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RESEARCH ARTICLE

Synthesis, Characterization and Thrombolytic Activity of N-Acetyl Cyanoacetyl Hydrazone Derivatives

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ABSTRACT

A series of novel compounds N-acetyl cyanoacetyl hydrazone were synthesized, characterized by IR, ^{1}H and ^{13}C NMR Data. Check the purity of all the synthesized compounds using thin layer chromatography. The synthesized compounds were subjected to thrombolytic activity. The thrombolytic activity was observed in 2 different concentrations of synthesized compounds. Our findings support the reported therapeutic use of these compounds as thrombolytic agents in the Indian system of medicine.

Keywords: N-acetyl cyano acetyl hydrazone, thrombolytic activity.

INTRODUCTION

Aromatic hetero cyclic chemistry is an enormous and complex subject of great industrial and academic significance. A number of molecules of life are derived from aromatic heterocycles and many pharmaceutical and agrochemical compounds are based on aromatic heterocycles. In recent decades, a large number of reports related to synthesis of N, O containing heterocycles have appeared owing to a wide variety of their biological activity. Organic chemistry and medicinal chemistry are becoming very vital chemistry. The primary objective of an organic chemist is to work towards isolation, characterization and synthesis of new compounds that are suitable for use as drugs. Medicinal or pharmaceutical chemistry is a discipline at the intersection of chemistry and pharmacology involved with designing, synthesizing and developing pharmaceutical drugs. However their derivatives having N-C linkage have been used in the fields of medicinal and pharmaceutical chemistry and reported to exhibit a variety of biological activities [1-4]. Hydrazones and their derivatives constitute an important class of compounds that has found wide utility in organic synthesis. The chemistry of carbon-nitrogen double bond of hydrazone is becoming the backbone of condensation reaction in benzo-fused N-heterocyclics also it constitutes an important class of compounds for new drug development [5].

A number of hydrazide hydrazone derivatives have been claimed to possess interesting bioactivity such as anti-microbial, antitubercular, anticonvulsant, analgesic, anti-inflammatory, antiplatelet aggregation, anticancer, antifungal, antiviral, antibacterial, and antimalarial activities. Formation of blood clot is thrombus and process is thrombosis that obstructs the flow of blood through circulatory system. Body uses platelets and fibrin to form blood clot as first step of repairing process after injury [6]. There are many drugs that are used to dissolve a clot and to treat heart attack, stroke, deep vein thrombosis and occlusion of peripheral artery such as streptokinase, S-Kinase etc [7]. Circulatory platelets are aggregated to the site of injury and become the major component for thrombus development. Thrombosis is a critical stage for arterial disease associated with myocardial infarction and stroke responsible for considerable morbidity and mortality. Moreover, for cancer patients, venous thrombosis is the second leading cause of death [8]. For treatment of these diseases, thrombolytic agents like tissue plasminogen activator (t-PA), Urokinase (UK), Streptokinase (SK) are used. In India among the thrombolytic agent, UK and SK are widely used. They have high risk of hemorrhage and severe anaphylactic reactions. Moreover, various treatments with SK is restricted due to immunogenicity [9-12]. Developing of improved recombinant variants of these drugs is disturbing due to unavailability of thrombolytic drugs [13-17].

Myocardial infarction due to arterial thromboembolism (ATE) is currently the leading causes of death under cardiovascular diseases (CVDs) in developed countries. The American Heart Disease foundation estimates more than 30% of all deaths in the world are from CVDs, thus the study highlights that a person has greater chance of dying from heart disease than cancer, AIDS, diabetes and accidents combined [18]. Indicating that ATE as the leading cause of morbidity and mortality world-wide. ATE typically forms under high shear conditions of blood flow and consists of platelets bound by small amounts of fibrin. ATE is the most common cause of cardioembolic events which includes myocardial infarction, ischemic stroke and Limb gangrene [19]. The treatment of acute myocardial infarction has changed during the past decade as newer approaches have become accessible, as prevention of complications has been the cornerstones for treatment. The management of ischemic heart diseases is now flanked by newer, more aggressive forms of therapy, which includes the early administration of thrombolytic drugs, highlighting clinical advantage of thrombolytic therapy for its ability to produce clot lysis, which directly restores nutritive myocardial perfusion [20]. Thrombolytic drugs like tissue plasminogen activator (t-PA), urokinase, streptokinase etc. play a crucial role in the management of ATE. The t-PA likes streptokinase and urokinase which are widely used as thrombolytic drugs have marked clinical drawback; these agents have a narrow therapeutic index and require continuous monitoring. Also, these agents have significant risk of haemorrhage, and produce anaphylactic reaction and lacks specificity. These entire therapeutic shortcomings of presently available streptokinase and urokinase and other t-PA indicate the need for better thrombolytic agents with clinical advantage [21].

Thrombus (blood clot) developed in the circulatory system due to failure of hemostasis causes vascular blockage and leads to serious consequences in thrombolytic diseases such as acute myocardial or cerebral infarction which may cause death[22]. Thrombolytic drugs are used to dissolve blood clots in a procedure termed thrombolysis. Alteplase, anistreplase, streptokinase, urokinase and tissue plasminogen activator (t-PA) are commonly used thrombolytic agents to dissolve clots. Heparin and Aspirin are only moderately efficient for acceleration of lysis and prevention of reocclusion, but are safe. Continued investigation in this area will provide new insights and promote progress towards the development of the ideal thrombolytic activity which is characterized by maximal coronary arteral thrombolysis with minimal bleeding. Selective third generation thrombolytic activity such as monoteplase, tenecteplase, reteplase etc. result in a greater angiographic potency in patients with acute myocardial infarction, although so far, mortality rates have been similar to those few drugs that have been studied in large-scale trials[23-26].

MATERIALS AND METHODS

All the chemicals (solvents and reagents) were purchased from foreign companies (Hi-media and Sigma/Aldrich) and were used as such with no further purification and distillation. Local chemical has not been used in the research work. The purity of these chemicals was 98-99.9%. The other reagents used were ammonium acetate and cyanoacetic hydrazide (Merck). Analytical grade solvents like ethanol, methanol, ethyl acetate, chloroform ($CHCl_3$) and N-hexane were used as such without further distillation. The synthesized compounds were scaled for yield and purified by recrystallization with suitable solvent system. IR spectra were recorded in AVATAR-330 FT-IR spectrophotometer (Thermo Nicolet) and only noteworthy absorption levels (reciprocal centimeters) are listed. ^1H NMR spectra were recorded on BRUKER AMX 300 MHz and 300 MHz NMR recorded on a BRUKER AMX 300 MHz NMR spectrometer operating at 100 MHz. For recording ^1H NMR spectrum of compound, solution were prepared by dissolving about 10mg of the compound in 0.5 ml of $CDCl_3$ was used as solvent while for ^{13}C NMR spectra, about 50 mg of the compound was dissolved in the same volume of the respective solvents. TMS (Tetra methyl silane) was used as a internal standard.

Preparation of N-acetyl 3-methyl-2,6-diphenylpiperidin-4-one cyanoacetyl hydrazone derivatives

A mixture of 3-methyl-2,6-diphenylpiperidin-4-one (0.1 mol), cyanoacetic hydrazide (0.1 mol) in the presence of few drops of concentrated acetic acid in methanol was refluxed for 2 hours. After the completion of reaction, the reaction mixture was cooled to room temperature. The solid product was separated by filtration and washed with warm water and recrystallized by methanol to afford 3-methyl-2,6-diphenylpiperidin-4-one cyanoacetyl hydrazone. Then cyclized by 0.1 mol of acetic anhydride.

In vitro thrombolytic activity

Thrombolytic activity determined by the method of Fatema Tabassum *et al* (2017)

Preparation of streptokinase (SK)

About 5 ml sterile distilled water was added to the commercially available lyophilized SK vial of 15, 00,000 I.U. and mixed properly. This suspension was used as a stock from which 100 µl (30,000 I.U) was used for *in vitro* thrombolysis study.

Collection of blood

Whole blood was drawn from healthy human volunteers without a history of oral contraceptives or anticoagulant therapy and 1 ml of blood was transferred to the previously weighed sterile eppendorf tubes and was allowed to form clots.

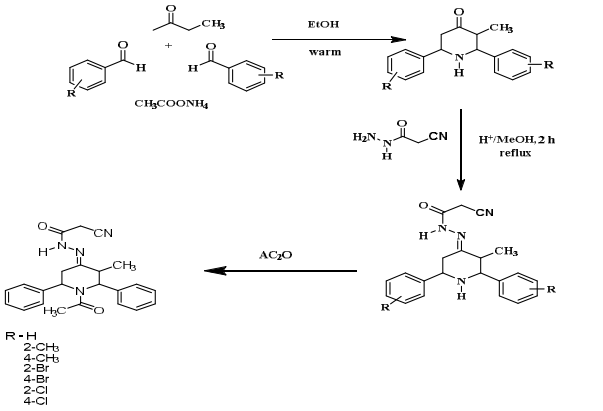
Procedure

3ml venous blood drawn from own blood was distributed in four different pre- weighed eppendorf tubes and incubated at 37^0 c for 45minutes. After clot formation, serum was completely removed without disturbing the clot and each tube having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube-weight of tube alone). To each eppendorf tube containing pre-weighed clot, 100µl (100µg/ml) of sample was added and another eppendorf tube containing pre-weighed clot, 200µl (200µg/ml) of sample was added. As a negative control, 100µl of distilled water was added to the control tube. For positive control, 100µl of streptokinase (SK) was added. All the tubes were then incubated at 37^0C for 90minutes and observed for clot lysis. After

incubation, fluid released was removed and tubes were again weighted to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clotlysis. The equation for calculating weight of clot is given below.

Clot weight = Weight of clot filled tube –Weight of empty tube

$$\% \text{ of Clot Lysis} = \left(\frac{\text{Weight of clot after lysis}}{\text{Weight of clot before lysis}} \right) \times 100$$



RESULTS AND DISCUSSION

N-acetyl 3-methyl-2,6-diphenylpiperidin-4-one cyanoacetylhydrazone (S1): Yield. 79.65%. Mp. 160-163ºC. FT-IR (KBr) ν_{max} (cm^{-1}): 3062-2935(C-H Aliphatic & Aromatic stretching), 1720(C=O),1495 (C=O piperidin moiety), 1647 (C=N), 2261 (C≡N), 3424-3269(N-H). ^{13}C NMR(300 MHz, $CDCl_3$) δ ppm :141.48(C-2 ipso carbon), 141.17 (C-6 ipso carbon), 127.59-128.72(Aromatic carbons), 209.53(C=O Piperidin moiety), 23.13 (CH_3 carbon of Piperidin moiety), 173.10(C=N), 126.82(C≡N), 43.07(CH_2 carbon of cyanoacetohydrazone moiety) , 76.65(C-2), 77.50(C-6), 46.23(C-3), 23.46(C-5), 13.63(3-CH_3).^{1}H NMR(300 MHz, $CDCl_3$), δ ppm: 7.25-7.10 (Aromatic Protons), 6.17 (N-H Hydrazone Moiety), 3.59 (CH_3 –Protons in Piperidin moiety), 3.28 (CH_2 –Protons in hydrazone moiety), 0.86 (3-CH_3), 3.90 (H-6a), 3.53 (H-2a), 2.23 (H-5a), 2.90 (H-5e), 2.58 (H-3a Proton).

N-acetyl 3-methyl-2, 6 (bis-*o*-bromo phenyl) piperidin-4-one cyanoacetyl hydrazone (S2): Yield. 79.65%. Mp. 179-181ºC. FT-IR (KBr) ν_{max} (cm^{-1}): 3099-2931 (C-H Aliphatic &Aromatic stretching), 1681 (C=O), 1567 (C=N), 2265 (C≡N), 3308-3179 (N-H). ^{13}C NMR(300 MHz, $CDCl_3$) δ ppm: 130.10 (C-2 ipso carbon), 130.62 (C-6 ipso carbon), 128.59-129.54 (Aromatic carbons), 166.34 (C=O), 159.54 (C=N), 125.18 (C≡N), 25.11 (CH_2 carbon of cyanoacetohydrazone moiety), 65.86 (C-2), 59.64 (C-6), 39.14 (C-3), 25.23 (C-5), 12.19 (3-CH_3). ^{1}H NMR (300 MHz, $CDCl_3$), δ ppm: 7.53- 7.31 (Aromatic protons) 10.79 (N-H, Hydrazone Moiety), 2.50 (N-H Piperidin moiety), 3.35 (CH_2 –Protons in hydrazone moiety), 0.91((3-CH_3), 3.88 (H-6a), 3.35(H-2a), 2.50 (H-5a), 3.35 (H-5e), 2.51(H-3a Proton).

N-acetyl 3-methyl-2, 6 (bis-*o*-chloro phenyl) piperidin-4-one cyanoacetyl hydrazone (S3) : Yield. 80.69%. Mp. 142-145ºC. FT-IR(KBr) ν_{max} (cm^{-1}): 3070-2939((C-H Aliphatic &Aromatic stretching), 1633 (C=O),1470 (C=O piperidin moiety) 1416(C=N), 2262 (C≡N), 3419-3259 (N-H). ^{13}C NMR(300 MHz, $CDCl_3$) δ ppm: 139.58 (C-2 ipso carbon), 133.81(C-6 ipso carbon), 128.17-132.84 (Aromatic carbons), 172.49(C=O Piperidin moiety), 21.55(CH_3 carbon of Piperidin moiety), 139.79(C=N), 128.17(C≡N), 22.97(CH_2 carbon of cyanoacetohydrazone moiety) , 48.05(C-2), 58.59(C-6), 39.15(C-3), 39.43(C-5), 13.52(3-CH_3). ^{1}H NMR(300 MHz, $CDCl_3$), δ ppm: 7.23-7.48 (Aromatic Protons), 7.50 (N-H

Hydrazone Moiety), 3.17 (CH_3 –Protons in Piperidin moiety), 3.20 (CH_2 –Protons in hydrazone moiety), 1.17 (3-CH_3) 3.73(H-6a), 3.3(H-2a), 2.98 (H-5a), 3.4(H-5e), 2.67(H-3a Proton).

N-acetyl 3-methyl-2,6 (bis-*o*-methyl phenyl) piperidin-4-one cyanoacetyl hydrazone (S4): Yield. 82.6%. Mp. 179-181^oC. FT-IR(KBr) ν_{max} (cm^{-1}): 3024-2977((C-H Aliphatic &Aromatic stretching), 1716 (C=O),1490 (C=O piperidin moiety) 1650(C=N), 2337 (C≡N), 3428-3066 (N-H). ^{13}C NMR(300 MHz, $CDCl_3$) δ ppm: 137.42 (C-2 ipso carbon), 136.89(C-6 ipso carbon), 126.75-131.45(Aromatic carbons), 172.49(C=O Piperidin moiety), 22.62(CH_3 carbon of Piperidin moiety), 140.90(C=N), 126.22(C≡N), 24.28(CH_2 carbon of cyanoacetohydrazone moiety) , 52.14(C-2), 57.53(C-6), 39.14(C-3), 22.78(C-5), 18.94(3-CH_3). ^1H NMR(300 MHz, $CDCl_3$), δ ppm: 6.88-7.31(Aromatic Protons), 7.42 (N-H Hydrazone Moiety), 2.32(CH_3 –Protons in Piperidin moiety), 3.21 (CH_2 –Protons in hydrazone moiety), 1.106 (3-CH_3) 3.73(H-6a), 3.3(H-2a), 2.98 (H-5a), 3.4(H-5e), 2.67(H-3a Proton).(3-CH_3), 3.89 (H-6a), 3.11 (H-2a), 2.39 (H-5a), 3.07(H-5e), 2.57 (H-3a Proton), 2.33 (*o*-CH_3 protons).

N-acetyl 3-methyl-2,6 (bis-p-bromo phenyl) piperidin-4-one cyanoacetyl hydrazone (S5): Yield. 79.65%. Mp. 186-189 ^oC.IR (cm^{-1}): 3025-2852 (C-H Aliphatic & Aromatic stretching), 1674 (C=O), 1568 (C=N), 2267 (C≡N), 3440-3184 (N-H). ^{13}C NMR (δ ppm): 139.98 (C-2 ipso carbon), 140.49(C-6 ipso carbon), 126.49-129.77 (Aromatic carbons), 164.48 (C=O), 158.05 (C=N), 114.35 (C≡N), 24.16 (CH_2 carbon of cyanoacetohydrazone moiety), 76.57 (C-2), 56.12 (C-6), 44.89 (C-3), 34.56 (C-5), 11.15 (3-CH_3) 19.20 (*o*-CH_3). ^1H NMR (δ ppm) : 7.32-7.13 (Aromatic Protons), 10.09 (Hydrazone Moiety), 2.09 (N-H Piperidin moiety), 3.50 (CH_2 –Protons in hydrazone moiety), 0.92 (3-CH_3), 3.89 (H-6a), 3.11 (H-2a), 2.39 (H-5a), 3.07(H-5e), 2.57 (H-3a Proton), 2.33 (*o*-CH_3 protons).

N-acetyl 3-methyl-2,6 (bis-p-chloro phenyl) piperidin-4-one cyanoacetyl hydrazone (S6) : Yield. 80.69% . Mp.148-151^oC. IR (cm^{-1}): 3100-2875(C-H Aliphatic & Aromatic stretching), 1687 (C=O), 1592 (C=N), 2261 (C≡N), 3295-3198 (N-H). ^{13}C NMR (δ ppm): 140.78 (C-2 ipso carbon), 141.63(C-6 ipso carbon), 130.55-130.75 (Aromatic carbons), 164.36 (C=O), 155.48 (C=N), 114.18 (C≡N), 23.96 (CH_2 carbon of cyanoacetohydrazone moiety), 67.56 (C-2), 58.98 (C-6), 44.27 (C-3), 35.72 (C-5), 11.31 (3-CH_3). ^1H NMR (δ ppm): 7.26-7.51(Aromatic Protons), 9.83 (N-H Hydrazone Moiety), 2.09 (N-H Piperidin moiety), 3.77 (CH_2 –Protons in hydrazone moiety), 0.89 (3-CH_3), 3.90 (H-6a), 3.51 (H-2a), 2.18 (H-5a), 2.97 (H-5e), 2.55 (H-3a Proton).

N-acetyl 3-methyl-2,6 (bis-*p*-methyl phenyl) piperidin-4-one cyanoacetyl hydrazone (S7) : Yield. 78.69%. Mp.139-141 ^oC. FT-IR(KBr) ν_{max} (cm^{-1}): 3026-2963(C-H Aliphatic & Aromatic stretching), 1701 (C=O), 1638 (C=N), 2266 (C≡N), 3195-3097 (N-H). ^{13}C NMR(300 MHz, $CDCl_3$) δ ppm: 139.33 (C-2 ipso carbon), 139.83 (C-6 ipso carbon), 126.42-129.38 (Aromatic carbons), 164.87 (C=O), 158.07 (C=N), 114.13 (C≡N), 24.56 (CH_2 carbon of cyanoacetohydrazone moiety), 68.92 (C-2), 60.46 (C-6), 45.34 (C-5), 36.16 (C-3), 12.10 (3-CH_3), 21.13(*p*-CH_3) . ^1H NMR(300 MHz, $CDCl_3$), δ ppm : 7.14-7.36 (Aromatic Protons), 9.01(N-H, Hydrazone Moiety), 2.06 (N-H Piperidin moiety), 3.73 (CH_2 –Protons in hydrazone moiety), 0.89 (3-CH_3), 3.87 (H-6a), 3.49 (H-2a), 2.24 (H-5a), 2.83 (H-5e), 2.57 (H-3a Proton), 2.06 (p-CH_3 protons).

In vitro thrombolytic activity of synthesized compounds

Thrombolytic therapy, also known as clot busting drug, is a breakthrough treatment which has saved untold lives. It has been used in the clinical area to treat venous and arterial thromboembolic complaints which are a foremost cause of death.The *in-vitro* thrombolytic activity of the synthesized compounds was determined by clot lysis study. The activity of the compounds was determined by comparison with the thrombolytic activity of Streptokinase. The test compound was measured for the decrease in clot weight at different concentrations. The *in-vitro* thrombolytic activity of the N-acetyl cyanoacetyl hydrazone derivatives were determined by clot lysis study. The activity of the compounds was determined by comparison with the thrombolytic activity of Streptokinase. The test compounds were measured for the decrease in clot weight at different concentrations 100 and 200 µl, respectively, streptokinase

(30,000 IU) was employed as positive control and distilled water as negative control. The results were plotted conc. vs percentage clot lysis were obtained by regression analysis.

The results of the *in vitro* thrombolytic activity were encouraging and the tested compounds exhibited substantial aggregation inhibition. All the seven tested N-acetyl cyanoacetyl hydrazone derivatives exhibited substantial clot lysis, with percentage value ranging from 40.5 to 78.45% in comparison to 84.57 % clot lysis exhibited by the reference standard streptokinase (30,000 IU). In the present study, thrombolytic activity analysis of compound S1 (100µl and 200µl) showed removal of clot by 40.57% and 69.19%, respectively, with that of positive Control streptokinase (SK) of 84.57% and negative control of 26.74% clot lysis. The thrombolytic activity analysis of compound S2 (100µl and 200µl) showed removal of clot by 46.59% and 74.93%, respectively, with that of positive Control streptokinase (SK) of 84.57% and negative control of 26.74% clot lysis. The thrombolytic activity analysis of compound S3 (100µl and 200µl) showed removal of clot by 47.62% and 76.21%, respectively, with that of positive Control streptokinase (SK) of 84.57% and negative control of 26.74% clot lysis. The thrombolytic activity analysis of compound S4 (100µl and 200µl) showed removal of clot by 47.85% and 78.45%, respectively, with that of positive Control streptokinase (SK) of 84.57% and negative control of 26.74% clot lysis.

The thrombolytic activity analysis of compound S5 (100µl and 200µl) showed removal of clot by 41.35% and 72.83%, respectively, with that of positive Control streptokinase (SK) of 84.57% and negative control of 26.74% clot lysis.The thrombolytic activity analysis of compound S6 (100µl and 200µl) showed removal of clot by 44.28% and 73.32%, respectively, with that of positive Control streptokinase (SK) of 84.57% and negative control of 26.74% clot lysis.The thrombolytic activity analysis of compound S7 (100µl and 200µl) showed removal of clot by 45.64% and 74.21%, respectively, with that of positive Control streptokinase (SK) of 84.57% and negative control of 26.74% clot lysis.The clot lysis at 100 µl 200 µl of compound S4 was 47.85%, 78.45% in 37^0c at 45 min respectively while standard shows 84.57%. The highest dose as 200 µl of compound has significant activity and near to the standard. The result shows the o-substituted compounds have higher activity than p-substituted compounds. The order of activity for o-substituted compounds is S4>S3>S2 (o- methyl >o- Cl >o- Br). The order of activity for p-substituted compounds is S7>S6>S5 (p- methyl >p- Cl >p- Br). From the result it was observed that, compound S4 shows good Thrombolytic activity than other synthesized compounds due to the presence of electron with drawing group [27].

CONCLUSION

The structures of the synthesized compounds were established on the basis of their analytical and spectral data (IR, ^{1}H NMR, ^{13}C NMR). The result from the study showed that the synthesized compounds have excellent thrombolytic activity that was comparable to the activity of Streptokinase. As from the research findings of the under taken in vitro clotlysis study, we demonstrated that the compounds showed mainly moderate thrombolytic activity. Our findings support the reported therapeutic use of this compound as clot lysis or thrombolytic agent in the Indian system of medicine. This is only a preliminary study and the synthesized compounds should be thoroughly investigated pharmacologically to exploit their medicinal and pharmaceutical potential.

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REFERENCES

1. Hasan MU, Arab M, Pandiarajan K, Sekar R, Marko D, *Magn. Reson. Chem*, 1985, 23:292.
2. Pandiarajan K, Sabapathy Mohan RT, Krishnakumar R, *Indian J. Chem*, 1987; 26B:624.

3. Pandiarajan K, Sekar R, Anantharaman R, Ramalingam V, *Indian J. Chem*,1991; 30B:490.
4. Pandiarajan K, Tamilselvi P, Gurumani V, *Indian J. Chem*, 1994; 33B:971.
5. Prasad S et al, Development of an in vitro model to study clot lysis activity of thrombolyticrugs, Thrombosis, 2006; 4:1-4.
6. http://en.wikipedia.org/wiki/Thrombosis (http://www.newsmedical.net/health/What-is-Thrombosis.aspx)
7. file://%20my%20project%20paper%20rima/Jan/CV%20Pharmacology%20%20Thrombolytic%20%28Fibrinolytic%29%20Drugs.htm
8. Furie B and Furie BC: Mechanisms of thrombus formation. *NewEngland Journal of Medicine* 2008; 359(9):38-49.
9. Collen D: Coronary thrombolysis: streptokinase or recombine anttissue type Plasminogen activator? *Annals of Internal Medicine*1990; 112(7):529-38.
10. Mucklow JC: Thrombolytic treatment. Streptokinase is moreeconomical than alteplase. *British Medical Journal* 1995; 311(7018):1506.
11. Rouf SA, Moo-Young M, and Chisti Y: Tissue-type plasminogen activator: characteristics, applications and production technology.Biotechnology Advances 1996; 14(3): 239-66.
12. Jennings K: Antibodies to streptokinase. *British Medical Journal*1996; 312(7028): 3934.
13. Adams DS, et al.: A synthetic DNA encoding a modified human urokinase resistant to inhibition by serum plasminogen activator inhibitor. *Journal of Biological Chemistry* 1991; 266(13): 8476-82.
14. Lijnen HR *et al*.: On the mechanism of fibrin-specific plasminogen activation by staphylokinase. *Journal of Biological Chemistry* 1991;266(18):11826-32.
15. Marder VJ: Recombinant streptokinase: opportunity for an improved agent. *Blood Coagulation and Fibrinolysis* 1993; 4(6):1039-40.
16. Nicolini FA et al.: Sustained reflow in dogs with coronary thrombosis with K2P, a novel mutant of tissue-plasminogen activator. *Journal of the American College of Cardiology* 1992;20(1): 228-35.
17. Wu DH, *et al*.: Coiled coil region of streptokinase gamma-domain is essential for plasminogen activation. *Journal of Biological Chemistry* 2001; 276(18):15025-33.
18. Farnsworth NR: *Ethnopharmacology and future drug development*: the North American experience. J of Ethnopharmacology, 1993; 38(2-3): 14552.
19. Murray CJ, Lopez AD. Global mortality, disability, and the contribution of risk factors: Global Burden of Disease Study. Lancet 1997; 349: 1436–42.
20. Meschia JF et al. Thrombolytic treatment of acute ischemic stroke. Mayo Clin Proc 2002; 77: 542-51.
21. Godfrey EM et al. Don't be a clot: A radiologist's guide to haemostasis including novel antiplatelet and anticoagulant therapy. Clin Rad 2011; 66: 693-700.
22. Sobel BE, Braunwald E. *The management of acute myocardial infarction. In: Braunwald E (ed) Heart disease: A textbook of cardiovascular medicine,* Braunwald E, 2nd ed. Philadelphia: WB Saunders, 1984, pp 1301-33.
23. Sherwani SK, Bashir A, Haider SS, Shah MA, Kazmi SU. Thrombolytic Potential of Aqueous and Methanolic Crude Extracts of Camellia sinensis (Green Tea): *In vitro*study. *Journal of Pharmacognosy and Phytochemistry*. 2013; 2(1):125-129.
24. Fathima SN, Ahmad SV, Kumar BR. Evaluation of *In Vitro* Thrombolytic Activity of Ethanolic Extract of CURCUMA CAESIA Rhizomes, *International Journal of Pharma Research & Review.* 2015; 4(11):50-54.
25. Sayeed MA, Kabir H, Rashid MMU, Bhuiyan MFA, Ahamad FM, Rashid MA. Thrombolytic Activity of Methanolic Extracts of *Desmodium paniculatum* (L.) and Sarcochlamys pulcherrima (Roxb.), Bangladesh Pharmaceutical Journal. 2014; 17(1):67-69.
26. Collen D. Coronary thrombolysis: streptokinase or recombinant tissue-type plasminogen activator. Ann Intern Med. 1990; 112(7): 529-538.
27. Maheswari R, Manjula J. Synthesis characterization and biological applications of benzohydrazide derivatives. *International journal of applied research.* 2015; 1(10): p.587-592.

Table 1. Analytical and spectral data of Synthesized compounds

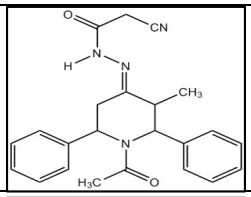
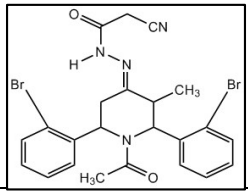
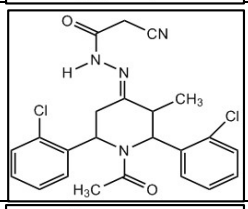
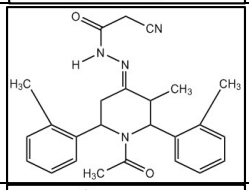
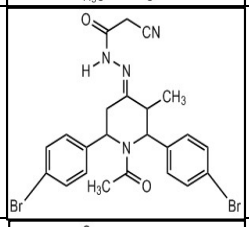
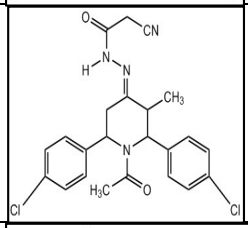
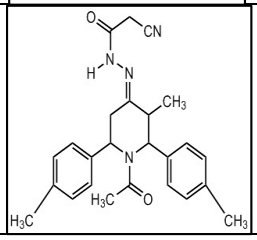
Compound	Structure	M.Formula	M.pt
S1		$C_{23}H_{22}N_4O_2$	160-163ºC
S2		$C_{23}H_{26}Br_2N_4O$	179-181 ºC.
S3		$C_{23}H_{20}Cl_2N_4O_2$	142-145ºC.
S4		$C_{25}H_{28}N_4O_2$	179-181ºC
S5		$C_{23}H_{26}Br_2N_4O$	186-189ºC
S6		$C_{23}H_{20}Cl_2N_4O_2$	148-151ºC
S7		$C_{25}H_{28}N_4O_2$	139-141ºC

Table 2. Thrombolytic activity of Synthesized compounds

	% of clot lysis			
Samples	**Control**	**100 (µg/ml)**	**200 (µg/ml)**	**Standard**
S1	26.74 ± 1.87	40.57 ± 2.83	69.19 ± 4.84	84.57 ± 5.91
S2	26.74 ± 1.87	46.59 ± 3.26	74.93 ± 5.24	84.57 ± 5.91
S3	26.74 ± 1.87	47.62 ± 3.33	76.21 ± 5.33	84.57 ± 5.91
S4	26.74 ± 1.87	47.85 ± 3.85	78.45 ± 5.54	84.57 ± 5.91
S5	26.74 ± 1.87	41.35 ± 2.89	72.83 ± 5.09	84.57 ± 5.91
S6	26.74 ± 1.87	44.28 ± 3.09	73.32 ± 5.13	84.57 ± 5.91
S7	26.74 ± 1.87	45.64 ± 3.19	74.21 ± 5.19	84.57 ± 5.91

Plate.1: Thrombolytic activity test photos

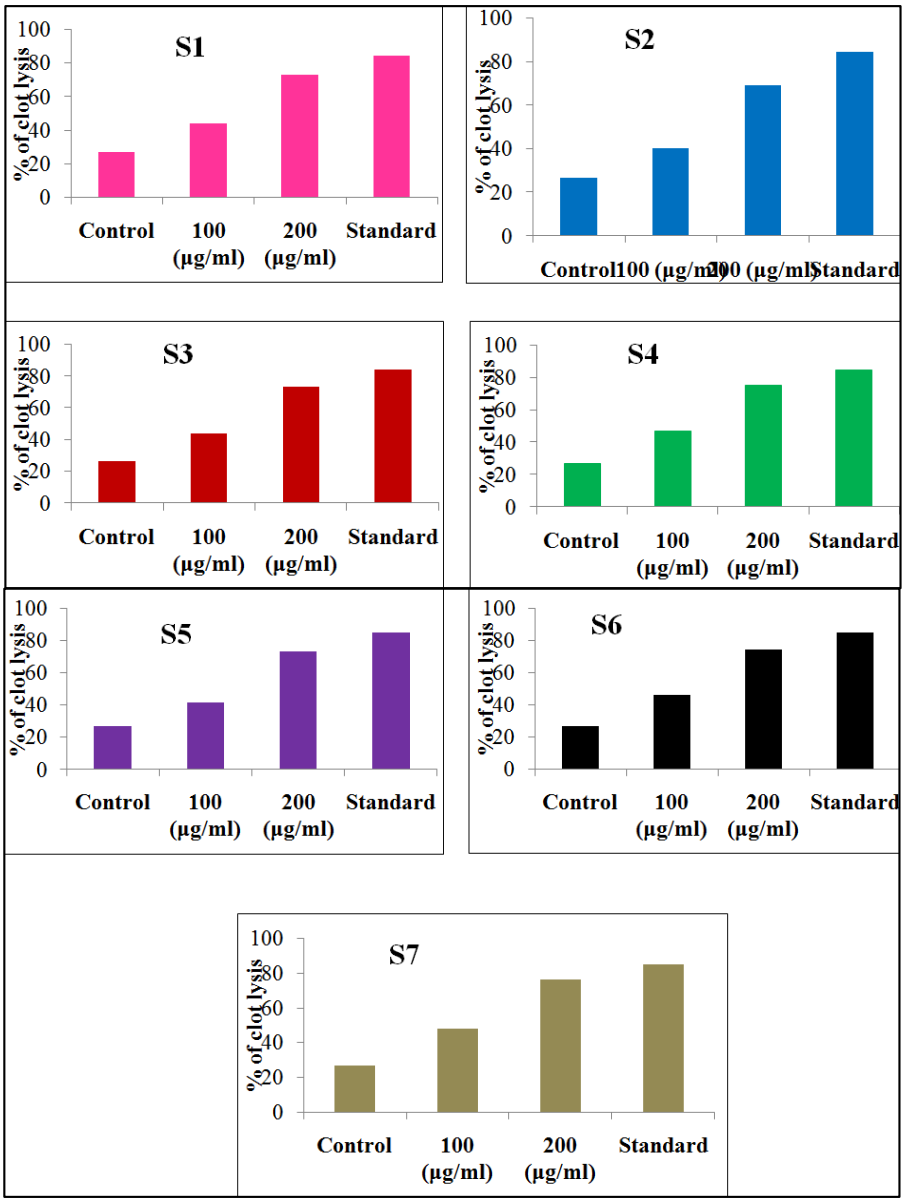


Plate.2: Thrombolytic activity graph

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RESEARCH ARTICLE

Anthropocene Water-Body Changes in Coastal Towns, Puri, Odisha: Micro-Scale Geospatial Analysis

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ABSTRACT

Scarcity of portable water is becoming acute in the globe and also in India. The coastal agglomerations are under anthropogenic pressure influenced by erratic climate change. There is continuous loss or decline of swamps, paleo channels, and water bodies existed along the coastal cities like Chennai, Calcutta and Mumbai, Puri and Mahabalipuram etc. These ponds, talabs, swamps, sweet water lakes maintain the ecological balance of these towns as the ground water table is unfit for use under salinity ingress. Image processing is done by ERDAS IMAGINE software. Vectorization of water bodies of the study area are done by Arc GIS software. The year wise area of the water bodies are calculated for analysis purposes. It is noticed that there is loss of fresh water bodies in 16sq km town with area of water bodies of ≈6.03 Km^2 in 2003 has been reduced by 69% by 2018 which is alarming for its up-surging illegal users, slum dwellers, encroachers and accommodating floating pilgrim population. The loss of fresh water body in the Balukhand shall have high impact on the reserve forest and wild life sanctuary. The drying up of Samang swamp and built up environment in Baliapanda area shall have negative impact on coastal ecosystem and ground water.

Keywords: Salinity ingress, Inland waterbody, coastal towns, Geospatial, Remote sensing & GIS Studies

INTRODUCTION

The globe is moving towards water crisis as water bodies in past have been triggered by modernization and anthropogenic pressure.600 millions in India are under water quandary, 70% of India's water supplies beyond norms, ≈ 200000 fatalities are due to water pollution (NITTI Aayog). Many cities in India are under drinking water

crisis and others are in process as urbanizationis fast growing. It is predicted that the demand shall exceed supply for drinking water shall exceed by 2030. Cities like cities Chennai, Ahmadabad, Hyderabad, Bengaluru, Vijayawada, Amravati, Coimbatore, Sholapur, Kochi Shimla, are poignant towards desperate portable water paucity. Ground water recharge has been under strain due to urbanization, concrete/ asphalt cover and inadequate drainage system.
The major crisis of recharge of ground water (GW) has invited urban floods in cosmopolis like Hyderabad in 2000, Ahmadabad city (FY2001), Delhi municipal corporation (FY's 2002,2003, 2009, and 2010), Chennai M.C. (2004 and 2015), Mumbai and greater Mumbai(FY 2005), Surat (2006), Kolkata city (FY 2007), Jamshedpur city (FY 2008), Delhi MC in 2009 and Guwahati (2010), Bhubaneswar city (2014), Assam and MP in (2016). In spite of these major urban floods most of the cities in east and west coast of India get flooded in rains but the ground water table and area of water bodies in these cities are diminishing .But the above cities are under water crisis in summer due to fall of GWT which urge for rejuvenation creation of new water fronts in these coastal townships. The aim of the paper intends that Puri town should not face the water disaster of 2017 of Cape town of South Africa Kate Wheeling, 2019 [1]. The main causes can be ascribed are population rise, climatic anomalies, erratic southwest monsoon, dearth precipitation, diminishing ground water table, poor water supply system and above all abnormal wastage due to poor planning in corporations and municipalities of the country where adequate funds are available for water supply management and their distribution and water loss concerns. The large gap between the demand and supply is chiefly managed by mafias of water those who illegally draw water by bore wells and their numbers are hundreds and thousands in a city. The urban water bodies in coastal environment act as catalysts to activate sustainable ecosystem and urges for forming strategic plans to restore them Bindu et. al. 2016 [2].

Small townships, ULBs and NAC's along the coast line are hassled for drinking water as the ground water is alkaline for a sizable depth. The supply water is alkaline and not fit for portability and human use. The only sources are reservoirs, water bodies, dug wells and ponds for availability of water for human use.Poongothaiet.al., 2014 [3] reported that there is a change in water bodies, forest where land has been deteriorated and suggested proper water shade management. Generally the sediment loss lesser gain for a water shed changes the water spread area which need immediate action. The water scarcity in small townships are due to seasonal scarcity, accessibility, natural barriers, water quality, poor planning, and institutional lacunas and poor Government restrictions Manasi, S. et al., 2009 [4]. Coastal ULB's along west coast of India generally depend upon their fresh water bodies and aquifers. The water supply security is challenged by steep rise in urban water demand, diminution or decline of water quality, erratic climate change, paradigm of changing priorities of old resources, industrial and agricultural uses with completion among users and water mafias World bank, 2018 [5]

Area of study

Small cities like Puri, has the disadvantage of growing urban vs. urban inequality, and migration from rural to urban context availing the benefits of economic growth, Andrea Hagn 2016 [6]. Puri district lat 19°28' and 20° 10' N and long 85° 09' and 86°25' E, is one among the major six coastal districts of Odisha (Fig -1). Puri district has geographical area of 3479Km^2. The district is shown in SOI Topo sheet 74 E & I and 73 H & L. Nayak P. C. et al, 2017 [7].Puri Municipality (19.28 N to 20.13 Lat. and 84.29 E to 86.25 Long.) was formed in the year1881. The topography of the town is of cronch shape (so called Shankha-khetra) which an area of 16.84 Km^2 with population 200564 (2011 census). As it is a place of pilgrimage and tourist place average daily floating population of about 100K persons are to be added. The decadal growth rate of the town is @23.9%, http://purimunicipality.nic.in/QLPdf/AboutUs. The horizontal and vertical growth is in progress of the town is observed in 21st century, AMRUT: SLIP [8].

