respectively. Banskota, [25] opined that, microalgae have huge quantity of antioxidant protection like in vitro and in vivo due to presence of alpha tocopherol β-carotene, and phenolic acids. The various groups of phenolic compounds were present in the microalgae and their concentration and may vary species to species like medicinal plants, fruits, herbs and etc. Manivannan et al. [26] stated that the antioxidants characteristics are high in microalgae than the other terrestrial and aquatic plants. Table-1 shows the phytochemical constituents of *A. paludosa* with various extracts cultured under TMRL medium. Among the four extracts (methanol, ethanol, ethyl acetate, hexane) tested, methanol result all phytochemical components except steroids, saponin and tannins. Ethanol extracts shows terpinoids, phenols, and glycosides where as ethyl acetate results steroids and glycosides. However, hexane extract



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did not react with any phytochemical constituents. The results showed that the *A. paludosa* extracts contained diversified phytochemicals with various bioactivities. The necessary of alkaloids, saponins and tannins were utilized as antibiotics to treat common pathogenic diseases [27].

**CONCLUSION**

The present study dealt with growth, proximate compositions, antioxidant activities and phytochemical constituents of *A. paludosa* extracts. The results showed that the *A. paludosa* possess higher growth, proximate compositions, phytochemicals, which directly or indirectly can apply to human health treatment activities. And also this findings suggest that the *A. paludosa* have better antioxidant properties that would be deliberated in the medication, food supplements, cosmetics, and aqua feed industries for future economical and ecofriendly applications.

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**REFERENCES**

1. Mimouni, V., Ulmann, L., Pasquet, V., Mathieu, M., Picot, L., Bougaran, G., & Schoefs, B., 2012. The potential of microalgae for the production of bioactive molecules of pharmaceutical interest. *Curr Pharm Biotechno.*, 13(15): 2733-2750.
2. Widowati, I., Zainuri, M., Kusumaningrum, H. P., Susilowati, R., Hardivillier, Y., Leignel, V. and Mouget, J. L., 2017. Antioxidant activity of three microalgae *Dunaliella salina*, *Tetraselmis chuii* and *Isochrysis galbana clone Tahiti*. *Earth Environ. Sci.*, 55: 12067
3. Caldwell, G. S., 2009. The influence of bioactive oxylipins from marine diatoms on invertebrate reproduction and development. *Mar Drugs*, 7: 367–400.
4. Falkowsky, P.G., 1980. Primary productivity in the sea. *Environ Sci Res.*, Plenum Press, New York/London. 19, 433-60.
5. Murdock, J.H., and Wetzel, D.L., 2009. FT-IR microspectroscopy enhances biological and ecological analysis of algae. *Appl. Spectrosc.*, 44: 335–361.
6. Shelly, K., Heraud, P. and Beardall, J., 2002. Nitrogen limitation in *Dunaliella tertiolecta* (chlorophyceae) I to increased susceptibility to damage by ultraviolet-b radiation butal increase repair capability. *J. Phycology.*, 38(4): 713-720.
7. Guedes, A., Gao, M. S., Seabra, R., Ferreira, A. C., Tamagnini, P., Moradas-Ferreira, P., & Malcata, F. X., 2013. Evaluation of the antioxidant activity of cell extracts from microalgae. *Marine Drugs*, 11(4): 1256-1270.
8. Young, I.S., and Woodside, J.V., 2001. Antioxidants in health and disease. *J. Clin. Pathol.*, 54: 176-186.
9. Preeti, K., Suresh, and Abhishek. K., 2013. Research Journal of Pharmaceutical, Biological and Chemical Sciences Free Radicals: Generation, Defenses and Implications in Various Diseases 1. *Biochem.*, 269: 337–341.
10. Perumal, P., BalajiPrasath, B., Santhanam, P., Shenbaga Devi, A., Dinesh Kumar, S., and Jeyanthi, S., 2015. Isolation and intensive culture of marine microalgae. In: *Advances in Marine and Brackishwater Aquaculture* (Ed. P. Santhanam. A. R. Thirunavukkarasu and P. Perumal). Springer Publisher 1-16 pp.
11. Santhanam, P., S. Dinesh Kumar, S. Ananth, S. Jeyanthi, R. Sasirekha and C. Premkumar., 2017. Effect of culture conditions on growth and lipid content of marine microalga *Nannochloropsis* sp. strain (PSDK11). *Indian J Geo-MarSci.*, 46: 2332-2338.





**Krishnaveni et al.**

12. Doyle, J.J. and Doyle, J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem Bul.*,19: 11–15.
13. Sanjeet, K., Dagnoko, S., Haougui, A., Ratnadass, A., Pasternak, D. and Kouame, C., 2010. Potential and progress on its improvement. *Afr. J. of Agric. Res.*, 5(25): 3590-3598.
14. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, 193: 265–275.
15. Folch, J., M. Lees, and G.H. Sloane-stanley., 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*,97:383-394.
16. DuBois, M., Gilles, K., Hamilton, J., Rebers, P., & Smith, F. 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.*, 28(3): 350–356.
17. Brand-Williams, W., Cuvelier, M.E., Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity. *Food Sci. Technol.*, 28 (1): 25–30.
18. Halliwell B, Gutteridge JM, Aruoma OI., 1987. The deoxyribose method: A simple “test-tube” assay for determination of rate constants for reactions of hydroxyl radicals. *AnalBiochem.*, 165(1):215-9.
19. Liu F, Ooi VE, Chang ST., 1997. Free radical scavenging activities of mushroom polysaccharide extracts. *Life Sciences*, 60(10):763-71.
20. Gu, N., Lin, Q., Li, G., Tan, Y., Huang, L. and Lin, J., 2012. Effect of salinity on growth, biochemical composition, and lipid productivity of *Nannochloropsis oculata* CS 179. *Eng LifeSci.*, 12(6), pp.631-637.
21. Allakhverdiev, S.I., Nishiyama, Y., Miyairi, S., Yamamoto, H., Inagaki, N., Kanesaki, Y. and Murata, N., 2002. Salt stress inhibits the repair of photodamaged photosystem II by suppressing the transcription and translation of psbA genes in *Synechocystis*. *Plant Physiol.*, 130(3), pp.1443-1453.
22. Chrismadha, T. and Borowitzka, M.A., 1994. Effect of cell density and irradiance on growth, proximate composition and eicosapentaenoic acid production of *Phaeodactylum tricornutum* grown in a tubular photobioreactor. *J. Appl. Phycol.*, 6(1), pp.67-74.
23. Yao, C., Ai, J., Cao, X., Xue, S. and Zhang, W., 2012. Enhancing starch production of a marine green microalga *Tetraselmis subcordiformis* through nutrient limitation. *Bioresour. Technol.*, 118, pp.438-444.
24. Borowitzka, M. A., 2013. High-value products from microalgae their development and commercialisation. *J. Appl. Phycol.*, 25:743–756.
25. Banskota, A. H., 2019. Antioxidant properties and lipid composition of selected microalgae. *J. Appl Phycol.*, 31:309-318.
26. Manivannan, K.; Anantharaman, P. and Balasubramanian, T., 2012. Evaluation of antioxidant properties of marine microalga *Chlorella marina* (Butcher, 1952). *Asian Pac. J. Trop. Biomed.*, S342-S346.
27. Mir, M. A.; Parihar, K.; Tabasum, U. and Kumari, E., 2016. Estimation of alkaloid, saponin and flavonoid, content in various extracts of *Crocus sativa*. *J. Med. Plants Stud.*,4(5): 171-174.

**Table-1. Phytochemical constituents of *Amphiprora paludosa* with various extracts cultured under TMRL medium**

Phytochemical constituents	Extracts			
	Methanol	Ethanol	Ethyl acetate	Hexane
Alkaloids	+	-	-	-
Flavonoids	+-	-	-	-
Steroids	-	-	+	-
Terpenoids	+	+	-	-
Saponin	-	-	-	-
Phenols	+-	+	-	-
Tannins	-	-	-	-
Glycosides	+	+	+-	-

Note: + denotes present; - denotes absent.





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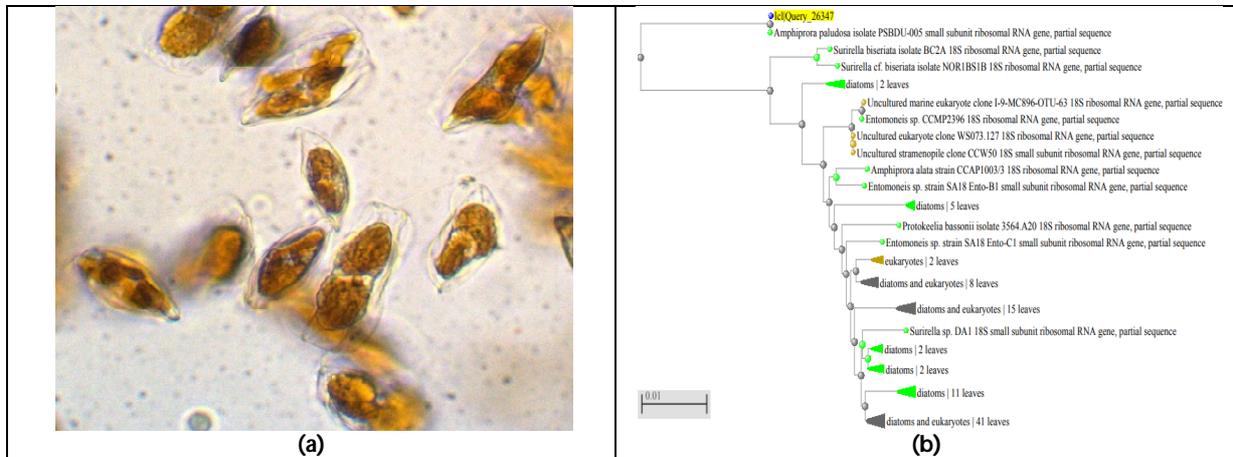


Fig 1. Microscopic view (a) and phylogenetic tree (b) of *Amphiprora paludosa*

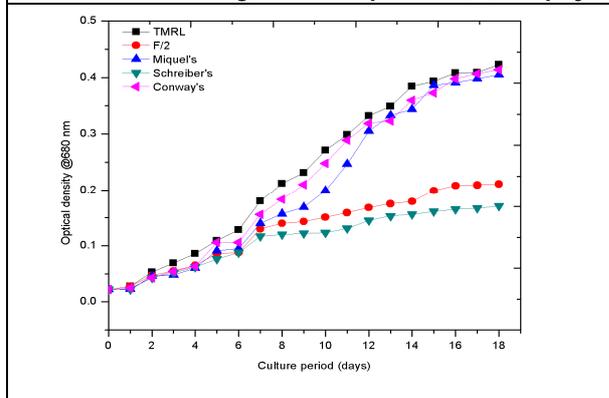


Fig2. Growth curve of *Amphiprora paludosa* under various culture medium

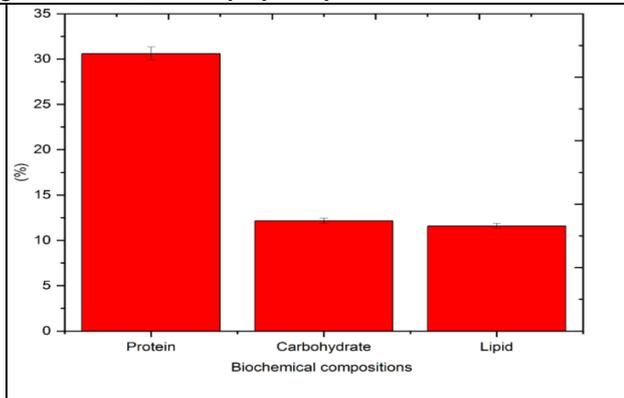


Fig 3. Proximate compositions of *Amphiprora paludosa* cultured under TMRL medium. Values shown are averages of three triplicates  $\pm$  standard deviation, and the standard deviations were calculated from three repetitions.

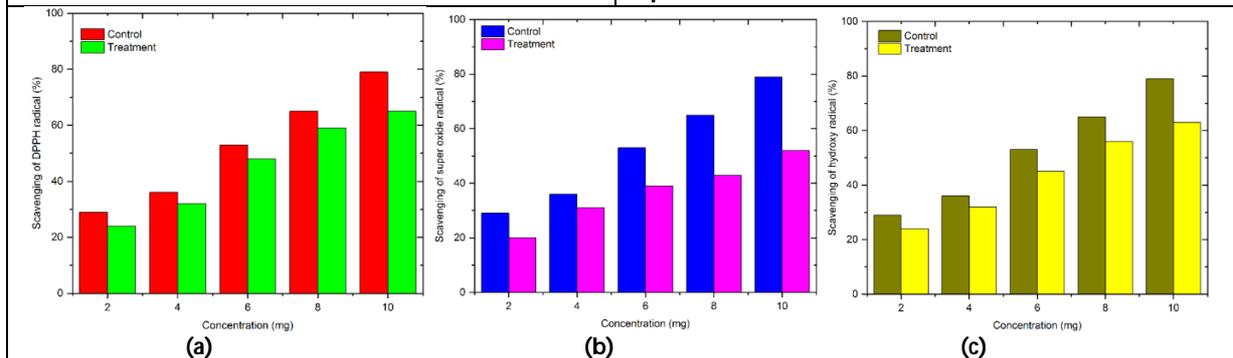


Fig 4. Scavenging of DPPH radical, super oxide radical, and hydroxy radical of *Amphiprora paludosa* cultured under TMRL medium. Control was assessed against ascorbic acid as standard and treatment was assessed against *A. paludosa* sample.





## ***In-vitro* Wound Healing Activity of Chloroform Extract of *Hedyotis herbacea* and Ursolic Acid**

K.Anand Babu<sup>1\*</sup>, S. Sivakrishnan<sup>2</sup> and S. Jasemine<sup>1</sup>

<sup>1</sup>Department of Pharmaceutical Chemistry, College of Pharmacy, Kannur Medical College, Anjarakandy, Kannur-670612, Kerala, India

<sup>2</sup>Department of Pharmacy, Faculty of Engineering and Technology, Annamalai University, Annamalai Nagar, Chidambaram – 608002, Tamil Nadu, India

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### **\*Address for Correspondence**

**K.Anand Babu**

Associate Professor

Department of Pharmaceutical chemistry

College of Pharmacy, Kannur Medical College

Anjarakandy, Kannur -670612, Kerala, India.

E.Mail: anandbabu23@rediffmail.com



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### **ABSTRACT**

The Leaves of *Hedyotis herbacea* Linn. which was claimed to have wound healing activity was extracted with various solvents such as Petroleum ether, Chloroform, Ethyl acetate, and Ethanol. The extracts were screened for Ursolic acid by HPTLC. The literature review suggests that Ursolic acid was scientifically validated for wound healing activity. HPTLC evaluation showed that chloroform extract contained a rich fraction of Ursolic acid. So the Ursolic acid, rich fraction of chloroform extract of *Hedyotis herbacea*(CEHH), and isolated constituent of Ursolic acid were subjected to cytotoxicity and scratch assay by utilizing H9c2(2-1)cells. Based on the cytotoxicity study nontoxic concentrations such as 40 µg/ml and 80µg /ml were chosen for further study. The Report suggests that 80µg /ml of chloroform extract was having the better percentage of wound closure than an isolated constituent of Ursolic acid.

**Key words:** Wound healing, *Hedyotis herbacea*, Chloroform extract and Ursolic acid.

### **INTRODUCTION**

Cell migration is that the movement of cells from one area to another usually in response to a chemical signal. It is a key property for achieving functions like wound repair, cell differentiation, embryonic development, and therefore the metastasis of tumours [1 & 2]. Throughout this assay, a cell-free area is made during a convergent monolayer by physical exclusion or by removing the cells from the area through mechanical, thermal, or chemical impairment. The exposure to the cell-free area induces the cells to migrate into the gap [3 & 4]. The main principle of cell migration





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assay is the ability of cells to grow after the wound formation. The fundamental steps involve creating a "wound" in a cell monolayer, capturing the images at the beginning and at regular intervals during cell migration to close the wound, and comparing the images to quantify the migration rate of the cells. It's notably appropriate for studies on the results of cell-matrix and cell-cell interactions on cell migration [5]. Cell movement is calculated by measuring the decrease of the uncovered region at different time points until the "wound" is closed [6].

*Hedyotis herbacea* Linn (family- Rubiaceae) is an erect, glabrous annual shrub found in temperate and tropical regions of Africa and Asia. A paste of leaves is considered as emollient and applied to abscesses and wound [7 & 8]. The whole plant of *Hedyotis herbacea* contains the interesting triterpenoid of nature, Ursolic acid in a good amount. It provoked us to identify and determine the presence of ursolic acid in the leaves [9]. The aim of the research work is to find out the *in-vitro* wound healing effectiveness of *Hedyotis herbacea* by comparing Ursolic acid rich fraction (CEHH) and isolated constituent of UA by using H9c2(2-1) cells

## MATERIALS AND METHODS

Petroleum ether, Chloroform, Ethyl acetate, Ethanol, EDTA and DMSO were purchased from Merck (India). Cell lines were purchased from National Centre for Cell Science (NCCS), Pune. Dulbecco's modified eagle medium (DMEM), Foetal bovine serum (FBS), Phosphate buffered saline (PBS) and Trypsin procured from Invitrogen.

### Preparation of leaves extract

Leaves were dried in shade and ground to obtain a fine powder. The crude powder was subjected to extraction with various solvents such as Petroleum ether, chloroform, ethyl acetate and ethanol. The crude powder was kept in a contact with respective solvents for 7 days at room temperature with occasional shaking by the maceration technique. Then the extract was filtered and the filtrate was concentrated at reduced pressure to obtain the residue.

### Isolation of Ursolic acid:[10]

The crude chloroform extract (2g) of *Hedyotis herbacea* was subjected to column chromatography using chloroform with increasing concentration of ethanol, as eluent. Twenty-five fractions were collected, of which fractions 20-24 contained the major compound with a few minor constituents. The fractions were combined and then subjected to re-column chromatography using ethyl acetate/chloroform (10:90) as the eluent. Ten fractions (30 ml each) were collected. Fractions 4-7 contained the desired compound (20 mg).

### HPTLC Estimation

Various extracts of *Hedyotis herbacea* were examined to determine the Ursolic acid rich fraction. So, a High-performance Thin-Layer Chromatographic method was developed for quantitative estimation of Ursolic acid in *Hedyotis herbacea*. The Separation was performed on silica gel 60 F254 HPTLC plates using toluene: ethyl acetate: formic acid (7 :3: 0.1) as mobile phase for elution of markers from the extract. The determination was carried out by using densitometric absorbance-reflection mode at 516 nm for Ursolic acid. The HPTLC reports suggested that CEHH contain higher amount of Ursolic acid than other extracts. So, chloroform extract of *Hedyotis herbacea* was chosen for *in vitro* assay ("In press" (ijpsr/ra-15083/07-20)- Quantification of Ursolic acid and minerals from *Hedyotis herbacea* linn. IJPSR, vol. 12, issue 7; July 2021)

### Cytotoxicity studies for H9c2(2-1) cell line by MTT method

#### Cell lines and culture medium

H9c2(2-1) cell line was purchased from National Centre for Cell Science (NCCS), Pune. Stock cells was cultured in DMEM supplemented with 10% inactivated foetal Bovine Serum (FBS), penicillin(100IU/ml), streptomycin (100µg/ml) in a humidified atmosphere of 5% CO<sub>2</sub> at 37°C until confluent. The cell was dissociated with cell



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dissociating solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The cells were checked for viability and centrifuged. Further, 50,000 cells /well was seeded in a 96 well plate and incubated for 24 hrs at 37°C, 5 % CO<sub>2</sub> incubator.

**Procedure for MTT assay: [11]**

The monolayer cell culture was trypsinized and therefore the cell count was adjusted to 5x10<sup>5</sup> cells/ml using respective media containing 10% FBS. To each well of the 96 well microtiter plate, 100µl of the diluted cell suspension (50,000cells/well) was added. After 24 hrs, when a partial monolayer was formed, the supernatant was removed, washed the monolayer once with medium and 100µl of different test concentrations of test drugs were added on to the partial monolayer in microtiter plates. The plates were then incubated at 37°C for 24hrs in 5% CO<sub>2</sub> atmosphere. After incubation the test solutions in the wells were discarded and 100 µl of MTT (5mg/10ml of MTT in 1X PBS) was added to each well. The plates were incubated for 4 hrs at 37°C in 5% CO<sub>2</sub> atmosphere. The supernatant was removed and 100 µl of DMSO was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 590 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (IC<sub>50</sub>) values is generated from the dose-response curves for each cell line.

**Calculating Inhibition**

**% Inhibition** = ((OD of Control – OD of sample)/OD of Control) x 100.

**Statistical evaluation****IC50 Value: [12]**

The half maximal inhibitory concentration (IC<sub>50</sub>) is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. This quantitative measure indicates how much of a particular drug or other substance (inhibitor) is needed to inhibit a given biological process (or component of a process, i.e. an enzyme, cell, cell receptor or microorganism) by half. The IC<sub>50</sub> of a drug can be determined by constructing a dose-response curve and examining the effect of different concentrations of antagonist on reversing agonist activity. IC<sub>50</sub> values can be calculated for a given antagonist by determining the concentration needed to inhibit half of the maximum biological response of the agonist. IC<sub>50</sub> values for cytotoxicity tests were derived from a nonlinear regression analysis (curve fit) based on sigmoid dose response curve (variable) and computed using Graph Pad Prism 6 (Graph pad, San Diego, CA, USA).

**In vitro scratch assay****Preparation of test solutions and Procedure: [13 & 14]**

32mg/ml stock of the samples was taken and serial two-fold dilutions were made to obtain 50µg/ml to 100µg/ml using DMEM media. 0.25% Trypsin-EDTA solution used to detach cells from the tissue culture. Pellet cells in a 15ml conical tube was centrifuged, aspirated the supernatant, and re-suspend cells in culture media. Plated the appropriate number of cells in a 6-well plate for 100% confluence in 24hrs. Tests was used to determine the time and number of cells to achieve 100% confluence due to the cell type and the size of the well being used. In a sterile environment (typically a biosafety hood) 200µl pipette tip was pressed firmly against the top of the tissue culture plate and swiftly made a vertical wound down through the cell mono layer (2 mm Scratch was made). A different sized pipette tip was used to made the desired wound size. Carefully aspirated the media and cell debris. Slowly added the enough culture media along with desired concentrations of samples against the well wall to cover the bottom of the well and care taken to avoid the detaching additional cells. Initial picture was taken by following the generation and inspection of the wound. Placed the tissue culture plate in an incubator set at the appropriate temperature and CO<sub>2</sub> concentration (typically 37°C and 5% CO<sub>2</sub>). At several time points, e.g. every 24hrs, removed the plate from the incubator and place it under an inverted microscope to take a snapshot picture and its checked for wound closure. Depending on the cell type, wound closure time may change. Image J software was used to analyze





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the results of snapshot pictures, measure the distance of one side of the wound to the other using a scale bar. Analyze and clearly present wound closure over time using a bar graph. percentage growth inhibition was calculated by below mentioned formula.

$$\% \text{ Inhibition} = \frac{(\text{Mean of Control} - \text{Mean of Sample})}{\text{Mean of Control}} \times 100$$

## RESULTS

In the attempt to carry out the wound healing property of CEHH and Ursolic acid, *in vitro* scratch wound-healing assay to the monolayer of HaCaT cells was chosen for the study. MTT assay was performed to know the cell viability and IC 50 was calculated and it was depicted in table no.1 and figure no.1&2. IC 50 was not calculated for Ursolic acid due to lesser inhibition whereas IC 50 for chloroform extract is 240.3µg/mL. Henceforth non-toxic concentrations such as 40µg/ml and 80µg/ml of both the samples were taken for scratch assay and cell migration was calculated and the results were shown in table no.2 and figure 3-7. Percentage wound closure of untreated control at 24hrs and 48hrs were 50.50% and 74.50% respectively. Percentage wound closure of Ursolic acid and CEHH with a concentration of 80µg/ml at 48hrs was 84.02% and 90.77% respectively. This result suggests that chloroform extract is having significant effect than an isolated constituent.

## DISCUSSION

Pentacyclic triterpenoids are a category of C30 isoprenoid compounds found widely in distinct parts of a plant, such as the leaves, pollen, seeds, and fruits. They accelerate wound healing mainly due to their effects on the production and activity of inflammatory mediators and growth factors, and thus produce wound contraction and increase the rate of epithelialization. A Natural pentacyclic triterpenoid the Ursolic acid is widely distributed in various plants that have become an integral part of the human diet. Isolated Ursolic acid from methanolic extract of *Shorea robusta* leaves has been reported to have healing activity in rats using distinct wound models [15]. Based on the literature review Ursolic acid was identified and quantified in *Hedyotis herbacea*, which was collected from Tirunelveli, Tamil Nadu by HPTLC with the help of standard Ursolic acid. The extract rich in Ursolic acid content, the Chloroform extract was chosen to isolate the Ursolic acid. Isolated Ursolic acid and Chloroform extract was assayed for *in-vitro* wound healing activity by using H9c2(2-1) cells. The results showed that CEHH has more potential wound healing activity than isolated Ursolic acid.

Nevertheless, Ursolic acid isolated from other plants has exhibited better wound healing activity than its plant extracts. But in the present study, the Ursolic acid rich extract of the plant exhibited better activity than the isolated compound. So, the present study reveals that compounds present in the extract, other than Ursolic acid, are producing an additive effect. The isolation and wound healing activity of other compound require further study.

## CONCLUSION

CEHH showed an IC50 value of 240.3µg/ml in H9c2(2-1) cells, whereas Standard Ursolic acid did not show significant inhibition, hence IC50 was not calculated. For the wound healing Assay, non-toxic concentrations such as 40µg/ml and 80µg/ml for both the samples were considered. CEHH with concentrations of 40µg/ml and 80µg/ml have shown better migration of cells which was seen in Fig 6 and 7 respectively. Where as Ursolic acid with concentration 40µg/ml and 80µg/ml did not show effective migration of cells comparatively. Hence it is proved that CEHH 80µg/ml was having effective migration of cell. Though Ursolic acid was proved to be a good wound healing agent, in the present study the Ursolic acid rich extract as a whole has better activity than the isolated compound.





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This may be due to the presence of other phytoconstituents present in the extract which may give a synergistic effect. The *In vivo* wound healing evaluation of CEHH is under progress for further confirmation of the above results.

## REFERENCES

- Hilary Sherman, Pilar Pardo and Todd Upton. Cell migration, Chemotaxis and Invasion assay Protocol: Corning Life sciences; 1-10
- James E. N. Jonkman, Judith A. Cathcart, Feng Xu, Miria E. Bartolini, Jennifer E. Amon, Katarzyna M. Stevens and Pina Colarusso. An introduction to the wound healing assay using live-cell microscopy. *Cell Adhesion & Migration* 2014; 8:5:440-451.
- Calvin R Justus, Nancy Leffler, Maria Ruiz-Echevarria, Li V Yang. In vitro cell migration and invasion assays. *Journal of visualized experiments* 2014; 88:51046.
- Nina Kramer, Angelika Walzl, Christine Unger, Margit Rosner, Georg Krupitza, Markus Hengstschlager, Helmut Dolznig. In vitro cell migration and invasion assays – A Review. *Mutation Research* 2013; 752:10–24.
- Rodriguez LG, Wu X, Guan JL. Wound-healing assay. *Methods of molecular biology* 2005;294:23-9.
- Yanling Che: Scratch Wound Healing Assay. *Bioprotocol* 2012; Vol 2, Issue:1-2
- T.Pullaiah: Encyclopedia of world medicinal plants 2006;1:1064.
- Indian Medicinal Plants. A Compendium of 500 Species 1994;3:124-126.
- Mohammad Pravez and Alok Kumar Patel. Wound healing activity of ursolic acid stearyl glucoside (UASG) isolated from *Lanata camara L.* *International Journal of Pharmaceutical Sciences and Research* 2014; 5(10):4439-4444.
- Ahmad Sazali Hamzah and Nordin Hj. Laji. Chemical constituents of *Hedyotis herbacea*. *ASEAN Review of Biodiversity and Environmental Conservation* 1998:1-6.
- Mark Frei. Cell Viability and Proliferation. *BioFiles* 6(5):17–21.
- [www.fda.gov](http://www.fda.gov)
- Neeraj Dholia. Montelukast Induces Mesenchymal Properties in A549 Lung Epithelial Cells. *Indian Journal of Natural Sciences* 2021; 12(65):30058-30065.
- Francois Berthiaume, Suneel Kumar and Yuying Tan. Neuropeptide Substance P Enhances Skin Wound Healing *In Vitro* and *In Vivo* under Hypoxia. *Bio medicines* 2021; 9,222:1-12.
- Lais C. Agra, Jamille N. S. Ferro, Fabiano T. Barbosa & Emiliano Barreto. Triterpenes with healing activity: A systematic review, *Journal of Dermatological Treatment* 2015;26(5):465-470.

**Table 1: IC<sub>50</sub> Value of Chloroform Extract of *Hedyotis herbacea***

H9c2(2-1)	Compound name	Conc. µg/ml	OD at 590nm	% Inhibition	IC <sub>50</sub> µg/mL
	Control	0	0.654	0.00	IC <sub>50</sub> is not calculated due to lesser inhibition
UA		10	0.620	5.20	
		20	0.588	10.09	
		40	0.546	16.51	
		80	0.503	23.09	
		160	0.455	30.40	
		320	0.374	42.81	
CE		10	0.602	7.95	240.3
		20	0.574	12.23	
		40	0.525	19.72	
		80	0.480	26.61	
		160	0.388	40.67	
		320	0.285	56.42	





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Table 2. % Wound Closure of the Control and Sample Treated Cells

Samples	Conc. µg/ml	Migration distance in mm			% Wound Closure at 24 hrs	% Wound Closure at 48 hrs
		0 hrs	24 hrs	48 hrs		
Control	0	2.00	0.99	0.51	50.50±0.1360	74.50±0.3600
Sample UA	40	1.92	0.87	0.40	54.69±0.2450	79.17±0.1415
	80	1.94	0.80	0.31	58.76±0.1415	84.02±0.2427
Sample CE	40	1.99	0.85	0.36	57.29±0.2356	81.91±0.1360
	80	1.95	0.74	0.18	62.05±0.1416	90.77±0.1387

Cell viability assay

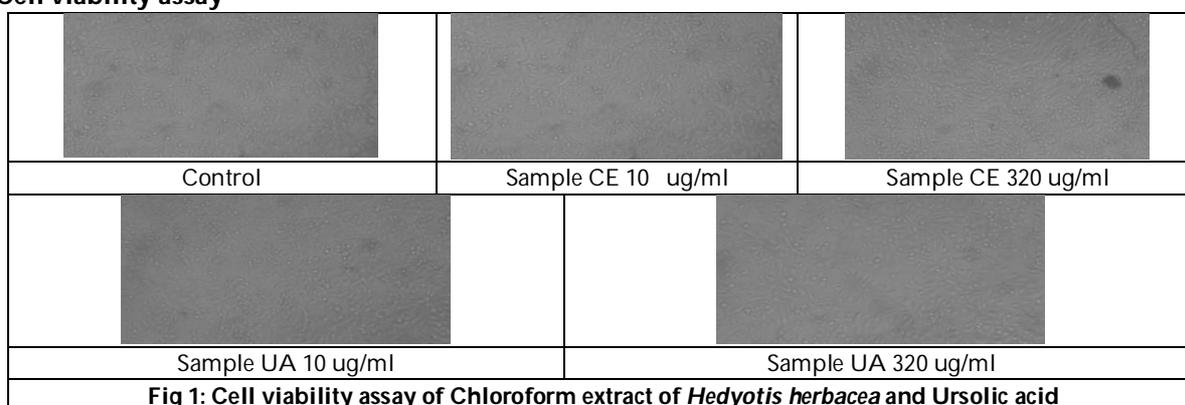


Fig 1: Cell viability assay of Chloroform extract of *Hedyotis herbacea* and Ursolic acid

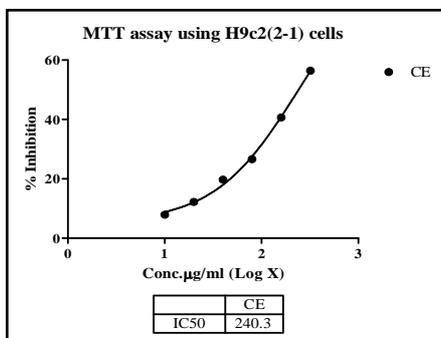


Figure no.2: Cell viability study of chloroform extract

Scratch assay

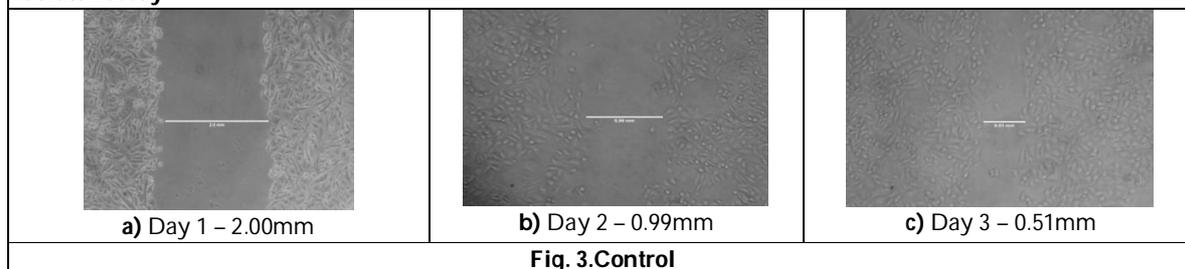


Fig. 3.Control





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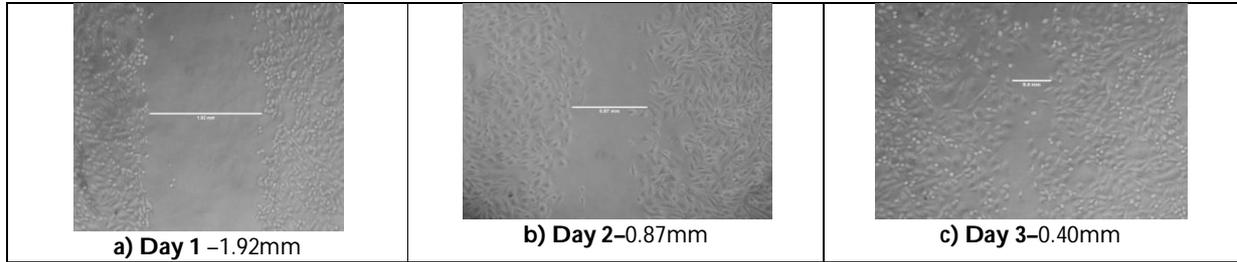


Fig. 4. Sample UA- 40µg/ml

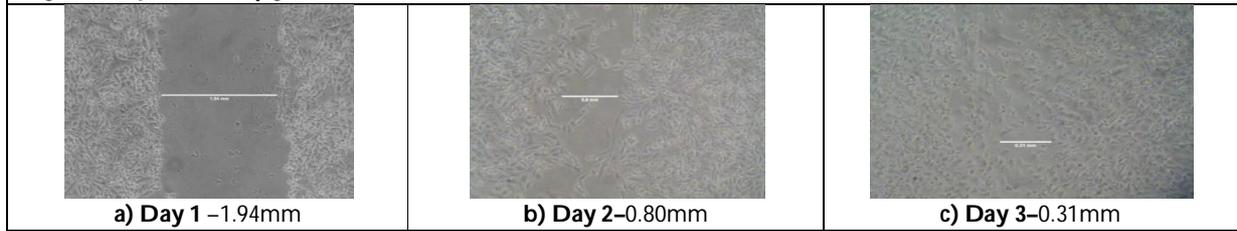


Fig 5: Sample UA-80µg/ml

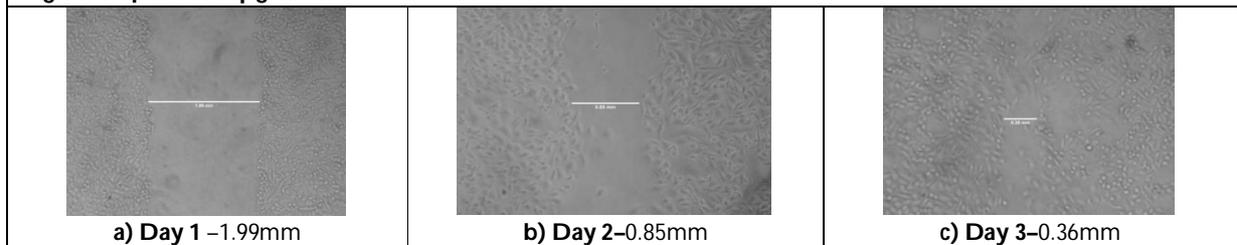


Fig 6: Sample CE- 40µg/ml

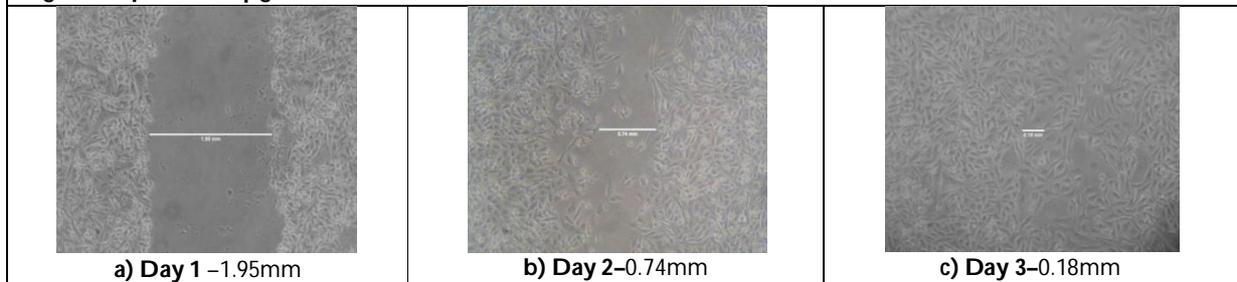


Fig 7: Sample CE-80µg/ml

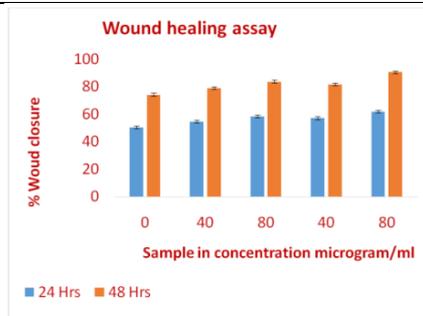


Fig no.8: Wound healing assay





## Anti Multi Fuzzy BH-Ideals in BH-Algebras

K. Anitha<sup>1\*</sup> and N. Kandaraj<sup>2</sup>

<sup>1</sup>Research Scholar, PG and Research Department of Mathematics, Saiva Bhanu Kshatriya College, Aruppukottai, Tamil Nadu, India.

<sup>2</sup>Associate Professor, PG and Research Department of Mathematics, Saiva Bhanu Kshatriya College, Aruppukottai, Tamil Nadu,

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### \*Address for Correspondence

#### K. Anitha

Research Scholar,  
PG and Research Department of Mathematics,  
Saiva Bhanu Kshatriya College,  
Aruppukottai - 626 101, Tamil Nadu, India.  
Email: anithamaths19@gmail.com



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### ABSTRACT

In this paper we speak approximately the Anti multi fuzzy BH-Ideals and associated homes in BH-Algebras. Multi Fuzzy set concept is an extension of fuzzy set concept. These offers with the multi-dimensional fuzziness. we introduce the perception of Anti multi-fuzzy BH-ideals, the Anti multi-level subset of BH-ideals. And additionally we outline a few associated Anti multi-fuzzy BH-ideals based on level subset of it.

**Keywords:** BH-algebra, Anti Fuzzy BH-ideal, Anti Multi-fuzzy BH-ideal. Anti Multi fuzzy closed ideal.

**Subject Classification:** AMS (2000), 06F35, 03G25, 06D99, 03B47

## INTRODUCTION

Y. Imai and K. Iseki [1,2&3] are brought lessons of summary algebras. BCK- algebras and BCI-algebras. It is understood that the elegance of BCK-algebras is a right subclass of the elegance of BCI-algebras. K. Iseki and S. Tanaka [4] are brought creation to concept of BCK-algebras. L.A. Zadeh [5] are brought fuzzy units. S. Sabu and T.V. Ramakrishnan [6] are brought Multi-Fuzzy units, The perception of BH-algebras is brought with the aid of using J.B. Jun, E.H. Roh and H.S. Kim [7]. Since then, numerous authors have studied BH-algebras. In particular, Q. Zhang, E.H. Roh and Y.B. Jun [8] studied the fuzzy concept in BH-algebras. K. Anitha and N. Kandaraj [9] are brought Fuzzy subalgebras on BH-algebras. K. Anitha and N. Kandaraj are brought Fuzzy ideals and Fuzzy dot ideals on BH-algebras. In this paper, we outline Anti multi-fuzzy ideals in BH-algebra and talk a number of their associated primarily based on level subsets and homomorphism.





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**PRELIMINARIES**

In this phase we talk the fundamental definitions of a BH-algebras.

**Definition: [ 1,2,3]** Let  $X$  be a nonempty set with a binary operation  $*$  and a constant  $0$ . Then  $(X, *, 0)$  is referred to as a BCI-algebras if it satisfies the subsequent conditions.

1.  $((x * y) * (x * z)) * (z * y) = 0$
2.  $(x * (x * y)) * y = 0$
3.  $x * x = 0$
4.  $x * y = 0$  and  $y * x = 0 \implies x = y \quad \forall x, y \in X$ .

**Example** Let  $X = \{0, a, b, c\}$  be a set with the subsequent cayley table.

$*$	0	a	b	c
0	0	0	0	0
a	a	0	a	0
b	b	b	0	0
c	c	c	c	0

Then  $(X, *, 0)$  is known as a BCI-algebras.

**Definition [1,2,3]** Let  $X$  be a nonempty set with a binary operation  $*$  and a constant  $0$ . Then  $(X, *, 0)$  is known as a BCK-algebras if it satisfies the subsequent conditions.

1.  $((x * y) * (x * z)) * (z * y) = 0$
2.  $(x * (x * y)) * y = 0$
3.  $x * x = 0$
4. If  $x * y = 0$  and  $y * x = 0 \implies x = y$
5.  $0 * x = 0$  for all  $x, y, z \in X$ .

**Example :** Let  $X = \{0,1,2,3\}$  be a set with the subsequent cayley table.

$*$	0	1	2	3
0	0	0	0	0
1	1	0	1	2
2	2	3	0	0
3	3	1	2	0

Then  $(X, *, 0)$  is known as a BCK-algebras.

**Definition:[7,8]** Let  $X$  be a nonempty set with a binary operation  $*$  and a constant  $0$ . Then  $(X, *, 0)$  is referred to as a BH-algebras if it satisfies the following conditions.

1.  $x * x = 0$
2.  $x * 0 = x$





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If  $x * y = 0$  and  $y * x = 0 \implies x = y$  for all  $x, y \in X$ .

**Example :** Let  $X = \{0,1,2,3\}$  be a set with the subsequent cayley table.

*	0	1	2	3
0	0	1	2	3
1	1	0	2	1
2	2	3	0	0
3	3	2	3	0

Then  $(X,*,0)$  is known as a BH-algebras.

**Definition :[8]** Let  $S$  be a nonempty subset of a BH-algebra  $X$ , then  $S$  is referred to as subalgebra of BH-algebra if  $x * y \in S$  for all  $x, y \in S$ .

**Definition [8]** Let  $X$  be a BH-algebra and  $I$  be a subset of  $X$ , then  $I$  is known as a ideal of  $X$  if Satisfies the following conditions.

1.  $0 \in I$
2.  $x * y \in I$  and  $y \in I \implies x \in I$  for all  $x, y \in I$
3.  $x \in I$  and  $y \in X \implies x * y \in I$

**Definition : [9]** Let  $\sigma$  be a fuzzy set in a BH-algebra  $X$ . Then  $\sigma$  is referred to as a fuzzy BH-subalgebra if  $\sigma(x * y) \geq \min\{\sigma(x), \sigma(y)\} \forall x, y \in X$

**Definition [7,8,10]** Let  $\sigma$  be a fuzzy set in a BH-algebra  $X$ . Then  $\sigma$  is referred to as a fuzzy BH-ideal if it satisfies the subsequent conditions.

1.  $\sigma(0) \geq \sigma(x)$
2.  $\sigma(x) \geq \min\{\sigma(x * y), \sigma(y)\}$
3.  $\sigma(x * y) \geq \min\{\sigma(x), \sigma(y)\} \forall x, y \in X$ .

**Definition [7,8]**

A mapping  $g: X \rightarrow Y$  of a BH-algebra is referred to as a homomorphism if  $g(x * y) = g(x) * g(y) \forall x, y \in X$ .

**Definition [6]** Let  $X$  be a nonempty set. Define a multi-fuzzy set  $B$  in  $X$  is a set of ordered sequences:

$$B = \{(x, \sigma_1, \sigma_2, \dots, \sigma_i, \dots): x \in X\}, \text{ where } \sigma_i: X \rightarrow [0,1] \text{ for all } i$$

**Remark 6]**

1. If the sequences of the membership functions have only  $k$ -terms(finite wide of terms)  $k$  is called the dimension of  $B$ .
2. The set of all multi-fuzzy sets in  $X$  of dimension  $k$  is denoted through  $M^kFS(X)$ .
3. The multi-fuzzy membership function  $\sigma_B(x)$  is a function from  $X$  to  $[0,1]^k$  such that for all  $x \in X$   $\sigma_B(x) = (\sigma_1(x), \sigma_2(x), \dots, \sigma_k(x))$
4. For the sake of simplicity, we denote the multi-fuzzy set as  $B = \{(x, \sigma_1(x), \sigma_2(x), \dots, \sigma_k(x), \dots): x \in X\}$  as  $B = (\sigma_1, \sigma_2, \dots, \sigma_k)$ .

**Definition [6]**

Let  $k$  be a positive integer and allow  $B$  and  $C$  in  $M^kFS(X)$ , where  $B = (\sigma_1, \sigma_2, \dots, \sigma_k)$  and  $C = (\rho_1, \rho_2, \dots, \rho_k)$  then we have got the subsequent members of the relations and operations:

1.  $B \subseteq C$  if and only if  $\sigma_i \leq \rho_k$ , for all  $i = 1, 2, \dots, k$





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2.  $B = C$  if and only if  $\sigma_i = \rho_k$ , for all  $i = 1, 2, \dots, k$
3.  $B \cup C = (\sigma_1 \cup \rho_1, \dots, \sigma_k \cup \rho_k) = \{(x, \max(\sigma_1(x), \rho_1(x)), \dots, \max(\sigma_k(x), \rho_k(x))) : x \in X\}$
4.  $B \cap C = (\sigma_1 \cap \rho_1, \dots, \sigma_k \cap \rho_k) = \{(x, \min(\sigma_1(x), \rho_1(x)), \dots, \min(\sigma_k(x), \rho_k(x))) : x \in X\}$ .

**Definition [6]:** Let  $B$  be a multi-fuzzy set in BH-algebra  $X$ . For any  $s = (s_1, s_2, \dots, s_k)$  where  $s_i \in [0,1]$  for all  $i$ , the set  $\cup(B; s) = \{x \in X / B(x) \geq s\}$  is referred to as the multi-level subset of  $B$ .

**Definition [6]**

Let  $B$  be a multi-fuzzy set in BH-algebra  $X$ . Then  $B$  is referred to as Anti multi-fuzzy closed ideal in  $X$  if it satisfies the subsequent conditions

1.  $B(x) \leq \max\{B(x * y), B(y)\}$
2.  $B(0 * x) \leq B(x)$

**Example:** Let  $X = \{0,1,2,3\}$  be a set with the subsequent cayley table.

*	0	1	2	3
0	0	1	2	3
1	1	0	1	1
2	2	2	0	2
3	3	2	2	0

Then  $B$  is known as Anti multi-fuzzy closed ideal in  $X$ .

**Definition [6]:** Let  $\sigma$  be a fuzzy set in a BH-algebra  $X$ . Then  $\sigma$  is referred to as Anti fuzzy BH-ideal if it satisfies the subsequent conditions.

1.  $\sigma(0) \leq \sigma(x)$
2.  $\sigma(x) \leq \max\{\sigma(x * y), \sigma(y)\}$
3.  $\sigma(x * y) \leq \max\{\sigma(x), \sigma(y)\} \forall x, y \in X$

**ANTI MULTI-FUZZY BH-IDEAL IN BH-ALGEBRAS**

In this segment we mentioned the Anti multi-fuzzy BH-ideal and its properties.

**Definition [6]** Let  $B$  be a multi-fuzzy set in BH-algebra  $X$ . Then  $B$  is known as a multi-fuzzy BH-ideal in  $X$  if it satisfies the subsequent conditions.

1.  $B(0) \leq B(x)$
2.  $B(x) \leq \max\{B(x * y), B(y)\}$
3.  $B(x * y) \leq \max\{B(x), B(y)\} \forall x, y \in X$

**Example:** Let  $X = \{0,1,2\}$  be a set with the subsequent cayley table.

*	0	1	2
0	0	1	2
1	1	0	1
2	2	2	0

Define a multi-fuzzy set  $B: X \rightarrow [0,1]$  with the aid of using  $B(0)=B(1)=(p_1, p_2)$  and  $B(2)=(q_1, q_2)$  where  $p_1, p_2, q_1, q_2 \in [0,1]$  with  $p_1 > q_1$  and  $p_2 > q_2$ . Then  $B$  is Anti multi-fuzzy BH-ideal in BH-Algebras.

**Theorem :** Let  $X$  be a BH-algebra. Then  $B$  is Anti muti-fuzzy BH-ideal in  $X$  if and only if  $B$  is a Anti multi-fuzzy subalgebra of  $X$ .





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**Proof:**

Let  $X$  be a BH-algebra.

Let  $B$  be Anti multi-fuzzy BH-ideal in BH-algebra  $X$ .

To show that  $B$  is Anti multi-fuzzy subalgebra in BH-algebra  $X$

We recognize that Every Anti multi fuzzy BH-ideal of a BH-algebra  $X$  is a Anti multi- fuzzy subalgebra of  $X$ .

Let  $B$  be Anti multi fuzzy subalgebra in  $X$ .

To show that  $B$  is Anti muti fuzzy BH-ideal in  $X$ .

Let  $x, y \in X$

By the use definition of BH-algebras conditions.

- 1)  $B(0) = B(x * x)$   
 $\leq \max \{B(x), B(x)\}$   
 $= B(x) \forall x \in X$
- 2)  $B(x) = B((x * y) * (0 * y))$   
 $\leq \max \{B(x * y), B(0 * y)\}$   
 $\leq \max \{B(x * y), \max \{B(0), B(y)\}\}$   
 $\leq \max \{B(x * y), B(y)\}$
- 3) It is in reality true.

Hence  $B$  is a Anti multi fuzzy BH- ideal in  $X$ .

**Theorem :** Let  $B_1$  and  $B_2$  be two Anti multi fuzzy BH-ideals of a BH-algebra  $X$ . Then  $B_1 \cup B_2$  is a Anti multi-fuzzy BH-ideal of  $X$ .

**Proof:**

Let  $B_1$  and  $B_2$  be two Anti multi fuzzy BH-ideals of a BH-algebra  $X$ .

To show that  $B_1 \cup B_2$  is a Anti multi-fuzzy BH-ideal of  $X$ .

Let  $x, y \in B_1 \cup B_2$ .

Then  $x, y \in B_1$  and  $x, y \in B_2$

By the usage of multi fuzzy set union definition conditions

1.  $B_1 \cup B_2(0) = (x * x)$   
 $B_1 \cup B_2 = \max\{B_1(x * x), B_2(x * x)\}$   
 $\leq \max \{\max\{B_1(x), B_1(x)\}, \max \{B_1(x), B_2(x)\}\}$   
 $= \max\{B_1(x), B_2(x)\}$   
 $= B_1 \cup B_2(x)$
1.  $B_1 \cup B_2(x) = \max\{B_1(x), B_2(x)\}$   
 $\leq \max\{B_1(x * y), B_2(y)\}, \max\{B_2(x * y), B_2(y)\}\}$   
 $= \max\{B_1(x * y), B_2(x * y)\}, \max\{B_1(y), B_2(y)\}\}$   
 $= B_1 \cup B_2(x * y), B_1 \cup B_2(x)(y)\}$
2.  $B_1 \cup B_2(x * y) = \max\{B_1(x * y), B_2(x * y)\}$   
 $\leq \max \{\max\{B_1(x), B_2(y)\}, \max\{B_1(x), B_2(y)\}\}$   
 $= \max \{\max\{B_1(x), B_2(x)\}, \max\{B_1(y), B_2(y)\}\}$   
 $= \max \{B_1 \cup B_2(x), B_1 \cup B_2(y)\}$

Hence the proof.





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**Definition** Let B be a multi fuzzy set in a BH-algebra X. Then B is referred to as Anti multi fuzzy closed ideal in X if it satisfies the subsequent conditions:

1.  $B(x) \leq \max\{B(x * y), B(y)\}$
2.  $B(0 * y) \leq B(x)$

**Example :** Let  $X = \{0,1,2,3\}$  be a set with the subsequent cayley table.

*	0	1	2	3
0	0	1	2	3
1	1	0	1	1
2	2	2	0	2
3	3	3	3	0

Let  $B: X \rightarrow I$  be a multi-fuzzy set described with the aid of using  $B(0) = B(1) = (0.6,0.8)$  and  $B(2) = B(3) = (0.3,0.4)$ . Then B is known as multi-fuzzy closed ideal in X.

**Theorem :** Every Anti multi-fuzzy closed ideal is a Anti multi fuzzy ideal in X.

Proof: Let B be a Anti multi fuzzy closed ideal in X.

To show that B is a Anti multi fuzzy ideal in X

It is sufficient to show that  $B(0) \leq B(x)$

Now ,  $B(0) \leq \max \{B(0 * x), B(x)\}$

Since through the use of Anti multi fuzzy closed ideal conditions

$$\begin{aligned}
 B(0) &\leq \max \{B(x), B(x)\} \\
 &= B(x)
 \end{aligned}$$

Clearly ii and iii are true.

**Remark**

The speak of the above theorem is not always true.

**Theorem**

If B is a Anti multi fuzzy BH-ideal in X, then the set  $U(B; s)$  is a BH-ideal in X for  $s = s_1, s_2, \dots, s_k$  where  $s_i \in [0,1]$ , for all i.

Proof:

Let B be a Anti multi fuzzy BH-ideal in X.

To show that  $U(B; s)$  is a BH-ideal in X

i) Since  $B(0) \leq B(x) \leq s$

ii) Let  $x * y \in U(B; s)$  and  $y \in U(B, s)$

Then  $B(x * y) \leq s$  and  $B(y) \leq s$

$$\begin{aligned}
 \text{Now } B(x) &\leq \max\{B(x * y), B(y)\} \\
 &\leq \max \{s, s\} = s
 \end{aligned}$$

This implies that  $x \in U(B; s)$

iii) Let  $x \in U(B; s)$  and  $y \in X$

$$\begin{aligned}
 \text{Choose } y \in X \text{ such that } B(y) &\leq s \\
 B(x * y) &\leq \max \{B(x), B(y)\} \\
 &\leq \max \{s, s\} = s
 \end{aligned}$$





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This implies that  $x * y \in U(B; s)$   
Hence  $U(B; s)$  is a BH- ideal in X.