As the time changing, Puri steps a rapid development with increasing urbanization and side by side climate and environmental condition is taking a sharp change. Present global warming, MSLR, erosion, drought, human adaptations (e.g., diverting water and building hydraulic structures that upshotis depleting of water provisions), and natural events such as floods and hurricanes water bodies got modified. The portable or sweet water level has been

depleted in the town and apprehended may exhaust its aquifer supplies in a decade if the two aquifers at Baliapanda and Balukhand exhausts Fig -2. Illicit drawl of GW by the hotels, and community shall compound with the present climate change.Sharma et. al., 2008 [9] reported from GIS studies that river, Tank and lake area in along Puri coast were 1323 Ha, 296 Ha and 444Ha in the year 1999 whereas in the year 2005 they were 1208, 471 and 313 Ha respectively which shows increase in Tank area and decrease in sweet water Samang lake area.

Importance of the City

Puri, the golden sand city of Lord Jagannath, plethora of temples, was communicated to Kolkata in 1822-1826 by Jagannath Old Sadak, became a municipality in 1881 (Smith D's approval), have the first overhead tank for water supply to the town in 1928 (Fig 3). The city is divided into sandy littoral tract and alluvial tract. Puri as one DHAM among the four abodes according to Hindu culture, fascinating golden sands, the clean beach with running crabs, car festival, religious festivals round the year, the panoramic Chilika Lake, the appliques of Pipili, the beach at Konark, the seventh wonder Konarktemple attracts tourist from India and foreign make Puri as the epitome of pilgrimage.

The Climate of the Town

Puri is prevailed by coastal humid, climate tropics .with average annual rainfall of 1372 mm/ year. The two sweet water zones (Baliapandaand Balukhand) are adjacent to thecoast. These two areas with unconsolidated formations of sandy bed have fresh water aquifers and a good source for water supply. The uncovered sandy area is water logged during rain and a potential recharge zone for ground water Mishra S P., 2015 [10].

The Drainage System of the City

The 1000 years old temple city is constructed on deltaic riverine plains over sandy ridges with gentle slopes, with drainage channels like Dhaudia Nadi (West), Kanchi Nal (North), Mangala Nadi (south) and the Bay of Bengal along east. Two major drains are crossing the town the lost Malini (Saradhar) Paleo River near Gundicha Temple and the depleted Musa River at the outskirt, Jana S et al., 2018 [11] and https://puriwaves.nirmalya.in/destinations/atharnala-bridge (Fig-4). A saline marshy swamp and the expanse of water lies in the NE direction called Samang Pat (Local name) having area 16 to 18Km2 before two to three decades now shrank to 3 to 4 Km^2. The GW Table (2001-2011) @ 0.08 mbgl to 5.13 mbgl and GWT long term table Pre-monsoon: rise is 0.001 to 0.303m/yr and fall at places of 0.0 to 0.554 m/yr. But there is . Post- monsoon: rise amounts t0 0.004 to 0.30 m/y rand there is fall at places it was 0.0 to 0.18 m/yr (Fall) (FY 2001-2011)CGWB report 2012 [12].

Anthropogenic activities

With Anthropocene epoch (mid of 20th century) many lacustrine areas have been lost/ down-sized or encroached by mafias for other utilities like settlement or farming and the remaining water body areas have decreased due to climate changes The causes for such a huge loss are due to human actions, nature, urban agglomeration. At present the stretch of water left are due to modernization, paucity in horizontal settlement area, community advancement and migration of rural community to urban for employmentin industries, lively hood, land reclamation, waste disposal, deforestation, have invited water body disappearance. With the horizontal growth of cities, the migratoryhave invited encroachment of water bodies. Evaluation of such water body loss (to compensate ground water recharge, detection of their changes and their preservation in time is the call of the day. By knowing the quantity of loss of water accumulated areas and the land cover data documentshelps in planning to protect them from future water crisis.

Objective of Study

The city and suburbs of Puri town have combated combined pressures from natural (cyclone and urban flooding)) and anthropogenic factors (land for settlement, agriculture and vegetation). For protectionof the deterioration and sustenance, it is essential forquantitative detection of water bodies' change and their driving forces need to be analyzed. The coordination between nature and anthropogenic action must be well judged before any infrastructural development and modernization. With unique capability, GIS reveals deeper insights into data, such as patterns, relationships, and situations helping users make smarter decisions. This method and its outcomesforcategorizations and post-cataloguing shall exhibit change detection of multi-temporal high spatial resolution satellite imagery of the Puri City, Odisha FY2003, FY=2014 and latest FY-2018. The purpose this study are (a) Toprotray a change detection methodology of the map and simultaneously monitor the spread reduction changes of the water bodies in 16years interval i.e. between 2003 and 2018; (b) To quantify the area of change and spatial distribution change for water bodies; and (c) To find the changes and causes of water body's changes.

METHODS

Satellite remote sensing provides a considerable opportunity to monitor land use and land cover (LULC) and detect changes because of the rapid, synoptic and repetitive capabilities of remote sensing. Multi-source satellite images provide efficient information on the land changes. Specifically, moderate-resolution multi-spectral sensors (e.g., Landsat, SPOT, and ASTER) have been used for studying the extent of flooding and affected land cover, detecting land changes. GIS (Geographic Information System) a geographic information system (GIS) is a framework for gathering, managing, and analyzing data. Rooted in the science of geography, GIS integrates many types of data. It analyzes spatial location and organizes layers of information into visualizations using maps and 3D scenes. With this unique capability, GIS reveals deeper insights into data, such as patterns, relationships, and situations—helping users make smarter decisions. The Puri city has faced combined pressures from natural (cyclone) and human factors. For protection purposes, quantitative water bodies change detection and a driving factors analysis are to find a gap between anthropic activities and natural systems Chang K. T., 2017.

METHODOLOGY

This project describes the methods and results of classifications and post-classification change detection of multi-temporal high spatial resolution satellite imagery of the Puri City, Odisha for 2003, 2014 and 2018. The main objectives of this study are as follows: (1) develop a change detection method to map and monitor water bodies changes between 2003 and 2018; (2) quantify the area of change and spatial distribution change for water bodies; and (3) To find the changes and causes of water bodies changes. The prominence attached with city water bodies are maintaining the ecosystem, other usages (drinking, bathing, industry and religion), aesthetics, water sports and tourism. Image processing has been done by ERDAS IMAGINE software i.e. layer staking, subset and supervised classification etc. Vectorization of water bodies of the study area are done by ArcGIS software. The shape files of the different years are taken for analysis. The year wise area of the water odies are calculated for analysis purposes.

Data processing and acquisition

For high spatial resolution and to detect area changes of water bodies, images that represent several stages within the same season are preferable. However, it was difficult to find time-series of high spatial resolution satellite images that were well matched within the same season for several reasons but primarily due to the weather conditions in this area. Two cloud-free images were acquired in January 2003, December 2014 covering the whole Puri town. One image is downloaded from Google earth. These images were selected under the constraints of limited suitable images in the archives (Table 1). All images were acquired from the USGS (GLoViS). All images were provided in the

Universal Transvers Mercator projection (UTM Zone, 45 NORTH) and WGS84 datum. These were carefully geo-referenced to a RMS error within 2 pixels using a 1st-order polynomial transformation method. Finally, they were clipped to the same extent (Tab-1, 2& 3) and Fig 6 (a, b, c, and d) (Bhatta B. D., 2008).

Bands, wavelengths and resolutions

The different attributes attached to different lands against different wavelengths and respective resolutions for Landsat 7 and Landsat 8 are given in Table- 4 and Table – 5. Puri Municipality was having spread area of 16.8Km^2 in later part of 20th century. The study area is considered 46.11Km^2 taken as our study area including the Samang area, the reserved forest and wild life sanctuary in the southern end including Gabakund cut (Nuanai) and Mangala cut. The water body has been lost / affected severely in the present 21st century.

RESULTS AND DISCUSSION

It is observed that the area of water bodies in 2018 is less in comparison to year 2003. The area has been decrease in the Puri town from 6.0285 to 1.8478 Km^2 and the % of loss of water bodies.Hence, the water bodies are decreasing and the 16years decrease is about 69%. The Landsat image shows the conversion of water body to human uses Table-6**.** The possible reasons for reduction in Lacustrine area are (a) Extension of the holy city to west of Puri town. (b) Construction of a barrage at Gobardhanpur in Brahmagiri block to stabilize the water logged area of Puri town. (C) Encroachment and conversion of water bodies to home stead Land. (d) In Samang Pat to home stead land and agricultural field.

Change in LU/LC of Samang Lake

With shifting of strand lines the paleo water bodies have left imprints of paleo channels, swamps and water bodies parallel to the coastline in the Puri district like outer channel of the Chilika lagoon, Samang Pat (Lake) and SAR Lake. These water bodies due to Anthrop-geo-climatic changes have converted either to an agricultural land or to home stead lands. The Samang Lake was covering about 16-20 Km^2 before 50-60 years back, the present swamp has been reduced to an area 1 -2 Km^{2}to accommodate for food and settlement of rising population. It was accessed from the Survey of India Topo Sheet (FY 1985-86) was 5.78 Km^2 in Fig 7(a) whereas the same area has been reduced to 1.05Km^2 summer FY 2018-19 which is super-imposed on yellow lines (old boundary and blue shading is of 2018-19). in Fig 7 (b)

Deteriorating Ecosystem

Balukhand reserve forest, protected area with wild life sanctuary lies in southern coastal zone of Puri town. The area is an assemblage of dune flora and fauna having more than 100 black bucks (endangered species) and large herd of spotted dears. The forest area has many natural sweet water bodies (≈30 numbers) sporadically distributed for use of the wild life animals. In addition a medical college, a Jail and many house complexes with coastal business centers are growing for the last 20years at the cost of either the shallow water body or the dune vegetation (cashew and casuarinas) https://www.triphobo.com/places/puri-india/balukhand-wildlife-sanctuary. The Puri-Konark Marne drive road has become a slaughter house for these wild animals. Want of food and water, the protected species are entering nearby localities. This distorted ecosystem with preservation of water bodies must be protected for sustenance of the ecology of the area. These sweet water zones and lost water bodies must be renovated.

The Illicit land invaders

The urban authorities of Puri Municipality have identified 46 slums (26 notified, & 20 illicit), in the city 62 out of 69 identified slums are categorized to access of water as the DPR claims Puri shall be a Slum-Free City (SUIDL 2013:14–15 [17]. These 69 slums are using unaccounted surface and ground water sources today. These slums are developed by water body filled up lands or over sandy zone along the coast and mostly dealing with garbage and wastes of the town. They are directly or indirectly accountable for decrease in the water body area of the town (Tab-7). The 69 slums are prescribed in DPR are: Apart from the house hold in the slums, there is average floating population of 100K people, their water demand amounts to 10-13MLD which must be supplied to sustain the status of the eco-city of the pilgrims.

Surface Water bodies

According to 2011 census, there are 41140 households at Puri town out of which 12250 houses have water supply connection and rest are dependent on street water supply or the ponds and tanks nearby. The 75% storm water received during monsoon seasons hasneed for immediate improvement and 25% mixes directly with sewage water which draws immediate attention. There are 18numbers of waterlogged areas only left in the town for ground water recharge only. The causes are due to erratic intensified rainfall, inadequate drainage network, encroachments. The gray water body are formed due choking of drains caused due to obstacles, uncovered drains, silting of drains, and disposal house hold waste to drain add congestions (Fig – 8).

Urban flooding

The soil of the town is sandy but the drainage system is insufficient to address the storm water disposal during heavy rain. There is accumulation of water on the Hospital square of depth 1m or even more. The fig-8 shows mixing of grey water of drains and the storm water at hospital square during 2016 on a heavily raining day during September. This water is the demand of the ground water table of Puri but drained with grey water to join Mahanadi.

Samang reservoir over the Dhaudia River

To ameliorate present crisis of water demand (75MLD) and shortage in supply, a sweet water reservoir is constructed on the river Dhaudia within Samang pat area. The reservoir covers ≈ 260Ha which narrate the improvement of water body within the town which is the major existing waterbody. The present demand of water for consumption is ≈35MLD for the town which was 33.2 MLD during 2011 PKDA Draft plan 2013 p-46 [16].

Strategic plan for improving the gap

To improve the surface water status there is need for renovation of Musa Nai and demolishing the encroach on the bed of the river, providing more culverts in the water logged areas like ring road from Bira-Harkrushnapur Chhack to Mangalaghat bridge. The separation of gray water from storm water, renovation of drains with cover are the other options.. Before 40-50years backthe coastal sand was having a large number of water bodies on the shore and the community centers (JAGA Ghar) were having multiple ponds maintained by the community for their uses which are at present either encroached or depleted. The Balukhanda reserved forest and protected area and wild-life sanctuary had 30 to 35 large tanks and natural water spreads which have been lost due to anthropogenic or climatic factors. After December Tsunami 2004, the 5Km long coast of Puri town from Mangala river mouth in south to Nuanai mouth in north is exhibiting continuous erosion and loss of fresh water bodies along the coast line. Over exploitation of coastal sweet water bodies are under threat of either loss or downsizing. There is constant threat of salinity ingress and the wells are unfit for use. The unauthorized withdrawal of sweet ground water by bore wells have posed

drinking water crisis within a decade. The existing sweet water aqifers at Balukhand and Baliapanda are inadequate to quench the thirst of authorized water users of 12K and unauthorized households of ≈ 30k houses of the city from a demand of 20 MLD 20 years back to 75MLD in the far end of 20's of 21st century.

CONCLUSION

From the GIS study of sweet surface water bodies for last 16years, from 2003 to 2018, there is continuous declining from 6.0285 Km^2 in 2003 to 3.213 Km^2 in 2014 (less ≈ 50%) to 1.8478 in 2018 and in total 69.35%. If the process continues, except few old sacred ponds like Narendra, Markanda, Swetaganga and Indradyumnaall the ponds shall be lost. The largest open air hotel of Lord Jagnnath shall not get water to prepare food stuffs. The paleo swamp Samang, the remaining Paleo channel Musa Nai shall be dried up and shall be encroached either as settlements or agricultural land. The depletion of ground water table shall be depleted further and the water crisis shall invite water disaster to the town. The slum dwellers need to be rehabilitated by vertically growing the township rather than horizontally. The illegal water users should be detected and heavy penalties need to be imposed as water tax by the local federal body to reduce reckless ground water consumption.

REFERENCES

1. Wheeling Kate, 2019, Major cities in India are starting to run out of water, The Week, 13th July, 2019, https://theweek.com/articles/850956/major-cities-india-are-starting-run-water.
2. Bindu C.A., Mohamed A. R., 2016, Water bodies as a catalyst to growth and development- The case of Kodungallur town, Kerala, International Conference on Emerging Trends in Engineering, Science and Technology (ICETEST - 2015), Procedia Technology 24 (2016), pp-1790 – 1800.
3. Poongothai .S, Sridhar. N, Shouria R.A, 2014 Change Detection of Land use/ Land Cover of a Watershed using Remote Sensing and GIS, International Journal of Engineering and Advance Technology(IJEAT) Volume-3 issue-6, Page-226-230.
4. Manasi S., Latha ., and Raju K V., 2009, Emerging trends in managing drinking water – case studies of coastal villages in Karnataka, working paper 212, ICES, PP- 1-24.
5. World bank, 2018, Water scarce cities :Thriving in a Finite World, International Bank for Reconstruction and Development / The World Bank 1818 H Street NW, Washington, pp-1-79 DC 20433, www.worldbank.org
6. Andrea Hagn, 2016, Of Slums and Politics in Puri, Odisha: The Localization of the Slum-Free Cities Mission in the Temple City, editor; Natasha Cornea and Anna Zimmer, Environment Politics in Urban India: Citizenship, Knowledges and Urban Political Ecologies
7. Nayak P. C., Vijaya Ku. P S. V., Rao R. S., Vijay T., 2017, Recharge source identification using isotope analysis and groundwater flow modeling for Puri city in India, Applied water Sc., Springer, Volume 7, Issue 7, pp 3583–3598
8. Atal Mission for Rejuvenation and Urban Transformation (AMRUT), 2015, AMRUT; Puri, under Service Level Improvement Plan (SLIP), a project report, Department of Housing and Urban Development, Govt. of Odisha.
9. Sharma V. S. and Ramesh R., Ramachandran S., 2008, Coastal LU/LC mapping of Puri to Konark; Coastal stretch of Odisha, The Indian Geographical Journal, Vol- 82(1), pp-37-42
10. Mishra S. P and Dwibedy S.,2015, Geo-Hydrology Of South Mahanadi Delta And Chilika Lake, Odisha , India, International Journal of Advance Research, Volume 3, Issue 11, pp- 430- 445
11. Jana S., Mohanty W. Ku., Gupta S., Rath, C. S. and Patnaik P., 2018, Palaeo-channel bisecting Puri town, Odisha: vestige of the lost river 'Saradha', Current Science, Vol. 115(2), Pp-30.
12. Central ground water Board, GOI, 2013, Ground water information booklet: Puri district, Odisha, CGWB, Min of W. R., Govt. of India, pp- 1-21,http://cgwb.gov.in/District_ Profile/Orissa/Puri
13. Ministry of Urban development, 2013, City HRIDAY Plan for Puri Volume I/IV, IPE Global Pvt. Ltd., in consortium with CRCI (India) Pvt. Ltd. Prepared for Puri Municipality Puri, Odisha FEBRUARY 2016 PP- 1-411

14. Bhatta, B.: Analysis of urban growth pattern using remote sensing and GIS: a case study of Kolkata, India. Int. J. Remote Sens. 30(18), 4733–4746 (2009)
15. Kang Tsung Chang. p.p, 2017, "Introduction to GIS" by McGraw-Hill Higher Education,
16. Centre for Environmental Planning and Technology (CEPT) and ORSAC,2013,Draft comprehensive development plan-2031,Vol-II, submitted to Puri Konark Development Authority (PKDA), pp-46
17. SUIDL. 2013. RAY, Pilot Project: In-Situ Upgradation of Tridev Nagar. Detailed Project Report (Final), Volume I: Report for Puri Municipality. New Delhi: Shristi Urban Infrastructure Development Ltd.

Table 1. Satellite Imagery Information

SATELITE	SENSOR	Acquisition Date	Spectral Resolution
Landsat-7	ETM	February 2003	15m and 30 m
Landsat-8	OLI_TIRS	January 2014	15m, 30 m, 100m

Table 2. Preparation and planning for digitization

Feature group	Feature class	Feature type	Symbol/colour
Water bodies 2003	River/pond/tanks//reservoir	Polygon	Violet ,red
Water bodies 2014	Natural tanks /water logged area	Polygon	Yellow, black
Water bodies 2018	Water logged area /natural tanks/ artificial tanks/pond/ rivers	Polygon	Blue , black

Table 3. Water bodies classification System

Level-i	Level-ii	Description
Waterbodies	Ponds	A body of standing water.
	River	Such as stream, blocked water tank, big water bodies, creek, brook, rill, rivulet.
	natural tanks	A body of deposit water, which has been created automatically due to rain or something.
	tanks(artificial tanks)	A body of water which has been created by human being by digging or something.
	Coastline	Where the water level meets the land.

Table 4. Different bands, wavelengths, colours and their resolution of Landsat- 7

Spectral selected band	Real wavelengths (µm)	Geometrical resolution(m)
Band – I	0.452µm to 0.514µm	30m
Band – II	0.519µm to 0.601µm	30m
Band – III	0.631µm to 0.692µm	30m
Band – IV	0.772µm to 0.898µm	30m
Band – V	1.547µm to 1.748µm	30m
Band – VI	10.31µm to 12.36µm	60m
Band – VII	2.06 5µm to 2.346µm	30m
Band – VIII	0.515µm to 0.896µm	15m

Table 5. Different bands, colours, wavelengths and their resolution of Landsat- 8

Spiral Band	Category	Wavelengths ((µm)	Resolution
Band 1	Coastal aerosol	0.43µm -0.45µm	30m
Band 2	Blue	0.45µm -0.51µm	30m
Band 3	Green	0.53µm -0.59µm	30m
Band 4	Red	0.64µm -0.67µm	30m
Band 5	Near Infrared(NIR)	0.85µm -0.88µm	30m
Band 6	SWIR -1	1.57µm -1.65µm	30m
Band 7	-SWIR-2	2.11µm -2.29µm	30m
Band 8	Panchromatic	0.50µm -0.68µm	15m
Band 9	Cirrus	1.36µm -1.38µm	30m
Band 10	Thermal Infrared (TIRS-1)	10.60µm -11.19µm	100m
Band 11	Thermal Infrared (TIRS-2)	11.50µm -12.51µm	100m

Table 6: The water body changes in Puri city during year 2003, 2014 and 2018

Feature group	Year	Area	unit
water bodies in 2003	2003	6.0285	sq.km
water bodies in 2014	2014	3.2130	sq.km
water bodies in 2018	2018	1.8478	sq.km

Table 7: The slums and the household need water in future as per DPR of Puri in 2016:

Category	Notified	Unauthorized	Total	House holds
Tenable	28	08	36	3,115 households
Semi Tenable	07	01	08	3,659 households
Untenable	11	14	25	Unknown
Total	46	23	69	

Source: https://journals.openedition.org/samaj/4226

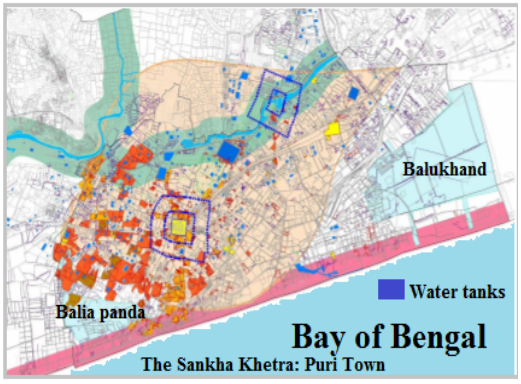

Fig 1. Index map for Sankhakhetra, Puri town. Source: HRIDAY plan modified

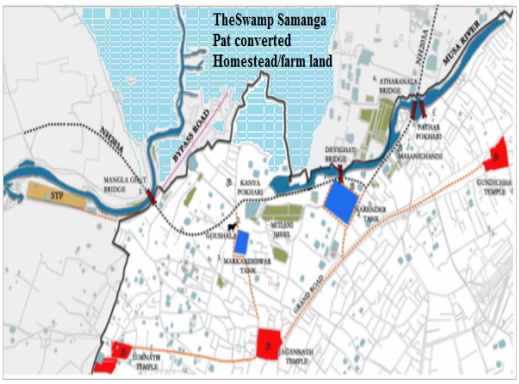

Fig 2. The tanks & sweet water aquifers zone of Puri Source: City HRIDAYA PLAN [13]- Puri Town (modified)

Fig. 3. The old Topo Map of Puri, the study area

Fig. 4. The lost Malini river near Gundicha Temple, & Jagannath Temple, Puri in past, Source: British Library

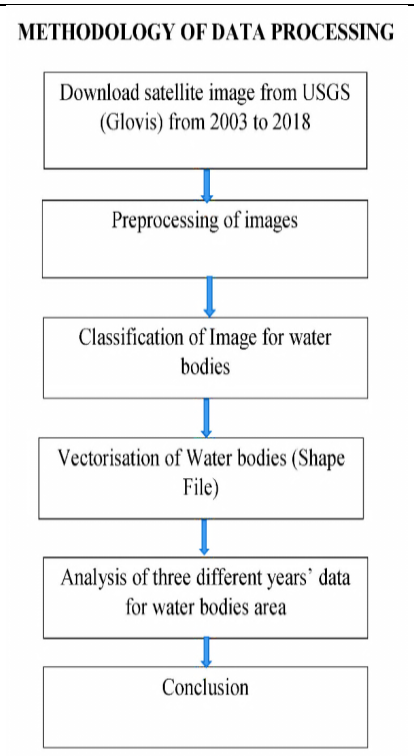

Fig 5. The methodology of data Processing

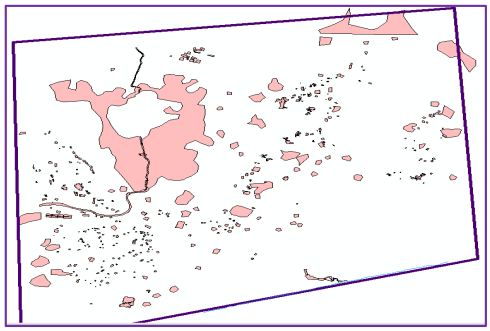

Fig 6 (a): Digitized image Puri Town FY 2003

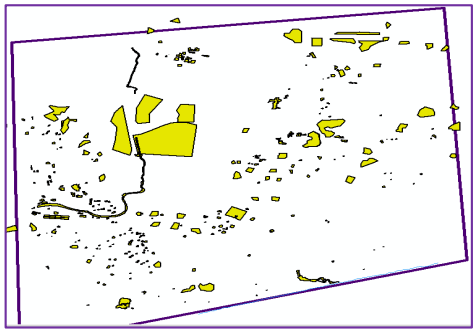

Fig 6. (b): Digitized image Puri Town FY 2014

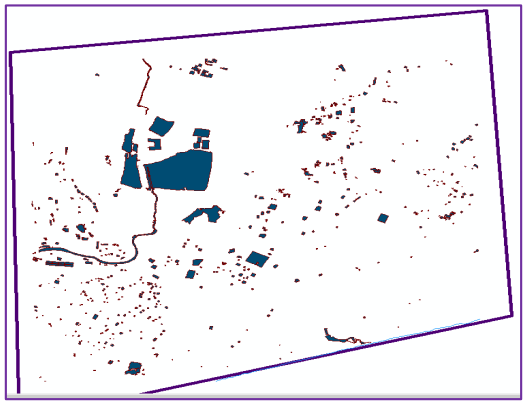

Fig. 6 c. Digitized of image Puri FY 2018

Fig. 6 d: Superposed image Puri (2003, 2014, 2018)

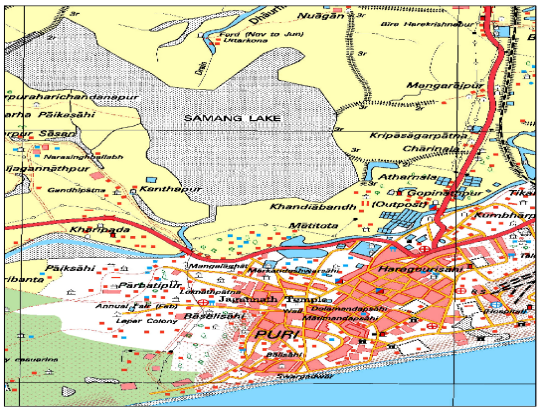

Fig. 7. (a): The Samang lake at Puri (FY 1985-86) SOI Topo Sheet

Fig. 7. (b) The Samang area left Fy 2018-19

Fig .8. Mixing of storm and grey water at Hospital square (Badadanda) Puri on a heavily raining day: 2016

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REVIEW ARTICLE

Sleeplessness as a Marker of Anaemia: A Review

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ABSTRACT

Anaemia is a condition in which the red blood cells are either reduced in number or volume or become deficient in haemoglobin. It affects the overall cardiovascular and mental health. Present day lifestyle habits such as reduced hours of sleep, iron deficient diet, etc. have caused an increase in the number of people suffering from anaemia. Acute total and short term sleep deprivation have been seen to cause an overall increase in heart rate, systolic and diastolic blood pressure and a decrease in the haemoglobin levels. Cognitive function have been found to be worse in anaemic, sleep deprived individual than in healthy controls. Sleep deprivation interferes in the restorative function of sleep .i.e. the clearance of metabolites that have accumulated during the day, thus causing toxic accumulation. Fatigue, that leads to reduced activity is a symptom of anaemia and can be used to explain insomnia related complaints.

Keywords: Anaemia, Sleeplessness, Sleep deprivation, Cardio-vascular health, mental health.

INTRODUCTION

Anaemia is one of the most common haematological disorders affecting humanity. It is a condition in which the RBC are either reduced in number, volume or are abnormally sized. The affected RBC usually have a lower amount of haemoglobin on their surface which decreases their efficiency of oxygen delivery to different tissues and organs of the body. The most noticeable outward symptom of anaemia are the paleness of skin and nail beds, insomnia, brittleness of nail. Symptoms of oxygen deficiency in tissue include pulsating noises in ear, dizziness, fainting and shortness of breath. The compensatory action of heart to make up the oxygen deficiency may cause its enlargement

and cause rapid heartbeat. Other symptoms include headache and chest pain. An estimated 1.62 billion people that corresponds to 24.8% of the world's total population suffered from anaemia in 2005. In 2011, an estimated 800 million children and women were affected by anaemia. The highest prevalence was in case of children .i.e. at 42.6% followed by pregnant women (38.4%) and non-pregnant women (29.4%). Thus a total of 273.2 million children and 528.7 million women (including both pregnant and non-pregnant are affected by anaemia worldwide. .Anaemia occurs due to variety of causes. Highest prevalence of anaemia was seen in South East Asia, Eastern Mediterranean and African regions (WHO 2015).

It usually occurs when other diseases interfere in the body's ability to produce healthy and adequate amount of RBC or due to abnormal increase in RBC breakdown and loss. There are various types of anaemia based on these causes. Anaemia due to faulty or decreased RBC production include sickle cell anaemia, iron deficiency anaemia, bone marrow and stem cell problems and vitamin deficiency anaemia. Rapid blood loss due to surgery, childbirth, trauma, ruptured blood vessel and chronic blood loss due to ulcer, cancer, tumour and menstruation also account for anaemia as the body falls short of the iron that flows away with the blood. Another prevalent cause of anaemia for the present generation are the modern lifestyle habits such as irregular sleep pattern, insufficient sleep, unbalanced diet, iron lacking diet, alcoholism, smoking , etc. . Irregular time table of sleep is a trend among the present young generation with less than 30% of adults report sleeping for less than 6 hours per night (Sunil Sharma and Mani Kavuru, 2010).. The impact of insufficient sleep are most profound during infancy as it influences optimal development of the brain. Insufficient sleep during adulthood ultimately develops into a condition called sleep deprivation and elongated periods of sleep deprivation lead to insomnia, which is most prevalent in older people. Recent researches have proved sleep deprivation as a symptom or marker of anaemia.Symptoms of sleep deprivation include difficulty falling asleep, not feeling well rested, daytime sleepiness, lack of concentration, headache, irritability, etc. Sleep loss also contributes to weight gain and obesity by increasing appetite and decreasing metabolism.

Sleep is a period when physiological, reversible and periodic changes occur in consciousness and behaviour of an individual. It is defined as "a reversible state when the interaction of the organism with the environment is lost temporarily, partially and periodically" (Murat et al, 2015). Irregular sleep pattern has profound cardiovascular, mental and metabolic implications. Sleep is a period of resetting when the rate of activity of various vital organs are reduced enabling them to work at a relaxed rate. For example, as we sleep the movement of the voluntary muscles of our body is greatly reduced. This allows the heart to focus on its own well-being and health and enables the brain to reorganise and recharge itself. Sleep plays a restorative role in allowing the clearance of metabolites that have accumulated throughout the day in the body, especially in the brain. It promotes better cognitive ability, memory reconsolidation, resets the levels of synaptic activity, prolongs alertness (Edinboro et al, 2017) and rejuvenates the brain for optimal function (Eugene and Masaik, 2015). Sleep is intricately related to various processes of our body and the circadian rhythm. The misalignment of the circadian rhythm due to irregularity is believed to be the main cause of metabolic disregulation ((Eugene and Masaik, 2015). Disregulation of metabolism either causes an increase or decrease in the BMI (Body Mass Index) which in turn leads to obesity and other metabolic diseases.

Insomnia may be a cause, consequence or marker of anaemia (Edinboro et al, 2018).The impact of insufficient sleep and irregular sleep pattern varies with age. In case of infants who are recommended a minimum of 11 hours sleep, sleep alteration and fragmentation is associated with significant amount of behavioural difficulties and neurocognitive effects. Studies have shown that infants with IDA show difference in sleep state organisation which interferes in the optimal functioning of brain. The peak period for IDA is 6-24 months when the central nervous system is rapidly developing and thus making it highly vulnerable (Peirano et al, 2013). Irregular sleep patterns in school children also has behavioural impacts.Iron deficiency in early life has been reported to cause behavioural and developmental symptoms by affecting transmitters such as serotonin, noradrenaline and dopamine. It also affects myelination and metabolic activity of neurons. It has also been shown that brain functions such as cognition and learning are more affected in patients with iron deficiency anaemia (Beard JL, 1999).Therefore, IDA at any age

impacts the memory and cognitive ability. IDA is known to impact sleep quality irrespective of the psychological symptoms such as depression and anxiety (Murat et al, 2015).Acute total and short term sleep deprivation results in increased systolic and diastolic blood pressure, increased heart rate and reduced level of haemoglobin. It also leads to increased level of CRP (C - reactive protein) which are inflammatory markers of cardiovascular disease. (Liu et al, 2018). In case of older people, individuals with non-iron deficient anaemia are more likely to experience insomnia like symptoms than those who are found to be non-anaemic (Edinboro et al, 2017) but in case of infants irregular and insufficient sleep is usually associated with iron deficiency anaemia (IDA) (Peirano et al, 2013).Iron deficiency anaemia is known to be more common in infants and younger aged individuals than in case of adults. Surveys indicated that a link between insomnia in older adults may have important implication for treatment of each condition such as anaemia; "anaemia may be a modifiable contributor to insomnia and vice versa" (Edinboro et al, 2018). Infancy and old age are the two age groups where sleep deficiency poses a greater risk of developing anaemia.

Sleep duration and it's importance

Sleep is being increasingly recognized as critical component of healthy development and health (Chaput et al, 2016; Chaput et al, 2017; St-Onge et al, 2017). Healthy sleep comprises adequate duration, good quality sleep, appropriate timing, no sleep fragmentation or disturbance and absence of any kind of sleep disorders. (Buysse DJ, 2014; Gruber et al, 2014). Chronic insufficient sleep has become very common nowadays and is a matter of great concern as it increases the chance of mortality and morbidity (Matricciani et al, 2011). Short sleep duration if continued as a regular habit is associated with adverse health outcomes such as obesity(Wu et al, 2014) , type II diabetes(Shan et al, 2015) , hypertension(Wang et al, 2015), cardiovascular disease(Wang et al, 2016), depression (Zhai et al,2015), and all-cause mortality(Shen et al,2016). (Chaput et al, 2018). However the ideal amount of sleep required each night can vary between individuals based on genetic factors and other reasons (Chaput et al, 2018). A person can be considered to be obtain adequate amount of sleep if they wake up feeling well rested and perform well throughout the day (Chaput et al, 2018).

Short sleep duration, poor sleep quality, late bedtimes, sedentary lifestyle is associated with increased food intake, poor diet quality and obesity in adolescents. A landmark study by Spigel et al showed that appetite hormone hormone levels were altered after partial sleep deprivation and the appetites of the subjects increased (Spigel et al, 2004). Sleeping either lesser or greater than the recommended range has negative implications at later age but is beneficial to infants and adolescents (Chaput et al, 2016). Women sleeping 5 hours per night or less gained about 1.16 kg and the women sleeping for 6 hrs. per night gained 0.61 kg weight (Patel et al, 2006). Sleeping less than 5 hrs was associated with higher body mass index (BMI) in elderly people. Sleep fragmentation also causes increased BMI and low haemoglobin level. A survey by Grander et al, showed that among the youngest respondents (16+ yrs), higher BMI is associated with shortest sleepers and lower BMI is associated with longest sleepers. (Gardner aet al, 2016). Another study revealed that long sleep increases a participants tendency to gain weight, provided they were already obese (Nagai et al, 2013).

Iron Cycle

Dietary iron enters into body through duodenal enterocytes. The basolateral transporter, ferroportin then transport ferrous iron. This ferrous ion Fe^{3+} is then available to bind to free sites of plasma a transferrin (Tf). In conditions such as thalassemia major (a kind of anaemia), Tf are fully saturated. Under normal conditions, body maintains one-third saturation of Tf .i.e. only a small fraction of it is used to form iron containing proteins (approx. 30mg). The remainder of iron in circulation is derive from reticuloendothileal macrophages that obtain iron through reticuloendothileal macrophages from the senescent RBC. Tf cells release approximately 30 mg of iron to produce haemoglobin for about 200 billion erythrocytes. However, only a small portion .i.e. less than 3 mg is used normally. Thus Tf are the most dynamic iron compartment, turning over about 10 times iron per day. Binding to Tf helps iron to overcome

insolubility and toxicity constraints in an organism (Sheftel et al, 2012). The amount of iron that should be present generally are 35.5%- 44.9% in adult females and 38.3% - 48.6% in adult male (mayoclinic). Excess iron accumulation in body causes complete saturation of Tf receptors and leads to a condition called hemochromatosis where excess iron is stored in liver, heart and pancreas and can lead to life threatening condition (mayoclinic). Iron accumulation in brain causes neurodegeneration. Neurological disorders such as Parkinsonism, spasticity, optic atrophy, retinal degeneration, etc. (Gregory and Hayflick, 2013). Due to the constriction of interstitial space of cerebral cortex, flow of CSF through interstitial space during waking is only 5% of flow found at sleep. As a consequence, the metabolites that have accumulated during the day are cleared as we sleep. Thus the restorative function of sleep is due to switching of brain into a state that facilitates clearance of metabolites (E.g. - Adenosine) (Xie et al, 2013). This constriction might also be affecting the clearance of accumulated iron. Tiring of the body due to less sleep might be reducing the efficiency of the Tf, thus affecting the efficiency of iron mobilisation in the body.

Observation

As per our review we have found that to determine the impacts of insufficient sleep and irregular sleep pattern on the overall health of individuals belonging to 3 different age groups. The age groups that have been considered include (a) infancy and childhood, (b) Adolescents, (c) Adults and elderly cohorts.

(a) Infancy and childhood

Out of all the several kinds of anaemia, iron deficiency anaemia (IDA) accounts for majority of the anaemic cases in infants and children. An estimated 20-25% of world's infant have IDA and the prevalence of anaemia among children less than 4yrs of age are estimated to range between 44-66%, and half of these cases are probably due to IDA (Brotanek JM et al, 2005). Iron deficiency in the body during early stages .i.e. from 6 to 24 months has been linked with impaired development of the CNS (central nervous system). It is a period when the development of CNS (Peirano et al, 2010), myelination of cellular and motor processes occur at a rapid rate. Thus IDA in the early months interferes in optimal development of brain, affects the cognitive ability, motor and emotional domains. Inadequate sleep has been known to affect the development of pre-frontal cortex (Dahl PE, 1996) in infants that controls the emotion domain. Evidence have suggested that IDA infants underwent altered CNS development (Roncagliolo M., 1998) and slower transmission in both auditory and visual systems (Peirano D. et al, 2013). In a study conducted by Peirano et al, night polysomnographic recordings were taken for 55 healthy full-term 4 yrs. children who had been followed since infancy (former IDA= 27, Non- anaemic controls= 28).Polysomnography consists of electro encephalon recording(EEG) with other physiological variables(eye movements, submental electromyogram, ECG, oxygen saturation, etc.)(Nunes and Bruni, 2015). Both the IDA and control infants chosen were assessed for IDA at 6, 12, 18 months. When the infants attained pre-school age, a sleep wake study was conducted.

(b)Infancy

Sleep pattern organisation occurs as follows: Stage 1- Non rapid eye movement (NREM 1), stage 2 (NREM 2), Stage 3-4 (SWS), and Rapid eye movement (REM). The study done on the children during infancy revealed that the duration of sleep stages and the latency between different stages varied from normal. This is because the organisation of sleep depends on various mechanisms that involves neural and humoral processes whose development gets affected if the infant suffers from iron deficiency in early months. (Peirano D. et al, 2008). In a study done in Nepal and Zanzibar revealed that 6 to 18 months old IDA infants were reported to sleep longer and wake up more frequently at night than the non-IDA infants (Kordas et al, 2008). Sleep spindles, produced during second stage of sleep, are generated by a deep brain system called the thalamic reticular nucleus in association with principal thalamic nuclei and are synchronised and coordinated by corticotical, corticothalamic, and thalamocortical loops.(Stride M et al, 1985). Spindles are thought to be markers of normal brain functioning, development and integrity and their absence

suggested cerebral dysfunction or pathology (Peirano et al, 2013). Sleep spindle development probably occur in the latter (6-24 months) period because infants with IDA at 6 months showed altered sleep spindle development. If connections between sleep spindles and learning can be applied to infancy, it may be possible that altered sleep spindle patterns in IDA afflicted infants restricts their cognitive ability and memory development (Lozoff B et al, 2006). The sleep spindles were analysed through EEG readings.

(c) Childhood

When the infants were examined at 4yrs of age, it was seen that they continued to show altered sleep organisation despite the iron therapy given to them at infancy. (Peirano et al, 2013). The sleep organisation was almost similar, this indicates that IDA at early stage had a permanent impact on sleep spindle development and subsequent cognitive ability. In a population based study done in Pennsylvania showed that one out of five pre-adolescent children were insomniac, with the highest prevalence (approximately 30%) observed in girls aged 11-12 yrs. This prevalence may be due to hormonal changes rather than due to anxiety or depression (Calhoun et al, 2014; Nunes and Bruni, 2015). Another population based study done in China using data that was collected twice with a 5 yr. interval in between reported an increase in insomnia prevalence from 4.2% to 6.6 %(Zhang et al, 2011; Nunes and Bruni, 2015).

(d) Possible reason

IDA causes behavioural and developmental symptoms by affecting transmitters such as serotonin, noradrenaline and dopamine.It also affects myelination and metabolic activity of neurons. It has also been shown that brain functions such as cognition and learning are more affected in patients with iron deficiency anaemia (Beard JL, 1999). Iron deficiency has long lasting effects on dopamine system development(Lozoff B et al, 2006; Lozoff B. 2007; Beard and Connor, 2003) and alters neurotransmission of dopamine in specific region of brain that are involved in sleep regulation (Fuller et al,2006; Mccarley RW,2007). Dopamine system plays a neuro-modulating role by regulating sleep, including REM sleep quality, quantity, and timing (Dzirasa et al, 2006; Monti Jm and Monti D, 2007; Crochet and Sakai, 2003). IDA also affects serotonin and noradrenergic transporter level (Beard Jl, 2005; Felt et al, 2006; Burhans et al, 2005). Disruption in normal iron levels at early stage affect the iron processing, storage and avaibility. This affects the quality, quantity, composition of myelin sheath. These alterations continue to persist even when normal iron levels are achieved by supplementation (Todorich et al, 2009). The slow auditory and visual information transmission is due to this defective myelination (Algarin et al, 2003). Sleep spindles seem to be involved in controlling auditory inputs to brain while sleeping, thus reduced number of sleep spindles in IDA infants might represent another way by which early IDA may influence the interaction between sleep and sensory pathway(Patrico et al, 2013). Due to the key role of iron in metabolism of monoamines in brain and their role in sleep physiology, it was stipulated that sleep quality might deteriorate in IDA (Murat et al, 2018). IDA in infants is associated with long lasting neurofunctional effects and these dysfunctions of brain continues to persist despite iron therapy. Since inadequate sleep also affects optimal brain development and cognition, the assessment of sleep pattern can enable better understanding of effects of IDA. (Peirano D. et al, 2008).

(e) Adolescents

Majority of the surveys to understand the link between anaemia and sleeplessness have been done on infants and elderly people. A population based survey done on adolescents in Norway showed that, the average number of sleep hrs. on weekdays was 6hrs 25min several of the subjects showed long latency to sleep onset .i.e., 30 min (Nunes and Bruni, 2015). Sleeplessness leads to fatigue and fatigue reduces the efficiency of various metabolic processes of the body. A study done on teenagers showing restless leg syndrome (RLS), usually caused due to sleep deprivation, and showed that these individuals had a lower body store of iron. After 4-5 months of iron therapy, there occurred

marked reduction in RLS, the mean sleep latency dropped from 143 to 23 min and sleep efficiency increased from 75.7 to 84 %.(Kryger et al, 2001).

(f) Impacts of insufficient sleep

Insufficient sleep during adolescence influences intrinsic factors such as pubertal hormonal changes, which may cause a shift towards evening chronotype as opposed to the morning chronotype of infancy. This may lead to asynchronisation between biological clock and environmental clock (Chaput et al, 2018). Thus the biological clock tends to fall behind the environmental clock and the early school start timings, which act as a time synchronizer for adolescents may actually be contributing towards development if sleep deficit in them(Kelley et al, 2015).

(g) Adults and elderly cohorts

A previous study based on self-reporting by individuals over 50 yrs. of age conducted by Jackoswa et al had revealed a sex wise distinction in the association between sleep pattern and anaemia. Men with disturbed sleep and longer or shorter sleep duration had lower levels of haemoglobin and were anaemic. In women, disturbed sleep contributed to anaemia but no link was found between sleep duration and anaemia in women (Jackowska et al, 2013). In a study done in Baltimore, revealed that anaemic individuals had a lower BMI. The study conducted by Edinboro et al, had excluded the aged people with IDA as they wereless in number and the result obtained was that anaemic individuals were more likely to suffer from insomniac condition than non-anaemic(Edinboro et al, 2018).It has been suggested that anaemia should be considered in the clinical assessment of insomniacpatients(Morin CM, 1999).

A study conducted in the Chinese population to determine the association between sleep duration and prevalence of anaemia among the participants. A total of 84,791 participants were considered from age 18-98 (Liu et al, 2018). They were divided into different categories based on their sleep duration. In showed that both short and long term sleep independently increased the prevalence of anaemia. All throughout the 7.9 yrs. for which the study was conducted, the relation between sleep and anaemia was seen to persist even after removal of other known markers of anaemia like smoking, drinking, diabetes, hypertension, obesity, etc. Both long and short sleep duration causes an increase in inflammatory markers and instances of inflammation in the body. In this study conducted on Chinese population, a sex-wise distinction of the risk of anaemia was found to be more in younger individuals than in elderly people. Individuals who reported short duration of sleep (<=5 h) or long sleep duration (>=9 h) were more likely to be have higher levels of CRP(C- reactive proteins) than those who slept for 7 h. Instance of having increased CRP levels increases with age(i.e. age above 60 yrs.) (Liu et al, 2018). CRP which increase in both total and partial sleep deprivation are the inflammatory markers that indicate increased cardiovascular risk (Ewert MD et al, 2004). It was also seen that participants below 60 yrs. of age who slept for <=5hrs (Hr. =1.24) and <=9hrs (HR=1.04) were more likely to develop anaemia compared to older participants aged above 60, who slept for similar duration. Women were especially at a higher risk (Liu et al, 2018). It was also seen that acute total and short term sleep deprivation is associated with an increase in systolic and diastolic blood pressure, heart rate and lower haemoglobin levels. Recent studies have indicated that patients with chronic heart failure are mostly anaemic(Mozaffarian et al, 2003;Kosiborod et al, 2003;Mancini et al, 2002; Ezekowitz et al, 2003;Tanner et al, 2001). In patients with chronic heart failure, treatment with erythropoietin (EPO) can increase haemoglobin level and exercise capacity in these patients, thus improving their health (Mancini et al, 2002)

The mechanism that associates insomnia and anaemia is not yet well understood. However, Jackowska et al (2013) noted that fatigue may mediate the insomnia – anaemia link.BMI and cardiovascular fitness are important factors in explaining fatigue and association with physical activity. Fatigue leads to reduced physical activity (Egerton et al, 2015). The body does not become tired enough to make us fall asleep at the proper time or for a proper duration of time. This affects the circadian rhythm and timing of sleep. Older adults therefore tend to have a hard time falling

asleep and more trouble staying asleep. A circadian shift occurs in this period that causes a change from evening chronotype during adolescence to morning chronotype during old age (Edwards et al, 2012). Sleep related disorders are thus more common at old age and contribute to the increased frequency of sleep related disorders in them (Ancoli et al, 1991; Bailes et al, 2005; Young et al, 1993; Ancoli and Ayalon, 2006).

DISCUSSION

Extensive study has been done in case of infants to understand how sleeplessness leads to anaemia and vice versa. The studies on infants revealed that sleeplessness in early months had a permanent impact on optimal brain development and functioning, which cannot be corrected by iron therapy at later stage. In elderly people, fatigue is thought to mediate the insomnia anaemia link. In general, old people have decreased efficiency in various functions of the body. Slight amount of sleep deprivation in them causes increased fatigue and accumulation of toxic materials, which contributes to further decrease in efficiency and ultimately leads to insomnia where the metabolism of the body gets down regulated. While analysing insomniacpatients, their medical history pertaining to anaemia should be considered and iron therapy can be used to cure insomnia in elderly people, provided they did not haveanaemia during infancy and early childhood.

Majority of the studies linking anaemia and sleeplessness have been restricted to these 2 age groups. Very few studies have focused on adolescent. Adolescence, in general is a period of extensive and rapid hormonal changes. Just like in infancy where rapid development occurs, adolescence period also involves development in terms of sexual maturity brought about by hormones. The main cause of sleeplessness in adolescents has been attributed to the hormonal change that they undergo in that age. Puberty is a period in which both the male and female undergo a dip in iron content of the body due to the extensive tissue growth and other changes occurring. However, girls are more prone to anaemia due to the regular menstrual bleeding and it is also known that anaemia can lead to sleep deprivation. Menstruation involves a variety of hormonal changes that might be influenced by sleep pattern and time as well as the iron content of body. A study had shown that iron therapy can help reduce sleeplessness in adolescents (Kryger et al, 2001).The study indicates the development of anaemic condition in the body. Further studies should be done to determine how the sleep pattern alteration and iron level in body influence the various hormones that are involved in bringing about pubertal changes.

It is seen that in during early stages of life, especially at infancy; sleeplessness and anaemia affects the memory and cognitive abilities and influences the development of brain (Peirano et al, 2010). As a person ages, sleeplessness due to anaemia or vice versa influence their overall metabolism. At old age, insomniac anaemic patients show a high prevalence of heart diseases (Mozaffarian et al, 2003; Kosiborod et al, 2003; Mancini et al, 2002; Ezekowitz et al, 2003; Tanner et al, 2001).This shows that anaemic condition and sleep deprivation at a particular stage of life influences the organ that is undergoing rapid development (such as the impact on brain at infancy) or is degrading rapidly (such as the influence on heart at old age). Research has suggested that need for sleep does not decrease with age, instead the ability to get adequate amount of sleep decreases with age (Chaput et al, 2018).

REFERENCES

1. Alex D. et al, 2012. The Long History of Iron in the Universe and in Health and Disease. Biochem Biophys Acta.1820(3): 161–187. (doi: 10.1016/j.bbagen.2011.08.002)
2. Algarín C. et al, 2003. Iron deficiency anaemia in infancy: long-lasting effect on auditory and visual functioning. Pediatr Res. 53(2):217-23.
3. Ancoli-Israel S. et al, 2016. Diagnosis and treatment of sleep disorders in older adults. Am J Geriatric Psychiatry.14(2):95-103. (DOI:10.1097/01.JGP.0000196627.12010.d1)

4. Ancoli-Israel S. et al, 1991. Periodic limb movements in sleep in community-dwelling elderly.Sleep.14(6):496-500. (DOI:10.1093/sleep/14.6.496)
5. Andy R. Eugene and Jolanta Masaik, 2015. The Neuroprotective Aspects of Sleep. MEDtube Sci. 3(1): 35-40.
6. Bailes S. et al, 2005. Diagnostic indicators of sleep apnea in older women and men: a prospective study.J Psychosom Res. 59(6):365-73. (DOI:10.1016/j.jpsychores.2005.06.067)
7. Kim WH, 2013. Prevalence of insomnia and associated factors in a community sample of elderly individuals in South Korea. Int Psychogeriatr. 25(10):1729-1739.
8. Beard JL. et al, 2003. Iron status and neural functioning. Annu Rev Nutr.23:41-58. (DOI:10.1146/annurev.nutr.23.020102.075739)
9. Bradley A. Edwards, Ph.D. et al, 2010. Aging and Sleep: Physiology and Pathophysiology. Semin Respir Crit Care Med.31(5): 618–633. (doi: 10.1055/s-0030-1265902)
10. Brotanek JM. et al, 2005. Iron deficiency, prolonged bottle-feeding, and racial/ethnic disparities in young children.Arch Pediatr Adolesc. 159(11):1038-42. (DOI:10.1001/archpedi.159.11.1038)
11. Burhans MS. et al, 2005 Iron deficiency: differential effects on monoamine transporters. Nutr Neurosci. 8(1):31-8. (DOI:10.1080/10284150500047070)
12. Carskadon MA. et al, 1993. Association between puberty and delayed phase preference.Sleep. 16(3):258-62. (DOI:10.1093/sleep/16.3.258)
13. Chaput JP. et al, 2016. Systematic review of the relationships between sleep duration and health indicators in school-aged children and youth.Appl Physiol Nutr Metab. 41(6 Suppl 3):S266-82. (DOI:10.1139/apnm-2015-0627)
14. Crochet S and Sakai K. 2003. Dopaminergic modulation of behavioural states in mesopontine tegmentum: a reverse microdialysis study in freely moving cats. Sleep. 26(7): 801-6. (DOI: 10.1093/sleep/26.7.801).
15. Dahl PE, 1996. The impact of inadequate sleep on children's daytime cognitive function. Semin Pediatr Neurol. 3(1):44-50.
16. Daniel J. Buysse, MD, 2014. Sleep Health: Can We Define It? Does It Matter?Sleep.37(1): 9–17. (doi: 10.5665/sleep.3298)
17. Dzirasa K. et al, 2006. Dopaminergic control of sleep wake-status. J Neuroscience. 26(41): 10577-89.
18. Ewert MD. et al, 2004. Effect of sleep loss on C - reactive protein, an inflammatory marker of cardiovascular risk. Journal of the American College of Cardiology. 43(4): 678-683.(https://doi.org/10.1016/j.jacc.2003.07.050).
19. Fuller PM. et al, 2006. Neurobiology of the sleep-wake cycle: sleep architecture, circadian regulation, and regulatory feedback.J Biol Rhythms. 21(6):482-93. (DOI:10.1177/0748730406294627)
20. Jackowska. et al, 2013. Sleep and biomarkers in the English Longitudinal Study of Ageing: Associations with C-reactive protein, fibrinogen, dehydroepiandrosterone sulfate and haemoglobin. Psychoneuroendocrinology. 38(9): 1484-1493. (https://doi.org/10.1016/j.psyneuen.2012.12.015)
21. Jean-Philippe Chaput. et al, 2017. Systematic review of the relationships between sleep duration and health indicators in the early. (0–4 years). BMC Public Health.17 (Suppl 5): 855. (doi: 10.1186/s12889-017-4850-2)
22. Jean-Philippe Chaput. et al, 2018. Sleeping hours: what is the ideal number and how does age impact this? Nat Sci Sleep.10: 421–430. (doi: 10.2147/NSS.S163071)
23. John Beard, 2003. Iron deficiency alters brain development and functioning. The Journal of nutrition. 133 (5): 1468S-1472S.
24. Justin A. Ezekowitz et al, 2003.Anaemia Is Common in Heart Failure and Is Associated With Poor Outcomes. Circulation. 107:223–225. (https://doi.org/10.1161/01.CIR.0000052622.51963.FC.)
25. Kordas K. et al, 2008. Maternal reports of sleep in 6-8 month-old infants from Nepal and Zanzibar: Association with iron deficiency anaemia and stunting. Early Hum Dev. 84(6): 389-98.
26. Kosiborod M. et al, 2003. The prognostic importance of anaemia in patients with heart failure. Am J Med. 114(2):112-9. (DOI:10.1016/s0002-9343(02)01498-5)
27. Kryger et al, 2002. Low body stores of iron and restless legs syndrome: a correctable cause of insomnia in adolescents and teenagers. Sleep Medicine. 3(2): 127-132. (https://doi.org/10.1016/S1389-9457(01)00160-5).

28. Lenis P Chen-Edinboro. et al, 2018. Association Between Non-Iron-Deficient Anaemia and Insomnia Symptoms in Community-Dwelling Older Adults: The Baltimore Longitudinal Study of Aging. The Journals of Gerontology: Series A. 73(3): 380–385.(https://doi.org/10.1093/gerona/glw332)
29. Lozoff B. et al, 2006. Long-lasting neural and behavioural effects of iron deficiency in infancy. Nutr Rev. 64(5 Pt 2):S34-43.(DOI:10.1301/nr.2006.may.s34-s43)
30. Lozoff B. 2007. Iron deficiency and child development. Food Nutr Bull. 28(4 Suppl):S560-71. (DOI:10.1177/15648265070284S409)
31. LisaMatricciani et al, 2021. In search of lost sleep: Secular trends in the sleep time of school-aged children and adolescents. Sleep Medicine Reviews. 16(3): 203-211. (https://doi.org/10.1016/j.smrv.2011.03.005)
32. Liu, X. et al, 2018.Night Sleep Duration and Risk of Incident Anemia in a Chinese Population: A Prospective Cohort Study. Sci Rep 8. 3975 (doi:10.1038/s41598-018-22407-5)
33. Marie-Pierre St-Onge. et al, 2016. Sleep Duration and Quality: Impact on Lifestyle Behaviours and Cardiometabolic Health. Circulation.134(18): e367–e386. (Doi: 10.1161/CIR.0000000000000444).
34. Mancini et al, 2002. Effect of Erythropoietin on Exercise Capacity in Patients With Moderate to Severe Chronic Heart Failure. Circulation. 107:294–299. (https://doi.org/10.1161/01.CIR.0000044914.42696.6A).
35. McCarley RW. 2007. Neurobiology of REM and NREM sleep. Sleep Medicine. 8(4):302-30. (DOI:10.1016/j.sleep.2007.03.005)
36. Meier-Ewert HK.et al, 2004.Effect of sleep loss on C-reactive protein, an inflammatory marker of cardiovascular risk. J Am Coll Cardiol. 43(4):678-83. (DOI:10.1016/j.jacc.2003.07.050)
37. Michael A. Grandneret al, 2015. The Relationship between Sleep Duration and Body Mass Index Depends on Age. Obesity (Silver Spring).23(12): 2491–2498. (doi: 10.1002/oby.21247)
38. Monti JM and Monti D. 2007. The involvement of dopamine in the modulation of sleep and waking. Sleep Med Rev. 11(2): 113-33.(DOI:10.1016/j.smrv.2006.08.003)
39. Morin CM. 1993.Psychological Assessment and Management. Insomnia.67.
40. Mozaffarian D. et al, 2003.Anaemia predicts mortality in severe heart failure: the prospective randomized amlodipine survival evaluation (PRAISE). J Am Coll Cardiol. 41(11):1933-9. (DOI:10.1016/s0735-1097(03)00425-x)
41. Nagai M. et al, 2013. Association between sleep duration, weight gain, and obesity for long period.Sleep Medicine.14(2):206-10. (DOI: 10.1016/j.sleep.2012.09.024).
42. Nunes and Bruni,2015. Insomnia in childhood and adolescence: clinical aspects, diagnosis, and therapeutic approach. Jornal de Pediatria. 91(6): S26-S35 (https://doi.org/10.1016/j.jped.2015.08.006).
43. Patel et al, 2006. Association between Reduced Sleep and Weight Gain in Women. American Journal of Epidemiology. 146(10): 947-954. (https://doi.org/10.1093/aje/kwj280).
44. Paul Kelley et al, 2015. Synchronizing education to adolescent biology: 'let teens sleep, start school later'. Learning, Media and Technology. 40(2): 210-226. (https://doi.org/10.1080/17439884.2014.942666)
45. Peirano P. et al, 2007. Iron deficiency anaemia is associated with altered characteristics of sleep spindles in NREM sleep in infancy. Neurochem Res. 32(10): 1665-72.
46. Reut Gruber et al, 2014. Position Statement on Paediatric Sleep for Psychiatrists. J Can Acad Child Adolesc Psychiatry. 23(3): 174–195.
47. Roncagliolo M. et al, 1998. Evidence of altered central nervous system development in infants with iron deficiency anaemia at 6 mo.: delayed maturation of auditory brainstem responses. Am J Clin Nutr. 68(3):683-690.
48. Semiz Murat et al, 2015. Assessment of subjective sleep quality in iron deficiency anaemia. Afr Health Sci. 15(2): 621–627. (doi: 10.4314/ahs.v15i2.40)
49. Shan Z. et al, 2015. Sleep duration and risk of type 2 diabetes: a meta-analysis of prospective studies.Diabetes Care.38(3):529-37.(Doi: 10.2337/dc14-2073.)
50. Spiegel K. et al, 2004. Brief communication: Sleep curtailment in healthy young men is associated with decreased leptin levels, elevated ghrelin levels, and increased hunger and appetite.Ann Intern Med. 141(11):846-50. (DOI:10.7326/0003-4819-141-11-200412070-00008)
51. Sharma and Kavuru, 2010. Sleep and Metabolism: An Overview. Int J Endocrinol. 2010: 270832. (doi: 10.1155/2010/270832)

52. Suzana Herculano-Houzel, 2015. Decreasing sleep requirement with increasing numbers of neurons as a driver for bigger brains and bodies in mammalian evolution. PROCEEDING OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES). (https://doi.org/10.1098/rspb.2015.1853)
53. Tanner et al, 2002. The prevalence of anaemia in chronic heart failure. International Journal of Cardiology. 28(1): 115-121.(https://doi.org/10.1016/S0167-5273(02)00273-5)
54. Todorich B. et al, 2009. Oligodendrocytes and myelination: the role of iron. Glia. 57(5):467-78. (doi: 10.1002/glia.20784.)
55. Thorlene Egerton et al, 2016. Fatigue May Contribute to Reduced Physical Activity Among Older People: An Observational Study. The Journals of Gerontology: Series A.71 (5): 670-676. (https://doi.org/10.1093/gerona/glv150)
56. Wang D. et al, 2016. Sleep duration and risk of coronary heart disease: A systematic review and meta-analysis of prospective cohort studies.Int J Cardiol.219:231-9. (DOI:10.1016/j.ijcard.2016.06.027)
57. Xiaoli Shen. et al, 2016. Night time sleep duration, 24-hour sleep duration and risk of all-cause mortality among adults: a meta-analysis of prospective cohort studies. Sci Rep.6: 21480. (doi: 10.1038/srep21480)
58. Xie L. et al, 2013. Sleep drives metabolite clearance from the adult brain.Science. 342(6156):373-337. (DOI:10.1126/science.1241224)
59. Yan Wang, MD. et al, 2015. Relationship between Duration of Sleep and Hypertension in Adults: A Meta-Analysis. J Clin Sleep Med.11(9): 1047–1056. (doi: 10.5664/jcsm.5024)
60. Yili Wu. et al, 2014. Sleep duration and obesity among adults: a meta-analysis of prospective studies. Sleep Medicine. 15(12): 1456-1462. (https://doi.org/10.1016/j.sleep.2014.07.018)
61. Young T. et al, 1993. The occurrence of sleep-disordered breathing among middle-aged adults. N Engl J Med. 328(17):1230-5. (DOI:10.1056/NEJM199304293281704)
62. Zhai L. et al, 2015. SLEEP DURATION AND DEPRESSION AMONG ADULTS: A META-ANALYSIS OF PROSPECTIVE STUDIES.Depress Anxiety.32(9):664-70. (DOI:10.1002/da.22386)

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RESEARCH ARTICLE

A Study on Designing and Development of Microbial Fuel Cell (MFC) for Electricity Generation from Gir Cow Urine

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ABSTRACT

In today's growing world, people are depending upon fossil fuel to fullfill energy requirement. But its long term use affects climate. Microbial Fuel Cell (MFC) is a new approach to generate energy from waste. Basically Microbial fuel cell is a bio-electrochemical system or fuel cell that is simply conversion of chemical energy into electric energy. Present study based on manufacturing of Microbial Fuel Cell by using Gir Cow Urine and Sea Water as waste with using varieties of electrodes to get maximum output voltage to generate electricity. From the obtained data Cuboidal Carbon Block (Size=21.98cm^3) material as electrodes as well as4% or 0.7M NaCl with 4% Agar-agar made salt bridge was selected. Where get maximum voltage output was 0.67V with standardized design of MFC set up. And also arranged MFC set up in series connection, which gave maximum output was 2.45V. For battery back up assembly were arranged rechargeable cell with capacity 1.2V. For development of MFC set up with scale up process were apply variations in anode chamber with Cow (Gir) dung and Methylene Blue as well as in cathode chamber withisolated bacterial strain to enhance electricity generation through Microbial Fuel Cell (MFC).

Key Words: MFC, Electrodes, Cow (Gir) Urine, Sea Water.

INTRODUCTION

According to current status of the world, there is great issues of waste disposal and fuel crisis (Reddy *et al.,* 2010). These problem can be solved by developing new microbial technologies, to create new energy resources. Microbial

fuel cell is help to develope new era to fullfill energy crisis. Microbial fuel cell known for many years and it is possible to generate electricity from waste. Today humankind is mostly dependent on energy with the advancement of science and technology. The present day energy scenario in India and at global level decreasing, thus it is must to the search of the alternative to the fossil fuels (Purswani*et al.,* 2014). Electricity is the sole of today's society and economy, with developing technologies there demand and requirement of electric energy increasing. The need for new and alternate sources of energy is increasing in daily life. In India rural areas suffer from problem of frequent power cut. Hence we worked on Cow urine power generated system for growing world. In our country widely agricultural activities occurs and people dependent on farm related activities as well as domestic animals like cow, bullock etc. So, it become easy to get Cow urine as renewable energy source. This motivates us to work on Cow urine power generated system as traditional renewable energy source. As well as there is most part of earth covered with sea water. Which is also a biowaste source and it can useful for electricity generation by microbial fuel cell. This system is easily accessible and pollution free. Here in this work we are showing small scale and simple model of generating electricity by using Cow urine and Sea water as a electrolytic solutions. Research is going on to develop alternative sources of energy for electricity generation using Gir Cow Urine and Sea Water in microbial fuel cell system (Pratyush*et al.*, 2015).

Use of Cow urine and Sea water for MFC Set up

Generally cow urine contains water - 95%, urea - 2.5% and Salts, Minerals, Hormones and Enzymes - 2.5%. Cow urine contains minerals, uric acid and salts which acts as a electrolytic solution (Pratyush*et al.*, 2015).Cow urine contains uric acid. This uric acid is a heterocyclic compound, which contain carbon, nitrogen, hydrogen and oxygen with the formula $C_5H_4N_4O_3$. Which forms ions and salts which known as urates and acid urates, like ammonium acid urate.

Uric acid is synthesized by oxidation of purine within the body system and excreted with urine. In mammals, uricase enzyme further oxidizes the uric acid to allantoin. Uric acid contains de-localized loan pair of electron. These pairs of electrons participate in generation of electricity from cow's urine (Hasan*et al.,* 2014), (Bisen*et al.,* 2015), (Rajak*et al.,* 2017). The pH of cow urine was in between 8 to 9. So, bio-batteries are conventional energy source to generate power by natural means (Ramalingam*et al.,* 2009), (Rajak*et al.,* 2018).

Basics of electricity generation in MFC

Here in the MFC system an Anode is a negative electrode (terminal) and Cathode is a positive electrode (terminal). Electrons produce in anodic chamber and move towards cathodic chamber through external circuit and generate current. Our source of electricity generation is Gir Cow urine (uric acid) and Sea water. Both contain ions, minerals etc. so both acts as a electrolytic solution. Where electrons move from anodic chamber (Gir Cow urine) to cathodic chamber contains Sea water, where some metal ions, salts help to maintain pH and generate electricity (Bisen*et al.*, 2015). MFC is a compact reactor which can generate electricity from the biomass through microbial metabolic activity (anaerobic oxidation). MFC are act as potential devicesto harvest bioenergy from the waste material or wastewater. A simple MFCset up consists of an anode in anodic chamber, a cathode in cathodic chamber, which is separated by a protonexchange membrane (PEM) or salt bridge. MFC works on a principle that biocatalysts oxidize organic substrates inthe anodic chamber and releases protons and electrons in the process along with the generation of CO_2.Where proton passes through salt bridge and electrons towards the cathode through external circuit. At the cathode, the electronscombine with protons and oxygen to form water.(Du Z *et al.,* 2007), (G. Bhargavi *et al.,* 2018).

MATERIALS AND METHODS

Materials

For double chamber microbial fuel cell

- Source of waste
- Gir Cow urine [From Vadtalswaminarayanmandirgaushala]
- Sea water [From Valsad (Tithal, Bhat), Surat (Dumus)]
- Sample size : 2000ml
- Plastic jars with 2000 ml capacity
- PVC pipe [Size =21.98cm^3]

Other materials

Gir Cow dung, Methylene blue solution, Insulated flexible wires, Crocodile clips, Glue gun, Flex kwik, Digital multimeter, Rechargeable battery (cell).

Methods

Sample collection

Gir Cow urine from Vadtalswaminarayanmandirgaushala and Sea water from Valsad (Tithal and Bhat), Surat (Dumas).

Double chamber Microbial Fuel Cell (MFC)

Salt bridge

Salt bridge used to connect both chamber of microbial fuel set up. PVC pipe (Size = 21.98cm^3) was used to construct salt bridge. (4%) Nacl + Agar powder used to prepare salt bridge, where 4 gmNaCl and 4 gm Agar powder dissolved into 100 ml distilled water. Here 4% NaCl means 0.7M NaCl with 4% Agar-agar. Digest agar by heatinguntill solution become transparent. It is must to proper digestion of agar. Then solution pour into PVC pipe. Put it for solidify (Parkash*et al.,* 2015).

Assembly of MFC

Two plastic jars with 2000ml volume carrying capacity were taken, both attached with salt bridge. Jars were sealed with glue and after drying filled chambers with respective waste, anode chamber with Gir Cow urine and cathode chamber with Sea water. It must be sealedproperly to prevent leakage (Anand Parkash*et al.,* 2015).

RESULTS

Here in this study Double chamber Microbial Fuel Cell (MFC) design selected. Where various waste sample analysed, also used different electrode material and also arranged set up with different salts like NaCl (Sodium chloride), KCl (Pottasium chloride) with different concentration. From all of these experiment for standardised set up of MFC were select Gir Cow urine and Sea water as waste sample, NaCl (Sodium chloride) with concentration 0.7M or 4% NaCl + 4% Agar powder made up salt bridge (Size = 21.98 cm^3) and Cuboidal carbon block (Size = 27.69cm^2) as electrode material. This MFC set up gave more output other than all setups at long time period. Where highest voltage generated on 35th day it was 0.67V. From 42nd day to 70th day it gave continous output of voltage. So, it was considered as saturated level or point of this particular setup. Then from 77th day output reduced. Here output level goes up-down due to electro-chemical reactions of components of waste. And here used Gir Cow urine and Sea water combination as waste sample. These both maintained pH of the system. Due to that it may help to maintain voltage generation at long time duration. So, these both waste confirm to design standard MFC setup assembly along with above described materials.Power density of this MFC setup was also highest on 35th day it was46.18698µW/cm^2. It

showed overall electricity output including resistance, voltage, current and electron transfer rate on the surface of electrode (cuboidal carbon block).

Results for MFC set up with variations in anode chamber,

Any significant change is not observed in the voltage by adding cow dung or methylene blue with compare to standard set up of fuel cell.

Results for MFC setup with Double salt bridge

Double salt bridge contained MFC setup also gave improved voltage output but it take long time for that. Initially it gave lower output. On 42nd day maximum output 0.77V.Then voltage dropdown. Here in this MFC setup it help to get acceptable output. But here voltage output range not maintained within small gap of range.

Results for MFC setup with variations in Cathode chamber

Isolated strains from Gir Cow dung and Sea water used for variations into cathode chamber. Strains were inoculated into cathode chamber and observed for voltage output changes either it help to maintain current flow or not. Here these strains gave maximum output on 14th and 21st day, it was between 0.24V-0.46V. This voltage generation was lower but it may able to gave improved output after long time duration.

Results for series arrangement set up of MFC

For Scale up setup were connect MFC setups into series arrangement (connection). The series arrangement assembly of MFC setup gave improved voltage output. By series arrangement setup it gave 2.45V maximum voltage. It was enough to glow one LED light. So, these help to detect that this type design of MFC assembly may help to generate electricity from waste. Battery back assembly where arranged with rechargeable cell into series connected MFC setup, where positive and negative terminals of the rechargeable cell (battery) and MFC setup were joint to complete circuit.

Elemental components into Gir Cow urine

The Gir Cow urine contain different elements which help to maintain pH and ionic condition. It leads electrochemical reaction and generate electricity. The Double chambered MFC set up withGir Cow urine and Sea water gave average 0.5V-0.7V output. In series arrangement it gave increased voltages and Glow a LED light.

CONCLUSION

From the experimental work studies it can be conclude that GirCow urine and Sea water both act as convensional and renewable source of energy. Here for this particular study where standardised a design of a Double chambered MFC setup. Also for development of MFC setup were performed variations into chambers (compartments) of MFC. To get maximum electricity generation were MFC setup arranged into series connection and also arranged battery backup assembly using rechargeable cell (battery).The standard confirmed MFC design was as like in given figure.

REFERENCES

1. Akshay D. Tharali, NamrataSain& W. Jabez Osborne, (2016), Microbial fuel cells in bioelectricity production, FRONTIERS IN LIFE SCIENCE, 2016, Vol. 9, Issue N0. 4, 254, 259-262.

2. Ali Masduqi. (2018), Physicochemical Properties of Sea Water and Bittern in Indonesia: Quality Improvement and Potential Resources Utilization for Marine Environmental Sustainability, www.researchgate.net, 5.
3. Anand Parkash, (2015), Design and febrication of a double chamber microbial fuel cell for voltage generation from biowaste, Journal of bioprocess &biotechniques, Vol. 5, Issue 8, 2.
4. Anand Parkash, Imran NazirUnar, Shaheen Aziz, SuhailSoomro, (2015), Utilization of sewage for voltage generation in duel chamber microbial fuel cell, www.researchgate.net, 5065.
5. Bruce E. Logan, John M. Regan, (2006), Microbial fuel cells - Challenges and harnessing the metabolic activity of bacteria can provide energy for a variety of applications, once technical and cost obstacles are overcome, Environmental Science & technology, 5175.
6. D. Singh, D. Pratap, Y. Baranwal, B. Kumar, R. K. Chaudhary, (2010), Microbial fuel cells: A green technology for power generation, Scholars research library, 131-133.
7. Du Z, Li H and Gu T A, (2007), state of the art review on microbial fuel cells: a promising technology for wastewater treatment and bioenergy, Biotechnol Adv.25,464–82.
8. FlavianaCalignano, Tonia Tommasi, Diego Manfredi& Alessandro Chiolerio, (2015), Additive Manufacturing of a Microbial Fuel Cell—A detailed study, www.nature.com/ Scientificreports, 2.
9. G Bhargavi, V Venu, S Renganathan, (2018), Microbial fuel cells: recent developments in design and materials, IOP Conf. Series: Materials Science and Engineering, 3.
10. L.Veeranjaneya Reddy, S. Pradeep Kumar, Young-Jung Wee, (2010), Microbial fuel cells (MFCs) – a novel source of energy for new millennium, Current research,Technology and education topics in applied microbiology and microbial biotechnology A. Mendez-Vilas (Ed.), 956.
11. Logan BE, (2004), Peer reviewed: extracting hydrogen and energy from renewable resources, EnvironSci Technol. 38(9), 160A–167A.
12. Munn, C. in *Marine microbiology: Ecology and Applications* 2nd edition. (eds Taylor and Francis Group), (Garland Science, 2011).
13. PratyushBisen, RajnarayanChoudhary, Samir Khule, (2015), Cow urine power generated system**,** International journal of advance research in science and engineering, Vol. 4, Issue 1, 81-84.
14. PratyushBisen, RajnarayanChoudharyand Samir Khule, (2015), Cow Urine Power Generated System, International Journal of Advance Research in Science and Engineering. 4(1), 80-85.
15. Raj Kumar Rajak, Bharat Mishra, (2017), Customized Cow's Urine Battery Using MnO_2 Depolarizer World Academy of Science, Engineering and Technology, International Journal of Energy and Environmental Engineering 11(11), 766-769.
16. Raj Kumar Rajak, Bharat Mishra, (2018),Studies on the Feasibility of Cow's Urine as Non-Conventional Energy Sources, World Academy of Science, Engineering and Technology International Journal of Energy and Environmental Engineering, Vol. 12, Issue 5, 372.
17. S. Ramalingam, T. Prabhu, K. Manimaran, S. Murugan and S. M. Nagarajan, (2009), Bio-Emf Device (Bed) Using Hibiscus Leaf as Electrolyte, Asian Jr. of Microbiol. Biotech.Env. Sc. 11(1), 1-2.
18. S.D.Purswani, S.S.Atkare, G. Bhumkar, M.B.Patil, (2014), Electricity generation from dairy waste water through microbialfuel cell technology, International journal ofengineering research and reviews, Vol. 2, Issue 4, 24.
19. Singh D, Pratap D, Baranwal Y, Kumar B, Chaudhary RK, (2010), Microbial fuel cells: a green technology for power generation, Ann Biolog Res. 1(3), 128–138.
20. WahidulHasan, Hafiz Ahmed, and Khosru M. Salim, (2014), Generation of Electricity Using Cow Urine, International Journal of Innovation and Applied Studies. 9(4), 1465-1471.