#### HOMOMORPHISM OF ANTI MULTI-FUZZY BH-IDEALS

In this segment we mentioned approximately the properties of Anti multi fuzzy BH-ideals under homomorphism.

##### Definition

Let  $g: X \rightarrow Y$  be a mapping of BH-algebra and B be a Anti multi fuzzy set Y then  $g^{-1}(B)$  is the pre-image of B under  $g$  if  $g^{-1}(x) = B(g(x)) \forall x \in X$ .

##### Theorem

Let  $g: X \rightarrow Y$  be a homomorphism of BH-algebra. If B is Anti multi fuzzy BH-ideal of Y.

Then  $g^{-1}(B)$  is a Anti multi fuzzy BH-ideal of X.

Proof:

Let  $g: X \rightarrow Y$  be a homomorphism of BH-algebra.

Let B is a Anti multi fuzzy BH-ideal of Y.

To show that  $g^{-1}(B)$  is a Anti multi fuzzy BH-ideal of X.

For any  $x \in X$ ,

By the usage of Anti multi fuzzy BH-ideal.

$$1) g^{-1}(B)(x) = B(g(x)) \leq B(0) \\ = B(g(0))$$

$$= f^{-1}(B)(0)$$

$$2) g^{-1}(B)(x) = B(g(x)) \leq \max\{B(g(x)) * B(g(y)), B(g(y))\}$$

$$= \max\{B(g(x * y)), B(g(y))\}$$

$$= \max\{g^{-1}(B)(x * y), g^{-1}(B)(y)\}$$

$$3) g^{-1}(B)(x * y) = B(g(x * y)) = B(g(x) * g(y))$$

$$\leq \max\{B(g(x)), B(g(y))\}$$

$$= \max\{g^{-1}(B)(x), g^{-1}(B)(y)\}$$

Hence  $g^{-1}(B)$  is a Anti multi fuzzy BH-ideal of X.

##### Theorem

Let  $g: X \rightarrow Y$  be an epimorphism of a BH-algebra. If  $g^{-1}(B)$  is a Anti multi fuzzy ideal in X then B is a Anti multi fuzzy ideal in Y.

Proof:

Let  $g: X \rightarrow Y$  be an epimorphism of a BH-algebra

Let  $g^{-1}(B)$  is a Anti multi fuzzy ideal in X

To show that B is a Anti multi fuzzy ideal in Y.

Let  $y \in Y$  there exists  $x \in X$  such that  $g(x) = yB(y)$





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$$\begin{aligned}
 &= B(g(x)) = g^{-1}(B)(x) \\
 &\leq g^{-1}(B)(0) \\
 &= B(g(0)) = B(0)
 \end{aligned}$$

That is  $B(0) \geq B(y)$

ii) Let  $x, y \in Y$  there exists  $a, b \in X$  such that  $g(a) = x, g(b) = y$

$$\begin{aligned}
 B(x) &= B(g(a)) \\
 &= g^{-1}(B)(a) \\
 &\leq \max\{g^{-1}(B)(a * b), g^{-1}(B)(b)\} \\
 &= \max\{B(g(a * b)), B(g(b))\} \\
 &= \max\{B(g(a) * g(b)), B(g(b))\} \\
 &= \max\{B(x * y), B(y)\}
 \end{aligned}$$

$$\begin{aligned}
 \text{iii) } B(x * y) &= B(g(a) * g(b)) \\
 &= B(g(a * b)) \\
 &= g^{-1}(B)(a * b) \\
 &\leq \max\{g^{-1}(B)(a), g^{-1}(B)(b)\} \\
 &= \max\{B(g(a)), B(g(b))\} \\
 &= \max\{B(x), B(y)\}
 \end{aligned}$$

Hence B is Anti multi-fuzzy BH-ideal in Y.

## REFERENCES

1. Y. Imai and K. Iseki, On axiom system of ropositinal calculi, XIV Proc, Japan Academy, 42(1966) 19-22.
2. K. Iseki, An algebra related with a propositinal calculi, Proc, Japan Acad 42(1966), 26-29
3. Iseki K: "On BCI-algebras, Math. Seminar Notes. 11(1980), 313-320
4. Iseki K and Tanaka S: " An introduction to theory of BCK-algebras", Math, Japan 23(1978), 1-26.
5. L.A.Zadeh, Fuzzy sets, Informatin and Control, 8(1965) 338-353.
6. S. Sabu and T.V. Ramakrishnan, Multi-fuzzy sets, International Mathematical Forum, 50 (2010) 2471-2476.
7. Y.B. Jun, E.H. Roh and H.S. Kim, On BH-algebras, Scientiae Mathematicae 1(1) (1998), 347-354
8. Q. Zhang, E.H. Roh and Y.B. Jun, On Fuzzy BH-algebras, J.Huanggang Normal Univ, 21(3)(2001), 14-19.
9. K. Anitha and N. Kandaraj, Fuzzy subalgebras on BH-algebras, *American International Journal of Research in Science, Technology, Engineering & Mathematics*, (2019), 27-36
10. K. Anitha and N. Kandaraj Fuzzy Ideals And Fuzzy Dot Ideals On BH-Algebras" *International Journal of Advanced Research in Engineering and Technology*, (2019) 359-361.





## Skin Cancer Detection: Microwave Imaging

Vimlesh Singh<sup>1\*</sup>, Priyanka Bansal<sup>1</sup>, Amarinder Kaur<sup>2</sup>, Amarjeet Singh Verma<sup>3</sup>, Jyoti Singh Rajput<sup>4</sup> and Sarthak Singhal<sup>5</sup>

<sup>1</sup>Electronics and Communication Engineering, FET, MRIIRS, Faridabad, Haryana, India.

<sup>2</sup>Electrical and Electronics Engineering, FET, MRIIRS, Faridabad, Haryana, India.

<sup>3</sup>Department of Dermatology, Rama Medical College, Hapur, Uttar Pradesh, India.

<sup>4</sup>Department of Pathology, NCR Institute of Medical Sciences, Meerut, Uttar Pradesh, India.

<sup>5</sup>Department of Electronics and Communication Engineering, MNIT, Jaipur, Rajasthan, India.

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### \*Address for Correspondence

**Vimlesh Singh**

Electronics and Communication Engineering,  
FET, MRIIRS, Faridabad,  
Haryana, India.



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### ABSTRACT

This paper deals with the study and simulation results of a wearable device for the diagnosis of cancer. This study is based on electromagnetic imaging behavior of skin dermal tissue dielectric property. Cancerous tissue had a high concentration of water content in comparison to healthy tissue. The sensor proposed in this paper has unique characteristics to couple with the tissue due to small size, easy fabrication & direct coupling with sample tissue. For an initial development and validation, and data availability consideration in the open literature, basal cell carcinoma (BCC) is used for simulations. Results validate that the proposed technique provides fast, easy fabrication and low-cost solution is accurate skin cancer detection.

**Keywords:** Skin, Microstripline, Cancer, Dielectric Properties

## INTRODUCTION

Recently media have highlighted the concern for increment in cancerous cases in the northern part of India, especially in the state of Punjab. Many researchers are attributing this rise to the local environment governing the area. Fortunately, though skin cancer has not till been a major concern, the recent spike in cases may alter this view [1-3]. Cancer developed in skin is classified based on type of skin cell in which it developed. The main categories of cell are:

- Melanoma (MM)
- Basal cell carcinoma (BCC)
- Squamous cell carcinoma (SCC)



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Basal cell & Squamous cell carcinoma are collectively named as Non-Melanoma Skin Cancer (NMSC). Basal cell epithelioma arises due to damage to the skin by the sun. It very rarely develops on the palm, sole or the mucous membrane. BCC is a slow-growing tumor but is highly destructive. It disfigures local tissues in case of delay in treatment. In clinical examination, it appears as flesh/pink colored widened venules vessels in form of clusters. The major affected area of BCC is the head or neck in maximum reported cases [4][5]. BCC has 26 different subcategories still four major categories identified by dermatologists are based on clinicopathologic nodular, fibroepithelial, superficial and morpheaform. Combination of all four types of BCC also occurs. BCCs appear majority in cases as amelanotic, in which the number of melanin present within the tumors.

The etiological factor shows that the development of BCC in humans is due to exposure to Ultraviolet(UV) light rays, especially UVA & UVB rays due to its wavelength. UVA rays cause premature aging and wrinkle formation on the skin due to deep penetration in the skin layer, whereas UVB causes sunburn on skin which plays a major role in the development of skin cancers. Research shows that UVA rays are approximately 500 times more in comparison to UVB rays. This also shows a higher level of risk of skin cancer in outdoor workers in exposure to UV rays [6-7]. Development of skin cancer not only due to UV rays exposure this can also develop due to several other types of exposure such as arsenic/ionized radiation, immune-suppression and genetic predisposition.

Pathophysiology analysis of BCC explains that delay diagnosis of Chronic sun exposure develops a high level of risk in clinical onset [8]. The formation of BCC due to UV exposure directly damage DNA and indirectly impact to reactive oxygen species (ROS) of cellular system and the immune system. UVA rays absorbed by melanin damage DNA by free radicals, whereas UVB rays damage RNA & DNA by changing mutation by CC/TT transition. Despite the past two decades' advancements, cancer treatment remains a challenge and a motivation for many researchers worldwide. New treatment possibilities are constantly arising and show promise in improving treatment effectiveness, survival rates, and the patient quality of life, while at the same time decreasing unwanted side effects.

**Diagnostic Procedures**

The current diagnostic procedure to identify melanoma skin is through self-examination by visualization of own by patients or by performing dermoscopy which is a non-invasive method, where the doctors use lenses and high intensity the light source, which enables us to distinguish typical skin cancer skin changes, to determine if lesions are benign or malignant and if they require excision for biopsy for a conclusive diagnosis. Prospective total skin photography is also used as one method of looking at the onset of melanoma in patients with several atypical moles since it is more frequent the case that melanin carcinoma arises as a new lesion rather than as a changed existing lesion. However, when one has hundreds of moles, many of which are located on the back, it is unlikely that either the patient or the doctor who sees the patient once every 6 months can investigate all lesions without expensive photography and detailed review. Existing imaging techniques for skin cancer diagnosis (such as total body photography and sequential imaging at the macroscopic level) have a low resolution. At the microscopic level, where higher resolution imaging can be achieved; invasive biopsy samples need to be imaged to determine the type and stage of skin cancer [5, 9, 10-13].

Techniques like optical coherence tomography, ultrasound, near-IR, and Raman spectroscopies, MRI, positron emission tomography, and X-ray techniques are gaining popularity as most of them render nonionizing solutions, upright resolution, adequate acquisition rates, and well-targeted contrast implementation. Microwave imaging provides a solution towards the detection of lesions in skin because of very good resolution and the ability to limit the depth of penetration in subcutaneous fat layer. Moreover, instead of unnecessarily penetrating deep into our vital organs which may be harmful, penetrating power can be restrained up to our subcutaneous fat layer. But, the development of EMI needs reliable information regarding the dielectric properties of the cutaneous tissues. Several research teams have examined and characterized the dielectric properties of different healthy tissues found in different body parts. But the study of unhealthy tissue dielectric properties is quite limited by the researchers. The tissues exhibit dielectric properties based on the criterion like the amount of water content in the cells, Na<sup>+</sup>, K<sup>+</sup> ion





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concentration, protein content, and other physiological properties. The healthy tissues can be distinguished from the unhealthy ones based on dielectric criteria. Due to the development of tumor in the unhealthy tissues, their physiological opus changes as there arise variations in the water content and salt, ion concentration in the cells. In succession, these criteria bring about differences in the dielectric properties of the unhealthy tissues. optical coherence tomography, ultrasound, near-IR, and Raman spectroscopy, MRI, positron emission tomography, and X-ray techniques are receiving the most attention [14,30-31].

**Principle of Operation**

Human organ system has complex dielectric characteristics in which change of water content identify various health and unhealthy tissues, so by estimating water content in biological tissue identify tissue condition. For biological tissue dielectric properties are represented as:

$$\epsilon_{\text{Biological}} = \text{Dielectric Permittivity } (\epsilon_{\text{Dielectric}}) - j \text{ Dielectric loss } (\epsilon_{\text{Dielectric Loss}})$$

As per Lichtenecker’s theory biological healthy and unhealthy tissue dielectric property [11] rewritten as:

$$\epsilon_{\text{Healthy}} = \text{electric Permittivity } (\epsilon_{\text{Water}}^a \cdot \epsilon_{\text{Organ}}^{1-a}) - j \text{ Dielectric loss } (\epsilon_{\text{Water}}^a \cdot \epsilon_{\text{Organ}}^{1-a})$$

$$\epsilon_{\text{unhealthy}} = \text{electric Permittivity } (\epsilon_{\text{Water}}^b \cdot \epsilon_{\text{Organ}}^{1-b}) - j \text{ Dielectric loss } (\epsilon_{\text{Water}}^b \cdot \epsilon_{\text{Organ}}^{1-b})$$

Where a & b are water content percentage in healthy & unhealthy biological tissue of skin respectively.

For unhealthy biological tissue effective dielectric property re-writes as [16]:

$$\epsilon_{\text{unhealthy}} = \text{Dielectric Permittivity } \left( \epsilon_{\text{Water}}^{\frac{b-a}{1-a}} \cdot \epsilon_{\text{Healthy}}^{\frac{1-b}{1-a}} \right) - j \text{ Dielectric loss } \left( \epsilon_{\text{Water}}^{\frac{b-a}{1-a}} \cdot \epsilon_{\text{Healthy}}^{\frac{1-b}{1-a}} \right)$$

The detection of Basal cell carcinoma (BCC) in early stage can investigated on basis of variation in dielectric property of skin tissue. Major innovation of this work is to design testing strip and analyze skin modeling with test strip. The Electromagnetic wave (EM) developed by the test strip penetrate the skin sample and reflect back to the receiving terminal. The reflected signal carries unique information at different frequency. The penetration of signal on healthy skin sample and unhealthy skin sample show variation in characteristics parameter which help in identification of condition of skin tissue. This can be achieved by evaluating relative permittivity and conductivity in respect to frequencies. After evaluating parameter test strip designed than, test strip analyzed with skin sample by electromagnetic simulator CST microwave studio. Modeling of complex dielectric properties of human skin modeled by Debye theory of frequency dependent dielectric variable[5, 17-18].

Permittivity in terms of frequency function of skin is modeled as:

$$\epsilon_{\text{model}}(\omega) = \epsilon_{\text{infinte Frequency}} + \frac{\epsilon_{\text{Static}} - \epsilon_{\text{relative}}}{1 + j\omega\tau_{\text{initial}}} + \frac{\epsilon_{\text{relative}} - \epsilon_{\text{infinte}}}{1 + j\omega\tau_{\text{final}}}$$

This complex dielectric property of human tissue consists of real and imaginary part.

$$\epsilon_{\text{model}} = \epsilon_{\text{Real}} - j\epsilon_{\text{imaginary}}$$

Effective conductivity of skin,

$$\epsilon_{\text{imaginary}} = \frac{\sigma_{\text{Effective}}}{2\pi f \epsilon_{\text{Free space}}}$$

Designed test antenna with waveguide port work as test strip sensor for sensing firing field to discriminate healthy tissue of skin and BCC.



**Vimlesh Singh et al****Proposed Antenna Design**

The monopole antenna proposed in this paper can be used for imaging of BCC in human skin at the range of 1GHz to 10GHz. To simulate antenna with multilayer human forearm three layers model of epidermis, adipose tissue and bone are taken as sample. To evaluate the accuracy of proposed test antenna with human model develop three layer mimic structure of epidermis, adipose tissue and bone. In proposed model cancerous tumor is embedded in adipose tissue. This tumor is between epidermis & adipose tissue. The permittivity and conductivity of each layer is tabulated in table-1 [17, 19-22].

To design proposed monopole antenna designed for fire redundant substrate (FR<sub>4</sub>) material for dielectric constant 4.4, substrate thickness 1.6mm and loss tangent 0.002 as shown in figure 1a & 1b. The dimension of proposed antenna is shown in figure 1a simulated by CST microwave studio [23]. To feed antenna wave guide port of 50Ω is used.

**RESULT AND DISCUSSION**

Simulated test antenna shown in figure 1a and 1b analyzed for 1GHz to 8GHz frequency range. Reflection coefficient for the test antenna is shown in figure-2 in which resonance appears at 1.5GHz in L-band, 4GHz in S-Band and 6.5GHz in C-band. Designed microwave antenna sensor, when placed in contact with biological sample material, on the permittivity of sample material, affects the resonating frequency and quality factor of the proposed antenna design. The electromagnetic properties of monopole antenna change with distance from effective area and size of effective area [24]. Permittivity, permeability and free carrier concentration are the function of electromagnetic properties of the biological skin sample. Moisture content present in biological skin samples is a measurable parameter of conductivity. To analyze these variations near field patterns of microwave antenna sensors are mapped with the density of tissue and permittivity of the biological sample [5, 18]. Various techniques are available to evaluate electromagnetic properties by near field sensors. The setup of proposed triple-band microwave antenna with human multi-layer skin model is shown in figure-3.

Electromagnetic property of multi layer phantom with the proposed strip is shown in figure-4a. The variation in reflection coefficient shows that with change in dielectric properties help to identify the abnormality in the skin tissue. Figure -4b shows equivalent model of phantom different layer with sensor strip. Figure 4a explain comparison between S<sub>11</sub> parameter with and without human model. Dielectric properties with frequency dielectric properties with reflection coefficient are shown in figure-5a & 5b. As dielectric properties of human body change with the moisture content present various tissues this will help in imaging any kind of abnormality in the tissue system [25-26]. Far field pattern of proposed microwave sensor test strip shows Omni directional pattern as shown in figure 6a, 6b & 6c. Simulation result explains capability of microwave sensor strip for diagnosis of all four type of tissue which are consider in phantom. Electric field pattern for frequency of 1.5GHz, 4GHz & 6.5GHz is shown in figure 7a, 7b & 7c. This explains that for different frequencies, electromagnetic properties of sensor is changed which help in detection of abnormality in the tissue. To achieve good resolution of image microwave sensor is placed on phantom directly [27-28].

**CONCLUSION**

Microwave sensor strip is simulated & designed in this paper to analyze skin abnormality by variation in dielectric properties of biological tissue. The proposed phantom in this paper is simulated for epidermis, tumor, adipose tissue and bone. Surface imaging by microwave sensor strip shows good resolution image at resonance frequency of 1.5GHz, 4GHz and 6.5 GHz. This early diagnosis provides primary care to patient and help in managing BCC. Result obtained from simulation of microwave sensor strip shows good agreement with available literatures. The result show feasibility of electromagnetic imaging non-invasive skin tissue scanning. The next stage is to verify the concept.



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This is performed by pre-clinical analysis using animal models constituting BCC and normal control skin [29]. This acts as a precursor to vitro study and research with human skin samples. In order to validate the studies' sensitivity and specifics a statistical study is advocated.

## REFERENCES

1. WHO world health statistics. GLOBOCAN 2000.Cancer Incidence, Mortality and Prevalence Worldwide version 1.0.IARC CancerBase No. 5.Lyon, IARC Press; 2001.
2. Howe HL, Wingo PA, Thun MJ, Riess LAG, Rosenberg HM, Feigal EG, et al. "Annual report to the nation on the status of cancer (1973 through 1998), featuring cancers with recent increasing trends". J Natl Cancer Inst. 93, pp 824-42, 2001.
3. Clive M. Alabaster "The microwave properties of tissue and other lossy dielectric " P.hd Thesis in Cranfield University college of defence technology Department of Aerospace, power and sensors, 2004.
4. Dai J, Lin K, Huang Y, Lu Y, Chen WQ, Zhang XR, He BS, Pan YQ, Wang SK, Fan WX. "Identification of critically carcinogenesis-related genes in basal cell carcinoma". Onco Targets Ther. 2018;11:6957-6967. [PMC free article]
5. Kamath P, Darwin E, Arora H, Nouri K. "A Review on Imiquimod Therapy and Discussion on Optimal Management of Basal Cell Carcinomas". Clin Drug Investig. 2018 Oct;38(10):883-899.
6. Martens MC, Seebode C, Lehmann J, Emmert S. Photocarcinogenesis and Skin Cancer Prevention Strategies: An Update. Anticancer Res. 2018 Feb;38(2):1153-1158
7. King Yuk Chan & Rodica Ramer "Novel concept of detecting basal cell carcinoma in skin tissue using a continuous-wave millimeterwave rectangular glass filled probe". Medical Devices: Evidence and Research.pp275-285, 2018.
8. Skoda AM, Simovic D, Karin V, Kardum V, Vranic S, Serman L. "The role of the Hedgehog signaling pathway in cancer: A comprehensive review". Bosn J Basic Med Sci. 2018 Feb 2018(1):8-20.
9. Chen LF, C. K. Ong, C. P. Neo, V. V. Varadan, Vijay K. Varadan. Microwave Electronics Measurement and Materials Characterization. Chichester: John Wiley & Sons Ltd, 2004.
10. R. Aminzadeh, M. Saviz, A. Shishegar, "Dielectric properties estimation of normal and malignant skin tissues at millimeter-wave frequencies using effective medium theory," IEEE EE Iranian Conf, pp. 1657-1661, 2014.
11. SA. Naqvi, B. Mohammed, A. Abbosh, "Fourth order debye model for the skin at the millimetre-wave band using heuristic genetic algorithm," IEEE IntSymp Antennas and Propagation, pp. 896-897, 2016.
12. Malyuskin O and Fusco V (2016) "High-resolution microwave near-field surface imaging using resonance probes". IEEE Transactions on Instrumentation and Measurement 65, 189-200.
13. A. Mirbeik-Sabzevari, R. Ashinoff, R. and N. Tavassolian, "Ultra-Wideband Millimeter-Wave Dielectric Characteristics of Freshly-Excised Normal and Malignant Human Skin Tissues," IEEE Transactions on Biomedical Engineering. vol. PP, no. 99, pp. 1-11, 2017
14. De Giorgi V, Savarese I, Gori A, Scarfi F, Topa A, Trane L, Portelli F, Innocenti A, Covarelli P. "Advanced basal cell carcinoma: when a good drug is not enough". J Dermatolog Treat. 2020
15. C. Gabriel, S. Gabriel, and E. Corthout, "The dielectric properties of biological tissues: I. literature survey", Physics in Medicine and Biology, vol. 41, no. 11, pp. 2231- 2249, 1996.
16. R. Simpkin, "Derivation of Lichteneker's Logarithmic Mixture Formula From Maxwell's Equations," IEEE Transactions on Microwave Theory and Techniques, vol. 58, no. 3, pp. 545-550, March 2010.
17. TeraView. Study to Distinguish Between Healthy Tissue and Basal Cell Carcinoma. 2017.
18. FatemehKazemi, FarahnazMohanna and JavadAhmadi-Shokouh. Detection of biological abnormalities using a near-field microwave microscope. International Journal of Microwave and Wireless Technologies. pp.1-9 2018.
19. Pozar DM. Microwave Engineering. 3 ed. New York: Wiley; 2004.
20. Balanis, C.A., Antenna Theory: 'Analysis and design' 3rd edn., Hoboken, NJ: John Wiley, 2005



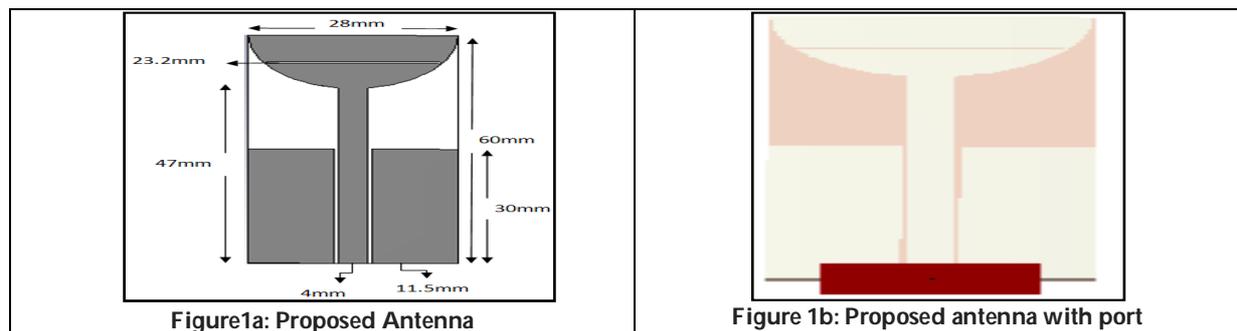


**Vimlesh Singh et al**

21. Hirth G. Improving public health relating to ultra-violet radiation exposure– innovations and plans at Arpansa. Science and the Art of Radiation Protection. Wollongong, Australia, 2017.
22. Malyuskin O and Fusco V Super-resolution defect characterization using microwave near-field resonance reflectometry and crosscorrelation image processing. Sensing and Imaging 18, pp.7–18, 2017.
23. CST Microwave Studio Suite 2011, CST Inc., 2007.
24. Koutsoupidou M, Irene S. Karanasiou, Constantine G. Kakoyiannis, Evangelos Groupas, Christophe Conessa, Nadine Joachimowicz & Bernard Duchêne. Evaluation of a tumor detection microwave system with a realistic breast phantom. Microwave and Optical Letters 59, pp. 6–10, 2017.
25. Bucci OM, Gennaro Bellizzi, Sandra Costanzo, Lorenzo Crocco, Giuseppe Di Massa & Rosa Scapaticci. Towards the assessment of detection limits in magnetic nanoparticle enhanced microwave imaging of breast cancer, Antennas and Propagation (EUCAP), 2017 11th European Conference, pp.19–24 March 2017.
26. Sugumaran S, Mohd Faizal Jamlos, Mohd Noor Ahmad, Chandar Shekar Bellan, Dominique Schreurs, Nanostructured materials with plasmonic nanobiosensors for early cancer detection: a past and future prospect. Biosensors and Bioelectronics 100, pp.361–373, 2017.
27. Mirbeik-Sabzevari A, Ashinoff R and Tavassolian N. Ultra-wideband millimeter-wave dielectric characteristics of freshly-excised normal and malignant human skin tissues. IEEE Transactions on Biomedical Engineering 99, pp.1–11, 2017.
28. Khokhar, Umair, Naqvi, S. A. R., Al-Badri, Noor, Bialkowski, K. and Abbosh, Amin. Near-field tapered waveguide probe operating at millimeter waves for skin cancer detection. 2017 - IEEE International Symposium on Antennas and Propagation & USNC/URSI National Radio Science Meeting, San Diego, CA, 2017.
29. Chaudhary SC, Waseem M, Rana M, et al. Naproxen inhibits UVB induced basal cell and squamous cell carcinoma development in Ptch1(±)/ SKH-1 hairless mice. Photochem Photobiol. 93(4):1016–1024. 2017.
30. Lulu Wang, Review Microwave Sensors for Breast Cancer Detection, sensor, , 18, 655, pp.1-17, 2018.
31. Maged A. Aldhaeabi, Khawla Alzoubi, Thamer S. Almoneef, Saeed M. Bamatraf, Hussein Attia, and Omar M. Ramahi, Review of Microwaves Techniques for Breast Cancer Detection, Sensors, 20(8), pp.1-38, 2020.

**Table:1 Dielectric property and conductivity of Human Tissue**

Sample	Permittivity ( $\epsilon$ )	Conductivity ( $\sigma$ )	Radius taken for sample (mm)
Epidermis[3]	40	0.91	6
Adipose Tissue[17]	27	2.14	5
Tumor[17]	50	3	1
Bone[17]	10	0.96	3





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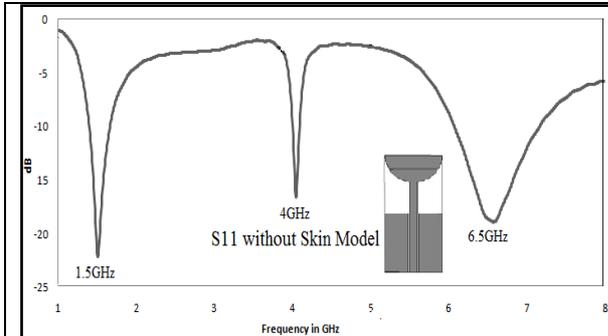


Figure: 2 S11 parameter of Proposed Antenna Sensor

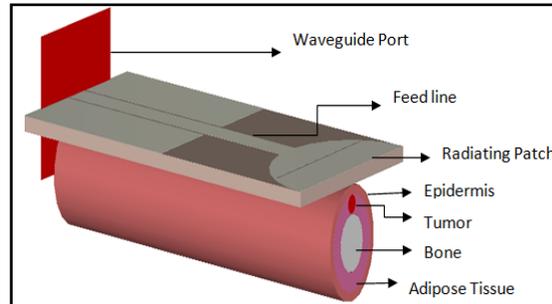


Figure 3: 3D model of Antenna sensor with phantom

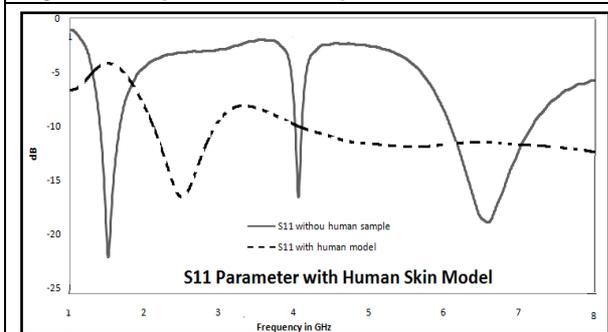


Figure: 4a S11 parameter with Human skin model

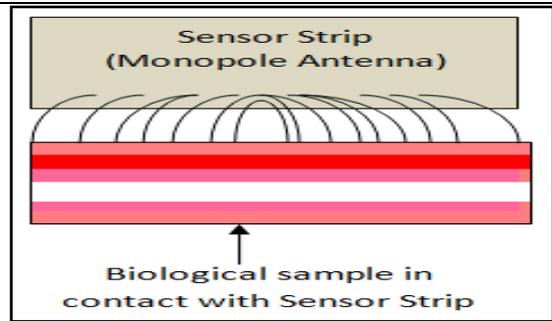


Figure 4b: Equivalent of model

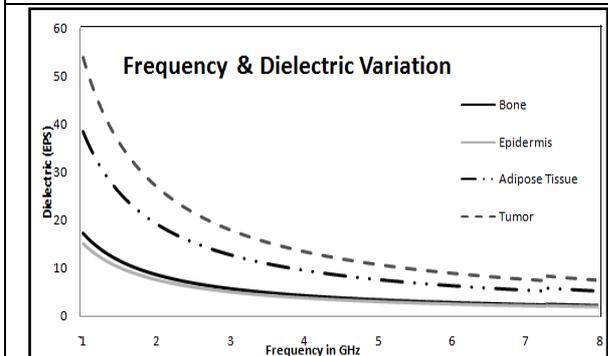


Figure: 5a Plot between Frequency & Dielectric of Human Tissue

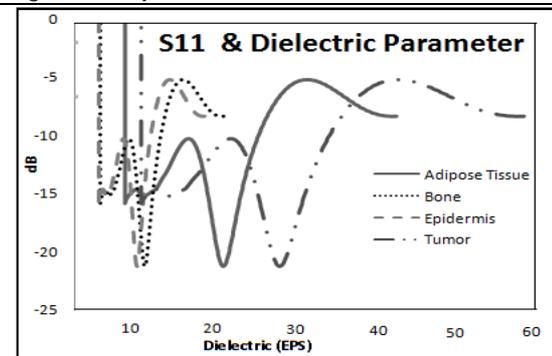


Figure 5b: Plot between S11 & Dielectric of Human Tissue

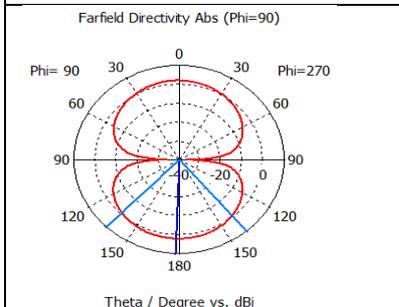


Figure: 6a Polar Plot at 1.5GHz

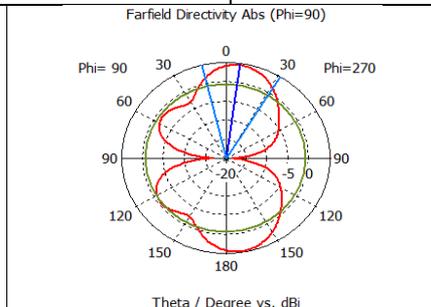


Figure: 6b Polar Plot at 4GHz

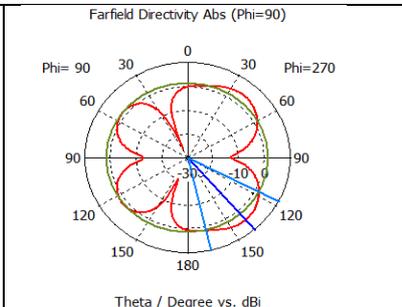
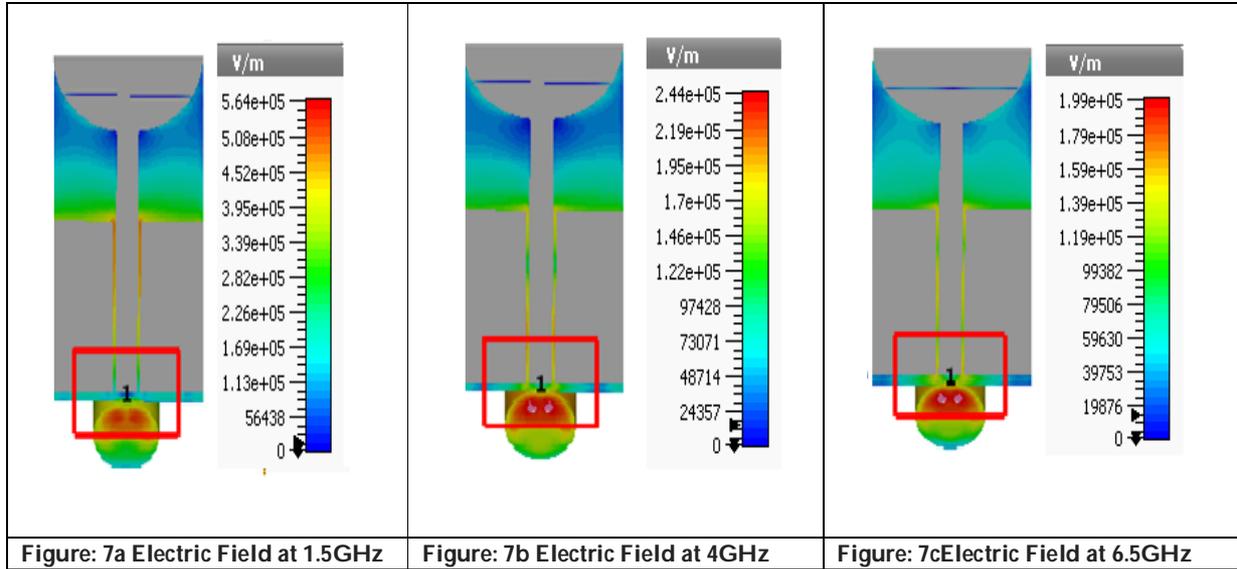


Figure: 6c Polar Plot at 6.5GHz





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## Marine Immune Booster: Seaweeds and Corals

Harshita Singh<sup>1</sup>, Pradipta Banerjee<sup>2\*</sup> and Preetha Bhadra<sup>3</sup>

<sup>1</sup>Department of Fisheries, Centurion University of Technology and Management, Odisha, India.

<sup>2</sup>Department of Biochemistry and Plant Physiology, Centurion University of Technology and Management, Odisha, India.

<sup>3</sup>Department of Biotechnology, Centurion University of Technology and Management, Odisha, India.

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### \*Address for Correspondence

#### Pradipta Banerjee

Department of Biochemistry and Plant Physiology,  
Centurion University of Technology and Management,  
Odisha, India.

E.Mail: pradipta.banerjee@cutm.ac.in



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### ABSTRACT

Modern lifestyles also created a new emphasis on beauty and personal care, attracting a large number of customers to cosmetics. The market for natural-ingredient skincare products is increasingly rising. Seaweeds are a significant source of in-demand active compounds that have a wide range of uses. Many scientific studies have shown the possible skincare properties of seaweed bioactive compounds, leading to an increase in the use of seaweed-derived ingredients in cosmetic products in recent years. The potential skincare properties of seaweed bioactive compounds are highlighted in this study. The study explains the mechanism behind skin disorders, which includes a large number of immune-boosting properties. Researchers have been studying the use and utilisation of invertebrate immune systems by corals and seaweeds in recent years, especially their potential for therapeutic applications of corals and seaweeds as immune boosters. Corals that have been shown to have therapeutic properties, particularly those derived from soft and hard corals. Researchers have already demonstrated many uses, including anti-inflammatory properties, anti-cancer properties, bone regeneration, and neurological benefits.

**Keywords :** Bioactive compounds, corals, seaweed.

### INTRODUCTION

Plants materials are used in Ayurvedic methods of the treatment. Generally, they are non-toxic and without any side effects (Babich, O., et.al (2020). Various parts of medicinal plants and trees such as Neem tree (*Azadirachta indica*), *Embllica officinalis* commonly known as Indian gooseberry or amla have been reported for acquiring immunomodulatory properties such as : modulation of cytokine secretion, histamine release, immunoglobulin secretion, cellular co-receptor expression function etc., (Mirunalini, S., et.al, (2010) and (M., Rahal,et.al.,2012),and

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some other plants are popular for their antiviral activities and immunity boosting capacity (Patil, A., &Kakde, M. , 2020) ,other than that the marine environment is a dynamic environment, which is having most valuable natural resources and provide majority of the food in the form of in fish, shellfish, corals and seaweeds contains 60 trace elements whose concentration is higher than terrestrial plants . Marine water contains enormous amounts of biodiversity, which makes it as a source of huge amounts and wide varieties of bioactive compounds with immune boosting (Singh, H. (2020). Aquatic plants are a major production component of mariculture in the Asia-Pacific region. About 13.5 million tonnes of aquatic plants were produced in 2003. China is the largest producer, producing just less than 10 million tonnes. The dominant cultured species is Japanese kelp (*Laminaria japonica*) and Corals are also considered to contain a diverse and abundant microbial community with immune-boosting properties and a variety of bioactive compounds.

**What are seaweeds**

The plant species which were found along the sea-shore are known as “seaweeds” or the term “Seaweed” refers to multi-cellular, marine algae that are large enough to be seen without the aid of a magnifying glass. Seaweed are large, multicellular algae, Most familiar type of marine algae , Some biologists prefer the name macrophytes or macroalgae (MDPI, M. M. (2018). Some species can reach a length of 60 meters. Algae from the red, brown, and green families make up from seaweeds. The multi-cellular algae Takes different structure, types of pigments and store food products into consideration Lack highly specialised structures and reproductive mechanisms like terrestrial plants they Vary in shapes and sizes.

They were reported all along the Indian coastal state waters in variable amounts. Among all these Indian coastal state Tamil Nadu and Gujarat State waters contribute more seaweed resources and seaweeds are mainly found along the coastal waters of the ocean. According to colouration and pigmentation in plants they were classified into three categories as mentioned above; i.e. Red, Brown, Green seaweeds. It is reported that the seaweed utilized for many different purposes all along the world.

The foliose blade (also known as the frond), the stem like stripe, and the holdfast that attaches the plant to a hard substrate make up the traditional structure of seaweed in other words holdfasts keep the kelp firmly anchored to the ground, preventing it from being carried away by the waves; must glue on to a rock or hard substrate, not soft, sifting sand; differs from roots in that nutrients can access any portion of the plant, allowing it to photosynthesize(CAS, C. A. (2015).As a result, the thallus refers to the whole plant. The thallus's arrangement varies greatly, resulting in a wide range of shape. The thallus morphology of seaweeds varies greatly, ranging from simple crusts, leafy blades, tubular and filamentous forms with simple branching to more complex forms. Seaweeds can vary in size from a few centimetres to several metres. The temperate giant kelps can reach a height of 60 metres in length .

The red seaweeds contain chlorophyll a and d, as well as phycobilins (phycoerythrin and phycocyanin), which give the thallus its distinctive red colour. In contrast to green seaweeds, red seaweeds can live in low-light environments and can be found even in the darkest depths of the ocean, where only blue light can penetrate. Brown algae contain chlorophyll a and c, as well as fucoxanthin, which gives them their brown colour. It needs sunshine, but not as much as green seaweed.

The brown algae *L. japonica* and *Undaria pinnatifida*, the red algae *Porphyra* , *Eucheuma*, *Kappaphycus*, and *Gracilaria*, and the green algae *Monostroma* and *Enteromorpha* are among the 200 species of seaweed used globally, with roughly ten species intensively grown. Seaweeds are grown for a variety of reasons, including direct consumption as food or medicine, production of the commercially valuable polysaccharides alginate and carrageenan, usage as fertilisers, and feeding of other aquaculture commodities like abalone and sea urchins. Iron, zinc, magnesium, riboflavin, thiamin, vitamin A, B, C, and K, among other minerals, are abundant in seaweeds. (TOI, T. O. (2020).



**Harshita Singh et al.****Production of seaweeds**

Seaweed cultivation has enormous potential as a mariculture diversification activity all along the Indian coast. In 2016, the global production of seaweed was expected to be 30.1 million wet tonnes (FAO 2018), with 95 percent coming from culture and the remaining 5% coming from natural sea beds. China, Japan, Korea, Indonesia, the Philippines, Malaysia, and Vietnam are the main seven contributors to seaweed production. In India, however, agar and alginate are derived from wild stocks of seaweeds, while carrageenan is derived from cultivable *Kappaphycus alvarezii*

**Phycocolloid production**

Polysaccharides generated from seaweeds are known as phycocolloids. Alginates, agars, and carrageenans are the three major phycocolloids. Brown seaweeds are used to extract alginates, while red seaweeds are used to extract agar and carrageenan

**Agar - (Red algae)**

*Gracilaria edulis* and *Gelidium micropterum* were used as raw materials, and there are currently 25 agar processors in operation, producing between 300 and 400 tonnes of agar each year.

**Carrageenan - (Red algae)**

One of the most commonly traded tropical red seaweeds, *Kappaphycus alvarezii* is used to extract the commercially important phycocolloid *kappa carrageenan* (-carrageenan). *Kappa-carrageenan* is utilised as an emulsifier, binder, thickening, and gelling agent in a wide range of products, including toothpaste, cosmetics, ice cream, pet meals, drinks, pharmaceuticals, personal care, and dairy products, due to its unusual gelling qualities.

The health consequences of carrageenan have been a source of debate. Carrageenan may cause inflammation, gastrointestinal ulcers, and harm to your digestive system, according to some studies. More human investigations are needed to confirm any relation between carrageenan and digestive issues. In the meanwhile, you may want to reduce your intake of carrageenan. (This substance is still approved by the Food and Drug Administration. Carrageenan was removed from the National Organic Standards Board's authorised list in 2016. This means that items containing carrageenan cannot be classified as "USDA organic."(Wilson et.al, 2017). The cultivation of *Kappaphycus alvarezii* in India began with the importation of a few grammes of biomass from Japan by CSIR-CSMCRI in the early 1990s; the importation followed the proper quarantine procedures for cultivation and consumption. Polythene bags, fish net bags, net enclosed open culture, and bamboo raft method were used as cultivation methods.

**Alginates – (Brown algae)**

*Sargassum spp.* and *Turbinaria spp.* are gathered from natural populations along the Gulf of Mannar coast and used to make alginate. Alginates have been employed in a variety of applications in the food and feed processing industries as thickening and gelling agents, as well as in the pharmaceutical sector as stabilizers, and in the textile business as water proofing, paper coating, and also used for wastewater treatment.

**Utilization of seaweed**

In Southeast Asian nations, the direct intake of seaweeds as food in the form of salads, soups, and a variety of other culinary preparations is highly widespread. Japan, China, and Korea are in the forefront in using seaweeds in human food preparations, whereas seaweeds are solely utilised in India to extract industrially essential phycocolloids such as agar, alginate, and carrageenan.



**Harshita Singh et al.****Seaweeds as Nutrition Supplement**

Seaweeds have a higher nutritional value than land-based plants. Seaweeds do not waste energy because they lack a circulatory system, leaves, roots, and stem. It's a delicacy in traditional Japanese and Chinese cuisine. The Chinese consume more seaweed species than any other ethnic group on the planet.

**Polysaccharides from seaweed**

*Sulphated galactans* and acidic polysaccharide are found in green algae (Chlorophyceae). *Ulva* species have a high polysaccharide content, with polysaccharides accounting for 65 percent of the dry weight. Alginic acid, fucoidan, and laminarin are produced by brown algae (Phaeophyceae). Agar, carrageenan, floridean, and *Porphyra* n are all produced by red algae (Rhodophyceae).

**Dietary fibres from seaweed**

Both water-soluble (alginic acid, agars, furonan, laminarin, and *Porphyran*) and water-insoluble (cellulose, mannans, and xylan) fibres are abundant in marine algae, and both contain vital nutrients and serve as functional foods. Obesity, cholesterol, and large intestine cancer can all be prevented with these fibres. Algae have higher levels of dietary fibres (approx. percentage dry weight basis) as mentioned in table.1 mentioned below.

**Proteins and amino acids from seaweeds**

The protein content of seaweeds varies greatly by phylum. Brown algae have a protein content of 5-16 percent, while green algae have a protein content of 10-30 percent and red algae have a protein content of 15-20 percent. Some red seaweeds, such as *Palmaria palmata* and *Porphyra tenera*, have protein content of 36 percent and 48 percent, respectively, which is comparable to soybean protein content of 35 percent. Protein content in *Ulva* species ranges from 15 to 20%. Alanine, aminobutyric acid, citrulline, hydroxyproline, ornithine, and taurine are the free amino acids. The basic amino acid composition of edible algae is almost identical (Paul Cherry, et.al 2019). *Porphy ratenera*, *Ulva pertusa*, and *Undaria pinnatifida*, for example, have a high arginine content. *Porphyra* species contain up to 70% dry weight protein, as well as all of the essential amino acids that humans cannot synthesise. *Porphy ratenera*, *Ulva pertusa*, and *Undaria pinnatifida*, for example, have a high arginine content. *Porphyra* species contain up to 70% dry weight protein, as well as all of the basic amino acids that humans cannot synthesise, such as leucine, lysine, methionine, threonine, tryptophan, and valine.

**Lipids and amino acids**

Polyunsaturated fatty acids, such as omega-3 and omega-6 acids, are found in seaweed lipids and are essential in the prevention of cardiovascular disease, diabetes, and osteoarthritis. Alpha-linolenic acid is abundant in green algae, while eicosapentaenoic acid and docosahexaenoic acid are abundant in brown and red algae. Omega-3 fatty acids such as alpha-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid are important for human physiology. Seaweeds have been found to contain a variety of sterols. Cholesterol, methylene cholesterol, and sitosterol are all found in green algae. Red algae contain desmosterol, cholesterol, sitosterol, fucosterol, and chalinasterol. Fucosterol is found in abundance in brown algae. Fucosterol makes up 83-97 percent of total sterol in *Laminaria* and *Undaria* species (0.66 - 2.32 mg/g dry weight). Desmosterol makes up 87-93 percent of total sterol in *Palmaria* and *Porphyria* species (0.08 - 0.33 mg/g dry weight).

**Minerals**

Seaweed is also a good source of a range of minerals that are good for you. Calcium, iodine, iron, potassium, phosphorus, and sodium are all abundant in seaweeds. *Porphyra* contains have a high iron content, ranging from 0.2–0.7 g/100 g. *Pseudo fallaciatenera* has a high manganese content, ranging from 33.2 to 409 g/g dry weight. Seaweeds are an excellent source of iodine, particularly in areas where food is scarce. Because of their high iodine content, *Laminaria* and *Saccharina* species have long been used to treat thyroid and goiter and there are different sources of mineral content in seaweed as shown in Table 2(MDPI, M. M. (2018).



**Harshita Singh et al.****Vitamins**

Vitamin A, B1, B2, and B12 are abundant in some red seaweeds, such as *Palmaria palmata* and *Porphy ratenera*. *Codium fragile* and *Gracilaria chilensis* have higher levels of  $\beta$ -carotene (provitamin A) than carrots. Vitamin B12 is found in abundance in *Ulva* and *Pyropia* sp. Brown algae have a higher concentration of vitamin E than green and red seaweeds. Tocopherols range from 200 to 600 mg/kg dry weight in *Ascophyllum* and *Fucus* sp.

**Seaweed as Medicine**

Seaweeds have higher concentrations of the inhibitor vitamins C and E than land plants. antioxidant protects against scurvy, whereas fat-soluble vitamin aids within the management of medicine problems caused by poor nerve conductivity and anaemia caused by aerophilicharm to red blood cells. Due to its blue pigment called as phycocyanin, that forms soluble complexes with iron and alternative minerals throughout digestion, prototist iron is a lot of without delay absorbed by the build than iron from higher land plants. The phenolic-rich extracts obtained from *Alaria*, *Ascophyllum*, *Palmaria*, and *Prototist* genus species are natural antioxidants that conjointly suppress biological process enzymes and have antidiabetic drug properties. Brown algae which is called *Laminaria* species (kelp) (Seaweed, B. O. (2021) produce up to 13 times more calcium than milk and are high in antioxidants. Kelps high in fucoxanthin and fucoidan are high in vitamin B, C, and K<sub>1</sub>, as well as magnesium, potassium, and iron (Ganesan, et.al (2019).

**Seaweed as an immune booster**

The brown, red, and green algae are the three primary divisions of marine algae based on their natural pigmentation. The presence of fucoxanthin pigment gives the phaeophyceae their colour, which has many physiological functions and biological properties such as anticancer influence and other immune strengthening effects. The presence of phycoerythrin and phycocyanin pigments, which have antioxidative and anti-inflammatory properties, gives Rhodophyceae its colour. The presence of chlorophyll pigment, which aids in the development of RBC and is also used for detoxification, gives Chlorophyceae its green colour (Khalid et al., 2018).

**Bioactive compounds in seaweed boost immunity**

Edible seaweeds are rich in bioactive compounds and act as a novel food helps in preventing diseases as well as having many therapeutic properties such as antiobesity, antiviral, antifungal, antibacterial, antidiabetic, antihypertensive, immune-modulatory, antihyperlipidemic, anticoagulant, anti-inflammatory, antiestrogenic, thyroid stimulating, neuroprotective, anticancer, antioxidant and tissue healing properties. The intake of seaweed helps reducing the risk of calcium deficiency. Seaweeds are a natural wealth of the sea, with exceptional immune boosting properties and diverse groups of bioactive compounds, as shown in Tables 3 (Khalid et al., 2018).

**Other uses of seaweed****Industrial use**

In many maritime nations, seaweeds are utilised as a food source, for industrial uses, and as a fertiliser. Currently, seaweeds are utilised for human consumption, fertilisers, and the extraction of industrial gums and chemicals. They might be utilised to create long- and short-chain compounds for medical and industrial purposes. Algae from the ocean may additionally be used as a supply of energy and Fermentation and transmutation could also be wont to take away doubtless helpful compounds from marine prototist (Niobiotics, S. U. (2011).

**Cosmetics: Seaweeds as a potential Source**

People nowadays prefer natural ingredients to artificial ingredients in cosmetics. Since natural products are safe to use and have no side effects, many customers seek out natural products to maintain a youthful appearance and healthy skin. As a result, the beauty industry has put a greater emphasis on ingredients extracted from natural resources such as plants, algae, bacteria, and their metabolites. A large range of organisms with numerous bioactive compounds are in high demand in the marine environment like seaweed (Jesumani, V., et.al, 2019).



**Harshita Singh et al.****As food**

Sea weeds are also consumed as a food source in the Far East and Australia. Sea weeds are consumed in large amounts by the people of Hawaii. Chile's indigenous people consume a lot of *Durvillea antarctica* and some *Ulva* plants. Certain green sea weeds are used in salads and soups by New Zealand natives. *Porphyra* spp., or *nori*, is a red alga (Bangioophyceae). Nori comes in sheets that can be toasted for a green colour before being flaked and used in sauces, soups, and broths. It is often simply soaked and eaten. Cold rice balls are wrapped in small, dry nori sheets and served as a popular lunchtime snack in Japan. Nori's nutritional value comes from its high protein content (25-35 percent dry weight), vitamins, and mineral salts, particularly iodine. It has 1.5 times the vitamin C content of oranges, and humans can absorb 75% of the protein and carbohydrates, which is very high for seaweeds. Apart from ascorbic acid, Chlorella has been found to contain a number of vitamins. Thiamin, niacin, pyridoxine, pantothenic acid, chlorine, biotin, vitamin B, and lipoic acid are among the vitamins present in Chlorella.

**As a source of agar**

The best agar is made from *Gelidium* of Rhodophyceae, also known as vegetative agar, and Japan is the largest producer of agar. It accounts for 95% of global demand. Agar was obtained from ten species belonging to *Gelidium*, *Sarconema*, *Hypnea*, and *Gracilaria*, and the yield of agar, setting temperature, and gel intensity of the product were obtained. Agar can be used in a variety of ways. It's used to make ice cream, jellies, and cakes, as well as measuring textiles and clearing a variety of liquids. Shaving creams, cosmetics, and shoe polishes are all made with it. In biological laboratories, agar has long been used for media preparation.

**Corals**

Corals are colourful and interesting invertebrate species that belong to the "*Cnidaria*" family of creatures. Other species in this group include jellyfish and sea anemones, which you may have seen in rock pools or on the beach. Polyp is a single coral animal living in a colony of hundreds to thousands of GM polyps. Coral polyps have evolved particular defensive mechanisms to protect themselves against predators and other natural pressures in the coral reef environment. The colony is generated through a process known as budding, in which the original polyp reproduces itself. "Hard coral" and "soft coral" are the two types of coral that are commonly found. (EPA, U. (2019) Hard corals, popularly known as "reef builders," are thought to number approx. over 800 species. Soft corals, such as sea fans, sea feathers and sea whips, do not have the calcareous rock structure of other corals, but have a supporting wooden core and a soft shell for protection. Soft corals live in colonies similar to bright plants. Their polyps are different from hard corals. Polyps have eight tentacles and have a typical feather-like appearance. From the equator to the north and south poles, soft corals have been found in caves or ledges in the water. Healthy coral reefs are beneficial. The community in different ways. Coral reef ecosystems provide food for people all over the world, coastal protection, tourism and fishery income. ( National history museum, N. (2018,)

**What are coral reefs?**

Coral reefs are the world's largest living structure and the only one that can be seen from space. Coral reefs as we know them now evolved on Earth over the last 200 to 300 million years, and the highly evolved form of symbiosis is likely the most unique aspect of corals over this evolutionary history. Corals are motile, which means they can move on their own. (Hawaii's Coral Reefs, H. (2020) Corals feed on plankton, which includes small crustaceans, mollusks, and reef animal larvae. Hard corals collect a lot of calcium from the water around them and use it to harden their structure for protection and growth. Coral reefs are formed by millions of tiny polyps forming massive carbonate structures that provide a framework and home for hundreds of thousands, if not millions, of different species. This symbiosis between plant and animal is the reason of the bright colours of coral that can be seen while diving on the reef. Corals fight for space on the floor, because the light is important. In a competitive environment with so many diverse species it continually tests its physiological tolerances. Coral reefs belong to a broader ecosystem which includes mangroves and seagull beds. When coral polyps lose their symbiotic algae, the zooxanthellae, "coral bleaching" occurs. The live tissues are practically transparent without their zooxanthellae, and you can see right through to the rocky skeleton, which is white, hence the name coral bleaching (Nancy Knowlton, O. F.