Table.1.Voltage with Nos. of setup into series arrangement (Connection)

Nos. of set up into Series	Voltage (V)
2	1.00
4	1.77
5	1.94
6	2.07

8	2.27
9	2.14
14	2.45

Table. 2. Components into Gir Cow urine

Parameter	Result	Unit	Method
N (Nitrogen)	0.36	%	Micro kjeldhal
P (phosphorus)	0.019	%	Yando-molibdate
K (Potassium)	0.64	%	Flame photometric
B(Boron)	11.16	ppm	MP-AES method
Ca (Calcium)	262.99	ppm	MP-AES method
Cu (Copper)	3.25	ppm	MP-AES method
Fe (Iron)	22.25	ppm	MP-AES method
Mn (Manganese)	2.40	ppm	MP-AES method
Zn (Zinc)	4.00	ppm	MP-AES method

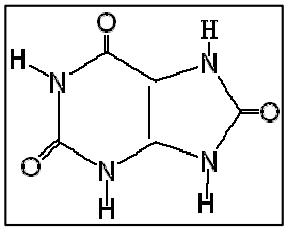


Figure.1. Structure of uric acid

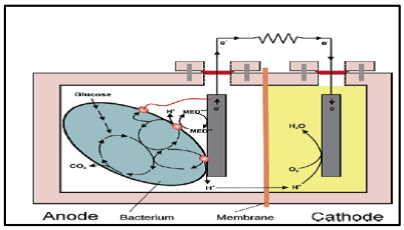


Figure 2.Working principle of Microbial Fuel Cell.(Bruce E. Logan *et al.*, 2006)

Figure.3. [Gir Cow] [Gir Cow urine] [Sea water]

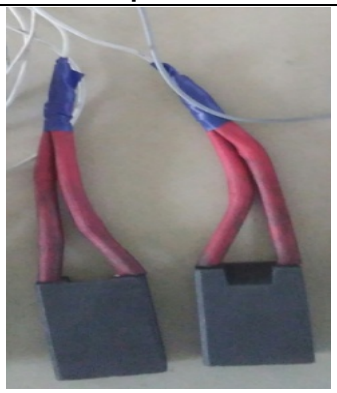
		
Figure.4.Rectangular carbon block	**Figure.5.Copper plate**	**Figure. 6. Aluminium mesh + Graphite sticks**
		
Figure.7. Aluminium mesh	**Figure.8.Round (small) carbon block**	**Figure.9.Cuboidal carbon block**
		
Figure 10. Digital multimeter with probes	**Figure 11. Rechargeable battery (cell)**	**Figure 12. Salt bridge with (4%) NaCl + Agar-agar**

Figure 13. Assembly of Double Chamber MFC setup

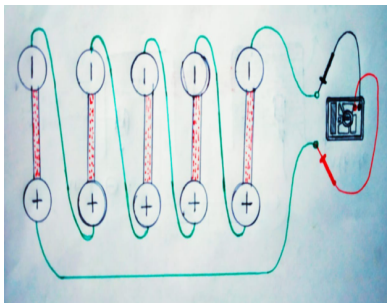


Figure 14. Series arrangement Design for MFC setup

Figure 15. Whole Series arrangement assembly of Microbial Fuel Cell set up

Figure 16. Glowing LED light in series arrangement

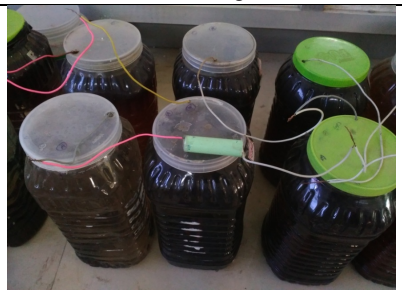


Figure 17. Battery back up assembly

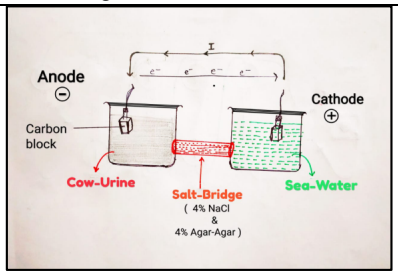
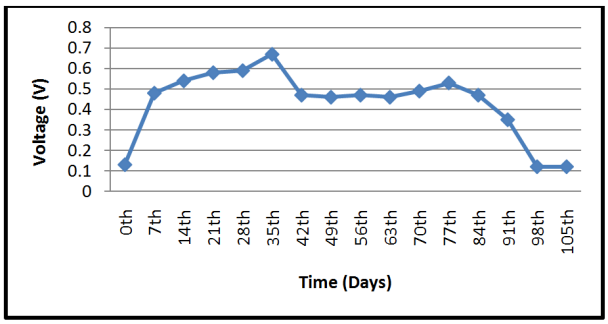
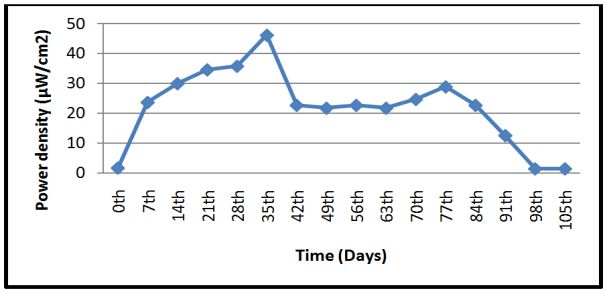


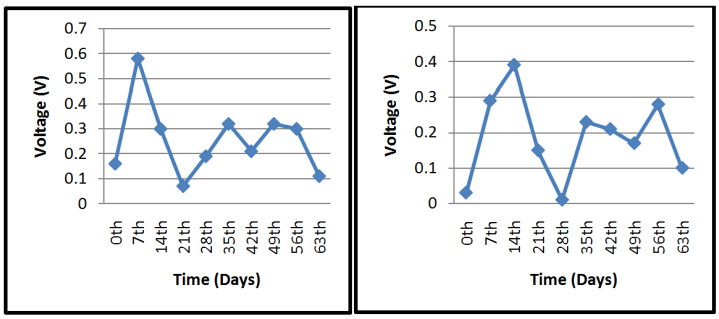
Figure 18. Standardize Design of Microbial Fuel Cell (MFC)



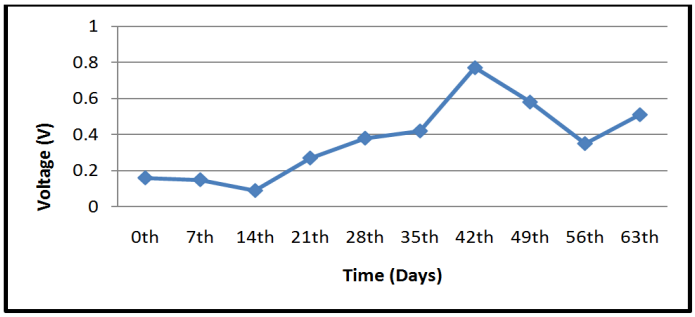
Graph 1.Voltages Vs Time (For setup with 0.7M or 4% NaCl + 4% Agar-agar)



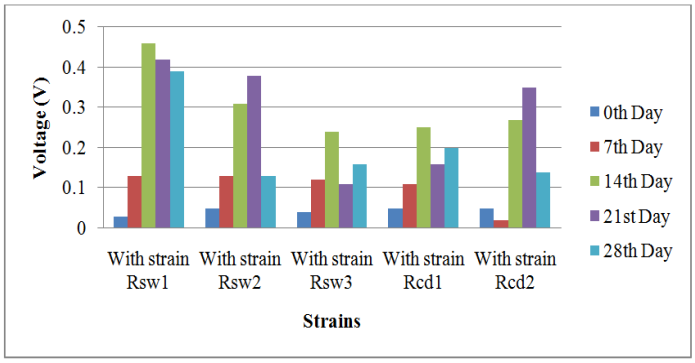
Graph 2. Power density Vs Time (For setup with 0.7 M or 4% NaCl + 4% Agar-agar)



Graph3 (a)With Gir Cow dung (b) (With Methylene blue)



Graph 4. Voltage Vs Time



Graph5 :Voltage Vs Strains

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RESEARCH ARTICLE

Non-Invasive Prediction of High-Risk Esophageal Varices Through Platelet Count to Spleen Diameter Ratio

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ABSTRACT

The aim of the study was to determine the positive predictive value (PPV) of platelet count to spleen diameter (PS/SD) ratio for the diagnosis of high-risk esophageal varices (HREV) taking endoscopic findings as gold standard exclusively in Hepatitis CVirus (HCV) related cirrhosis. A cross-sectional study was conducted at Outdoor patient department, Service Hospital, Lahore for a duration of six months from 25th June 2012 to 24th December 2012. A total of 200 HCV related cirrhotic patients were included in the study after attaining written informed consents. The data was collected using a pre-designed questionnaire inquiring patients' demographic details and ultrasonographic measurement of spleen diameter were observed. For PC/SD ratio, total blood count was obtained using Cell-DYN 1700. The risk of esophageal varices was predicted on the basis of the platelet count/spleen diameter ratio, patients with less than 830.8 (PC/SD ratio) received upper gastrointestinal tract endoscopy in order to determine the presence and grade of esophageal varices. Collected data was analyzed using SPSS Version 10. The mean age of the study patients was 56.9±9.4 years; 127(63.5%) males and 73(36.5%) females. Grade-I esophageal varices were found in 84 (42%) patients, grade-II in 77 (38.5%) and grade-III in 39(19.5%) patients. Positive cases of HREV on endoscopy were 146 and PC/SD ratio diagnose HREV in 182 cases, PPVof PC/SD ratio was 75.2%. It can be concluded from the study results thatPC/SD ratio is useful for the non-invasive diagnosis of HREV among patients with HCV related cirrhosis.

Keywords : High Risk Esophageal Varices, HCV, Platelet Count to Spleen Diameter Ratio, Liver Cirrhosis.

INTRODUCTION

HCV is one of the major health problems affecting approximately 130-170 million people worldwide i.e. around 2%-3% of the total world's population is affected by HCV [1]. End stage HCV leads to Liver cirrhosis, further complicated by numerous contributors like portal hypertension, ascites, hepatic encephalopathy, and esophageal varices [2]. Portal hypertension is a common complication of cirrhosis and incidence of subsequent varices is 5% per year. The incidence of progression from small to large esophageal varices is 20% per year, one third of such patient die of bleeding from varices with their first episode. Pakistan is suffering heavy burden of HCV related cirrhosis and locally it is the 10thleading cause of death. American college of gastroenterology and Bravo VI Consensus conference on portal hypertension recommends screening endoscopies in all such patient for esophageal varices and taking preventive measures for HREV [3].

As a developing nation we have limited endoscopic facilities in most of the health care centres. Based the recent guidelines, screening of esophageal varices is recommended for the patients diagnosed with cirrhosis. It is evident that decompensated liver cirrhosis patients undergo repeated endoscopy annually due to lack of detection of varices at the first attempt while in case of compensated cirrhosis, a repeated requirement is observed every 2-3 years [3]. Repeated endoscopies are associated with multiple side effects such as aspiration, perforation, and bacteremiaetc [4]. The lack of detection of these esophageal varices is not only due to decreased patient compliance and invasive nature of the procedure but also the cost-ineffectiveness of the procedure [5]. And based on these considerations the current focus of the medical and research field is to identify the non-invasive clinical, radiological, and biochemical parameters that can effectively determine the presence of portal hypertension and esophageal varices. A few identified non-invasive predictors for the detection of HREV among cirrhotic patients, that are being used both locally and internationally include portal vein diameter, right liver lobe/albumin ratio, platelet count, Child-Pugh classification and PC/SD ratio [2,6].

PC/SD ratio is by far the finest predictor of esophageal varices, which links the spleen size to thrombocytopenia i.e. the diminished platelet count is caused by hypersplenism (splenomegaly) which in turn is due to portal hypertension [7]. While the other identified parameters like aminotransferase-to-platelet ratio index (APRI), Fibroindex, and Fibrosis-4 score (FIB-4) predict the progression of portal hypertension based on the extent of fibrosis and cirrhosis [8]. PC/SD ratio has shown high effectiveness in detection of HREV with reported sensitivity of 77.9%, specificity 74.2%, PPV was 71.4% and negative predictive value (NPV)was 77.8%, using a cut-off value 830.8[9]. Although the diagnostic accuracy of PC/SD ratio is evident from the existing literature [6,7,9], and hence it is universally accepted and approved. But locally not much is known about the PPV of PS/SD for diagnosis of high-risk esophageal varicesamong local Pakistani population.The objective of our study was to determine thePPV of PC/SD ratio for the diagnosis ofHREV taking endoscopic findings as gold standard among HCV related cirrhosis.

METHODOLOGY

A cross-sectionalstudy was conducted at Outdoor patient department, Service Hospital, Lahore for a duration of six months from 25th June 2012 to 24th December 2012. A total of 200 HCV related cirrhotic patients were included in the study. Sample size was calculated with WHO software Sample Size Determination in Health Studies[10], keeping 95% confidence interval, 6.5% margin of error and taking expected percentage of PPV i.e. 71.4% of PC/SD ratio for the diagnosis of HREV taking endoscopic findings as gold standard. Male and female patients aged between 18-79 years, with overt liver cirrhosis proved by clinical and imaging methods, anti-HCV positive indicated by Elisa for more than six month and positive for HREV on PC/SD ratio were included in the study. Patients with past history of upper gastrointestinal bleeding, previous band ligation, those receiving prophylactic treatment for portal hypertension, diuretics, interferon, portal vein thrombosis/ hepatoma on ultrasound abdomen, acute febrile illness, aplastic anemia, megaloblastic anemia and Systemic lupus erythematosus (SLE) were excluded.

Data was collected using a pre-designed Performa after attaining written informed consent, patient's age, gender and biochemical parameters were measured. The blood count, PC and ultrasonographic measurement of spleen diameter on a longitudinal section in right lateral decubatous position was obtained to calculate the PC/SD ratio. The full blood count was assessed using CELL-DYN 3700 (Abbott Laboratories, Beckman Coulter, United States).Patients with less than 830.8 PC/SD ratio were taken for upper gastrointestinal tract endoscopy on the same day (Olympus video endoscope) to confirm the presence of HREV in these patients. Esophageal varices were detected and classified according to de Franches classification.

Patients with PC/SD ratio < 830.8 and also HREV on endoscopic examination were considered as true positive (TP) cases. While those with PC/SD ratio <830.8 but showed low risk esophageal varices upon endoscopic examination were considered as false positive (FP) cases.PPV of PC/SD ratio was also calculated by:

$$PPV = \frac{\text{True Positive}}{\text{True Positive + False Positive}} \text{ X } 100$$

Data was analyzed using SPSS Version 10, age was presented as mean ± SD while gender, HREV, positive predictive values of PS/SD ratio in the diagnosis of HREV were presented as frequency and percentages.

RESULTS

A total of 200 HCV patients with hepatic cirrhosis were included in the study with majority being males. The average age of the study population was 56.9±9.4years. 77(38.5%) patients were having grade II varices (medium risk), 84 (42%) had grade I varices (low risk) and 39(19.5%) were having grade III (high risk) varices. The mean platelet count of the study subjects was 80920±20840n/mm^{3}and the mean PC/SD ratio was 503±109n/mm^3/mm (Table 1). Based on the estimations 137 patients were presented with PC/SD ratio < 830.8 and also showed high risk esophageal varices on endoscopic examination, 45 patientshad PC/SD ratio <830.8 but showed low risk esophageal varices in endoscopy. While only 09 patients with >830.8 PC/SD ratio had high risk esophageal varices as indicated by endoscopy (Table 2).

DISCUSSION

The research demand in the field of endoscopy has increased rapidly in the past few years as it is not possible to perform endoscopy every year or two to detect esophageal varices among high-risk patients. Therefore, non-invasive predictors for estimating the risk of esophageal varices among Cirrhosis patients might help in reducing the requirement of endoscopic examination and also lower the overall management cost among cirrhotic patients.The validation of such parameters in local population will aid in distinguishing the high and low risk patients followed by endoscopic examinations only among patients with HREV. Globally, PC/SD ratio is considered as the primary non-invasive predictor of esophageal varices, evident from its high sensitivity and specificity among hepatic cirrhosis patients [12]. In ourstudy 73% of the cirrhotic patients had HREV as diagnosed by endoscopy, similar findings were reported by a Brazilian study i.e. 73.2% of the cirrhotic patients displayed HREV upon endoscopy [13].The predictor greatly decreases the management cost as it required two of the basic measurements (platelet count and spleen diameter) recorded during the routine assessment of the cirrhotic patient. Moreover, literature indicates that PC/SD ratio helps in indicating the HREV risk with minimum errors i.e. in case of biological variation [11, 14]. A contradictory article by Barcelona group, indicated that the PC/SD ratio was not associated to esophageal varices diagnosis [15]. Although theplatelet count was significantly associated with esophageal varices but due to overall insignificant findings the study suggested that PC/SD ratio as an inadequate predictor ofesophageal varices among cirrhotic patients. While based on our findings the results were in favor of PC/SD ratio, the PPV of PC/SD ratio for the diagnosis of HREV was 75.2%. Also, supported by a study conducted by Giannini and colleagues, demonstrating the

PPV of PC/SD ratio as 76.6% [16]. The sensitivity and specificity of PC/SD ratio for diagnosing the risk of HREV is approved by a number of studies. Therefore, taking support from previous literature and based on the current findings, we consider PC/SD ratio as an effective method for predicting HREV in patients with cirrhosis. As it is an ideal tool with high high sensitivity and specificity and is cost-effective. And hence, it should be considered during diagnosis of patients with a high risk of developing esophageal varices. Although the study results significantly supported the hypothesis but the study holds some limitations which must be considered and further research must be carried out. The sample size was limited the patients were included from only one centre, due to which the findings cannot be generalized for the entire local population. A multicenter study is required to validate the diagnostic use of this non-invasive parameter among local population suffering from HCV related cirrhosis.

CONCLUSION

In conclusion, the PC/SD ratio is a useful non-invasive method for detecting esophageal varices among cirrhosis patients and it not only reduces the number of unnecessary endoscopies performed without significant risk of missing the presence of esophageal varices. It is cost-effective in comparison to the repeated endoscopic procedures performed. The cirrhotic patients usually undergo annual / biannual ultrasonography as part of their routine examination therefore no additional expense would be entailed.

Conflicts of Interest

None.

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REFERENCES

1. Cuadros DF, Branscum AJ, Miller FD, Abu-Raddad LJ. Spatial epidemiology of hepatitis C virus infection in Egypt: analyses and implications. Hepatology. 2014;60:1150–1159.
2. Zhang W, Wang L, Wang L, Li G, Huang A, Yin P, Yang Z, Ling C, Wang L. Liver stiffness measurement, better than APRI, Fibroindex, Fib-4, and NBI gastroscopy, predicts portal hypertension in patients with cirrhosis. Cell BiochemBiophys. 2015;71:865–873.
3. De Franchis, R. (2010). Revising consensus in portal hypertension: Report of the Baveno V consensus workshop on methodology of diagnosis and therapy in portal hypertension. Journal of Hepatology, 53(4), 762–768.
4. Spiegel BM, Targownik L, Dulai GS, Karsan HA, Gralnek IM. Endoscopic screening for esophageal varices in cirrhosis: Is it ever cost effective? Hepatology. 2003;37:366–377.
5. Talwalkar JA, Kamath PS. Screening for esophageal varices among patients with cirrhosis of the liver. Am J Gastroenterol. 2001;96:3039–3040.
6. Elalfy H, Elsherbiny W, Rahman AA, Elhammady D, Shaltout SW, Elsamanoudy AZ, El Deek B. Diagnostic non-invasive model of large risky esophageal varices in cirrhotic hepatitis C virus patients. World journal of hepatology. 2016 Aug 28;8(24):1028.
7. Giannini E, Botta F, Borro P, Risso D, Romagnoli P, Fasoli A, Mele MR, Testa E, Mansi C, Savarino V, et al. Platelet count/spleen diameter ratio: proposal and validation of a non-invasive parameter to predict the presence of oesophageal varices in patients with liver cirrhosis. Gut. 2003;52:1200–1205.

8. Crisan D, Radu C, Lupsor M, Sparchez Z, Grigorescu MD, Grigorescu M. Two or more synchronous combination of noninvasive tests to increase accuracy of liver fibrosis assessement in chronic hepatitis C; results from a cohort of 446 patients. Hepat Mon. 2012;12:177–184.
9. Barrera F, Riquelme A, Soza A, Contreras Á, Barrios G, Padilla O, Viviani P, Pérez-Ayuso RM. Platelet count/spleen diameter ratio for non-invasive prediction of high riskesophageal varices in cirrhotic patients. Annals of hepatology. 2009;8(4):325-30.
10. Lwanga SK, Lemeshow S, World Health Organization. Sample size determination in health studies: a practical manual. Retrieved from: https://apps.who.int/iris/handle/10665/40062
11. Giannini E, Botta F, Borro P, Risso D, Romagnoli P, Fasoli A, Mele MR, Testa E, Mansi C, Savarino V, et al. Platelet count/spleen diameter ratio: proposal and validation of a non-invasive parameter to predict the presence of oesophageal varices in patients with liver cirrhosis. Gut. 2003;52:1200–1205.
12. de Mattos AZ, de Mattos AA. Platelet count/spleen diameter ratio: can it replace endoscopy for the screening of esophageal varices in cirrhotic patients. Eur J Gastroenterol Hepatol. 2012;24:1113.
13. Mattos ÂZ, Mattos AA, Vianna FF, Musskopf MI, Pereira-Lima JC, Maciel AC. Platelet count/spleen diameter ratio: analysis of its capacity as a predictor of the existence of esophageal varices. Arquivos de gastroenterologia. 2010 Sep;47(3):275-8.
14. Giannini EG, Zaman A, Kreil A, Floreani A, Dulbecco P, Testa E, Sohaey R, Verhey P, Peck-Radosavljevic M, Mansi C, Savarino V, Testa R. Platelet count/spleen diameter ratio for the noninvasive diagnosis of esophageal varices: results of a multicenter, prospective, validation study. Am J Gastroenterol. 2006;101:2511-9.
15. Berzigotti A, Gilabert R, Abraldes JG, Nicolau C, Bru C, Bosch J, García-Pagan JC. Noninvasive prediction of clinically significant portal hypertension and esophageal varices in patients with compensated liver cirrhosis. Am J Gastroenterol. 2008;103:115

Table 1. Demographic and clinical characteristics of study population

Parameters		(n=200)
Mean Age (Years)		56.9±9.4
Age Groups (Years)	20-40	51(25.5)
	41-60	121(60.5)
	61-79	28(14)
Gender	Male	127(63.5)
	Female	73(36.5)
Grading of Varices	I	84(42)
	II	77(38.5)
	III	39(19.5)
Platelet Count (n/mm^3)		80920±20840
PC/SD Ratio [(n/mm^3)/mm]		503±109

*PC/SD-Platelet count/diameter of the spleen.
*values are given as mean ± SD or n(%)

Table 2. Comparison of PC/SD ratio with endoscopy for diagnosis of HREV

PC/SD Ratio	Endoscopy	
	Positive	Negative
Positive	137(TP)	45(FP)
Negative	09(FN)	09(TN)

* PC/SD-Platelet count/diameter of the spleen; HREV-High-Risk Esophageal Varices.
*TP-True Positive; FP-False Positive; FN-False Negative; TN-True Negative.

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RESEARCH ARTICLE

Heterogeneous Catalyst Derived from Natural Resources for Biodiesel Production

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ABSTRACT

Biodiesel is fatty acid methyl ester (FAME) of long chain fatty acids derived from a varied range plant oils through transesterification reaction with methanol in presence of homogeneous or heterogeneous catalysts. In this paper, a study has been carried out for the preparation of calcium oxide from waste crab shells as a heterogeneous catalyst from natural resources for application in biodiesel synthesis. The waste crab shells (CS1) were processed, calcined at 900°C for 2h and characterized for thermogravimetric analysis (TGA), X-ray fluorescence (XRF), X-ray diffraction (XRD), and scanning electron microscope (SEM) for its suitability in transesterification reaction with non-edible oil. The biodiesel conversion was determined by Gas chromatography and ^{1}H Nuclear Magnetic Resonance (^{1}H NMR) Spectroscopy. The prepared catalyst (CS1) was utilized for biodiesel synthesis from simarouba glauca oil. The maximum yield of biodiesel was 95% under optimized reaction conditions of 12:1 methanol to oil molar ratio, 6% wt catalyst doses, 65±2 °C reaction temperature, 2 h reaction time and 600 rpm stirrer speed. The FAME produced was analyzed for various phyico-chemical properties as per standard specification of IS 15607-2016. The prepared ester was blended with BSVI Diesel fuel and characterized as per Diesel fuel specification IS1460-2017.

Key words: Biodiesel, FAME, catalyst, transesterification.

INTRODUCTION

Due to rapid development, the worldwide demand for biodiesel as an alternative to the conventional transport fuel, petro diesel for diesel engines, is increasing because of the limited reserves of fossil fuels, increasing prices of crude oils and environmental concerns. It has become apparent that biodiesel is destined to make a substantial contribution to the future energy demands of the domestic and industrial economies. India is the third biggest crude oil consumer

on the globe and meets about 85% of its needs through imports, exposing the economy to the risks of price and supply disruptions often caused by geopolitical tensions and trade wars. The prime minister has set a target to reduce such overseas energy purchases by 10 percentage points by 2022, through increased domestic output and greater use of alternate fuels. There is different potential feedstock for biodiesel production. Non-edible vegetable oils which is known as the second generation feedstock can be considered as promising substitutions for traditional edible food crops for the production of biodiesel[1]. Biodiesel is usually produced through the transesterification reaction between the plant oils and methanol in presence of acidic or basis catalyst. Commonly basic catalysts such as NaOH or KOH are commercially viable due to their fast and low temperature reaction to produce the biodiesel, but possesses various drawbacks i.e intolerance to high free fatty acid (FFA) content of oils and more waste water generation during processing. Heterogeneous catalytic process overcomes these drawbacks. Heterogeneous solid base catalyst such ascalcium oxide prepared from natural resources has several advantages over homogeneous catalyst in terms of its wealth from waste materials, catalyst re-usability (cost effective), tolerance to high acidic nature of feedstock and eco-friendly in nature. Till date, research carried out worldwide on various heterogeneous catalysts derived from renewable materials (natural resources) such as Musa balbisiana Colla, waste shell of T. striatula, waste freshwater mussel shell etc.and utilized for biodiesel production from plant oils [1]. But the use of calcium oxide as heterogeneous catalyst from waste crab shell not experimented till date for simarouba glauca oil in transesterification reaction.

In this paper, an attempt has been taken to study the feasibility of using calcium oxide derived from natural resources to produce fatty acid methyl ester from non-conventional simarouba oil via a one-step alkali transesterification reaction followed by analysis of neat B100 as well as 5% blend in BS VI grade diesel fuel.

MATERIAL AND METHODS

Materials

The waste crab shells collected from local restaurant of Chilika lagoon area was washed to remove all organic matter and muds with tap water followed by distilled water. The washed waste crab shells were sun dried and then crushed to small pieces in mixture-grinder. Thepre-weight quantity was calcined in muffle furnace at 900 ºC for 2 hrs at the heating rate of 20°C/minute. After calcination, the floppy white shells powdered by mortar for fine particles. The ash obtained was utilized as heterogeneous CaO catalyst for the reaction. Simarouba glauca seeds werecollected, washed and sun dried before extraction of oil. The seeds were removed from the hard kernel and crushed to small pieces for oil extraction by soxhlet extractor by using hexane as solvent. The extracted oil was analyzed for various physico-chemical properties such as specific gravity, viscosity, acidity and fatty acid composition by GC as per standard methods.

Catalyst characterization

XRF technique was used to determine the chemical composition in percentage for the raw and calcined shells. X-ray Diffraction (XRD) analysis was carried out to identify the crystalline phases of catalyst by the instrument (Shiadzu, xrd-700, Japan) with the 2θ range of 10°-80° at a scanning speed of 2°/min and step size of 0.02° with X-ray source of CuKα (λ= 1.5406 A°). Scanning electron microscope (SEM) was employed to determine the morphology of the materials.

Transesterification reaction

Transesterification reaction was carried out by taking about 100 ml of simarouba oil with methanol and the prepared catalyst in a 500ml three necked round bottom flask fiited with a water cooled condenser. Reaction was carried out in

a constant temperature bath of 60±5°C on heating cum magnetic stirrer system. The reaction was carried out in optimized condition such as 6% w/w catalyst doses, 1:12 molar ratio of oil to methanol, 2 hrs time period and 600rpm stirring speed. After reaction period, the FAME and glycerol were separated in two different layers in separating funnel. The glycerol portion was discarded and FAME portion analyzed as per standard specifications.

RESULTS AND DISCUSSION

Thermogravimetry is a technique to measure the change in weight of a material as a function of temperature and the experimental curves of CS1 catalyst is presented in Fig.1. The major decomposition started at a temperature of about 600°C and completed before 800°C. The weight loss observed within the temperature range of 500-800°C is about 61%. This endothermic event is related to the thermal decomposition process of $CaCO_3$ containing crab shells leading to the formation of CaO and CO_2. Therefore, the TGA profile shows that the calcination temperature of crab shells should be more than 800°C for 1 hr. It is also confirmed from XRD data.

The results of XRD analysis of CS1 catalyst was obtained with main peak at 2θ =37.423, d=2.40116 and hkl=200, this peak is the characteristic of calcium oxide. The particle size of the crystalline catalyst was also calculated from the XRD data using Scherrer's formula, $D= 0.89\lambda/\beta \cos\theta$. The particle size of calcined crab shell is 0.42nm. The presence of CaO indicated by other diffraction peaks of 2θ at 32.263, 53.945, 64.248, 67.461 and 79.730. this shows that complete conversion of $CaCO_3$ to CaO happened in material at calcinations temperature of 900^0C. X-Ray fluorescence (XRF) technique was used for determining the chemical compositions of raw and calcined crab shells. The raw material contains calcium carbonate about 92% by mass and on calcination at 900^0C, it is converted to CaO with 96% along with other metal oxides in traces.

The morphology of raw and calcined shells (CS1) was investigated by SEM at equal magnification of 500x are shown in Fig 3a and 3b respectively. SEM image shows that, the raw crab shell had a generally irregular crystal structure as shown in fig-3a, upon calcination, the morphology of CS has changed from bulky substances without any clear pores on its surface to porous particles of various sizes and shapes with higher surface area. This porosity is perhaps due to the fact that a large number of gaseous water molecules are released upon the decomposition of $CaCO_3$. Similar observations are also noticed by Gendy et al., The biodiesel produced by utilizing synthesized catalyst (CS1) with optimized reaction conditions and the FAME (B100) was tested as per IS 15607 specifications. The B100 blended with BSVI Diesel fuel in 5%v/v doses (B5) and typical parameters performed.The test results of neat B100 and B5 sample are shown in table-1.

CONCLUSION

Based on thefindings, it is concluded that the wastecurb shells analyzed for its suitability in transesterification of non-edible oil to produce biodiesel for blending with BSVI grade diesel fuel for better results w.r.t pollution reduction by automobiles and improve the economic standards. The waste crab shell act as a heterogeneous catalyst with promising source of calcium oxide content of > 95% as confirmed by XRF and XRD techniques and biodiesel yield of more than 96% in optimum condition. This catalyst is biodegradable, environmentally benign and is value addition to the waste generated in the society. This waste to wealth material can reduce the cost of biodiesel production and can acts a potential feedstock for industrial applications.

REFERENCES

1. P. L. Boey, G. P. Maniam, S. A. Hamid and D. M. H. Ali, Utilization of Waste Cockle Shell (Anadara Granosa) in Biodiesel Production from Palm Olein: Optimization using Response Surface Methodol. Fuel, 90, 2353-2358 (2011).

2. J. Boro, A. J. Thakur and D. Deka, Solid Oxide Derived from Waste Shells of Turbonilla Striatula as a Renewable Catalyst for Biodiesel Production, Fuel Process, Technol., 92, 2061-2067 (2011).
3. Boey P.L., Maniam G.P., Hamid S.A., Biodiesel production via transesterification of palm olein using waste mud crab (*Scylla serrata*) shell as a heterogeneous catalyst, *Bioresour.Technol*., 100, 6362-6368 (2009).
4. Birla A., Singh B., Upadhyay S.N., Sharma Y.C., Kinetics studies of synthesis of biodiesel from waste frying oil using a heterogeneous catalyst derived from snail shell, *Bioresour.Technol*., 106, 95-100 (2012)
5. N. Nakatani, H. Takamori, K. Takeda, and H. Sakugawa, Transesterification of soybean oil using combusted oyster shell waste as a catalyst, *Bioresour Technol*, **100**, 1510–1513, (2009).
6. M. Nurhayati and W. Utami, in *Mollusk Shell Waste of Anadara Granosa as aheterogeneousCatalyst for the Production of Biodiesel*, 36–39, Prosiding SeminarNasional Kimia UGMYokyakarta, W., 2013.
7. Nur Syakirah Talha, Sarina Sulaiman , Overview of catalysts in biodiesel production, ARPN Journal of Engineering and Applied Sciences, vol.11, No.1, January 2016.
8. Ngoya Tshizanga, Elizabeth Funmilayo Aransiole, Optimisation of biodiesel production from waste vegetable oil and eggshell ash, South African journal of chemical engineering, Elsevier, 23(2017),145-156
9. Chakraborty, R., Bepari, S., Banerjee, A., 2010. Transesterification of soybean oil catalyzed by fly ash and egg shell derived solid catalysts. Chem. Eng. J. Elsevier 165, 798-805.
10. Basumatary Sanjay, Heterogeneous catalyst derived from natural resources for biodiesel production: a review, Research journal of chemical sciences vol.3(6),95-101, june(2013).

Table. 1. Physico-chemical data of biodiesel (B100) and B5 blend

			Biodiesel (B100)		B5 Blend	
S.No.	**Properties**	**Test Methods**	**IS:15607-2016 specification**	**Biodiesel (FAME)**	**IS1460-2017 specification (BS VI)**	**B5 Data**
1	Appearance & color	Visual	To report	Clear yellow liquid	To report	To report
2	Density@15°C,g/cc	D4052	0.860-0.900	0.870	0.810-0.845	0.835
3	Kin.Viscosity @40°C,cSt	D445	3.5 – 5.0	4.836	2.0-4.5	2.712
4	Acid Value. mgKOH/g, Max.	D 974	0.50	0.15	0.20	0.005
5	Ester content,% by mass, Min.	GC	96.5	97.5	--	-
6	Pour Point,°C, max	D97	+10	12	15	-9
7	Cold Filter Plugging Point (CFPP),° C, max.	D6371	0	-3	15	-6
8	Flash Point,°C (PMCC),min.	D93	101	164	35	58.0
9	Total ulphur content, ppm	ISO13032	--	0	10	8

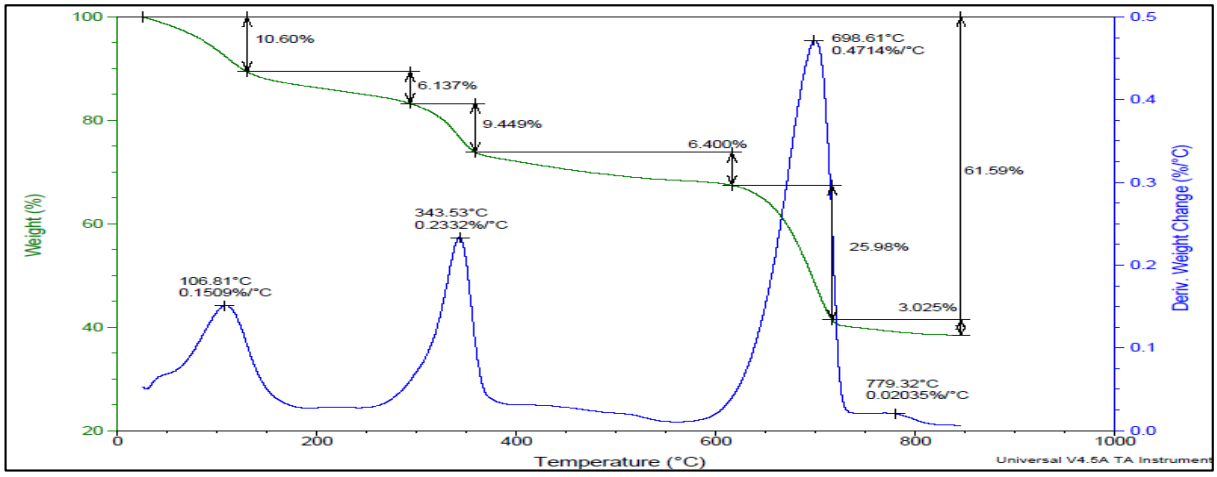

Fig. 1. TGA curve of raw material

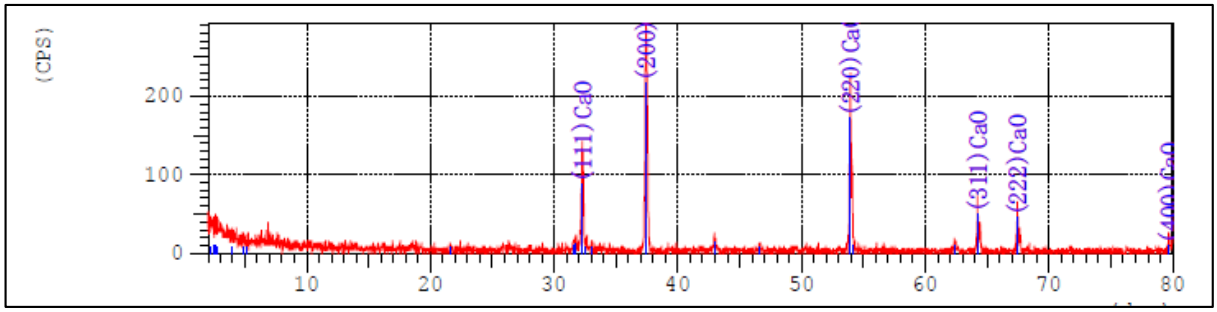

Fig. 2. XRD peaks of calcined crab shell

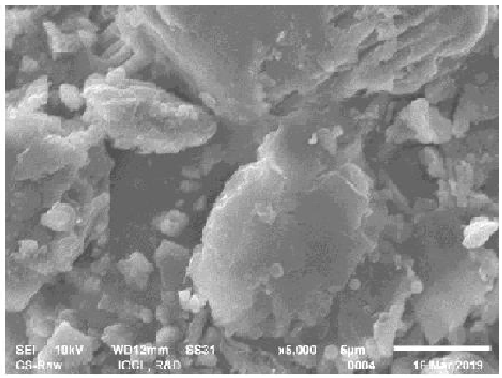

Fig 3a: SEM image of raw shell

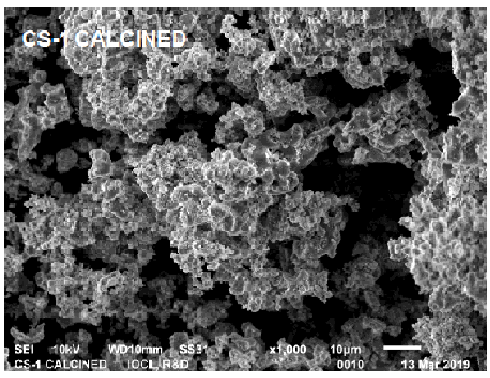

Fig 3b: SEM image of calcined shell

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RESEARCH ARTICLE

Effect of Zn Levels in Combination with Microbial Inoculation on Soil Exchangeable Ca and Mg and Their Uptake by Maize

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ABSTRACT

Field experiment was conducted in black soil in order to study the secondary nutrients availability such as calcium and magnesium and their uptake in black soil by using of Arbuscular Mycorrhizal (AM) fungi and Zinc Solubilizing Bacteria (ZSB) in combination with graded levels of $ZnSO_4$. Treatment consisted of two factors *viz.,* microbial inoculation (M_1: control, M_2: AM fungi, M_3: ZSB and M_4: M_2+M_3) and graded levels of $ZnSO_4$ (S_1: 0, S_2: 12.5, S_3: 25, S_4: 37.5, S_5: 50 Kg ha $^{-1}$ and S_6: 0.5% foliar spray @ 45 and 65 DAS) replicated three times in RBD. The results revealed that the exchangeable Ca and Mg content of soil were positively influenced by the microbial inoculation as well as the graded doses of $ZnSO_4$. These contents were decreased with the advancement of crop growth. Application of AM fungi + ZSB (M_4) had highest uptake of Ca and Mg by maize grain and stover. Graded levels of Zn application enhanced the Ca and Mg uptake correspondingly. However, the increase was not significant beyond 25 kg of $ZnSO_4$ ha^{-1}.

Keywords: Maize, AM fungi, Zinc Solubilizing Bacteria and Ca and Mg uptake.

INTRODUCTION

In India, maize is grown in a wide range of environments, extending from extreme semiarid to sub humid and humid regions. It is grown in about 9.09 M ha (Anonymous, 2014) and utilized for versatile purposes. It is a main source of calories and minerals for most rural populations (Suganya *et al.,* 2019). Calcium is the fifth most abundant element in

the earth's crust. Calcium is a non-toxic mineral nutrient, even in high concentrations and is very effective in detoxifying high concentrations of other mineral elements in plants. The metalloenzyme, amylase has calcium as metal. Calcium is major cation in the middle lamella of cell walls, of which calcium pectate is the principle constituent. Thus, calcium provides mechanical strength to tissues, enhancing cell division and plant growth, protein synthesis, carbohydrate movement and balancing cell acidity Calcium is not mobile in plants. It does not easily move from old leaves to young leaves. Calcium also has a positive effect on soil properties. This nutrient improves soil structure thereby increasing water penetration, and providing a more favorable soil environment for growth of plant roots and soil microorganisms. Magnesium is an essential constituent of chlorophyll in plants. Therefore, it is essential for photosynthesis. Magnesium is also the most common activator of enzymes concerned with energy metabolism. The functions of magnesium in plants are related to its mobility within the cells. As might be expected, plants that are deficient in Mg have an overall light green color. In maize, the veins are mainly white when concentrations are inadequate.

Indian soils are generally low in organic matter and have low cation exchange capacity. Being cations, both calcium and magnesium are subjected to cation exchange. Soils usually contain less magnesium than calcium because magnesium are not adsorbed as strongly by clay and organic matter as calcium and further magnesium are more susceptible to leaching than calcium. The importance of calcium and magnesium as plant nutrients is more realized in acid and alkali soils than in neutral soils. In alkaline soils, crops suffer due to lack of calcium and excess of sodium. The experimental soil is moderately alkali, the application of microbial inoculation like zinc solubilizing bacteria and AM fungi increased the availability of calcium and magnesium. Zinc solubilizing bacteria produce gluconic acid (Saravanan *et al*., 2007), it's solubilize native basic cations such as Ca and Mg in the soils. AM fungi mobilize the solubilized the native basic cations viz., Ca and Mg through the external mycelium (Ameeta and Savitha, 2013; Suganya and Saravanan, 2016). Keeping these in view the present study in contemplated to study the soil availability of secondary nutrients such as exchangeable Ca and Mg and their uptake by maize.

MATERIALS AND METHODS

Experimental soil

The field experiment was conducted at Cotton Research Station (TNAU), Perambalur. The soil was clay, deep black in colour belonging to *Typic Chromustert* (Peelamedu series). The initial soil was moderately alkaline, non saline, medium in organic status and low, low and medium in available N, P and K, respectively. The exchangeable Ca content was 27.5 cmol (p^+) kg^{-1} and exchangeable Mg content was 10.5 cmol (p^+) kg^{-1}. With regards to the available micronutrient status of the soil, DTPA-Fe, DTPA-Cu and DTPA-Mn were found to be in sufficient level but the availability of DTPA-Zn was found to be deficient.

Field experiments

Treatment consisted two factors *viz.,* microbial inoculation (M_1: control, M_2: AM fungi, M_3: ZSB and M_4: M_2+M_3) and graded levels of $ZnSO_4$ (S_1: 0, S_2: 12.5, S_3: 25, S_4: 37.5, S_5: 50 Kg ha^{-1} and S_6:0.5% foliar spray @ 45 and 65 DAS) replicated three times in RBD. Seeds of maize hybrids (NK 6240) were sown on the sides of the ridges by adopting a spacing of 60 x 25 cm along with vermiculite based mycorrhizal inoculum (*Glomus intraradices* TNAU-03-08) 2 g per hill at a depth of 5 cm. Zinc solubilizing bacteria was applied at 2 kg ha^{-1} after mixing it with 25 kg each of sand and farm yard manure. The recommended fertilizer prescription for maize *viz.,* 250:75:75 kg N, P_2O_5 and K_2O kg ha^{-1} was followed. The full dose of P and K were applied basally and N was applied at three splits *viz.,* basal (25%), vegetative (50%) and tasselling (25%) stages. Calculated quantities of $ZnSO_4$ were applied basally as per the treatment schedule. The soil and plant samples were drawn at vegetative (30^{th}), tasselling (60^{th}) and harvest stage (110^{th}) and analysed for Exchangeable Ca & Mg and their content respectively in soil and plant samples. The grain and stover yield were recorded treatment wise and Ca and Mg uptake were computed.

RESULT AND DISCUSSION

Soil exchangeable Ca and Mg

The soil exchangeable Ca and Mg content was found to be gradually reduced from the vegetative stage to the harvest stage and this reduction might be due to the crop removal and other transformation in soil (Table 2 & 3). A proportionate increase in soil exchangeable Ca and Mg content was noticed by the application of microbial inoculations combinations with Zn levels. During the crop growth, both the Ca and Mg concentrations were found decreased in all treatments at the growth stages advanced. Crop uptake, adsorption on clay complex and leaching could be attributed for the decrease in Ca and Mg contents of the soils. Application of AM fungi + ZSB (M_4) recorded highest exchangeable Ca and Mg than the other microbial inoculations (M_2) and (M_3) under both the soils. The reason attributed could be the microbial inoculation would have contributed to the acidification and solubilization of insoluble cations. Among the graded levels of Zn application, S_5 (50 kg of $ZnSO_4$ ha^{-1}) recorded highest exchangeable Ca and Mg in both the soils.

Ca and Mg uptake by Maize grain

The Ca and Mg uptake by maize grain was increased to the tune of 46.90% and 43.65% respectively over control (Table 4 & 5). The results corroborate with the findings of Suganya and Saravanan (2016), who reported the increased nutrient uptake due to Zinc solubilizing bacteria produce gluconic acid, its solubilize native basic cations such as Ca and Mg in the soils. AM fungi mobilize the solubilized the native basic cations viz., Ca and Mg through the external mycelium. Both mechanisms increased the availability of exchangeable Ca and Mg in soil. Hence, it's lead to increase the total uptake of Ca and Mg. From the table 6 and 7, it may be seen that interaction between microbial inoculation of AM fungus (M_2), Zinc Solubilizing Bacteria (M_3) and their combinations (M_4) under graded levels of $ZnSO_4$ application increased the Ca and Mg uptake in Maize crop grown in black soil. This reasoning is in agreement with the report of Suganya and Saravanan (2016). The uptake of a nutrient by the plant depends on two factors *viz*., the nutrients supply from the soil which is being reflected on the concentration of nutrient in different plant parts and the second being the dry matter accumulation. These two factors are mutually interdependent, one factor influencing the other and depending on the stage of crop, when the plants are younger, there will be sufficient supply of nutrients from the soil, some times more than the requirement and the plant absorb and grow in proportion to the nutrient supply and absorption. However, as the crop grows and accumulates dry matter, the nutrients supply may be sufficient or may not be equal to the rate at which the dry matter accumulates and hence the concentration of nutrients get diluted as the crop grows and attain maturity. In any case, the nutrient absorption from soil solution is a continuous phenomenon and any factor which influences the growth, dry matter accumulation and yield of crop is bound to influence the nutrient uptake pattern. Hence, the uptake values of crop can be directly linked to the growth and yield of a crop on one hand and the nutrient depletion from the soil on the other hand. Hence, the uptake Calcium by the plant as a whole at important physiological stages was computed from the concentration of nutrients and yield.

REFERENCES

1. Anonymous. 2014. Annual report 2013-2014. Department of agriculture and cooperation, Ministry of agriculture, Government of India, Krishi bhawan , New Delhi P. 37.
2. Ameeta, S. and Y. Savitha. 2013. Review on role of VAM fungi in crop plant-soil system. International journal of Agricultural Science and Research, 3(1): 17-24
3. Saravanan, V.S., Kalaiarasan, P.M. Madhaiyan, Thangaraju, M. 2007. Solubilization of insoluble zinc compounds by *Gluconacetobacter diazotrophicus* and the detrimental action of zinc ion (Zn^{2+}) and zinc chelates on root knot nematode Meloidogyne incognita. Lett, Appl Microbiol. 44: 235–241.

4. Suganya, A and A. Saravanan. 2016. Effect of Zn levels in combination with microbial inoculation on soil exchangeable Ca and Mg and their uptake by maize. International journal of agricultural science and research, 6(2): 1-4.
5. Suganya, A., A. Saravanan, M. Basker, P. Pandiyarajan and R. Kavimani. 2019. Effect of Zinc and Microbial Inoculation on Soil Enzyme Activities for Maize (Zea mays L.) in Black Soil. International Journal of Current Microbiology and Applied Sciences, 8(8): 1804-1814.

Table 1. Initial soil characters

Taxonomy	***Typic Chromustert***
Textural class	Clay
pH (1:2.5 soil : water)	8.34
EC (dS m^{-1})	0.30
Free $CaCO_3$ (g kg^{-1})	90.4
Organic Carbon (g kg^{-1})	5.10
CEC (c mol (p^+) kg^{-1})	36.2
Alkaline $KMnO_4$- N (kg ha^{-1})	221
Olsen- P (kg ha^{-1})	20.0
Neutral N NH_4Oac- K (kg ha^{-1})	245
Exchangeable Ca (c mol (p^+) kg^{-1})	27.50
Exchangeable Mg (c mol (p^+) kg^{-1})	10.50
DTPA Zn (mg kg^{-1})	0.80
DTPA Cu (mg kg^{-1})	2.52
DTPA Fe (mg kg^{-1})	10.2
DTPA Mn (mg kg^{-1})	3.45

Table 2. Graded levels of Zn with Arbuscular Mycorrhizal Fungi and Zinc Solubilizing Bacteria on Soil Exchangeable Ca (cmol (p^+) kg^{-1})

Treatments	Vegetative stage							Tasselling stage						
	S_1	S_2	S_3	S_4	S_5	S_6	Mean	S_1	S_2	S_3	S_4	S_5	S_6	Mean
M_1	17.53	18.34	19.14	19.56	19.69	17.73	**18.66**	17.33	18.12	18.20	19.06	19.14	17.73	**18.40**
M_2	18.06	18.68	19.83	19.84	19.93	18.19	**19.09**	17.74	18.27	19.05	19.18	19.43	18.01	**18.61**
M_3	18.29	18.74	19.39	19.57	19.80	18.31	**19.02**	17.99	18.22	19.26	19.37	19.42	18.11	**18.73**
M_4	18.66	19.18	19.38	19.17	19.35	18.68	**19.07**	18.33	19.03	19.25	19.63	19.70	18.19	**19.02**
Mean	**18.13**	**18.73**	**19.43**	**19.53**	**19.69**	**18.22**	**18.96**	**17.85**	**18.41**	**19.14**	**19.31**	**19.42**	**18.01**	**18.69**
		SEd		CD (0.05)					SEd		CD (0.05)			
M		0.08		0.16					0.07		0.14			
S		0.10		0.20					0.09		0.17			
M X S		0.19		0.40					0.17		NS			

Treatments	Harvest stage						
	S_1	S_2	S_3	S_4	S_5	S_6	Mean
M_1	17.05	17.91	18.66	19.18	19.20	17.08	**18.18**
M_2	17.45	18.08	18.79	19.46	19.53	17.45	**18.46**
M_3	17.78	18.21	19.71	19.74	19.79	17.79	**18.84**
M_4	18.18	18.77	19.30	19.34	19.42	18.19	**18.87**
Mean	**17.62**	**18.24**	**19.11**	**19.43**	**19.48**	**17.63**	**18.58**
		SEd		CD (0.05)			
M		0.11		0.23			
S		0.14		0.28			
M X S		1.19		NS			

Table 3. Graded levels of Zn with Arbuscular Mycorrhizal Fungi and Zinc Solubilizing Bacteria on Soil Exchangeable Mg (cmol (p^+) kg^{-1})

Treatments	Vegetative stage							Tasselling stage						
	S_1	S_2	S_3	S_4	S_5	S_6	Mean	S_1	S_2	S_3	S_4	S_5	S_6	Mean
M_1	4.74	4.85	5.06	5.17	5.17	4.80	**4.96**	4.74	4.91	5.06	5.18	5.19	4.80	**4.98**
M_2	4.90	4.94	5.13	5.22	5.23	4.91	**5.05**	4.88	4.93	5.08	5.17	5.19	4.92	**5.03**
M_3	4.93	5.07	5.16	5.28	5.28	4.94	**5.11**	4.93	5.00	5.10	5.21	5.22	4.95	**5.07**
M_4	4.93	5.09	5.18	5.30	5.31	5.00	**5.13**	4.93	5.01	5.08	5.18	5.19	4.97	**5.06**
Mean	**4.87**	**4.99**	**5.13**	**5.24**	**5.25**	**4.91**	**5.06**	**4.87**	**4.96**	**5.08**	**5.18**	**5.19**	**4.91**	**5.03**
		SEd		CD (0.05)					SEd		CD (0.05)			
M		0.01		0.03					0.02		0.03			
S		0.02		0.03					0.02		0.04			
M X S		0.03		NS					0.04		NS			

Treatments	Harvest stage						
	S_1	S_2	S_3	S_4	S_5	S_6	Mean
M_1	4.71	4.90	5.00	5.17	5.18	4.72	**4.94**
M_2	4.85	4.92	5.05	5.17	5.18	4.91	**5.01**
M_3	4.92	4.99	5.08	5.17	5.18	4.84	**5.03**
M_4	4.92	5.00	5.08	5.16	5.18	4.95	**5.05**
Mean	**4.85**	**4.95**	**5.05**	**5.17**	**5.18**	**4.85**	**5.01**
		SEd		CD (0.05)			
M		0.02		0.03			
S		0.02		0.04			
M X S		0.04		0.08			

Table 4. Graded levels of Zn with Arbuscular Mycorrhizal Fungi and Zinc Solubilizing Bacteria on Calcium uptake (kg ha^{-1}) by grain and stover

Treatments	Grain Uptake							Stover Uptake						
	S_1	S_2	S_3	S_4	S_5	S_6	Mean	S_1	S_2	S_3	S_4	S_5	S_6	Mean
M_1	1.36	1.86	2.00	2.49	2.55	1.70	**1.99**	18.6	27.7	32.6	34.0	34.4	27.4	**29.1**
M_2	1.64	1.87	2.18	2.53	2.70	1.71	**2.10**	27.8	32.8	35.7	39.0	38.0	30.4	**34.0**
M_3	1.77	1.90	2.38	2.60	2.58	1.78	**2.16**	30.0	34.4	37.9	42.2	40.6	32.2	**36.2**
M_4	1.90	2.35	2.50	2.90	2.77	1.97	**2.40**	32.6	38.8	41.2	46.3	55.2	36.7	**40.1**
Mean	**1.67**	**1.99**	**2.27**	**2.63**	**2.65**	**1.79**	**2.16**	**27.3**	**33.4**	**36.8**	**40.4**	**39.5**	**31.7**	**34.9**
		SEd		CD (0.05)							SEd			
M		0.05		0.10							1.48			
S		0.10		0.20							1.86			
M X S		0.20		0.43							3.71			

Table 5. Graded levels of Zn with Arbuscular Mycorrhizal Fungi and Zinc Solubilizing Bacteria on Magnesium uptake (kg ha^{-1}) by grain and stover

Treatments	Grain uptake							Stover uptake						
	S_1	S_2	S_3	S_4	S_5	S_6	Mean	S_1	S_2	S_3	S_4	S_5	S_6	Mean
M_1	4.43	6.42	7.08	7.38	7.40	6.22	**6.49**	9.29	14.14	16.46	18.17	18.50	12.94	**14.91**
M_2	5.56	6.45	7.20	7.37	7.35	6.00	**6.65**	11.95	14.85	16.53	18.75	19.40	13.01	**15.75**
M_3	6.01	7.09	7.31	7.49	7.55	6.17	**6.93**	12.99	15.16	18.03	20.46	20.47	13.47	**16.76**
M_4	6.35	7.41	8.30	10.03	10.15	6.45	**8.11**	13.17	16.55	18.36	23.40	24.17	14.99	**18.44**
Mean	**5.59**	**6.84**	**7.47**	**8.07**	**8.11**	**6.21**	**7.05**	**11.85**	**15.18**	**17.34**	**20.19**	**20.63**	**13.60**	**16.47**
		SEd		CD (0.05)					SEd		CD (0.05)			
M		0.52		1.10					0.95		2.06			
S		0.62		1.31					1.20		2.52			
M X S		1.24		2.62					2.38		5.04			

Table 6. Graded levels of Zn with Arbuscular Mycorrhizal Fungi and Zinc Solubilizing Bacteria on total calcium uptake (kg ha^{-1})

Treatments	Vegetative stage							Tasselling stage						
	S_1	S_2	S_3	S_4	S_5	S_6	Mean	S_1	S_2	S_3	S_4	S_5	S_6	Mean
M_1	5.6	8.1	9.8	9.6	10.1	6.7	**8.3**	12.5	18.9	21.2	29.2	32.1	16.4	**21.7**
M_2	6.9	7.1	9.2	10.3	11.1	7.3	**8.6**	15.7	21.6	25.4	27.1	30.9	19.2	**23.3**
M_3	7.2	9.2	9.6	9.9	12.0	8.0	**9.3**	17.9	22.6	24.3	26.8	29.8	21.4	**23.8**
M_4	8.3	9.4	10.4	12.3	14.9	10.1	**10.9**	22.7	26.8	28.2	30.6	33.1	23.4	**27.4**
Mean	**7.0**	**8.4**	**9.7**	**10.5**	**12.0**	**8.0**	**9.3**	**17.2**	**22.5**	**24.8**	**28.4**	**31.5**	**20.1**	**24.1**
		SEd		CD (0.05)					SEd		CD (0.05)			
M		0.37		0.8					0.86		1.8			
S		0.45		0.9					1.06		2.2			
M X S		0.91		NS					2.11		NS			

Treatments	Harvest stage						
	S_1	S_2	S_3	S_4	S_5	S_6	Mean
M_1	20.0	29.5	34.6	36.5	36.9	29.1	**31.1**
M_2	29.4	34.7	37.9	41.6	40.7	32.1	**36.1**
M_3	31.8	36.3	40.3	44.8	43.2	33.9	**38.2**
M_4	34.5	41.2	43.7	49.2	47.9	38.6	**42.5**
Mean	**28.9**	**35.4**	**39.1**	**43.0**	**42.2**	**33.4**	**37.0**
		SEd		CD (0.05)			
M		1.48		3.1			
S		1.81		3.8			
M X S		3.44		7.6			

Table 7. Graded levels of Zn with Arbuscular Mycorrhizal Fungi and Zinc Solubilizing Bacteria on total magnesium uptake (kg ha^{-1})

Treatments	Vegetative stage							Tasselling stage						
	S_1	S_2	S_3	S_4	S_5	S_6	Mean	S_1	S_2	S_3	S_4	S_5	S_6	Mean
M_1	2.2	2.7	3.5	3.8	4.1	2.8	**3.2**	9.4	10.6	14.3	18.8	21.0	9.9	**14.0**
M_2	2.3	2.8	3.4	4.6	5.0	2.7	**3.4**	11.2	13.7	17.5	20.9	23.9	11.9	**16.5**
M_3	2.8	3.4	4.1	4.4	5.4	3.2	**3.9**	12.2	15.6	16.4	20.4	22.7	13.9	**16.9**
M_4	3.4	4.0	5.0	5.7	6.9	3.8	**4.8**	15.2	19.7	23.4	27.1	29.4	18.4	**22.2**
Mean	**2.7**	**3.2**	**4.0**	**4.6**	**5.3**	**3.1**	**3.8**	**12.0**	**14.9**	**17.9**	**21.8**	**24.2**	**13.5**	**17.4**
		SEd		CD (0.05)					SEd		CD (0.05)			
M		0.21		0.4					0.85		1.8			
S		0.26		0.5					1.04		2.2			
M X S		0.51		NS					2.08		NS			

Treatments	Harvest stage						
	S_1	S_2	S_3	S_4	S_5	S_6	Mean
M_1	13.7	20.6	23.5	25.6	25.9	19.2	**21.4**
M_2	17.5	21.3	23.7	26.1	26.7	19.0	**22.4**
M_3	19.0	22.3	25.3	28.0	28.0	19.6	**23.7**
M_4	19.5	24.0	26.7	33.4	34.3	21.4	**26.6**
Mean	**17.4**	**22.0**	**24.8**	**28.3**	**28.7**	**19.8**	**23.5**
		SEd		CD (0.05)			
M		1.14		2.4			
S		1.43		3.0			
M X S		2.76		5.8			

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REVIEW ARTICLE

A Review on Ethnobotanical Research on Mayurbhanj District of Odisha: An Advance Step, After Primitive

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ABSTRACT

Now a day the development of traditional medicine system remains high. About 64% of total global population depend on traditional medicines. Mayurbhanj, is a hilly district, rich in ethnobotanical plants. Due to poor condition of modern healthcare facilities and poverty, indigenous people of the district fully or partially dependent on local medicinal plants. This paper deals with the study of various medicinal plants belongs to many genera and families are employed ethnomedicinally by the people in rural areas of Mayurbhanj district of Odisha. An attempt has been made to document traditional knowledge from that particular area of Mayurbhanj on the treatment of various diseases.

Keywords: Diseases, Ethnobotanical, Mayurbhanj, Traditional, Treatment.

INTRODUCTION

A review is a fundamental or scholar paper which contains the present knowledge involving the research fields, theoretical data and methodological contribution to a specific topic. Reviews are the secondary facts which do not have any kind of real experimental study. It is very much necessary to provide a proper direction to a researcher for a fundamental research work to reach a successful and meaningful result. A review contains the history, morphology, status and conservation of medicinal plants found in the forests of Odisha. Since a long period of time, medicinal plants have been used in human healthcare. Some experimental studies have been carried for the actual worth of the findings which leads to the production of plant based medicines. The value of the medicinal plant products in front of the global market is 100 billion per annum (Sofowora et al., 2013). Human society is completely depends upon the plants for food to shelter and also use them as medicines for various diseases. Nearly the human beings from every

corner of the world use plant parts as medicine and accumulate knowledge about them. That knowledge has been collected and represented orally and as evidence from one to other and generation to generation. The human being lives near to the natural environment are known through various names such as, Aboriginals, Jungle people or Hill tribe, etc. They bear vast knowledge about plants and plant parts which they apply over various diseases on their daily life. This knowledge is applicable for the production of various modern products, which is a method to learn about the plants. The ethonobotanical study provides fundamental information for the production of new drugs, food, pesticides, natural products, gene resources and chemicals (Prusti and Behera, 2007).

India is rich in bio diversity. According to the vegetation of India, variation can be observed in any particular area; it may be from the cultural heritages to the religious beliefs and from diverse climatic conditions to the growth of fauna and fauna. A report estimated by the World Medicine Situation 2011 that 70-95% population in developing countries use Traditional Medicine (TM) (Lu et al., 2011). Uses of Traditional Medicine (TM), plays an important role to fulfill the healthcare need in many cases. The Indian traditional Medicine(TM) systematically divided into several branches like; Ayurveda, Unani and sidddha, during 18th century by British; such as the homeopathic, allopathic and some chronic diseases also get practiced for the cure of several health issues (Kumar and Dua, 2016).For the core regions and for the people away from the convenient lifestyle and modern medicinal practices, the use of ethnomedicine is a most useful part. Human beings are also born with no knowledge on how to survive in their surrounding environment like other species exit in this earth and few instinct that would help them to survive during their first few days but much less later (Korkora and Nayak, 2018). As we know a society form from community and community form from families and a healthy family form from a healthy and strong mother. Ethnomedicinal use helps to maintain the primary health care of a mother, which plays a vital role on the formation of a healthy society. Now a days the social scientists are giving more interest in Ethnomedicinal studies due to the increase knowledge of life and culture of tribal communities. Belongingthe tribal and rural communities of India, many works has been reported from the current study (Bhadra and Tirkey, 1997; Choudhury, 2000).

Why we need ethnomedicines?

All system of medicines is form from ethnomedicine. Human being has acquired knowledge on health, preventive, promotive and curative properties of plant and animal products, minerals, etc., over thousands of years, through their experiment in their environment (Sen and Behera, 2017). According to World Health Organization, due to the lack of modern health care, about 65-80% of world's population in developing countries depend upon traditional medicines to fulfill their primary healthcare (Khan and Yadav, 2010; Calixto, 2013). India is a developing country, so still there are some regions away from all the technological facilities and where ethnomedicine is the only source for healthcare need. The rural and undeveloped areas of India are known to supply a very large proportion of medicinal plant requirements, such as; 33% of Allopathic drugs, 46% of Unani drugs and 80% of Ayurvedic drugs. The degradation of practice on botanical healing in the mid 90's, results the industrial and development of advanced allopathic medicine. The interest in the therapeutic use of natural products has been raising before last two years and the medicinal properties of plants in different parts of this world, now a days the scientific community also throwing interest about the ethnobotanical information in medicinal plants (Ayyanar, 2013; Yahia, 2014).

The herbal medicine get highest interest due to create awareness of limited sector of pharmaceutical products to control major disease and also the reason is that, the ethnomedicines are more safe, more accessible and more affordable than the pharmaceutical drugsbecause of the noticeable increase and irreversible reaction of modern drugs (Parekh and Chanda, 2006). All the Ethnomedicinal properties were derived from the plant, either by plant parts or by mixtures. The benefit of having plant medicines is that, are provide therapeutic benefits and are easily affordable in terms of treatment. Before 5000 B.C. the key of Indian traditional medicine system has been traced (Sharma and Sharma, 2013). Basic knowledge of the people in a locality or particular area has developed over time and based upon experience, practices, adapted to that locality and culture and also experienced by communities and individuals (Mohanty *et al.,* 2015). Folklore and folk sayings are the methods of spreading Indigenous knowledge orally from

generation to generation (Agarwal, 1981; Singh et al., 2002). Also the indigenous knowledge also helps in the establishment of a complex wealth of knowledge and skills (Cotton, 1996; Ballick and Cox, 1996). Through the surviving tradition, the intimate relationship between human beings and plants has been passed to us (Jain, 1968).

The knowledge about the traditional medicine has been passed from generation to generation through oral communication. So, now with the passage of time and development of modern healthcare techniques, the traditional knowledge is under serious threat. The local traditional knowledge helps a lot to documenting, analyzing and publicizing the knowledge on the interaction between biodiversity and human society and for the permanent use of traditional medicines before it lost &conservation of knowledge, documentation of indigenous knowledge of native people through ethnobotanical studies is much more important (Mallick and Mahana, 2018). Since the beginning of the human life in this earth, plants have been used as medicines for its curative value. From thousands of years nature has been regarded as the source of medicine and a huge number of drugs have been isolated from the natural sources. The traditional medicine system plays a key role in the health care system and also 80% of the world population mainly depends upon the traditional medicine for their primary health care (Owoabi et al., 2007).

Some medicinal plant contains inherent active ingredient which can be used to relieve pain (Okigbo et al., 2008). In many developing countries the medicinal plant parts, mixture and traditional medicine are used as therapeutic agent for maintain good health (UNESCO, 1996). Many synthetic analogues form from prototype compounds isolated from plants and also the modern pharmaceutical drugs still contain at least 25% compounds obtained from plants (Lucy and Edgar, 1999). Due to the rising cost of the prescribed drugs in the maintenance of individual health and the bio prospecting of new plant derived drugs, the special interest on medicinal plants as a re-emerging health aid has been powered. The continuously growing identification of medicinal plant is the result of developing faith of herbal medicine (Kala, 2015). The antioxidant, antipyretic anti microbial effects of the phytochemicals in plants are responsible for the therapeutic properties of plant and the plants also produce some bio active compounds mostly used as medicines for various systems of animals including man and act as an inhibitor for metabolism of microbes infection and the microbes may be pathogenic or symbiotic (Cowman, 1999; and Adesokan et al., 2008). The reorganization of bioactive compounds in medicinal plants, their isolation, purification and characterization of active ingredients in crude extract by different analytical methods is important and these compounds play a regulating role in determining host-microbe interaction in favor of the host.

The medicinal plants would be the best source to produce a variety of pharmaceutical drugs, according to the World Health Organization. For the better understand of their properties, safety and potency, such medicinal plants should be explored (Nascimento et al., 2000). Over harvesting of some selected high value medicinal plant populations is the result of immediate rising demand of plant based drugs (Nautiyal et al., 2002). Some medicinal plant species are more prone to extinction because of their slow growth rate, low population density and narrow geographic ranges (Jablanski, 2004).

A lot of newly s lot of newly synthesized drugs originate from natural plant products (Vuorelaa et al., 2004). All parts of medicinal plant and the knowledge about medicinal plants are collected by local and folk communities all over the world and normally these are collected in low quantities (Uniyal et al., 2006). Some plant parts are also collected in large quantities, which are supplied to the market as the raw material for herbal medicines or pharmaceutical drugs. Since ancient times, nature has been regarded as the origin of the curative agent. Because of the side effects herbal medicine have collected high reputation all over the world. For traditional & modern medicines, nutritive supplements, pharmaceutical products and chemical entities for synthetic drugs, medicinal plants are used as the bio resource (Ncube et al., 2004). Natural products are the reservoir of potential drug substance, that's why there has been an advanced of interest in the study of natural products, since past decade (Joshi et al., 2004). Approximately 85% of new compounds of medicinal action have been discovered from all over India and it is so effective from plant based sources. Ayurveda, Siddha and Homeopathy, are the three major traditional medicinal systems of India, depend completely on plants for conventional formulations. According to the World Health Organization (WHO),

the total international herbal drug marker as US $62 billion and likely rise to US $5 trillion around the year 2050. In case of India, Ayurveda alone accounts for rate Rs. 3500 cores (US $ 813 million) to the internal market annually. Some plants have the potential to treat many diseases which are referred to as medicinal plants. To observe the diversity of medicinal plants for further utility and conservation is the main aim for their study. Phytochemical, pharmacognostical, pharmacological and clinical research are based on the active principle of ethnobotanical database which also serves as a base for new compounds. So the cultivation and maintenance of threatened plant is mostly needed (Madharia and Jahan, 2015). Koraput contains maximum number of sacred grooves, where only little scientific documentation of plant species in them is done. Nearly 94 scared plant species distributed in 63 genera be owned to 43 different families from 6 different groves of floral diversity have been documented in a systematic manner, which are use as medicinal. Out of all the grooves contain 48 trees, 26 shrubs & 21 herbs. According to the tribal, some plant species are used as herbal medicines (39%), some bears religious important (23%) and some are food plants (13%). And some plants under threat categories have also been recorded (Panda *et al.,* 2014).

A variety of local flora, explore, identification, ethnobotany and conservation of wild and cultivated plant species are there in the city of Rourkela, Odisha, India. Along with the botanical name, vernacular name, family and habitat, a total of 154 plant species under 128 genera and 55 families were identified. Out of the 154 plant species 53 are medicinal, 44 are ornamental and 32 are edible, where 24 are weeds. To preserve and maintain the floral diversity of Rourkela, proper conservation planning is needed, which support the traditional knowledge of plants. For the conservation of plant diversity and environmental survey along with potential applications in drug discovery and oriental medicine, the documentation of all useful floras with ethnobotanical properties is important (Kumar et al., 2018). Pregnancy is a very sensible phase of a women's life, including a several pregnancy related problems like nausea, vomiting, constipation, heartburn, etc. With the help of ethnomedicine we are able to study the health of women during pregnancy, child birth & postpartum period, under maternal healthcare (Mainasara et al., 2017). According to the ancient knowledge in many rural areas in the world, some plant parts play significant role as medicine during pregnancy, child birth and postpartum phase. It was found that out of the total maternal mortality, 99% belongs to the developing countries where as 830 women die due to some problems related to pregnancy and child birth, according to a study in 2018. The health of the child can be affected because of the mother having problems due to several factors like mental stress, emotional weakness, physical problems, etc. and the major problems are malnutrition status like anemia and menstrual irregularities. For all the problems, herbal treatment can effective or can give batter result. Women shoulder all the responsibilities in the home, so they should provided with all the mental and physical care (Sethuraman et al., 2006).

Due the medicinal properties human beings always depend upon the plant from ancient age and the use of plant accordingly varies globally from one to another culture (Simpson, 1995). From thousands of years the traditional knowledge about plant medicines providing remedies to our society and it still continuing with new remedies. The medicinal plant therapy is not like any other erroneous branch of medicinal therapy, because the medicinal plant therapy is based on the empirical findings of hundreds and probably thousands of years of use (Tekwu et al., 2013). Regarding these days, plant medicines gaining very much attention from the scientists and pharmaceutical industries for the formation of advanced, effective and fine drugs for our day to day life (Dogruoz et al., 2008; Samee et al., 2009 and Karsha and Lakshmi, 2010).The use of plants as medicines have been learn by predisposition, observing the activities if birds, animals, etc. but if the wild plants were planted in herbal gardens, which are collected from the wild locality for the preparation of medicines, then the pharmaceutical companies can synthesized them chemically by isolating bioactive compounds from them. Slowly over study and trails human recognize that which plant and plant proportion serves the best and this knowledge also helpful for animals as like human beings (Mokwala, 2007).
Now a day 50% of all the synthetic drugs in the world are related to the natural products and their derivatives, over which half of the total value is contributed by the higher plants (Farnsworth *et al.,* 1985; Cragg and Newman, 2005). In case of traditional medicine mainly the crude form like- tinctures, teas, poultices, powers and other formulations are used (Balick and Cox, 1996). The knowledge or information about traditional medicine are generally collected from folk talks and recorded in herbal pharmacopoeias (Balunas and Kinghorn, 2005). To understand the tradition of

ancient knowledge, the concept of impurities and cleansing are essential & the imbalance between these elements is commonly known as the cause of illness and the goal of treatment is to restore balance with in the body (Magner, 1992). Symptoms and the pattern of imbalance detected by the professionals, in case of certain diseases and the ailments being treated (Kapoor, 1990; Padua et al., 1999).

Plants used in ethnomedicine

Plants are like blessings to human beings. Directly or indirectly we always depend upon plants to fulfill our basic needs in every sector. The medicinal property of plants is much more beneficial to cure human disease as well as animal also. All the plant parts, it may be the leaves, bark, fruit, flower, etc. are used to cure disease through various methods. Sometimes the leaves are dried and sometimes the juice of leaves used as medicine. Such as sometimes the direct fruit and also the fruit powders are used. Mainly the rural people are depending upon plants or plant parts to fulfill their medicinal need. The plants contains the properties like Phytohormones, antioxidant compounds are highly useful, which is unknown by the rural people. So using that traditional knowledge modern healthcare system establishes the synthetic medicines for human benefit. Some scientists start collecting these knowledges against various critical diseases like- diabetes, jaundices, Diarrhoea, etc.,

CONCLUSION

Medicinal plants have been a wellspring of wide assortment of naturally active compounds for a long time and utilized broadly as unrefined material or as pure compounds for curing different ailment. Plant-based products have been perceived for a long time as a wellspring of restorative specialists. These have assumed an indispensable role in the disclosure of new compound for drug discovery. There is a developing rise sought after for natural and other traditional remedies for treating different diseases among various communities through the world. Detailed study of medicinal plants is required for the revelation and improvement of novel bioactive compound that would help in diminishing human sufferings.

REFERENCES

1. Adesokan, A. A, Yakubu, M. T, Owoyele, B.V., Akanji, M. A., Soldoye, A. & Lawal, O. K. (2008).Effect of administration of aqueous and ethanolic extracts of *Enantia chlorantha* stem bark on brewer's yeast induced pyresis in rats. African Journal of Biochemistry Researh, 2(7),145e150.
2. Agarwl, S. R. (1981).Trees, flowers and fruits in Indian folk songs, folk proverbs and folktales. Glimpses of Indian Ethnobotany, 2,3e12.
3. Ayyanar, M. (2013).Traditional Herbal Medicines for Primary Healthcare among Indigenous people in Tamil Nadu, India. Journal of Traditional Medicine and clinical Naturopathy, 2,140.
4. Balick, M. J. & Cox, P.A. (1996).Plants, People, and Culture: the Science of ethnobotany. American library, 209e219.
5. Bhadra, R. K. & Tirkey, J. B. (1997).Health culture practices in rural society. Case studies of castes and tribes of North Bengal. "Health Studies in Anthropology, Department of Anthropology, Dibrugarh University, Dibrugarh, 79e94.
6. Balunas, M. J. & Kinghorn, D. A. (2005).Drug discovery from medicinal plants. Life Science, 78(5),431e441.
7. Calixto, J. B., (2005).Twenty-five years of research on medicinal plants in Latin America: a personal view. Journal of Ethnopharmacology, 100(1-2),131e134.
8. Choudhary, S. (2000).Indigenous beliefs and practices of herbal medicine among the few Arunachalis. Resarun, Gov. of Arunachal Pradesh, Department of Cultural Affairs, 26,72e81.
9. Cotton, C. M. (1996).Ethnobotany; Principles and Applications. Journal of Medecinal Chemistry, 40(13),336e347.

10. Cragg, G. M. & Newman, D. J. (2005).Biodiversity: a continuing Source of novel Drug leads. Pure and Applied Chemistry, 77(1),7e24.
11. Cowman, M. M. (1999).Plant products as antimicrobial agents. Clinical and Microbiology Review, 12(4),561e582.
12. Zeybek, Z., Dogruoz, N. & Karagoz, A. (2008).Antibacterial Activity of Some Plant Extracts. European Journal of Biology, 67(1),17e21.
13. Farnsworth, N. R., Akerele, O., Bingel, A. S., Soejarto, D. D. & Guo, Z. (1985).Medicinal Plants in Therapy. Bulletin of the world health organization, 63(6),965e981.
14. Jain, S. K. & Sastry, A. R. K. (1980).Threatened plants of India- A State of Art Report. Botanical Survey of India (BSI), New Delhi 148.
15. Jain, S. K. (1968).Medicinal plants (India-The Land and the People). National Book Trust of India. New Delhi, 1e3.
16. Jamkhande, P. G., Wattamwar, A. S., Kankudte, A. D., Tidke, P. S. & Kalaskar, M.G. (2016).Assessment of *Annona reticulata* L. leaves fractions for *in vitro* antioxidative effect and antimicrobial potential against standard human pathogenic strains. Alexandria Journal of Medicine, 52(1),19e25.
17. Joshi, K., Chavan, P., Warude, D. & Patwardhan, B. (2004). Molecular markers in herbal drug technology. Current Science, 87e25.
18. Kumar, K. P. S., Bhowmik, D., Chiranjib, Tiwari, P.& Kharel, R. (2010).Indian traditional herbs *Adhatoda vasica* and its Medicinal application. Journal of Chemical and Pharmaceutical Research, 2(1),240e245
19. Kala, C. P. (2005). Indigenous uses, population density and conservation of threatened medicinal plants in protected areas of the Indian Himalayas. Conservation Biology, 19(2),368e378.
20. Kapoor, L. D. (1990).Handbook of Ayurvedic Medicinal Plants. CRC Press, Boca Raton, Florida, USA.
21. Karsha, P. V. & Lakshmi, O. B. (2010).Antibacterial activity of Black Pepper (*Pipper nigrum* Linn.) with special reference to it's mode of action on Bacteria. Indian Journal of Natural Product and Resource, 1(2),213e215.
22. Kartnig, T. (1988).Clinical applications of Centella asiatica (L.) Urb. Herbs, spices, and medicinal plants: recent advances in botany, horticulture, and pharmacology (USA).
23. Khan, M. H. & Yadav, P. S. (2010).Antidiabetic plants used in ThoubalDistric of Manipur, Northern India. Indian Journal of Traditional Knowledge, 9(3),510e514.
24. Korkora, J. P. & Nayak, J. K. (2018).Nutritional status of tribal mothers and children (0-5 years) of Nabarangpur district, Odisha: An anthropological approach. International Journal of current Research and Development, 3(2),691e697.
25. Kumar, N. K. & Dua, P. K. (2016).Status of regulation of traditional medicine formulations and natural products: Whither is India?. Current Science, 111,293e301.
26. Kumar, S., Das, G., Shin, H. S., Kumar, P. & Patra, J. K.(2018).Diversity of Plant species in the still city of Odisha, India: Ethnobotany and Implications for Conservation of Urban Bio-Resources. Brazilian Archives Biology and Technology,61, 1e3.
27. Lu, Y., Hernandez, P., Abegunde, D. & Edejer, T. (2011).The World medicines situation. Geneva: Medicine expenditures World Health Organization (WHO).
28. Lucy, H. & Edgar, J. D. (1999).Medicinal plants: A reemerging health aid. Electronic. Journal of Biotechnology, 2(2),1e15.
29. Madharia, P. & Jahan, A. (2015).Ethnomedicinal plants and their conservation in Chhattisgarh State: Review and Perspectives. Journal of Environmental Science, Toxicology and Food Technology,1(4),46e50.
30. Magner, L. N. (1992).A History of Medicine. Vol. 4. New York: Marcel Dekker, 203e205.
31. Mainasara, M. M., Maishanu, H. M., John, P. B. & Sanusi, S. B. (2017).Medicinal plants used in Traditional Maternal Healthcare Delivery in Five selected Villages of Zango Kataf Local government Area of Kaduna State, Nigeria. International Journal of Science and Healthcare Research, 2(2),24.
32. Mahalik, G., Satapathy, K. B. & Sahoo, S. (2015).Ethnobotanical survey of plants used in treatment of urinary disorders in Dhenkanal district of Odisha, India. Journal of Environmental Science, Toxicology and Food Technology, 9(8),58e63.