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(2020). Mangroves are salt-tolerant trees with submerged roots that serve as nurseries and breeding grounds for marine life prior to their migration to the reef. (NOAA, C. (2019) Mangroves also filter pollutants from land-based run-off, stabilise the shoreline, protect the coastal zone from storms, and trap and create nutrients for food. Sea grasses are flowering marine plants that function as primary producers in the food web. Turtles, seahorses, manatees, fish, foraging sea life such as urchins and sea cucumbers, and a variety of juvenile sea animal species rely on them for food and habitat.

**Types of corals**

Fringing reefs, barrier reefs, atolls, and patch reefs are the four types of coral reefs identified by scientists.

**Fringing reefs**

Near emergent land, fringing reefs can be found. They're shallow, narrow, and new to the area. A navigable waterway (which is sometimes wrongly referred to as a "lagoon") might separate them from the coast.

**Barrier reefs**

Barrier reefs are larger and are located further from the shoreline. They are separated from the coast by a body of water that can be several miles long and tens of metres deep. On top of a barrier reef, sandy islands with a distinctive flora pattern have occasionally evolved. These islands' coastline is fragmented by passages that have taken over previous riverbeds.

**Atolls**

Atolls are similar to barrier reefs, except they're round and enclose a shallow lagoon with no land in the center. Large, ring-shaped reefs off the coast with a lagoon in the centre are known as atolls. The emergent area of the reef is frequently covered with accumulated sediments, and coconut trees are the most common flora found on these reefs. Atolls form near the sea surface on underwater islands or sinking or subsiding islands. (Biology discussion, B. D. (2016)

**What is coral calcium?**

Coral calcium is a supplement made from the sands of coral reefs. These sand deposits, which were once part of a coral reef, are usually obtained directly from the shore or nearby shallow waters. The coral sand deposits are refined to eliminate any toxins or other undesirable substances before being ground into a powder to make coral calcium (Biology discussion, B. D. (2016). This powder is available unprocessed or in capsule form. Calcium carbonate makes up the majority of coral calcium supplements, although small quantities of magnesium and other trace minerals may also be present. Coral calcium has been used as a bone graft material for over 30 years since its composition is close to that of human bone. It's now often used to avoid or treat low calcium levels in people who don't get enough calcium from their diet. While it is said to have several additional health benefits, the majority of these have yet to be proven by researchers.

**Pharmaceutical uses of corals**

Coral reefs are sometimes referred to as the 21st century's medicine cabinets (Ravishankar C.N. et.al (2018). New treatments for cancer, arthritis, human bacterial infections, Alzheimer's disease, heart disease, viruses, and other disorders are being developed using coral reef flora and animals. Corals evolved chemical defences to protect themselves from predators because they are immobile animals. The therapeutic potential of these compounds is still being investigated by scientists. Medical treatments, nutritional supplements, pesticides, cosmetics, and other commercial items could all be derived from coral reef ecosystems in the future. Corals have been discovered to be useful in the development of novel therapeutic products by the pharmaceutical sector. For example, many corals promote the fusion of shattered bones. The components of a Caribbean sponge are utilised to make Zidovudina, an HIV-treatment drug.



**Harshita Singh et al.****Corals as immune booster**

Anti-cancer properties are found in the *Sinularia* genus of soft corals. *Sinularia crassa* steroid inhibits liver cancer while also suppressing pro-inflammatory activities. Soft coral yields 13-acetoxysarcocrassolide, which has cytotoxic properties against bladder cancer cells. Michaelides and lobomichaelides are newly discovered cembranolides from soft corals. *Lobophytum michaelae* has anticancer properties. Eunceafusca contains three diterpenes that have been shown to have anti-inflammatory properties. One of the diterpenes obtained from the same plant had both antibacterial, anticancer and anti-inflammatory properties other than this corals have many other bioactive components which show immune boosting properties (Cooper, E., et.al (2014,)) and the Coral calcium has a long list of health benefits, including stronger bones and less arthritis symptoms, as well as cancer and Parkinson's disease prevention and other benefits also. Recent progress and challenges for seaweed bioactives as a nutraceutical in terms of biocompatibility and bioavailability, as well as therapeutic properties (Britannica (2020).

**Bioactive compounds in corals boost immunity**

Bioactives found in seaweed, such as polysaccharides, pigments, fatty acids, polyphenols, and peptides, have been shown to have a variety of biological properties that could aid in the production of functional foods and nutraceuticals. There is a wide range of potent bioactivities, such as anti-cancer, cytotoxic, anti-inflammation, anti-bone loss, etc are present in corals. In an in-vitro and in-vivo model system, these bioactives are investigated for antioxidant, antibacterial, anticancer, antidiabetic, antitumor, antiviral, anti-inflammatory, and anticoagulant properties.

**Other uses of corals**

The coral polyp is a small, plant-like marine invertebrate with an exterior skeleton similar to coral. Generations of these organisms living as colonies on top of one another can create huge structures. Coral has been utilised in carvings, cabochons, and other jewellery pieces since the beginning of time and also the Corals are commonly used as souvenirs, home decor, and costume jewellery (Gregoriotti, G. (2019), but they are living animals that feed, grow, and reproduce. Leave corals and other aquatic life on the reef because it takes them decades or longer to construct reef structures. Coral reefs are among the world's most biologically diverse and economically important habitats, but they are under threat from a growing number of factors, including global climate change, unsustainable fishing, and pollution. In addition, the United States is the world's largest recorded user of *Corallium*, red and pink corals commonly used in jewellery. In the marketplace, finished pieces of jewellery and art made from this form of coral will fetch anywhere from \$ 20 to \$ 20,000 (Rp.1,459.63 to Rp.1458201.02 INR). Continued market demand is leading to the global depletion of these fragile corals. (US Department of Commerce, N. (2011).

**CONCLUSION**

Enhanced aquatic vegetation or phytoplankton formation, as well as algal blooms, disrupt the normal functioning of aquatic ecosystems all over the world. When toxic microalgae and cyanobacteria play a role in the eutrophication process, a variety of ecological, economic, and public-health issues can arise. The majority of the bioactive compounds derived from seaweeds have been used for biocidal (anti-fungal, anti-bacterial) and medicinal purposes. However, only a few studies by researchers have looked into their anti-cyanobacterial and algicide properties and with other immune boosting effects. It's important to encourage people to eat seafood because of the immune-boosting properties found in seaweed. We should also encourage people to take coral supplements and strive to eat less high-fat, high-sugar foods, such as fast food. Exploitation of the marine ecosystem occurs as a result of the widespread use of available marine organisms in both medicinal and human use. As a result, we must consider the future generation's better survival.





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## REFERENCES

- Babich, O., Sukhikh, S., Prosekov, A., Asyakina, L., & Ivanova, S. (2020). Medicinal plants to strengthen immunity during a pandemic. *Pharmaceuticals*, 13(10), 313. doi:10.3390/ph13100313
- Singh, H. (2020). Common Marine Organisms: A Novel Source of Medicinal Compounds. *International Journal of Bioresource Science*, 7(2). doi:10.30954/2347-9655.02.2020.1
- Patil, A., & Kakde, M. (2020). Medicinal plant as a natural immunity booster for COVID19- A review. *Indian Journal of Integrative Medicine*, 2(2), 24-27. Retrieved from <https://mansapublishers.com/IJIM/article/view/2458>
- FAO, P. (2015, April 22). Introduction to Eucheuma seaweed. Retrieved May 15, 2021, from <http://www.fao.org/3/AC287E/AC287E01.htm#ch1.1>
- Mirunalini, S., & Krishnaveni, M. (2010). Therapeutic potential OF PHYLLANTHUS emblica (amla): The ayurvedic wonder. *Journal of Basic and Clinical Physiology and Pharmacology*, 21(1). doi:10.1515/jbcpp.2010.21.1.93
- M., Rahal, A., Deb, R., K. Latheef, S., Abdul Sama, H., Tiwari, R., . . . Dhama, K. (2012). Immunomodulatory and therapeutic potentials of HERBAL, TRADITIONAL/INDIGENOUS AND ethnoveterinary medicines [Abstract]. *Pakistan Journal of Biological Sciences*, 15(16), 754-774. doi:10.3923/pjbs.2012.754.774
- Cooper, E. L., Hirabayashi, K., Strychar, K. B., & Sammarco, P. W. (2014). Corals and their potential applications to integrative medicine. *Evidence-Based Complementary and Alternative Medicine*, 2014, 1-9. doi:10.1155/2014/184959
- Khalid, S., Abbas, M., Saeed, F., Bader-UI-Ain, H., & Suleria, H. (2018, November 05). Therapeutic Potential of Seaweed Bioactive Compounds. Retrieved January 14, 2021, from <https://www.intechopen.com/books/seaweed-biomaterials/therapeutic-potential-of-seaweed-bioactive-compounds>
- Wilson, D. R., Kathleen Pointer. (2017, October 12). *Should You Remove Carrageenan from Your Diet?* Retrieved May 17, 2021, from <https://www.healthline.com/health/food-nutrition/carrageenan>.
- Seaweed, B. O. (2021, January 20). Seaweed. Retrieved May 22, 2021, from <https://www.biologyonline.com/dictionary/seaweed-d3>
- Seaweeds Cultivation and Utilisation, T. F. (2018, October 11). Seaweeds Cultivation and Utilisation - Prospects in India. [https://tifac.org.in/images/pdf/pub/TIFACReports/newreports/seaweed\\_w.pdf](https://tifac.org.in/images/pdf/pub/TIFACReports/newreports/seaweed_w.pdf)
- Ravishankar C.N., S. M. (2018, February 22). Seaweeds as a Source of Micro and Macro Nutrients Biochemistry and Nutrition Division. Retrieved May 22, 2021, from <https://krishi.icar.gov.in/jspui/bitstream/123456789/20485/1/Seaweeds%20as%20a%20source%20of%20micro%20and%20macro%20nutrients.pdf>
- Auckland University of Technology, S. F. (2013, February 22). Lipid , fatty acid and sterol content analysis of seaweeds. Retrieved May 24, 2021, from <https://openrepository.aut.ac.nz/bitstream/handle/10292/5780/LiY.pdf?sequence=3&isAllowed=y>
- Paul Cherry, Cathal O'Hara, Pamela J Magee, Emeir M McSorley, Philip J Allsopp, Risks and benefits of consuming edible seaweeds, *Nutrition Reviews*, Volume 77, Issue 5, May 2019, Pages 307–329, <https://doi.org/10.1093/nutrit/nuy066>
- MDPI, M. M. (2018, July 15). Minerals from marine algae origin: Health benefits. Retrieved May 24, 2021, from <https://www.mdpi.com/1660-3397/16/11/400/pdf>
- Britannica, T. Editors of Encyclopaedia (2020, March 16). Coral. Encyclopedia Britannica. <https://www.britannica.com/animal/coral>
- Gregorietti, G. (2019, January 29). Jewelry. Encyclopedia Britannica. <https://www.britannica.com/art/jewelry>
- Cooper, E., Hirabayashi, K., Strychar, K., & Sammarco, P. (2014, March 13). Corals and Their Potential Applications to Integrative Medicine. Retrieved January 19, 2021, from <https://www.hindawi.com/journals/ecam/2014/184959/>
- US Department of Commerce, N. (2011, November 29). Does coral jewelry make a good gift. Retrieved May 24, 2021, from <https://oceanservice.noaa.gov/facts/coral-jewelry.html>
- Niobiotics, S. U. (2011, November 11). Uses of seaweeds. Retrieved May 24, 2021, from <http://www.niobioinformatics.in/seaweed/use/uses%20of%20seaweeds.htm#:~:text=The%20present%20uses%20o>





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- f%20seaweeds,of%20industrial%20gums%20and%20chemicals.&text=Marine%20algae%20may%20also%20be,extracted%20by%20fermentation%20and%20pyrolysis.
21. Ganesan, A., Tiwari, U., & Rajauria, G. (2019, August 10). Seaweed nutraceuticals and their therapeutic role in disease prevention. Retrieved May 25, 2021, from <https://www.sciencedirect.com/science/article/pii/S2213453019300254>
  22. And Ana M. M. Gonçalves , A. J. (2020, August 6). Seaweed's Bioactive Candidate Compounds to Food Industry and Global Food Security. Retrieved May 25, 2021, from <https://www.mdpi.com/2075-1729/10/8/140/pdf>
  23. Jesumani, V., Du, H., Aslam, M., Pei, P., & Huang, N. (2019, December 06). Potential use of seaweed bioactive compounds in skincare-a review. Retrieved May 25, 2021, from <https://dx.doi.org/10.3390%2Fmd17120688>
  24. TOI, T. O. (2020, June 22). What is SEAWEED, benefits & how to use it. Retrieved May 25, 2021, from <https://timesofindia.indiatimes.com/life-style/food-news/what-is-seaweed-benefits-how-to-use-it/photostory/76499913.cms>
  25. CAS, C. A. (2015, November 22). Sensational seaweed. Retrieved May 25, 2021, from <https://www.calacademy.org/educators/lesson-plans/sensational-seaweed>
  26. The Ocean Portal Team Reviewed by Nancy Knowlton, O. F. (2020, February 19). Corals and coral reefs. Retrieved May 25, 2021, from <https://ocean.si.edu/ocean-life/invertebrates/corals-and-coral-reefs>
  27. Biology discussion, B. D. (2016, February 24). Essay on coral reefs. Retrieved May 25, 2021, from <https://www.biologydiscussion.com/essay/essay-on-coral-reefs/21524>
  28. Hawaii's Coral Reefs, H. (2020, March 11). Coral reef lesson. Retrieved May 26, 2021, from <https://hilo.hawaii.edu/affiliates/prism/documents/WhatIsCoralLessonOne.pdf>
  29. NOAA, C. (2019, November 11). Coral Structure and Function. Retrieved May 26, 2021, from <https://coast.noaa.gov/data/SEAMedia/Lessons/G3U3L2%20Coral%20Structure%20and%20Function.pdf>
  30. EPA, U. (2019, October 22). Coral reefs. Retrieved May 26, 2021, from <https://nepis.epa.gov/Exe/ZyPURL.cgi?Dockkey=400004AS.TXT>
  31. National history museum, N. (2018, June 23). Why are coral reefs important? Retrieved May 26, 2021, from <https://www.nhm.ac.uk/discover/quick-questions/why-are-coral-reefs-important.html#:~:text=Coral%20reefs%20provide%20an%20important,found%20living%20on%20one%20reef.>
  32. Peñalver, R., Lorenzo, J. M., Ros, G., Amarowicz, R., Pateiro, M., & Nieto, G. (2020). Seaweeds as a Functional Ingredient for a Healthy Diet. *Marine drugs*, 18(6), 301. <https://doi.org/10.3390/md18060301>

**Table 1. Shows the dietary fibre content of various seaweeds (Peñalver, R , et.al(2020).**

Types of seaweed	Dietary fibre (% in dry weight basis) (Approximate %)		
	Soluble	Insoluble	Total
<b>Phaeophyceae( brown algae )</b>			
<i>Himantaliaelongata</i>	21.70	6.00	32.70
<i>Sargassumfusiforme</i>	33.90	16.30	49.20
<i>Laminariadigitata</i>	32.50	5.63	38.00
<i>Undariapinnatifida</i>	31.00	5.30	35.30
<b>Chlorophyceae ( green algae )</b>			
<i>Ulva lactuca</i>	22.40	17.60	38.00
<b>Rhodophyceae ( red algae )</b>			
<i>Pyropiatenera</i>	19.00	6.90	35.81





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**Table 2. Seaweed Mineral Content**

Minerals	Approx. Mineral contents in algae ( mg/100 dry matter)			
	<i>Palamariapalamata</i>	<i>Undariapinnatifida</i>	<i>Laminariasps.</i>	<i>Ulva sps.</i>
Calcium	500 – 3000	360 – 1200	1100 – 3000	860 – 560
Iodine	11 – 100	25	300 – 1000	5 – 26
Iron	16 – 150	08	5 – 70	7– 100
Magnesium	160 – 600	1000 – 2000	500 – 3000	2000 – 5300
Phosphorous	370	300 – 600	150 – 800	90 – 270
Potassium	8000 – 9000	5600 – 6400	1400 – 10700	730 – 1033
Sodium	1600 – 2600	1700 – 5000	900 – 7000	900 – 5800

**Table 3. List of bioactive compounds that promote immunity**

IMMUNE BOOSTING EFFECTS	BIOACTIVE COMPOUNDS
Cardiovascular disease protection	Carotenoids, DHA, EPA.
Anti-inflammatory activity	Bioactive peptides, sulfated polysaccharides, fucoidan, terpenes.
Anti-oxidant activity	Fucoxanthin, phcoerythrobilin.
Anti-viral activity	Sulfated Glucuronogalactan, Sulfated galactans, carrageenan.
Anti - cancer activity	Fucoidans, glucans, stypoldione, carotene, fucoxanthin.
Anti - coagulant activity	Sulfated polysaccharides.

**Table 4. List of bioactive compounds with health claims from different coral reef(K., & Sammarco, P.2014)**

SCIENTIFIC NAME	BIOACTIVE COMPOUNDS	HEALTH CLAIMS
<i>Sinularia granosa</i>	9,11-Secosterol	Anti-inflammation and Cytotoxicity
<i>Sinularia crassa</i>	Crassalone A	Cytotoxicity
<i>Sinularia querciformis</i>	11-epi-Sinulariolide, sinularin, acetate	Anti-bone loss, Anti-inflammation, anti-neuroinflammation and antinociception
<i>Cladiellakrempfi</i>	Kremptielins	Anti-inflammation and cytotoxicity
<i>Capnella imbricata</i>	capnellenes	anti-neuroinflammation and antinociception
<i>Scleronephthyagracillimum</i>	Sclerosteroids	Anti-inflammation and Cytotoxicity
<i>Paraminabeaacronocephala</i>	Paraminabic acid	Anti-inflammation and Cytotoxicity
<i>Lemnaliacervicomitenuis</i>	Lemnalol	Anti-inflammation and antinociception





## Predictors of Science, Technology, Engineering and Mathematics (STEM) Career Preference

Nestor P. Elano Jr<sup>1</sup>, Emmylou A. Borja<sup>2</sup> and Ruel T. Buba<sup>2</sup>

<sup>1</sup>Surigao City National High School, Surigao City, Surigao Del Norte, Philippines.

<sup>2</sup>Surigao State College of Technology-Surigao City, Philippines.

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### \*Address for Correspondence

**Nestor P. Elano Jr**

Surigao City National High School,  
Surigao City, Surigao Del Norte,  
Philippines.

E.Mail: nestorjr2112@gmail.com



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### ABSTRACT

This study aimed to develop a predictive model of STEM career preference. It investigated what significantly predict STEM career preference among socio – economic profile, personal interest, family influence, school facilities and equipment, social orientation, and self – efficacy in STEM. It also determined the preferred career of the respondents. A predictive research design was utilized where data were gathered from 137 STEM graduates of the three divisions in the province of Surigao del Norte using a modified researcher-made questionnaire and analyzed using frequency count and percent, mean and standard deviation, and logistic regression. Findings revealed that most of the respondents are males whose mothers either have Non-STEM occupations or jobless and fathers either have Non-STEM occupations or jobless. Their average family monthly income is Php 31,051.84. Career preference is perceived to be influenced by personal interest, family influence, school facilities and equipment, social orientation, and self – efficacy in STEM. STEM related careers are mostly preferred by the respondents. Mother's occupation, family influence and self – efficacy in STEM are significant predictors of STEM career preference. Based on the identified predictors, the predictive model for STEM career preference is:  $Logit (Enrolling\ in\ STEM\ Course) = -1.58(MO) - 2.10(FI) + 2.60(SE)$ . In conclusion, STEM SHS graduates preferred to enroll STEM related careers in college and a SHS STEM student would most likely enroll in STEM if his/her mother is jobless or has Non-STEM occupation, has independence in choosing a course in college, and strongly believes that he/she can deal with STEM-related activities.

**Keywords:** predictors, career preference, predictive model, technology, engineering.





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## INTRODUCTION

Effective STEM education spurs significant and positive effects towards productivity (Croak, 2018) that can potentially reduce job-skill mismatch. Moreover, careers in STEM drive economic growth (Powers, 2017). With this growing demand of globalization, the Philippine government enacted RA 10533 also known as the “Enhanced Basic Education Act of 2013” which gives every student an opportunity to receive quality education that is globally competitive based on a pedagogically sound curriculum that is at par with international standards. This law requires students additional two (2) years in Senior High School (SHS) wherein they have an avenue to hone and master basic skills and prepare them for the demands of the economy especially in the STEM industry. The acquired skills in SHS will be irrelevant if students will pursue a non - STEM career programs in college. This can be avoided if contributory factors to career choice are taken into accounts during SHS years. In fact, Nyamwange (2016) revealed that students’ interest precedes career choice. Mau and Li (2018) added that socioeconomic status, math interest and self – efficacy are most vital in choosing a career. Moreover, students with low mathematical self – efficacy most likely do not choose STEM careers (Blotnick, Franz-Odendaal, French and Joy, 2018). Furthermore, interest in STEM either intensifies or diminishes depending on the students learned abilities overtime (Wiebe, Unfried& Faber, 2018).

Based on the foregoing premises, it is imperative to formulate a science career predictive model using the pre – identified factors in Senior High School so that alignment between learned skills in SHS and preferred STEM career programs in college is achieved thereby addressing the job – skill mismatch and ensuring quality STEM workforce to fuel economic progress in the future.

### Theoretical and Conceptual Framework

This study is rooted from the Social Cognitive Career Theory (SCCT) developed by Robert W. Lent, Steven D. Brown, and Gail Hackett in 1994 which is based on Albert Bandura’s general social cognitive theory, an influential theory of cognitive and motivational processes. It aimed at explaining three interrelated aspects of career development: (1) how basic academic and career interests develop, (2) how educational and career choices are made, and (3) how academic and career success is obtained. The theory incorporates a variety of concepts (e.g., interests, abilities, values, environmental factors) that appear in earlier career theories and have been found to affect career development.

Figure 1 shows the connection of the variables of the study through a research paradigm. Box No. 1 represents the factors or influencers such as socio – economic profile of the respondents such as sex, parents’ occupation in term of STEM related and non – STEM related, parent’s highest educational attainment, monthly income, personal interest (PI), family influence (FI), school facilities and equipment (SFE), social orientation (SO) and self – efficacy in STEM (SE) that influence STEM career preference. These factors will be tested to determine a predictive model of STEM career preference which is in the second box. Consequently, this model will be utilized to determine STEM career preference of Senior High School Students in college.

### Statement of the Problem

This study aimed to create a model that significantly predicts career preference of SHS STEM students. Specifically, it seeks answers to the following questions:

- What is the socio – economic profile of the respondents as to:
  - Sex,
  - Parents’ Occupation,
  - Highest Educational Attainment of Parents, and
  - Family Monthly Income?





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- To what extent do the respondents perceive the influence in Career Preference in terms of:
  - Personal Interest,
  - Family Influence,
  - School Facilities and Equipment,
  - Social Orientation, and
  - Self – Efficacy in STEM?
  
- What are the career preference of STEM graduates as to :
  - STEM Related and
  - Non – STEM Related?
  
- Do the socio – economic profile of the respondents and the factors stated in problem 2 significantly predict STEM career preference of the students?
- What model can be formulated to significantly predict STEM career preference of the students?

## METHODS

This study made use of a predictive research design which was deemed appropriate because this was used to determine a predictive model that was formulated to predict STEM Career Preference of the students. The study was conducted online among SHS STEM graduates from the three (3) schools division of Surigao del Norte during the school year 2018 - 2019. The respondents of this study were the senior high school graduates of STEM strand in School Year 2018-2019 from the public schools of the three divisions of Surigao del Norte who were freshmen college students for Academic Year 2019-2020. They were chosen as a reference to gather authentic data which this study used in creating a predictive model of STEM career that will be beneficial to SHS STEM students. The population size was 226. However, given the challenges of the pandemic and online constraints, the researcher only gathered 137 samples. This study used a modified researcher-made questionnaire with some items derived from the study of Penedilla & Rosaldo, (2017). The following statistical tools were used in the study, Frequency and Percentage Count, Mean and Standard Deviation and Logistic Regression.

## RESULTS AND DISCUSSIONS

### On the Socio-Economic Profile

Table 1 presents the socio-economic profile of the respondents in term of sex, mother and father's occupation, parent's highest educational attainment and monthly income. For their sex, there are 76 or 55.47% males and 61 or 44.53% females. There are 29 or 21.17% who have mothers with STEM – related occupations and 108 or 78.83% non – STEM related occupations/jobless. On the other hand, 14 or 10.22% who have fathers with STEM – related occupations and 123 or 89.78% non – STEM related/jobless. As to their parent's highest educational attainment, there are 3 or 2.19% are elementary level, 4 or 2.92% are high school level, 6 or 4.38% are high school graduates, 33 or 24.09% are college level, 73 or 53.28% are college graduates, and 18 or 13.14% are graduate level or higher. The average monthly income of parents is Php. 31, 051.84 with a SD of 39532.41. This could be said that these families belong to the middle-income class (Albert, Santos & Vizmanos, 2018).

### On the Perceived Influence to Career Preference

The perceived influence to career preference is shown in tables 2, 3, 4 and 5. Table 2 shows the perceived influence of personal interest to career preference. This table reflects an average of 3.16 with SD=0.49 which suggests that the respondents agree that the personal interest can influence career preference. The respondents agree that all 6 items support that personal factor is influential. Among the items under personal interest, Item 2 "My personal choice in



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Senior High School” got the highest mean value of 3.34 with SD=0.78. Item 4 “My childhood dream” got the lowest mean value of 2.97 with SD=0.95. The results are proven by Blotnicky et al. (2018) that students with greater interest in technical and scientific skills were more likely to consider STEM career for it will suit their skills. The perceived family influence to career preference is presented in table 3. The respondents generally agree that family influence affects career preference. This is based for the average mean of 2.76 with SD=0.7. Item 3 “The financial support of my family” got the highest mean of 3.4 with SD=0.80. However, Item 5 “The dominant profession in my family” got the lowest mean value of 2.49 with SD=1.04 which based on the responses, is not influential in choosing a career in STEM. The results as to family influence are in line with the study of Cridge and Cridge (2015) stated that parents play a vital role in their children’s life, including choices of career, they often guide their children to develop skills and observe their children’s academic progress. Parents can also affect students’ interest in STEM careers, because they can look more closely at the lives of their parents who are involved in STEM careers and gather information from them. The perceived influence of school facilities and equipment to career preference is shown in Table 4.

As presented in the table, the obtained average mean is 3.35 with SD=0.44. This means that respondents perceived school environment as influential to career preference. Respondents mostly agree that using new facilities and equipment excites them. This is due to the highest attained mean value of 3.49 with SD=0.57. The quality of facilities in Senior High School were enjoyed by the respondents given the computed mean value of 3.09 and SD=0.78. This is the item that has the lowest mean value in school environment factor. School environment and curriculum that support active involvement in scientific activities or STEM engender aspirations and interest in related STEM careers could influence preference of learners (Sahin, 2015). Students pursuit their STEM career in college if students engaged in STEM activities in terms of knowledge, attitude and interest in STEM; and the desire to engage in STEM careers by the use of appropriate facilities and equipment to carried out science field work, science camps, learning in science centers, museums, zoos, and robotics competitions (Gwen et al., 2016). Perceived influence of social orientation to career preference in presented in table 5.

The table reflects an obtained average mean of 2.63 with SD=0.81. This means that generally, respondents perceived social orientation as a factor in choosing a career in STEM. The respondents agree that majority of the items in this factor are contributory. Most of the respondents agree that the support of their relatives contributes to their choice of career in STEM. This is supplemented with the highest obtained mean value of 2.85 with SD=0.89. Nonetheless, the respondents do not agree that friends in Senior High School enrolled in career programs affect their choice of getting into the same career. This is supported by a computed mean value of 2.49 with SD=1.06. Moreover, previous studies stated that support from friends also influence the ways of students thinking and it is the key in developing strong expectations in STEM careers (Cridge&Cridge, 2015). Peers who share an interest in STEM will help each other develop their vision as a scientist in the future. The attitude of friends, their achievements and norms have a strong influence on motivation and choice of programs (Nugent et al., 2015). This finding reinforces that friends can affect students in the selection of STEM careers (Halim, 2018). The perceived influence of self – efficacy to career preference is shown in table 6. It can be gleaned in the table that the respondents agree that self – efficacy influences career preference due to the average mean value of 3.30 with SD=0.40. In fact, they agree on all six items are influential given their obtained mean values. Among all the items, they agree that their belief of excelling in STEM – related programs is most influential. This is supported by the highest mean value of 3.43 with SD=0.60. The respondents agree that they can usually find a solution when confronted by STEM problems. This is due to the lowest computed mean value of 3.19 with SD=0.56.

Results of the present study is aligned with recent findings by Nugent et al., (2015) stated that students are more likely to pursue careers in which they are confident of their capabilities and less likely to be drawn to careers where they doubt their skills and performance. Self-efficacy has been shown to be a predictor of pursuing a college major in STEM. Furthermore, Compeau et al. (2016) show that self-efficacy along with knowledge of STEM careers are significant factors in whether or not adolescents pursue STEM careers.





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### On Career Preference

The career preference of the respondents is reflected in table 7. The table shows that 113 or 82.5% of the respondents are enrolled in STEM programs. On the other hand, the table presents also that only 24 or 17.5% of the 137 respondents are enrolled in non – STEM programs. Based on the study of Mangu et al. (2015), students who enrolled in STEM in high school were interested in pursuing a STEM career. Nearly 70% of students surveyed revealed that they were either somewhat likely or very likely to pursue a STEM career and 30% were less likely to do so. Majority of STEM graduates pursue STEM – related career programs.

### Significant Predictors of STEM Career Preference

The significant predictors among sex, socio-economic status, mother and father's occupation, parent's highest educational attainment, monthly income, personal interest, family influence, school equipment and facilities, social orientation, and self – efficacy in relation to STEM career preference is presented in Table 8. It can be gleaned in the table that mother's occupation is a significant predictor of STEM career preference. This is supported by  $B=-1.58$  with  $p=0.02$  which is less than 0.05 level of significance. The coefficient value of mother's occupation is negative ( $B=-1.58$ ). This suggests a negative correlation between mother's occupation and STEM career preference. This further means that respondents whose mothers have non-stem occupations are most likely to enrol in STEM. This result is true in the study of Peters, Abukmail and Willis (2019) which found a significant relationship between mother's work and students' choice of employment. Moreover, in the same study above, revealed that parent whether worked in a STEM career field and student STEM career preference after high school were found to be not significant. Based on the results, family influence (FI) significantly predicts STEM career preference. This is due to  $B=-2.10$  with  $p=0.01$  which is less than 0.05 level of significance. Like mother's occupation, the coefficient of FI is also negative ( $B=-2.10$ ). This proposes a negative correlation between FI and STEM career preference. This also means that SHS STEM students with low family influence would most likely enroll in STEM programs.

This result contradicts the studies of Mtemeri (2017) and Kim, Ahn and Fouad (2015) which found that family influence is a vital factor in career intentions and growth. However, the result of this study may hold true because students may perceive their high family influence as a dictation rather than a motivation thereby pursuing a different career. Conversely, students with low family influence have their freewill intact so they have the freedom in pursuing a career of their choice – STEM career in the case of this study. Another significant predictor of STEM career preference is self – efficacy in STEM (SE) given with  $B=2.60$  with  $p=0.02$  which is also less than 0.05 level of significance. Unlike MO and FI, SE has a positive value of coefficient ( $B=2.60$ ). This means that there is a positive correlation between SE and STEM career preference. Those with high self-efficacy would most likely enrol in STEM programs. Indeed, self-efficacy in STEM is a vital predictor of STEM career (Nugent, et. al., 2015; Halim, 2018 & Mau and Li, 2018). Lastly, sex, father's occupation, parents' highest educational attainment, monthly income, personal interest, school equipment and facilities, and social orientation are not significant in predicting STEM career preference. This is supported by their respective  $p$  – values which are all greater than 0.05 level of significance.

### Predictive Model

Based on the significant predictors in Table 9, the predictive model is given by:

$$\text{Logit (Enrolling in STEM Program)} = -1.58(MO) - 2.10(FI) + 2.60(SE)(1)$$

Where,

MO – Mother's Occupation (0 or 1)

FI – Average Perceived Family Influence

SE – Average Perceived Self-Efficacy

Table 9 shows that the predictive model (1) significantly predict enrolment to STEM programs. This is based on the obtained Chi-square value of 34.514 which has a  $p$ -value of 0.002 at 14 degrees of freedom. Since the  $p$ -values is less





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than 0.05, the null hypothesis is rejected. This implies that if the model is used, the likelihood that a senior high school student will enroll in STEM program in college can be predicted through probabilities. There are eight sample sets in the table that represent the probability of enrolling in STEM program using the predictive model. The significant predictors as to Mother's occupation with coefficient (B) of -1.58, average perceived family influence with coefficient of -2.10, and average perceived self-efficacy with coefficient of 2.60 are factored into the computations. The Xi represents the value of the significant predictors which represent sample values or different combinations of possible outcomes. Probability of enrolling STEM Program will depend on the coefficient (B) as well as the combination of possible outcomes (Xi).

Set 1 shows 0.9997 probability. This represents that there is a very high probability of the students to enroll in STEM Programs if the possible outcomes (Xi) were: mother occupation is Non-STEM/ Jobless/ Deceased (0), very low (1) average perceived family influence, and very high average perceived self-efficacy (4). Set 2 shows 0.9988 probability. This indicates that there is also a very high probability of the students to enroll in STEM Programs if the possible outcomes (Xi) were: mother's occupation is STEM related (1), very low (1) average perceived family influence, and very high (4) average perceived self-efficacy. Set 3 also represents very high probability of the students to enroll in STEM Programs which is 0.8807 since the possible outcomes (Xi) were: mother's occupation is Non-STEM/ Jobless/ Deceased (0), very high (4) average perceived family influence, and very high (4) average perceived self-efficacy.

Set 4 shows 0.6039 probability. This represents that there is a high probability of the students to enroll in STEM Programs if the possible outcomes (Xi) were: mother's occupation is STEM related (1), very high (4) average perceived family influence, and very high (4) average perceived self-efficacy. Set 5 shows 0.6224 probability. This indicates that there is a high probability of the students to enroll in STEM Programs if the possible outcomes (Xi) were: mother's occupation is Non-STEM/ Jobless/ Deceased (0), very low (1) average perceived family influence, and very low (1) average perceived self-efficacy. Set 6 shows 0.2540 probability. This indicates that there is low probability of the students to enroll in STEM Programs if the possible outcomes (Xi) were: mother occupation's is STEM related (1), very low (1) average perceived family influence, and very low (1) average perceived self-efficacy. Set 7 shows 0.0030 probability. This indicates that there is very low probability of the students to enroll in STEM Programs if the possible outcomes (Xi) were: mother's occupation is Non-STEM/ Jobless/ Deceased (0), very high (4) average perceived family influence, and very low (1) average perceived self-efficacy. Set 8 shows 0.0006 probability. This indicates that there is very low probability of the students to enroll in STEM Programs if the possible outcomes (Xi) were: mother's occupation is STEM related (1), very high (4) average perceived family influence, and very low (1) average perceived self-efficacy. Thus, the combination of the possible outcomes (Xi) in set 1, 2, and 3 shows very high probability of the respondents to enroll in STEM programs since the probability results were close to 1.00 while the possible outcomes (Xi) in set 7 and 8 had very low probability for the respondents to enroll in STEM program since the results were near zero.

Although 88% of parents believe they can help guide their children's learning, less than 28% discuss the value of a STEM education with their children ("Let's Talk Science Canada Annual Report," 2015). Nonetheless, Peters et.al. (2019) stated that influence of parents was dominant as compared to other family members. This result supports the finding of Tzu Ling (2019) that there was a significant relationship between the mother's employment and future career of students.

## FINDINGS

Based on the results of the study, it was found that:

1. Most of the respondents are males whose parents were college graduates and have Non-STEM occupations. Their average family monthly income is Php 31,051.84.
2. Career preference is perceived to be influenced by personal interest, family influence, school facilities and equipment, social orientation and self – efficacy in STEM.





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3. Most of the respondents were enrolled in STEM related programs.
4. Mother's occupation, family influence, and self – efficacy in STEM are significant predictors of STEM career preference.
5. Based on the identified predictors, the predictive model for STEM career preference is:  

$$\text{Logit (Enrolling in STEM Course)} = -1.58(MO) - 2.10(FI) + 2.60(SE)$$

## CONCLUSION

The study concluded that male students are more inclined and preferred to pursue STEM – related programs in college as long as their parents support them financially regardless of their occupation. Career choice is a multifaceted decision between one's ideals and aspirations, skill inclination, opinions of family member, peer decision, and appeal of the school in terms of availability and quality of facilities and equipment. Further, a SHS STEM graduate would most likely enroll in STEM-related programs in college if he/she is inclined to STEM, his/her mother has non-STEM work, and has a supportive family.

## REFERENCES

1. Akosah-Twumasi, P., Emeto, T. I., Lindsay, D., Tsey, K., & Malau-Aduli, B. S. (2018). *A Systematic Review of Factors That Influence Youths Career Choices — the Role of Culture*. *Frontiers in Education*, 3. doi:10.3389/educ.2018.00058;
2. Albert, Ph.D., J. R. G., Santos, A. G. F., & Vizmanos, J. F. V. (2018). Unmasking the Middle – class: Profile and Determinants. Accessed from <https://psa.gov.ph/sites/default/files/1.1.3%20Unmasking%20the%20Middle%20Class%20in%20the%20Philippines%20Aspirations%2C%20Lifestyles%2C%20and%20Prospects%20for%20Sustainable%20Consumption.pdf> on March 20, 2020;
3. Alhaddab, Taghreed Ahmed (2015). "Matched, Somewhat-Matched or Mismatched? Predictors of Degree-Job Match among STEM Graduates". Seton Hall University Dissertations and Theses (ETDs). 2117. Accessed from <https://scholarship.shu.edu/dissertations/2117> on November 20, 2019;
4. Blotnick, K. A., Franz-Odenaal, T., French, F., & Joy, P. (2018). A study of the correlation between STEM career knowledge, mathematics self-efficacy, career interests, and career activities on the likelihood of pursuing a STEM career among middle school students. *International Journal of STEM Education*, 5(1). doi:10.1186/s40594-018-0118-3;
5. BukasPaladFoundation of the Philippines. Retrieved from <https://bukaspaladfoundation.org/the-story-of-bukas-palad/> on November 10, 2019;
6. Cecilia, Ernie (2019). Addressing The Nagging Jobs - Skills Mismatch. The Manila Times. Retrieved from <https://www.manilatimes.net/2019/08/08/campus-press/addressing-the-nagging-jobs-skills-mismatch/596570/> on November 20, 2019;
7. Compeau, S. (2016). The calling of an engineer: High school students' perceptions of engineering. Retrieved from <http://qspace.library.queensu.ca/jspui/handle/1974/13924> on July 20, 2020; Cridge, B. J. & Cridge, A. G. (2015). Evaluating how universities engage school students with science: A model based on the analysis of the literature. *Australian University Review*, 57(1), 34-44. Retrieved from <https://files.eric.ed.gov/fulltext/EJ1053519.pdf> on July 20, 2020; Croak, Mallory (2018). "The Effects of STEM Education on Economic Growth" Honors Theses. 1705. Retrieved from <https://digitalworks.union.edu/theses/1705> on November 10, 2019;
8. Deming, D. J., & Noray, K. L. (2018). STEM Careers and Technological Change. NBER Working Paper Series, 25065. Retrieved from <https://www.nber.org/papers/w25065.pdf> on November 17, 2019;
9. Depasupil, William (2017). 1 million graduates face job-skill mismatch. The Manila Times. Retrieved from <https://www.manilatimes.net/2017/03/14/news/top-stories/1-million-graduates-face-job-skill-mismatch/317111/317111/> on November 20, 2019;





**Nestor P. Elano Jr et al.**

10. Employer's Confederation of the Philippines (2018). Retrieved from <http://www.fsi.gov.ph/wp-content/uploads/2018/02/JRAM-Job-Skills-Mismatch-Mabini-Dialogue.pdf> on November 10, 2019;
11. GMA News Online (2020). Retrieved from <https://www.gmanetwork.com/news/money/economy/733653/who-is-middle-class-here-s-the-classification-from-philippine-gov-t-think-tank/story/> on February 28, 2020;
12. Gwen, N. Bradley, B., Neal, G. & Greg, W. (2016). Robotics camps, clubs& competitions: results from the us robotics projects. *Robotics & Autonomous System*, 75, 686-691. Retrieved from <http://stellar.edc.org/sites/stellar.edc.org/files/Nugent%20et%20al.%202014%20-%20TRTWR.pdf> on July 20, 2020;
13. Halim, L., Rahman, N. A., Ramli, N. A. M., & Mohtar, L. E. (2018). *Influence of students' STEM self-efficacy on STEM and physics career choice*. doi:10.1063/1.5019490;
14. Humayon, A. A., Raza, a., Khan, R. A. & Ansari, N. (2018). Effect of Family Influence, Personal Interest and Economic Considerations on Career Choice amongst Undergraduate Students in Higher Educational Institutions of Vehari, Pakistan. *International Journal of Organizational Leadership*, 7(2), 129-142. doi: 10.33844/ijol.2018.60333;
15. Kazi, AsmaShahid&Akhlq ,Abeeda (2017). Factors Affecting Students' Career Choice. *Journal of Research and Reflections in Education*, Vol., No. 2, pp 187-196. Retrieved from [https://www.researchgate.net/profile/Asma\\_Shahid\\_Kazi/publication/325987918\\_Factors\\_Affecting\\_Students'\\_Career\\_Choice/links/5ba0ab3c299bf13e6038e19d/Factors-Affecting-Students-Career-Choice.pdf](https://www.researchgate.net/profile/Asma_Shahid_Kazi/publication/325987918_Factors_Affecting_Students'_Career_Choice/links/5ba0ab3c299bf13e6038e19d/Factors-Affecting-Students-Career-Choice.pdf) on November 27, 2019;
16. Kim, S., Ahn, T., & Fouad, N. (2015). Family Influence on Korean Students' Career Decisions. *Journal of Career Assessment*, 24(3), 513–526. doi:10.1177/1069072715599403;
17. Kızılay, E., Yamak, H. & Kavak, N. (2019). STEM Career Preferences of the Anatolian High School Students from Different Socioeconomic Levels. *Journal of Educational Issues*, 5(2). Retrieved from <http://www.macrothink.org/journal/index.php/jei/article/view/15069> on November 22, 2019;
18. KoyunluÜnlü, Z., Dökme, İ (2018). Multivariate Assessment of Middle School Students' Interest in STEM Career: a Profile from Turkey. *Res SciEduc* 50, 1217–1231 (2020). Retrieved from <https://doi.org/10.1007/s11165-018-9729-4> on November 28, 2019;
19. Lent, R. W., Brown, S. D. and Hackett, G. (1994). "Toward a Unifying Social Cognitive Theory of Career and Academic Interest, Choice, and Performance" [Monograph]. *Journal of Vocational Behavior* 45:79-122. Retrieved from <https://eric.ed.gov/?id=EJ487458> on December 8, 2019;
20. Let's Talk Science Annual Report(2015). Spotlight on Science Learning - Exploring Parental Influence: Shaping Teen Decisions Regarding Science Education. Retrieved from <http://letstalkscience.ca/Portals/0/Documents/RPS/Spotlight/LTS-Exploring-parental-influence-EN.pdf> on July 20, 2020;
21. Mangu, DM, Lee, AR, Middleton, JA, Nelson, JK (2015). Motivational factors predicting STEM and engineering career intentions for high school students. In *Frontiers in education conference (FIE)*, 2015. 32614 2015. IEEE, (pp. 1–8). IEEE Retrieved from [http://ieeexplore.ieee.org/xpls/abs\\_all.jsp?arnumber=73440650](http://ieeexplore.ieee.org/xpls/abs_all.jsp?arnumber=73440650) on July 20, 2020;
22. Mau, W.-C. J., & Li, J. (2018). Factors Influencing STEM Career Aspirations of Underrepresented High School Students. *The Career Development Quarterly*, 66(3), 246–258. doi:10.1002/cdq.12146;
23. Mtemeri, Jeofrey (2017). Factors Influencing The Choice Of Career Pathways Among. Retrieved from [https://s3.amazonaws.com/academia.edu.documents/60193072/pdf\\_research20190802-128266-1y0ellz.pdf?response-content-disposition=inline%3B%20filename%3DPdf\\_research.pdf&X-Amz-Algorithm=AWS4-HMAC-SHA256&X-Amz-Credential=AKIAIWOWYYGZ2Y53UL3A%2F20200227%2Fus-east-1%2Fs3%2Faws4\\_request&X-Amz-Date=20200227T133039Z&X-Amz-Expires=3600&X-Amz-SignedHeaders=host&X-Amz-Signature=90ae1a89eab34238b6b4f3dfee749aabd26239ac4bdaf0a57eeb5f640d37660c](https://s3.amazonaws.com/academia.edu.documents/60193072/pdf_research20190802-128266-1y0ellz.pdf?response-content-disposition=inline%3B%20filename%3DPdf_research.pdf&X-Amz-Algorithm=AWS4-HMAC-SHA256&X-Amz-Credential=AKIAIWOWYYGZ2Y53UL3A%2F20200227%2Fus-east-1%2Fs3%2Faws4_request&X-Amz-Date=20200227T133039Z&X-Amz-Expires=3600&X-Amz-SignedHeaders=host&X-Amz-Signature=90ae1a89eab34238b6b4f3dfee749aabd26239ac4bdaf0a57eeb5f640d37660c) on November 21, 2019;
24. Nugent, G., Barker, B., Welch, G., Grandgenett, N., Wu, C.R. & Nelson, N. (2015). A Model of Factors Contributing to STEM Learning and Career Orientation, *International Journal of Science Education*, DOI: 10.1080/09500693.2015.1017863;





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25. Nyamwange, Josephine (2016). Influence of Student's Interest on Career Choice among First Year University Students in Public and Private Universities in Kisii County, Kenya. Vol. 7. Journal of Education and Practice. Accessed from <https://files.eric.ed.gov/fulltext/EJ1092415.pdf> on November 10, 2019;
26. Pajares, G. G. et al. (2018). The Sectoral and Skills Mismatch between the Senior High School Program and the Top In-Demand Jobs and Projected In - demand Jobs in the Province of Cebu, Philippines. Vol. 9(2). International Refereed Journal. Retrieved from <http://dx.doi.org/10.18843/rwjasc/v9i2/24> on November 20, 2019;
27. Penedilla, J. S. & Rosaldo, L. B. (2017). Factors Affecting Career Preferences Among Senior High School Students in Tacloban City. Retrieved from [https://s3.amazonaws.com/academia.edu.documents/54766619/Asademia\\_Career\\_pref.pdf?response-content-disposition=inline%3B%20filename%3DFACTORS\\_AFFECTING\\_CAREER\\_PREFERENCES\\_AMO.pdf&X-Amz-Algorithm=AWS4-HMAC-SHA256&X-Amz-Credential=AKIAIWOWYYGZ2Y53UL3A%2F20200228%2Fus-east-1%2Fs3%2Faws4\\_request&X-Amz-Date=20200228T132655Z&X-Amz-Expires=3600&X-Amz-SignedHeaders=host&X-Amz-Signature=4d4ec301793d18f0d0933e0460b30d5e45526124cc1ada98439bc0132aa9d21b](https://s3.amazonaws.com/academia.edu.documents/54766619/Asademia_Career_pref.pdf?response-content-disposition=inline%3B%20filename%3DFACTORS_AFFECTING_CAREER_PREFERENCES_AMO.pdf&X-Amz-Algorithm=AWS4-HMAC-SHA256&X-Amz-Credential=AKIAIWOWYYGZ2Y53UL3A%2F20200228%2Fus-east-1%2Fs3%2Faws4_request&X-Amz-Date=20200228T132655Z&X-Amz-Expires=3600&X-Amz-SignedHeaders=host&X-Amz-Signature=4d4ec301793d18f0d0933e0460b30d5e45526124cc1ada98439bc0132aa9d21b) on November 20, 2019;
28. Peters, M., Abukmail, A. & Willis, J. (2019). STEM College-Bound: Relationship of Familial Factors. In K. Graziano (Ed.), *Proceedings of Society for Information Technology & Teacher Education International Conference* (pp. 1318-1323). Las Vegas, NV, United States: Association for the Advancement of Computing in Education (AACE). Retrieved from <https://www.learntechlib.org/primary/p/207815/> on February 27, 2020;
29. Popa, R. A. and Ciascai, L. (2017). Students' Attitude Towards STEM Education. Vol. 10, No. 4. *Acta Didactica Napocensia*. Retrieved from <https://eric.ed.gov/?id=EJ1164986> on February 28, 2020;
30. Powers, Anna (2017). The Secret to Future Economic Growth and Prosperity? More Women in STEM. *Forbes*. Retrieved from <https://www.forbes.com/sites/annapowers/2017/08/29/tapping-into-the-untapped-potential-to-drive-economic-growth/#5c1c6d2163a0> on November 11, 2019;
31. Raabe, I. J., Boda, Z., & Stadtfeld, C. (2019). *The Social Pipeline: How Friend Influence and Peer Exposure Widen the STEM Gender Gap*. *Sociology of Education*, 003804071882409. doi:10.1177/0038040718824095;
32. RA 10533 - "Enhanced Basic Education Act of 2013". Retrieved from <https://www.officialgazette.gov.ph/2013/05/15/republic-act-no-10533/> on November 10, 2019;
33. RA 10687 - "Unified Student Financial Assistance System for Tertiary Education (UniFAST) Act" of 2013. Retrieved from <https://www.officialgazette.gov.ph/2015/10/15/republic-act-no-10687/> on November 10, 2019;
34. RA 10931 - Universal Access to Quality Tertiary Education Act. Retrieved from <https://www.officialgazette.gov.ph/downloads/2017/08aug/20170803-RA-10931-RRD.pdf> on November 10, 2019;
35. Republic Act No. 10533. "Enhanced Basic Education Act of 2013". Retrieved from <https://www.officialgazette.gov.ph/2013/05/15/republic-act-no-10533/> on November 10, 2019;
36. Sahin, A., Gulacar, O., & Stuessy, C. (2015). *High School Students' Perceptions of the Effects of International Science Olympiad on Their STEM Career Aspirations and Twenty-First Century Skill Development*. *Research in Science Education*, 45(6), 785–805. Retrieved from <https://eric.ed.gov/?id=EJ1088142> on July 20, 2020;
37. Syed, M., Zurbruggen, E. L., Chemers, M. M., Goza, B. K., Bearman, S., Crosby, F. J., ... Morgan, E. M. (2018). The Role of Self-Efficacy and Identity in Mediating the Effects of STEM Support Experiences. *Analyses of Social Issues and Public Policy*. doi:10.1111/asap.12170;
38. Tamara A. Franz-Odenaal, Karen Blotnick, Frederick French & Phillip Joy (2016). Experiences and Perceptions of STEM Subjects, Careers, and Engagement in STEM Activities Among Middle School Students in the Maritime Provinces, *Canadian Journal of Science, Mathematics and Technology Education*, 16:2, 153-168, DOI: 10.1080/14926156.2016.1166291;
39. The Manila Times (2019). STEM track prepares HS students for work. Retrieved from <https://www.manilatimes.net/2019/08/29/campus-press/stem-track-prepares-hs-students-for-work/607355/> on November 20, 2019;
40. Tzu-Ling, H. (2019). Gender differences in high-school learning experiences, motivation, self-efficacy, and career aspirations among Taiwanese STEM college students. *International Journal of Science Education*, 41(13), 1870–1884. doi:10.1080/09500693.2019.1645963;





**Nestor P. Elano Jr et al.**

41. U.S. Department of Commerce Economics and Statistics Administration Office of the Chief Economist. STEM Jobs: 2017 Update. Retrieved from <https://files.eric.ed.gov/fulltext/ED594354.pdf> on November 17, 2019;

42. U. S. Joint Economic Committee Democrats, (n.d.). Retrieved from [https://www.jec.senate.gov/public/\\_cache/files/6aaa7e1f-9586-47be-82e7-326f47658320/stem-education---preparing-for-the-jobs-of-the-future-.pdf](https://www.jec.senate.gov/public/_cache/files/6aaa7e1f-9586-47be-82e7-326f47658320/stem-education---preparing-for-the-jobs-of-the-future-.pdf) on November 18, 2019;

43. Wiebe, E., Unfried, A., & Faber, M. (2018). The Relationship of STEM Attitudes and Career Interest. EURASIA Journal of Mathematics, Science and Technology Education, 14(10), em1580. Retrieved from <https://doi.org/10.29333/ejmste/92286> on November 11, 2019;

44. Xue, Y & Larson, R. C. (2015). STEM crisis or STEM Surplus? Yes and yes, Monthly Labor Review, U. S. Bureau of Labor Statistics. Retrieved from <https://doi.org/10.21916/mlr.2015.14> on November 10, 2019.

**Table 1. Socio-Economic Profile of Respondents**

	Profile	f(n=137)	Percent
Sex	Male	76	55.47
	Female	61	44.53
Mother's Occupation	STEM	29	21.17
	Non-STEM/Jobless	108	78.83
Father's Occupation	STEM	14	10.22
	Non-STEM/Jobless	123	89.78
Parents Highest Educational Attainment	Elementary Level	3	2.19
	High School Level	4	2.92
	High School Graduate	6	4.38
	College Level	33	24.09
	College Graduate	73	53.28
Monthly Income	Graduate Level or Higher	18	13.14
	Mean		31051.84
	SD		39532.41

**Table 2. Perceived Influence of Personal Interest to Career Preference**

Statement	Mean	SD	Description
<i>I chose my career program today because it is...</i>			
1. based on the school where I considered since Senior High School.	3.10	0.78	Agree
2. my personal choice in Senior High School.	3.34	0.78	Agree
3. connected to my favorite subject in Senior High School.	3.08	0.81	Agree
4. my "childhood dream"	2.97	0.95	Agree
5. an in – demand career.	3.28	0.71	Agree
6. suited to my talent.	3.20	0.67	Agree
<b>Average</b>	<b>3.16</b>	<b>0.49</b>	<b>Agree</b>





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**Table 3. Perceived Family Influence to Career Preference**

Statement	Mean	SD	Description
<i>I chose my career program today based on...</i>			
1. the recommendation of my family.	2.90	0.89	Agree
2. idea that it will not compromise the reputation of the family.	2.86	0.86	Agree
3. the financial support from my family.	3.04	0.80	Agree
4. the wish of my family.	2.79	0.87	Agree
5. the dominant profession in my family.	2.49	1.04	Disagree
6. values and belief of my family.	2.59	0.96	Agree
<b>Average</b>	<b>2.78</b>	<b>0.73</b>	<b>Agree</b>

**Table 4. Perceived Influence of School Facilities and Equipment to Career Preference**

Statement	Mean	SD	Description
<i>I chose my career program today because...</i>			
1. I always wanted to be exposed an environment that has good facilities and equipment.	3.42	0.62	Agree
2. I enjoyed the quality of facilities in Senior High School.	3.09	0.78	Agree
3. I want to experience using quality facilities and equipment.	3.42	0.62	Agree
4. I gained new perspectives from SHS on the relevance of facilities and equipment in quest for knowledge.	3.25	0.76	Agree
5. I want to reinforce my skills in using good equipment and facilities.	3.43	0.58	Agree
6. Using new facilities and equipment excites me.	3.49	0.57	Agree
<b>Average</b>	<b>3.35</b>	<b>0.44</b>	<b>Agree</b>

**Table 5. Perceived Influence of Social Orientation to Career Preference**

Statement	Mean	SD	Description
<i>I chose my career program today because...</i>			
1. my friends in Senior High School were enrolled also in the same program.	2.49	1.06	Disagree
2. of the influence of my teacher in Senior High School.	2.52	0.94	Agree
3. of the influence of my relatives.	2.64	1.00	Agree
4. of the influence in my community.	2.66	0.93	Agree
5. of the support of my relatives.	2.85	0.89	Agree
6. of the recommendation of my friends.	2.64	1.00	Agree
<b>Average</b>	<b>2.63</b>	<b>0.81</b>	<b>Agree</b>





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**Table 6. Perceived Influence of Self-Efficacy to Career Preference**

Statement	Mean	SD	Description
<i>I chose my career program today because...</i>			
1. I believe that I can excel in STEM related program.	3.43	0.60	Agree
2. I can manage to solve difficult problems in STEM if I try hard enough.	3.38	0.53	Agree
3. It is easy for me to stick to my aims and goals through STEM programs.	3.34	0.64	Agree
4. I am confident that I can deal with unexpected requirements in STEM.	3.24	0.55	Agree
5. I rely on my coping mechanism to remain calm when facing difficulties in STEM.	3.23	0.53	Agree
6. I can usually find a solution when facing problems in STEM.	3.19	0.56	Agree
<b>Average</b>	<b>3.30</b>	<b>0.40</b>	<b>Agree</b>

**Table 7. Career Preference of the Respondents**

Program	Frequency	Percent
STEM	113	82.5
NON-STEM	24	17.5
<b>Total</b>	<b>137</b>	<b>100.0</b>

**Table 8. Significant Predictors of STEM Career Preference**

Independent Variable	B	df	p	Interpretation
Male (1)	-.66	1	0.25	Not Significant
Mother's Occupation STEM (1)	-1.58	1	0.02	Significant
Father's Occupation STEM (1)	.78	1	0.44	Not Significant
High School Level (1)	-19.40	1	1.00	Not Significant
HEA High School Graduate (2)	-1.36	1	1.00	Not Significant
HEA College Level (3)	-20.64	1	1.00	Not Significant
HEA College Graduate (4)	-18.73	1	1.00	Not Significant
HEA Graduate Level or Higher (5)	-19.26	1	1.00	Not Significant
Monthly Income	.00	1	0.80	Not Significant
Personal Interest	1.19	1	0.12	Not Significant
Family Influence	-2.10	1	0.01	Significant
School Equipment and Facilities	.16	1	0.86	Not Significant
Social Orientation	.50	1	0.40	Not Significant
Self-Efficacy in STEM	2.60	1	0.02	Significant
Constant	13.76	1	1.00	Not Significant

**Table 9. Significance of Predictive Model**

Chi-square	df	p	Decision on Ho	Interpretation
34.514	14	.002	Rejected	Significant

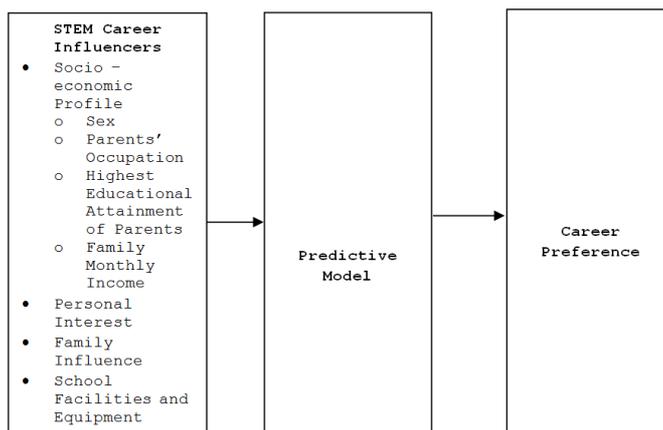




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**Table 10. Sample Computations of Probabilities**

Predictor	B	Xi	Bxi	$\Sigma Bxi$	Odds	Probability
MO	-1.58	0	0.00	8.29	3984.50	0.9997
FI	-2.10	1	-2.10			
SE	2.60	4	10.39			
MO	-1.58	1	-1.58	6.71	823.21	0.9988
FI	-2.10	1	-2.10			
SE	2.60	4	10.39			
MO	-1.58	0	0.00	2.00	7.38	0.8807
FI	-2.10	4	-8.39			
SE	2.60	4	10.39			
MO	-1.58	1	-1.58	0.42	1.52	0.6039
FI	-2.10	4	-8.39			
SE	2.60	4	10.39			
MO	-1.58	0	0.00	0.50	1.65	0.6224
FI	-2.10	1	-2.10			
SE	2.60	1	2.60			
MO	-1.58	1	-1.58	-1.08	0.34	0.2540
FI	-2.10	1	-2.10			
SE	2.60	1	2.60			
MO	-1.58	0	0.00	-5.79	0.003	0.0030
FI	-2.10	4	-8.39			
SE	2.60	1	2.60			
MO	-1.58	1	-1.58	-7.37	0.001	0.0006
FI	-2.10	4	-8.39			
SE	2.60	1	2.60			



**Figure 1. Schematic Diagram**





## Comparative Assessment of Proximate Composition, Phytochemical and Antioxidant Properties of Culture Important Marine Microalgae

N. Krishnaveni, P. Santhanam\*, S. Dinesh Kumar, A. Gowthami, K. Nanthini Devi and P. Perumal

Marine Planktonology & Aquaculture Lab., Department of Marine Science, School of Marine Sciences, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India.

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### \*Address for Correspondence

**P. Santhanam**

Marine Planktonology & Aquaculture Lab.,  
Department of Marine Science, School of Marine Sciences,  
Bharathidasan University,  
Tiruchirappalli, Tamil Nadu, India.  
E-mail: santhanamcopepod@gmail.com



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### ABSTRACT

Microalgae are considered as one of the most promising raw materials for the development of high value products for aquaculture, pharmaceuticals, nutraceuticals, and cosmetic industries, as well as being potential sources of protein, vitamins, and minerals for human consumption. Hence, the present study focus to assess and compare the proximate compositions, phytochemical and antioxidant properties of three different microalgae from three families such as Chlorophyceae (*Isochrysis galbana*), Cyanophyceae (*Synechocystis* sp.) and Bacillariophyceae (*Nitzschia microcephala*). The extraction were carried out using different solvents such as methanol, ethyl acetate and hexane. In vitro free radical quenching activity of extracts were investigated with 1,1-diphenyl-2-picryl hydrazyl, hydroxyl radicals and hydrogen peroxide activity and compared with ascorbic acid as positive controls. The highest protein (37.32%), and lipid (22.18%) contents were noticed high in *I. galbana* and carbohydrate (15.1%) were high in *N. microcephala*. The scavenging activity against DPPH, hydroxyl and hydrogen peroxide were high in *I. galbana* followed by *Synechocystis* sp. and *N. microcephala*. The present findings suggest that these microalgae are a natural source which can be used for healing purpose of various disease in human and animals and could be potential raw material for the animal and human food.

**Keywords:** *Isochrysis galbana*; *Synechocystis* sp.; *Nitzschia microcephala*; phytochemicals; antioxidant.



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## INTRODUCTION

The sea is habitat to half of the global biodiversity and is the largest remaining reservoir of bioactive compounds [1]. Microalgae are microscopic organisms, typically found in freshwater, estuarine, and marine environment. Marine microalgae can be categorized into five different family such as Chlorophyceae (Green algae), Dinophyceae (Dinoflagellates), Bacillariophyceae (Diatoms), Cyanophyceae (Blue-green algae) and Prymnesiophyceae (Coccolithophores) [2, 3]. The first reports of culturing microalgae for use as feed in aquaculture were published a 100 years ago [4] and, since then, the use of microalgae for this purpose has developed rapidly. Today, microalgae are widely used as one of the most important feed sources for different groups of commercially important aquatic organisms in both freshwater and marine aquaculture [5]. Algae are used in various food, cosmetic, aquaculture, nutraceutical, medical, pharmaceuticals and agricultural industries. Algae are presently produced and sold as health food all over the world. Algal biomass is commonly used for aquaculture feed, as well as for other animal feed. Extracts from algae can be used for production of cosmetics and many different pharmaceutical products [6-8]. Microalgae have great potential to provide protein, lipids, vitamins, carotenoids, and energy in feed.

Generally, microalgae contain 30–40% protein, 10–20% lipid, and 5–15% carbohydrate in the late logarithmic growth phase [9]. Microalgae are an attractive and viable alternative source of high-value chemical compounds such as fatty acids, pigments, phytochemicals, fine chemicals and antioxidants [10]. These compounds could be used for protecting humans from many diseases. Many biologically and pharmacologically active substances have been isolated from algae which are difficult to be synthesized chemically [11]. Fatty acids derived from microalgae have long been commercialized for animal feed, pharmaceuticals, and food supplement products. The long-chain polyunsaturated fatty acids (LC-PUFAs) especially omega-3 and omega-6 fatty acids such as arachidonic acid (AA, C20:4), eicosapentaenoic acid (EPA, C20:5), and docosahexaenoic acid (DHA, C22:6) have received much attention because of their role in human health and nutrition [12-14]. Many algae have the ability to survive harsh environmental conditions due to different adaptation strategies [15-16]. Generally microalgae contain high amount of secondary metabolites. When algae apply stress reactions for handling more difficult environmental conditions, algal species use very different methods for managing the change in the environment. Depending on their ability to handle the various types of stress, the algae will produce different secondary metabolites in order to increase their chance of survival. Metabolites are organic compounds involved in the metabolism of living cells. Some metabolites are not part of the primary metabolic processes of growth, reproduction or general maintenance of the cell systems, but are produced for use in secondary cell functions. These are called secondary metabolites, and are often produced as a reaction to environmental stress [17].

Photodamage of the photosynthetic systems is a continuous process that occurs during light conditions in all photosynthetic eukaryotes. When the solar energy absorbed by the antenna pigments exceeds the capacity of the photosynthetic system to process the energy, this will lead to generation of long lived excited triplet state chlorophyll which can interact with oxygen to cause the formation of reactive oxygen species (ROS). ROS are toxic products of the metabolism, ROS can damage DNA, proteins and lipids in all living organisms; oxidative stress leads to severe health problems in humans and animals. Oxidative damage is linked to aging, atherogenesis, cancer, neurodegenerative diseases, infant retinopathy, macular degeneration and renal failure, along with other problems [18, 19]. The secondary defense system targets to quench the ROS before they cause damage to the photosystems [20]. Microalgae are often exposed to high oxygen levels and irradiance stress and, as a result, these organisms have developed defense systems in the form of antioxidants to prevent damage to the cells. Antioxidants are produced by algae in large amounts under certain stressful conditions in order to protect the photosynthetic cells from oxidative stress [19]. The aim of the present study is to evaluate the biochemical, phytochemicals and antioxidant properties of the methanol, ethyl acetate and hexane extracts of the three important microalgae such as *I. galbana*, *Synocystis* sp. and *N.microcephala*.



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## MATERIALS AND METHODS

### Culture Condition and Utilization of Biomass

Microalgal strains such as *I. galbana*, *Synechocystis* sp. and *N. microcephala* taken from microalgae culture facilities of Marine Planktonology and Aquaculture Laboratory (MPAL), Department of Marine Science, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India. The stock culture was maintained using Conway's medium [21], BG11 medium [22] and TMRL medium [23] and the method of cultivation was followed according to standard method [23]. During the growth, the algal cells passed through different phases (e.g., lag, exponential, stationary, death). At the stage of stationary phase microalgae culture were harvested by centrifugation at 3000rpm for 10min and washed using distilled water. The biomass were dried at 60°C and stored in vacuum desiccator for further analysis.

### Preparation of Extract

The extracts were bought from exclusive solvents such as methanol, ethyl acetate, and hexane. 1mg/ml of the microalgae powder was taken for secondary metabolites extraction and soaked in different solvents and stored in room temperature for 24 hours. After the extraction, the solvent filtered through Whatman No.1 (0.45 µm) filter paper. The crude extracts were stored at 4°C for further analyses.

### Evaluation of Phytochemicals and Proximate Composition

The phytochemical compounds viz., tannins, flavonoids, phenol, terpenoids, steroids, saponins, and alkaloids were estimated according to Sanjeet *et al.* [24]. The protein content was estimated according to the method of Lowry *et al.* [25]. Carbohydrate was determined according to the method of Dubois *et al.* [26]. Lipid was estimated by using the chloroform-methanol method as described by Folch *et al.* [27].

### Evaluation of Antioxidant Activities

The free-radical scavenging activity of the microalgal extracts was evaluated using standard procedures and Ascorbic Acid was used as the reference compound. All analysis were run in triplicates and the average was taken. The potential of marine microalgal extract to scavenge the stable radical DPPH (1, 1-diphenyl-2-picrylhydrazyl) was measured by the method described by Brand-Williams *et al.* [28] with slight modifications. The hydroxyl radical scavenging activity of different crude extract was determined by the method of Halliwell *et al.* [29]. The hydrogen peroxide scavenging sample was determined by adopting the method of Ruch *et al.* [30]. The percentage scavenging effect was calculated using the following formula:

Scavenging effect (%) =  $(A_{cont} - A_{test}) / A_{cont} \times 100$

## RESULTS AND DISCUSSION

The microalgae *I. galbana*, *Synechocystis* sp. and *N. microcephala* were resulted carbohydrate content of 10.29%, 13.21% and 15.1% respectively. Protein was noticed higher (37.32%) in *I. galbana* followed by *Synechocystis* sp. (29.48%) and *N. microcephala* (26.85%). The recorded lipid content in *I. galbana*, *Synechocystis* sp. and *N. microcephala* were 22.18%, 6.24% and 8.2% respectively. Among the three microalgae strains tested, the highest protein and lipid was noticed high in *I. galbana* when compared to *Synechocystis* sp. and *N. microcephala*. The carbohydrate content was found to be higher in *Synechocystis* sp. than the *N. microcephala* and *I. galbana* (Fig. 1). In current scenario, microalgae consider as major live feed in aquaculture and food supplement because they have higher nutritional value and there is an ability to synthesize poly unsaturated fatty acids which can help the fish and shrimp larvae growth and survival [31]. Among the many parameters, growth rate and biochemical composition of microalgae are consider to be a major factor in nutritional value of the certain species which is used for aquaculture purpose [32]. Vasileva *et al.* [33] opined that the, nitrogen is one of the important components for microalgae growth and is related to protein biosynthesis. This trend suggests that the addition of nutrient solutions (without nitrogen) of the Conway's culture



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medium created an imbalance situation that affected the production of protein in microalgae. The high availability of phosphorus, trace metals and other components might have stimulated the synthesis of non-nitrogenaceous substances, such as ATP and carotenoids. The low availability of nitrogen in the stationary growth phase of N-experiments lead to a low protein content, which is in accordance with earlier study of Setta *et al.*[34]. Furthermore, biochemical composition of microalgae may vary among species depending on culture conditions, environmental factors or different harvesting phases [35, 36].

The results of phytochemicals screening have found secondary metabolite. Preliminary qualitative phytochemical analysis of three marine algal strains were extracted with three different solvents. The phytochemicals present in the algal strains were identified as flavonoids, alkaloids, steroids, saponins and phenol. Methanol extracts showed the presence of a larger group of molecules followed by ethyl acetate and hexane extracts (Table 1). Similar qualitative phytochemical results were obtained by some earlier authors [37-41]. They are reported that the microalgae have many biological and therapeutic properties [42, 43]. Hence, the microalgae is expected to participate in many medicinal applications. From present findings it can declare that the polarity level are playing major role in extracting the secondary metabolites product [44,45].

Most commonly known phytochemicals with antioxidant property are phenolics, flavonoids and tannins which counteract the body's reactions to allergens, viruses and carcinogens. They show many useful therapeutic roles such as antiallergic, anti-inflammatory, antimicrobial and anticancer activity [46]. The present study focused the screening on antioxidant activity of different solvent extracts (methanol, ethyl acetate and chloroform) of three different microalgal strains including *I. galbana*, *Synechocystis* sp and *N. microcephala*. DPPH is a stable free radical at room temperature, which accepts an electron or hydrogen radical to become a stable, diamagnetic molecule [47]. In the DPPH assay, antioxidants reduce the DPPH radical to the non-radical form, DPPH-H; hence, absorption is reduced and the color of the DPPH solution changes from purple to yellow. The degree of reduction in absorbance at 517 nm is a measure of the radical-scavenging potential of a compound [48,49]. The maximum DPPH radicals scavenging activity was observed in ethyl acetate extract of *I. galbana* when compared to *Synechocystis* sp. and *N. microcephala*. The scavenging potential was increased with increasing concentrations of the *I. galbana* (2m-10mg), being comparable to that of an ascorbic acid (vitamin C) standard. The *I. galbana* at a concentration of 10mg brought about the highest percentage of inhibition (69%) while ascorbic acid (10mg) brought about 79% inhibition (Fig. 2).

The hydroxyl radical, an extremely reactive free radical formed in biological systems, permits uninhibited accumulation of H<sub>2</sub>O<sub>2</sub> in living systems, leading to the development of oxygen free radicals, such as peroxide and hydroxyl radicals, which cause marked damage to cell membranes [50]. In the current study, focused on the scavenging effect of an H<sub>2</sub>O<sub>2</sub> using a potential microalgal extract to inhibit OH<sup>-</sup>-mediated deoxyribose damage was assessed by means of an iron (II)-dependent DNA damage assay in the current study. The methanolic extract of *I. Galbana* was found to be exhibit maximum OH<sup>-</sup> radical-scavenging activity at a concentration of 10 mg. The capacity to scavenge OH<sup>-</sup> radicals increased with increasing concentrations of the extract and of ascorbic acid (Fig. 3).

The highly reactive hydroxyl radical ( $\bullet$ OH) generated via a biologic Fenton reaction (hydrogen peroxide with Fe<sup>2+</sup> and Cu<sup>2+</sup> causes cytotoxic effect through the alteration of [Ca<sup>2+</sup>] homeostasis [51]. The results suggested that the searching of hydrogen peroxide scavenging activity. The reducing ability was found to be highest for *methanolic* extract of *I. galbana* followed by *Synechocystis* sp *I.* and *N. microcephala* respectively (Fig. 4). The present findings clearly reveal that the marine microalgae *I. galbana* has promising antioxidant activity as the free radical scavenging activity assay has shown values less deviant to the standard values.





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## CONCLUSION

An *in-vitro* assays done using the different solvents of *I. galbana*, *Synechocystis* sp. and *N. microcephala* indicated that the microalgal extract are the efficient source of natural antioxidant which could be a potential one in reducing the oxidative stress naturally. By comparing the results, the solvent extracts of *I. galbana* contains high amount of bioactive compounds, antioxidant activity and free radical scavenging activity with high reducing power capacity. Scavenging ability of the ethyl acetate solvent extract of *I. galbana* were mostly observed in DPPH radical. They also rich source of nutrition which can provide a healthy life for both the environment and living organism which could be considered for future applications in medicine, dietary supplements, cosmetics or food industries.

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## REFERENCES

1. Aneiros, A., Garateix, A., 2004. Bioactive peptides from marine sources: pharmacological properties and isolation procedures. *J. Chromatogr.*, 803, 41–53.
2. Mobin, Saleh, MA., and Firoz Alam., 2017. Some promising microalgal species for commercial applications: A review. *Energy Procedia.*, 110: 510-517.
3. Christiaan van den Hoek, D. M., Jahns, H. M., 1995. *Algae: an Introduction to Phycology*; Cambridge University Press: Cambridge, UK., 640.
4. Allen EJ, Nelson EW., 1910. On the artificial culture of marine plankton organisms. *J Mar Biol Assoc UK* 8: 421–474.
5. Duerr EO, Molnar A, Sato V., 1998. Cultured microalgae as aquaculture feeds. *J Mar Biotechnol* 6:65–70.
6. Apt, KE., Behrens, PW., 1999. Commercial developments in microalgal biotechnology. *J Phycol.*, 35:215–226.
7. Luiten EE, Akkerman I, Koulman A, Kamermans P, Reith H, Barbosa MJ, Sipkema D, Wijffels RH, 2003. Realizing the promises of marine biotechnology. *Biomol Eng.*, 20: 429–439.
8. Yamaguchi K, 1997. Recent advances in microalgal bioscience in Japan, with special reference to utilization of biomass and metabolites: A review. *J Appl Phycol* 8:487–502.
9. Brown M, Jeffrey S, Volkman J, Dunstan G, 1997. Nutritional properties of microalgae for mariculture. *Aquaculture*, 151:315–331.
10. Gonzalez, del Val A., Platas, G., Basilio, A., Cabello, A., Gorrochategui, J., Suay, I., 2001. Screening of antimicrobial activities in red, green and brown macroalgae from Gran Canaria (Canary Islands, Spain). *Int Microbiol.*, 4: 35-40.
11. Kaushik. P., Chauhan, A., 2008. *In vitro* antibacterial activity of laboratory grown culture of *Spirulina platensis*. *Indian J Microbiol.*, 48:348-352.
12. Borowitzka, MA., 2013. High-value products from microalgae their development and commercialisation. *J Appl Phycol.*, 25:743–756.
13. Spolaore, P., Joannis-Cassan, C., Duran, E., Isambert, A., 2006. Commercial applications of microalgae. *J Biosci Bioeng.*, 101:87–96.





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14. Qi, B., Fraser, T., Mugford, S., Dobson, G., Sayanova, O., Butler, J., Napier, J.A., Stobart, A.K., Lazarus, C.M., 2004. Production of very long chain polyunsaturated omega-3 and omega-6 fatty acids in plants. *Nat Biotechnol.*, 22:739–745.
15. Barsanti L, Coltelli P, Evangelista V, Frassanito AM, Passarelli V, Vesentini N, Gualtieri P., 2008. Oddities and curiosities in the algal world, In: Evangelista V, Barsanti L, Frassanito AM, Passarelli V, Gualtieri P (Eds.), *Algal toxins: nature, occurrence, effect and detection*. Springer, Dordrecht. pp. 353–391.
16. Seckbach J., 2007. Algae and cyanobacteria in extreme environments. Springer, Dordrecht, Netherlands.
17. Kari Skjånes, Céline Rebours & Peter Lindblad., 2013. Potential for green microalgae to produce hydrogen, pharmaceuticals and other high value products in a combined process. *Crit. Rev. Biotechnol.* 2013; 33(2): 172–215.
18. McCord JM., 2000. The evolution of free radicals and oxidative stress. *Am J Med* 108: 652–659.
19. Granot E, Kohen R., 2004. Oxidative stress in childhood—in health and disease states. *Clin Nutr.*, 23: 3–11.
20. Havaux M, Dall'osto L, Bassi R., 2007. Zeaxanthin has enhanced antioxidant capacity with respect to all other xanthophylls in Arabidopsis leaves and functions independent of binding to PSII antennae. *Plant Physiol.*, 145: 1506–1520.
21. Walne, P. R., 1970. Studies on the food value of nineteen genera of algae to juvenile bivalves of the genera *Ostrea*, *Crassostrea*, *Mercenaria*, and *Mytilus*. *Fish. Invest.* 26, 1–62.
22. Stanier, R. Y., Kunisawa, M. M. & Cohen-Bazire, G., 1971. Purification and properties of unicellular blue-green algae (order Chroococcales). *Bacteriol. Rev.*, 35:171–201.
23. Santhanam, P., S. Dinesh Kumar, S. Ananth, S. Jeyanthi, R. Sasirekha and C. Premkumar., 2017. Effect of culture conditions on growth and lipid content of marine microalga *Nannochloropsis* sp. strain (PSDK11). *Indian J. Geo-Mar. Sci.* 46: 2332-2338.
24. Sanjeet, K., Dagnoko, S., Haougui, A., Ratnadass, A., Pasternak, D. and Kouame, C., 2010. Potential and progress on its improvement. *Afr. J. of Agric. Res.*, 5(25): 3590-3598.
25. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, 193: 265–275.
26. Dubois, M., Gilles, K., Hamilton, J., Rebers, P., & Smith, F., 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chemi.*, 28(3): 350–356.
27. Folch, J., M. Lees, and G.H. Sloane-stanley., 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 97:383-394.
28. Brand-Williams, W., Cuvelier, M.E., Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity, *Food Sci. Technol.*, 28 (1): 25–30.
29. Halliwell, B., and Gutteridge, J.M., 1995. The definition and measurement of antioxidants in biological systems. *Free Rad. Biol. Med.*, 18(1): 125-126.
30. Liu F, Ooi VE, Chang ST, 1997. Free radical scavenging activities of mushroom polysaccharide extracts. *Life Sci.* 60(10):763-71.
31. Patil, N. K., Sharanagouda, U., Javed, J. H., Kim, C. K. and Karegoudar, T.B., 2003. Degradation of salicylic acid by free and immobilized cells of *Pseudomonas* sp. strain NGK1. *J. Microbiol. Biotechnol.*, 13(1): 29-34.
32. Castro Araujo, S., and Virginia, M.T.G., 2005. Growth and biochemical composition of the diatom *Chaetoceros* cf. *wighamii* brightwell under different temperature, salinity and carbon dioxide levels. I. Protein, carbohydrates and lipids. *Aquaculture*, 246 (1-4): 405-412.
33. Vasileva, I., Marinova, G. and Gigova, L., 2015. Effect of nitrogen source on the growth and biochemical composition of a new Bulgarian isolate of *Scenedesmus* sp. National Youth Conference 'Biological Sciences for A Better Future', Plovdiv, October, 2015.
34. Setta, B.R.S., Barbarino, E., Passos, F.B. and Lourenço, S.O., 2014. An assessment of the usefulness of the cyanobacterium *Synechococcus subsalsus* as a source of biomass for biofuel production. *Lat. Am. J. Aquat. Res.*, 42(2): 364-375.
35. Brown, M. R., Jeffrey, S. W., Volkman, J. K. and Dunstan, G. A., 1997. Nutritional properties of microalgae for mariculture. *Aquaculture*, 151(1-4): 315-331.





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36. Becker, W., 2004. Microalgae in human and animal nutrition. In: Handbook of Microalgal Culture. Richmond, A., ed., *Appl. Phycol. Biotechnol.* New York, 312-351.
37. Pratt, R., Daniels, T. C., Eiler, J. J., Gunnison, J. B., Kumler, W. D., Oneto, J. F., and Smith, J. H. C., 1944. Chlorellin, an antibacterial substance from Chlorella. *Science (Washington)*, 351-2.
38. Prashanth Kumar V. P., K. H., and Venkatesh, Y. P., 2015. Structural analyses and immunomodulatory properties of fructo-oligosaccharides from onion (*Allium cepa*). *Carbo. Poly.* 117: 115-122.
39. Singh, P., 2010. Chemical profile, Antifungal, Antiaflatoxicogenic and Antioxidant activity of Citrus maxima Burm and Citrus sinensis (L.) Osbeck essential oils and their cyclic monoterpene, DL-limonene. *Food and Chem Toxicol.*, 48 (6): 1734-1740.
40. Uma, R., Sivasubramanian, V., and Niranjali Devaraj, S., 2011. Preliminary phytochemical analysis and in vitro antibacterial screening of green micro algae, *Desmococcus olivaceus*, *Chlorococcum humicola* and *Chlorella vulgaris*. *J Algal Biomass Utiln.*, 2 (3): 74-81.
41. Bhagavathy, S., P. Sumathi, P., and Jancy Sherene Bell, I., 2011. Green algae *Chlorococcum humicola*-a new source of bioactive compounds with antimicrobial activity. *Asian Pac J. Trop. Biomed.*, 1(1): S1-S7.
42. Prasad, N. D., Rao, B. G., Rao, B. S., Rao, T. M. and Praneeth, D. V. S., 2012. Quantification of phytochemical constituents and in-vitro antioxidant activity of *Mesua ferrea* leaves. *Asian Pac. J. of Tropical Biomed.*, 2, S539–S542.
43. Anusha, P., Bai, R. S., 2017. Phytochemical profile and antimicrobial potential of methanolic extracts of bark and leaf of *Quassia indica* (Gaertn) Nooteb. *J. Phytopharm.* 6(5): 269–276.
44. Ghasemzadeh, A. and Ghasemzadeh, N., 2011. Flavonoids and phenolic acids: Role and biochemical activity in plants and human. *J.Meds.Plants.Res.*, 5(31): 6697–6703.
45. Senguttuvan, J., Paulsamy S., Karthika, K., 2014. Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, *Hypochaeris radicata* L. for in vitro antioxidant activities. *Asian Pacific J. Trop. Biomed.*, 4(1): 359–367.
46. Balch, J.F., Balch, P.A., 2000. Prescription for Nutritional Healing: A Practical A-to-Z Reference to Drug-Free Remedies Using Vitamins, Minerals, Herbs & Food Supplements. *Prescription for Nutritional Healing; 5th edition*, New York: Penguin Putnam Inc., 267 – 270.
47. J.R. Soare, T.C.P. Dinis, A.P. Cunha, L. Almeida, 1997. Antioxidant activities of some extracts of *Thymus zygis*, *Free Radic.*, Res. 26 (5) 469–478.
48. W. Brand-Williams, M.E. Cuvelier, C. Berset, 1995. Use of a free radical method to evaluate antioxidant activity, *LWT- Food Sci. Technol.*, 28 (1) 25–30.
49. R. Amarowicz, R.B. Pegg, P. Rahimi-Moghaddam, B. Barl, J.A. Weil, 2004. Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies, *Food Chem.*, 84 (4) 551–562.
50. E. Rollet-Labelle, M.-J. Grange, C. Elbim, C. Marquetty, M.-A. Gougerot- Pocidallo, C. Pasquier, 1998. Hydroxyl radical as a potential intracellular mediator of polymorphonuclear neutrophil apoptosis, *Free Radic. Biol. Med.*, 24 (4) 563–572.
51. Mujeeb F, Bajpai P, Pathak N, 2014. Phytochemical evaluation, antimicrobial activity, and determination of bioactive components from leaves of *Aegle marmelos*. *Biomed Res. Int.* 497606.

**Table-1. Phytochemical constituents of *Isochrysis galbana*, *Synechocystis* sp. and *Nitzschia microcephala* with various extracts.**

Phytochemical	<i>Isochrysis galbana</i>			<i>Synechocystis</i> sp.			<i>Nitzschia microcephala</i>		
	Methanol	Ethyl acetate	Hexane	Methanol	Ethyl acetate	Hexane	Methanol	Ethyl acetate	Hexane
Alkaloids	+	+-	-	+	+	+-	+	+	+-
Flavonoids	+	+	+-	+	+	+	+	+-	-
Steroids	+	+-	-	+	+	-	+	+	-
Terpenoids	+-	-	-	-	-	-	-	-	-
Saponin	+	-	-	+	+	-	+	+	-
Phenols	+	+	+	+	+	+-	+	+	+-
Tannins	-	-	-	-	-	-	-	-	-
Glycosides	+-	-	-	-	-	-	+	+-	-





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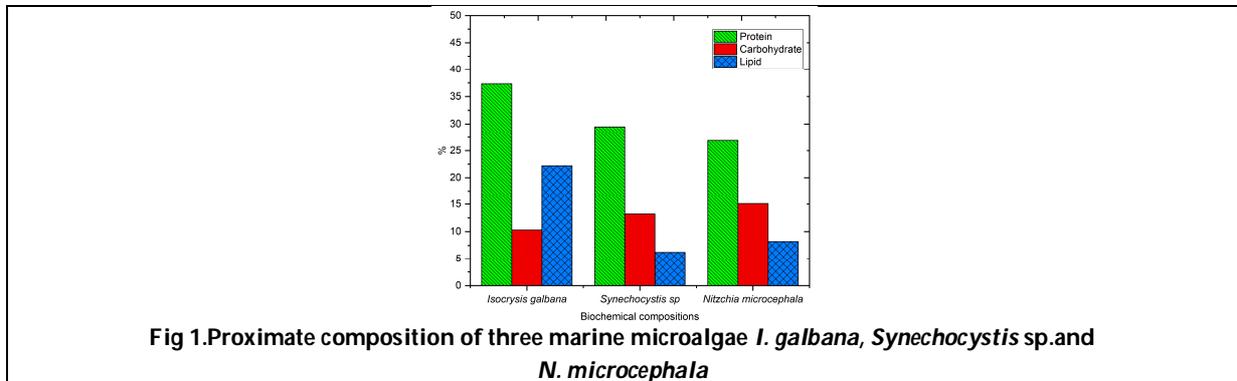


Fig 1. Proximate composition of three marine microalgae *I. galbana*, *Synechocystis sp.* and *N. microcephala*

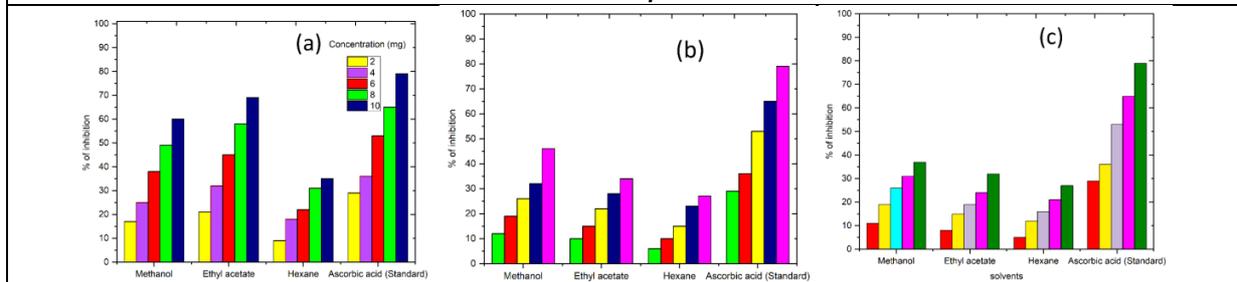


Fig 2. Scavenging activity of DPPH radical against microalgae *I. galbana* (a), *Synechocystis sp.* (b) and *N. microcephala* (c) with different solvents

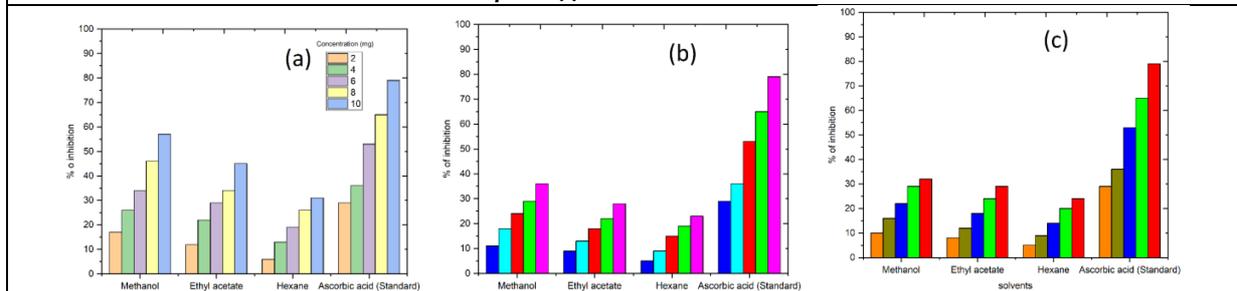


Fig 3. Scavenging activity of hydroxyl radical against microalgae *I. galbana* (a), *Synechocystis sp.* (b) and *N. microcephala* (c) with different solvents

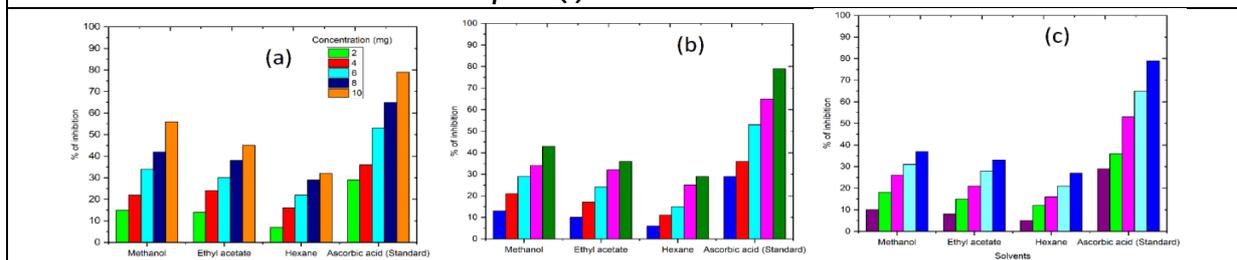


Fig 4. Scavenging activity of hydrogen peroxide radical against microalgae *I. galbana* (a), *Synechocystis sp.* (b) and *N. microcephala* (c) with different solvents.





## An Optimized Algorithm to Detect Diabetic Retinopathy and Cardiovascular Disease in Early Stages using Artificial Intelligence

P. Ajitha\*

Assistant Professor, Dept. of Software Systems and Computer Science (PG), KG College of Arts and Science, Coimbatore, Tamil Nadu, India.

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### \*Address for Correspondence

**P. Ajitha**

Assistant Professor,  
Dept. of Software Systems and Computer Science (PG),  
KG College of Arts and Science,  
Coimbatore, Tamil Nadu, India.  
Email: ajitha.p@kgcas.com, ajitha.mca@gmail.com



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### ABSTRACT

An optimized algorithm is designed and implemented that detects diabetic retinopathy, cardiovascular disease in early stages for clinical management. Scanned images of retina are used to process the images for recognizing the patterns of diabetic retinopathy and cardiovascular diseases. An algorithm is designed with Artificial Intelligence and Particle Swarm Optimization for better accuracy and prediction.

**Keywords:** unsupervised algorithms, artificial intelligence clusters, machine learning

### INTRODUCTION

Artificial Intelligence (AI) simulates the human mind where, the intelligence is trained to the machines by observing or processing the data. It adapts the cognitive abilities of the human mind and also process numerous amount of big data and predicts for the accurate diagnosis. Particle Swarm Optimisation (PSO) is an optimization algorithm to find best solutions where computational procedure to select effective and best element from collection of accessible alternatives. Computations are done iteratively and calculated for the normalized mutual data with metrics, similarity indexes, and evolutionary techniques. Every individual element or data is considered as particle and ability to store information as memory and remember previous points to optimize the accurate solutions from lists of computations. Feasible solutions can be computed through the real valued multidimensional data and the values of each particle. Artificial Intelligence processes these computational elements and finds out the prediction of particle through various iterations. PSO is an Artificial Intelligence technique to employ various iterations through computation and finds out the accurate data for predicting the constraints provided in the algorithm. Of all the behavior of the natural systems PSO is one of the Artificial Techniques in closest with genetic algorithms. Using Artificial Intelligence an evolving technology with numerous computing to perform pattern recognition based on the

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input data. Diabetic Retinopathy [DR] by detecting earlier and screening the disorder reduces the human intervention. DR requires continues screening for treatment and specialized care [4]. With AI by ability to process imaging of medical type provides an diagnosis in earlier stages [5]. Automatic analyses will lead to faster and accurate predictions in big volume of data. In this paper section 2, describes the review literature, section 3 presents the proposed methodology. section 4, provides results and discussions by providing conclusion in section 5.

### Review Literature

Conventional Particle Swarm Optimisation (PSO) provides higher accuracy compared to the benchmark backward propagation for training preceptors and predicting the outcomes of construction claims as discussed in [3]. PSO can be easily and readily adopted for fast convergence rates and multi layer perceptrons optimization techniques with low optimization cost. Prevalence of Diabetic Retinopathy was 30.5%. Risk of Cardiovascular disease was significantly higher in Proliferative Diabetic Retinopathy (PDR). vs No Diabetic Retinopathy (NDR), ( $18.7 \pm 10.0\%$  vs.  $11.3 \pm 8.4\%$ ,  $p = 0.01$ ). The prevalence of non proliferative diabetic retinopathy (NPDR) ( $32\%$  vs.  $17.8\%$ ,  $p = 0.002$ ) and PDR ( $20\%$  vs.  $4.1\%$ ,  $p = 0.04$ ) was more in high risk group for CVD in comparison with low risk group for CVD. After adjustment for traditional risk factor for CVD, the risk for CVD remained markedly increased in the presence of DR [2]. DR is associated with estimated risk of CVD in type 2 diabetic patients. In correlation to this data, the necessity for predicting diabetic retinopathy and cardiovascular disease for early prevention will lead to the significant improvement in saving the lives of people. Artificial Intelligence provides a significant contribution in treating cardiovascular along with the areas of ophthalmology. Family of algorithms of AI and Machine Learning (ML) can elucidate iterative patterns to optimise tasks like classification and prediction[6]. To provide a better diagnosis, an optimising of natural behaviour of algorithms like PSO provides better and effective prediction of early stages of DR and CVD.

### Proposed Methodology

The proposed methodology deals with the algorithm for detecting diabetic retinopathy along with the cardiovascular disease using Artificial intelligence and Particle Swarm Optimisation. Diabetic retinopathy needs frequent screening of the patients which increases the cost and also resources. By using the scanned images of the retina of the patients, the values are extracted and iterated through particles with the threshold of identifying the best value in predicting the DR along with CVD. Algorithm RetiCard detect through PSO uses iteration of particles with the computation of mepbest. Computations involves, swarming of particles towards the evolution of medical imaging data. Only, retinal images are provided as input. After, proper preprocessing of the image by removing noises and extracting the image values. The proposed algorithm, RetiCard Detect through PSO first retrieves the values through extracting pixilated values of the images. Preprocessing with retina by removing outliers are considered as input. Iterations of the values of images are processed by computing the particles of big data. meupbest process individual values by finding threshold and iterating with calculating the value. Computations are made of the particles which move through data of values which is closest and nearest to the particles of optimisation. The actions of swarms in the direction of threshold values provide a accurate diagnosis. The inter and intra base pairing directions between the molecular and secondary form for nucleic acids involves computation in the imaging and retrieving the value properties of the same. Pattern recognition in this form results in the loop in basing of the parings.

The values are iterated and computed through iterative development and contemporary constraints. These constraints are threshold values with computing the mepbest. The resulted values are provided as a training set. Machine detects or learns through patterns. Artificial intelligence, emulates these behavior by training the data with threshold values of iterations and deciding which swarms of particles provides the new possible accurate diagnosis. The training data provides intelligence and decides accurate results towards the predicting of diabetic retinopathy through temperature. Temperature, blood pressure can be identified through retinal images. Based on these values, the computations of swarms with particles are done and predicted. Equation 1, mentions the particles i.e individual element of data that are used for predicting the diabetic and cardio diseases. These computations are provided in the loop through, iterations for calculating the values that identifies in which level the threshold stands. Whatever the





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value that exceeds it, is targeted as data with diseases. Equation 2, loops the value for computation to provide optimized results in the particles that are provided as input. Predicting, with threshold values for optimized value with meupbest. The section 4, discusses the results of implementing the algorithm RetiCard through PSO.

## RESULTS AND DISCUSSIONS

An image of retina that is used for processing is displayed in the figure 1. Retina image is processed for retrieving the temperature, blood pressure, age and muscular in retina to provide clear information on the disease related to it. Fig 2, provides the patterns in retina so that the values can be retrieved for predicting and computations. The values provided are based on the computations with threshold by providing the equation 1. After, providing these data as training data and simulated to provide a better and accurate results of diagnose. Training data provides, the iterations with target error and number of particles. Equation 2, on training provides accurate diagnosis and particles are optimized with values that's are swarmed towards the thresholds. Second level of iterations, target error and particles provides prediction at nearest values of target, with values like age.

Table 1, mentions the swarms of particles that are iterated and where swarms are mutated with computational threshold values. Figure 3, provides age and threshold values that determines the DR and CVD. The green color line curves towards the upper values by providing particles that swarm towards the computational thresholds. Respectively, blue and green values also deignies towards it. Figure 4, provides age distribution through computations of iterations where the swarms are mutated or processed through it. Age wise distribution is swarmed with particles of values along with evolutionary computing of values that are extracted through images by detecting it the values prefaced. Figure 5 iteration values with target errors and swarms that are computed for accurate prediction. Figure 6, provides overall value distribution and iteration computations. Finally, figure 7 mentions the detections of Diabetic retinopathy and cardio vascular diseases through iterated value calculation and predicting with scattered values by providing the age wise distribution. Results and discussions graph and table provides the prediction of DR and CVD by using PSO technique with optimized result which has low cost and high accuracy.

## CONCLUSION AND FUTURE WORKS

Particle Swarm Optimisation provides accurate prediction with computation of data in correlation to the associated comorbidity for early detection and prevention. The proposed algorithm in this paper RetiCard Detect through PSO shows the prediction of diabetic retinopathy and cardiovascular disease. Predicting by utilizing the behaviors of natural method resulted in accurate diagnosis. Swarming of particles with numerous iterations can provide for the values in distribution of age, muscular degeneration. Artificial Intelligence with proper legal and ethical codes in place may result in the saving the lives of people by utilizing it efficiently. Future works may move towards the Artificial intelligence in both the images and text based data. This paper considers only the medical imaging with proper filtering of noises and removing outliers.

## REFERENCES

1. Aman Chandra Kaushik, Shiv Bharadwaj, Ajay Kumar, Avinash Dhar and Dongqing Wei, "New Trends in Artificial Intelligence: Applications of Particle Swarm Optimization in Biomedical Problems,.".2018.
2. Shoeibi N, Bonakdaran S., "Is There any Correlation Between Diabetic Retinopathy and Risk of Cardiovascular Disease? , Curr Diabetes Rev. 2017;13(1):81-86. doi: 10.2174/1573399812666151012115355. PMID: 26456361.
3. Chau K. (2006), "A Split-Step PSO Algorithm in Predicting Construction Litigation Outcome". In: Yang Q., Webb G. (eds) PRICAI 2006: Trends in Artificial Intelligence. PRICAI 2006. Lecture Notes in Computer Science, vol 4099. Springer, Berlin, Heidelberg. [https://doi.org/10.1007/978-3-540-36668-3\\_163](https://doi.org/10.1007/978-3-540-36668-3_163)





**Ajitha**

4. Padhy, Srikanta Kumar; Takkar, Brijesh; Chawla, Rohan; Kumar, Atul , "Artificial intelligence in diabetic retinopathy", Indian Journal of Ophthalmology: July 2019 - Volume 67 - Issue 7 - p 1004-1009.doi: 10.4103/ijo.IJO\_1989\_18
5. Doi K. , "Diagnostic imaging over the last 50 years research and development in medical imaging science and technology", Phys Med Biol. 2006;51:R5–27
6. Benjamins, J.W., Hendriks, T., Knuuti, J. *et al.* "A primer in artificial intelligence in cardiovascular medicine". *Neth Heart J* 27, 392–402 (2019). <https://doi.org/10.1007/s12471-019-1286-6>
7. Ajitha, "Classification of outliers for predicting the heart disease using distributed data mining with AI", International Journal of Scientific and Technology Research, 9(2).2020
8. Wachowiak MP, Smolíkóv R, Zheng Y, Zurada JM, Elmaghraby AS. "An approach to multimodal biomedical image registration utilizing particle swarm optimization". IEEE Transactions on Evolutionary Computation. 2004;8:289-301.
9. Shadman Nashif, Md Rakib Raihan, Md Rasedul Islam, Mohammed Hasan Imam, "Heart Disease Detection by Using Machine Learning Algorithms and a Real-Time Cardiovascular Health Monitoring System", World Journal of Engineering and Technology, 6(4), 854-873. January 2018.

**Algorithm: RetiCard Detect through PSO**

```
//input an retina image called r
r = retina image to retrieve temp, bp and muscular values
iter = iterations of the retrieved values
p = particles /data for processing the iterations
mepbest = iterations/particles * (∑ xij * p (i))
iterate the process with finding threshold in
    p(i) * ∏j<ni ∂ (x + a)n = ∑k=0n (n)k xk an-k / mepbest ----- equation 1
threshold with particles optimizing
for r in iterate , meupbest
meupbest = p(i) ** (x + a)n = ∑k=0n (n)k xk an-k ----- equation 2
training the particles and iteration of above steps
test the data for prediction
predict meupbest, particle for optimizing
end
```

**Table 1 – Computational particles with iteration**

Inform the number of iterations: 100  
 Inform the target error: 0.98  
 Inform the number of particles: 100

Prediction /care at [-41.55695141 22.43614801] meupbest is [-41.55695141 22.43614801]  
 Prediction /care at [22.08197007 42.8092406 ] meupbest is [22.08197007 42.8092406 ]  
 Prediction /care at [-3.12113027 0.20007175] meupbest is [-3.12113027 0.20007175]  
 Prediction /care at [-2.49909654 -13.17466117] meupbest is [-2.49909654 -13.17466117]  
 Prediction /care at [ 27.15561923 -41.78813566] meupbest is [ 27.15561923 -41.78813566]  
 .....  
 .....  
 Prediction /care at [-46.01626374 -34.44098502] meupbest is [-46.01626374 -34.44098502]  
 Prediction /care at [ 41.51887621 -17.56320221] meupbest is [ 41.51887621 -17.56320221]  
 The best solution is: [ 0.28743302 -0.88426944] in n\_iterations: 2





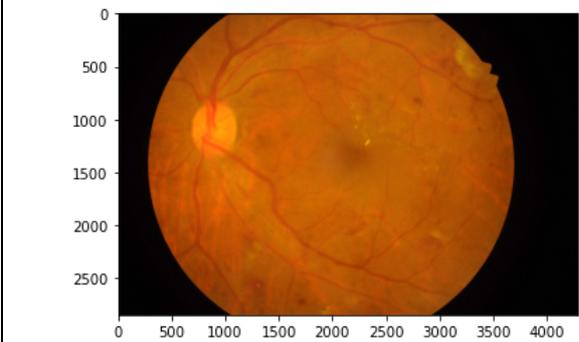
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Inform the number of iterations: 100

Inform the target error: 0.98

Inform the number of particles: 100

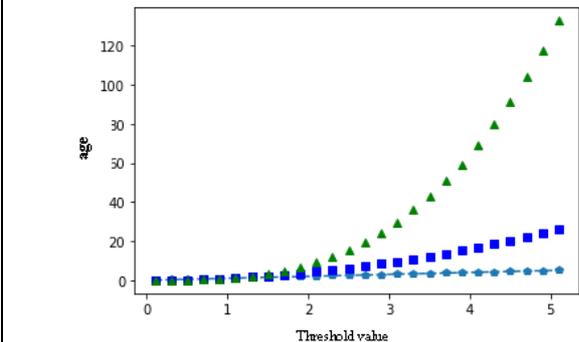
645.973406736464 [-17.73441896 -18.17866307]  
 2962.9248833008037 [-44.95853017 -30.67010675]  
 2223.5129615651776 [ 45.45661955 -12.49834793]  
 865.8914777680203 [28.65583411 -6.61321779]  
 3728.5552466569425 [40.11639456 46.0242342 ]  
 198.33283569914656 [13.76309892 -2.81246225]  
 3593.7632649079023 [-46.91345371 37.30805712]  
 .....  
 .....  
 1.8241612308695792 [0.87624535 0.23739276]  
 29.998042605470527 [ 5.38116208 -0.20282332]  
 23.08658605917278 [-2.76463737 -3.80044291]  
 18.21939220552724 [3.44619395 2.31152319]



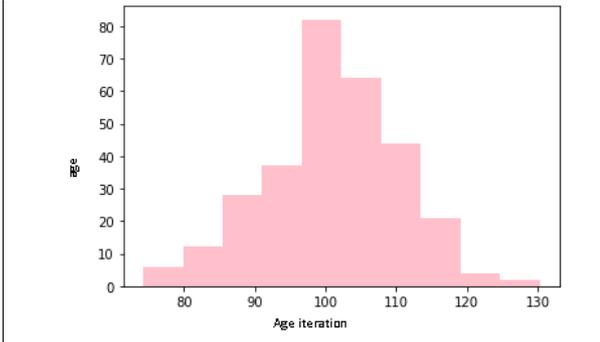
**Fig 1 : Retina Image**



**Fig 2 : Pattern in retina**



**Fig 3 : Age Vs Threshold**

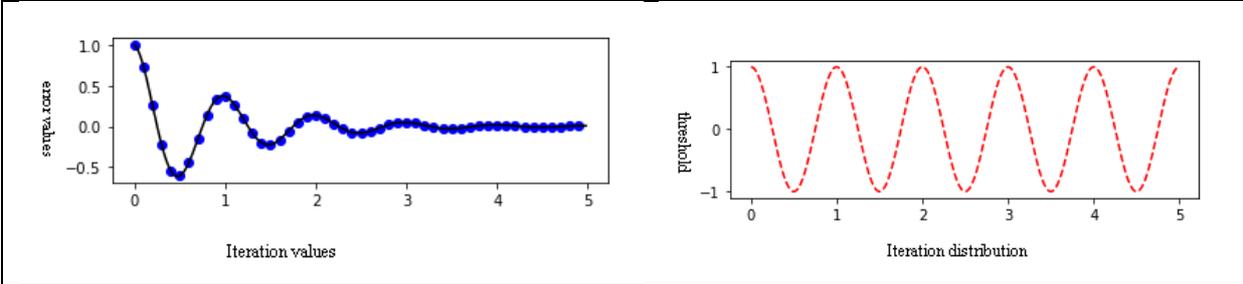


**Fig 4: Age distribution with iteration**



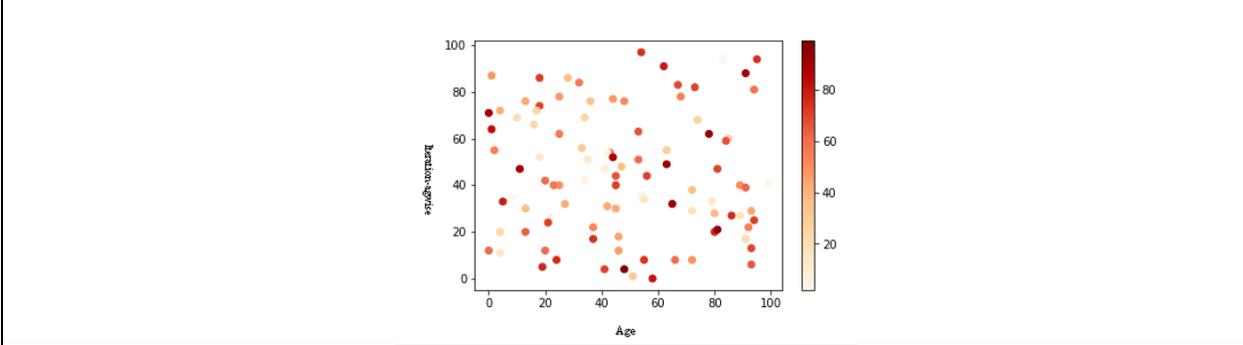


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**Fig 5 : iteration values Vs error values**

**Fig 6 : iteration values distribution**



**Fig 7: Age and iteration distribution**





## Analysis of Frequency and Temperature Dependent Dielectric and Conductivity Study of SrBi<sub>2</sub>Ta<sub>2</sub>O<sub>9</sub> Ceramic

G. K. Sahu<sup>1</sup>, S. Behera<sup>1\*</sup> and S.R. Mishra<sup>2</sup>

<sup>1</sup>Department of Physics, School of Applied Sciences, Centurion University of Technology and Management, Odisha, India.