33. Mallick, S. M. & Mahana, A. (2018).A survey of ethnomedicinal plants used by the Paudi Bhuiyan tribals of adjoining areas of Khandadhar waterfall, Bonai subdivision of Sundergarh district, Odisha. India. International Journal of Biology Research,3(2),11e16.
34. Mohanty, N., Panda, T., Sahoo, S. & Rath, S. P. (2015).Herbal folk remedies of Dhenkanal district, Odisha, India. International Journal of Herbal Medicine, 3(2),24e33.
35. Mokwala, P. W. (2007).Antibacterial activity of Plants that are used in the treatment of Heart water in Livestock and the Isolation and Identification of Bioactive Compounds from *Petalidium oblongifolium* and *Ipomoea adenioides*. Ph.D. Thesis. University of Pretoria.
36. Nautiyal, M. C., Prakash, V. & Nautiyal, B. P. (2002).Cultivation techniques of some high altitude medicinal herbs. Ann. For., 10,62e67.
37. Nascimento, G. G., Locatelli, J., Freitas, P. C. & Silva, G. L. (2000).Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Brazilian journal of microbiology, 31(4),247e256.
38. Ncube, N. S., Afolayan, A. J. & Okoh, A. L. (2008).Assessment Techniques of Antimicrobial properties of natural compounds of plant origin; current methods and Future trends. African Journal of Biotechnology, 7(12),1797e1806.
39. Okigbo, R. N., Eme, U. E. & Ogbogu, S. (2008).Biodiversity and conservation of medicinal and aromatic plants in Africa. Biotechnol. Molecular Review, 3(6),127e134.
40. Owoabi, J., Omogbai, E. K. I. & Obasuyi, O. (2007).Antifungal and antibacterial activities of the ethanolic and aqueous extract of Kigella Africana (Bignociaceae) stem and bark. African Journal of Biotechnology, 6,882e885.
41. Panda, D., Pradhan, S., Palita, K. S. & Nayak, K. J. (2014).Medicinal weed diversity and Ethnomedicinal weeds used by tribal's of Koraput, India. Ecology, Environment and Conservation, 20,S35eS38.
42. Patra, S. & Samal, P. (2018).Medicinal plants- Therapeutic Potential in Today's context. International Journal of Current Microbiology and Applied Sciences, 7(8),3841e3848.
43. De Padua, L. S., Bunyapraphatsara, N. & Lemmens, R. H. M. J. (1999).Plant resources of South-East Asia. Backhuys publishers, Leiden, Netherlands, 12(1),210e218.
44. Parekh, J. & Chanda, S. (2006).In-vitro antimicrobial activities of extracts of *Launaea procumbens* Roxb. (Labiateae), *Vitis vinifera* L. (Vitaceae) and *Cyperusrotundus* L. (Cyperaceae), African Journal of Biomedical and Research, 9,89e93.
45. Prusti, A. B., Behera & K. K. (2007).Ethno-Medico Botanical Study of Sundargarh District, Odisha, India. Ethnobotanical Leaflets, 11,148e163.
46. Rao, P. S., Navinchandra, S. & Jayaveera, K. N. (2012).HPTLC analysis of the essential oil from Pimenta dioica leaf. Journal of Applied Pharmaceutical Science, 2(12),141.
47. Samee, H., Li, Z. X., Lin, H. & Khalid, J. (2009).Anti-allergic effect s of ethanol extracts from brown seaweeds. Journal of Zhejiang University Science B, 10(2),147e153.
48. Sen, S. K. & Behera, L. M. (2017).Some Traditional Ethnomedinal Plants Used For Child Healthcare in Bargarh District of Western Odisha (India). World Journal of Pharmaceutical and Medicinal Research, 3(1),136e140.
49. Sethuraman, K., Lansdown, R. & Sullivan, K. (2006).Women's empowerment and domestic violence: the role of sociocultural determinants in maternal and child under nutrition in tribal and rural communities in South India. Food and Nutrition Bulletin, 27(2),128e143.
50. Simpson, B. B. & Ogorzaly, M. C. (1995).Economic Botany: Plants in Our World. (2nd Edition). Mcgraw-Hill, New York.
51. Sofowora, A., Ogunbodele, E. & Onayade, A. (2013).The role and place of medicinal plants in the strategies for disease prevention. African journal of traditional, complementary and alternative medicines, 10(5),210e229.
52. Singh, K. R., Dwivedi, B. S. & Singh, R. (2002).Traditional wisdoms of farmers: an experience towards the sustainable development of livestock. Indian Journal of Traditional Knowledge, 1,70e74.
53. Sharma, A. K. (2013).A Critical Appraisal of Headache vis-à-vis Shiro Roga. Journal of Homeopathy and Ayurvedic Medicine, 2,131.
54. Tekwu, E. M., Beng, V. P. & Kuete, V. (2013).African medicinal plants acting on the reproductive, cardiovascular and central nervous system. Medicinal Plant Research in Africa, 19,805e841.

55. UNESCO. (1996). Culture and Health, Orientation Texts- World Decade for Cultural development 1988-1997, Document CLC/DEC/PRO , Paris, France 129.
56. Uniyal, S. K., Singh, K. N., Jamwal, P. & Lal, B. (2006).Traditional plant of medicinal plants among the tribal communities of Chhota Bhangal, Western Himalaya. Journal of Ethnobiology and Ethnomedicine, 2(1),14.
57. Uprety, Y., Asselin, H., Boon, K. E., Yadav, S. & Shrestha, K. K. (2010).Indigenous use and Bio-efficacy of Medicinal plants in the Rasuwa District, Central Nepal. Journal of ethnobiology and Ethnomedicine, 6,3.
58. Vuorelaa, P., Leinonenb, M., Saikkuc, P., Tammelaa, P., Rauhad, J. P. & Wennberge, T. (2004).Natural products in the process of finding new drug candidates. Current Medicinal Chemistry, 11(11),1375e1389.
59. Yahia, O.A. (2014).Local knowledge of trees utilization and their existing threats in Rashad district of Nuba Mountains, Sudan. Journal of Forest and Environmental Science, 30(4),342e350.

Table 1. List of Plants used in ethnomedicine

Sl. No.	Plant Name	Diseases	Plant parts	References
1	*Areca catechu*	Nervous system disorder	Nut	Mahalik et al., (2015).
2	*Bombax ceiba*	Against ulceration of kidney	Fruit paste	Mahalik et al., (2015)
3	*Adhatoda zeylanica*	Use to cure Diarrhea, asthma, tuberculosis	Leaves, Root Flower	Kumar et al., (2010)
4	*Argemone Mexicana*	Against Diarrhoea	Leaves	Priya and Rao, (2012).
5	*Bauhinia variegate*	Against Dysuria & gall bladder stone.	Fresh bark and Root	Mahalik et al., (2015)
6	*Phyllanthus fraternus*	To dissolve stones and check burning urination	Plant extracts	Mahalik et al., (2015).
7	*Annona reticulata*	Contains antipyretic activity	Leaves	Jamkhande et al., (2016)
8	*Centella asiatica*	Use to cure stomach and duodenal ulcers	Leaves	Kartnig et al., (1988)

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REVIEW ARTICLE

Inventory and Morphometry of Anurans in Terrestrial Environs of Lake Mainit, Philippines

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ABSTRACT

The study determined the species diversity, endemism, abundance, conservation status and morphometrics of anurans in the terrestrial areas around Lake Mainit. Records displayed that there were 14 species under 8 genera of 6 families of anurans of among those species, 11 were extant in the Philippines, 10 least concerned, 2 species near threatened (Limnonectes magnus and Limnonectes acanthi), 1 is considered endangered (Platymantis paengi), then, 1 data deficient by IUCN. Threatened and endangered species were endemic in the Philippines. Inventories on anurans around Lake Mainit evidently highlighted a healthy environment. Though it is observed by fact that there were varied species of anurans around Lake Mainit which are considered vulnerable, near threatened, critically endangered, yet, these species were only found in the areas identified the least concern by IUCN and mostly endemic. The results inventorially express that morphometrics of the species of anurans greatly conspired well in a good environment. Hence, this eventually substantiated the needs of the aquatic and forest habitats around Lake Mainit. Thus, a conservation policy on forest habitats' protection and conservation of selected fauna are needed in the area. Zoning for access and no access zones within the buffer zone around Lake Mainit should be established for the protection of these species of anurans for their reproduction and development.

Keywords: Anurans, species, avifauna, reptiles, mammals.

INTRODUCTION

The Philippines is one of the most biologically diverse countries in the world. Its biological wealth, represented by its flora and fauna, is enormous and has extraordinary high rate of endemicity (Britanico, Dizon, Bello, Quimbo & Duque-Piñon, n.d.) In fact, the country has 576 species of birds, 195 (34%) of which are endemic, 174 mammalian species, 111(64%) of which are endemic, and 258 reptilian and amphibian species, 214(73%) of which are endemic

(Lasco, Veridiano, Habito & Pulhin, 2013); (Garcia, Lasco, Ines, Lyon & Pulhin, 2013). Flora and fauna are the plants and animal life of a region in a period of time. That may sound simple, but the ecosystem created by the interdependence of these two life forms is not simple at all. The very air we breathe and the food we eat, the medicines that cure us, and the water that keeps us alive would not exist were it not for flora and fauna. All things in an ecosystem are interdependent and should be conservedWilkinson, C. (2012); (Wilkinson, Saarne, Peterson & Colding, 2013); (Dennell, Louys, O'Regan& Wilkinson, 2014).

Lake Mainit is the fourth largest fresh water lake in the country. It is the second largest in Mindanao approximately having an area of 17,340 hectare (Camacho, Abella & Tayamen, 2001); (Hurtado, Agbayani, Sanares & de Castro-Mallare, 2001). The name itself reflects the pride of Mainitnons as one the four (4) lakeshore municipalities politically and geographically acclaimed by the provinces of Agusan del Norte and Surigao del Norte. Lake Mainit as watershed comprises an area about 87,072 hectares and supports 99 barangays in the eight municipalities (Natad, J-LMDA-EMP 2005). Notwithstanding the fact that numerous taxonomic groups are not yet completely investigated the current knowledge on the biodiversity of selected fauna specific to anurans around Lake Mainit this study has been conducted. There were already studies in terrestrial fauna that includes anurans conducted before but there is a need to verify if the data still the same at present. This study determined the species diversity, endemism, abundance, conservation status of anurans around Lake Mainit.

METHODOLOGY

Study Areas

The study is within the Lake Mainit watershed area in the province of Surigao del Norte and Agusan del Norte. Two sites were chosen, site 1 at Barangay Cantugas, Mainit, Surigao del Norte and site 2 at Sitio Dinarawan, Barangay San Pablo, Jabonga, Agusan del Norte. Each site covered lowland and upland areas.

Site 1 is located at Barangay Cantugas ,Mainit,Surigao del Norte. There were nine stations established in the lowland area with the following coordinates: 09^0 35.050’ N and 125^0 28.201E’, 09^0 35.089’ N and 125^o 28.051E’, $09^0$35.O68’ N and $125^0$27.905’E, 09°34.994' N and 125°27.796'E, 09°34.982'N and 125°27.653'E,09°34.982'N and 125°27.653'E, 09°34.972'N and 125°27.512E,09°35.001'N and125°27.389' E, 09° 34.970'N and 125°27.215'E and 09°34.932'N and 125°27.100'E respectively. The type of vegetations observed were the dipterocarps and patches of premium tree species such as *Pterocarpus indicus*, *Dracontomelon dao* and *Diospyrus philippensis*. Some rare wildlife flora and fauna are also found in the area such as *Rafflesia mixta,* Kalau and primate species as indicators of a regenerating tropical rainforest. Coconut trees intercropped with falcata and some agricultural crops were also observed. It has stony substrate and the ground covers are mostly ferns, grasses and wildlings.

Same with the lowland area nine stations were put up in the upland with the coordinates as follows: 09.38877°N and 125.49719°E, 09.38852°N and125.49789°E , 09.38981° N and 125.50005°E,09.39032°N and 125.50070°E ,09°34.639'N and125°27.106E' , 09°34.737'N, and 125°26.853'E, 09°34.714'N and125°26.759'E, and 09° 34.740'N and 125°26.651'E. The area are found to have patches of abandoned coconut and abaca plantation, dominated by endemic tree species and some notable wildlife and elevated ranging 805 meters above sea level, a very steep slope with humus and rocky substrate, ground covers are mostly ferns, mosses, lianas and wildlings.

Site 2 is located in Sitio Dinarawan, Barangay San Pablo,Agusan del Norte. The area is adjacent to the lake with a very steep slope at lower portion and gradually becoming rolling at the ridge with intermittent water system and disturbed habitat due to hunting and gathering of forest products by forest occupants. The lowland area is shrubby forest, some portions are planted with falcata, coconut and other agricultural crops such as banana, pineapple and papaya. It has rocky substrate, elevation ranging from 99 to 221 meters above sea level, ground covers are mostly

ferns, grasses, vines and wildlings. Nine stations were also established in the area with the following coordinates : North09.38877°,09.38852°,09.38981°,09.39032°,09.39020°,09.38551°09.38563°,09.38452°, and 09.38446°,

East125.49719°,125.49789°,125.50005°,125.50070°,125.50082°,125.50143°,125.50056°,125.49989°, and 125.49848°. While the upland area were observed to have vegetation like ferns, mangium and endemic species of forest trees .The substrates found were humus and sandy substrate. The most notable species is Mancono "the Philippine Iron wood". The coordinates of the nine stations are as follows: North: 09.38772°, 09.38661°, 09.38762°, 09.38864°, 09.39007°, 09.39185°09.39297°,09.39375° and 09.39365° and 125.49293°, 125.49105°, 125.49052°, 125.49095°, 125.49187°, 125.49242°, 125.49168°, 125.49078° and 125.48997° East of the area.

Sampling Methods, Conservation Status and Data Analysis

Time constrained survey was used across different sampling locations between 9 am to 3 pm during day time and 8 pm to 12 midnight during night timewhere chances of encountering anurans were high. The sampling was done employing man-hour of search during day and night time using a combination of field transect surveys pitfall traps or funnel traps A 10-hour was spent in each site for 5 days sampling. All observed anurans were noted and captured individuals were photographed, recorded, measured for morphometry and released. The following morphometries were recorded (all in mm) using a Vernier caliper: snout-vent length (SVL, from snout tip to posterior margin of vent); head length (HL, from tip of snout to posterior margin of jaw articulation); eye diameter (ED); and eye-tympanum distance (ETD, from posterior margin of eye to anterior margin of tympanum). Body weights (BW) were measured using a weighing scale in grams (g) and morphological description was based on species account guidelines (Lehtinen & Georgiadis, 2012); (Buckley, 2001).

Conservation status of the species captured was noted and were classified as endangered (EN), critically endangered (CR), vulnerable (VU), data deficient (DD), and least concern (LC) according to the International Union for Conservation of Nature Red list categories (IUCN 2016). The identified anurans were also classified for their distribution status. PAST software was used to measure the biodiversity indices. SPSS version 17 was used in principal component analysis to determine the similarities and differences in abundance of anurans.

RESULTS AND DISCUSSION

Diversity of Anurans, Species Richness, Abundance, Conservation and Distribution Status of Anurans

Diversity Indices and Inventory of anurans are presented in Tables 1 and 2. There were 14 species under 8 genera of 6 families of anurans documented. Among the species recorded, there are 11 species extant in the Philippines, 10 are considered least concern, 2 species near threatened (*Limnonectes magnus* and *Limnonectes acanthi*), 1 endangered (*Platymantis paengi*) and 1 data deficient by IUCN. The near threatened and endangered species are all endemic to the Philippines. The highest diversity (H'= 1.938) was recorded in upland habitat of Cantugas which could be related to its high species richness (9), abundance (70) and evenness (0.77191) with low dominance (0.1653). While the lowest was in the lowland habitat of Jabonga with H' index of 1.374 that could be associated with its low species richness (5) and high dominance value (0.2961). In general, the diversity of anurans in Lake Mainit was high.

Morphometries of Anurans

The presentation of the morphometries of anurans around Lake Mainit was in descriptive manner as shown in Tables 3. Morphometries of species captured were only documented in the present study.Total lengths of the *Bullimus bagobus, Rattus tanezumi* and *Eonycteris spelaea* captured in Cantugas were much higher than those captured in Jabonga. There were 12 measurements done in captured anuran species found around Lake Mainit. The morphometrics of the 14 species of anurans captured in Cantiugas were mostly higher than those captured in Jabonga (Tables 3).

CONCLUSIONS

The study reflected that anurans in the terrestrial areas around Lake Mainit were evidently create healthy environmentits diversity of anurans are generally high. Though there are some species of anurans found around Lake Mainit which are considered vulnerable, nearly threatenedand endangered, critically endangered yet this diverse species of anurans are generally found in the area are identified least concern by IUCN. Most of the species are endemic to the Philippines. The morphometrics of the species of anurans are good indicators of a good environment. Nevertheless, aquatic and forest habitats found around Lake Mainit needs to be protected. Thus, a conservation policy on forest habitat protection and conservation of selected fauna is needed in the area. Thereby it is recommended also that zoning for access and no access zones within the buffer zone around Lake Mainit should be established to protect those species of fauna for their reproduction and development

REFERENCES

1. Britanico, S. C. R., Dizon, J. T., Bello, R. T., Quimbo, M. A. T., & Duque-Piñon, C. Legal Challenges in Securing Land Tenure and Property Rights in Lake Sebu, South Cotabato, Philippines;
2. Buckley, D. (2001). Evolution of viviparity in salamanders (Amphibia, Caudata). *e LS*;
3. Camacho, A. S., Abella, T., & Tayamen, M. M. (2001). Fish genetics research and development in the Philippines. In *Fish Genetics Research in member countries and Institutions of the International Network in Genetics in Aquaculture. Conference Procedures* (pp. 71-76);
4. Dennell, R. W., Louys, J., O'Regan, H. J., & Wilkinson, D. M. (2014). The origins and persistence of Homo floresiensis on Flores: biogeographical and ecological perspectives. *Quaternary Science Reviews*, *96*, 98-107;
5. Garcia, K., Lasco, R., Ines, A., Lyon, B., & Pulhin, F. (2013). Predicting geographic distribution and habitat suitability due to climate change of selected threatened forest tree species in the Philippines. *Applied Geography*, *44*, 12–22. https://doi.org/10.1016/j.apgeog.2013.07.005;
6. Hurtado, A. Q., Agbayani, R. F., Sanares, R., & de Castro-Mallare, M. T. R. (2001). The seasonality and economic feasibility of cultivating Kappaphycus alvarezii in Panagatan Cays, Caluya, Antique, Philippines. *Aquaculture*, *199*(3-4), 295-310;
7. Lasco, R. D., Veridiano, R. K. A., Habito, M., & Pulhin, F. B. (2013). Reducing emissions from deforestation and forest degradation plus (REDD+) in the Philippines: will it make a difference in financing forest development?. *Mitigation and adaptation strategies for global change*, *18*(8), 1109-1124;
8. Lehtinen, R. M., & Georgiadis, A. P. (2012). Observations on parental care in the glass frog Hyalinobatrachium orientale (Anura: Centrolenidae) from Tobago, with comments on its natural history. *Phyllomedusa: Journal of Herpetology*, *11*(1), 75-77;
9. Wilkinson, C. (2012). Social-ecological resilience: Insights and issues for planning theory. *Planning Theory*, *11*(2), 148-169;
10. Wilkinson, C., Saarne, T., Peterson, G. D., & Colding, J. (2013). Strategic spatial planning and the ecosystem services concept–an historical exploration. *Ecology and Society*, *18*(1);
11. Natad, J.S. (2013). Community Satisfaction on Lake Mainit Development Alliance. Master of Public administration. Bukidnon State University, Graduate School City of Malaybalay;
12. IUCN. (2010). IUCN Red List of Threatened Species. Version 2010.1. Electronic Database accessible at www.iucnredlist.org;
13. Lake Mainit Development Alliance – Environmental Management Plan, 2014;
14. Lake Mainit Key Biodiversity Area. 2010. Fauna of Lake Mainit Ecosystems. Biodiversity Conservation and Management Plan. Vol.1, Chapter 4.

Table 1. Diversity Indices of Anurans around Lake Mainit

SITES Diversity indices	Cantugas		Jabonga	
	Lowland	**Upland**	**Lowland**	**Upland**
Taxa S	7	9	5	7
Individuals	114	70	80	29
Dominance_D	0.2592	01653	0.2961	0.2699
annon_H	1.561	1.938	1.374	1.556
Evenness_e^H/S	0.6804	0.7719	0.7903	0.6772

Table 2. Species Richness, Abundance, Conservation and distribution status of various species of Anurans found selected environs around Lake Mainit, Surigao del Norte, Philippines.

Family	Scientific Name	Common Name	Conservation Status (IUCN)	Distribution Status	Sites			
					Cantugas		Jabonga	
					Low land	Up land	Low land	Up land
Dicrogloss idae	*Fejervarya cancrivora*	Crab-eating frog	Least Concern	Brunei Darussalam; Cambodia; China; India; Indonesia; Lao People's Democratic Republic; Malaysia; Philippines; Singapore; Thailand; Vietnam	5	3	6	8
	Fejervarya moodiei	Brackish frog	Data Deficient	Extant Philippines	0	9	0	3
	Fejervarya vittigera	Luzon wart frog	Least Concern	Extant (resident) Philippines	3	0	0	1
	Limnonectes magnus(Stejneger, 1909)	Mindanao Fanged Frog	Near Threatened	Extant (resident) Philippines	6	6	35	12
	Limnonectes woodworthi	Woodworth's wart frog	Least Concern	Extant (resident) Philippines	6	0	0	0
	Limnonectes acanthi	Busuanga Wart frog	Near Threatened	Extant (resident) Philippines	0	0	0	1
Leptodactylidae	*Leptodactylus leptodactyloides*	Slender-fingered toadlet	Least Concern	Bolivia, Brazil, Colombia, Ecuador, French Guiana, Guyana, Peru, Suriname, and Venezuela	0	5	0	0
Megophryidae	*Megophrys stejnegeri* (Taylor, 1920)	Mindanao horned frog	Least Concern	Extant (resident) Philippines	33	17	0	0

Ceratobatrachidae	*Platymantis corrugatus*	Rough-back Forest frog	Least Concern	Extant (resident) Philippines	0	0	0	2
	Platymantis paengi	Panay limestone frog	Endangered	Endemic to the Philippines	0	1	0	0
Ranidae	*Pulchrana grandocula*	Big-eyed frog	Least Concern	Extant (resident) Philippines	18	13	28	0
	Pulchrana similis	Laguna del Bay frog	Least Concern	Extant (resident) Philippines	43	14	8	2
	Staurois tuberilinguis	Green-spotted rock frog	Least Concern	Malaysia, Brunie and Indonesia	0	0	9	0
Rhacophoridae	*Rhacophorus pardalis*	Panther Flying Frog	Least Concern	Extant - Brunei Darussalam; Indonesia; Malaysia; Philippines	0	2	0	0
				Total Abundance (Captured)	**114[a]**	**70[b]**	**86[b]**	**29[c]**
				Total Number of Species	**7[b]**	**9[a]**	**5[c]**	**7[b]**

Table 3. Average Morphometric of various anuran species found around Lake Mainit.

Scientific Name	**Morphometrics**											
	Sampling Sites											
	Cantugas	**Jabonga**	**Cantugas**	**Jabonga**	**Cantugas**	**Jabonga**	**Cantugas**	**Jabonga**	**Cantugas**	**Jabonga**	**Cantugas**	**Jabonga**
	SVL		**ES**		**HL**		**HW**		**TD**		**FmL**	
Fejervarya cancrivora	61.1	64.0	10.5	12.1	25.3	24.8	25.0	23.8	3.4	3.7	28.3	226.0
Fejervarya moodiei	65.0	62.7	12.0	9.3	27.0	16.7	29.0	24.3	3.0	7.7	34.0	100.0
Fejervarya vittigera	84.0	89.0	12.3	13.0	33.7	33.0	29.7	35.0	4.7	6.0	37.7	51.0
Leptodactylus leptodactyloides	53.6		10.0		20.4		23.2		3.0		22.8	
Limnonectes acanthi		75.0		12.0		31.0		26.0		9.0		37.0
Limnonectes magnus	48.2	54.3	9.0	8.7	24.5	21.8	23.0	19.2	3.2	3.8	29.8	494.0
Limnonectes wood worthi	79.3		14.2		29.3		29.2		4.7		36.3	
Megophrys stejnegeri	76.8		8.5		28.4		33.1		2.8		26.8	
Platymantis corrug atus		61.5		12.0		27.5		27.0		5.0		69.0

Polypidates paengi	75.0		13.0		29.0		30.0		4.0		36.0	
Pulchrana grandocula	77.2	20.6	6.8	3.8	29.2	7.7	26.2	6.9	2.0	1.4	35.2	262.0
Pulchrana similis	89.9	60.4	10.9	8.1	34.6	22.8	30.5	20.4	4.2	3.8	38.2	93.0
Rhacophurus pardalis	31.0		5.0		10.0		11.0		2.0		16.0	
Staurois tuberilinguis		12.4		2.0		4.8		3.9		0.9		64.0

Table 3. (cont.)

Scientific Name	**Morphometrics**											
	Sampling Sites											
	Cantugas	**Jabonga**	**Cantugas**	**Jabonga**	**Cantugas**	**Jabonga**	**Cantugas**	**Jabonga**	**Cantugas**	**Jabonga**	**Cantugas**	**Jabonga**
	TbL		**TrL**		**TFOL**		**PhL**		**EAD**		**FAL**	
Fejervarya cancrivora	29.7	30.6	14.8	15.2	31.8	29.7	12.2	15.8	4.7	9.8	11.9	13.0
Fejervarya moodiei	32.7	33.0	16.3	23.0	32.2	34.3	16.2	15.3	5.0	6.7	13.3	12.0
Fejervarya vittigera	39.3	19.0	20.3	24.0	39.3	45.0	22.0	22.0	5.0	8.0	16.0	19.0
Leptodactylus leptodactyloides	19.0		11.8		16.4		13.0		7.0		13.8	
Limnonectes acanthi		36.0		23.0		51.0		2.0		9.0		16.0
Limnonectes magnus	30.2	26.2	15.5	13.9	29.5	25.2	16.5	13.4	6.2	5.7	9.7	11.2
Limnonectes woodworthi	33.7		17.5		33.2		16.7		5.8		15.5	
Megophrys stejnegeri	25.8		14.8		27.7		18.9		10.9		16.8	
Platymantis corrugatus		35.0	41.0	20.5	64.0	32.0	31.0	15.5	24.0	12.0	25.0	12.5
Polypidates paengi	40.0		19.0		35.0		20.0		6.0		16.0	
Pulchrana grandocula	36.8	10.0	15.6	5.2	36.6	9.3	24.6	5.6	2.6	1.8	12.0	4.3
Pulchrana similis	39.9	27.2	18.8	13.6	38.8	26.5	27.2	17.8	5.1	4.4	13.9	10.4
Rhacophurus pardalis	18.0		9.0		14.0		9.0		4.0		6.0	
Staurois tuberilinguis		8.1		4.2		5.9		3.9		1.3		2.6

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RESEARCH ARTICLE

Diffusion of Clean Energy Products: A Conceptual Framework

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ABSTRACT

Globally, 1.3 billion people live in poverty, under $2 dollars a day (UNDP, 2018). Many of them lack basic access to modern energy. Lack of access to modern energy undermines key development indicators such as education, health and livelihoods which hampersin achieving socio-economic development in the region. In 2015, UN General Assembly adopted the agenda for Sustainable Development Goals.Sustainable Development Goals aimed to ensure “access to affordable, reliable, sustainable and modern energy for all” (Chetan et al., 2016). Energy access is multidimensional which includes householdsfor lighting needs, fulfilling cookingand productive use for livelihoodneeds. In the above context access to affordable, reliable, sustainable and modern energy through the diffusion of clean energy products has been focused to urban areas, whose benefits do not scroll down to the rural areas of the society. Hence, there is a need to understand how these clean energy products can reach people residing in rural areas. For achieving the same, their is a need to develop a conceptual frameworkfor diffusion of clean energy products in rural areas. The conceptual framework links the factors and actors affecting the diffusion of clean energy products in rural areas. Section 4.1 focuses on the actors necessary for diffusion of clean energy products in rural areas. Section 4.2 focuses on the linkages between identified actors and factors. Finally, in section 4.3 the conceptual framework for diffusion of clean energy products in rural areas has been discussed.

Keywords: modern energy, products, framework, diffusion, clean .

INTRODUCTION

Actors for Diffusion of Clean Energy Products

According to Oxford Dictionary (2019), an "actor is a person who performs on the stage, on television or in filmmovies, especially as a profession" but in the present study actors are the different stakeholders which helps in diffusion of clean energy products. According to Suurs (2009) and Wieczorek et al., (2013), "Actors are the organisations that contribute to technology diffusion either directly or indirectly". Actors also consist of "end users, NGOs, financing institutions, manufacturers, regulators, consultants and start-ups, which helps in diffusion of technological products" (Jacobsson, 2004; Wieczorekand Hekkert,2012).

"Diffusion of clean energy products requires a collective, long term process and involvement of multiple actors to achieve new solutions to social challenges" (Anderson et al., 2015). In the present context actors are those without which the diffusion will not take place. Thus there is a need to identify those actors. Rogers (1995), in his book "Diffusion of Innovation" had identified some actors which were responsible for the diffusion to take place. It was found that early adopters act as opinion leaders. Opinion leaders along with the consultants, experts in the technical organisations and researchers working in technological and professional institutes were the actors responsible for the diffusion to take place. Silva (2008), undertook a case study on deployment of renewable energy technologies. It was found that there are several actors influencing the deployment of renewable energytechnology product and process. According to them, Government at central and local level, NGOs, local community and academic institutions were the actors responsible for the deployment of renewable energy technology product and process. World Business Council for Sustainable Development (2010), presented an enabling framework for clean energy diffusion. According to WBCSD, actors such as stable government policies, undertaking capacity building programs and involvement of educational institutions were responsible for diffusion to take place.

Sung and Park (2018),devised a framework for transition of renewable energy technology to any region. It was found that the transition process is governed by five actors; government, public, marketsand the traditional energy industry were responsible for renewable energy diffusion. Government includesboth at state and central govt, public agencies, policy-makers, bureaucrats, sub-governmental organisations, etc. Public include households, families, neighbours, communities, community groups, civil societies, academia, etc. Market include markets, firms, business, consumers, infrastructure, bank groups and NGOs, non-profit associations, activists, volunteers, benefactors, researchers, philanthropists, etc. respectively.Lastly the traditional energy industry includes the energy companies, coal mining, oil and gas companies, trade associations, etc (ibid). SELCO Foundation along with the Renewable Energy Working Group (2012), did a study and suggested an eco-system approach for diffusion of off-grid solar products in rural context. They found that for diffusion of off-grid solar products products, it is required to have a comprehensive approach to see the market as an ecosystem. For having the ecosystem approach in place, there should be the involvement of all the members with their respective roles defined. Members of the ecosystem include the end-users, service provider, facilitator, financial institutions and implementing agency.

End users are the under served rural consumers, who need to be aware on the advantage of using the off-grid solar products. Provision of accessibility to take loans from the bank and availability of government subsidy needs to be informed to the end-users. Service provider is another member of the ecosystem. To reach the target consumers their should be a strong rural network with reliable after-sales service. Facilitator is another member of the ecosystem. Here the role of the facilitator is to push the end-user on creating awareness on the use of off-grid solar products and facilitates the financing institutions in lending off-grid solar products to the end-users. Facilitator acts as the bridge between the end-users, service providers and the financing institutions. Financial institutions is another member of the ecosystem. They are commercial and rural banks who provide loans for adoption of off-grid solar products. Banks provide loans and its re-payment process needs to be easy. Implementing agency is another member of the ecosystem. It sets guidelines for solar lending and acts as a source of financing. They gave that MNRE, NABARD here act as the implementing agency.

In 2015, SELCO foundation in collaboration with WWF undertook a study to identify the possibleactors required for diffusion of clean energy products in rural areas in Indian context. According to the study, enterprise, institutions,

government, communityand households play a major role forclean energy diffusion. Apart from the above, the role of capacity developers, technology and service providers, civil society, policy makers and regulators and knowledge networks were also highlighted for the diffusion of clean energy products (SELCO and WWF, 2015). After identification of various actors, it is required to understand their roles. There is a need to understand their interest and intentions. This can be achieved by understanding the stakeholder analysis. A stakeholder can be an individual, a group of individuals, an organisation who may affect, be affected by, or think itself to be affected by any decision, or outcome (PM, 2013). Stakeholders are basically of two types, primary and secondary stakeholders. Primary stakeholders are the rural consumers who are most affected in the process and secondary stakeholders are the intermediaries in the process which include the facilitating institutions, implementing agency, manufacturers, financing institutions etc. Stakeholders are assessed on the basis of their interest in the activities of the organisation and the extent of power to influence the activities concerned (Mendelow, 1981).

In the present context, stakeholders are the actors of the ecosystem. They include rural consumers, facilitating institution, implementing agency, manufacturers and financing institutions. Financing institutions can be Government, Banks, Donors, Funding Agency, etc.Table 4.1 focuses on roles of each actors in the diffusion process. From the earlier chapters which discussed about the factors such as finance, capacity building, technology and infrastructure and presently the actors are all essential members of the ecosystem. The members of the ecosystem do not operate in isolation. They are all linked with each other. Fig 4.4 gives the clean energy ecosystem for diffusion of clean energy products. Their exist a link between each factor and actor. These linkages between the actors-factors, actors-actors and factors-factors have been discussed in the next section.

Linkages between Actors and Factors

In this section an attempt has been made to understand the linkages between actors and factors, actors and actors and factors and factors.Factors include finance related, capacity building related, technology related and infrastructure related. Actors include rural consumers, implementing agency, facilitating institutions, manufacturers and financing institutions. Table 4.2 focuses on the linkages between actors and factors, table 4.3 focuses on the linkages betweenactors and actors and table 4.4 focuses on the linkages between factors and factors.

(ia) Finance Related- Rural Consumers Linkage

Rural consumers do not have adequate financing capability. However, they need some initial investment, and hence they prefer to use products with low initial cost. As a result, rural consumers need to have access to the facility for provision of loans, savings mechanisms provided by the bank, post office and other financing institution. Provision of loans might not help in complete diffusion of clean energy products, however, if combined with easy repayment methods can ease the affordability for rural consumers. Easy repayment methods such as say EMIs can be tracked down by appointing collection agents. They can also act as service delivery agents, thus helping in diffusion of clean energy products.

(ib) Finance Related- Implementing Agency Linkage

People's institutions such as SHGs and cooperatives are the implementing agency. Generally, the funds from the financing institutions are disbursed to implementing agencyin rural areas through facilitating institutions. As a result, the implementing agency gets to connect with facilitating institutions. It understands various financial schemes and subsidy available for diffusion of clean energy products. Implementing agency help in getting collective approach towards diffusion of any product and services in rural areas. They bring in professionalism in its approach, which helps in optimum utilisation of fund and other resources. This also helps in building good relation with the facilitating institutions and financing institutions. And finally, it helps in good linkage and funding for the rural consumers, which ultimately helps in diffusion of clean energy products in rural areas.

(ic)Finance Related- Facilitating Institution Linkage

Facilitating institutions act as a support system for the diffusion of clean energy products in rural areas. NGOs is considered as one of the facilitating institutions. Facilitating institution assist the implementing agency in making financial linkages with the bank, micro-finance institutions, cooperative banks, regional rural banks, etc. Facilitating institution provides handholding support, mentors and create market linkage to implementing agency. It supports the implementing agency to develop project reports and business plans, to receive funds/loans from banks and other financing institutions. Sometimes the facilitating institutions helps the consumer for financing through banks, MFIs, cooperatives, regional rural banks, etc.Facilitating institution, implementing agency sustain and scale through CSR grants, funds from different corporates and other funding agencies.

(id) Finance Related- Manufacturer Linkage

Manufacturer, focuses on investing capital in designing and developing clean energy products and providing services to consumers in rural areas. Manufacturers require financial support to develop new product, undertake innovative monitoring and service delivery. They create demand for adoption of clean energy products in rural areas in particular, which in turn will help them in increasing of their revenue by selling clean energy products.

(ie) Finance Related- Financing InstitutionsLinkage

For diffusion of clean energy products among the rural consumers, financinginstitutions like government provides clean energy products at subsidised rates. It provides Central FinanceAssistance (CFA)to facilitating institutions, implementing agency, state nodal agencies, (SNAs), etc. for deployment of clean energy products (MNRE, 2017).Financinginstitutions like banks, donors, funding agencies, etc. provide loans, CSR grants for undertaking different projects in the clean energy area. It facilitates several schemes, custom and excise duty benefits to the manufacturers of clean energy products which helps in lowering the cost of setting up manufacturing units, thus helping in diffusion of clean energy products in rural areas.

(iia) Capacity Building Related- Rural Consumers Linkage

For diffusion of clean energy products, rural consumers need to be aware on the usefulness, advantage of using a clean energy products, availability and trained on its usage. Capacity building plays an important role in awareness and training. It focuses on developmental approach of rural consumers, leading to transformation in usage and diffusion of clean energy products. Awareness through door to door campaign, frequent meetings and interactions with local people, opinion leaders and other relevant stakeholders need to be conducted. It not just helps in creating awareness on the availability and usage of clean energy products but also helps in creating awareness on the availability of financing options offered by local banks and other financing institutions.

(iib) Capacity Building Related- Implementing Agency Linkage

Implementing agency create avenues for sharing information and knowledge transfer. They conduct group discussions, group meetings to understand and share their problems and try to find out possible solutions. They interact with the consumers, understand the problems and try to bring solutions. They pass on the information of their community problems to the facilitating institutions. Creating awareness on protection against the use of low quality products. They support the facilitating institution to provide training by conducting workshops on technical and managerial aspects related to diffusion of clean energy products. They develop training materials in local language for easy understanding to the consumers.

(iic) Capacity Building Related- Facilitating Institution Linkage

Facilitating institutions act as a training centre for the implementing agency for creating awareness on the clean energy products, its operations and maintenance. It provides training on both technical and managerial aspects. It provide handholding support to implementing agency by bringing in other actors to provide support for diffusion to take place. It supports in undertaking entrepreneurial growth among local youths and other stakeholders.