<sup>2</sup>Department of Chemistry, Gandhi Institute for Education and Technology, Baniatangi, Khorda, Odisha, India.

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### \*Address for Correspondence

**S. Behera**

Department of Physics,  
School of Applied Sciences,  
Centurion University of Technology and Management,  
Odisha, India.

Email: saubhagyalaxmi.behera@cutm.ac.in



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### ABSTRACT

A Bismuth layered structure SrBi<sub>2</sub>Ta<sub>2</sub>O<sub>9</sub> ceramic is prepared by the solid-state reaction technique at calcination temperature of 1000°C. X-ray diffraction analysis is used to analyze the phase purity with orthorhombic structure and A<sub>2</sub>1<sub>1</sub>amspace group. The fracture surface morphology of the sintered pellet is visualized by scanning electron microscopy. Diffusive phase transition behavior is existed in the temperature dependence dielectric study at T<sub>c</sub>= 305°C. The electrical ac and dc conductivity study shows the negative temperature coefficient of resistance behavior. Activation energy from the Arrhenius plot is studied to discuss the fatigue property

**Keywords:** Bismuth layered structure; Dielectric parameters; Conductivity study

### INTRODUCTION

Strontium Bismuth Tantalate (SrBi<sub>2</sub>Ta<sub>2</sub>O<sub>9</sub>, SBT) is one of the promising materials of Aurivillius compounds with excellent ferroelectric properties of long fatigue endurance and large remanent polarization (P<sub>r</sub>) [1,2]. The electrical properties of the SrBi<sub>2</sub>Ta<sub>2</sub>O<sub>9</sub> system is widely studied in bulk materials and thin films [3-9]. Bulk SBT ceramics having a composition (Sr/Bi/Ta=1/2/2) have been investigated widely, and are mostly prepared by the conventional solid-state reaction methods at high sintering temperatures of 1250°C. However, the solid-state method requires calcination at high temperature, which causes coarsening of the powder. It has also been reported that Sr-deficient and Bi-excess SrBi<sub>2</sub>Ta<sub>2</sub>O<sub>9</sub> shows an increase in Curie temperature T<sub>c</sub> with increasing compositional deviation and a marked increase in remnant polarization [10-12].

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However, in this high temperature sintering methods, a procedure requiring long reaction times and high temperatures, and involving serious problems, such as stoichiometry losses and uncontrolled particle size. To avoid these problems, decrease of sintering temperature of SBT ceramics is an urgent task to make it useful for device fabrication. So the low calcination temperature can be obtained by a heat and trial method by checking the phase formation by subsequent interval of temperature. Mixed oxide precursors can be calcined at different temperature ranging from 800-1000°C interval of 50°C for 2 and 4 hrs durations for phase study. So we have synthesized the SBT ceramic by mixed oxide route in step increasing of calcination temperature up to 1000°C starting from 800°C. The calcination temperature is fixed at 1000°C basing on the absence of impurity phases. In this paper, we have explained the dielectric and conductivity study of bismuth layered ferroelectric SrBi<sub>2</sub>Ta<sub>2</sub>O<sub>9</sub> (SBT) ceramic. To the best of the author's knowledge, this is the first report on the dielectric and conductivity studies of SBT ceramic synthesized by cost effective mixed oxide route at 1000°C.

### Experimental methods

Bismuth layered ferroelectric SrBi<sub>2</sub>Ta<sub>2</sub>O<sub>9</sub> (SBT) was prepared by solid state route. SrCO<sub>3</sub> (99.0%) (LobaChemie, Mumbai), Bi<sub>2</sub>O<sub>3</sub> (99.9%) and Ta<sub>2</sub>O<sub>5</sub> (99%) (Merck, Germany) were used as starting raw material. Initially, stoichiometrically measured precursors were mixed and calcined at 1000°C for 2 hrs in the programmable furnace. The calcined powder was mixed with 5% polyvinyl alcohol and pressed into disk under 60MPa for 3 mins. Finally, disks were sintered in the furnace at 1100°C for 3hrs with a heating rate of 30°C/min. The structural studies of the sintered pellet were examined by x-ray diffraction (XRD) (Powder X-Ray Diffractometer, Rigaku miniflex), scanning electron microscope (SEM) (JEOL JSM6480, USA). For electrical measurement, the pellet was coated with silver electrode on both sides using a silver paste. The impedance and dielectric data were collected from the computer interfaced Solartron 1260A Gain/Phase analyser over the temperature range from room temperature to 400°C at 1°C/min.

## RESULT AND DISCUSSION

### Phase analysis, Morphology and elemental analysis

Fig.1 (a) shows the well-defined and prominent peaks corresponding to the standard data of JCPDS no 49-0609 and the diffraction peaks are allocated to the single phase layered structure (m = 2) with A2<sub>1</sub>am orthorhombic symmetry without any secondary phase. The lattice parameters are calculated by indexing the intense peaks by "Powd" software and are a=5.5394(10) Å, b=5.5491(9) Å, c=25.1768(31) Å and V=773.901(36) Å<sup>3</sup>. Orthorhombic distortion (b/a) values is 1.0017. Fig.1 (b) shows the fracture structure of sintered SBT ceramic. The clear grain growth and overall dense ceramic with little amount of porosity in the sample is observed. The relative density of the pellet is 92% is calculated by Archimedes principle.

### Temperature dependence dielectric study

Fig 2 shows the temperature variation of dielectric constant of the compound at various frequencies. The dielectric constant ( $\epsilon_r$ ) increases gradually up to the maximum value ( $\epsilon_m$ ) with the rise in temperature upto 300°C ( $T_c$ ), and then decreases with the further increase in temperature which suggests a phase transition from ferroelectric to paraelectric state. The increment of dielectric constant above phase transition at low frequency (i.e., 1 10 kHz) is due to the space charge polarization which acts as a dipole. The T

The modified Curie–Weiss law [13] has been used to investigate the diffuseness of the ferroelectric phase transition, which is described by the following equation:

$$\frac{1}{\epsilon} - \frac{1}{\epsilon_m} = \frac{(T-T_m)^\gamma}{C'} \quad (\text{at } T > T_m) \quad (2)$$

where  $\gamma$  ( $1 < \gamma < 2$ ) is the relaxation strength and  $C'$  is a constant. The value of  $\gamma$  is 1 for the case of a normal ferroelectric and  $\gamma = 2$  (quadratic) is valid for an ideal ferroelectric relaxor [14]. A plot of  $\ln(1/\epsilon - 1/\epsilon_m)$  vs  $\ln(T - T_m)$  are shown in the Fig. 3 and the calculated diffusiveness is 1.675.





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An intermediate value of  $\gamma$  confirms the diffuse phase transition in the material. The compositional fluctuation due to randomness of structure due to presence of more cations at one or more crystallographic direction causes diffuseness in the transition [14].

### AC and DC conductivity

Fig.4 (a) shows the AC conductivity versus angular frequency plot of the sample sintered at 1100°C for 30 mins. The plot between  $\log \sigma_{ac}$  versus  $\log \omega$  shows the linear behavior upto phase transition temperature. As the temperature increases the  $\log \sigma_{ac}$  curves flatten out at low frequencies. This frequency dependence of conductivity obeys Jonscher's power-law [15],  $\sigma_{ac} = \sigma_{dc} + A\omega^s$  ( $0 < s < 1$ ), where  $\sigma_{dc}$  is frequency independent term and  $\sigma_{ac}$  is the pure dispersive component of ac conductivity,  $s$  is the frequency component and  $A$  is frequency independent but temperature-dependent term. It has been observed that low-frequency dispersion obeying the power-law feature  $\sigma(\omega)$  and changes its slope governed by  $s$ . The frequency at which change in slope takes place is known as hopping frequency ( $\omega_p$ ). At higher frequencies, the conductivity becomes more or less temperature dependent. The experimental data are fitted with the above equation for the given temperatures value of  $s$  is less than 1 obtained from non-linear fitting and confirmed the translational motion of the bound charge carriers [16]. Table 1 gives the data correlating  $A$  and  $s$  with temperature. The increasing trend of  $\sigma_{ac}$  in the low-frequency range may be due to the disordering cations between the neighboring sites and the presence of space charges that vanishes at higher temperatures and frequencies.

### Dc conductivity

Fig. 4 (b) shows the variation of dc conductivity with respect to inverse of absolute temperature. The value of bulk conductivity of the material was evaluated from the complex impedance plots of the sample at different temperatures using the relation:  $\sigma_{dc} = t / R_b A$ , where  $R_b$  is the bulk resistance,  $t$  the thickness and  $A$  is the surface area of the sample respectively. The dc conductivity increases with rise in temperature confirming the negative temperature co-efficient of resistance (NTCR) behavior. This plot follows the Arrhenius relation:  $\sigma_{dc} = \sigma_0 e^{-\frac{E_a}{k_B T}}$ . The activation energy estimated from the conductivity variation was found to be 0.38 eV.

## CONCLUSION

Bismuth layered  $\text{SrBi}_2\text{Ta}_2\text{O}_9$  ferroelectric material has been synthesized by solid state reaction technique and the dielectric and conductivity study has been studied. The temperature and frequency dependence dielectric study shows the diffuse phase transition behavior at 300°C. AC conductivity follows the Joncher's power law and the DC conductivity has been calculated. The activation energy is calculated from dc conductivity plot.

## REFERENCES

1. Lu CH, Chang DP, J. Phys. Chem. Solids. 2008;69:480
2. Panda AB, Tarafdar A, Sen S, Pathak A, Pramanik P, J. Mater. Sci. 2004;39:3739
3. Wu Y, Forbess MJ, Seraji S, Limmer SJ, Chou TP, Cao G, Mater. Sci. Eng. B 2001;86:70
4. Torii Y, Tato K, Tsuzuki A, Hwang HJ, Dey SK, J. Mater. Sci. Lett. 1998;17:827
5. Seong N, Yang C, Shin W, Yoon S, Appl. Phys. Lett. 1998;72:1374
6. Dat R, Lee JK, Auciello O, Kingon AI, Appl. Phys. Lett. 1995;67:572
7. A-Paz de Araujo C, Cuchiaro JD, Memillan LD, Scott MC, Scott JF, Nature London 1995;374:627
8. Amanuma K, Hase T, Miyasaka Y, Appl. Phys. Lett. 1995;66:221
9. Wang C, Fang QF, Zhu ZG, Appl. Phys. Lett. 2002;80:3578
10. A.D Rae, J.G Thompson and R.L Withers. *Acta Crystallogr. Sect. B Struct. Sci.*, **48** (1992), 418
11. T.C Chen, C.-L Thio and S.B Desu. *J. Mater. Res.*, **12** (1997), 2628
12. Irie H, Miyayama M, Appl. Phys. Lett. 2001;79:251



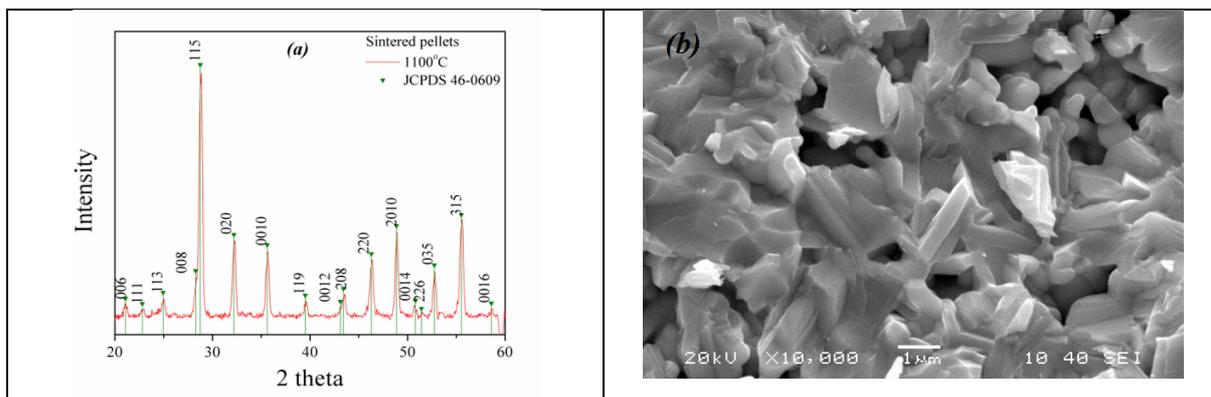


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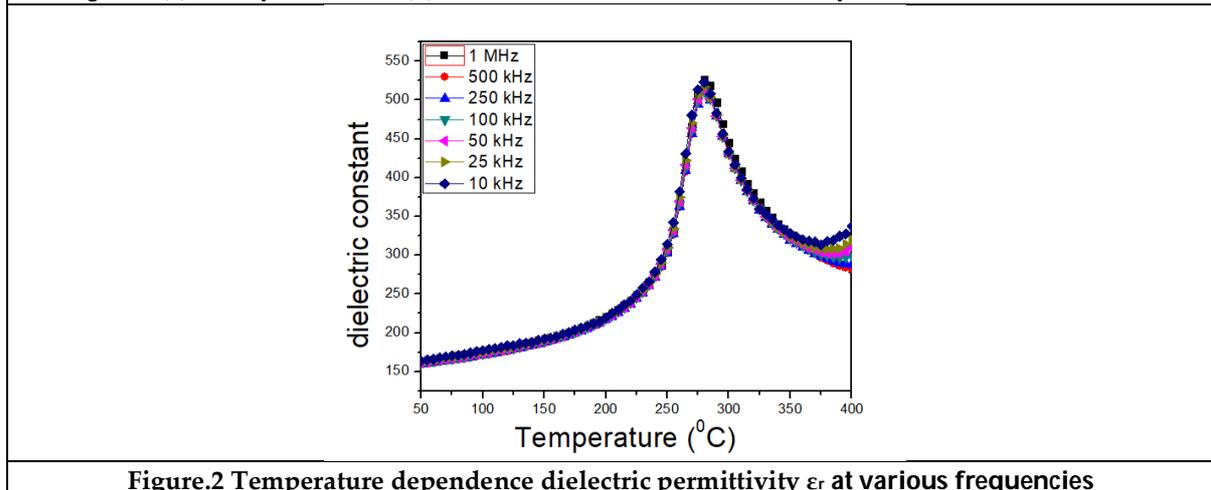
13. Uchino K, Nomura S, Integr. Ferroelectr. 1982;44:55
14. G. N. Bhargavi, A. Khare, T. Badapanda, M. S. Anwar, N. Brahme, Journal of Materials science: Materials in electronics, Vol. 29, (2018), PP. 11439–11448.
15. Jonscher AK, Dielectric Relaxation in Solids, Chelsea, London, 1983.
16. K. Funke, Jump relaxation in solid electrolytes Solid State Chem. 22(1993) 111.

**Table.1. Fitting parameters of Joncher’s power law from AC conductivity**

Temperature °C	A x 10 <sup>-10</sup>	s
325	1.7032	0.45728
350	2.7669	0.43127
375	2.7215	0.4504
400	2.5655	0.47594
425	3.0209	0.48477



**Figure.1(a) XRD pattern and (b) SEM of microwave sintered SBT pellet at 1100°C for 30 mins**



**Figure.2 Temperature dependence dielectric permittivity  $\epsilon_r$  at various frequencies**





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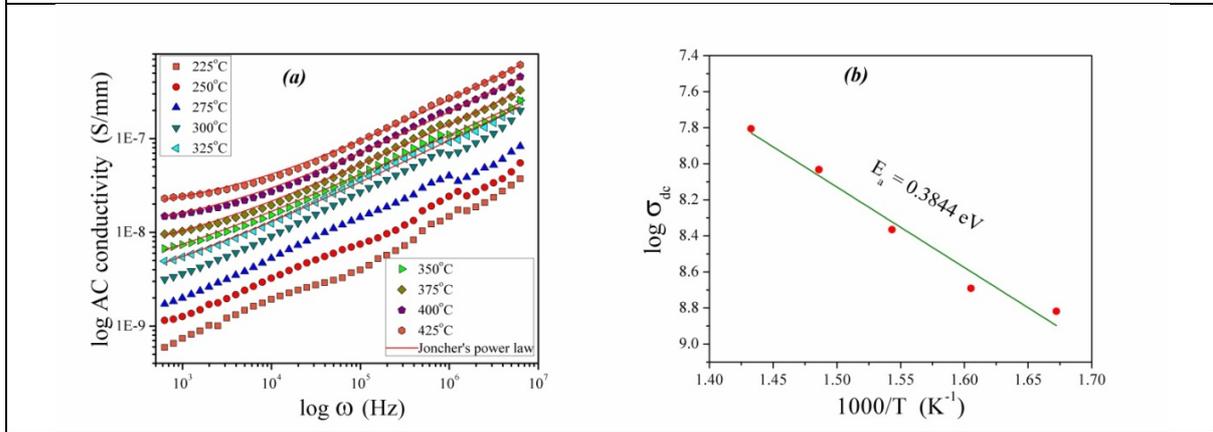
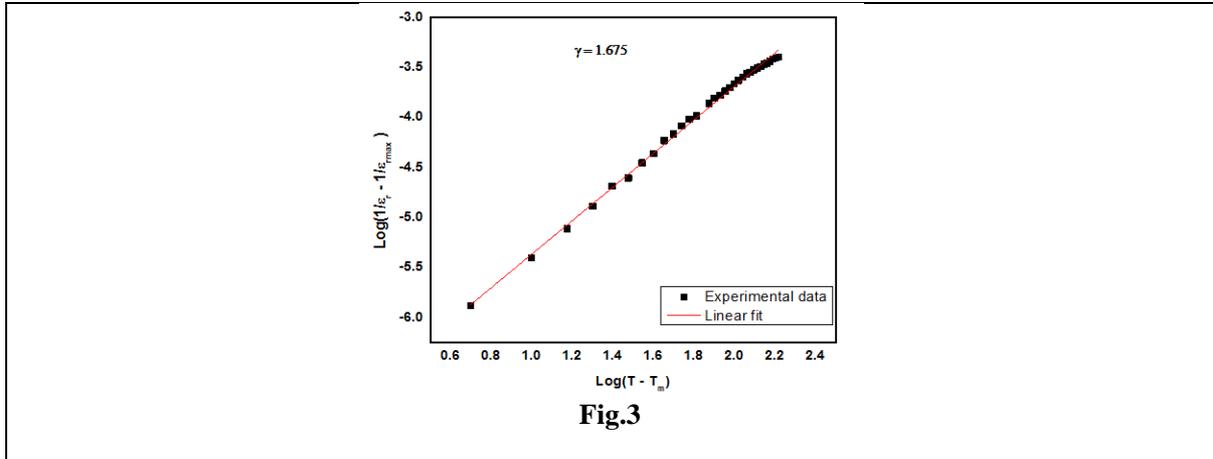


Figure.4 (a) AC conductivity versus angular frequency of sintered SBT pellet and (b) Arrhenius plot of DC conductivity.





## COVID-19 Deters Life, Restricts Education: Module, G-suite, YouTube, Facebook, Zoom, etc. substantiated Students' Learning

Edilmar P. Masuhay<sup>1\*</sup>, Ludy G. Alsong<sup>1</sup>, Siony M. Cordova<sup>2</sup>, Gil M. Alegre<sup>2</sup>, Michael G. Alsong<sup>3</sup>, Nona D. Saratorio<sup>2</sup>, Bernadette P. Bagaipo<sup>4</sup> and Edlyn A. Chan<sup>4</sup>

<sup>1</sup>Bachelor of Technology Livelihood Education (BTLED) Department, SSCT-Mainit Campus, Philippines.

<sup>2</sup>Bachelor of Agricultural Technology (BAT) Department, SSCT-Mainit Campus, Philippines.

<sup>3</sup>Mainit National High School, Quezon, Mainit, Surigao del Norte, Philippines.

<sup>4</sup>SSCT-Main, Surigao City, Philippines.

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### \*Address for Correspondence

**Edilmar P. Masuhay**

Bachelor of Technology Livelihood Education (BTLED) Department,  
SSCT-Mainit Campus, Philippines.

E.Mail: yahusamedilmar@gmail.com



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### ABSTRACT

Using phenomenal descriptive qualitative research secondary information and literature related to modus operandi on the learning-teaching process during Covid-19 were analyzed thematically. Findings showcase that school administrators and teachers confused about how to substantiate their students' learning because their life was threatened by the said virus. By then, this study generated three themes: *First*, Confused teaching strategies; Thereby this learning-teaching was strategized thru module blended with some electronics applications such as G-Suite, Facebook, YouTube, Zoom, etc. during teaching, discussions, and monitoring. *Second*, Students' capacity on adapting online classes: The discussion, class monitoring, tackling all of those assignments, and learning activities should be done through with the module provided as their ultimate guide. Aside from Module, G-suite, Facebook, YouTube, Zoom were utilized as means or tools during the learning-teaching process. *Third*, Implications for teacher's management: The quality Module is reflective, this mainly guides students on how to sustain their activities hence, and this module has an emphasis on self-face and experiential learning. However, every teacher was determined and accountable for their strategies in teaching. The study practically leads the Administrators and Educators to discover the most rightful decision and desirable learning-teaching methods during the crisis.

**Keywords:** Covid-19; Life; Education; Module; G-Suite; YouTube; Facebook; Zoom.





## INTRODUCTION

Covid-19 challenged everyone and consequentially ruins man's life: their economic, education, and health condition. According to Anderson, Heesterbeek, Klinkenberg & Hollingsworth (2020) that the Governments will not be able to minimize both deaths from coronavirus disease 2019 (COVID-19) and the economic impact of viral spread. The ongoing epidemic of coronavirus disease 2019 (COVID-19) is devastating, despite of extensive implementation of control measures (Ji, Ma, Peppelenbosch & Pan, 2020). Nicola (2020) declared that the COVID-19 pandemic has resulted in over 4.3 million confirmed cases and over 290,000 deaths globally. It has also sparked fears of an impending economic crisis and recession. Social distancing, self-isolation, and travel restrictions reduced the workforce. Viner, et.al. (2020) reported that in response to the coronavirus disease 2019 (COVID-19) pandemic, there were 107 countries had implemented national school closures dated March 18, 2020. Rundle, Park, Herbstman, Kinsey & Wang (2020) proclaimed that the COVID-19 pandemic is causing substantial morbidity and mortality, straining health care systems, shutting down economies, and closing school districts.

Though it is presented that that literature above distinctly informed the worst scenarios brought by this virus, but for the sake of education, economic, health instructions, and discussion. Purposively, the author studied a descriptive qualitative research that reviewed specified articles of few relatives on modus operandi in the learning-teaching process and so those administrators, educators, lecturers, and students with their intent should be guided or took the best judgment, desirable means in pursuing teaching during Covid-19, thus the following literature, below were critically reviewed.

### Previous studies relative to Modus Operandi in the Learning-Teaching process

Harvey (2002) stressed that substantial questions on how to improve students should be addressed, rather than focusing on accountability, it is suggested by him that the quality of education should be examined, monitored, explored, and analyzed. Wherein, evaluation is to be examined in detail, though, evaluation is legitimate but failed to ask substantial questions about the nature of learning. Thus, quality monitoring with the method is conventional to research-learning theory, nature and styles of learning, and classroom innovations. Moreover, it is advocated that higher education teachers adopt an explicit 'transformation' approach to substantial questions about improving student learning".

McPhee (2002) in the University of Glasgow understood that Problem-based learning (PBL) by Menon (1997) pointed out that this theory is not new, and originated by Dewey that by evolutions the applications of this methodology has been discussed and undertaken in some other county such that of United Kingdom e.g. the models of initial teacher education, and described that this PBL research was carried out. And that this modus operandi provides some discussion about the applicability in teacher education".

Ansell, Sørensen & Torfing (2020) highlighted that this pandemic disease threatened the public sector into a difficult situation. He even accorded that this epic is not only complex but turbulent problems because these were characterized by surprising emergence of inconsistent, unpredictable, and uncertain events. This scenario demands robust governance solutions that are sufficiently adaptable, agile, and pragmatic to upheld a particular goal or function in the face of continuous disruptions. Entirely, this drawn that the consequences should focus on robust governance for public administration and leadership.

## METHODS

The study is a descriptive review of qualitative research that described certain phenomenon [(Maxwell, 1992); (Boydell, Stasiulis, Volpe & Gladstone, 2010); (Patton & Westby, 1992); (Laanterä, Pölkki & Pietilä, 2011)] and using Observations and Critical thinking as tools to analyze premises. Thus, profoundly pondered this study and





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generated overall concept and logical interpretation to convene the readers or else provides justifications, arguments that simply answer their doubts onwards to the teaching-learning in a midst of crisis because of Covid-19. (Stahl & Stahl, 1991; Baker & Campbell, 2004; Fisher, 2011). The study showcase eventually the effects of this epic towards the teaching-learning process of certain State Universities and Colleges (SUCs) which set to be an example or basis. Mainly, theories of Harvey (2002); McPhee (2002), and Ansell, Sørensen & Torfing (2020) were reviewed to critically analyzed on how their theories augmented the ability of teachers to uphold their duties within the scheme of Covid-19, and introduced the previous studies of Xu, Elomri, Kerbache, & El Omri (2020) to resolute against this pandemic.

## RESULTS AND DISCUSSIONS

This Covid-19 has ruined the educational schemes, specifically in the case of the Surigao State College of Technology (SSCT) one of the State Universities Colleges (SUCs) in the Philippines. Whereby, the President of this institution has progressively ordered that all faculty should prepare a module; created a skeletal team as workforce; for humanitarian reasons allows Job orders and Guest lecturers to continually in the service; non-designated teachers were allowed to work at home and prepared logbook on their daily time records. But moreover the College President, issued office orders in some other times that proclaimed schedules of face-to-face and modified class program (blended learning). Then, lately issued again another memorandum order to suspend the face-to-face classes but continue the teaching-learning process using G-suite, Facebook, and YouTube. The study generated three themes:

### Confused teaching strategies

These scenarios established conflicted opinions among faculty, staff, and students yet, the management were determined and enlightened everyone to be more attentive of the office orders thereby imposed that due to local transition of disease relative to the "Modified Enhance Community Quarantine" of the Executive Order No. 56, Series of 2020, Office of the Mayor, Surigao City (MyWay, n.d.). This is also consonant to the Situational report No. 48, of the National Disaster Risk Reduction and Management Council (NDRMMC), Republic of the Philippines (NDRRMC, n.d.), see screen shoot shot in Figure 1.

### Students' capacity on adapting online classes

Subsequently, these report tended some of the students to drop out from the lists, and this scenario shortly impaired among faculty and students in joining G-suites, Facebook group chats, viewing blogs in You Tubes, attending meetings in Zoom besides the signals of internet is unstable, and their financial instability constrained the e-learning activities (Masuhay, 2020). Uncertainties duels in the mind and hearts of teachers and students which to prefer or prioritize either their lives safety or to secure their future endeavors.

### Implications for teacher's management

Teachers' teaching was confused about what they cared off if this is all about their accountability to students or complying with the institutional standards substantiated for their students learnings. Yet, teachers tried to be responsive on how to substantiate their students learning with their creativity and teaching-learning tactics, and electronic classroom innovations of this era as what Harvey (2002) emphasized. Thus, rigid meetings and discussions were done to solicit and strategies their modus operandi in teaching and monitoring. Aside from module, some electronics applications have been adopted such as G-Suite, Facebook, YouTube, Zoom, etc. just to employ quality teaching and discussions, class monitoring.

Although McPhee (2002) over-emphasized it that "Problem-based learning (PBL) is not new, as it is originated by Dewey and cited by Menon (1997) as pointed out that it has been around for a considerable time, by century. Yes, it is also agreeable that this theory is sustainable in this present day hence, though teachers, parents, and students hardly attained their responsibilities in terms of monetary and budgetary prerequisites during the learning process,





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with time constraints, and suppressed their good intentions in attending classes or pursuing e-learning activities this scenario progressively brought them to stay at home and limit their expenses e.g. transportation and stipend, boarding house rentals. Then, took their studies sincerely with module as their ultimate guide, wherein G-suite, Facebook, YouTube, Zoom, etc. are their tools for the teaching-learning process, during submission of outputs, and class monitoring. This study is confirmed to what Ansell, Sørensen & Torfing (2020) claims that this Covid-19 is not only a complex problem, but also turbulent problem characterized by the surprising emergence of inconsistent, unpredictable, and uncertain events, this is also relative to the report of Xu, Elomri, Kerbache, & El Omri (2020) that "The Covid-19 pandemic is not the first disaster that abruptly damaged Global Supply Chains (GSCs). Several other natural catastrophes, such as the 2011 mega-earthquake in Japan (Takami, WON & Kawamura, 2013), the 2003 SARS outbreak in China (Heymann, 2004), and the 2004 tsunami in Indonesia (Paris, 2009) have led to shortages of parts and products. It is worth noting that production is recovered from these disasters in a matter of weeks. However as shown in Figure 2, based on the scope and magnitude, the Covid-19 are different from those of all previous events.

## CONCLUSION

Although, the study showcase literature that demeans to sustain education momentarily for safety reasons, as if there is no other precious thing than life. The Surigao State College of Technology (SSCT) from one of the SUCs in the Philippines were still committed in complying their institutional standards and substantiated their students' learnings during this pandemic. The philosophies of Harvey (2002); McPhee (2002); Ansell, Sørensen & Torfing (2020); and Xu, Elomri, Kerbache, & El Omri (2020) confirming to the teaching-learning process of SSCT and so, their teachers found to be flexible to their modus operandi in teaching while committed to substantiate their students learning.

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## REFERENCES

1. Anderson, R. M., Heesterbeek, H., Klinkenberg, D., & Hollingsworth, T. D. (2020). How will country-based mitigation measures influence the course of the COVID-19 epidemic?. *The Lancet*, 395(10228), 931-934;
2. Ansell, C., Sørensen, E., & Torfing, J. (2020). The COVID-19 pandemic as a game changer for public administration and leadership? The need for robust governance responses to turbulent problems. *Public Management Review*, 1-12;
3. Baker, D., & Campbell, C. (2004). Fostering the development of mathematical thinking: Observations from a proofs course. *Problems, Resources, and Issues in Mathematics Undergraduate Studies*, 14(4), 345-353;
4. Boydell, K. M., Stasiulis, E., Volpe, T., & Gladstone, B. (2010). A descriptive review of qualitative studies in first episode psychosis. *Early intervention in psychiatry*, 4(1), 7-24;
5. Fisher, A. (2011). *Critical thinking: An introduction*. Cambridge University Press;
6. Harvey, L. (2002). Evaluation for what?. *Teaching in higher education*, 7(3), 245-263;



**Edilmar P. Masuhay et al.**

7. Heymann, D. L. (2004). The international response to the outbreak of SARS in 2003. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, 359(1447), 1127-1129;
8. Ji, Y., Ma, Z., Peppelenbosch, M. P., & Pan, Q. (2020). Potential association between COVID-19 mortality and health-care resource availability. *The Lancet Global Health*, 8(4), e480;
9. Laanterä, S., Pölkki, T., & Pietilä, A. M. (2011). A descriptive qualitative review of the barriers relating to breast-feeding counselling. *International Journal of Nursing Practice*, 17(1), 72-84;
10. Masuhay, E. P. (2020). Adversaries on Covid-19 Set Forth an Argument Onward To Educational Endeavor: Resulting To Develop a Modular Concept in the Learning Process. *American International Journal of Social Science Research*, 5(2), 26-37;
11. Maxwell, J. (1992). Understanding and validity in qualitative research. *Harvard educational review*, 62(3), 279-301;
12. McPhee, A. D. (2002). Problem-based learning in initial teacher education: taking the agenda forward. *The Journal of Educational Enquiry*, 3(1);
13. MyWay. (n.d.). Retrieved October 4, 2020, from <https://int.search.myway.com/search/GGmain.jhtml?p2=%5EY6%5Echr827%5ETTAB03%5EPH&ptb=FA9A7724-813D-4169-AA96-D1312DB1A4B6&n=7867ff00&ln=en&si=&trs= wtt&brwsid=&st=tab&tpr=sc&searchfor=%E2%80%9CModified+Enhanced+Community+Quarantine%E2%80%9D+of+the+Executive+Order+No.+56%2C+Series+of+2020%2C+Office+of+the+Mayor%2C+Surigao+City&ots=1601811171772;>
14. NDRRMC, Office of Civil Defense, (N.d.). Retrieved October 4, 2020, from [https://www.ndrrmc.gov.ph/;](https://www.ndrrmc.gov.ph/)
15. Nicola, M., Alsafi, Z., Sohrabi, C., Kerwan, A., Al-Jabir, A., Iosifidis, C., & Agha, R. (2020). The socio-economic implications of the coronavirus pandemic (COVID-19): A review. *International journal of surgery (London, England)*, 78, 185;
16. Paris, R., Wassmer, P., Sartohadi, J., Lavigne, F., Barthomeuf, B., Desgages, E., & Gomez, C. (2009). Tsunamis as geomorphic crises: lessons from the December 26, 2004 tsunami in Lhok Nga, west Banda Aceh (Sumatra, Indonesia). *Geomorphology*, 104(1-2), 59-72.
17. Patton, M., & Westby, C. (1992). Ethnography and research: A qualitative review. *Topics in Language Disorders*;
18. Rundle, A. G., Park, Y., Herbstman, J. B., Kinsey, E. W., & Wang, Y. C. (2020). COVID-19–Related School Closings and Risk of Weight Gain Among Children. *Obesity*;
19. Stahl, N. N., & Stahl, R. J. (1991). We can agree after all! Achieving consensus for a critical thinking component of a gifted program using the Delphi technique. *Roeper Review*, 14(2), 79-88;
20. Takami, H., WON, N. I., & Kawamura, T. (2013). Impacts of the 2011 mega-earthquake and tsunami on abalone *Haliotis discus hannai* and sea urchin *Strongylocentrotus nudus* populations at Oshika Peninsula, Miyagi, Japan. *Fisheries Oceanography*, 22(2), 113-120;
21. Viner, R. M., Russell, S. J., Croker, H., Packer, J., Ward, J., Stansfield, C., & Booy, R. (2020). School closure and management practices during coronavirus outbreaks including COVID-19: a rapid systematic review. *The Lancet Child & Adolescent Health*;
22. Xu, Z., Elomri, A., Kerbache, L., & El Omri, A. (2020). Impacts of COVID-19 on global supply chains: facts and perspectives. *IEEE Engineering Management Review*, 48(3), 153-166.





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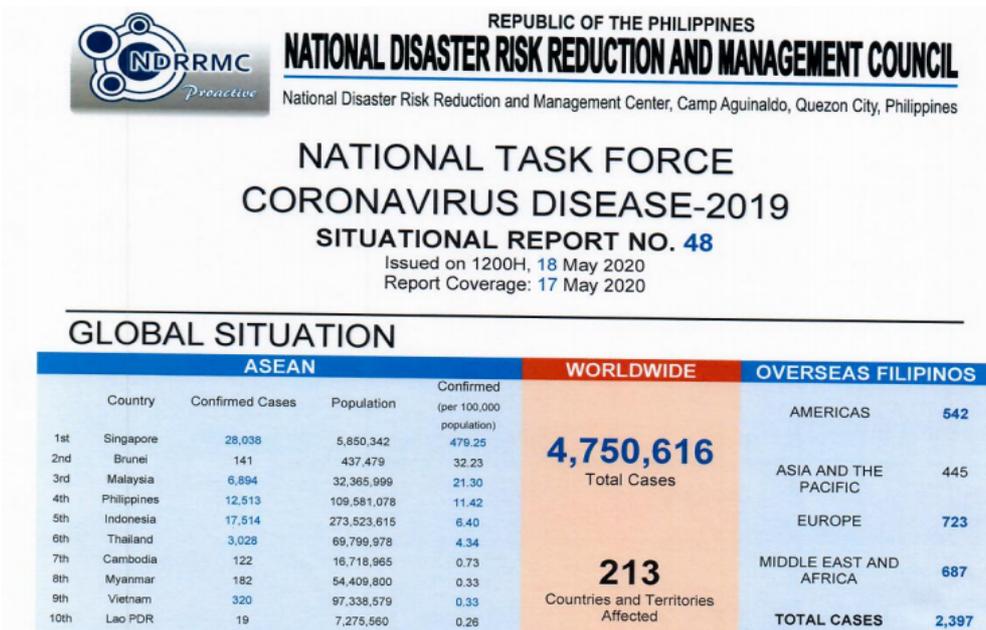


Figure 1. Situational report No. 48, of the National Disaster Risk Reduction and Management Council (NDRMMC), Republic of the Philippines(NDRRMC, n.d.).

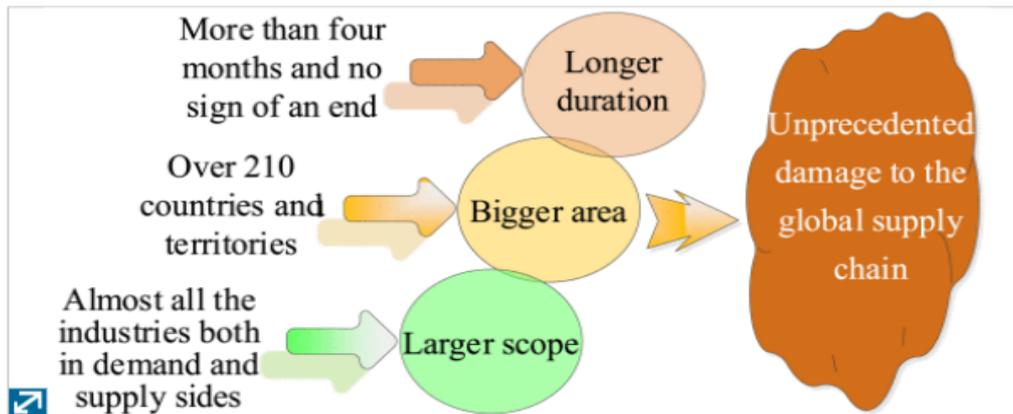


Figure 2. Features of the damage of the Covid-19 pandemic o GSCs (Xu, Elomri, Kerbache & El Omri, 2020).





## A Review on the Studies Conducted on *Coleus amboinicus*

Deepa Jose<sup>1</sup>, Sini Baby<sup>2</sup>, Devika Firose<sup>3</sup> and Aswathy Surendran<sup>3\*</sup>

<sup>1</sup>Professor, Vice Principal, Nirmala College of Pharmacy, Muvattupuzha, Ernakulam, Kerala, India.

<sup>2</sup>Associate Professor, Nirmala College of Pharmacy, Muvattupuzha, Ernakulam, Kerala, India.

<sup>3</sup>B.Pharm Student, Nirmala College of Pharmacy, Muvattupuzha, Ernakulam, Kerala, India.

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### \*Address for Correspondence

#### Aswathy Surendran

B.Pharm Student,

Nirmala College of Pharmacy,

Muvattupuzha, Ernakulam,

Kerala, India.

E.Mail: aswathysurendran243@gmail.com



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### ABSTRACT

*Coleus amboinicus* Lour belonging to the family *Labiatae*, is a perennial subshrub, erect and nearly reaching a meter in height. This plant is believed to be indigenous to the Moluccas. It was long ago introduced into many areas of the Old-World tropics and some of the Pacific islands. It was often used as a substitute for borage or thyme because of its aromatic leaves. *Coleus amboinicus* (CA) is known to exert different medicinal properties such as antimicrobial, analgesic, antidandruff, diuretic, fungitoxic and antioxidant activities.

**Keywords:** *Coleus amboinicus*, biological activities, nanoparticles, inotropic effects, catalytic activity.

### INTRODUCTION

Nature is a source of medical agents. A large number of modern drugs have been isolated from natural sources based on their use in traditional medicines. Plants from the genus *Coleus* have been used in traditional medicine in many parts of world. The major reported phytoconstituents of the *Coleus* species are flavonoids, glycosides, phenolic compounds and volatile constituents [1] *Coleus amboinicus* (*Plectranthus amboinicus*) belongs to the family *Lamiaceae* (*Labiatae*). It is a semi-succulent plant with a pungent oregano-like flavor and odor (Fig: 1, 2). Tropical region is mainly selected for the cultivation of *Coleus amboinicus* where it is used as a spice and ornamental plant. Indian borage, country borage, French thyme, Indian mint, Mexican mint, Cuban oregano, soup mint and Spanish thyme are the common names in English [2]. It is used as medicine in treating various diseases, such as diarrhoea, flatulence, constipation, cough, chronic asthma, bronchitis and malaria and also used as food and food additives.. *In vitro* studies reveals that the extract of *C. amboinicus* has antioxidant capacity, antibacterial, cytotoxic, anti-





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inflammatory and hepatoprotective activity. *In vivo* studies using animals and human experiments showed that this plant possess anti-diabetic activity, immunomodulator, analgesic and lactagogue property [3].

#### Taxonomic Classification [4]

Kingdom: Plantae  
Division: Magnoliophyta  
Class: Magnoliopsida  
Subclass: Asteridae  
Order: Lamiales  
Family: Lamiaceae  
Genus: Coleus  
Species: amboinicus

#### CULTIVATION

*Coleus amboinicus* is a fast-growing plant, grows easily in a well-drained, semi-shaded location [5]. *C. amboinicus* is usually propagated by stem cuttings. It is a fast-growing plant. Commonly grown in gardens and indoors in pots. Stem cuttings is preferred propagation because it rarely seeds or sets seeds. It cannot withstand temperatures lower than 0 °C and is stressed even when it is colder than 10 °C. The plant grows best in rich, compost soil with neutral pH and high humidity, but if there is excess water in the ground its roots start to rot [6].

#### PHARMACOLOGICAL ACTIVITIES

##### Antimicrobial Activity

An endophytic fungus was isolated from the leaves of *Coleus amboinicus* Lour., by Ding et al. (2010). Preparative Thin Layer Chromatography is used for the isolation and purification of compounds. Antimicrobial activity was evaluated against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Salmonella typhi* and *Staphylococcus mutans*, by determining its IC<sub>50</sub> and MBC (Minimum Bactericidal Concentration) values. Modified microdilution method was used to determine the IC<sub>50</sub> and MBC values of isolated compounds. By spectroscopic data the structure of the bioactive compound was deduced (Fig: 3).

The crystal showed a melting range of 202.37°C – 203.40°C. Structure elucidation was conducted based on analysis of FT-IR, LC-MS, 1H-NMR and 13C-NMR data. The study concluded that hemiterpenoid compounds isolated from an endophytic fungus *Atheliarolsii* showed potential antimicrobial activities [7]. Francisco Fábio et al determined the effectiveness of *Plectranthus amboinicus* (Lour.) Spreng against MRSA clinical isolates. Broth microdilution and bioautography was used to determine the *in vitro* antimicrobial activity of the hydroalcoholic extract (HE), the ethyl acetate (EA) fraction ciprofloxacin and its sub fractions against MRSA clinical isolates. *In vitro* drug combination studies were assessed by the microdilution checkerboard method. Bacterial suspensions were added to Citodex in order to induce abscess formation, and inoculated subcutaneously into male Swiss mice. The treatment protocol consisted of intraperitoneal administration of 2 doses of HE, the EA fraction or vancomycin into mice at 3 and 12 h after infection. Lowest minimal inhibitory concentrations (MIC, 0.25 to 0.5 mg/mL) were presented by the EA fraction and its subfractions. Plant samples were bactericidal at 100 mg/mL and bacteriostatic at 2x and 4x MIC. The EA fraction presented an additive effect with ciprofloxacin and synergism with vancomycin. In the HE and EA fraction-treated groups, reduction of abscess volume, bacterial cell counts in abscess slurries, and inflammatory scores was expressed. The samples can be used in treating the animals in a dose-dependent fashion. The above study revealed the effectiveness of *P. amboinicus* fractions against MRSA clinical isolates [8].



**Deepa Jose et al.****Antidandruff Activity [9]**

Leaves of *Coleus amboinicus* and *Eucalyptus globules* were used for performing antidandruff activity. The essential oils from the shade dried leaves were obtained by hydro distillation procedure. The chemical composition of hydro distilled essential oils was analyzed by Gas Chromatography-Mass spectrometry (GC-MS). The pure essential oils and the essential oils diluted with coconut oil were used to check antidandruff activity against the dandruff causing fungus *Malassezia furfur* by agar well diffusion assay. The results of GC-MS explained the presence of eight compounds from *Coleus amboinicus* essential oil. The major component was identified as Thymol (41.3%) in *Coleus amboinicus*. In the antidandruff activity study, an inhibitory zone of 31mm was observed for *Coleus amboinicus*. Ketoconazole based shampoo was used as a standard.

**Anti Epileptic Activity [10]**

Fresh leaf juice of *C. Amboinicus* was used to evaluate antiepileptic activity on male albino rats of wistar strain. Generalized tonic clonic seizures were induced in the animals by using an electroconvulsimeter at 150 mA intensity of current for 0.2 sec using a pair of ear clip electrodes. A pretest was done by inducing MES (Maximum electric shock) in the above-mentioned strength and animals showing all the three phases of flexion, extension followed by clonus were selected. The selected animals were grouped into 6 groups (n=6). 24 hrs after pretest, MES was induced as discussed in the above manner one hour after treatment with fresh leaf juice of *C. Amboinicus*. The test juice significantly stimulated Na<sup>+</sup>/K<sup>+</sup> and Ca<sup>2+</sup>- ATPases levels in the rat whole brain in a dose dependent manner. It also showed dose dependent significant (p<0.05) increase in the GABA levels in the rat whole brain. Hence *C. Amboinicus* leaves may have the capacity to inhibit different types of seizures induced by the experimental models either by interaction with Na<sup>+</sup> and Ca<sup>2+</sup> channels or by enhancing the GABA activity.

**Lactogenic Activity [11]**

The lactogenic property of ethyl acetate fraction of *Coleus amboinicus* L leaves was assessed and identified compounds acted as 'milk booster' using LC- MS approach. Quantity of milk produced from the rats treated with commercial milk booster, ethyl acetate and water fractions of leaves and kaempferol were used to evaluate the lactagogue activity. Milk production was measured by weigh –suckle-weigh method and ELISA method was used to determine prolactin serum level. Mammary gland, liver, intestines and kidney tissues were subjected to Histopathological analysis. The study revealed that the ethyl acetate fraction of *Coleus amboinicus* L leaves and its bioactive compounds can stimulate and improve milk production.

**Antioxidant Activity [12]**

Roshan D. Patel et al., 2010 extracted coarsely powdered, air dried leaves of *Plectranthus amboinicus* (Lour) Spreng using petroleum ether, chloroform, ethanol and water by cold maceration in increasing order of their polarity. In addition to this, the fresh powder was defatted with pet ether and extracted with 95% ethanol (72 hours) and water (24 hours) separately. The extracts showed a significant antioxidant activity when they were assessed by various in vitro methods such as 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Nitric Oxide screening method and reducing power assay (Fig: 4)

**Antimalarial Activity [13]**

According to Periyanyagam K et al., aqueous extract of the leaves of *P. amboinicus* shows antimalarial activity. They carried out *in vivo* study of the extract on *Plasmodium bergheyoellii* on laboratory infected albino mice and compared with standard drug chloroquine. Decrease of parasitaemia at 250 mg/kg and 500 mg/kg of aqueous extract for 24 hrs, 48 hrs, 72 hrs and 96 hrs were observed. The decrease of parasitaemia after 96 hrs was 100%, 67.9% and 76.2% for standard, 250 mg/kg and 500 mg/kg of aqueous extract respectively.



**Deepa Jose et al.****Diuretic Activity [14]**

Roshan Patel et al., evaluated the ethanolic and aqueous extract of *Plectranthus amboinicus* for its diuretic activity in male albino rats by the method described by Lipschitz *et al.* At the dose level of 500 mg/kg b.wt, they reported that, there was a significant diuresis ( $P < 0.01$ ) on treatment with single dose of the ethanolic extract ( $1.37 \pm 0.06$ ) and aqueous extract ( $1.18 \pm 0.07$ ) of *Plectranthus amboinicus* (Lour). After the administration of dose, it was comparable to significant ( $P < 0.01$ ) diuresis produced by standard furosemide ( $1.41 \pm 0.08$ ), and when compared with the control rats ( $0.63 \pm 0.10$ ) after 5 hours. In order to obtain cumulative urinary excretion the urine output was stimulated throughout the study period, such that the cumulative urinary excretion was found significant ( $P < 0.01$ ) in rats treated with furosemide and extracts when compared with the control after 24 h of drug administration.

**Treatment of Rheumatoid Arthritis [15]**

Jia-Ming Chang et al., investigated the therapeutic efficacy of *P. amboinicus* in treating Rheumatoid Arthritis (RA) with the help of collagen-induced arthritis animal model. By immunization with bovine type II collagen, arthritis was induced in Lewis rats. In the Serum the presence of anti-collagen IgG, IgM and C-reactive protein (CRP) were analyzed. Production of TNF- $\alpha$ , IL-6 and IL-1 $\beta$  from peritoneal exudates cells (PEC) were analyzed to understand the inflammation condition of treated animals. *P. amboinicus* significantly blocked the footpad swelling and arthritic symptoms in collagen-induced arthritic rats, and the serum anti-collagen IgM and CRP levels were consistently decreased. In the high dose of *P. amboinicus* the production of pro-inflammatory cytokines TNF- $\alpha$ , IL-6 and IL-1 $\beta$  were also reduced. As per the results they reported that *P. amboinicus* shows the ability to treat collagen-induced arthritis in rats.

**Positive Inotropic effect [16]**

According to R.C. Hole et al., the aqueous leaf extract of *Coleus amboinicus* produces positive inotropic effect on isolated perfused frog heart preparation. They extracted fresh leaves of *C. amboinicus parent* (CAP) as well as tissue culture-raised plant (CAT) with boiling distilled water. The frogs heart was perfused with Ringer solution (pH 7.8). Graded doses of CAP and CAT (50–800  $\mu$ g) were added and responses were recorded. In order to study the interaction of CAP and CAT with other agents, the hearts were perfused with solution containing propranolol ( $1 \times 10^{-7}$  M), atropine ( $1 \times 10^{-7}$  M), verapamil ( $1 \times 10^{-7}$  M), imidazole ( $1 \times 10^{-4}$  M) and lignocaine ( $2.5 \times 10^{-4}$  M) for 15 min and the responses to CAP and CAT were recorded. In another set of experiments, the perfusion fluid was replaced by frog Ringer containing one half of the sodium chloride (55.6 mM) or double the sodium chloride (222 mM) concentration. Equimolar sucrose solution was added and the responses for CAP and CAT were taken.

From the responses CAP and CAT induced positive inotropic effects were not altered by propranolol, the beta-receptor antagonist, or by atropine, the alpha-receptor antagonist. The data revealed that CAP as well as CAT induced positive inotropism does not involve any adrenergic receptors. It is clear that, the parent and the tissue culture plant extracts produce positive inotropic effects which may be attributed to increase in sodium influx, thereby causing greater intracellular availability of calcium.

**Antibacterial Activity [17]**

Krithi et al., evaluated the antibacterial efficacy of *Plectranthus amboinicus* against *Streptococcus mutans* through Disc Diffusion Test and Minimum Inhibitory Concentration. *Streptococcus mutans* was found to be sensitive to ethanolic and aqueous extracts of *Plectranthus amboinicus*.

**Wound Healing Activities**

*Plectranthus amboinicus* has the ability to increase the healing process of wounds. It is a powerful immune stimulant which aids the removal of microorganisms that retard healing process and for the maintenance of path of normal progress. It also shows anti-inflammatory property. *Plectranthus amboinicus* contain a high content of zinc, which supports important functions to promote and facilitate the healing of wounds<sup>[9]</sup>.



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Smitha Shenoy et al., evaluated the healing of burn wounds in wistar rats using ethanolic extract of the leaf of *Plectranthus amboinicus* in comparison with silver sulfadiazine. Under ketamine anaesthesia burn wounds were made on each rat. The wounds in the five groups of rats were treated topically with petroleum base, silver sulfadiazine, 1%, 2% and 3% ointment of ethanolic extract of *Plectranthus amboinicus*, respectively, once daily for 21 days. The rate of wound contraction was significantly more in *Plectranthus amboinicus* treated groups in comparison to the control. The mean period of epithelization was significantly reduced in *Plectranthus amboinicus* treated group when compared to control ( $P < 0.01$ ) and silver sulfadiazine ( $P = 0.02$ ) treated group. The study concluded that ethanolic extract of *Plectranthus amboinicus* promoted healing of burn wound in wistar rats [18].

K Muniandy et al., studied the ethanolic and aqueous extract of *C. aromaticus* leaves and roots (100 mg/kg per day for 10 days) for their wound healing activity in Monosodium Glutamate induced diabetic mice using excision and dead space wound models. To measure the progress of wound area "Excision wound model" was performed on five different groups of mice for ten consecutive days. On four different groups of mice "Dead space wound model" was carried to determine wet and dry granulation tissue and to examine the histological appearances of granulation tissue after ten days. Ethanolic extract treated wounds showed 76.6% of wound area reduction when compared with control that exhibited 55.9% of wound area reduction. The wounds treated with ethanolic extract epithelised faster when compared to controls. The results revealed that *Coleus aromaticus* can exhibit wound healing activity in induced diabetic mice [19].

**Anti-Inflammatory and Antitumor Activities [20]**

Ana Pavla et al evaluated the anti-inflammatory and antitumor activities of the hydroalcoholic extract from leaves of *P. amboinicus*. Carrageenan-induced paw edema method was used to determine the anti-inflammatory activity. By using the Sarcoma-180 and Ehrlich ascite carcinoma the antitumor effect was evaluated in an in vivo experimental study. There was statistically significant decrease ( $p < 0.05$ ) of edema paw in the doses of 150, 250 and 350 mg/kg (i.p.) of the hydroalcoholic extract of *P. amboinicus*. Similarly, the administration of *P. amboinicus* at the doses of 100, 150, 250 and 350 mg/kg (i.p.) inhibited the growth of sarcoma-180 and Ehrlich ascite carcinoma tumors in mice. The results suggested that the hydroalcoholic extract of *P. amboinicus* possesses anti-inflammatory and antitumor activities.

**Analgesic and Anti-Inflammatory Properties [21]**

Yung-Jia Chiu et al., investigated the analgesic and anti-inflammatory properties of the aqueous extract from *Plectranthus amboinicus* in vivo and in vitro. It inhibited pain induced by acetic acid and formalin, and inflammation induced by carrageenan. The anti-inflammatory effect was related to modulation of antioxidant enzyme activities in the liver and decrease of the Malondialdehyde (MDA) level and the production of tumor necrosis factor alpha (TNF- $\alpha$ ), and cyclooxygenase 2 (COX-2) in edema-paw tissue. In vitro studies explained that aqueous extract from *Plectranthus amboinicus* inhibited the pro inflammatory mediators in RAW 264.7 cells stimulated with lipo polysaccharide (LPS). It also blocked the degradation of I $\kappa$ B- $\alpha$  and nuclear translocation of NF- $\kappa$ B p65 subunit.