(iid) Capacity Building Related- Manufacturer Linkage

Here the role of the manufacturer is to focus on the capacity development in the entire supply chain, including the consumers. It also includes their own employee development. They provide training on different grounds ranging from technical to service aspects. This helps the manufacturers to diffuse the clean energy products in the rural areas by understanding the needs, problems of the rural consumers and providing them customised product.

(iie) Capacity Building Related- Financing Institutions Linkage

Financinginstitutions like government, banks, donors, funding agencies, etc. undertakes awareness and sensitising programs for development of rural community to adopt clean energy products. It provide training on several aspects ranging from socio-economic development, market linkage, legal, intellectual property rights, administration, managerial and environmental aspects. Financing institutions along with the NGOs and other community engagement platforms, develop training materials in local language for the rural consumers, for easy understanding and knowledge gain ultimately leading to diffusion of clean energy products.

(iiia)Technology Related- Rural Consumers Linkage

In today's technology driven world, even the rural consumers prefer those products which are convenient to use, portable, easy to operate, require less maintenance, etc. The manufacturers make optimum utilisation of technology for delivering the features those are relevant for the target audience. The clean energy products are to be reliable, efficient and affordable, without compromising on the quality. This will help in solving the problems of rural consumers. Further, this will result in a push and pull strategy, where the consumers requires the product for solving their problems and it will also help the manufacturers or dealers to sell their products in rural areas.

(iiib)Technology Related- Implementing Agency Linkage

Rural consumers need reliable, efficient and affordable clean energy products for its effective utilisation. There are high chances that the manufacturer market their low quality, unreliable and inefficient clean energy products and convince rural people to procure their products at very low cost. Hence the implementing agency needs to be equipped with certain level of knowledge and develop an understanding on selecting the appropriate clean energy products available in the market. Implementing agencyneed to go for right kind of product assessment and select the best product available without compromising on the feature and quality aspect. Further, it need to use technological assets such as solar street light, solar operated rice huller, solar operated cold storage, etc. for providing end to end solutions to common problems of the community as a whole. Community has to monitor the usage of these clean energy products and also undertakes its maintenance and service for its longevity and productivity.

(iiic) Technology Related-Facilitating Institution Linkage

Here the facilitating institution adopt partnership approach with other actors such as academic and research institution to develop clean energy products either in the form of tangible product or use of a solution existing off-the shelf to create a new arena for its application. They test the product and do suitable iterations based on the feedback

of the implementing agency. Facilitating institution becomes a hub where researchers, experts, consultants, along with the implementing agency meet and collaborate to develop technological innovations which can change the future of developing solutions to complex social problems.

(iiid) Technology Related- Manufacturer Linkage

Here the manufacturer focuses on design and development of high quality, energy efficient clean energy products. They collaborate with R&D institutions, academic institutions, etc. to create a platform for technology transfer, patenting, new product design and development. They focus on establishing high end technology machines, for manufacturing of clean energy products. They develop and establish quality standards for each clean energy product they manufacture. Quality standards focuses on product features, reliability and efficiency of the clean energy product.

(iiie) Technology Related- Financing Institution Linkage

Financinginstitution like government, banks, donors, funding agencies, etc. play an important role in technology aspect. It promote and supports high end technology transfer initiatives from other countries in the field of clean energy development. It help in supporting and building a conducive environment for growth of R&D in manufacturing energy efficient clean energy products. It provide financial support for setting up testing centres, laboratories, associated services such as quality standards and accreditation for clean energy products, thus helping in design and development of clean energy product prototypes. All these initiatives helps in the diffusion of clean energy products.

(iva)Infrastructure Related- Rural Consumers Linkage

For diffusion of clean energy products, rural consumers need to have the access to basic physical infrastructure and community engagement platforms. Physical infrastructure includes transport of goods and services from one place to another, provision of telecommunication facility like mobile towers, that will act as a supporting system for the diffusion to take place. Use of clean energy products create an asset for the rural consumers.

(ivb)Infrastructure Related- Implementing Agency Linkage

Implementing agency need to have access to basic utilities and physical infrastructure for diffusion of clean energy products. Implementing agency need to create a physical space where the entire community can sit together and discuss on the issues and the feasible and possible solutions. Such infrastructure can not only help identify the problems but also understand the probable solutions and what kind of help can be taken from whom. This platform can also be used to explore the clean energy products, its features, quality, usage and cost benefits directly from the manufactures. Such infrastructure can help community to take a collective decision on the issues and solutions, even for the usage of clean energy products. Diffusion of clean energy products can only take place when their is last mile quick delivery of the service associated with the product. Implementing agencyneed to coordinate with manufacturers of clean energy products to provide spares of clean energy product and go for quick replacement of defective parts of clean energy product, etc. It need to provide supporting infrastructure such as availability of a warehouse for storage of clean energy products, stationeries and other items associated with it. For service delivery, the implementing agency can establish IT enabled communication system, so that the rural consumers can call the service providers to solve any problem related to malfunction of clean energy products.

(ivc) Infrastructure Related- Facilitating Institution Linkage

The role of facilitating institution is to provide assistance in acquisition of the basic needs and amenities for the implementing agency. Presence of local community centres, NGO's regional office etc. acts as a platform for discussion with rural consumers to identify their issues, implement various developmental programs, create awareness, etc. among the consumers. They articulate the problem, needs and bring the attention to those who can bring change. They help in defending both environmental and developmental rights.

(ivd) Infrastructure Related- Manufacturer Linkage

Manufacturers alone cannot be effective without the existence of a robust supply chain involving facilitating institutions and the implementing agency in the rural areas.

(ive) Infrastructure Related- Financing Institution Linkage

Financinginstitutionlike government, banks, donors, funding agencies, etc. supports in technology transfer, patenting and new product development which promotes diffusion. It helps in setting up manufacturing units using advance technology that supports the ecosystem. After understanding the linkages between actors and factors, table 4.3 focuses on the linkages betweenactors and actors.

(ia) Rural Consumers- Rural Consumers Linkage

No link can be establish between rural consumers and rural consumers.

(ib) Rural Consumers- Implementing Agency Linkage

Same link as established between implementing agency and rural consumers.

(ic) Rural Consumers- Facilitating Institution Linkage

Same link as established between facilitating institution and rural consumers.

(id) Rural Consumers- Manufacturer Linkage

Same link as established between manufacturer and rural consumers.

(ie) Rural Consumers- Financing Institutions Linkage

Same link as established between financing institution and rural consumers.

(iia) Implementing Agency- Rural Consumers Linkage

People's institutions such as SHGs and cooperatives are implementing agency. Rural consumers make an integral part of the implementing agency. They form a voluntary association with common interest to achieve a collective social and economic goals. They try to understand, share their problems and find out possible solutions. Such groups are organised for mutual help and benefit. They become the basis for action and change.

(iib) Implementing Agency- Implementing Agency Linkage

No link can be establish between implementing agency and implementing agency.

(iic) Implementing Agency- Facilitating Institution Linkage

Same link as established between facilitating institution and implementing agency.

(iid) Implementing Agency- Manufacturer Linkage

Same link as established between manufacturer and implementing agency.

(iie) Implementing Agency- Financing Institution Linkage

Same link as established between financing institution and implementing agency.

(iiia) Facilitating Institution-Rural Consumers Linkage

NGOs is considered as one of the facilitating institutions. Facilitating institution play an active role in people's development in rural areas. It selectively utilise local talent, train rural youth and use them for the community development. It undertakes personality development programs, skill development programs, educational programs, integrate various development projects, etc. helping the rural consumers in empowering them. It helps in alleviating them from poverty by opening the path of income generation. The facilitating institution act as a catalyst in helping the rural unemployed youth to acquire training through various programs so that they can also become job providers instead of job seekers. Facilitating institution help the rural consumers in providing livelihood security, making them understand various agricultural development policies and its implementing mechanism. It helps in providing better sanitation, establishing schools and other basic amenities. It bring in other actors, work at local and regional level with the participation and involvement of the implementingagency.

(iiib) Facilitating Institution-Implementing Agency Linkage

Facilitating institution create market and finance linkage for the implementing agency. It assist the implementing agency in selling the rural products in urban market by attaching them with business houses, corporates, marketers, etc. It assist the implementing agency in making financial linkages with the bank, micro-finance institutions, cooperative banks, regional rural banks, etc. The facilitating institution sometimes examines creditworthiness of the implementing agency so that banks can lend money to the implementing agency. It guides, mentors and provide handholding support to the implementing agency to undertake various developmental programs in the rural areas. Itprovide training to the rural consumers, members of the implementing agencyon various aspects related to their community development. It create avenues for sharing information and knowledge transfer. It goes for undertaking various awareness programs, conduct campaigns and sensitise the rural consumers, members of implementing agency to protect against the use of low quality products and adopt high end technological products.

(iiic) Facilitating Institution- Facilitating InstitutionLinkage

No link can be establish between facilitating institution and facilitating institution.

(iiid) Facilitating Institution- Manufacturer Linkage

Same link as established between manufacturer and facilitating institution.

(iiie) Facilitating Institution- Financing Institution Linkage

Same link as established between financing institution and facilitating institution.

(iva) Manufacturer- Rural Consumers Linkage

Rural consumers have emerged as opinion leaders in influencing the brand and making product decisions in a market.They have become growth drivers of new products coming into the market.They demand products with high quality, and low price. Rural consumers seek value for the product they buy. Hence, the manufacturers need to find out not the right consumer for their products but rather the right products for their consumer. They need to sale high

quality products at affordable price to the rural consumers. High quality products needs to be accompanied with quick service delivery.

(ivb) Manufacturer- Implementing Agency Linkage

Manufacturers alone cannot be effective without the existence of a robust supply chain involving the implementing agency in the rural areas. They need to use the implementing agency for distribution of their products in rural areas. Itneed to establish a direct contact with the manufacturers for procurement of the products and can become their channel partners. Manufacturers need to make the implementing agency as their rural stockist and proponents of products and service providers. They can act as product pushers rather then channel members. Members of the implementing agency can be a rural agent and get commission to push the product onto the doorsteps of the rural consumers.

(ivc) Manufacturer- Facilitating Institution Linkage

Manufacturer produce and channelise the products to the facilitating institution at a marketable price. They provide customised products as and when demanded of the facilitating institution.

(ivd) Manufacturer- Manufacturer Liankage

No link can be establish between manufacturer and manufacturer.

(ive) Manufacturer- Financing Institution Linkage

Same link as established between financing institution and manufacturer.

(va) Financing Institution- Rural Consumers Linkage

Financing institutions can be Government, Banks, Donors, Funding Agency, etc.Financing institution need to provide loans, saving mechanism, etc. to the rural consumers. They train the rural consumers and provide them technical support on how to manage the account, utilisation of loans, and repayment procedures. Financing institution need to develop easy repayment methods which can ease the affordability for rural consumers. It need to make the rural consumers aware on various schemes, policies, subsidy available on financial products.

(vb) Financing Institution- Implementing Agency Linkage

Financing institutions provide monetary assistance to the implementing agencies with payable interest or sometimes as grantdepending on the kind of projects or study they undertakes. Here the implementing agency opens a savings account with the bank, and the bank lends to the implementing agency, which in turn gives loans to its members in accordance with the group's policy. The loan is granted in the name of the implementing agency and all members of the group are collectively responsible for the repayment to the bank. The loans have no collateral security as group cohesion and peer pressure act as security for the bank loan.

(vc) Financing Institution- Facilitating Institution Linkage

Financing institution sometimes provide bulk lending, soft loan, grants to the facilitating institution which can act as micro-finance institution (MFI). They stimulate the credit demand of the rural consumers. It also provide technical support for the beneficiaries to ensure proper utilisation of loans and repayment.

(vd) Financing Institution- Manufacturer Linkage

Financing institution help the small as well as the medium size manufacturers financially by providing them loans at low interest rate. It provide both fixed capital and working capital loans to the manufacturers. It provide information on various schemes, subsidy available for starting a new enterprise. It help in the establishment of new projects,

expansion, diversification, modernisation and renovation of existing manufacturing units. It provide loans to manufacturing units for acquisition of specific machinery and equipments.

(ve) Financing Institution- Financing Institution Linkage

No link can be establish between financing institution and financing institution. After understanding the linkages between actors and actors, table 4.4 focuses on the linkages between factors and factors.

(ia) Finance Related- Finance Related Linkage

No link can be establish between finance and finance.

(ib) Finance Related- Capacity Building Related Linkage

Same link as established between capacity building and finance.

(ic) Finance Related- Technology Related Linkage

Same link as established between technology and finance.

(id) Finance Related- Infrastructure Related Linkage

Same link as established between infrastructure and finance.

(iia) Capacity Building Related- Finance Related Linkage

It focuses on managing finance related activities effectively. Knowing of basic and updated knowledge on finance related products though training will help the individuals, organisation, etc. in empowering the person, strengthening the organisation and achieve better output. It will lead to improved financial practices, enhance fundraising and developing new earning revenue.

(iib) Capacity Building Related- Capacity Building Related Linkage

No link can be establish between capacity building and capacity building.

(iic) Capacity Building Related- Technology Related Linkage

Same link as established between technology and capacity building.

(iid) Capacity Building Related- Infrastructure Related Linkage

Same link as established between infrastructure and capacity building.

(iiia) Technology Related- Finance Related Linkage

Use of improved financial services, practices for acquiring technology led products will lead to diffusion. Availability of clean energy products through EMI options for rural consumers will lead to diffusion of clean energy products in rural areas.

(iiib) Technology Related- Capacity Building Related Linkage

Here emphasis is given on provision of training to rural consumers and enhancing their skill set. Provision of training, demonstration of clean energy product, etc. will help in easy understanding about the product, their usability, operation and maintenance of the product, ultimately leading to diffusion of clean energy products in rural areas. It will laid the foundation for technology transfer, information exchange, adoption of best practices related to the development and application of clean energy in rural areas.

(iiic) Technology Related- Technology Related Linkage

No link can be establish between technology and technology.

(iiid) Technology Related- Infrastructure Related Linkage

Same link as established between infrastructure and technology.

(iva) Infrastructure Related- Finance Related Linkage

Infrastructure through development of roads, telecommunication, etc. will help in easy diffusion. Financing institutions need to provide funds, comprehensive financial solutions for infrastructure development in rural areas more focusing on clean energy aspect. It need to work with facilitating institutions, implementing agency, multilateral development organisations, etc. in financing funds, setting up industry, etc. emphasising more on development and application of community based technological assets like solar grid, solar rice huller, solar cold storage, street light, etc. for rural development.

(ivb) Infrastructure Related- Capacity Building Related Linkage

Capacity building of an individual, organisation, etc. by acquiring adequate knowledge and skills through provision of training, demonstration, conducting workshop, etc. helps in diffusion. Availability of a physical space in facilitating institution or implementing agency, for discussion will help in developing and fostering an environment for quick decision making to adopt clean energy product, building policies, programs, etc. for diffusion of clean energy products in rural areas.

(ivc) Infrastructure Related- Technology Related Linkage

Infrastructure and technology are required for rural development. People in rural areas need to use clean energy products for improving quality of life. Establishment of quick and reliable technology enabled service for maintenance for clean energy products will help in diffusion of clean energy products in rural areas.

4.3 Conceptual Framework for Diffusion of Clean Energy Products in the Rural Areas

For diffusion of clean energy products in rural areas it is necessary to develop a framework that cater to the needs of rural consumers and provide context specific solutions. The framework is represented in Fig 4.5. It represents different actors and their interactions. Key actors are manufactures, facilitating institutions, financing institutions, implementing agencyand rural consumers. A brief description of actors is presented below. Manufactures are basically the manufacturing enterprises which produces clean energy products, spare parts, etc. Their objective is to produce and channelise the clean energy products to the facilitating institutions at marketable price. Facilitating institutions helps in diffusion of clean energy product in the rural areas. Facilitating institutions can be the Non-Government Organisation (NGO). They work at the local and regional level with the participation and involvement of the implementing agency. They bring other actors such as universities, private vocational training institutions, self employment training institutions, working in the local area to provide training and operational support to the implementation agency. Facilitating institutions guide, mentor and provide handholding support to the implementing agency. They create market and financial linkages between different stakeholders with the implementation agency.

Financing institutions helps in diffusion of clean energy product in the rural areas. Financing institutions can be Government, Banks, Donors, Funding Agency, etc. It depends on the kind of projects or study the facilitating institution or the implementing agency undertakes. Financing institutions provide monetary assistance to facilitating institutions, implementing agencies, state nodal agency etc. with payable interest or sometimes as grant depending on the kind of projects or study they undertakes. Financing institutions supports different R&D institutions for design, development and deployment of clean energy products. It develops various policies, schemes, subsidy for promotion of clean energy products (MNRE, 2017). Financing institutions includes rural banks, micro finance institution (MFI), non-banking financial company (NBFC), private and public banks, National Bank for Agricultural and Rural Development (NABARD), Small Industries Development Bank of India (SIDBI), etc. Other financing

institutions include cooperative credit institutions, state co-operative bank, central co-operative banks, primary agricultural co-operative credit societies, and large-sized agricultural multipurpose co-operative societies, regional banks and commercial banks working in rural development sector. Implementing agency helps in diffusion of clean energy product in the rural areas. Implementing agency can be people's institutions such as SHGs and cooperatives. It improves the quality of life of rural people as a whole. It identify the problems and take collective actions to resolve a common problem of the community.

It focuses on empowering the individuals, by enriching them with adequate skills and imparting knowledge. It understand the needs of the rural people and focus on their problems by conducting meetings, interacting with them. It brings in possible solutions after consulting with facilitating institutions. It seeks guidance and handholding support from the facilitating institution. They carry out the product assessment and implement the diffusion process. Product assessment and process implementation focuses on product features and process of diffusion. Product assessment deals with the appropriateness of the technical features of the product. Features include product output (eg. lumen, l/h), storage capacity of the battery, hours of operation, price of the product, etc. These features are taken into consideration for assessing the product and deciding which product can fulfil the needs of the rural consumers. In addition it focuses on selecting the appropriate vendor, manufacturer to provide high quality, reliable, efficient and affordable clean energy product for the diffusion process.

After selection of appropriate clean energy product, frequent interactions is held with the rural consumers, local youths, key opinion leaders and other relevant people from all grounds. They are explained on the benefits of using a clean energy product, financial viability associated with it, subsidy available on adopting a clean energy product. Live demonstration of clean energy product is conducted in order to create a sense of urge and motivating the rural consumers to own it. More emphasis is given on how the use of clean energy products will help in income generation and generating employment opportunity, which will lead to the diffusion of clean energy products among them. Local service providers can be community service providers (CSPs) of the implementing agency, local entrepreneurs, local youths, etc. of the area. They provide after sales service to the rural consumers. Rural consumers provide feedback on their specific needs.

With continuous increase in purchasing power and saturation in urban markets, the focus of attention of manufacturers, dealers has shift fromurban consumers to rural consumers in particular in selling their products. Rural consumers are the most important stakeholder in the diffusion process. They are the target audience as they are the ultimate decision makers. They decide whether to adopt the product or not. They seek value for each product they buy. Manufacturers needs to focus on developing high quality, value driven clean energy products for ultimate diffusion to take place in rural areas. After getting an understanding on various actors, factors and their linkages that support in diffusion of clean energy products in rural areas, the conceptual framework for diffusion of clean energy products was applied in the rural areas.

REFERENCES

1. Human Development Reports. (n.d.). Retrieved from http://hdr.undp.org/en/2018-MPI
2. Chetan, S.S. (2018). Dawn of Solar PV Cooking. Akshay Urja. pp. 22-26.
3. Oxford English Dictionary, 7th Edition, 115th impression, (2019). pp. 152, 325, 499 and 571.
4. Suurs, R.A.A. (2009). Motors of sustainable innovation: Towards a theory on the dynamics of technological innovation systems. Unpublished doctoral dissertation. University of Utrecht, Utrecht.
5. Wieczorek, A. J., Negro, S. O., Harmsen, R., Heimeriks, G. J., Luo, L., & Hekkert, M. P. (2013). A review of the European offshore wind innovation system. Renewable and Sustainable Energy Reviews, 26, pp. 294–306.
6. Wieczorek, A. J., & Hekkert, M. P. (2012). Systemic instruments for systemic innovation problems: A framework for policy makers and innovation scholars. Science and Public Policy, 39(1), pp. 74–87.

7. Anderson, T.,Curtis. A.,and C. Wittig. (2015). Definition and Theory in Social Innovation: The Theory of Social Innovation and International Approaches. ZSI Discussion Paper, Nr. 33, Zentrum für Soziale Innovation, Wien, Austria.
8. Silva, C.E. (2008). Factors Influencing the Development of Local Renewable Energy Strategies : The cases of Lolland and Samsø Islands in Denmark.
9. Sung, B., & Park, S.-D. (2018). Who Drives the Transition to a Renewable-Energy Economy? Multi-Actor Perspective on Social Innovation. Sustainability, 10(2), pp. 448.
10. Selco Foundation and Renewable Energy Working Group (REWG) (2012). Ecosystem Creation for Off-Grid Solar: Achieving Diffusion Across India. pp. 1-10.
11. (n.d.). Retrieved from https://pmstudycircle.com/2012/03/stakeholders-in-project-management-definition-and-types/
12. Mendelow, A.L. Environmental Scanning- The Impact of the Stakeholder Concept, (1981).
13. (n.d.). Retrieved from https://mnre.gov.in/file-manager/annual-report/2017-2018/EN/pdf/chapter-4.pdf.

Table 4.1: Role of Each Actor

Actors	Role
Rural Consumers	• They buy the clean energy products • They use the clean energy products and maintain it
Facilitating institution	• Create market and financial linkages • Provide handholding support to implementing agency • Provide knowledge on technical and managerial aspects to the rural consumers • They bring in other actors
Implementing agency	• They identify the problems within the community and develop a common thinking • Take collective actions to resolve the common problem of the community • Develop work plans by involving local people, local resources • They are guided, mentored by the facilitating institutions • They implement, monitor different social, economic development programs for rural people • They create avenues for sharing information and knowledge transfer • Create awareness programs through conducting meetings, interactions, etc. with the community for rural development. • Get connected with facilitating institution
Manufacturers	• Produce clean energy products • Focuses on standardisation and quality aspect • Sells and import clean energy products to the market • Develops delivery mechanism along with serviceability
Financing institutions (Government/Banks/Donors/Funding Agencies, etc.)	• Focuses on achieving sustainable goals • Preparation of financial policies, schemes, subsidy for promotion of clean energy • Building a conducive environment for growth of R&D in manufacturing energy efficient clean energy products • Provide financial assistance to facilitating institution, implementing agency, state nodal agencies, etc. • Provide financial support for setting up testing centres, laboratories, etc. • Provide training to professionals on clean energy aspects

Table 4.2. Actors and Factors Linkages

Factors and Actors	(i) Finance Related	(ii) Capacity Building Related	(iii) Technology Related	(iv) Infrastructure Related
(a) Rural Consumers	• Provision of loans • Having an easy repayment method • Financing institutions providing saving mechanisms through post office, banks and informal group saving • Appointing of collection agents-cum- service delivery to consumers	• Creating awareness to use clean energy products through door-to-door campaigning, meetings, interactions etc • Creating awareness on availability of financing options	• Use of reliable, energy efficient and affordable products with services • Emphasis on quality aspect • Push and pull strategy	• Accessibility to basic physical infrastructure and community engagement platforms • Assets of the consumers
(b) Implementing Agency	• Get to connected to facilitating institutions • Understands various financial schemes and subsidy available • Getting collective approach • Developing professionalism for utilisation of fund and resources	• Conducting meetings and follow up with the consumers and understanding their needs • Sensitising about benefits of clean energy product and services to consumers • Creating awareness on protection against low quality products	• Protection from low quality products • Community based technological assets like solar grid, solar rice huller, solar cold storage, street light, etc. • Product assessment and procurement • Regular repair and maintenance	• Access to basic utilisation and physical infrastructure • Create physical space • Quick service delivery • Supporting infrastructure • Implementing IT enabled communication system
(c) Facilitating Institution	• Assist in making financial linkages • Provide handholding support, mentors and create market linkage • Supports the implementing agency to develop project reports and business plans, to receive funds/loans from banks and	• Facilitating training on technical and managerial aspects • Providing handholding support to implementing agency • Bring in other actors • Supports in undertaking entrepreneurial growth among	• Adopt partnership approach with different actors • Test the product and carry out suitable iterations • Becomes a hub • Collaborate to develop technological innovations	• Facilitating availability of basic physical amenities • Presence of NGO, implementing agency, local office, community centres etc.

	other financing institutions • Sustain and scale through CSR grants, funds from different corporates and other funding agencies • Facilitating consumer financing through banks, MFIs, cooperatives, regional rural banks, etc.	local youths and other stakeholders		
(d) Manufacturer	• Require financial support for design, development and production • Revenue generation through demand and sales	• Providing training • Employee development	• Design and development of high quality products • Collaborate with other actors • Develop and establish quality standards	• To establish a robust supply chain for service delivery
(e) Financing Institutions (Government/Banks /Donors/Funding Agencies, etc.)	• Provides financial assistance • Develop financial policies, schemes, subsidy • Provide custom and excise duty benefits to the manufacturers • Provide loans, CSR grants for undertaking different projects in the clean energy area	• Creating awareness and sensitising about importance of clean energy policies • Facilitates in conducting seminars, workshops, conferences etc. for policy makers, manufacturers, suppliers on clean energy policies, future prospects, targets programs and subsidy	• Taking up high end technology transfer from other countries, creating an conducive environment for growth of R&D on energy efficient products. • Establishment of testing centres both at state and regional level • Developing quality standards and accreditation for clean energy products	• Facilitating creation of required infrastructure for technology transfer, patenting, new product development , etc. • Helps in setting manufacturing units using advance technology that supports the ecosystem

Table 4.3. Actors and Actors Linkages

Actors and Actors	(i) Rural Consumers	(ii) Implementing Agency	(iii) Facilitating Institution	(iv) Manufacturer	(v) Financing Institutions
(a) Rural Consumers	No Linkage	• Association of people • Integral part and elementary unit • Understanding of needs	• Undertakes rural development programs • Achieve socio-economic development	• Buy and sell products • Provide service and maintenance	• Loans, saving mechanism, other financing products • Proper utilisation of loans and repayment
(b) Implementing Agency	Same as (iia)	No Linkage	• Support services • Create market and financial linkages	• Establishment of a robust supply chain	• Financial assistance with payable interest • Grants, CSR initiatives
(c) Facilitating Institution	Same as (iiia)	Same as (iiib)	No Linkage	• Produce and channelise products at marketable price	• Bulk, lending, soft loans, etc. • Information on schemes, subsidy, etc. available
(d) Manufacturer	Same as (iva)	Same as (ivb)	Same as (ivc)	No Linkage	• Provide financial support • Loans for fixed capital and working capital
(e) Financing Institutions (Government/Banks/Donors/Funding Agencies, etc.)	Same as (va)	Same as (vb)	Same as (vc)	Same as (vd)	No Linkage

Table 4.4. Factors and Factors Linkages

Factors and Factors	(i) Finance Related	(ii) Capacity Building Related	(iii) Technology Related	(iv) Infrastructure Related
(a) Finance Related	No Linkage	Arranging and managing finance	• Availability of EMI	• Provision of funds, financial solutions
(b) Capacity Building Related	Same as (iia)	No Linkage	• Locally trained manpower • Ease of operation of products	• Enhancing employee capability • Availability of physical space

Factors and Factors	(i) Finance Related	(ii) Capacity Building Related	(iii) Technology Related	(iv) Infrastructure Related
(c) Technology Related	Same as (iiia)	Same as (iiib)	No Linkage	• Basic physical infrastructure • Communication
(d) Infrastructure Related	Same as (iva)	Same as (ivb)	Same as (ivc)	No Linkage

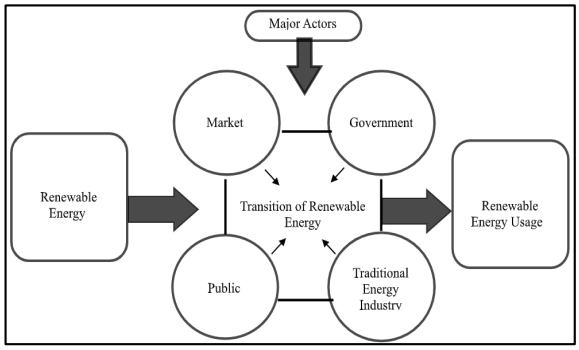

Fig 4.1.Renewable Energy Transition Framework (Adapted from Sung and Park, 2018)

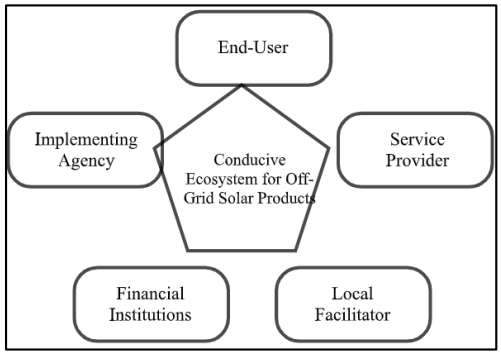

Fig 4.2. Ecosystem for Off-Grid Solar Products(Adapted from SELCO and REWG, 2012)

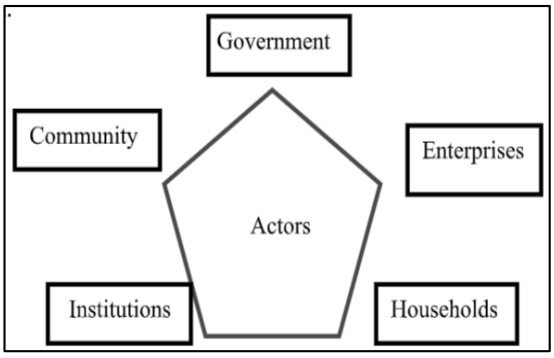

Fig 4.3: Actors for Diffusion of Clean Energy Products (Adapted from SELCO and WWF, 2015)

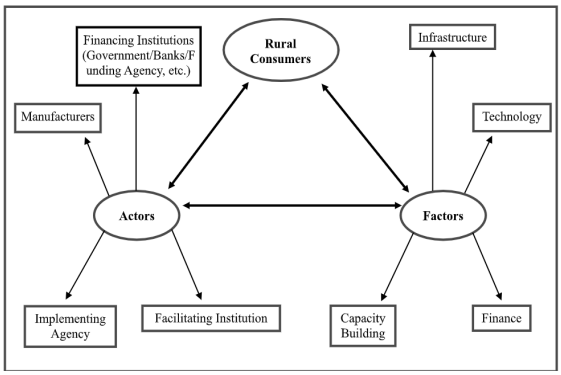

Fig 4.4: Clean Energy Ecosystem

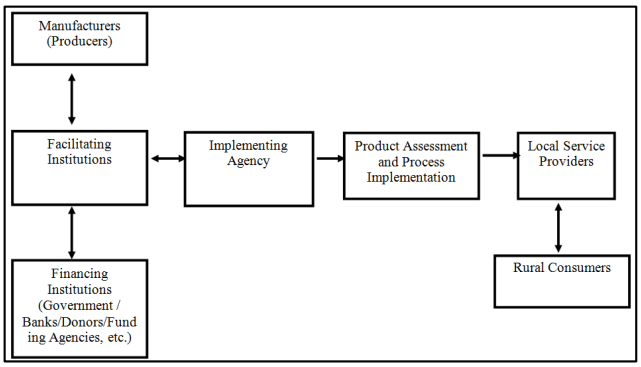

Fig 4.5: Framework for Diffusion of Clean Energy Products in Rural Areas

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RESEARCH ARTICLE

Integrated Plant Protection Measures and Micronutrient Foliar Spray on Growth and Yield of Green Gram (*Vigna radiata* L.)

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ABSTRACT

The productivity of green gram (*Vigna radiata* L., family Fabaceae**)** is mainly dependent on high yielding varieties with desired quality attributes, nutrient received, other favorable cultural practices and prevalent climatic condition in the cultivated area. The present study was undertaken in Krishi Vigyan Kendra (KVK), Angul, located in Panchmahala to study the effect of micronutrient foliar spray with oregon 80 on growth and yield of green gram variety IPM 02-03in two villages i.e., Sankhapur and Partara. For this study soil samples analysis was done on prior to cultivation of green gram in order to know the suitability soil composition for growth and yield. Carbon, potassium, sulphur, boron, ferrous and zinc content of different soil samples collected were found to be maximum of 0.53%, 196 kg/ha, 4.86 ppm, 0.53 ppm, 4.25 ppm and 0.51 ppm respectively from Sankhapur village. pH of soil samples ranges from 5.31 to 5.64 which is not optimum for growth and yield of green gram. Green gram thrives in nutrient rich soil with optimum level of pH 6 to 7.5. In this study, micronutrient like oregon 80 was sprayed along with other plant protection measures to promote growth and increase in yield. Increase in numbers of nodules per plant, dry weight of nodules/plant was noticed in the sub plot which was subjected to both micronutrients foliar application and other crop protection measures like application of pesticides, insecticides and fungicides namely immidacloprid, carboxin, thiram to reduce the disease and pest infestation in green gram crop. Increase in net return of 88.56% was achieved with micronutrient foliar application in improved variety IPM 02-03 variety.

Keywords: Green gram, Fabaceae, immidacloprid, micronutrient foliar sprayand oregon 80.

INTRODUCTION

Green gram is one of the important pulse crops in India. It is known for its rich source of protein, iron and high fiber content. Green gram is a dicotyledonous plant with the scientific name *Vigna radita* (L.) R. Wilczek commonly known as moong bean belonging to family Fabaceae. Use of improved crop management packages can invariably increase the productivity by 50 to 100 per cent. In addition to other management practices such as irrigation and plant protection, green gram responded to plant population and mineral nutrition especially, when applied in balanced amount by micronutrient foliar spray.

Nutrients are important and crucial elements, which are required for the plant for its growth and development. Plant growth regulators are chemical substances and when applied in small amounts, they bring rapid changes in the phenotypes of the plant and also influence the plant growth, right from seed germination to yield to senescence. Growth regulators can improve the physiological efficiency including photosynthetic ability and can enhance effective partitioning of accumulates from source and sink in the field crops. Foliar application of growth regulators and chemicals at the flowering stage may improve the physiological efficiency and may play a significant role in raising the productivity of the crop (Solaimalai et al., 2001). Hence the present study was undertaken in green gram to study the effect of fertilization through foliar spray on the growth and yield of green gram. The objectives of the study is to analyze the soil samples collected from two villages of Angul district, to know know the soil fertility status required for growth and yield prior to cultivation. Micronutrients application through foliar application was done to observe the response of green gram and to estimate the yield/ production rate. Increased number of pods, seed yields, test weight and protein content were observed in green gram when sown after application with 30 kg N and 60 kg P_2O_5/ha (Nadeem et. al, 2004). Increased grain yield of green gram was also obtained with application of 2t compost and 1t gliricidia per hectare (Sharma et al., 2004). Oad et. al., (2003) stated that application of 100 kg P and K induced to get maximum green gram seed yield.

MATERIALS AND METHODS

Field study was conducted during *Rabi* 2018-19 at two villages i.e. Sankhapur and Partara of Angul district under the management and extension activity of Krishi Vigyan Kendra (KVK), Angul, located in Panchmahala (Fig. 1).These two villages were selected by KVK, Angul for cluster frontline demonstration (FLDs) as these villages are easily accessible for monitoring and collecting feedback from farmers. Soil testing was done prior to sowing of seeds.

Soil testing

Soil testing was done to optimize crop production and to know the nutritional content of the soil and accordingly nutritional balance to be done by application of fertilizer. Random soil samples from these villages were collected from the experimental plot up to the depth of 6 to 30 cm. Chemical analysis of soil was done to study the physico chemical properties of soil. The samples were collected before the addition of manure from a depth of 6 inches. The samples were taken by cutting the land in 'V' shape.

Field preparation

Before sowing of the seeds, the fields were prepared. The left over residues of the previous crops were cleaned up and the fields were ploughed by use of rotavator. Cow dung manure was added. Subplots were made for carrying different parameter testing.

Sowing of seed

Seeds of IPM 02-03 variety were procured from Orissa University of Agriculture and Technology, Bhubaneswar. Sowing of seeds of green gram was done in mild winter as a *Rabi* crop in different villages i.e. Partara, Sankhapur. Seeds were treated before sowing with a mixture of carboxin and thiram at the rate of 3gm per kg of seed to disinfect from soil borne pathogenic fungi present on seed surface. The seeds were subjected to *Rhizobium* culture at the rate of 20g per kilogram of seed was sown in small sub plots using traditional hand sowing method (Table 1).

Foliar spray

Integrated pest management (IPM) measures were given in their recommended doses through foliar application during various stages of crop growth (Table 1) in all the sub plots. No foliar application of micronutrient i. e. oregon 80 was given to the sub plots, where IPM 02-03 variety was grown as control (A_0). Foliar spraying of oregon 80 (a new form of liquid fertilizer) and other IPM measures were sprayed using hand sprayer in the early morning or late afternoon to prevent evaporation in other subplots.The spraying process was carried on in regular intervals in different cultivated lands to treat the diseases and obtain higher seed yield. Study was conducted to monitor the effect of foliar application of micronutrients along with other plant protection measures on growth and yield of green gram. Observations were taken at regular interval in randomized block design (RBD) method with three replications during 2018 and 2019 at KVK, Angul.

RESULTS

It was evident from table 2 total six soil samples were collected from two villages namely Sankhapur and Partara. Four samples were collected from Sankhapur village, and two samples were from Partara village. These samples were tested to measure the soil fertility status. Different parameters like pH, electrical conductivity (EC), organic carbon, available Nitrogen (N), Phosphorous (P), Potassium (K), Sulphur(S), Boron (B), Ferrous (Fe), and Zinc (Zn) were measured and depicted in Table 2.

It was observed in Table 2 that pH of the samples of Sankhapur village varied from 5.38 to 5.64 and pH of 5.58 and 5.31 were noted for the two samples collected from Partara village. Percentage of organic carbon and available nitrogen content in terms was found to be maximum of 0.58 and 0.283 in one sample collected from Partara village in comparison to other soil samples collected. But Carbon, Potassium, Sulphur, Boron Ferrous and Zinc content was found to be maximum of 0.53%, 1.96 ppm, 4.86 ppm, 0.53 ppm, 4.25 ppm and 0.51 ppm respectively from the soil sample D from Sankhapur village. Soil fertility status of one soil sample collected from Partara village showed minimum organic carbon (0.38%), P (4.46), N (0.15%) and K (1.34 ppm); Sulphur (2.29 ppm), Boron (0.32 ppm), Ferrous (0.21ppm) and Zinc (0.38 ppm).