**Antinociceptive And Antipyretic Effects [22]**

Roshan Dhruv Patel et al studied the Antinociceptive and Antipyretic Effects of *Plectranthus amboinicus* (Lour) Spreng using acetic acid-induced writhing and tail immersion tests in mice and yeast-induced pyrexia in rats. Significant ( $P < 0.01$ ) antinociceptive and antipyretic effects were produced by both extracts (500 mg/kg, p.o.).

**Anti-Gastric Ulcer Activity [23]**

Rama Mohan Gupta et al performed a study to evaluate Anti gastric ulcer activity of *Plectranthus amboinicus* (Lour) in pylorus ligation-induced ulcer model in wistar albino rats in which ability of different extract of *Plectranthus amboinicus* (Lour) was tested at dose level of 200mg/kg body weight orally and compared with Ranitidine (30mg/kg)



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as standard. The study concluded that the various extracts of *Plectranthus amboinicus* (Lour) (200mg/kg) showed highly significant anti-ulcer activity.

**Hepatoprotective Activity [24]**

Smita Shenoy *et al.*, evaluated the hepatoprotective activity of ethanolic extract of *Plectranthus amboinicus* against paracetamol induced hepatotoxicity in wister rats in six groups. Gum acacia and paracetamol were given to two control groups orally respectively. To the three test groups paracetamol were given orally followed by 300, 600 and 900 mg/kg of ethanolic extract of *plectranthus amboinicus* respectively. The sixth group (standard hepatoprotective) is treated with paracetamol followed by N-acetylcysteine 100 mg/kg orally. The hepatoprotective activity was determined by estimating serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, tissue malondialdehyde levels and by histopathological analysis of the liver tissue. Analysed the results using one-way ANOVA followed by Dunnett's multiple comparison test. *Plectranthus amboinicus* in doses of 600 mg/kg and 900 mg/kg undoubtedly ( $p < 0.05$ ) altered paracetamol induced changes in the serum and tissue enzyme levels to near normal values. It also improved the liver histopathology profile. The study indicated that the ethanolic extract of *Plectranthus amboinicus*, possesses hepatoprotective activity against paracetamol induced hepatotoxicity in rats.

**Skin Conditions [25]**

*Plectranthus amboinicus* is the most commonly cited species for the treatment of burns, wounds, sores, insect bites and allergies. It is used in Brazil for the treatment of skin ulcerations caused by *Leishmania braziliensis* and also it is used in Saudi Arabia for diaper rash and itching, as well as an antiseptic dressing.

**Nanochemical studies****Biological synthesis of gold nanoparticles by using the leaf extract of *Coleus amboinicus* Lour**

By using UV–Vis spectroscopy, XRD, TEM and SAED analysis the nanoparticles were structurally characterized. 4.6 to 55.1 nm were the size range of gold nanoparticles. *C. amboinicus* leaf extract caused bio-reduction of gold ions which resulted in the synthesis of spherical, truncated triangle, triangle, hexagonal and decahedral nanoparticles. With the help of SPR band centered at 536 nm of UV–Vis absorption spectra revealed the reduction of gold ions to gold nanoparticles in 1 h. Based on the fcc structure of gold nanoparticles intense peaks corresponding to (111), (200), (220) and (311) Bragg's reflection were observed in XRD analysis. The formation of spherical, triangle, truncated triangle, hexagonal and decahedral morphologies with an average size of  $20.5 \pm 11.45$  nm were revealed in HR-TEM analysis. The colour change of pale yellowish reaction mixture to pink-ruby red after 1 h indicated the formation of gold nanoparticles. It is due to coherent reaction [26].

**Extracellular synthesis of silver nanoparticles using the leaf extract of *Coleus amboinicus* Lour**

The size and shape of silver nanoparticles are typically measured using analytical techniques such as TEM, scanning electron microscopy (SEM) or atomic force microscopy (AFM) [27]. *C. amboinicus* leaf extract act as a bio reducing and stabilizing agent for the synthesis of silver nanoparticles and its ability to modulate the size and shape. UV–vis absorption spectra explained the synthesis of silver nanoparticles from 103 M aqueous AgNO<sub>3</sub> in the presence of *C. amboinicus* leaf extract at 437 nm in 6 hrs. Intense peaks corresponding to (1 1 1), (2 0 0), (2 2 0) and (3 1 1) Bragg's reflection was showed on XRD analysis of the nanoparticles based on the face-centered cubic (fcc) structure of silver. HR-TEM analysis revealed the formation of anisotropic nanostructures of triangles, truncated triangles, decahedral and little spherical morphologies in the range of 2.6– 79.8 nm in size with an average of 35.8 nm. The change of colour to yellowish brown shows the formation of silver nanoparticles due to the coherent oscillation of electron gas at the surface of nanoparticles resulting in surface plasmon resonance (SPR).The study clearly indicated that, silver metal ions have been reduced from anisotropic nanostructures to isotropic spherical nanoparticles with increase in the concentration of leaf extract of *C. amboinicus* [28].





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**Ultrasonically driven green synthesis of palladium nanoparticles by *Coleus amboinicus***

To reduce the palladium chloride into palladium nanoparticles (PdNPs@CA) ultrasonically driven bio-reduction method was used using *coleus amboinicus* extract. The UV–vis spectrum confirmed the formation of Pd nanoparticles by the disappeared peak at 480 nm of PdCl<sub>2</sub> solution. Formation of phase pure cubic Pd nanoparticles with the crystallite size range of 40–50 nm were confirmed using XRD. In order to determine the catalytic behaviour with ultrasonic frequency of 40 kHz and power of 150 W, synthesized PdNPs@CA were explored in Suzuki-Miyaura coupling reaction and its recycling ability was determined. Biaryl compound is obtained by reacting Aryl halides with aryl boronic acids with excellent reaction yields in the presence of PdNPs@CA only using PEG-400 as a green solvent [29].

**Insecticidal Activity [30]**

The leaf oil of *Coleus amboinicus* from India is rich in thymol and is a potent insecticide against *O. obesus*, a pest of sugarcane fields. The oil was insecticidal to white termites (*Odontotermes obesus* Rhamb.) with 100% mortality at a dose of  $2.5 \times 10^{-2}$  mg/cm<sup>3</sup> for 5 h exposure. This oil was also more active than the synthetic insecticides, Thiodan and Primoban-20, against termites, although it was ineffective against *Tribolium castaneum*, a stored product pest.

**CONCLUSION**

*Coleus amboinicus* is a traditional medicinal plant having multiple pharmacological actions. The studies revealed a wide range of biological properties and the studies also proved that *C.amboinicus* is effective in curing respiratory, skin, cardiovascular, digestive, urinary and various other diseases. It also possesses a powerful wound healing property, antimicrobial activity antidandruff activity, anti-epileptic activity, lactogenic activity, antioxidant activity, antimalarial activity, diuretic activity, positive inotropic effect, antibacterial activity, anti-inflammatory, antitumor activities, analgesic, antinociceptive, antipyretic effects, hepatoprotective activity, skin conditions. *C.amboinicus* also possess insecticide activity and its oil is rich in thymol. *Coleus amboinicus* leaf extract can be used for synthesis of gold, palladium, silver nanoparticles. One of the main advantages is that, it shows more effective when given in combination with other medicinal plants. The plant has huge future prospects in meeting the global demand for natural, cost-effective and safer bioactive molecules in pharmaceutical and nutraceutical industries.

**REFERENCES**

1. Himesh Soni& Akhilesh Kumar Singhai. Recent updates on the genus Coleus: Review. Asian Journal of Pharmaceutical and Clinical Research. Vol 5, Issue 1, 2012 ISSN - 0974-2441
2. "Plectranthus amboinicus (Indian borage), Datasheet, Invasive Species Compendium". Centre for Agriculture and Biosciences International. 23 November 2017. Retrieved 13 March 2020.
3. Adnan Khattak,Torbangun (*Coleus amboinicus*Lour) Extracts Affect Microbial and Fungus Activities.,Journal of Nutritional Therapeutics, January 2013
4. Punet Kumar, Sangam, Nitin Kumar. *Plectranthusamboinicus*: a review on its pharmacological and pharmacognostical studies. American journal of physiology. 2020;10(2):55-62.
5. Khan MC. Current Trends in *Coleus Aromaticus*: An Important Medicinal Plant. Booktango; 2013.
6. GreethaArumugam, Mallappa Kumara Swamy, Uma Rani Sinniah. *Plectranthusamboinicus* (Lour.) Spreng: botanical, phytochemical, pharmacological and nutritional significance. Molecules. 2016;21(4):369.
7. PujiAstuti, RollandoRollando, SubagusWahyuono, AriefNurrochmad. Antimicrobial activities of isoprene compounds produced by an endophytic fungus isolated from the leaves of *Coleus amboinicus* Lour. Journal of Pharmacy &Pharmacognosy Research. 2020;8(4):280-
8. de Oliveira FF, Torres AF, Gonçalves TB, Santiago GM, de Carvalho CB, Aguiar MB, Camara LM, Rabenhorst SH, Martins AM, Valença Junior JT, Nagao-Dias AT. Efficacy of *Plectranthusamboinicus* (Lour.) Spreng in a murine

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- model of methicillin-resistant *Staphylococcus aureus* skin abscesses. Evidence-Based Complementary and Alternative Medicine. 2013 Jan 1;2013.
9. P.Selvakumar, B. Edhayanaveena, S.D. prakash. Studies on the antidandruff activity of the essential oil of *Coleus amboinicus* and *Eucalyptus globulus*. Asian Pacific Journal of Tropical Disease. 2012 ;2: S715-9.
  10. B. PushpaKumari, D. Sujatha, Ch. Gopi Chand, K. Divya, I. Malleswari, D. Ranganayakulu. Evaluation of antiepileptic activity and probable mechanism of action of *Coleus amboinicus* in MES and PTZ models. Journal of Pharmacy Research. 2012;5(3):1587-91.
  11. R M Damani, L Kustiyah, M Hanafi, A C Iwansyah. Evaluation lactogenic activity of ethyl acetate fraction of torbangun (*Coleus amboinicus* L.) leaves. In IOP Conference Series: Earth and Environmental Science 2017 (Vol. 101, No. 1, p. 012007). IOP Publishing.
  12. Roshan D. Patel, Naveen K. Mahobia, Manjul P Singh, Anita Singh, Naheed W. Sheikh, Gulzar Alam, Sudarshan K. Singh. Antioxidant potential of leaves of *Plectranthusamboinicus* (Lour) Spreng. Der Pharmacia Lettre. 2010;2(4):240-5.
  13. Periyannayagam K, Nirmala Devi K, Suseela L, Uma A, Ismail M. In vivo antimalarial activity of leaves of *Plectranthusamboinicus* (Lour) Spreng on *Plasmodium bergheiyoelii*. The Journal of communicable diseases. 2008 ;40(2):121-5.
  14. Roshan Patel, Naveen K Mahobia, Ravindra Gendle, Basant Kaushik, Sudarshan K Singh. Diuretic activity of leaves of *Plectranthusamboinicus* (Lour) Spreng in male albino rats. Pharmacognosy research. 2010;2(2):86.
  15. Jia-Ming Chang, Chun-Ming Cheng, Le-Mei Hung, Yuh-Shan Chung and Rey-Yuh Wu. Potential use of *Plectranthusamboinicus* in the treatment of rheumatoid arthritis. Evidence-Based Complementary and Alternative Medicine. 2010 Mar;7.
  16. Hole RC, Juvekar AR, Roja G, Eapen S, D'Souza SF. Positive inotropic effect of the leaf extracts of parent and tissue culture plants of *Coleus amboinicus* on an isolated perfused frog heart preparation. Food Chemistry. 2009 May 1;114(1):139-41.
  17. Nikhil, Krithi, & Yavagal, P. C. (2015). Antibacterial efficacy of *plectranthusamboinicus* extracts against *streptococcus mutans*- an invitro study. International Journal of Ayurveda and Pharma Research, 3(11).
  18. Shenoy S, Amberkar M, Amuthan A. Effect of ethanolic extract of *Plectranthusamboinicus* Leaf on healing of burn wound in Wistar rats. International Journal of Applied Biology and Pharmaceutical Technology 3(3):32-5. 2012
  19. Muniandy K, Hassan Z, Mohd IM. Wound healing activity of *Coleus aromaticus* in experimentally induced diabetic mice. Asian Journal of Microbiology, Biotechnology and Environmental Sciences. 15(4):627-638: 2013.
  20. Gurgel AP, da Silva JG, Grangeiro AR, Oliveira DC, Lima CM, da Silva AC, Oliveira RA, Souza IA. In vivo study of the anti-inflammatory and antitumor activities of leaves from *Plectranthusamboinicus* (Lour.) Spreng (Lamiaceae). Journal of Ethnopharmacology. 2009 Sep 7;125(2):361-3.
  21. Chiu YJ, Huang TH, Chiu CS, Lu TC, Chen YW, Peng WH, Chen CY. Analgesic and anti-inflammatory activities of the aqueous extract from *Plectranthusamboinicus* (Lour.) Spreng. both in vitro and in vivo. Evidence-Based Complementary and Alternative Medicine. 2012 Jan 1;2012.
  22. Patel R, Mahobia N, Singh S, Gendle R, Kaushik B, Patel V. Antinociceptive and Antipyretic effects of *Plectranthusamboinicus* (Lour) Spreng Leaves. Deccan J Nat Prod. 2010; 1:9-15.
  23. Devi MR, Subramanian NS, Gupta VR, Prasad BG, Reddy CM. Anti-gastric ulcer activity of *Plectranthusamboinicus* (Lour) in wistar albino rats. J Chem Pharm Res. 2010;2(3):374-80.
  24. Shenoy S, Kumar H, Nayak V, Prabhu K, Pai P, Warriar I, Madhav V, Bairy KL, Kishore A. Hepatoprotective Activity of *Plectranthusamboinicus* Against Paracetamol Induced Hepatotoxicity in Rats. International Journal of Pharmacology and Clinical Sciences. 2012;1(2).
  25. Catherine W. Lukhoba, Monique S.J. Simmonds, Alan J. Paton. *Plectranthus*: A review of ethnobotanical uses. Journal of ethnopharmacology. 2006 ;103(1):1-24.
  26. Kannan Badri Narayanan, Natarajan Sakthivel. Phytosynthesis of gold nanoparticles using leaf extract of *Coleus amboinicus* Lour. Materials characterization. 2010;61(11):1232-8.
  27. Oldenburg SJ. Silver nanoparticles: properties and applications. Sigma-Aldrich Co., nd. 2014.





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28. Kannan BadriNarayanan, NatarajanSakthivel. Extracellular synthesis of silver nanoparticles using the leaf extract of *Coleus amboinicus* Lour. Materials Research Bulletin. 2011;46(10):1708-13.
29. Chinna Bathula, Subalakshmi K, Ashok Kumar K, Hemraj Yadav, Sivalingam Ramesh, Surendra Shinde, Nabeen K Shrestha, K.Mallikarjuna, Haekyoung Kim. Ultrasonically Driven Green Synthesis of Palladium Nanoparticles by *Coleus Amboinicus* for Catalytic Reduction and Suzuki-Miyaura Reaction. Colloids and Surfaces B: Biointerfaces
30. Singh G, Singh OP, Prasad YR, De Lampasona MP, Catalan C. Studies on essential oils, Part 33: chemical and insecticidal investigations on leaf oil of *Coleus amboinicus* Lour. Flavour and fragrance journal. 2002 ;17(6):440-2.

Table 1. Studies Conducted on <i>Coleus amboinicus</i>	
ACTIVITY EVALUATED FOR:	ACTIVITY EVALUATED BY:
ANTIMICROBIAL ACTIVITY	PujiAstuti et al; in the year 2020 evaluated the Antimicrobial activities of isoprene compounds produced by an endophytic fungus isolated from the leaves of <i>Coleus amboinicus</i> Lour. And also, de Oliveira FF et al; in the year 2013 conducted antimicrobial studies on this plant.
ANTIDANDRUFF ACTIVITY	P.Selvakumar et al; in 2012 studied antidandruff activity of the essential oil of <i>Coleus amboinicus</i> and <i>Eucalyptus globulus</i> .
ANTI EPILEPTIC ACTIVITY	B. PushpaKumari et al; in the year 2012 evaluated antiepileptic activity and probable mechanism of action of <i>Coleus amboinicus</i> in MES and PTZ models.
LACTOGENIC ACTIVITY	R M Damanik et al; in the year 2017 evaluated lactogenic activity of ethyl acetate fraction of torbangun ( <i>Coleus amboinicus</i> L.) leaves.
ANTIOXIDANT ACTIVITY	Roshan D. Patel et al; in the year 2010 determined Antioxidant potential of leaves of <i>Plectranthus amboinicus</i> (Lour) Spreng.
ANTIMALARIAL ACTIVITY	Periyannayagam K, et al; in the year 2008 studied antimalarial activity of leaves of <i>Plectranthus amboinicus</i> (Lour) spreng on <i>Plasmodium bergheiyoelii</i> .
DIURETIC ACTIVITY	Roshan Patel et al; in 2010 determined Diuretic activity of leaves of <i>Plectranthus amboinicus</i> (Lour) Spreng in male albino rats.
TREATMENT OF RHEUMATOID ARTHRITIS	Jia-Ming Chang, et al; in the year 2010 evaluated the Potential use of <i>Plectranthus amboinicus</i> in the treatment of rheumatoid arthritis.
POSITIVE INOTROPIC EFFECT	Hole RC et al; in the year 2009 studied the Positive inotropic effect of the leaf extracts of parent and tissue culture plants of <i>Coleus amboinicus</i> on an isolated perfused frog heart preparation.
ANTIBACTERIAL ACTIVITY	Krithi et al; in the year 2015 determined Antibacterial efficacy of <i>plectranthus amboinicus</i> extracts against <i>streptococcus mutans</i> - an invitro study.





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WOUND HEALING ACTIVITIES	Shenoy S et al; in the year 2012 determined the effect of ethanolic extract of <i>Plectranthus amboinicus</i> Leaf on healing of burn wound in Wistar rats. And in 2013 Muniandy K et al; carried out studies on Wound healing activity of <i>Coleus aromaticus</i> in experimentally induced diabetic mice.
ANTI-INFLAMMATORY AND ANTITUMOR ACTIVITIES	Gurgel AP et al; in 2009 studied anti-inflammatory and antitumor activities of leaves from <i>Plectranthus amboinicus</i> (Lour.) Spreng (Lamiaceae).
ANALGESIC AND ANTI-INFLAMMATORY PROPERTIES	Chiu YJ et al; in the year 2012 evaluated Analgesic and anti-inflammatory activities of the aqueous extract from <i>Plectranthus amboinicus</i> (Lour.) Spreng.
ANTINOCICEPTIVE AND ANTIPYRETIC EFFECTS	Patel R et al; in the year 2010 studied Antinociceptive and Antipyretic effects of <i>Plectranthus amboinicus</i> (Lour) Spreng Leaves.
ANTI-GASTRIC ULCER ACTIVITY	Devi MR et al; in the year 2010 determined Anti-gastric ulcer activity of <i>Plectranthus amboinicus</i> (Lour) in wistar albino rats.
HEPATOPROTECTIVE ACTIVITY	Shenoy S et al; in the year 2012 studied Hepatoprotective Activity of <i>Plectranthus amboinicus</i> Against Paracetamol Induced Hepatotoxicity.
INSECTICIDAL ACTIVITY	Singh G et al; in the year 2002 carried out insecticidal investigations on leaf oil of <i>Coleus amboinicus</i> Lour.



**Fig.1. *Coleus amboinicus***



**Fig.2. *Coleus amboinicus* Leaf and stem**





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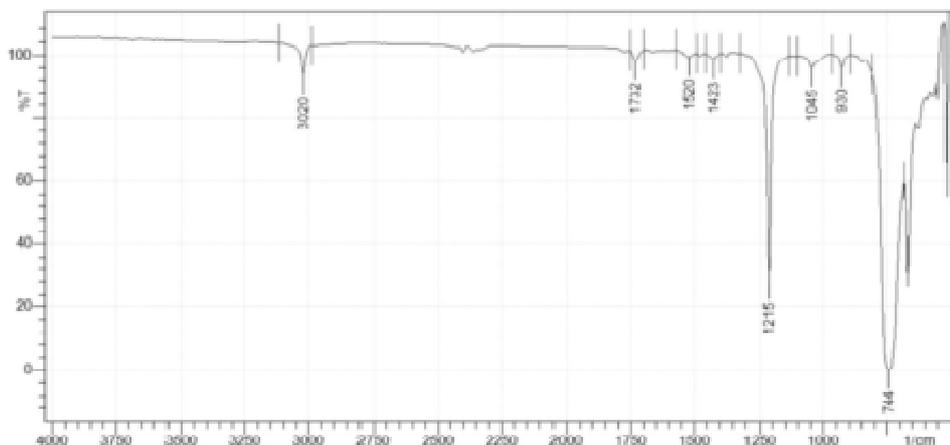


Fig. 3. FT-IR (KBr) Spectrum of isolated compound

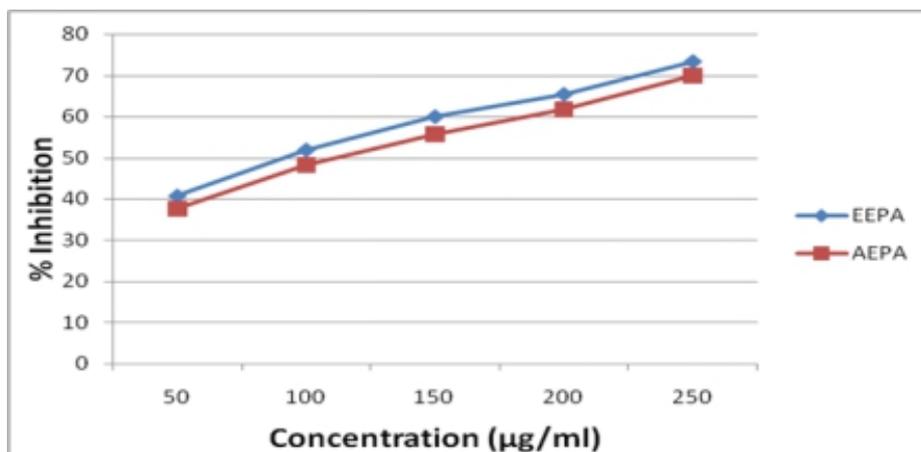


Fig. 4. Antioxidant Potential of ethanolic and aqueous extract of *Plectranthus amboinicus* by Reducing Power Method





## Ethnoveterinary Practices of Medicinal Plant Resources among the Farmers of Western Part of Tamil Nadu State, South India

K.Dhanasekaran<sup>1</sup>, T.Sundari<sup>2,3</sup>, R.Kavitha<sup>2</sup>, P.Subramaniam<sup>1</sup> and V.Balakrishnan<sup>1\*</sup>

<sup>1</sup>PG and Research Department of Botany, Arignar Anna Government Arts College, Sanyasikaradu, Namakkal, Tamil Nadu, India.

<sup>2</sup>PG and Research Department of Chemistry, Sri Sarada College for Women (Autonomous), Salem, Tamil Nadu, India.

<sup>3</sup>Department of Chemistry, K.S.R. College of Engineering, Tiruchengode, Tamil Nadu, India.

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### \*Address for Correspondence

#### V.Balakrishnan

PG and Research Department of Botany,  
Arignar Anna Government Arts College,  
Sanyasikaradu, Namakkal,  
Tamil Nadu, India.

E.Mail: palanivbalu@gmail.com



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### ABSTRACT

Tamil Nadu is one among the agriculture state in India. Agriculture practices is a major role in the state especially Kongu region. Western districts are culturally significant districts in Tamil Nadu state, especially for agriculture and industry. We identified the medicinal plant resources for ethnoveterinary aspects through farmer's community of Erode, Namakkal, Salem, Tiruppur, Coimbatore and The Nilgiris districts. Medicinal plant resources such as leaves, stems, fruits, roots, seeds and succulent sour are commonly used in the form of extracts, decoctions and powders to cure various diseases and to improve the animal health care systems. The medicinal plant species are identified with binomial name, family, parts used, local name, cure the diseases and mode of preparation are provided. Totally 72 medicinal plant species were recorded in Western districts for ethnoveterinary practices among the farmers.

**Keywords:** Ethnoveterinary, Western districts, Agriculture, Industry, medicinal plants.

### INTRODUCTION

Nearly 80% of the peoples from the Asian and African populations need to full fill their medicine from the plant resources especially for health care [1-2]. In our Nation started that India generated Rs 16,000 crores of revenue from the herbal industrial sector. In India rural peoples utilize 70% of countries population depends on the traditional medicinal system. Now a day's China is leading in the traditional medicinal system from 30 to 50 % of their needs.

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India is having a rich reservoir of cattle genetic resources not only for population strength but also for genetic breeds. Tamil Nadu has also rich experience in practicing ethnoveterinary medicine aspects [3]. The animal husbandry system in livestock is raised could effect the exposure to various parasites. The ethnoveterinary knowledge and plant-based anthelmintics. Still, farmers are crudely used in many traditional medicines. Now a days, the demonstration of ovicidal, larvicidal and adulticidal activities of traditional medicinal plant extracts and to determine the therapeutic doses remain in the preliminary studies. Medicinal plants have been used for the treatment of various diseases all over the world, due to their phytochemical substances and used for the therapeutic purposes or as precursors for the synthesis of drugs [4]. It is a new, safe, convenient and environment friendly product with the development of immune resistance and to enhance the level of immunity [5].

Even though 50 percent of modern drugs are based on natural products and play a significant role in the development of drugs in the pharmaceutical industries [6]. Bacterial resistant to most all antibacterial agents have been reported [7]. The resistance is due to its application of antimicrobial drugs are commonly used in the treatment of various infectious diseases. However, medicinal plants and herbs having effective bioactive metabolites with pharmacological effects can represent natural drugs and less harmful. Common herbal medicines and chemicals are affecting pathogenic protozoa. Medicinal plants display anti-inflammatory, analgesic, antioxidant and antiviral activities [8]. Venkatesan *et al.* [9]. reported that the medicinal values of mangroves. There is an increasing concern throughout the world about the uncontrolled exploitation and depletion of natural resources [10]. India has the very richest repository of traditional knowledge on the medicinal uses of plants [11]. Livestock production systems in India have been mostly primitive and unorganized. The animals are adjunct to crop production, which is necessarily a major occupation with the farmers [11].

Meanwhile, more investigations are needs to evaluate the botanical efficiency. In rural peoples of India have wide knowledge about veterinary plants and enhance their skills, methods of preparation, practices, knowledge and beliefs of the small holders about to take care of livestock system. Herbal preparations are an alternative source and cheap as well as very safe when compared to the modern animal health care system [12-14]. Several reports say that, livestock significantly contribute generate revenue for poor household peoples, those who are living below the poverty line especially in rural India [15-17]. Large number of medicinal plant resources grown in India in plains as well as hill regions or mountains. These medicinal plants are the most commonly used ingredients for the preparation of medicine to treat livestock [18]. Prince Nakula and Sahadeva both are physicians of horses and cows. In India, the traditional veterinary system of medicine is originated from the Mahabharata period. During the battle, more animals got injured and suffered from different illnesses. During this period, medicinal plants are a good source of medicine for the treatment of animal resources and protected from various illnesses especially diseases [19].

Different literature worldwide says that tribal doctors, barefooted doctors, herbal doctors, tribal medicine men, folk healers, folk and laymen practitioners of throw who are used medicinal plants traditionally [20]. The international level organization such as IUCN, WHO and WWF have approved and recognized them as traditional health care practitioners (WHO,1993) [21]. Swarup and Patra [22] reported that in 2008, Indian Council of Agriculture Research (ICAR), New Delhi, collected and documented nearly 595 veterinary traditional medicines from various sources. A vast number of modern drugs have been isolated and synthesized from natural resources such as medicinal plants [23]. Health care systems of animals mainly depends on peoples knowledge, skills, methods, regular practices, mode of medicine preparation by using medicinal plant resources through ethno veterinary practices [24]. The present study was undertaken with the farmers of Western districts of Tamil Nadu State for ethnoveterinary uses of medicinal plants.



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## MATERIALS AND METHODS

Ethnoveterinary studies with the farmers of Western parts of Tamil Nadu State, India was carried out during the year July 2018 – March 2019 totally 72 plant species belongs to 71 families generally used for different diseases of cattles were collected and reported.

### Western parts of Tamil Nadu

The present investigation of the usage of ethnoveterinary medicinal plants from the farmers of Western parts of Tamil Nadu State. The Western parts covering six districts such as Namakkal, Salem, Erode, Tiruppur, Coimbatore and The Nilgiris (Fig.1). These Western districts covering larger agriculture area and the population of livestock are high.

### Erode District

Erode district is in the Kongu Nadu region (Western part) of Tamil Nadu State. Erode district is surrounded by Salem, Namakkal, Karur, Tiruppur and Coimbatore districts respectively. Especially the climate is mostly dry and characterized by good rainfall. There are major rivers such as Bhavani, Kaveri, Palar, Amaravathi and Noyyal flow in the Erode district and mainly used for irrigation in agriculture purposes. The Erode District Latitude 11°20' 32.7228"N, Longitude 77°43' 41-3940"E. It contains nine taluks such as Andhiyur, Bhavani, Erode, Gobichettipalayam, Kodumudi, Modakurichi, Perundurai, Sathyamangalam and Thalavadi. All the regions contain 14 unions of Erode district. We collected the data from all the 14 unions among the farmers of Erode district, Tamil Nadu State.

### Namakkal District

Namakkal district was bifurcated from Salem district and started from 1997. The district is surrounded by Salem, Karur, Trichy and Erode. The geographical area of the district is 3368.21 sq kms which lies between 11.00° and 11.360° north latitude and 77.280° and 78.300 ° east longitudes. Namakkal district consist of 8 taluks such as Kolli Hills, Namakkal, Rasipuram, Tiruchengode, Paramatti Velur, Sendhamangalam, Komarapalayam and Mohanur.

### Coimbatore District

Coimbatore district have 3 revenue divisions 11 revenue taluks such as Coimbatore South, Coimbatore North, Perur, Madukkarai, Annur, Mettupalayam, Sulur, Pollachi, Kinathukadavu, Anaimalai and Valparai and 295 revenue villages. According to 2011 census status, Coimbatore district is the second most urbanized district of Tamil Nadu after Chennai. It's covering 29.63% of rural population. Coimbatore bordering to state of Kerala. It's surrounded by Western Ghats mountain range (Nilgiri biosphere reserve). The latitude and longitude is 11.0168° N, 76.9558° E.

### The Nilgiri district

The latitude and longitude of Nilgiri is 11.4916° N and 76.7337° E. Nilgiri is one of the oldest mountain range and located at the tri-junction of Tamil Nadu, Kerala and Karnataka states. The Nilgiri district comprises 6 taluks such as Udhamandalam, Kundah, Coonoor, Kotagiri, Gudalur and Pandalur, then consists of 88 Revenue villages.

### Tiruppur district

The latitude and longitude is 11.1085° N and 77.3411° E. Tiruppur district comprises three revenue divisions, 13 blocks and 265 panchayat villages. The Tirppur district consist of 7 taluks such as Tiruppur, Avanashi, Dharapuram, Palladam, Udumalaipettai, Kangeyam and Madathukulam.



**Dhanasekaran et al.,****Salem district**

Salem is a geologist paradise, surrounded by hills and the landscape dotted with hillocks. Salem has a vibrant culture dating back to the ancient Kongu Nadu. Salem district comprises of four revenue divisions, 13 taluks viz. Salem, Salem West, Salem South, Yercaud, Gangavalli, Attur, Pethanaickenpalayam, Valapady, Sankari, Edapadi, Mettur, Omalur and Kadayampatti and 640 revenue villages and 4 municipalities. The latitude longitude is 11.6643° N, 78.1460° E.

**Method of Data Collection**

The ethnoveterinary practices data were collected from July 2018 to March 2019. Farmers were interviewed in groups using open-ended questions at the dipping tanks or individually thereafter. Information was also gathered from previous more done in Dindigul district [11]. Medicinal plants were collected under the supervision of farmers and only when the same plant species was designated by at least two individuals for the same pathology was the information judged and acceptable (Fig 2 and 3). The information was documented through the ethnoveterinary survey, interview with farmers and field work. The information was included data about the botanical name, family, local name, part of the plant used mode of preparation (decoction, paste, powder, juice) and medicinal value. The information was confirmed through various discussions with respondents who practiced the use of the documented plants for veterinary applications.

**Identification of medicinal plants**

An Ethnoveterinary study was conducted and the information gathered and plant local name, plant parts used for medicine, method of the application was recorded with the help of aged farmers and senior local peoples. The Botanical name of the plant species, local name, family name was authenticated [25]. medicinal plant identification was carried out and confirm through a plant taxonomist also.

**Preservation of plant specimens**

The standard method was followed with record to the collection of plant materials, drying, mounting, preparation and preservation of plant specimens [26]. Voucher specimens of medicinal plants in triplicate were collected, prepared and identified. Plants with their correct nomenclature were arranged alphabetically by family name, vernacular name and ethnomedicinal uses. The identification and nomenclature of the listed plants were based on the Flora of Presidency of Madras [27] and the Flora of Tamil Nadu Carnatic [28]. All the preserved specimens were deposited at the Herbarium of Arignar Anna Government Arts College, Namakkal, Tamil Nadu.

**RESULTS AND DISCUSSION**

For the identification of new drugs and use of traditional ethnoveterinary medicine is must to documented and recorded and presence the traditional knowledge on medicinal plants especially treating for humans and animals. Further we enhance the botanical knowledge for exact identification of medicinal plant species. During ethnoveterinary survey among farmers of Western districts of Tamil Nadu state 72 plant species covering 71 Families. The botanical name of the plants provided in alphabetical order (Table 1). Mostly the way of administration of these herbal medicine remedies were oral method and followed by topical application then drops to ear and eyes for better treatment methods. The dose may be finalized by depend on the age or level of sick of the animal.

The plant species is equally used for to use various diseases such as mildew, fungal diseases, diarrhea, fever, snake bite, skin ulcer, cough, eye disease, wound healing, ulcer, bone fracture, scorpion bite, foot infections, swelling, pneumonia and mastitis. Meanwhile few medicinal plants used as antiseptic, cholesterol control, increase immunity and pain killer. Some medicinal plant species are gradually used to improve animal health care system such as good for cattle healthcare, oxygen circulation, edible, cooling stomach disorders, improve nutrition, cardiologic and good for digestive system, etc. The results are discussed with the relevant ethnoveterinary studies.



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The most extensively utilized the plant parts in the preparation of herbal medicine for various ailments is the leaf and followed by root. Among the different plant parts, the leaves were most commonly used (54%), root (10%), seeds (8%), oil (1%), Fruit (3%), bark (3%), whole plant (9%), rhizome (2%), bulb (1%), tuber (1%), stem (1%), latex(1%) and flowers (5%) (Fig.4). Medicinal and herbal plants have been commonly used by the farmers as well as village elder peoples. The common usage of herbal remedies and healthcare preparations are most common, because of natural products with medicinal properties. Regarding, current ethno-medicine in India with such great background it is very popular among the peoples and a good source of information to find out new natural remedies. Therefore, medicinal plants with antioxidant activity have been shown to counteract these situations and healthy source of health promotion [29]. Hassan *et al.* [30] stated that the emerging potential drug resistance of pathogens coupled with high cost and more side effects of antibiotics have the interest of researchers and general population towards ethnomedicinal plants for the potential discovery of useful phytochemicals.

Particularly can arise from traditional knowledge is described as a cumulative body of knowledge, practice and belief, evolving through adaptive processes and handed over through generations to generations by cultural transmission [31]. A decade now ethnoveterinary medicine has experienced a review and several reports published. The development and interest in traditional healthcare practices have been encouraged by the recognition of some efficacious ethnoveterinary medicinal products [32-35]. Collection and marketing of the wide range of non-timber products such as edible fruits, nuts, seeds and medicinal. One important factor is that several healers from different regions are the same plant to treat the same disease and those diseases which the plant is used against represents public health problems. India is rich in ethnic diversity and traditional knowledge that has resulted in a considerable body of ethnobotanical research of which our study has revealed a deep understanding of medicinal plants supported by preparation of various drugs and to enhance the conservation aspect also.

While the knowledge on the usefulness of these plants remains high, poor methods of exploitation, agriculture and over exploitation are putting most species under the pressure of extinction. Plant and plant derived products are part of health care system since ancient human civilization. Documentation of the indigenous knowledge through ethnobotanical studies is important for the conservation, utilization of medicinal and biological resources. Therefore establishment of the local names and indigenous uses of plants has significant potential societal benefits. Knowledge in utilizing these medicinal plant resources is characteristic and different from tribe to tribe. Folk medicine is no more an attraction to the younger generation and many young people migrate to urban for education and job opportunities. Plants have always played a major role in the treatment of human traumas and disease worldwide.

Ethnoveterinary practices are most common in our near countries Pakistan, China, Nepal and Sri Lanka due to the poor socioeconomic status of the rural farmer community [36]. The high level of utilization of these medicinal plants in ethnoveterinary investigation have been reported in various countries worldwide that might be due to their higher abundance in different regions, due to strong traditional knowledge of local peoples and farmers [37]. From the ecological point of view, herbal formulation that involves whole plant, stem, roots, bulb and leaves etc., have effect on plant life or to survive the mother plant resources. Lack of awareness on conservation strategy could also cause a depletion of this valuable resource. Few similar findings are also reported related to ethnoveterinary studies [38-41]. Many plant species is commonly used in traditional system of medicine for current health care system [42-43].

## CONCLUSION

Our ethnoveterinary survey reveals that most of the medicinal and herbal plant species are widely used in Tamil Nadu state for improving better animal health maintenance. The field work reflects to developing significant knowledge about the traditional medicine system among the farmers community in Western Districts of Tamil Nadu



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State. Peoples are ready to document and conscience the ethnoveterinary practice by *ex-situ*- further studies needed for pharmacology and toxicology aspects against the listed medicinal plants.

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## Appendix A. Questionnaire

1. Name of the farmer
2. Village name or village Panchayat
3. Age
4. Gender
5. Educational qualification
6. Occupation
7. Local name of the plants used locally for ethanoveterinary practices
8. Diseases cured by the medicinal plants
9. How can you prepare the plant based herbal products?
10. Any experience in traditional medicine preparations?
11. Aware of medicinal plant application through orally/ externally.
12. How many cattles in your home and its types?
13. Are you learned from ethanoveterinary practices from your father and grandfather?
14. How many years of experience for the herbal product preparation?
15. Are you know about the plant parts is toxic or non toxic?

## REFERENCES

1. Rastogi S, Pandey MK, Prakash J, Sharma A, Singh GN. Veterinary herbal medicines in India. Phcog Rev. 2015; 9:155-63.
2. Kunle OF, Eghavevba HO, Ahmadu PO. Standardization of herbal medicines- A review. Int J Biodivers Conserv. 2012; 4:101-12.
3. Senthamselvi S, Jagatheskumar S, Balasubramaniam V. Studies on the ethanoveterinary medicinal plants among the farmers of Dharapuram Taluk, Tiruppur district, Tamil Nadu, Kong.Res. J. 2015; (2) 2:155-159.
4. Sofowora A. Medicinal plants and traditional Medicine in Africa. John Wiley & Sons Limited, New York. 1982.
5. Githiori JB, Athanasiadou S, Thamsborg SM. Use of plants in novel approaches for control of Gastrointestinal Helminths in Livestock with emphasis on small ruminants. Vet.Parasitol. 2006; 139(4): 308-320
6. Jeyachandran R, Mahesh A. Antimicrobial evaluation of *Kigelia Africana* (Lam.). Research Journal of Microbiology. 2007; 2(8): 645-649.
7. Truitti MMDCT, Sarragiotto MHM, de Abreu Filho BA, Nakamura CV, Dias Filho BP. *In vitro* antibacterial activity of a 7-O-beta-Dglucopyranosyl-nutanocoumarin from *Chaptalia nutans* (Asteraceae). Mem. Inst. Oswaldo Cruz. 2003; 98:283-286.
8. Stintzing FC, Carle R. Cactus stems (*Opuntia* spp.): A review on their chemistry, technology and uses. Molecular Nutrition Food Research. 2005; 49(2):175-194.
9. Venkatesan K, Balakrishnan V, Ravindran KC, Devanathan V. Ethnobotanical report from mangroves of Pichavaram, Tamilnadu State, India, Sida. 2005; 1(4): 2243-2248.





**Dhanasekaran et al.,**

10. Philip Robinson J, John Britto S, Balakrishnan V. Regeneration of plants through somatic embryogenesis in *Emilia zeylanica* C.B.Clarke a potential Medicinal herb, Botany Research International, 2009; 2(1): 36-41.
11. Balakrishnan V, Philip Robiuson J. Manickasamy A., Ravindran KC. Ethnoveterinary Studies among farmers in Dindigul District of Tamil Nadu, India, Global Journal of Pharmacology. 2009; 3(1): 15-23.
12. Phondani PC, Maikhuri RK, Kal CP. Ethnoveterinary uses of medicinal plants among traditional herbal healers in Alaknanda catchment of Uttarakhand, India. Afr J Tradit Complement Altern Med. 2010; 7:195-206.
13. Chattopadhyay MK. Herbal medicines. Curr Sci 1996; 71:5.
14. Kamboj VP. Herbal Medicine. Curr Sci 2000;78:35-39.
15. Thornton PK, Kruska RL, Henninger N, Kristjanson PM, Reid KS, Atieno F, Odero A, Ndegwa T. Mapping poverty and livestock in the developing world. International Livestock Research Institute (ILRL), Nairobi, Kenya. 2002pp.124.
16. ADB (Asian Development Bank) Gender issues in Livestock. <http://www.adb.org> (accessed on 26 February 2016). 2000.
17. Hearth S. Women in livestock development in Asia. Journal of Commonwealth Veterinary Association. 2007; 24(1): 29-37.
18. Patil OP, Ashwini GP, Rajesh AR, pravin CP, Prashant KD, Sanjay BB, Dilip AP, Shital S N. Recent advancement in discovery and development of natural product combretastatin- inspired anticancer agents. Anticancer Agents in medicinal chemistry. 2015; 15(8): 955-969.
19. Raikwar A, Maurya P Ethnoveterinary medicine: in present perspective. Int. J. Agric. Sc. and Vet. Med. 2015; 3(1): 44-49.
20. Deora GS, Rathore MS. Ethno-Veterinary Medicine (EVM) and traditional Practices in Animal Health Care System (AHCS) in the Southern part of Rajasthan, India, In.J of Ayur and Her Med. 2017; 7(4); 2746-2751.
21. WHO. Health criteria and other supporting information. Volume 2. Geneva. 1993.
22. Swarp D, Patra RC, Naresh R, Kumar P, Shekhar R. Blood load levels in lactating cows reared around polluted localities: transfer of load into milk, Sci.Total. Environ. 2005; 347(1-3):106-110
23. Yadav SS, Bhukal R, Bhandoria MS, Ganie SA, Gulia SK, Raghav TBS. Ethnoveterinary Medicinal Plants of Tosham Block of District Bhiwani (Haryana) India. J App Pharm Sci. 2014; 4 (06): 040-048.
24. McCorkle CM. An introduction to Ethnoveterinary research and development. *J.Ethnobiol.* 1996; 6 (1) : 129-149.
25. Matthew PH. *Morphology*. 2nd edition. Cambridge, England: Cambridge University. 1991.
26. Jain SK. The role of Botanist in folklore research. Folklore 1964; 5, 145–150.
27. Gamble J.S. The Flora of the Presidency of Madras. Adlard & Son, Ltd., London, 1935.
28. Matthew K M. The Flora of the Tamil Nadu Carnatic. The Rapinat Herbarium, vol. 3. St. Josephs College, Tiruchirapalli, India, IXXXIV (2154), 1983.
29. Kafash-Farkhad N, Asadi-Samani M, Rafieian- Kopaei M. A review on phytochemistry and pharmacologica effects of *Prangos fetulacea* (L.) Lindl. Life sci J. 2013; 10(8) : 360-367.
30. Hassan HU, Murad W, Tariq A, Ahmad A. Ethnoveterinary study of medicinal plants in Malakand Valley, District Dir (Lower), Khyber Pakhtunkhwa, Pakistan. Irish Veter. J. 2014; 67.
31. Berkes F, Colding J, Folke C. Navigating Social–Ecological Systems: Building Resilience for Complexity and Change. Cambridge University Press, Cambridge, UK. 2003.
32. Zschocke S, Rabe T, Taylor JL, Jäger AK, van Staden J. Plant part substitution-a way to conserve endangered medicinal plants. J Ethnopharmacol. 2000;71: 281-292.
33. Masika PJ, VanAverbeke W, Sonandi A. Use of herbal remedies by small scale farmers to treat livestock diseases in central Eastern Cape Province, South Africa. Journal of South African Veterinary Association. 2000; 71, 81–91.
34. Tabuti JRS, Lye KA, DHillion SS. Traditional herbal drugs of Bulamogi, Uganda: plants use and administration. Journal of Ethanopharmacology. 2003; 88 : 19-44.
35. Masika P J, Afolayan AJ. An Ethnobotanical Study of Plants Used for the Treatment of Livestock Diseases in the Eastern Cape Province, South Africa, *Pharm. Biol.* 2003; 41: 16-21.





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Table.1. Ethno veterinary practices followed by farmers of western districts, Tamil Nadu State.

S.No	Botanical Name	Family	Local name	Parts used	Cure for diseases	Mode of application
1	<i>Abrus precatorious</i> L.	Fabaceae	Kundumani	Root, leaves	Eye complains, ulcer, dysentery	Extracts of root and leaves used as medicine
2	<i>Abutilan indicum</i> L.	Malvaceae	Thuththi	Leaves, root	Increase semen, pile complaints	Leaves and roots freshly given by orally
3	<i>Acacia nilotica</i> L.	Mimosaceae	Karuvelam	Leaves	Diarrhea, teeth issues	Decoction of leaves and bark is used.
4	<i>Acalypha indica</i> L.	Euphorbiaceae	Kuppai maeni	Leaves	Asthma and pneumonia	Extracts of fresh leaves are given
5	<i>Acanthospermum hispidum</i> L.	Asteraceae	Kanthimul	Leaves, seed	Diarrhea, fever	Decoction is given orally thrice in a day.
6	<i>Achyranthes aspera</i> L.	Amaranthaceae	Naayuruvi	Leaves	Cough, aundice, asthma, anemia	Fresh leaves are given orally
7	<i>Acorus calamus</i> L.	Araceae	Vasambu	Whole plant	Skin diseases	Whole plant powder applied in the skin.
8	<i>Adhatoda vasica</i> Nees.	Acanthaceae	Adathoda	Leaves, bark	Fever, cough	Leaves and bark extracts made a decoction and provided orally.
9	<i>Aegle marmelos</i> L.	Rutaceae	Vilvum	Seed, leaves	Ulcer and weak heart	Seeds and leaves decoction twice a day
10	<i>Aloe vera</i> L.	Liliaceae	Soaththukatra alai	Leaves	Skin diseases, stomach disorders	Fresh sour of leaves are given orally in empty Stomach
11	<i>Andrographis paniculata</i> F.	Acanthaceae	Chiriyangai	Leaves, rhizome	To treat infection and some diseases	Leaves and rhizome are freshly given to cattle's
12	<i>Aristolochia bracteolata</i> Lam.	Aristolochiaceae	Aadu thinna paalai	Leaves	Cold, fever and intestinal diseases	Decoction of leaves are given orally
13	<i>Azardirecta indica</i> A.Juss	Meliaceae	Vaambu	Seed, leaves, root, bark	Antiseptic and stomach diseases	Extracts of root and bark are given orally thrice a day for 5 days.
14	<i>Calotropis gigantea</i> (L.) R.Br	Asclepiadaceae	Vella Erukku	Bark, root, latex	Its used purgation and vomiting therapy	Bark and root extract is given orally
15	<i>Caparis tomentosa</i> Lam.	Capariaceae	Sengam	Root	Diarrhea, swelling	Root extracts are used and given orally.
16	<i>Cassia auriculata</i> L.	Caesalpiniaceae	Avaarai	Stem, leaves	Reduce sugar level, stomach diseases	Dry to stem and leaves and make it a powder to use as a tea.
17	<i>Cassia occidentalis</i> L.	Caesalpiniaceae	Payaverai	Leaves	Reduce sugar level	Extracts of leaf with lemon juice given orally twice in a day.





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18	<i>Cassia siamea</i> Lam.	Caesalpinaceae	Sarakondrai	Leaves	Fodder plant, water discarder	Decoction is given orally.
19	<i>Cassia sieberiana</i> DC.	Caesalpinaceae	Aavaram	Roots	Venereal diseases, pain killer	Roots are soaked in water then the water is given thrice a day.
20	<i>Cassine glauca</i> Rottb.Kuntze	Celastraceae		Leaves, Fruits, bark	Antodote, stimulant, cold, Antirheumatic	The powder of leaves, fruits and bark is used.
21	<i>Cissus populnea</i> Guill & Perr	Vitaceae	Vandhukolli	Climber	Anti-fungal properties	The plant powder is used.
22	<i>Cissus quadrangularis</i> L.	Vitaceae	Pirandai	Leaves	Stomach problems	Leaves make it chutney and to eat
23	<i>Cissus quandrangularis</i> L.	Vitaceae	Pirandai	Shoots	Stomach diseases skin diseases	Shoots are made into paste and given orally.
24	<i>Coccinia indica</i> L.	Cucurbitaceae	Koavai	Leaves, fruit	Eye issues, Diabetes treatment	Extract of leaves provided for eye reddish. Fruits are given orally
25	<i>Cucumis pubescens</i> Willd.	Cucurbitaceae	Thummati	Fruits	Pain reliever, Cure ulcer, urinary disorder and infection, skin care and stomach related issues	The fresh fruit id given orally. Dry fruits are also providing orally.
26	<i>Cuminum cyminum</i> L.	Apiaceae	Seeragam	Seed	Antiseptic, reduce cholesterol, increase immunity	Seeds are boiled in water and given orally.
27	<i>Curcuma aromatica</i> Salisb.	Zingiberaceae	Kasturi manjal	Rhizome	Inflammation of the under(mastitis) in cow	Rhizome powder with coconut oil and made a paste applied on mastitis.
28	<i>Cynodon dactylon</i> Pers.	Poaceae	Arugan	Aerial parts Leaves	Stomach worm and related diseases	The fresh juice is prepared from the true leaves and to take empty stomach .
29	<i>Eclipta prostrata</i> L.	Asteraceae	Karisalaankanni	Leaves	Skin diseases, eye disorders	Powder is used to make paste and applied.
30	<i>Euphorbia hirta</i> L.	Euphorbiaceae	Ammaan pacharisi	Whole plant, latex	Wound healing	The latex and whole plant paste is used for wound healing
31	<i>Euphorbia hirta</i> L.	Euphorbiaceae	Ammanpaccarisi	Latex	Heamorrhagic	Collect the late and used
32	<i>Ficus benghalensis</i> L.	Moraceae	Aalam	Latex,root	Bone fracture	Latex and root paste





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						applied on bone fracture.
33	<i>Ficus religiosa</i> L.	Moraceae	arasam	Leaves	Wound healing ,ulcer	Powder is mixed with hot water and given orally.
34	<i>Ficus sur</i> Forssk.	Moraceae	Arasa maram	Leaves, Stem, Bark	Pregnant cattles, good oxygen circulation	Decoction is provided orally.
35	<i>Flueggea virosa</i> Voigt.	Moraceae	Vellaipula	Leaves	Effective snake bite, treatment for pneumonia, diarrhea	Decoction of leaves given orally.
36	<i>Heliotropium indicum</i> L.	Boraginaceae	Dhetkodukki	Leaves	Skin ulcer	Extracts of leaves given in empty stomach.
37	<i>Hibiscus rosa-sinensis</i> L.	Malvaceae	Sembaruthi	Bark, flower	Skin, hair diseases	Dry Powder from Ban flower extract is used.
38	<i>Hibiscus trionum</i> L.	Malvaceae	Semparuthi	Flower	Decrease hair falls, it contains mucilage	Flowers are boiled – Powder used as a shampoo.
39	<i>Lanea coromandelica</i> Hutt.	Anacardiaceae	uthiyamaram	Bark	Cure injury	Bark powder is used.
40	<i>Leucas aspera</i> Spreng.	Lamiaceae	Thumbai	Leaves, flower	Treat scorpion bite	Fresh leaves and flower extracts are used
41	<i>Lippie nodiflora</i> L.	Verbenaceae	Poduthalai	Leaves	Anorexia and digestion	Leaf extract is provided orally twice in a day.
42	<i>Madhuca indica</i> J.F. Gmel	Sapotaceae	Iluppai	Flower	Stomach diseases	Dry flower Powder is used.
43	<i>Mangifera indica</i> L.	Anacardiaceae	Maa	Leaves, fruit	Throat disorders	Leaves, fruit extracts, made a decoction and provide orally
44	<i>Melia dubia</i> L.	Meliaceae	Malai vaambu	Leaves	Antiseptic and skin diseases	Decoction of leaves is used
45	<i>Mentha longifolia</i> L.	Lamiaceae	Puthina	Areal parts	It cure mildew and fungal diseases	The leaves along with Lemon juice are boiled and extracts is given orally. Some time extracts applied on the surface.
46	<i>Moringa oleifera</i> Lampk.	Moringaceae	Murungai	Leaves, pod, root	Improve nutrition	Fresh leaves, pod, make a tea and provide orally
47	<i>Murraya koengii</i> L.	Rutaceae	Karuvaepilai	Leaves	Anti-diabetic, eye disorders	Dry powder of leaves used always
48	<i>Musa paradisiaca</i> L.	Musaceae	Vaazhai	Leaves, rhizome	Body heat	Fruit, leaves are given orally Rhizome boiled in water and given orally





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49	<i>Nerium oleander</i> Sol.	Apocynaceae	Arali	Latex	Treatment for cancer	Collect the latex and given orally thrice a day
50	<i>Ocimum tenuiflorum</i> L.	Lamiaceae	Thulasi	Leaves	Cough, fever	Fresh leaves given orally
51	<i>Oryza sativa</i> L.	Poaceae	Vaikol	Leaves Whole plant	Good food and digestion for cattle	Plant body is used as a fodder.
52	<i>Passiflora foetida</i> L.	Passifloraceae	Siruppunaikkali	Whole plant	Used in tea, cough, also edible	A plant with water extracts and made a tea.
53	<i>Pedaliium murex</i> L.	Pedaliaceae	perunerunji	Leaves, seed	Foot infections	Powder of seeds and leaves are used
54	<i>Phyllanthus niruri</i> L.	Euphorbiaceae	Keelanelli	Root	Cough and fever	Root paste is given
55	<i>Pongamea glabra</i> Vent.	Fabaceae	Pungan	Bark	Wound healing	Bark powder applied on the wound.
56	<i>Psidium gujava</i> L.	Myrtaceae	Koyya	Leaves, fruit	Stomach problems	Fresh leaves and fruits are given orally
57	<i>Ricinus communis</i> L.	Euphorbiaceae	Amanakku	Leaves	Cooling, stomach disorders	Extracts of leaves is given by orally.
58	<i>Ricinus communis</i> L.	Euphorbiaceae	Aamanakku	Seed	Worm infection, skin disorders	Seeds grounded and applied on the skin disorder.
59	<i>Saba senegalensis</i> Pichon.	Apocynaceae	Mahohany	Bark and fruits	Diarrhea	Leaf Decoction is provided orally.
60	<i>Solanum nigrum</i> SW.	Solanaceae	Manathakkali	Leaves, fruit	Ulcer and stomach diseases	Fresh leaves and fruit are given orally for 3 days
61	<i>Solanum trilobatum</i> L.	Solanaceae	Thoodhuvai	Fruit, leaves	Antioxidant and hepatoprotective	Fresh leaves and fruits are given orally
62	<i>Strychnos spinosa</i> Lam.	Loganiaceae	Etti	Leaves	Dermatitis, skin diseases	Leaf extract is applied on the skin.
63	<i>Syzygium cumini</i> Walp.	Myrtaceae	Naval	Fruit, seed, bark	Diarrhea	Fruit and seed and bark powder given orally
64	<i>Tamarindus indica</i> L.	Fabaceae	Puliyam	Leaves, fruit	Pressure and sugar control	Leaves and fruit extracts are given orally
65	<i>Terminalia laxiflora</i> Engl & Diels	Combretaceae	Poo maruthu	Roots	Eye diseases	Decoction is used as eye drop.
66	<i>Tribulus terrestris</i> L.	Zygophyllaceae	Nerunchi	Leaves, seed	Foot wound, ulcer	Leaves and seeds make it powder and to treat diseases
67	<i>Tridax procumbens</i> L.	Asteraceae	Mookuththipoo	Leaves	Wound healing properties and antiseptic	Leaves are made in to paste and applied on wounds.





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68	<i>Vitex negundo</i> L.	Verbinaceae	Nocchi	Root, leaves	Pests and cough remedy	Fresh root and leaves extracts is used
69	<i>Waltheria indica</i> L.	Sterculiaceae	Kanakalaoa	Leaves	Diarrhea with blood, tonic	Leaves are given orally.
70	<i>Zingiber officinale</i> L.	Zingiberaceae	Inji	Rhizome	Antiseptic and good digester	Rhizome make it paste
71	<i>Ziziphus mucronata</i> Willd.	Rhamnaceae	Nariilanthai	Roots	Diarrhea	Root decoction to given orally.
72	<i>Zizyphus jujupa</i> L.	Rhamnaceae	Ilanthai	Fruit, leaves	Cardiotonic, and wound healing	Fruits are eatable. Leaves are used as paste for wound healing.



**Fig.1.Map shows different districts of Western Part of Tamil Nadu State**





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Fig. 2. Ethnoveterinary survey among the farmers of Western Districts of Tamil Nadu State.

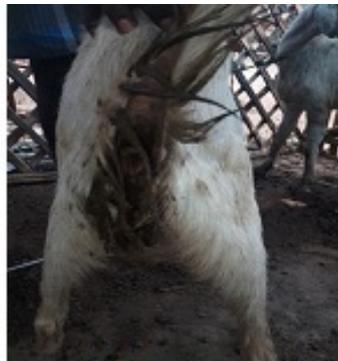




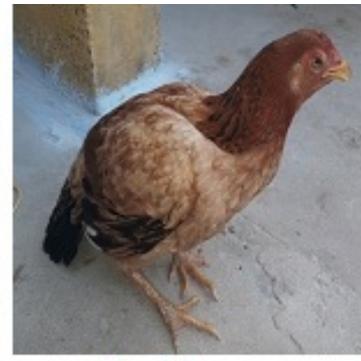
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Nose watering



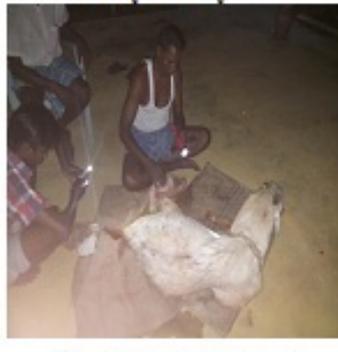
Dysentery



Eye Diseases



Interview with farmer



Dog biting treatment



Wound



Hair falling



Field Survey



Mastitis



Interview with farmer



Eye watering



Field Survey

Fig.3. Ethnoveterinary survey among the farmers of Western Districts of Tamil Nadu State





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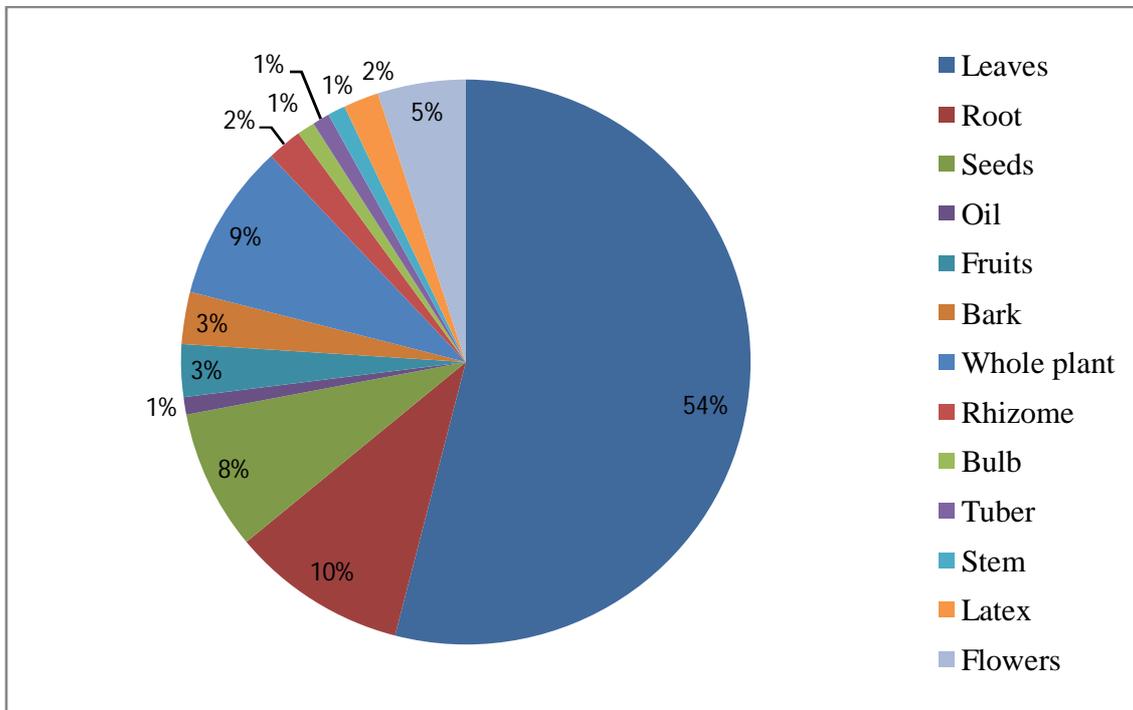


Fig. 4. Different plant parts significantly used for the various treatments.





## A Comprehensive Review on the Synthesis Mechanisms and Applications of ZnO Nanostructures

Debasrita Dash and Dojalisa Sahu\*

School of Applied Sciences, Centurion University of Technology and Management, Odisha, India.

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### \*Address for Correspondence

**Dojalisa Sahu**

School of Applied Sciences,  
Centurion University of Technology and Management,  
Odisha, India.

E.Mail: dojalisa.sahu@cutm.ac.in



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### ABSTRACT

Amongst the semiconducting metal oxides, zinc oxide (ZnO) is treated as a novel material with enormous applications. Several synthesis methods such as; co-precipitation, sol-gel, hydrothermal, vapour deposition (PVD, CVD), spin coating, plasma etc. have been adopted to produce diverse morphologies of ZnO nanostructures. The resulting nanostructures of different size in the nanometre regime are treated as suitable material to fabricate gas sensor with high sensitivity and selectivity. These gas sensors are used to detect many hazardous and toxic gases. ZnO is also regarded as a good photocatalyst for degradation of harmful dyes and a non toxic material for reducing environmental pollution. To enhance the photocatalytic properties of ZnO, different metallic dopants are also incorporated. In this review article, we have focused to study and discuss the recent proceedings on ZnO based photocatalysts and gas sensors. The usefulness of nano ZnO for the above applications and the detailed mechanism of photodegradation and gas sensing have also been discussed. A gas sensor has many characteristic properties which certify its quality which are detection limit, stability, selectivity, response time, recovery time and recyclability. This review paper precisely describes synthesis techniques for preparation of ZnO, working mechanism and its application as photocatalyst and gas sensor.

**Keywords:** Zinc Oxide, Photocatalyst, Gas Sensor, Synthesis, Applications.

### INTRODUCTION

Nanotechnology is a multidisciplinary area with greater research possibilities and exploration. It has assorted array of usage involving from health care unit to ecological issues. It is defined as arrangement of matter with at least one dimensional sized ranging from 1 to 100 nanometers. Nano composite is considered as one of significant part of

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nanotechnology owing to its surface morphology. The reckless ever-increasing population in urban place ensued from fast development of industrialization leads to air pollution. The survival of living species is at danger due to removal of secondary waste from industrial units and automobile exhausts endorse air pollution. Consequently, pure air has become need of our atmosphere [1]. The crucial need of current time is to develop gas sensor which is the realistic way to identify toxic gases in environment [2, 3]. A device which has the capability to detect the existence or concentration of a particular toxic and explosive gases is called as a gas sensor. Depending on the gas concentration, the sensor creates a potential difference by altering the resistance of the material inside the sensor which can be measured as output voltage. In present time, gas sensor has application in diverse area like in fuel cells, automobile manufacturing unit, mining industries, oil refineries and fertilizer industries [4-7]. The gas sensor having characteristic of low cost, reliable, minute power consuming ability, enhanced sensitivity and diminished size is considered as appropriate applicant toward gas sensor application [8,9]. In previous years, lot of experimentation has done in the field of nanoscience to develop metal oxide semiconductor based gas sensor having outstanding characteristics is considered as ideal gas sensor [10-13]. Now a day's extensive work is done to explore nanostructures with enhanced performance capacities with enlarged surface area and diminished dimension. The size of nanostructures is reliable on the factor responsible for the sensing response [14, 15]. Among various metal oxide semiconductor based nanostructures gas sensor zinc oxide has gained attention globally. In the array of low cost sensor ZnO took a significant position due to its enhanced selectivity, sensitivity properties [16]. ZnO based gas sensor is engaged in different usage owing to its enhanced sensing response, good selectivity, simple synthesis technique, cost effective, good thermal and chemical stability and non-toxic in nature. ZnO nanomaterial is intrinsically n-type semiconductor having wide band gap value of 3.37 eV, a large exciton binding energy of 60mev and electron mobility. Besides from the gas sensing applications of ZnO nanomaterial is widely used in the photovoltaic cells [17]. Among the synthesis of righteous chemical and biological sensors ZnO is proved to be latent applicant due to its enhanced photoelectric reaction with chemical and thermal stability [18].

ZnO has several benefits such as cost effective, toxic free, simple means of large scale fabrication made it an efficient environmental-friendly tool. Apart from this ZnO is a chemo resistive sensor which depicts that chemisorbed oxygen ions is highly responsible for the variation on its resistance that are adsorbed on the ZnO surface in the presence of air. The resistance of ZnO is raised by the extraction of electrons from the conduction band of ZnO due to the formation of chemisorbed oxygen. On the process of interaction of chemisorbed oxygen ions on the surface of ZnO with reducing gases, oxygen ions donate free electrons to the Conduction band of ZnO which leads to the deduction of resistance. In addition to this, working temperature and gas concentration highly influence sensing response of ZnO based gas sensor [19,20]. ZnO based gas sensor rely on working temperature need adequate amount of heat and light energy on the sensor surface to exceed the barrier of activation energy which increase sensing response followed by redox reaction [21, 22]. It was seen that with large value of operating temperature, the variation occurs on the surface morphologies of ZnO nanocomposite. Furthermore, by rising the temperature above optimum operating temperature the chemisorbed oxygen ions on the sensor surface reduces which paved path for the reduction of sensing responses [23]. So, the operating temperature always kept low to have good capability of sensing response. So, it is urging to develop ZnO nanostructures working at low temperature providing characteristics like good sensing response, large response time, durable, selectivity and reproducibility. By keeping in mind, enhancement of gas sensing properties of zinc oxide, several modifications are done in ZnO which comprises doping of metal into ZnO [24-29]. Now-a-days, lot of exploration is done in the field of gas sensing application of ZnO based nanomaterial owing to its ample properties. For example, gas sensor is developed due to interaction of reversible chemisorption process of reactive gases on the surface of ZnO with varying conduction [30]. The current review paper presents the various fabrication methods to produce different range of morphologies of ZnO nanomaterial which have good control over the surface to volume ratio that establishes improvement in gas sensing applications [31]. The nanostructures comprise of nanoplate, nanorod, nanosphere, nanowires, nanotubes, nanoflowers, nanofibres, nanoneedles, nanoribbon, ellipsoids, urchins, helices, combs [32]. The different types of morphologies of metal oxide based semiconductor is employed to detect diverse range of toxic gases present in



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atmosphere such as ammonia, nitrogen dioxide, hydrogen, hydrogen sulphide, carbon dioxide, ethanol, carbon monoxide and toluene [33]. The gas sensing mechanism is also discussed.

**Fabrication techniques of ZnO nanostructures:**

An ample number of methods are employed to synthesize ZnO nanomaterial with different size and morphologies which help in gas sensing performance. The array of nanostructures produced include one dimensional like nanowire, nanorod, nanofibre, nanoneedle, nanoribbon, nanotubes, and two dimensional comprises nanosheets, nanoplates whereas three dimensional consists of nanoflower, den dimers and urchins. Chemical vapour deposition technique is known as vacuum deposition method helpful in creating good quality, enhanced performance solid materials. This synthesis method is used to produce films by semiconductor manufacturing unit. In this method water substrate is exposed to one or more volatile precursor and reaction undergoes on the presence of substrate surface to manufacture needed deposit. During this process nano and microstructure is formed on the heated substrate as vapour. The factors like position of substrate, growth temperature, deposition time, gas flow rate and pressure are found significant while deposition occur on ZnO nanostructure [34]. Hence the regulation in deposition factor with appropriate optimizing gives the way to the formation of nanostructures of varying shape and size. This known mechanism is said to be vapour liquid solid method. The elementary steps involved in this process are assimilation of reactant gas and inert gas is allowed to flow in particular rate into the reaction chamber, movement of gas species into the substrate, adsorbent of reactant on the substrate surface and then the reacting material undertake the chemical reaction. In this process one and two dimensional nanostructures are formed.

Radio frequency sputtering is one of the synthesis procedures to obtain ZnO nanostructure on the chosen reacting material. This technique is said to be effectual for deposition process due to its outstanding bond formation ability and maintaining of homogeneity through the substrate. It works on the simple principle which tells that the atoms are discharged from the source substance and get deposited on substance surface. Argon is used as gas on the deposition process. The main parameter influencing sputtering process are RF power, temperature of substrate, flow of argon gas, distance between target and substrate and gas pressure [35]. It was found that ZnO nanorods are fabricated by using this process. Hydrothermal synthesis method is generally used to fabricate nanomaterial at low temperature. This synthetic approach leads to the chemical reaction occur in solution in the varying temperature range from room temperature to very high temperature. In this method the synthesis of ZnO nanomaterial take more time, less temperature and in the presence of autoclave. The development of nanozinc oxide is followed by the generation of nucleation site. This technique has lot of advantages regarding to the growth, composition etc. It requires less temperature, varying shape and size, altered pressure and enhanced crystallinity [36-39]. This method is used to fabricate one dimensional nanostructures including nanofibers and nanotubes having broad length and enhanced surface area [40]. This technique works on the principle of capillary electro spinning which shows that electric current passes through the capillary tube and collector leads to the formation of nanofibers [41]. Sol-gel method is a wet chemical synthesis technique which comprises production of an inorganic colloidal suspension known as sol and generation of three dimensional gel-like networks in a continuous liquid phase is called gel. It is used to fabricate glassy as well as ceramic material. This technique is simple, cost-effective, and versatile and use bottom up approach for the fabrication of particle. This method encompasses hydrolysis of reacting material in solvents. The ZnO thinfilms are produced by taking metal salt and inorganic salt as reacting material [42, 43]. Another method is used to fabricate ZnO is said to be template method. In this process, a template with definite shape and size is required for the development of nanostructures via pore or channel followed by nucleation and growth process. Templates are classified as hard template which use CNT and porous alumina whereas soft template includes flexible polymer and biomolecule. This technique is used to fabricate ZnO hollow sphere [44]. This method faces drawbacks like high maintenance cost and very slow rate of growth.



**Debasrita Dash and Dojalisa Sahu****Gas sensing applications of ZnO nanostructures**

A device which has the capability to detect the existence or concentration of a particular toxic and explosive gases is called as a gas sensor. Metal oxide semiconductors show good sensing activity. The change in electrical conductivity arises from the chemical reaction between the adsorbed oxygen onto the metal oxide surface and the gas molecules. The adsorbed oxygen molecule on the surface of metal oxides like ZnO nanoparticles is ionized to  $O^{2-}$ ,  $O^-$  and  $O_2^-$  by acquiring electrons from the conduction band (CB). So it exhibits resistivity towards air environment [45]. The reducing gases such as carbon monoxide, ammonia, dihydrogen and ethanol at modest temperature, the oxygen species on the nanowire surface react with the investigated gas resulting in the decrease in oxide ion on the surface and rise of concentration in electrons. Hence, ZnO nanowire sensors exhibits enhanced conductivity in reducing gases.

**Sensitivity**

It is defined as the response of gas sensor per unit change in the concentration of gas in terms of conductance and resistance.

**Selectivity**

The selectivity of gas sensor toward definite molecule depicts the ratio of its response comparing with governing intrusive molecule.

**Accuracy**

It is the measurement of degree of exactness in comparison with its true value.

**Speed of Response**

The time needed for sensor to reach out 90% of total response of the signal with the addition of target gas.

**Recovery Time**

The time essential for a sensor to come back to 90% original baseline after removal of the target gas.

**Detection Limit**

This is the specific temperature at which lowest trace of gas can be examined.

**Stability**

It is the capability of the sensor to maintain its performance for definite period of time.

**Resolution**

The lowest concentration difference that can be differentiated when continuous variation of composition.

For environmental application, highly costly and time consuming portable monitor constructed on chemo resistive sensor device is used following all recent laws of environment. Admst all the sensors, metal oxide semiconductor is treated as significant weapon in environmental monitoring due to properties like cost effective, strong, durable, light weight and advantages of having good material sensitivity and quick response time. It is also used to measure and regulate small amount of toxic gases like carbon monoxide and nitrogen dioxide [46]. The prerequisite for the metal oxide using chemo sensor for enhanced automobile application are ability to endure enhanced temperature, tough nature, and effectual steps of packing and diminished dimension. Mostly the temperature range of 600-900 °C is applied during package. Another factor is the measurement of the rate of the reaction in the order of the fraction of seconds and service life is about 5-10 years. So, new technologies are introduced in automobile manufacturing unit to monitor motor functioning, array of emission gases and detect high pollution levels in vehicles [47].



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Biomedical apply enhanced sensitive chemo resistive gas sensors for the identification of small trace of test gas approaching from biochemical processes taking place in the human body which is used as indicator in lot of patho-laboratories. Besides the test gas is examined and measured in mixture of intrusive gases and high content of humidity. These sensors are used for the clinical diagnostics, breath analysis and regulation of gases [48]. Gas sensor with enhanced ability for detecting number of gases present in the human breath is crucial for breath analysing system. The key ingredient of human breadth is nitrogen which is commonly found in air, carbon dioxide generated by the respiration process, water vapour obtained from evaporation of fluid present in body and unconsumed oxygen. For above health diagnostic procedure, advanced technologies are required for the detection of specific gas and its amount present in the breath which is connected with different disease and metabolism. Recently lot of the research are done in this area to establish relations between components of the breadth and diverse diseases like regulation of liver functioning applying ammonia, measurement of the metabolism of cholesterol by using isoprene and regulation of carboxyhaemoglobin in blood by carbon monoxide [49-51].

**Photocatalytic property of ZnO nanostructures**

Ecological contamination via water pollution has become a matter of great concern now-a-days. Due to rapid growth of urbanization, a lot of coloured waste originating from the dyes used for colouring different products has been discharged as effluent by many industries. Living beings face many problems by consuming water from natural water bodies like river into which the wastewater coming out of textile, paper, carpet, leather, distillery and printing industries is discharged. Major problems like carcinogenicity, mutagenicity, toxicity, coloration of natural water and threat to aquatic life in eco-system have been evolved due to the dye exposure to environment [52-55]. So, elimination of these dyes is a major research problem as the chemical and physical method cannot completely destroy the contaminants. Thus, complete conversion of these pollutants to simpler products is necessary by the use of an inexpensive and environment-friendly process. The method of photocatalysis can degrade these dye pollutants into simpler products like  $H_2O$ ,  $CO_2$  and mineral acids with no secondary pollution. Semiconducting materials are the part of a most important technology in the field of environmental cleaning among all the advanced oxidation processes. Metal semiconductor materials such as;  $TiO_2$ ,  $ZnO$ ,  $Fe_2O_3$ ,  $CdS$  and  $ZnS$  are widely used as photo catalyst [56, 57]. Further, these semiconductor nano crystals usually show extraordinary size and shape dependent material properties and a lot of progress in this field with controllable synthesis and related unique optical properties like photoluminescence has been reported [58]. Optically active ions are doped into the II-VI semiconductor nanocrystals acting as important host materials and exhibiting good luminescence efficiency even at room temperature [59]. Amongst various semiconductors, zinc oxide ( $ZnO$ ) shows improved photocatalytic degradation efficiency in degrading organic dyes in comparison to other metal oxides [60, 61].  $ZnO$  nanoparticles are very useful for water detoxification due to production of  $H_2O_2$  more efficiently and shows high rate of mineralization. It provides high surface for reaction due to higher number of active sites and being inexpensive and non-toxic.

There are many studies which provide a basis for the application of  $ZnO$  as a photocatalyst to improve azo dye pollution. However, the rapid recombination rate of the photo-induced carriers in  $ZnO$  generally slows down the photocatalytic activity which is unfavourable for commercial application in the photocatalytic degradation of contaminants [62, 63]. To improve its photocatalytic activity, metal doping has been carried out because the doping metal can efficiently trap the photo-induced charge carriers [64, 65]. It has been reported that metals are very suitable and widely used for doping into  $ZnO$  including transition-metal cations, noble metals and lanthanide ions [64, 66, 67]. Yang et al. [68] and Yu et al. [69] have reported the photocatalytic activity of  $ZnO:Eu^{3+}$  nanomaterials synthesized by hydrothermal method. Faraz et al. reported the essentiality of samarium doping in enhancing the photocatalytic properties [70]. Selvaraj et al. discussed how Gd doping can be useful in achieving better degradation of methylene blue by  $ZnO$  photocatalyst under visible light [71]. Quasi-stable energy states are created in the band gap by the doping of metal oxides with metals, transition metals and lanthanides which affects the optical and electronic properties [72, 73].



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Under the irradiation of UV light, electrons are excited from the valence band to the conduction band and forms holes in valence band [74]. The above process takes place in both the metal oxides existing in the nanocomposite. For enhancement of photocatalytic efficiency, two major parameters need to be satisfied namely (i) generation of more number of carriers and (ii) separation of charge carriers prohibiting the recombination [75]. In the present case, the coupling of the two metal oxides donates more and more charge carriers and due to the accumulation of charge carriers on either side of heterojunction, separation is achieved in between the charge carrier [76]. The holes attached to the ZnO surface react with surface hydroxyl groups or water molecules to generate more and more hydroxyl radicals ( $\text{OH}^\bullet$ ) [77]. On the other side, the accumulated electrons on tin oxide surface react with the dissolved oxygen molecules to give super oxide radical ( $\text{O}_2^{\bullet-}$ ) anions. The  $\text{O}_2^{\bullet-}$  radicals further react with water molecules to give hydrogen peroxide and hydroxyl radicals. The strong oxidizing property of  $\text{OH}^\bullet$  radicals triggers the decomposition of organic dyes like MO, MB and CR. The detailed reaction mechanism involved in the above process has been described elsewhere [77].

**CONCLUSION**

This study presents a short review on the synthesis and application of ZnO nanoparticles. Different methods of preparation of nano ZnO has been discussed here which are used to synthesize different morphologies of ZnO like nanoparticles, nanorods, nanoflowers and nanowires. Besides other important applications of ZnO, the gas sensing properties and photocatalytic degradation of ZnO nanoparticles have been discussed elaborately. By keeping in mind the factors like selectivity, operation time etc, the zinc oxide is proved to be excellent metal oxide gas sensor. In future, metal oxide nanomaterials such as ZnO are going to form main ingredient for production of gas sensors with enhanced gas sensing properties. As a wide band gap semiconducting material, ZnO shows good photocatalytic degradation property and this can be further improved by the incorporation of metallic dopant ions.

**REFERENCES**

1. T. Hübert, L. Boon-Brett, G. Black, U. Banach. *Sensors Actuators B*. 157 (2011) 329–352.
2. B.B. Uma, S.P. Uday, G. Oinam, A. Mondal, T.K. Bandyopadhyay, O.N. Tiwari, *Carbohydr. Polym.* 179(2018) 228–243.
3. J. F. Song, Z.Z. Lin, S. Ge, J. Li, X.M. Qiu, R.S. Zhou, S.Z. Li, Z.Guo, *Sci. Adv. Mater.* 9(2017) 2054–2065.
4. P. Bhattacharyya, P.K. Basu, H. Saha, S. Basu, *Sensors Actuators B*. 124(2017) 62–67.
5. R. Ghosh, A. Midya, S. Santra, S.K. Ray, P.K. Guha, *ACS Appl. Mater. Interfaces* 5 (2013)7599–7603.
6. F.J. Sansone, *Mar. Chem.* 37(1992) 3–14.
7. K. Wetchakun, T. Samerjai, N. Tamaekong, C. Liewhiran, C. Siriwong, V. Kruefu, A. Wisitsoraat, A. Tuantranont, S. Phanichphant, *Sensors Actuators B*. 160(2011)580–591.
8. U. Özgür, Y.I. Alivov, C. Liu, A. Teke, M.A. Reshchikov, S. Doğan, V. Avrutin, S.J. Cho, H.A. Morkoç, *J. Appl. Phys.* 98(2005) 041301.
9. D. C. Look, *Mater. Sci. Eng. B* 80, 383 (2001).
10. A. Dey, *Mat. Sci. Eng. B*. 229(2018) 206–217.
11. S.H. Hahn, N. Barsan, U. Weimar, S.G. Ejakov, J.H. Visser, R.E. Soltis, 436 (2003)17–24.
12. X. He, J. Li, X. Gao, L. Wang, *Sensors Actuators B*. 93(2003) 463–467.
13. G. Korotcenkov, V. Brinzari, V. Golovanov, Y. Blinov, *Sensors Actuators B*. 98(2004) 41–45.
14. B. Shouli, L. Dianqing, H. Dongmei, L. Ruixian, C. Aifan, C.C. Liu, *Sensors Actuators B*. 150 (2010)749–755.
15. Y. Li, Z. Tao, N. Luo, G. Sun, B. Zhang, H. Jin, H. Bala, J. Cao, Z. Zhang, Y. Wang, *Sensors Actuator B*. 290 (2010) 607–615.
16. Z.L. Wang, *ACS Nano*. 2 (2008) 1987–1992.
17. W. Raza, K. Ahmad, *Mater. Lett.* 212 (2008) 231–234.
18. X.L. Xu, Y. Chen, S.Y. Ma, W.Q. Li, Y.Z. Mao, *Sensors Actuators B*. 213 (2015)222–233.





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19. G.E. Kim, S. Noh, S.H. Kang, Y.D. Kim, T.K. Kim, *Sci. Adv. Mater.* 9 (2017) 2126–2130.
20. M. Kumar, V. Singh Bhati, S. Ranwa, J. Singh, M. kumar, *Sci. Rep.* 7 (2017) 236.
21. A. Ulyankina, I. Leontyev, M. Avramenko, D. Zhigunov, N. Nina Smirnova, *Mater. Sci. Semicond. Proc.* 76 (2018)7–13.
22. S. Ranwa, P.K. Kulriya, V.K. Sahu, L.M. Kukreja, M. Kumar, *Appl. Phys. Lett.* 105 (2014) 213103.
23. Z. Jing, J. Zhan, *Adv. Mater.* 20 (2008)4547–4551.
24. Y. Zhang, D. Li, L. Qin, P. Zhao, F. Liu, X. Chuai, P. Sun, X. Liang, Y. Gao, Y. Sun, G. Lu, *Sensors Actuators B.* 255(2018) 2944–2951.
25. O. Lupan, V. Postica, F. Labat, I. Ciofini, T. Pauporté, R. Adelung, *Sensors Actuators B.* 254 (2018)1259–1270.
26. X. Yang, S. Zhang, Q.yu, L. Zhao, P. Sun, T. Wang, F. Liu, X. Yan, Y. Gao, X. Liang, S. Zhang, G. Lu, *Sensors Actuators B.* 281 (2019)415–423.
27. T. Wu, Z. Wang, M. Tian, J. Miao, H. Zhang, J. Sun, *Sensors Actuators B.* 259 (2018) 526–531.
28. J.H. Kim, J.H. Lee, Y. Park, J.Y. Kim, A. Mirzaei, H.W. Kim, S.S. Kim, *Sensors Actuators B.* 294 (2019)78–88.
29. V.S. Bhati, S. Ranwa, M. Fanetti, M. Valant, M. Kumar, *Sensors Actuators B.* 255 (2018) 588–597.
30. N. Wang, X. Cao, Q. Wu, R. Zhang, L.Wang, P. Yin, L.Guo, *J. Phys. Chem. C.*113 (2009)21471–21476.
31. J. Zhang, B. Zhao, Z. Pan, M. Gu, A. Punnoose, *Cryst. Growth Des.* 15 (2015) 3144–3149
32. D. Nunes, A. Pimentel, L. Santos, P. Barquinha, L. Pereira, E. Fortunato, R. Martins, Eds. Elsevier. (2019)21–57
33. C. Wang, L. Yin, L. Zhang, D. Xiang, R.Gao, *Sensors.* (2010)
34. C. Yang, P. Wu, W. Gan, M. Habib, W. Xu, Q. Fang, L. Song, *AIP Adv.* 6 (2016) 055310.
35. S. Ranwa, P.K. Kulriya, V.K. Sahu, L.M. Kukreja, M. Kumar, *Appl. Phys. Lett.* 105 (2014) 213103.
36. K.H. Tam, C.K. Cheung, Y.H. Leung, A.B. Djurišić, C.C. Ling, C.D. Beling, S. Fung, W.M. Kwok, W.K. Chan, D.L. Phillips, L. Ding, W.K. Ge, *J. Phys. Chem. B.* 110 (2006)20865–20871.
37. S. Baruah, L. Dutta, *Sci. Technol. Adv. Mater.* 10 (2009) 013001.
38. P.S. Cho, K.W. Kim, J.H. Lee, *J. Electroceram.* 17 (2006) 975–978.
39. A. Umar, M.S. Akhtar, A. Al-Hajry, M.S. Al-Assiri, N.Y. Almelhad, *Mater. Res. Bull.* 47(2012) 2407–2414.
40. J. Guo, Y. Song, D. Chen, X. Jiao, J. Dispers. *Sci. Technol.* 31 (2010) 684–689.
41. S. Wei, M. Zhou, W. Du, *Sensors Actuators B.*160 (2011) 753–759.
42. L. Znaidi, *Mat. Sci. Eng. B.*174 (2010) 18–30.
43. H.T. Kim, S.Y. Lee, C. Park, *Vacuum.* 143 (2017)312–315.
44. Y. Zhang, L. Li, P. Qin, P. Zhao, F. Liu, X. Chuai, P. Sun, X. Liang, Y. Gao, Y. Sun, G. Lu, *Sensors Actuators B.* 255 (2018) 2944–2951.
45. J. Xu, Y. Chen, D. Chen, and J. Shen, *Sensors and Actuators B* 113(2006), pp.526-528.
46. N. Yamazoe, N. Miura *Sensors Actuators B Chem.* 20 (1994)95–102.
47. G.F. Fine, L.M. Cavanagh, A. Afonja, R. Binions, *Sensors.* 10 (2018)5469–5502.
48. A. Cederquist, E. Gibbons, A. Meitzler, *SAE Tech Pap.* (1976).
49. M. Righettoni, A. Amann, S.E. Pratsinis, *Mater Today.* 18 (2015)163–171.
50. I. Ohsawa, M. Ishikawa, K. Takahashi, M. Watanabe, K. Nishimaki, K. Yamagata, K. Katsura, Y. Katayama, S. Asoh, S. Ohta, *Nat Med.* 13 (2007)688–694.
51. W. Shin, T. Itoh, N. Izu, *Synthesiol Engl Ed.* 8 (2015) 211–219.
52. Y. Hauming, O. Jing, T. Aidong, X. Yu, L. Xianwei, D. Xiaodan, Y. Yongmei, *Mat. Resr. Bulln.* 41 (2006) 1310.
53. P.C. Vandevivere, R. Bianchi, W. Verstraete, *J. Chem. Technol. Bio. Technol.* 72 (1998) 289.
54. V. Augugliaro, C. Baiocchi, A. Bianco Prevot, E. Garcia-Lopez, V. Loddo, S. Malato, G. Marci, L. Palmisano, M. Pazzi, E. Pramauro, *Chemosphere* 49 (2002) 1223.
55. J. Li, P.L. Bishop, *Wat. Sci. Technol.* 46 (1-2) (2002) 207.
56. D.D. Liu, Z.S. Wu, F. Tian, B.C. Ye, Y.B. Tong, *J Alloys Compd* 676(2016) 489–498
57. S. Chidambaram, B. Pari, N. Kasi, S. Muthusamy, *J Alloys Compd* 665(2016)404–410
58. R. N Bhargava, D.Gallagher, X.Hong, A. Nurmikko, *Phys.ReV. Lett.* 72, 416 (1994).
59. W.Chen, J. O Malm, V.Zwiller, Y. N.Huang, S. M. Liu, R.Wallenberg, J. O.Bovin, L. Samuelson, *Phys. ReV.B,* 61, 11021(2000).





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60. H.J. Lee, J.H. Kim, S.S. Park, S.S. Hong, G.D. Lee. *J Ind Eng Chem* 25(2015) 199–206
61. S.A. Ansari, M.M. Khan, M.O. Ansari, J. Lee, M.H. Cho, *J Phys Chem C* 117(51)(2013) 27023–27030
62. M.T. Uddin, Y. Nicolas, C.L. Olivier, T. Toupance, L. Servant, M.M. Müller, H.J. Kleebe, J.R. Ziegler, W. Jaegermann, *Inorg. Chem.* 51(2012) 7764–7773.
63. S. Anandan, A. Vinu, K.L.P. Sheeja, Lovely, N. Gokulakrishnan, P. Srinivasu, T. Mori, V. Murugesan, V. Sivamurugan, K. Ariga, *J. Mol. Catal. A* 266(2007) 149–157.
64. P. Jongnavakit, P. Amornpitoksuk, S. Suwanboon, N. Ndiege, *Appl. Surf. Sci.* 258 (2012) 8192–8198.
65. J. Zhao, L. Wang, X.Q. Yan, Y. Yang, Y. Lei, J. Zhou, Y.H. Huang, Y.S. Gu, Y. Zhang, *Mater. Res. Bull.* 46(2011) 1207–1210.
66. M.D.L. Ruiz Peralta, U. Pal, R.S. Zeferino, *Appl. Mater. Interfaces* 4(2012) 4807–4816.
67. Y. Zhou, S.X. Lu, W.G. Xu, *Environ. Prog. Sustain. energy* 28(2009) 226–233.
68. L.L. Yang, Z. Wang, Z.Q. Zhang, Y.F. Sun, M. Gao, *J. Appl. Phys.* 113(2013) 033514.
69. F.H. Li, H. Liu, L.X. Yu, *J. Nanosci. Nanotechnol.* 13 (2013) 5115–5118.
70. M. Faraz, F. K. Naqvi, M. Shakir and N. Khare, *New J. Chem.*, 42 (2018) 2295–2305.
71. S. Selvaraj, M. Krishna Mohan, M. Navaneethan, C. Muthamizhchelvan, *Materials Science in Semiconductor Processing*, 103 (2019) 104622.
72. R. Wang, J. H. Xin, Y. Yang, H. Xu, L. Hu, *J. Appl. Surf. Sci.*, 227, 312–317.
73. K. Vanheusden, W. L. Warren, J. A. Voigt, C. H. Seager, D. R. Tallant, *Appl. Phys. Lett.*, 67, 1280–1282.
74. A.M. Al-Hamdi, U. Rinner, M. Sillanpää, *Process Safety and Environmental Protection*. 107 (2017) 190–205.
75. A. Hamrounia, H. Lachheba, A. Houasa, *Mater. Sci. Eng. B* 178 (2013) 1371–1379.
76. A. Hamrounia, N. Moussaa, F. Parrinob, A.D. Paolab, A. Houasa, L. Palmisano, *J. Mol. Catal. A: Chem.* 390 (2014) 133–141.
77. D. Dash, N.R. Panda, D. Sahu, *Nano Exp.* 2 (2021) 010007.

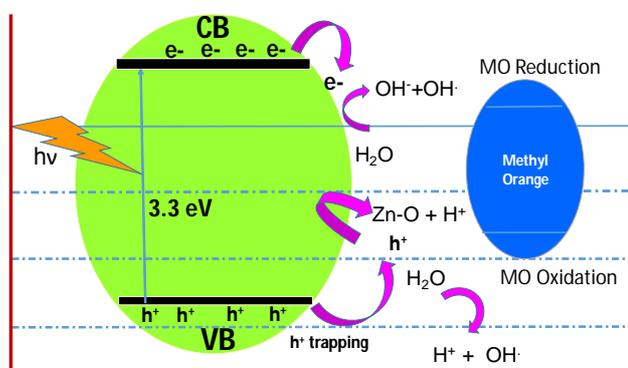


Figure-1: Mechanism of photodegradation of toxic dye (methyl orange) by ZnO.





## Predictors for Assessing the Elements that Challenge the Dancer's Limits: A Short Review

Sakshi Sadhu<sup>1\*</sup>, Ramesh Chandra Patra<sup>2</sup> and Ammar Suhail<sup>3</sup>

<sup>1</sup>Research Scholar/Assistant Professor, Department of Physiotherapy, Lovely Professional University, Phagwara, Punjab, India.

<sup>2</sup>Assistant Professor, Department of Physiotherapy, Lovely Professional University, Phagwara, Punjab, India.

<sup>3</sup>Assistant Professor, Department of Physiotherapy, Lovely Professional University, Phagwara, Punjab, India.

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### \*Address for Correspondence

#### Sakshi Sadhu

Research Scholar/Assistant Professor,

Department of Physiotherapy,

Lovely Professional University,

Phagwara, Punjab, India.

Email: Sakshi.22851@lpu.co.in



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### ABSTRACT

The main objective of this paper is to lay down the real meaning of a proverb, i.e. "*Prevention is better than cure*". Dance was and will be an integral part of the Indian society. It is associated with our cultural roots. The life of the dancers is full of struggle in terms of challenges they are facing in their career time. They suffer physically as well as mentally due to the stress, which results in the injuries. They cannot even express their state, as there is always a fear of replacement so they keep on suffering. There is limitation in the evidence in India related to the dance particularly the assessment component so the predictors is needed to find out the injury at the initial level so that we can prevent the injuries in dancers. This will help in prevent the career loss for them.

### INTRODUCTION

Dance medicine has seen an exponential increase over last decade. It is defined as "the field of medicine which is specialized in evaluating and treatment of performing artist"(1). Traditionally it has moved into the field of sports medicine so it has become a specialized branch of sports medicine. The reason can be attributed due to the body movements used during dance that places a high physical and physiological demand on the all the systems of the body in terms of muscular and joint flexibility, stability, muscle strength, coordination, sensory motor integrity, etc.

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Therefore, the dancers are believed to be as both athletes and artists (2). The word dance has its origin from the word "Danson" which means to drag or to stretch. It is the art of expressions, accompanying the graceful movements with or without the inclusion of musical backing. It develops the natural feeling of unity (3). The one who performs this art of expressions and movements, referred as "Dancer" or "Performer". The origination of this aesthetic art is still a mystery but we have the evident traces from the agricultural period (5,000 to 9,000 years ago) (4). In India Dance has a deep roots, it is believed to be the creation of God Brahma which inspired the Rishi Bharat Muni to write about "Natyashastra" (considered as the Bible of Indian classical dance systems), forming the foundation stone of every Indian classical dance form. Over the period, there are several phases and transitions in the dance forms of India and today there are around seven main kinds of traditional dance forms i.e.: Bharatanatyam, Mohiniattam, Manipuri, Kathak, Kathakali, Kuchupudi, and Odissi. The classical dance of India has two main portions, one is the pure movement and other is mimetic portion (5). The dance in India is forming the important part of cultural heritage but we are still lacking the researches related to the medical and psychological problems faced by the performers.

### **Challenges encountered among the dancers**

The dancer is considered as performing athlete, as there is enormous vividness of movements involved. It involves series of transition changing from one posture to another, considered as the complex process having various direct and indirect effects. The professionally expert dancers need to be aesthetically pleasing as well as technically sound. For the reason the dancers need to be physically sound and fit as well they should be able to handle the stress placed on them. According to the review by Koutedakis (2004), concluding that, as the aesthetic goals are important by the physical fitness always remains a concern in the life of dancers like the athletes. Classical dancers have earned recognition worldwide but the health perspective is still lacking attention. There is a huge demand on the physical fitness level of the dancers (6). Despite widespread practice of many traditional Indian dance styles, intensive preparation, and a long career as a performer, the health concerns of Indian classical dancers are being least investigated. Adam et al, Patterson et al, and Hamilton et al., in their studies, in reference to ballet dancers described that the negative stressors dancers' lives predicted an augmented injury rate (7–9). Noh et al., suggested that the negative stressors are associated with a heightened risk of injury in ballet dancers due to which the dances experienced lack of confidence and they start to doubt their ability as a dancer (10). Psychology plays important role in dancing, which describes the effect of psychological coping skills in reducing the injury extent and rate. So it's evident, the life of dancer is full of stress like the roller coaster ride and always being in the confusion about their capabilities, low self esteem and completion stress, and many other factors which become the reason of injury and restrict the career and dream of being a successful artist (11).

### **Musculoskeletal Injuries among the traditional dancers**

Yiannis Koutedakis, et al, 2008 in his study concluded that the dancers are at greater risk of injuries due to increasing use of efficient and advance ways of training in dance so to end this problem, advanced techniques are being used. The most common sites of musculoskeletal pain in female Bharatanatyam dancers were the knee (48.8%), low back (24%), ankle (12.4%), and shoulder-neck complex (7.5%), according to a study of musculoskeletal problems in female Bharatanatyam dancers (12). Another study of Bharatanatyam dancers from two separate dance schools showed that the knee, followed by the foot, elbow, hip, and shoulder, was the body part most vulnerable to injury. Based on the evidence it has been confirmed that greater prevalence of lower extremity injuries among dancers. Ryan and Stephens claim that 90% of all professional dancers suffer from different types of dancing injuries during their career. According to Bronner et al., lower extremity injuries accounts for 58% of all dancing injuries, out of which 34% affect the ankle and foot region (13). Some scholars report that lower limb injuries represent around 70% of all dancing injuries. In a study conducted by Paul and Kapoor in Indian traditional dancers, knee injuries were the most common type of injuries among these dancers it was due to the different dance positions that the knee experience torque from large twisting and rotation (14). Another knee joint injury explanation in Bharatanatyam dancers was given by Fry et al. which explains that extension if knee far beyond then the toe, puts excessive shearing stress on the knee joint (15). In a more recent study, a relationship was found between the tight iliotibial band and quadriceps muscle with lower extremity injuries among Bharatanatyam dancers. They discussed the importance of flexibility



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and stretching program for the prevention of injuries in dancers with an advice of including stretching exercise in their daily routine(16) . There are myriads of dancing players that place them at a risk of MSI. Some of the major factors leading to dance injuries are biomechanical abnormalities, agonist-antagonist muscular strength imbalances, lack of flexibility of the due to incorrect performance of dancing movements, Dancers who are not able to attain an ideal dancing position may develop compensatory strategies which may further lead to various musculoskeletal injuries. Negus et al. noted that if the dancer attempts to attain an ideal position in dancing without adequate range of motion or flexibility, he or she is more prone to injuries. Nair et al discussed in their survey related to the musculoskeletal injuries in dancer that around 73.5 % classical dancer population has described injury in their career in which back, knee and ankle considered as most prone area(17).

### **Predictors of Musculoskeletal Injuries**

The injury reason was attributed to various factors like muscle imbalance, flooring, diet, inadequate exercises etc. Some of the typical postures in Bharatanatyam and other classical dance forms are intensely challenging in terms of muscle control and balance, forcing the dancers' stability to the test and exposing them to pain. Second, classical dancers (both Indian and Western) often adopt postures that require forced knee turnout, resulting in medial contractile and non-contractile structures overstretching. As a result, the medial structures weaken, and the lateral knee stabilizers become more active, resulting in biomechanical imbalances in the patellofemoral joint. It is possible that this is the case. The classical dancers (both Indian and Western) commonly assume postures that involve forced turnout at the knee resulting in overstretching of medial contractile and non-contractile structures. This causes weakening of the medial structures and subsequent increased activity of the lateral knee stabilizers, leading to biomechanical imbalances of the patellofemoral joint. This could be the reason for patellofemoral joint syndrome as has been commonly reported among Bharatanatyam dancers. The muscle tightness can be also one of the reasons, which leads to overuse injury or even produce early wear and tear changes in weight-bearing joints (17).

Biomechanics is the branch, which deals the motion of human body by studying the forces acting on the body and the effects produced. It applies the principle of mechanics and engineering. is the study of the human body in motion. There are several equipments which are used in this field to measure and record time, motion and force, thereby advancing knowledge in such areas as developmental biomechanics, biomechanics of exercise and sports (including dance), and rehabilitative biomechanics. Motion capture, electromyography, dynamography, and dynamometry are the most common techniques used in the biomechanics around the world. In other words, biomechanics is the scientific discipline that studies the mechanical codes of human movement, such as muscle fiber recruitment, by using state-of-the-art technologies and techniques. In the history of the physics, Aristotle was the first to research and write about complex movements like running, walking. Archimedes supported this by the by the research of, movements in water. This study of biomechanics started by the 1970 and first used in the field of sports. It is very import tool in predicting out the any movement related fault so it can help in finding out the reason behind the injury caused by certain movements and avoiding them(12).

### **CONCLUSION**

With the increasing number of dancers in the country so do the rate of injuries are increasing but still we lack the right predictors to find out the way to analyze or predict the injury initially so that we can predict the injury at the initial level only and we can prevent them. Therefore, biomechanical analysis plays an important key towards this initiative. As all the individuals are biomechanically different and what we perceive as superior technique is uniquely reserved for a specific individual. Nevertheless, biomechanics can help dance educators to detect the root causes of faults (e.g., anatomical imbalances) that arise during particular movements, secure the best possible use of their dancers' natural abilities (talent), and avoid movements that may cause injuries. So need for analyzing the biomechanics of traditional form of the dance forms arise so that we can protect our dancers and we will be able to save our heritage.



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**REFERENCES**

1. Miller C. Dance Medicine: Current Concepts. *Phys Med Rehabil Clin N Am*. 2006;17(4):803–11.
2. Sharma M, Nuhmani S, Wardhan D, Muaidi QI. Comparison of lower extremity muscle flexibility in amateur and trained bharatanatyam dancers and nondancers. *Med Probl Perform Art*. 2018;33(1):20–5.
3. dance | Infoplease [Internet]. [cited 2021 Apr 5]. Available from: <https://www.infoplease.com/encyclopedia/arts/performing/dance/dance>
4. villier, G., & Grego J. *A History of Dancing from the Earliest Ages to our own Times*. New York: D. Appleton.
5. Rayapureddy L, Rayapureddy R. The Role of Indian Dances on Indian Culture. *Int J Eng Manag Res*. 2017;7(2):550–9.
6. Koutedakis Y, Jamurtas A. The dancer as a performing athlete: Physiological considerations. *Sport Med*. 2004;34(10):651–61.
7. Adam MU, Brassington GS, Steiner H, Matheson GO. Psychological Factors Associated with Performance-Limiting Injuries in Professional Ballet Dancers. *J Danc Med Sci*. 2004;8(2):43–6.
8. Patterson EL, Smith RE, Everett JJ PJ. Psychosocial factors as predictors of ballet injuries: Interactive effects of life stress and social support. *J Sport Behav*. 1;21(1):10.
9. Hamilton LH, Hamilton WG, Meltzer JD, Marshall P MM. Personality, stress, and injuries in professional ballet dancers. *Am J Sport Med*. 17(2):263-.
10. Noh YE, Morris T AM. Psychosocial factors and ballet injuries. *Int J Sport Exerc Psychol*. 3(1):79-90.
11. Medicine D. *And the Dance Goes On*. 2016;(October).
12. Koutedakis Y, Owolabi EO, Apostolos M. Dance biomechanics: a tool for controlling health, fitness, and training. *J Dance Med Sci*. 2008;12(3):83–90.
13. Bronner S, Ojofeitimi S, Rose D. Injuries in a modern dance company: Effect of comprehensive management on injury incidence and time loss. *Am J Sports Med*. 2003;31(3):365–73.
14. Paul JK, Kapoor S. Indian Anthropological Association Dance related Injuries among Bharatanatyam Dancers Author (s): Joyce K . Paul and Satwanti Kapoor Published by : Indian Anthropological Association Stable URL : <http://www.jstor.org/stable/41932561> Dance related injuri. 2018;28(2):21–33.
15. Prakash R, Williams B, Schmitt D, Gosselin-Ildari A, Canizares R. Musculoskeletal effects and injury risk in collegiate Indian classical and ballet dancers. 2015;1–44.
16. Anbarasi V, Rajan D V, Adalarasu K. Analysis of Lower Extremity Muscle Flexibility among Indian Classical Bharathnatyam Dancers. *Int J Med Heal Sci*. 2012;6(6):167–72.
17. Nair SP, Kotian S, Hiller C, Mullerpatan R. Survey of Musculoskeletal Disorders Among Indian Dancers in Mumbai and Mangalore. *J Dance Med Sci*. 2018;22(2):67–74.





## Smart ATM Transactions with Advanced Level Security using OTP Technique

Ramachandran A<sup>1\*</sup>, Ashfauk Ahamed AK<sup>2</sup>, EKSubramanian<sup>3</sup> and J.Jegan<sup>4</sup>

<sup>1</sup>Assistant Professor, Department of Computer Science and Engineering, B.S.Abdur Rahman Crescent Institute of Science and Technology, Chennai, Tamil Nadu, India.

<sup>2</sup>Assistant Professor, Department of Computer Applications, B.S.Abdur Rahman Crescent Institute of Science and Technology, Chennai, Tamil Nadu, India.

<sup>3</sup>Assistant Professor, Department of Computer Science and Engineering, Saveetha School of Engineering, Saveetha Institute of Medical and Technical Science Chennai, Tamil Nadu, India.

<sup>4</sup>Associate Professor, Department of Computer Science and Engineering, Sreenivasa Institute of Technology and Management Studies, Chittoor, Andhra Pradesh.

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### \*Address for Correspondence

#### Ramachandran A

Assistant Professor,

Department of Computer Science and Engineering,

B.S.Abdur Rahman Crescent Institute of Science and Technology,

Chennai, Tamil Nadu, India.



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### ABSTRACT

The day-to-day life financial transactions are related to Automatic Teller Machine (ATM). Authentication is an important aspect in system control in electronic machine. In general transaction mechanism uses in ATM card swiping with Personal Identification Number (PIN) for customer verification process. The most popular authentication mechanism is classical PIN method. Recently the advanced technology is introduced like fingerprint, face recognition, Iris recognition, palmprint technology, GPS, Sensor etc... for the security purpose and the combination of multilevel security mechanisms are implemented in ATM authentication process. The major five attributes namely accuracy, cost, speed, user flexibility and security are analyzed in the smart ATM transactions with advanced level security using One Time Password (OTP) technique. The proposed system has two objectives for transactions process. The first objective is smart transaction using Wi-Fi technology and second objective is an advanced level security mechanism using OTP. The proposed system is better than that the existing system based on the five major attributes. The major advantage of proposed system is a customer need not worry about the swipe and PIN.

**Keywords:** ATM, PIN, OTP, Security, Authentication



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## INTRODUCTION

Due to rapid theft and fake transaction occurs in ATM machine and threats also being posed to destroy this multilevel security. The various financial sectors namely bank, post office and finance related companies are expecting the higher level of security in ATM transaction. The existing ATM transaction model uses a card swipe and a PIN process. The hackers easy to hacking and theft the customer money through the swiping and static PIN methods [1]. To overcome, the smart ATM transactions with advanced level security using OTP technique is used. In database stored the information about a customer account details, debit card details. The random OTP will extend the security level for ATM transactions. The initial process of transaction, a user will connect the debit card through Wi-Fi connectivity device. The data received from user sides, which is compared with the stored database. If the initial verification process is completed it will forward to next process of random OTP generation. The OTP will be sent to the corresponding customer registered mobile number. If the second verification process done by customer and machine, the transaction can proceed. The mixture of Wi-Fi technology and random OTP generation process reduce the chances of theft free a user from an addition burden of recalling intricate passwords.

### Benefits of Wi-Fi and OTP

The purpose of Wi-Fi transaction is most flexible to customer. The customer can easy to communicate with machine without any interrupt. In general swiping or card holding method will taking much more time compared with proposed system and also sometime card may damage or lose by the customer. To overcome, Wi-Fi technology is better than that the existing technology. Shimal and Jhunu [2] presented the two-level authentication were PIN and OTP for customer transaction security. In general PIN methods used for all type of data transactions. The OTP method is advanced level of security for data transactions and it's used when a customer wishes to exceed the withdrawal limit.

### Types of Authentication Process

The ATM authentication process are classified into various categories namely single factor, two factor, three factor and multi factor. In Table 1 has been shown single-factor authentication process. The two-factor authentication process has been shown in Table 2. The three-factor authentication process has been shown in Table 3 and multifactor authentication process has been shown in Table 4.

### Biometrics Comparison

The biometric security is enhanced security for ATM transactions. In table 5 has been shown comparison of various biometric metrics namely cost, accuracy, performance, flaws and stability [1].

### The Proposed Method

**Problem statement :** The best public financial assist is an ATM banking and security is one of the major factors. The security enhancement is most important of ATM transactions. To overcome, the proposed system to solve problems like card fraud, skimming, card data stealing / trapping. The proposed system incorporating the advanced technology with random OTP technique. While generating random OTP, will send to the customer mobile number.

**Advanced Level Security:** The public is a bank customer or client when they have registered their details in the bank and the bank has created a profile for them. The customer details are maintained by database format. The authentication details are required to allow access for customer to carry out the transactions namely withdrawal, balance checking, mini statement, money transfer etc...

**Objective of Proposed System:** The high level of model of the proposed system has been shown in Figure 1. There are two authentication process handled by bank server. The first authentication process for bank employees and





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second authentication process for customers. The customer authentication process is maintained for transaction details. The advanced level authentication mechanisms used are Wi-Fi with random OTP generation. The ATM was interfaced with a Wi-Fi reader for easy to access and OTP enhancing the advanced level of security. The entire details will be verified on the banking system. If any authentications input is mismatched with banking system, it will destroy the process.

**Customer Registration Process:** The bank collects the customer personal information like first name, middle name, last name, age, date of birth, Aadhar number, photograph, mobile number etc... in customer registration phase [3]. The customer registration process has been shown in Figure 2.

**Wi-Fi Authentication Process:** The Wi-Fi authentication process is an initial stage for customer transaction has been shown in Figure 3. The client needs to carry out a transaction, Wi-Fi as an input for transaction and recognized the data automatically converted into binary codes. The binary code compared with bank database and find the matching of customer records. If the customer data matches with bank data bases, then the customer is authorized to carry out to the next OTP generation process.

**OTP Authentication Process:** The next level of security process is an OTP authentication process for customer transaction has been shown in Figure 4. The client needs to carry out a transaction, random OTP generation as an input for transaction and recognized the data automatically converted into binary codes. The binary code compared with bank database and find the matching of customer records. If the customer data matches with bank data bases, then the customer is authorized to carry out to the transaction process.

### Random Number Generation

The random number generation followed by method 1 and 2. The first method is generation of sequence of Pseudo-Random Numbers representing ( $Y_n$ ) in equation (1). The random number will generate dynamically on the time of customer transactions.

$$Y_{n+1} = (a \times Y_n + C) \text{ mod}(m) \quad (1)$$

The values are  $a$  – choices,  $C$  – increment and  $m$  – modulus. To overcome the first method random sequence OTP generation method has enhanced to the second method. The formula for new random number generator has been shown in equation (2). The value  $C$  and is substitute in the random number generators increment by 1.

$$C = (b \times X_n + d) \text{ mod}(m)$$

$$Y_{n+1} = (a \times Y_n + C) \text{ mod}(m) \quad (2)$$

The random number ( $Y_{n+1}$ ) generated will be the OTP. The value of ' $m$ ' should be a large prime number in order to distinct unrelated numbers. Though the overhead is increased due to computation, but the repetition of a sequence is completely eliminated [3].

**Factor Comparison:** The five important attributes namely accuracy, cost, speed, user flexibility and security are analyzed in the smart ATM transactions with advanced level security using One Time Password (OTP) technique compared with other security parameter in Table 6. The advanced level security has enhanced in ATM transactions. In table 5 has been shown comparison of four types of factor are compared with basic metrics namely cost, accuracy, performance, flaws and stability [4-15]. The comparison factors can be classified into four types like single, Two, Three and Multi-factor. The comparison factors pictorial representation has been shown in Figure 5. The values are Low, Medium and High. Low is denoting the numeric value one, medium is two and high is denoting three.

**Future Scope:** The biometric security is challenging technique compared with proposed OTP. The flaws of OTP generation technique like mobile network and GSM mobile server problems. To overcome the proposed system, need to be implement the other security technique.



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## CONCLUSION

The proposed system will be developed for ATM security enhancement. The system shows the metric and quality analysis of algorithms used based on the metrics of existing algorithms. The random generation algorithm is very accurate, require less computation time, less volume and user flexibility. The comparison of proposed system is better than that existing system.

## REFERENCES

1. Karovaliyya, Saifali Karediab, Sharad Ozac, Dr.D.R.Kalbanded, "Enhanced security for ATM machine with OTP and Facial recognition features Mohsin", International Conference on Advanced Computing Technologies and Applications (ICACTA2015) Procedia Computer Science 45,2015.
2. D. Shimal and D. Jhunu, "Designing a biometric strategy (fingerprint) measure for enhancing ATM security in Indian e-banking system," International Journal of Information and Communication Technology, 2011.
3. Abiodun Esther Omolara<sup>1</sup>, Aman Jantan, Oludare Isaac Abiodun, Humaira Arshad, NachaatAbdElatif Mohamed, "Fingereye: improvising security and optimizing ATM transaction time based on iris-scan authentication", International Journal of Electrical and Computer Engineering (IJECE) Vol. 9, No. 3, June 2019.
4. Machine Moses O. Onyesolu, Amara C. Okpala, "Improving Security Using a Three-Tier Authentication for Automated Teller", International Research Journal of Engineering and Technology (IRJET) Volume: 05 Issue: 05, May 2018.
5. Mohammed Hamid Khan Shah and Anchor, "Securing ATM with OTP and Biometric", International Journal on Recent and Innovation Trends in Computing and Communication, Volume: 3 Issue: 4, 2015.
6. LusekeloKibona, "Face Recognition as a Biometric Security for Secondary Password for ATM Users. A Comprehensive Review", IJSRST, Volume 1, Issue 2 2015.
7. Hossein Reza Babaei, Ofentse Molalapata and Abdul-Hay Akbar Pandor, "Face Recognition Application for Automatic Teller Machines", Malaysia International Conference on Information and Knowledge Management (ICIKM 2012) IPCSIT vol.45, IACSIT Press, Singapore, 2012.
8. Nawaya, J. J, Jemimah N, Oye. N, "Designing a Biometric (Finger) Using Multispectral Imaging Biometric Authentication Measures for Enhancing ATM Security in Nigeria", IJCSMC, Vol. 8, Issue. 11, November 2019, pp.38 – 47.
9. Ojekudo Nathaniel, Macarthy Osuo-Genseleke, "A Comparative Study of PIN Based and Three-factor Based Authentication Technique for Improved ATM Security", International Research Journal of Engineering and Technology, Volume: 05 Issue: 05, May 2018.
10. B. V. Prasanthi, Mahaboob Hussain, Prathyusha Kanakam, "Palm Vein Biometric Technology: An Approach to Upgrade Security in ATM Transactions", International Journal of Computer Applications (0975 – 8887) Volume 112 – No. 9, February 2015.
11. B V Prasanthi, U Padma Jyothi, Sridevi Bonthu, T Vamsi Krishna, "Security Enhancement of ATM System with Fingerprint and DNA Data", International Journal of Advanced Research in Computer Science and Software Engineering, Volume 4, Issue 12, December 2014.
12. Mithun Dutta, KangkhitaKeam Psyche, Shamima Yasmin, "ATM Transaction Security Using Fingerprint Recognition", American Journal of Engineering Research, Volume-6, Issue-8, pp-41-45, 2017.
13. Aru, Okereke Eze, Ihekweaba Gozie, "Facial Verification Technology for Use In Atm Transactions", American Journal of Engineering Research, Volume-02, Issue-05, pp-188-193, 2019.
14. Muhammad-Bello B.L, Alhassan M.E., Ganiyu, S.O., "An Enhanced ATM Security System using Second-Level Authentication", International Journal of Computer Applications, Volume 111 – No 5, February 2015.
15. De Luca, Marc Langheinrich, Heinrich Hussmann, "Towards Understanding ATM Security – A Field Study of Real World ATM Use", Symposium on Usable Privacy and Security (SOUPS) 2010, July 14–16, 2010.







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**Table 4. Multi-factor Authentication Process**

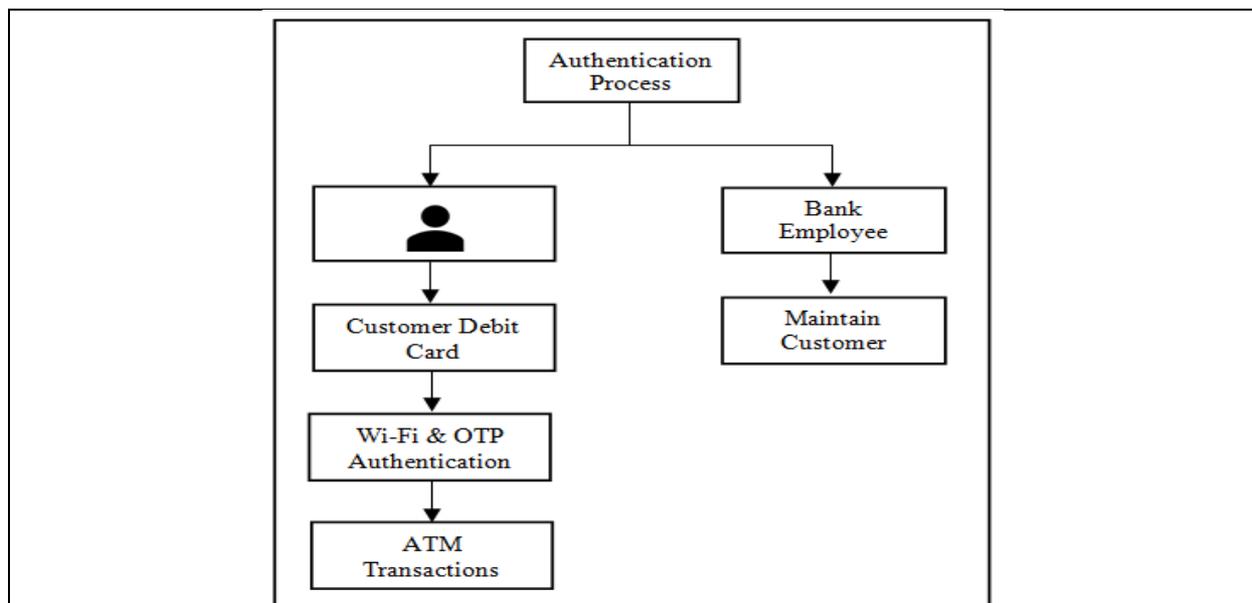
Authentication								
Swiping	PIN	OTP	Wi-Fi	Finger	Iris	Retina	Face	Palm
•	•	•		•				
•	•	•			•			
•	•	•				•		
•	•	•					•	
•	•	•		•				•
	•	•	•	•	•			
	•	•	•		•	•		
	•	•	•			•	•	
	•	•	•					•

**Table 5. Comparison of biometric technologies**

Biometrics	Cost	Accuracy	Performance	Flaws	Stability
Iris	High	High	High	Lighting	High
Retina	High	High	High	Glasses	High
Face	Medium	Medium	Medium	Beard, glasses, age	Medium
Fingerprint	Low	Medium	Medium	Dirt, dryness	High

**Table 6. Comparison of Factors**

Factors	Cost	Accuracy	Security	User Flexibility	Speed
Single	Low	Medium	Medium	High	High
Two	Medium	High	Medium	Medium	High
Three	High	High	High	Low	Medium
Multi	High	High	High	Low	Medium



**Figure 1. High Level Model of Proposed System**



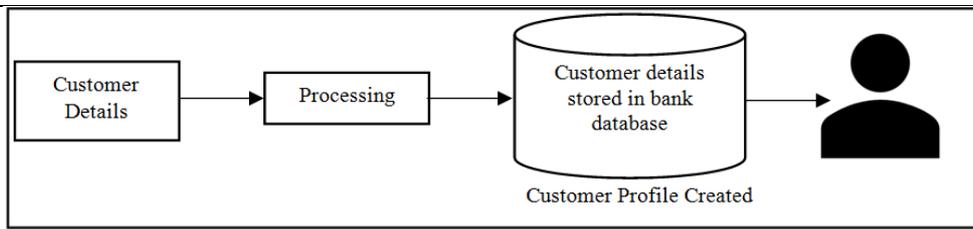


Figure 2. Customer Registration Process

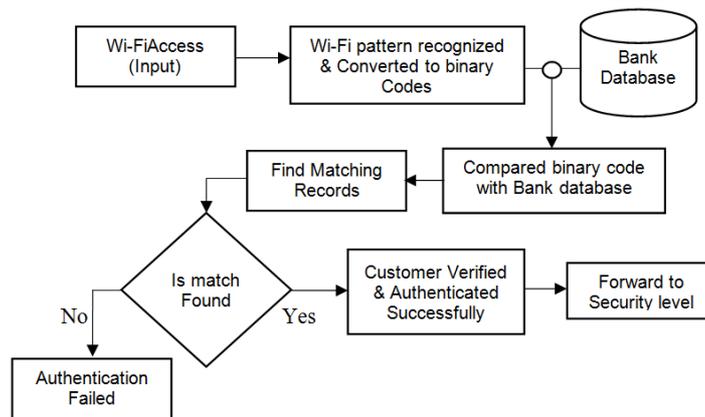


Figure 3. Wi-Fi Authentication Process

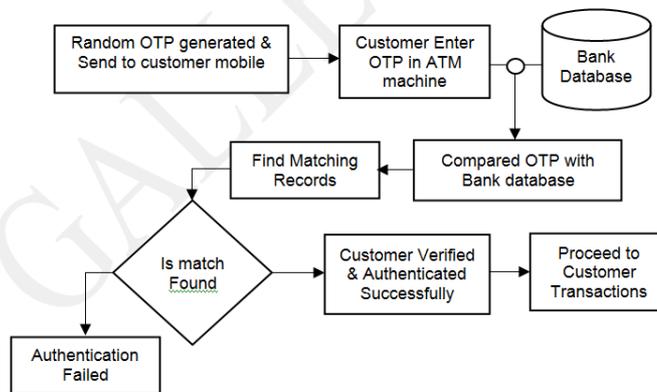


Figure 4. Wi-Fi Authentication Process

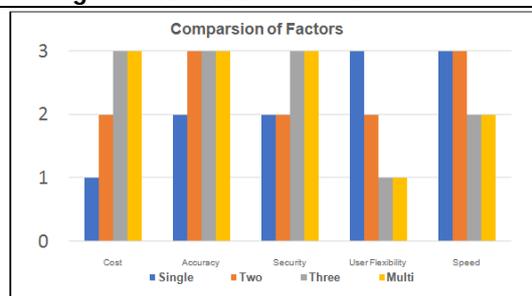


Figure 5. Factor Comparison





## Preliminary Phytochemical Investigation and *in Vitro* Hepatoprotective Activity of *Eclipta prostrata* (L.)

Umesh A<sup>1,3\*</sup>, Kumudhavalli MV<sup>2</sup> and Venkateswarlu BS<sup>2</sup>

<sup>1</sup>Department of Pharmacognosy, Grace College of Pharmacy, Palakkad, Kerala, India.

<sup>2</sup>Department of Pharmacognosy, Vinayaka Mission's College of Pharmacy, Vinayaka Mission Research Foundation-Deemed to be University (VMRFDU), Sankari Main Road, Ariyanoor, Salem, Tamil Nadu, India.

<sup>3</sup>Research Scholar, Vinayaka Mission Research Foundation-Deemed to be University (VMRFDU), Sankari Main Road, Ariyanoor, Salem, Tamil Nadu, India.

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### \*Address for Correspondence

**Umesh A**

Department of Pharmacognosy,  
Grace College of Pharmacy,  
Palakkad, Kerala-678004, India.  
E.mail: umeshpharma21@gmail.com



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### ABSTRACT

*Eclipta prostrata* (L.) is a medicinally important plant species to treat various diseases such as hepatic diseases. Liver diseases are a major health problem worldwide, making it necessary to develop new molecules to help counteract or prevent liver diseases. The present study aimed to evaluate the *in vitro* hepatoprotective activity. Ethanolic leaf extract was prepared by using the soxhlet extraction method. The plant extract has been shown the presence of different phytoconstituents. *in vitro* hepatoprotective activity was determined by the MTT assay method HEP G2 (Human Hepatic cells), ethanol were used as toxic agent. The study concluded that the ethanolic leaf extract of *Eclipta prostrate* (L.) has significant hepatoprotective activity due to the presence of wedelolactone, coumarin.

**Keywords:** *Eclipta prostrata* (L.), *in vitro* hepatoprotective activity, MTT Assay

### INTRODUCTION

The herb *Eclipta prostrata* (L.) L.(Asteraceae) is commonly known as Bhringaraja has been widely used in India for the traditional treatment of liver disorders [1]. Liver is an important vital organ it regulates different functions in the body, metabolism, secretion, storage, and detoxifying. Various types of liver disorders are characterized by cirrhosis, tumors, jaundice, metabolic and degenerative lesions, and liver cell necrosis. The liver is continuously exposed to an elevated amount of toxic agents because the portal vein supplies blood to this organ after intestinal absorption [2].

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Herbal medicines are popular remedies for diseases and are used by the vast majority of the population worldwide [3]. Natural remedies from medicinal plants are considered to be an effective and safe alternative treatment for liver toxicity [4]. The preliminary Phytochemical investigations are very important in identifying new sources of therapeutically important compounds like alkaloids, saponins, flavonoids, steroids, phenolic compounds, coumarin luteolin, wedelolactone, triterpenoids, proteins, amino acids, reducing sugar [5]. The plants are also having different pharmacological activities like Analgesic and Anti-inflammatory activity, antimicrobial effect, Antihyperglycemic effect, Antioxidant properties, hypolipidemic effect, Neuropharmacological effects, Hair growth and Alopecia, Immunomodulatory activities, Anticancer activity [6].

## MATERIALS AND METHODS

### Collection and Authentication

Fresh leaves of *Eclipta prostrata* were collected from Kanchipuram, Tamilnadu, India, and authenticated at Botanical Survey of India, Southern Regional Centre, Coimbatore. (No: BSI/SRC/5/23/2021/Tech/12)

### Preparation of Extract

The collected plant materials were gently washed and then shade dried under room temperature. After complete drying, the dried plant materials were pulverized followed by sieving to obtain coarse powder. The powdered plant material (500 g) was extracted with ethanol (95.99%) using a soxhlet extractor. The extract obtained was evaporated under vacuum using a rotary evaporator and concentrated [7].

### Preliminary Phytochemical Screening

Phytochemical investigation of ethanolic extract of *Eclipta Prostrata* (L.) L was carried out qualitatively to test for the presence of alkaloids, phenols, flavonoids, Phytosterols, proteins, amino acids, tannins, saponins [8, 9].

### Detection of Alkaloids

**Mayer's Test:** Filtrates were treated with Mayer's reagent (Potassium mercuric iodide solution ) Formation of a cream color precipitate indicates the presence of alkaloids.

**Hager's Test:** Filtrates were treated with Hager's reagent (saturated solution of picric acid) Formation of yellow color precipitate indicates the presence of alkaloids.

### Detection of Saponins

**Foam Test:** The extract were shaken with little quantity of water. If the foam persists for 10 minutes it indicates the presence of saponins.

### Detection of Carbohydrates

**Benedict's \$:** Filtrates were treated with Benedict's reagent and heated on a water bath. The formation of a red color precipitate indicates the presence of reducing sugars.

**Molisch's Test:** Filtrates were treated with 2 drops of alcoholic  $\alpha$ -naphthol solution and 2ml conc.  $H_2SO_4$  was added carefully through the sides of a test tube. Formation of purple to violet color ring appears at junction indicates the presence of carbohydrates.

### Detection of Phytosterols

**Liebermann bur chard's Test:** Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride. Boiled and cooled. Conc. Sulphuric acid was added carefully along the sides of the test tube. The brown ring is formed at the junction of two layers indicates the presence of phytosterols.





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**Detection of Flavonoids**

**Shinoda Test:** A small quantity was dissolved in alcohol to these pieces of magnesium followed by concentrated hydrochloric acid were added drop wise and heated. The magenta color indicates the presence of flavonoids.

**With Aqueous Sodium Hydroxide Solution:** Small quantity of extract was dissolved in aqueous sodium hydroxide and appearance of yellow color indicates the presence of flavonoids.

**Detection of Proteins and Amino Acids**

**Xanthoprotein Test:** The extracts were treated with few drops of conc. nitric acid solution. The yellow color indicates the presence of proteins.

**Ninhydrin Test:** The extracts were treated with 0.25% ninhydrin reagent was added and boiled for few minutes. The Blue color indicates the presence of amino acids.

**Detection of Phenols**

**Ferric Chloride Test:** Extracts were treated with ferric chloride solution. Bluish black color indicates the presence of phenols.

**Detection of Tannins**

**Gelatin Test:** To the extract, 1% gelatin solution containing sodium chloride was added. The Formation of white color precipitate indicates the presence of tannins.

**In Vitro Hepatoprotective Activity****Determination By MTT Assay****Cell lines and Maintenance**

HEP G2 (Human Hepatic cells) cell line was purchased from National Centre for Cell Sciences (NCCS), Pune, India and maintained in Dulbecco's Modified Eagles medium (DMEM) (Sigma Aldrich, USA). The cell line was cultured in a 25 cm<sup>2</sup> tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate, and the antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphotericin B (2.5µg/ml). Cultured cell lines were kept at 37°C in a humidified 5% CO<sub>2</sub> incubator.

The viability of cells was evaluated by direct observation of cells by microscope and followed by the MTT assay method.

**Cells Seeding In 96 Well Plate**

Two days old confluent monolayer of cells were trypsinized and the cells were suspended in a 10% growth medium, 100µl cell suspension (5x10<sup>3</sup> cells/well) was seeded in 96 well tissue culture plate and incubated at 37°C in a humidified 5% CO<sub>2</sub> incubator.

**Preparation of Compound Stock**

1mg of the sample was weighed and completely dissolved in 1mL DMEM using a cyclomixer. The extract solution was filtered through a 0.22 µm Millipore syringe filter to ensure sterility. Ethanol (40%) was added to induce toxicity.

**Cytotoxicity Evaluation**

After sufficient growth attaining, Ethanol (40%) was added to induce toxicity and incubated for one hour, prepared extracts in DMEM were five times serially diluted by two-fold dilution (6.25µg, 12.5µg, 25µg, 50µg, 100µg, in 500µl of DMEM) and each concentration of 100µl was added in triplicates to the respective wells and incubated at 37°C in a humidified 5% CO<sub>2</sub> incubator.

**Cytotoxicity Assay by Direct Microscopic observation**

Entire plates were observed in an inverted phase-contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and microscopic observations were recorded as images. To observe any detectable changes in the

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morphology of cells, rounding or shrinking of cells, granulation, and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.

#### Cytotoxicity Assay by MTT Method

15mg of MTT (Sigma, M-5655) were reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization. After 24 hours of the incubation period, the sample content in wells was removed and 3 0µl of reconstituted MTT solution were added to all test and cell control wells, the plates were gently shaken well, then incubated at 37°C in a humidified 5% CO<sub>2</sub> incubated for 4 hours. During the incubation period, the supernatant was removed and 100µl of MTT Solubilization Solution (DMSO) was added and the wells were mixed gently by pipetting up and down in order to solubilize the formazan crystals. The absorbance values will measure by using a microplate reader at a wavelength of 540 nm [10].

The % of growth inhibition was calculated by using the formula

$$\% \text{ of viability} = \frac{\text{Mean OD Samples}}{\text{Mean OD of Control Group}} \times 100$$

## RESULTS AND DISCUSSION

The preliminary Phytochemical screening of *Eclipta prostrata*(L.) leaf extract reveals the presence of phenols, alkaloids, proteins, amino acids, tannins, flavonoids, Phytosterols, saponins. It has been reported that ethanol causes apoptosis, cell injury and necrosis. A dose-dependent increase in the percentage viability was observed when ethanol exposed HepG2 cells were treated with different concentrations of the plant extract. The ethanolic leaf extract showed minimum percentage (64) of cell viability in the concentration of 6.25 µg/ml. Ethanol showed 45.08 percentage cell viability. Ethanolic leaf extract showed a maximum Percentage (74.61) of cell viability in the concentration of 50µg/ml. Higher concentrations such as 100 µg/ml shown reduced hepatoprotection than lower concentrations suggesting possible cytotoxicity at higher concentrations.

## CONCLUSION

The viability of ethanol-treated cells was reduced to 45.08 when compared with untreated controlled samples having 100% viability. The ethanolic leaf extracts effectively ethanol toxicity up to 50µg/ml in a dose-dependent manner, 50µg/ml effectively restored with cell viability to 75%. From the basis of the results of the study, it can be concluded that the ethanolic leaf extract of *Eclipta prostrata* (L.) has significant hepatoprotective activity.

## ACKNOWLEDGMENT

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## REFERENCES

1. Deepak K Ahirwar and Saxena RC. Hepatoprotective activity of ethanolic extract of *Eclipta alba* in albino rats. *Biomedical and Pharmacology Journal*, 2008; 1(1): 235-238.
2. Groneberg DA, Grosse-Siestrup C, Fischer A. *In vitro* models to study hepatotoxicity. *Toxicol Pathol.* 2002; 30: 394-9.
3. Kuruvilla A. Herbal formulation as pharmacotherapeutic agents. *Ind J Exp Biol.*2002; 40: 7-11.
4. Thirumali T, David E, Viviyan Theresa S, Elumalai EK. Restorative effect of *Eclipta alba* in CCl<sub>4</sub> induced hepatotoxicity in male albino rats. *Asian Pac J Trop Dis* 2011; 1(4): 304-307.





**Umesh et al.**

5. Kishor MP, Meenakshi M. Screening of antibacterial and antioxidant activities of leaves of *Eclipta prostrata* (L). *Scientific and Research and Essay*, 2007; 24: 101-104.
6. Satish A Bhalerao, Deepa R Verma, Nikhil C Teli and Vaibhav R Murukate. *Eclipta alba* (L.): AN OVERVIEW. *International Journal of Bioassays*, 2013; 02(11): 1443-1447.
7. Harborne JB, Williams CA, Advances in flavonoid research since 1992. *Phytochemistry*, 2000; 55: 481-504.
8. Karthikumar S, Vigneswari K, Jegatheesan K. Screening of antibacterial and antioxidant activities of leaves of *Eclipta Prostrata* (L). *Scientific Res. Essay*, 2007; 2:10-104.
9. Khandelwal KR, Practical Pharmacognosy, 4<sup>th</sup> Edition, Vallabh Prakashan, Delhi, 2001, 107-108.
10. Laura B Talarico *et al.*, The role of heterotypic DENV-specific CD8 + T Lymphocytes in an immunocompetent mouse virus infection. *EBioMedicine*. 2017.

**Table 1. Phytochemical screening of *Eclipta prostrata*(L.) ethanolic leaf extract**

S.No	Phytoconstituents	Ethanolic leaf extract
1	Alkaloids	+
2	Saponins	+
3	Phytosterols	+
4	Flavonoids	+
5	Proteins	+
6	Phenols	+
7	Tannins	+
8	Coumestans	++
9	Carbohydrates	-

+ Indicates presence of phytoconstituents

- Indicates absence of phytoconstituents

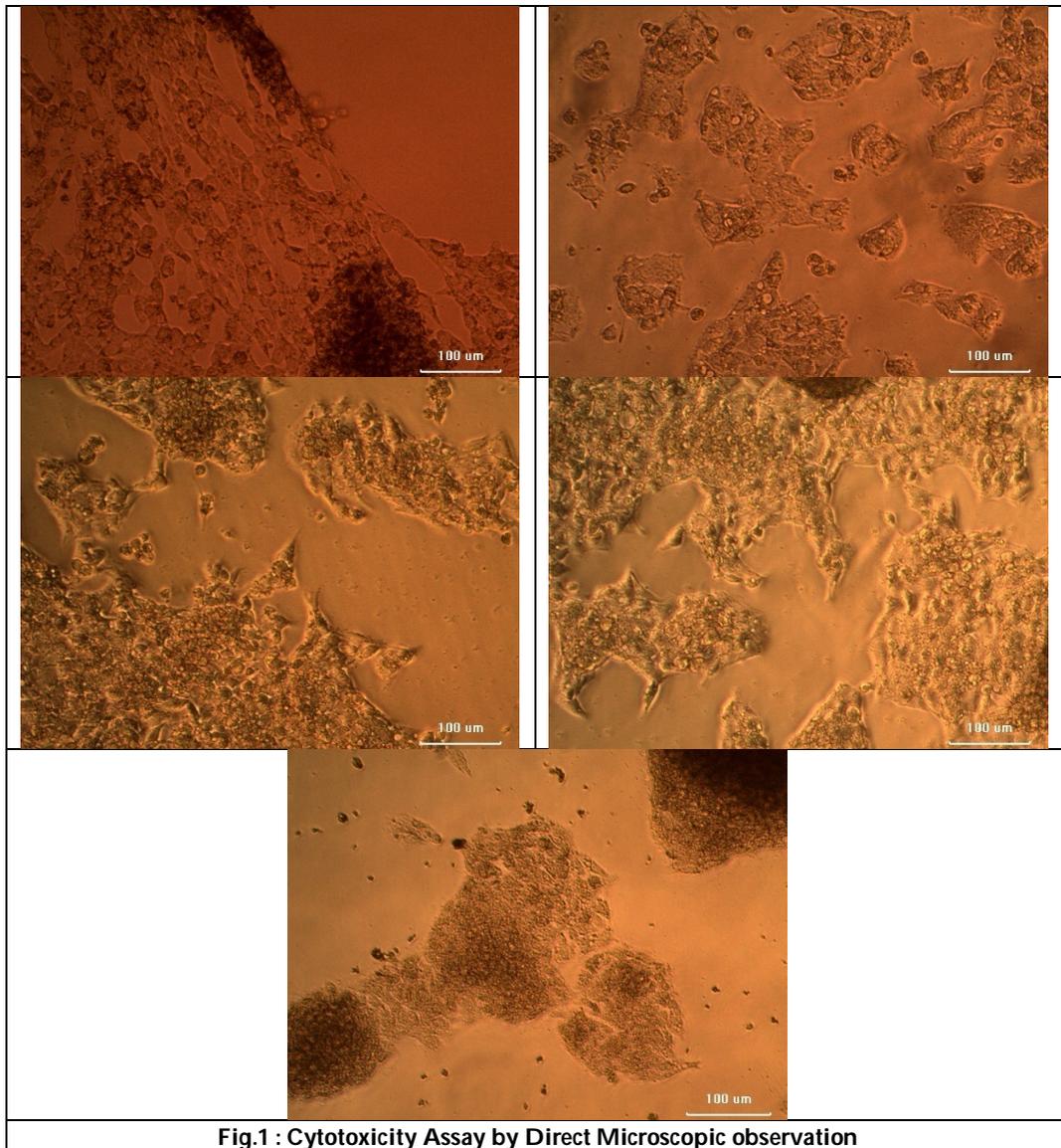
**Table 2. Percentage of cell viability on liver cells**

Sample Concentration (µg/ml)	OD I	OD II	OD III	Average Absorbance @ 540nm	Percentage Viability
<b>CONTROL</b>	0.4300	0.4317	0.4337	0.4318	100.00
<b>Ethanol</b>	0.1967	0.1909	0.1964	0.1947	45.08
<b>Sample code: Ethanolic Leaf Extract</b>					
6.25	0.2851	0.2715	0.2725	0.2764	64.00
12.5	0.2876	0.2775	0.2744	0.2798	64.81
25	0.2913	0.2816	0.2853	0.2861	66.25
50	0.3278	0.3168	0.3219	0.3222	74.61
100	0.2463	0.2482	0.2471	0.2472	57.25





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## High Order OSCM for Two-Point Boundary Value Problem

Santosh Kumar Bhal\* and Balaji padhy

Centurion University of Technology and Management, Odisha, India.

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### \*Address for Correspondence

**Santosh Kumar Bhal**

Centurion University of Technology and Management,  
Odisha, India.

E.mail : Santosh.bhal@cutm.ac.in



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### ABSTRACT

An orthogonal spline collocation technique (OSCM) has been utilized for a two-point boundary value problem in this paper. We utilize cubic monomial premise capacities to approximate the solution for linear and boundary value problems. A few mathematical trials are then performed and network refinement examination is carried out to figure the request for the intermingling of the mathematical strategy. We then, at that point attempt to show that the symmetrical spline collocation techniques (OSCM) provide the ideal request of intermingling at the bunches as analyzed against the current mathematical strategies.

**Keywords:** Orthogonal cubic spline collocation strategies (OCSCS), Two-point boundary value problem, Cubic monomial premise capacities, and Almost block diagonal (ABD) matrix

### INTRODUCTION

Numerous numerical methods exist to approximate the solutions of nonlinear partial differential equations. The stability and efficiency of orthogonal spline collocation methods over B-splines have made the former more preferable than the latter. As against finite element methods, determining the the approximate solution and the coefficients of stiffness matrices and mass is relatively fast as the evaluation of integrals is not an requirement.

#### Spaces of Piece-wise Polynomial Functions

For successful implementation of oorthogonal spline collocation methods, the selection of subspace  $S_n$  plays a vital role in efficient computation of the collocation approximations.

It is normally picked to be a space of piece-wise polynomial capacities. To characterize such spaces, let  $P_r$  indicate the arrangement of polynomials of degree  $\leq r$ . Let

$$\pi : 0 = x_0 < x_1 < x_2 < \dots < x_N < x_{N+1} = 1$$

denote a partition of  $I$ , and set

$$I_j = [x_{j-1}, x_j], \quad j = 1, \dots, N + 1,$$





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$h_j = x_j - x_{j-1}$  and  $h = \max_j h_j$ . Now define

$$M_k^r(\pi) = \{v | v \in C^k(I), v|_{I_j} \in P_r, \quad j = 1, \dots, N + 1\}$$

where  $C^k(I)$  indicates the space of capacities which are  $k$  times continuously differentiable on  $I, 0 \leq k \leq r$ , and  $v|_{I_j}$  signify the limitation of the capacity  $v$  to the interval  $I_j$ . We denote by  $M_k^{r,0}(\pi)$  the space  $M_k^r(\pi) \cap \{v | v(0) = v(1) = 0\}$ .

It is to see that  $M_k^r(\pi)$  and  $M_k^{r,0}(\pi)$  are linear spaces of dimensions  $N(r - k) + r + 1$  and  $N(r - k) + r - 1$ , respectively. Two examples of commonly used piece-wise polynomial spaces are treated in the following material of this paper.

**The Space of Piece-Wise Hermite Cubic Function  $M_1^3(\pi)$ :**

This space has dimension  $2N + 4$ . We express the basis function on  $[x_{i-1}, x_i]$ , with  $x_i = ih$ ,

$$vl_{i-1}(x) = -2 \left[ \frac{x_i - x}{h} \right]^3 + 3 \left[ \frac{x_i - x}{h} \right]^2, \quad vl_i(x) = -2 \left[ \frac{x - x_{i-1}}{h} \right]^3 + 3 \left[ \frac{x - x_{i-1}}{h} \right]^2,$$

and

$$sl_{i-1} = -h \left\{ \left[ \frac{x_i - x}{h} \right]^3 - \left[ \frac{x_i - x}{h} \right]^2 \right\}, \quad sl_i = h \left\{ \left[ \frac{x - x_{i-1}}{h} \right]^3 - \left[ \frac{x - x_{i-1}}{h} \right]^2 \right\}.$$

The functions  $v_i$  and  $s_i$  are known as the value function and the slope function respectively, related with the point  $x_i \in \pi$ . If the approximate solution is expressed as

$$u_h(x) = \sum_{j=0}^{N+1} \{ \alpha_j vl_j(x) + \beta_j sl_j(x) \},$$

then

$$\alpha_j = u_h(x_j), \quad \beta_j = u'_h(x_j), \quad j = 0, \dots, \dots, N + 1.$$

Since

$$vl_j(x_i) = \delta_{ij}, \quad vl'_j(x_i) = 0 \text{ and } sl_j(x_i) = 0, \quad sl'_j(x_i) = \delta_{ij}, \quad i, j = 0, \dots, N + 1,$$

where  $\delta_{ij}$  is the Kronecker delta function with  $\delta_{ij} = 1$ , if  $i = j$  and  $\delta_{ij} = 0$ , if  $i \neq j$ . A basis for  $M_1^{3,0}(\pi)$  is is gotten by precluding the capacities  $vl_0(x)$  and  $vl_{N+1}(x)$ .

**Monomial Bases**

Assuming that on each sub-interval  $[x_{i-1}, x_i], i = 1, 2, \dots, N$ , the collocation approximation  $u_h \in M_1^4(\pi)$  has the form:

$$u_h(x) = y_{i1} + (x - x_{i-1})y_{i2} + (x - x_{i-1})^2 z_{i1} + (x - x_{i-1})^3 z_{i2} + (x - x_{i-1})^4 z_{i3},$$

so that,

$$u'_h(x) = y_{i2} + 2(x - x_{i-1})z_{i1} + 3(x - x_{i-1})^2 z_{i2} + 4(x - x_{i-1})^3 z_{i3},$$

and

$$u''_h(x) = 2z_{i1} + 6(x - x_{i-1})z_{i2} + 12(x - x_{i-1})^2 z_{i3}.$$

A noteworthy observation is that the continuity is not built into the approximate solution. With this representation of  $u_h$ , the collocation equations on the sub-interval  $[x_{i-1}, x_i], i = 1, 2, \dots, N$ , are of the form

$$V_i y_i + W_i z_i = q_i,$$

where  $V_i$  and  $W_i$  are  $2 \times 3$  matrices,  $y_i = (y_{i1}, y_{i2})^T, z_i = (z_{i1}, z_{i2}, z_{i3})^T$ . In addition,  $u_h(x)$  and  $u'_h(x)$  are required to be continuous at  $x = x_i$ , which leads to

$$y_{i+1} = C_i y_i + D_i z_i, \quad i = 1, \dots, N.$$

For more details of the above basis functions, we refer to [1,2,4,8,10].

The prevalence of the orthogonal spline collocation strategy is because of its reasonable effortlessness, wide pertinence, and simplicity of execution. The orderly joining of limit and interface conditions in OSCM adds to the rundown of benefits of favouring this technique. The semi-discrete framework is addressed by RADAU 5 programming library [8-9] which is appropriate for tackling differential algebraic equations (DAEs).

Orthogonal spline collocation (OSC) strategy for straight two-point boundary value problem (BVPs) for customary differential conditions (ODEs) was depicted and first broke down in the original paper of deBoor and Swartz [3,5,9,11], which established the framework for the detailing and investigation of OSC techniques for a wide





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assortment of issues and the improvement of programming bundles for their answer; see [7]. The persistent time OSC strategy and the discrete-time Crank–Nicolson OSC technique for linear parabolic initial–boundary value problems (IBVPs) in one space variable and diagram utilizations of OSC to different conditions in a single space variable, for example, Schrodinger-type conditions were first figured and broke down in [8], and Cerutti and Parter [8] integrated with the aftereffects of [10] and those of Douglas and Dupont. Following the methodology of Douglas and Dupont, Houstis [11] considered OSC for nonlinear second-request exaggerated issues.

$$u_{xx} + u_x + u = 0, \quad x \in (0,1) \quad \text{--- (1)}$$

Subject to boundary condition

$$u(0) = \alpha, \quad u(1) = \beta$$

where  $\alpha$  and  $\beta$  are known constants. Optimal order  $H^1$  and  $H^2$  error estimates are derived. As the OSC linear system turns out to be complicated for the given case it is solved by a direct method based on the capacitance matrix technique. On a uniform partition, the total cost of the capacitance matrix method for computing the OSC solution is  $O(N^3)$ . Since the capacitance system is first formed explicitly and then solved by Gauss elimination, fourth-order accuracy of the approximations and the super convergence of the derivative approximations at the mesh points are demonstrated by the results calculated from the numerical experiments. Resulting system (1), we use orthogonal cubic spline collocation method and approximate the discrete solution using monomial basis functions or piece-wise Hermite cubic basis functions. The semi-discrete system is then integrated in time using RADAU 5 [8-9] time integrator.

#### Numerical Example

Consider the problem  $u_{xx} + u_x + u = \sin(x), \quad x \in (0, \pi)$

With boundary conditions  $u(0) = u(\pi) = 0$

Below, we have shown the error in tabular form.

#### CONCLUSION

An orthogonal cubic spline collocation method (OCSCM) to one-dimensional fourth-order linear boundary value problems has been developed as several numerical experiments are performed to obtain the fourth order convergence at the grid points.

#### REFERENCES

12. P. Danumjaya, A. K. Pani, Orthogonal cubic spline collocation method for extended Fisher-Kolmogorov equation, *J. Compt. Appl. Math.*, 174, 2005, 101-117.
13. P. Danumjaya, A. K. Nandakumaran, Orthogonal cubic spline collocation method for the Cahn-Hilliard equation, *J. Compt. Appl. Math.*, 182, 2006, 1316-1329.
14. P. Danumjaya, Orthogonal cubic spline collocation method for the Fisher-Kolmogorov equation, *Industrial Mathematics*, Narosa Pub., 2006, 87-96.
15. A.V. Manickam, K. M. Moudgalya, A. K. Pani, Second order splitting and orthogonal spline collocation methods for Kuramoto-Sivashinsky equation, *Compt. Math. Appl.*, 35, 1998, 5-25.
16. A.V. Manickam, A. K. Pani, S. K. Chung, A second order splitting combined with orthogonal cubic spline collocation method for the Rosenau equation, *Numer. Methods PDEs*, 14, 1998, 695-716.
17. U. Ascher, S. Pruess, R. D. Russel, On spline basis selection for solving differential equations, *SIAM J. Numer. Anal.*, 20, 1983, 121-142.





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18. G. Fairweather, D. Meade, A survey of spline collocation methods for the numerical solution of differential equations, *J.C. Diaz (Ed.), Mathematics for Large Scale Computing, Lecture Notes in Pure and Applied Mathematics, Marcel Dekker, New York, 120, 1989, 297–341.*
19. E. Hairer, C. Lubich, M. Roche, The Numerical Solution of Differential Algebraic Systems by Runge-Kutta Methods, in Lecture notes in Mathematics, *Springer, New York, 1409, 1989.*
20. E. Hairer, G. Wanner, Solving Ordinary Differential Equations II: Stiff and Differential Algebraic Problems, *Springer, New York. 1991.*
21. P. M. Prenter, Splines and Variational Methods, John Wiley & Sons, 1989.
22. C. de Boor, B. Swartz, Collocation at Gauss points, *SIAM J. Numer. Anal.*, 10, 1973, 582–606.

**Table :1 Numerical Example Error in Tabular Form.**

N	$L^\infty(u)$	order	$L^\infty(u')$	order
10	1.4753e-04		1.9765e-03	
20	8.6752e-06	4.0575e+00	1.9086e-04	3.3165e+00
30	1.3896e-06	4.5851e+00	3.0784e-05	4.5329e+00
40	4.5764e-07	3.7777e+00	1.8123e-05	1.8566e+00
50	1.8756e-07	3.9555e+00	6.9834e-06	4.2715e+00
60	9.5537e-08	3.6999e+00	3.7684e-06	3.3990e+00





## Cutting Force Measurement in Turning of Aluminium Alloy using Taguchi Method

Amit Kumar Mohanta<sup>1</sup>, Dillip Kumar Mohanta<sup>1</sup> and Mohammed Siddique<sup>2\*</sup>

<sup>1</sup>Dept. of Mechanical Engineering, Centurion University of Technology and Management, Odisha, India.

<sup>2</sup>Dept. of Mathematics, Centurion University of Technology and Management, Odisha, India.

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### \*Address for Correspondence

**Mohammed Siddique**

Dept. of Mathematics,

Centurion University of Technology and Management,  
Odisha, India

Maharashtra, India.

E.mail: siddique1807@gmail.com



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### ABSTRACT

It is very difficult to predict accurately the cutting forces encountered in metal cutting operation due to large number of variables involved. Some estimate of cutting forces is desirable to enable decide input parameters to be within the capacity of the machine tool to prevent unacceptable deflections and hence to produce quality products at a high production rate and low production cost. The paper discusses how the cutting force value is influenced by the most important input variables like cutting speed ( $v$ ), feed ( $f$ ) and depth of cut ( $d$ ) in CNC turning operation of Al- 6061 with uncoated carbide insets. Inputs and outputs are analyzed to determine optimum factor level. From experimental observation it was observed that cutting speed had remarkable effect in decreasing cutting force followed by feed and depth of cut had minor role. From result obtained by Taguchi method, it was concluded that, cutting speed, feed and depth of cut of 125m/min, 0.06 mm/rev and 0.2mm respectively are the optimal parameters to be maintained during machining. The confirmation test was carried out at optimum conditions.

**Keywords:** Turning input variables, Cutting Force Speed; Taguchi Method.

### INTRODUCTION

One of the most promising techniques for detection of cutting conditions like tool wear, chip formation, and surface quality, involves the measurement of cutting forces. In the area of contact between tool and material, a cutting force appears which can be resolved into 3 orthogonal components. We should minimize the cutting force or tangential cutting force component  $F_z$  because more unevenness leads to functional discrepancies [1].



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A statistical methodology for assessing the results of several variables in an experiment is design of experiments (DOE). Taguchi DOE employs modified and standardized quality improvement application principles. [2]. To make DOE more efficient, Taguchi devised a set of number tables known as orthogonal arrays. Experiments are carried out using orthogonal arrays. The results are interpreted using ANOVA, which is based on the variation from the targets rather than absolute values [3]. Taguchi DOE is able to select the most consistent output or design condition, resulting in higher product quality. Many researchers have presented the data for cutting force. A strain gauge-based dynamometer was devised and developed by S.Yaldiz and F. Unsacar in 2005. The results of machining experiments conducted at various cutting parameters demonstrate that the dynamometer can be used to accurately measure cutting forces [4]. The effects of cutting speed, feed rate, depth of cut, and machining time on machine ability variables such as machining force and surface roughness was investigated by Samuel G L et al.,. Considering tool wear during turning of AISI 4340 high strength low alloy steel with coated carbide inserts utilizing response surface methodology (RSM). The results show that combining a low feed rate, a shallow cut depth, and a short machining time with a high cutting speed reduces machining effort [5].

The relevance of measuring cutting force while making a quality product is grasped as a result of this literature review, and the characteristics that have the most impact on cutting force are recognized as cutting speed, feed rate, and depth of cut. Cutting force is measured using several types of dynamometers, and cutting force is predicted using various methods. The effort now has two goals: using the Taguchi parameter approach to discover optimal cutting force parameters and evaluating the effects of various input parameters. The smaller the best quality has been used in order to get optimal combination.

**Details of Research**

The cylindrical Al-6061 work piece is having a diameter 50 mm and length 150 mm. Aluminium alloy 6061 is an aluminum, magnesium and silicon alloy. It is a precipitation-hardened general-purpose aluminum alloys containing Al (95.85%), Mg (0.8%) and Si (0.40%). K45 cemented carbide inserts having outstanding hardness; wear resistance and toughness were used. CNC turning machine was used for turning operations by ACE designer. Kistler dynamometer with Multichannel charge amplifier was used for measuring cutting force.

**Experimentation**

Table 1 represents three different cutting parameters with their levels. MINITAB 14 programs performs analysis. L27 Orthogonal Array was used for experimentation [6, 7] consisting of 27 combinations of input process parameters. Table 2 represents plan of experimentation and response (cutting force) tools. MINITAB 14 was used for analysis of responses.

**RESULT AND DISCUSSION**

Results of Analysis of variance (ANOVA) conducted is shown in Table 3. Cutting speed has direct impact on the force compared with another parameter. The regression equation for it as follows.  $F_c = 96.6 - 0.653 \cdot v + 531 \cdot f + 103 \cdot d$   
Response Table for Means is given in Table 4. The  $R^2$ -value (coefficient of determination) is obtained as 94.8%, along with  $S = 0.1200$ ,  $R\text{-Sq (adj)} = 89.6\%$

Residual plots represent residual follow in a normal distribution throughout and indicating that ANOVA has behaved correctly. The cutting speed and feed rate had the percentage contribution of 90.012% and 8.27% respectively and depth of cut contributes only 1.718%. Figure 2 represents main effect plot. The optimal setting for cutting force minimization was considered as feed of 0.06 mm/rev, cutting speed of 125 m/min, and depth of cut of 0.2 mm. A confirmatory test was performed by choosing a combination of parameters except the optimum settings.





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The variation in experimental cutting force and estimated/predicted values is observed to be 10% by ANOVA. Thus it is concluded that the experimental analysis of Taguchi was carried out in a proper manner.

## CONCLUSION

The optimum process parameters obtained are 125 m/min, 0.06 mm/rev, and 0.2 mm as cutting speed, feed and depth respectively to reduce cutting force. Analysis of variance reveals that cutting speed, followed by feed, is the parameter most effective for cutting force. The graph shows that the proposed model's measured values have a good correlation.

## REFERENCES

1. M.C. Shaw, Metal Cutting Principles, Oxford University Press, London, (1984).
2. R.H.Locner, J.E Matar, 1990, Designing for quality, Productivity Press
3. G.R.Henderson, 2006, "SixSigma: Quality Improvement with MINITAB". John Wiley and Sons, England, ISBN: 10: 0470011556, pp: 452.
4. Gaitonde V. N., Karnik S. R., Figueira L., & Davim J. P. (2009). Machine ability investigations in hard turning of AISI D2 cold work tool steel with conventional and wiper ceramic inserts. International Journal of Refractory Metals and Hard Materials, 27(4), 754-763.
5. Suresh R., Basavarajappa S., Gaitonde V. N., & Samuel G. L. (2012). Machine ability investigations on hardened AISI 4340 steel using coated carbide insert. International Journal of Refractory Metals and Hard Materials, 33, 75-86.
6. P. J Ross, 1996, Taguchi Techniques for Quality Engineering, McGraw-Hill Book Company, New York.
7. S. S Mahapatra, A. Patnaik, P.K Patnaik, 2006, "Parametric Analysis and Optimization of Cutting Parameters for Turning Operations based on Taguchi Method "Proceedings of the International Conference on Global Manufacturing and Innovation, pp.1 –8, July.

**Table 1: Turning parameters with their different levels**

Parameters	Levels		
Cutting Speed (v)	100	125	150
Feed (f)	0.06	0.12	0.18
Depth of Cut (d)	0.2	0.6	0.8

**Table 2: Experimental Data (Input and Output)**

SI No	Inputs			Output
	Cutting Speed v	Feed f	Depth of cut d	Cutting Force Fz
1	100	0.06	0.2	61.34
2	100	0.06	0.6	102.44
3	100	0.06	0.8	168.56
4	100	0.12	0.2	134.79
5	100	0.12	0.6	200.47
6	100	0.12	0.8	235.41
7	100	0.18	0.2	145.26
8	100	0.18	0.6	192.66





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9	100	0.18	0.8	165.22
10	125	0.06	0.2	47.09
11	125	0.06	0.6	72.04
12	125	0.06	0.8	86.22
13	125	0.12	0.2	88.67
14	125	0.12	0.6	141.86
15	125	0.12	0.8	169.95
16	125	0.18	0.2	104.68
17	125	0.18	0.6	190.14
18	125	0.18	0.8	194.38
19	150	0.06	0.2	68.19
20	150	0.06	0.6	141.2
21	150	0.06	0.8	121.97
22	150	0.12	0.2	103.58
23	150	0.12	0.6	106.03
24	150	0.12	0.8	121.49
25	150	0.18	0.2	136.18
26	150	0.18	0.6	133.03
27	150	0.18	0.8	180.69

**Table 3: Mean Variance**

Source	DF	Seq SS	Adj SS	Adj MS	F	P
V	2	4.8993	4.8993	2.22678	109.2	0.000
F	2	0.92537	0.92537	0.23981	20.93	0.001
D	2	0.06535	0.06535	0.04183	1.82	0.101
v*f	4	0.00211	0.00211	0.00382	0.13	0.660
v*d	4	0.1092	0.1092	0.03059	1.32	0.247
f*d	4	0.043	0.043	0.01404	0.59	0.484
Residual Error	8	0.15661	0.15661	0.01994		
Total	26	6.20094				

**Table 4: Response Table for Means**

Level	V	F	d
1	1.287	2.057	1.737
2	1.834	1.797	1.865
3	2.333	1.599	1.852
Delta	0.891	0.303	0.027
Rank	1	2	3





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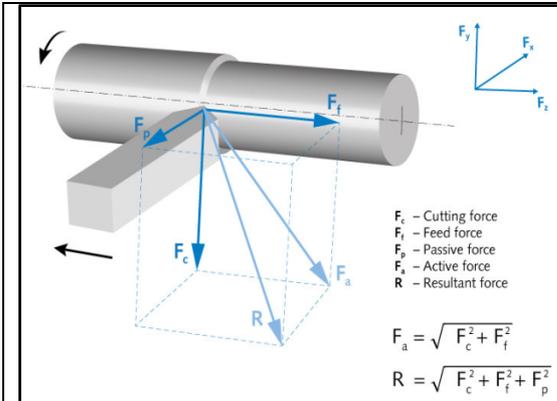


Fig 1. Cutting Force and Components

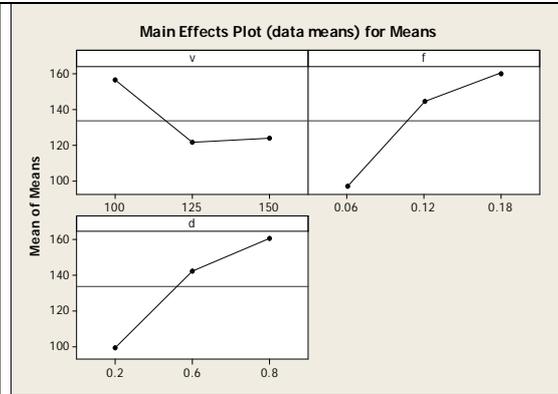


Fig. 2. Main Effect Plot

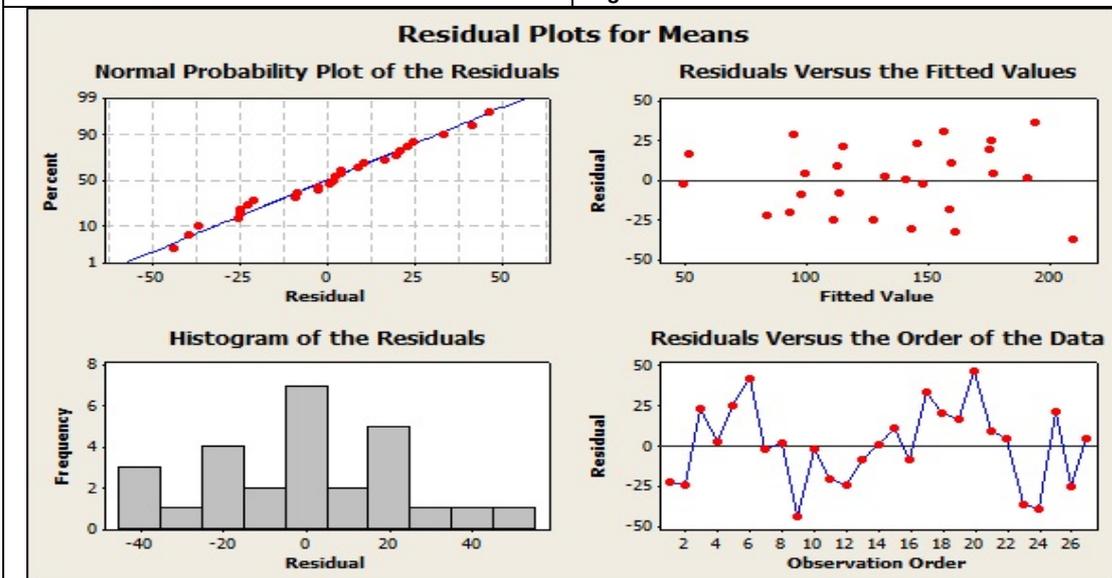


Fig.3. Residual Plots for Means