Effect on growth attributes

It was observed in Table 3 that there was promising difference in plant height. It was observed that maximum growth of the plants was observed in the plots where foliar application was done with oregon 80 over untreated seedlings. This was resulted because of higher absorption of micronutrients than the absorption of micronutrients from the soil. Highest plant height of 19.45 cm was recorded in the plots where seedlings were raised with oregon 80 treated fields in comparison with the controlled plants with average plant height of 15.75 cm after 20 days of showing. Average plant height was also recorded after 40 and 60 days after showing and the result depicted in table 3 indicated that the average plant height was found to be less with 33.27, 51. 24 cm. in control plants than the plants exposed with micronutrients foliar spray viz. oregon80 with 43.34 and 68.76 cm respectively. The average numbers of root nodules per plant were calculated by randomly taken ten plants after 20, 40 and 60 days of plant growth for

both control and foliar treated plants. The data revealed that the number of root nodules per plant was 21.23, 39.31 and 30.77 in control plants and 27.36, 51.66 and41.24 in oregon 80 foliar sprayed plants indicating increase in root nodules formation per plant which will enhance the nitrogen fixing ability and thereby will induce plant growth and seed yield. Dry weight of nodules per plant was calculated and depicted in table 3. It was observed that the dry weight of nodules per plant in control plants were 0.024, 0.042 and 0.031gm. per plant which were less against plant that were treated with Oregon 80 with 0.029, 0.054 and 0.039 gm. per plant after 20 DAS, 40 DAS and 80 DAS respectively indicating that oregon 80 had exerted impact on higher nodule weight per plant.Application of plant protection measures like immidacloprid, mixture of prophenophos and cyper methrine, dimethoate and thiomethoxam to control as well as micronutrient (oregon 80) foliar applied plants showed variation of plant growth , number of nodules per plant and dry weight of nodules in gms per plant on 40 DAS and 60DAS. The height of plant was more of 49.43 cm in oregon 80 treated plants incomparison to control plants with 44.63cm. Variation in plant height was also noticed in 60 DAS in control sub plots with all the plant protection measures except treatment with oregon 80 and all the plant protection measures with micronutrient foliar sprayed sub plot plants with 47.48 and 56. 23cm respectively. Maximum number of nodules per plant was noticed in 40 DAS in both control sub plots with all the plant protection measures except treatment with oregon 80 and all the plant protection measures including micronutrient foliar sprayed sub plot plants with 46.46 and 49.86 nodules respectively followed by 60 DAS and 20 DAS

Minimum numbers of nodules were detected in 20 DAS control plants with all the plant protection measures except treatment with oregon 80. Dry weight of nodules per plant was maximumu in 40 DAS plants in both control sub plots with all the plant protection measures except treatment with oregon 80 and all the plant protection measures including micronutrient foliar sprayed sub plot plants with 0.059 and 0.073 gm per plant respectively. In 60 DAS, the plants showed less number of nodules per plant might be because of integrated nutrient supply along with nitrogen became a limiting factor during pod formation as the nodules begin to disintegrate and a decline in photosynthetic activity of plants was also found to be reduced. From the statistical analysis it was observed from Table 4 that plant height increased significantly with 0.1 with application of micronutrients foliar spray along with other plant protection measures. Number of root nodules per plant was also found to be increased significantly with coefficient of variation of 0.50 with foliar micronutrients application than in conventional method of cultivation.

Data in Table 5 showed that the benefit- cost ratio (B: C) of control plots was 1:56 and plant protection applied plots was found to be 1.88 with a net return of 88.56%. Net return of treated plots was Rs. 19750.5 in comparison to control plot without any application of plant protection measures including micronutrient spray (Fig. 2). Most of the nutrients are absorbed through leaves. Therefore, foliar feeding practice is found to be more effective for increasing the production rate with regular plant protection programs. This investigation was also found to be effective in higher seed yield than the control plants which proved that foliar spray is important to increase the productivity of green gram.

DISCUSSION

Applications of nitrogen, phosphorus and potassium at the rate of 125 per cent along with 2 % DAP foliar spray and one percent SOP recorded higher nitrogen, potassium and sulphur uptake by green gram and higher soil available N (Sathyamoorthi et. al., 2008a, 2008 c). It is evident from the review that the foliar spray of DAP is more important for increasing the yield along with other chemicals like KCL and K_2SO_4. K_2SO_4 at the rate of one per cent foliar spray showed significant increase in seed yield by 12.2 per cent (Chandra Babu et al., 1985). Radhamani et al., (2003) reported that spraying of 2% DAP in combination with NAA at the rate of 40 ppm at 50 % flowering stage increased the number of pods per plant and number of pods and seed yield. Sathyamoorthiet. al, (2008 b) recorded higher length of root and pods per plant as well as number of seeds per pod on application of 125 per cent of NPK. Qureshi *et al* (2011) observed inoculation of *R. phaseoli* and *B. megaterium* with 20 kg nitrogen and 50 kg phosphorous per

hectare enhanced green gram growth, number and mass of root nodules and pod per plant.The combined inoculation of *Rhizobium* and PSB along with application of sulphur at the rate of 30 kg per hectare with gypsum and 2% DAP foliar spray of DAP recorded significant number of pod per plant, number of seeds per pod, seed yield and test weight (Ghose and Joseph, 2008).

CONCLUSION

It was observed that pH of the samples of Sankhapur village varied from 5.38 to 5.64 and pH of 5.58 and 5.31 were noted for the two samples collected from Partara village respectively. Percentage of organic carbon and available nitrogen content in terms of kg/ha was found to be more of 0.58 and 283 kg/ha in one sample collected from Partara village in comparison to other soil samples collected. But Carbon, Potassium, Sulphur, Boron Ferrous and Zinc content was found to be maximum of 0.53%, 196 kg/ha, 4.86 ppm, 0.53 ppm, 4.25 ppm and 0.51 ppm respectively from the soil sample D from Sankhapur village. Total area of 20 hectares was taken for demonstration variety IPM 2-3 in one cluster with a total of 50 farmers in Sankhapur and Partara village. Cost benefit ratio (B: C) of control plots was 1:56 and plant protection applied plots was found to be 1.88 with a net return of 88.56%. Net return of treated plots was Rs. 19750.5 in comparison to control plot without any application of plant protection measures including micronutrient spray.From this study it can be concluded that plant growth substances help to bring rapid changes in the phenotypes such as height of the plant, number of root nodules per plant which is responsible for atmospheric nitrogen fixation and also improves the growth, translocation of nutrients to economic parts and ultimately improve the productivity of the crops. Results indicated that plots with micronutrient foliar spraying of oregon 80 influenced the growth parameter, nodulation and seed yield. Most plant nutrients are absorbed through leaves. Therefore, foliar feeding practice is found to be more useful in early maturing crops in combination with regular plant protection programs.

Conflict of interests

There is no conflict of interest associated with this publications and no financial support has been received for this work.

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Author's Contributions

In this research work, both the authors contributed effectively. Aliva Das was carrying out all the experimental work in KVK, Angul, Odisha as the project assistant of ICAR. ; Sagarika Parida designed the experiments, supervised as the guide, analysed the data and wrote the manuscript

REFERENCES

1. Chandra Babu R, Sadasivam R, Natarajaratnam N, and Sreerangasamy SR. Effect of potassium nutrition on growth and yield of black gram. Madras Agric. J. 1985; 72(10): 595-596.
2. Ghosh MK and Joseph SA. Influence of biofertilizers, foliar application of DAP and sulphur sources on yield and yield attributes of summer green gram (*Vigna radiata* L. Wilczek). Legume Res.2008; 31(3): 232-233.
3. Nadeem MA, Ahmad R and Sarfraz Ahmad M.Effect of seed inoculation and different fertilizer levels on the growth and yield of mung bean (*Vigna radiata* L.). J. agron.2004; 3(1):40-42.
4. Oad FC, Shah AN, Jamro GH and Ghaloo SH. Phosphorus and potassium requirements of mung bean (*Vigna radiata*). Pak. J. Applied Sci.2003; 3 (6): 428-431.

5. Qureshi MA, Shakir MA, Iqbal A, Akhtar N and Khan A. Co-inoculation of phosphate solubilizing bacteria and Rhizobia for improving growth and yield of mung bean (*Vigna radiata* L.). The J. Animal and plant Sci.2011; 21(3): 491-497.
6. Radhamani S, Balasubramanian A, and Chinnusamy C. Foliar nutrition with growth regulators on the productivity of rain fed green gram. Agric. Sci. Digest. 2003; 23(4): 307-308.
7. Sathyamoorthi K, Mohamed Amanullah M, Vaiyapuri K and Somasundaram E. Growth and yield of greengram [*Vigna radiata* (L.) Wilczek] as influenced by increased plant density and nutrient management. Internat J. Agric. Sci. 2008a: 4 (2): 499 – 505.
8. Sathyamoorthi K, Mohamed Amanullah M, Somasundaram E and Vaiyapuri K.Yield attributes and yield of greengram (*Vigna radiata* (L.) wilczek) as influenced by increased plant density and nutrient management. Internat. J. agric. Sci.2008b;4 (2): 719 – 724.
9. Sathyamoorthi K, Mohamed Amanullah M, Somasundaram E and Vaiyapuri K. Influence of increased plant density and nutrient management on the yield and economics of greengram (*Vigna radiata* (L.) wilczek).Internat. J. Plant Sci. 2008c; 3 (2): 450-455.
10. Sharma, K.L., K. Srinivas, U.K. Mandal, K.P.R. Vittal, J. K. Grace and G.R. Maruthi Sankar. 2004. Integrated nutrient management strategies for sorghum and greengram in semi-arid tropical alfisol. Indian J. Dryland Agric. Res. & Dev. 19(1): 13-23.
11. Solaimalai, A., C. Sivakumar, S. Anbumani, T. Suresh and K. Arumugam. 2001. Role of plant growth regulators in rice production-a review. Agric. Rev. 22: 33-40.

Table 1. Application of Micronutrients and other plant protection measures and their effect

Treatment	Micronutrients/Plant Protection measures	Prepared Doses	Recommended Dose	Effect
Seed treatment (before sowing)	Carboxin + thiram	2gm/kg of seed	2gm/kg of seed	Protected from early seed borne diseases
	Rhizobium culture	20gm/kg of seed	20gm/kg of seed	Induces root nodule formation
Seedling of 15 DAS	Oregon 80	2 ml/lit of water	1 lit/ha	Promising growth with higher yield
Seedling of 20 DAS	Immidacloprid	3 ml /10 ml. of water	1 lit/ha	control of aphids
Seedling of 25 DAS	Prophenophos + Cypermethrine	2ml/1lit of water	1 lit/ha	control of Foliage beetle
Vegetative stage	Thiamethoxam	5 gms/15 lit of water	125g/ha	control white fly during vegetative stage
Plants before flowering	Dimethoate	2 ml/lit	1 litre/ha	control of early sucking pests

Table 2. Soil properties of experimental plot of Sankhapur and Partara village, Angul District

Sample no.	pH	EC (dSm^{-1})	Organic Carbon (%)	Nitrogen (%)	Phosphorous (ppm)	Potassium (ppm)	Sulphur (ppm)	Boron (ppm)	Ferrous (ppm)	Zinc (ppm)
A(Sankhapur)	5.38	0.148	0.46	0.179	7.8	1.58	3.14	0.35	0.34	0.45
B(Sankhapur)	5.53	0.152	0.52	0.209	10.2	1.76	3.25	0.39	0.35	0.46
C(Sankhapur)	5.42	0.136	0.45	0.225	7.5	1.65	3.05	0.42	0.27	0.44
D(Sankhapur)	5.64	0.176	0.53	0.265	15.6	1.96	4.86	0.53	4.25	0.51
E(Partara)	5.58	0.172	0.58	0.283	10.8	1.83	4.37	0.48	4.12	0.48
F(Partara)	5.31	0.09	0.38	0.152	4.46	1.34	2.29	0.32	0.21	0.38

Table 3. Effect of Oregon 80 and other plant protection measures on growth parameters of green gram

Treatment	Plant height(cm)			No.of nodules/plant			Dry weight of nodules (gm/plant)		
	20 DAS	40 DAS	60 DAS	20 DAS	40 DAS	60 DAS	20 DAS	40 DAS	60 DAS
A_0	15.75	33.27	51.24	21.23	39.31	30.77	0.024	0.042	0.031
B_0		43.34	68.76	27.36	51.66	41.24	0.029	0.054	0.039
A1	-	44.63	47.48	23.27	46.46	32.57	0.027	0.059	0.042
B1	-	49.43	56. 23	28.57	49.86	47. 27	0.031	0.073	0.051

Table 4. Standard deviation and coefficient of variation of Oregon 80 and other plant protection measures on growth parameters of green gram

Attributes of Plant	Cultivation Practices	Standard deviation	Coefficient of variation
Plant height	A_0	17.75	0.53
	B_0	24.66	0.56
	A_1	26.63	0.86
	B_1	34.95	0.1
Root nodule/plant	A_0	9.04	0.29
	B_0	12.19	0.30
	A_1	11.67	0.34
	B_1	22.13	0.50
Dry weight of nodules/plant	A_0	0.009,	0.28
	B_0	0.013	0.31
	A_1	0.016	0.38
	B_1	0.021	0.41

Table 5. Comparative expenditure and returns in Rs/ha in between control and treated sub plots

Production in control plot				Production in plant management measures with Oregon 80 plot				Net returns Increase (%)
Gross expenses Cost (Rs/ ha)	Gross return (Rs/ ha)	Net Return (Rs/ha)	B:C ratio	Gross Cost (Rs/ ha)	Gross return (Rs/ ha)	Net Return (Rs/ha)	B:C ratio	
18850.0	29324.5	10474.5	1.56	22508	42258.5	19750.5	1.88	**88.56**

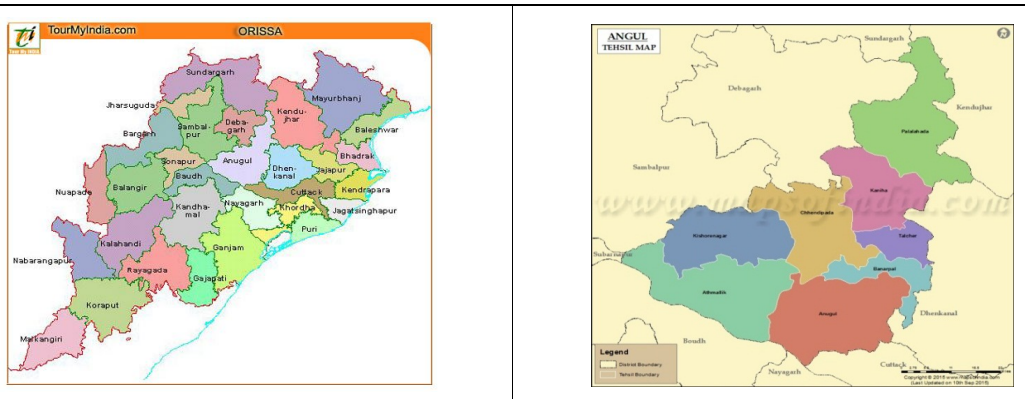

Fig. 1. Odishamap showing Angul district map

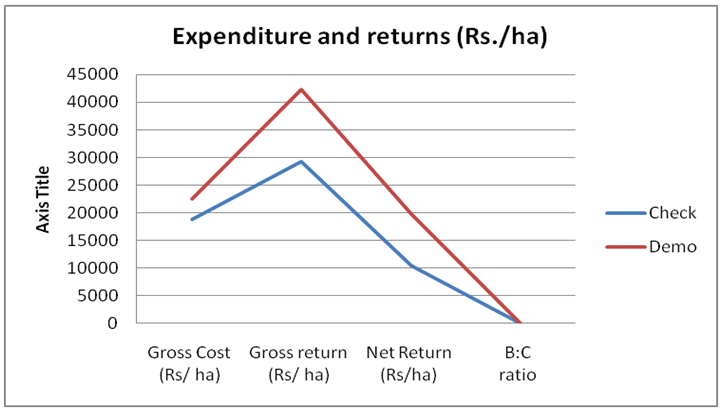

Fig. 2. Expenditure and returns in Rs/ha in between check and demonstrated varieties

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RESEARCH ARTICLE

Potential Risk Factors Influencing the Development of End-Stage Renal Disease

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ABSTRACT

End-stage renal disease (ESRD) is the irreversible decline of the kidney function, which has affected huge population proportions in recent years. The associated symptoms and comorbidities precipitate the condition, increasing severity leading to mortality. The aim of this study was to determine the frequency of ESRD and its risk factors among the patients presenting to the medical unit of Saidu Group of Teaching Hospitals (SGTH), Swat. A cross sectional study was conducted from June to December 2018, at the medical unit of SGTH, Swat. Total 300 patients either male or female, having clinical profiles potentially predictive of ESRD and aged ≥15 years enrolled in the study after obtaining written informed consent. Patients were subjected to abdominal or pelvic ultrasound and estimation of Glomerular filtration rate (eGFR) to confirm the presence of ESRD. The diagnosed ESRD cases were then scrutinized to evaluate the associated risk factors such as uncontrolled diabetes, hypertension, glomerulonephritis and renal calculi. Data was analyzed using SPSS Version 20.0. Out of 300 patients enrolled, 56.7% were presented with persistent vomiting, anuria (44.7%), oliguria (39.7%), dyspnea (35.5%) and acidotic breathing (35%) while 296 of them were diagnosed with ESRD upon clinical investigations. These symptoms continued to appear for 19-22 days in the majority of ESRD cases (43.2%). Glomerulonephritis (37.1%), Uncontrolled Hypertension (29.7%), Uncontrolled Diabetes Mellitus (25.6%) and Renal Calculi (7.43%) were the significant risk factors for ESRD ($p<0.05$). No statistical significance exists between age, gender, duration of symptoms and ESRD ($P>0.05$). ESRD was highly prevalent among the studied population. Glomerulonephritis and Hypertension were found to be the common risk factors leading to ESRD, the consistent change in the intensity of the disease risk and symptoms highlights the need to build up strategies confronting the ESRD associated mortality and morbidity rate.

Keywords: End Stage Renal Disease, Frequency, Risk Factors, Symptoms of ESRD.

INTRODUCTION

Globally, the mortality and morbidity rate associated with non-communicable diseases have excelled rapidly as compared to communicable diseases i.e. majority population undergoes premature deaths due cardiovascular or renal disorders [1]. It is estimated that the disease burden is high among the low to middle income countries [2]. The kidney disease is a universal threat as this health crisis has increased rapidly since 2005, i.e. out of 58 million deaths, 35 million were associated with chronic kidney disease (CKD) [3]. Approximately 10% of the world's population is suffering from renal burden, million people die due to inappropriate and unaffordable treatment [4]. ESRD is the last stage kidney disease causing irreversible damage and is fatal if not treated with dialysis or renal transplant in time. According to the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF KDOQI) classification, ESRD is the stage 5 CKD. The estimated glomerular filtration rate (eGFR) among ESRD patients is < 15 mL/minute per 1.73 m2 body surface area [5]. Mostly people with untreated ESRD die, due to poor diagnosis, progressive uremia, CVD complications or if not provided dialysis [6-8]. ESRD patients mostly encounter fluid retention, altered bone and mineral metabolism and anaemia etc [9].

For the patients and their families throughout the world particularly in developing countries like Pakistan, ESRD is a devastating medical, economic and social issue [10]. The major risk factors include uncontrolled hypertension, family history of dialysis or renal transplant, familial kidney diseases, acquired renal pathologies, acquired immunodeficiency syndrome (AIDS) and drug intoxication [11]. A local study reported that the common risk factors responsible for ESRD include chronic glomerulonephritis (37%), uncontrolled diabetes (10%), uncontrolled hypertension (12%) and renal calculi (5%) 4. Many of the other risk factors like chronological age, gender, racial background, obesity, proteinuria, decreased hemoglobin (Hb), nocturia, hyperuricemia, smoking and drug abuse are indirect modifiers of ESRD [12].

The social and religious beliefs have greatly impacted the disease management, as locally organ donation and transplant are not appreciated religiously. The epidemiological assessment of the disease burden is hard and inadequate in Pakistan, due to the lack of central registry for ESRD [13]. The aim of the current study is to measure the frequency of ESRD and its risk factors, in order to monitor the disease progression and provide timely intervention.

METHODOLOGY

This cross-sectional study was conducted at the medical unit of SGTH, Swat from June to December 2018. A sample of 300 was calculated using the World Health Organization (WHO) sample size calculator (5% frequency of renal calculi as a factor leading to ESRD; Confidence level of 95%, 2.5% margin of error)4. Both male and female patients aged ≥15 years, with clinical features highly indicative of ESRD were included in the study. While patients already diagnosed with ESRD, liver diseases or history of renal transplant were excluded from the study sample. The study proposal was approved by the institutional Ethics Committee and written informed consent was obtained from each patient enrolled. The data including detailed history and baseline demographics like age, gender, symptoms and duration was collected. To confirm the presence of ESRD, abdominal ultrasound was performed by a consultant radiologist at the radiology department and blood samples were also drawn in order to estimate the Glomerular Filtration Rate (GFR). The ESRD patients were then scrutinized to observe the leading risk factors.

SPSS version 20.0 was used for statistical analysis, quantitative variables like age and duration of symptoms observed while frequencies and percentages were calculated for all qualitative variables like gender, ESRD, symptoms and risk factors. The baseline characteristics and risk factors of ESRD were compared using the Chi-square test where $p<0.05$ was considered significant.

RESULTS

Out of 300 patients, there were 184 (61.3%) males and 116(38.6%) females with a mean age of 43.47 ± 16.60 years. ESRD was confirmed among 98.6% patients enrolled as per the clinical suspicion. Persistent vomiting (56.7%), anuria (44.7%) and oliguria (39.7%) are the most common symptoms observed and the mean duration of the symptoms was 20.50 ± 2.7 days (Table 1). Age wise distribution shows insignificant association, ESRD was most common among patients aged 46 to 60 years (34.1%). Male ESRD cases were greater in number as compared to females i.e. 61.1% vs 38.8%, although the relationship was insignificant (p=0.572). ESRD diagnosis was more frequent among the patients observed with the symptom duration of either 19 to 22 days (43.2%) or 23 to 26 days (29.7%). There was a significant association in between risk factors and presence of ESRD ($p<0.05$), most common risk factor among patients with ESRD was glomerulonephritis (37.1%) followed by uncontrolled hypertension (29.7%), uncontrolled diabetes mellitus (25.6%) and renal calculi (7.43%) (Table 2).

DISCUSSION

It is important to understand the need for symptomatic management and effective treatment of ESRD, as the disease risk is growing rapidly and hence much work is required in the field to improve the quality of life (QoL) of the patients suffering from renal disorders [14]. The patients of ESRD are known to be treated with dialysis and renal transplant, where majority cases undergo hemodialysis, followed by kidney transplants and peritoneal dialysis9. It is evident that early detection leads to decreased incidence rate of ESRD, for which regular checkup and screening are highly recommended [15]. Patients suffering from ESRD suffer from a number of comorbid conditions such as accelerated hypertension and atherosclerotic disease of the large arteries etc [16]. The frequency of patients developing ESRD in association to hypertension, is increasing rapidly and accounts for 25% of the incidence rate [17]. Moreover, literature indicates that the creatinine clearance rate declines with aging which is also correlated with high blood pressure [18].

This study was aimed to determine the frequency of ESRD and its related risk factors. We also compared the age, gender, duration of symptoms and frequency of risk factors in association with ESRD. The study results indicated the presence of ESRD among 98.6% of the patients enrolled, with the risk profile indicating glomerulonephritis as the major risk factor among majority cases followed by uncontrolled hypertension, uncontrolled diabetes mellitus and renal calculi. A longitudinal study conducted in China to evaluate the risk factors for ESRD also reported that the key risk factors were proteinuria, high blood pressure, obesity, glomerulonephritis, diabetes mellitus and renal calculi19. Moreover, they also presented the correlation of indirect factors affecting ESRD like gender, race, body mass index (BMI), and serum creatinine level, which were not included in our study [19]. Similar to our findings, a follow up study reported sustained elevation in creatinine level and reduced GFR [20].

No significant association of age, gender and duration of symptoms was observed in relation to ESRD, while significant impact of risk factors was observed among the ESRD patients ($P<0.05$). The disease prevalence is more in the older age group i.e. 34.1% of the patients detected with ESRD were 46 to 60 years old which is also, supported by a by conducted by Ishani and her colleagues [21]. The results may vary based on the genetic, ethnic and environmental factors affecting the respective population. This study highlighted many of the potential predictors of ESRD and also presented the symptomatic profile of the patients. But the limitations that must be considered include, the medication use and other contributing factors like circulating inflammatory markers were not assessed. Moreover, the sample was collected from a single center and hence our results are not generalizable to the overall Pakistani population.

CONCLUSION

The prevalence of ESRD was high among the studied population, which highlights the need to understand and work on the disease severity, morbidity, and mortality. Building on this foundation, interventional studies are required to control the disease risk and alleviate symptoms in order to improve QoL of the patients in addition to effective treatment.

Conflicts of Interest

The authors have no conflicts of interest.

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REFERENCES

1. Global facts: About kidney disease, National Kidney Foundation. [Updated March, 2015] Available at: https://www.kidney.org/kidneydisease/global-facts-about-kidney-disease
2. Couser WG, Remuzzi G, Mendis S, Tonelli M. The contribution of chronic kidney disease to the global burden of major noncommunicable diseases. Kidney Int. Dec 2011;80(12):1258-1270.
3. Levey AS, Atkins R, Coresh J, et al. Chronic kidney disease as a global public health problem: approaches and initiatives - a position statement from Kidney Disease Improving Global Outcomes. Kidney Int. 2007;72(3):247-259.
4. World Kidney Day: Chronic Kidney Disease. 2015; http://www.worldkidneyday.org/faqs/chronic-kidney-disease/
5. National Kidney Foundation. Kidney Disease Quality Outcomes Initiative (K/DOQI). [Updated June, 2010]. Available at: http://www.kidney.org/professionals/kdoqi/guidelines.cfm
6. United States Renal Data System. Annual data report atlas of chronic kidney disease & end-stage renal disease in the United States. USRDS 2009.
7. UK Renal Registry. [Updated 2 June 2010]. Available at: http://www.renalreg.com.
8. Powe NR, Jaar B, Furth SL, Hermann J, Briggs W. Septicemia in dialysis patients: incidence, risk factors, and prognosis. Kidney Int. 1999;55(3):1081-1090.
9. Abbasi MA, Chertow GM, Hall YN. End-stage renal disease. BMJ Clin Evid. 2010; 2010
10. Sakhuja V, Kohli HS. End-stage renal disease in India and Pakistan: incidence, causes, and management. Ethn Dis. 2006;16(2):S2-S6.
11. Tonelli M, Wiebe N, Culleton B, House A, Rabbat C, Fok M, McAlister F, Garg AX. Chronic kidney disease and mortality risk: a systematic review. J Am Soc Nephrol. 2006;17(7):2034-2047.
12. Levin A, Hemmelgarn B, Culleton B, Tobe S, McFarlane P, Ruzicka M, Burns K, Manns B, White C, Madore F, Moist L. Guidelines for the management of chronic kidney disease. Can Med Assoc J. 2008;179(11):1154-1162.
13. Sakhuja V, Sud K. End-stage renal disease in India and Pakistan: burden of disease and management issues. Kidney Int. 2003;63:S115-118.
14. Murtagh FE, Addington-Hall J, Higginson IJ. The prevalence of symptoms in end-stage renal disease: a systematic review. Adv Chronic Kidney Dis. 2007;14(1):82-99.
15. Ojo A. Addressing the global burden of chronic kidney disease through clinical and translational research. Trans Am Clin Climatol Assoc. 2014;125:229-246.

16. Weisstuch JM, Dworkin LD. Does essential hypertension cause end-stage renal disease? Kidney Int Suppl. 1992;36:S33-S37.
17. O'Callaghan-Gordo C, Shivashankar R, Anand S, Ghosh S, Glaser J, Gupta R, et al. Prevalence of and risk factors for chronic kidney disease of unknown aetiology in India: secondary data analysis of three population-based cross-sectional studies. BMJ Open. 2019;9(3):e023353-e9.
18. Jones ESW, Rayner BL. Hypertension, end-stage renal disease and mesangiocapillary glomerulonephritis in methamphetamine users. South Afri Med J. 2015;105(3):199-201.
19. Hsu C-y, Iribarren C, McCulloch CE, Darbinian J, Go AS. Risk factors for end-stage renal disease: 25-year follow-up. Archiv Int Med. 2009;169(4):342-350.
20. Rule AD, Bergstralh EJ, Melton LJ, Li X, Weaver AL, Lieske JC. Kidney stones and the risk for chronic kidney disease. Clin J Am Soc Nephro. 2009;4(4):804-811.
21. Ishani A, Grandits GA, Grimm RH, Svendsen KH, Collins AJ, Prineas RJ, Neaton JD. Association of single measurements of dipstick proteinuria, estimated glomerular filtration rate, and hematocrit with 25-year incidence of end-stage renal disease in the multiple risk factor intervention trial. J Am Soc Nephrol. 2006;17(5):1444-1452.

Table 1. Baseline characteristics of the study population

Variables		**(n=300)**
Mean Age (Years)		43.47±16.60
Age Group (Years)	≤30	89(29.7)
	31-45	74(24.7)
	46-60	102(34.0)
	≥60	35(11.7)
Gender	Male	184(61.3)
	Female	116(38.6)
Prevalence of ESRD	Yes	296(98.6)
	No	4(1.36)
Symptoms of ESRD	Dyspnea	106(35.5)
	Persistent Vomiting	170(56.7)
	Acidotic Breathing	105(35)
	Anuria	134(44.7)
	Oliguria	119(39.7)
Mean duration of symptoms (days)		20.53±2.719

**Values are given as mean±SD or n(%)*

**ESRD-End Stage Renal Disease.*

Table 2. Prevalence of ESRD stratified with baseline characteristics and risk factors

Variables		**ESRD**		**P-value**
		Present	Absent	
Age group (years)	*≤30*	88(29.7)	1(25)	0.647
	31-45	72(24.3)	2(50)	
	46-60	101(34.1)	1(25)	
	≥60	35(11.8)	-	
Gender	*Male*	181(61.1)	3	0.572

	Female	115(38.8)	1(25)	
Duration of symptoms	*15-18 days*	80(27.0	-	0.132
	19-22 days	128(43.2)	1(25)	
	23-26 days	88(29.7)	3(75)	
Risk factors	*Uncontrolled Diabetes Mellitus*	76(25.6)	-	0.010
	Uncontrolled Hypertension	88(29.7)	-	
	Glomerulonephritis	110(37.1)	2(50)	
	Renal Calculi	22(7.43)	2(50)	

*p<0.05 is considered significant

*values are presented as n(%)

*ESRD-End Stage Renal Disease

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RESEARCH ARTICLE

Improvising Livelihood of Fish-Farmers by Modulating Limnological Characteristics of Ponds

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ABSTRACT

With an ever increasing food demand, contribution of fish farming is significant. This study primarily focuses on making use of unexploited ponds present in the community aiming for introducing fish farming, resulting in an increment in the local economy. Aquaculture being the fastest growing food production sector, we focused on community-based pond aquaculture on the eastern coastal of India. Essentially, this diagnostic approach demonstrates how sustainability challenges can be countered at the community level. Physico-chemical parameters including dissolved O_2, pH, temperature, nitrate-nitrogen content, alkalinity was measured across three different ponds and finally fish farming was initiated. The pH found to be between 7.4 and 8.2. The dissolved O_2 ranged in between 6 to 7.8, the pH varied between 7.2 to 7.9. The temperature across the ponds ranged in between 18.2 to 34.3°C. Nitrate-nitrogen ($\mu g\text{-}l^{-1}$) and the total alkalinity (mg $CaCO_3.l^{-1}$) ranged between 1.18 to 5.4 and 80.45 to 196.15 respectively. Mostly, we emphasized the need for augmented knowledge and hands on training on effective aquaculture practice. The ponds were stabilized empowering the people with the techniques for composite fish farming of Indian major carps (*Catla catla, Labeo rohita and Cirrhinus mrigal*) as candidates for harvesting. Encouraging community based fish may thus be valuable in bringing about optimistic changes in the overall livelihood gains for the community people.

Key words: Community, aquaculture, fish, alkalinity, ponds.

INTRODUCTION

Essentially, in an ecosystem, ecology is considered to be the primary unit of ecology. It provides information concerning the utilization and recycling of mineral elements and the availability of solar energy. Mostly, the living

organisms present in an ecosystem contribute to the biotic component while the nonliving factors including the physical and chemical factors constitute the abiotic components of an ecosystem. Water is considered to be a vital natural resource and a critical agricultural input (Huang et al., 2015). Water usage is essential for sustainable agricultural escalation and boost of food availability (Grafton et al., 2015). However, strategies for increasing agricultural productivity need to be focussed. Culture of fish, particularly composite fish culture can be an imperative tool for sustainably recuperating agricultural productivity and for strengthening rural economies (Nagabhatla et al. 2012; Dey and Prein 2006; Dey et al., 2005). Water resources are continuously deteriorating everyday at a quicker rate primarily due to hasty population and urbanization load. Declining water quality is currently a global issue (Mahananda et al., 2010). The water purity varies from place to place in nature (Patil 2013).

Essentially, the interaction between physical, chemical and biological components of a habitat determines the quality of water of an ecosystem. Mostly, aquatic biota influences the physico-chemical characteristics of an aquatic ecosystem (Sharma et al., 2009). Limnology essentially deals with inland aquatic ecosystems. Primarily, the growth and survival of fresh water inhabitants depend on the quality of water (Boyd, 1989; Boyd, 1990; Philips, 1991; Jhingran, 1985). Fish plays an important role in agriculture sector of India. It provides livelihood to more than 60 million people and earns more than 6800 crore rupees through export. Extensive limnological studies have been carried out (Olopade, 2013; Nikolosky, 1963). The quality of water predominantly depends on the physical, chemical and biological characteristics of water (Zweig et al. 1999; Adeniji and Ovie 1982; Das and Padhi, 2014; Padhi et al. 2015). Mostly, the present study is focused on the determination of quality of water in order to utilize the ponds for aquaculture. The Main objectives of the study was to determine physical, chemical and biological characteristics of ponds in order to utilize them for fish culture and thus generate employment opportunity for gainful earning among rural people by creating awareness among them through training on aquaculture practices. Composite fish farming is the technique to culture different types of compatible and non competitive fishes in the same ecosystem so as to allow them to grow by feeding by making optimum use of different zones (surface, bottom and column) of the ponds without impeding the growth, development and maturity of one another. This is a very profitable method of aquaculture and hence importance has been laid to train the populace for gainful employment.

MATERIALS AND METHODS

Site of study

For the purpose of study, three ponds (P1, P2 and P3) in three villages in the eastern coastal state of India were chosen for investigation, and such ponds were not utilized for fish cultivation earlier.

Physico-chemical parameters measurement

The parameters chosen were water temperature, pH, dissolved oxygen, total alkalinity, nitrate nitrogen and plankton of water sample. Temperature was recorded using thermometer (accurate up to 0.01 degree Celsius), pH by pH meter, and alkalinity by using phenolphthalein and methyl orange indicators. Dissolved oxygen was measured by Wrinkler's method and nitrate nitrogen were measured by following standard procedures (APHA-2005) using water testing kits (NICE), during the period from November 2018 to October 2019.

Preparation of Ponds for Composite Fish Culture

The ponds were manually cleaned by making free from undesirable plants and weeds manually. Liming of the ponds was done in order to modulate the acidity of soil and water to speed up the decomposition of organic matter; which acts as disinfectant and also as an essential nutrient. Fertilizing the ponds was done after 3 days of liming for bloom of phytoplankton and growth of zoo plankton by manuring with organic manures like cow dung and oil cake (500 kg/ha) which carry almost all nutrients required for fish growth.

Introducing fingerlings in the pond

After cleaning, liming and fertilizing the ponds during March-April 2018, the fingerlings of 50-100 gm size (approx) purchased from govt. hatcheries were stocked in the ponds for 15 days after fertilization of ponds. Fingerlings of *Catla, Rohu* amd *Mrigal* in the ratio of 4:3:3 were selected to get good yield in mixed farming during July 2018.

RESULTS AND DISCUSSION

The maintenance of good water quality is essential for both survival and optimum growth (Gupta and Gupta 2006). The water quality standards vary significantly due to different environmental conditions, ecosystem and intended human users EPA 2006. The quality of aquaculture products and their suitability for human consumption may also be affected by water quality (Zweig et al. 1999). Keeping these factors in view, the ponds under study were maintained for aquaculture imparting training to local people also in order to empower them for gainful employment. The water temperature in pond 1 varied from 18.4 to 34.2, in pond 2 from 18.3 to 34.2 and in pond 3 from 18.2 to 34.3 (Table 1 and Figure 1). pH in pond 1 varied from 7.2 to 7.9, in pond 2 from 7.4 to 7.9 and in pond 3 from 7.3 to 7.9 (Table 2 and Figure 2). Total alkalinity showed 80.45 to 174.2 in pond 1, from 91.05 to 196.15 in pond 3 and varied from 89.1 to 184.12 (Table 3 and Figure 3). Dissolved oxygen values varied from 6.0 to 7.6 in pond 1, from 5.9 to 7.8 in pond 2 and from 6.0 to 7.5 in pond 3 (Table 4 and Figure 4). Nitrate nitrogen varied from 1.18 to 4.1 in pond 1, from 2.7 to 4.9 in pond 2 and from 2.7 to 5.1 in pond 3 (Table 5 and Figure 5). Taking all these factors into consideration, the three ponds under study were prepared for fish culture by cleaning, liming, fertilizing, stocking and artificial feeding for harvesting after one year. After having studied, the pond health, treatment was done preparing the ponds for aquaculture following standard prescribed guidelines for pre stocking, stocking and harvesting.

Catla catla (surface feeder), *Labeo rohita* (column feeder) and *Cirrhina mrigala* (bottom feeder) and with different feeding habits occupying different zones of the ponds utilize the available food of the ponds in all the zones profitably. Regularly the weeds were removed by physical methods, manuring was done along with providing artificial diet like oil cakes, waste vegetables etc., the fish were caught and weighed at intervals. The fish were caught by netting. The weight of fish, ranged from 500gm-1000gm. Fishes below 500gm were again left in the ponds for further growth. The average yield of fishes and their cost at site was from P1-277 kg, from P2-247 kg and from P3-257 kg @ Rs 100/kg and the total sale price was Rs. 78,100 and the net profit was 51,100 in the year under study (2018-2019) (Table 6). Awareness programmes were organized periodically in the villages in order to create awareness among people to motivate them for fish cultivation. Training programmes on composite fish farming were organized for villagers and students. The technical expertise so gained is believed to be utilized by the beneficiaries for gainful earning. During the period of study care of the ponds was monitored by a group of peer volunteers from each village who have assisted in managerial activity and watch of the ponds in their respective villages. The profit of the sale proceeds of fish was being used as seed money by the volunteers for cultivation of fish for livelihood besides other engagements. Thus the objectives have been achieved through training and interaction sessions generating confidence among the villagers for aquaculture for their livelihood.

Author contribution statement

Gagan Kumar Panigrahi and Pradip Kumar Prusty conceived the idea. Gagan Kumar Panigrahi, Annapurna Sahoo and Pradip Kumar Prusty performed the experiments. Gagan Kumar Panigrahi, Annapurna Sahoo and Pradip Kumar Prusty analyzed the results. All the authors have significant contribution in writing the manuscript.

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Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Adeniji, H.A. and Ovie, S.I. (1982). Study and appraisal for the water quality of the Ase Oli and Niger Rivers. NIFFER Annual Report, 15-20.
2. APHA. (2005). Standard methods for examination for water and waste water, 17th edition. American Public Health Association. Washington DC.
3. Boyd, C.E. (1989). Water quality management and aeration in shrimp farming. Fishes and Allied Aquaculture Department Series. No. 2. Birmingham Ala Aubum University Press.
4. Boyd, C.E. (1990). Water quality in ponds for aquaculture. Albama agricultural experiment station, Auburn University.
5. Das, S.K. and Padhi, S.N. (2014). In Application of Biology for Self Employment. Ed. I Vol. 1, Nanda Kishore Publication, Bhubaneswar, India.
6. Dey, M. M., Rab, M. A., Paraguas, J. P., Piumsombun, S., Bhatta, R., Alam, M. F., & Ahmed, M. (2005). Fish consumption and food security: a disaggregated analysis by types of fish and classes of consumers in selected Asia countries. Aquaculture Economics and Management, 9, 89–111.
7. Dey, M. M., & Prein, M. (2006). Community-based fish culture in seasonal floodplains. Naga, 29(1 & 2), 21–27.
8. EPA, (2006). "Water Quality Standards Review and Revision, Washington DC.
9. Gupta, S.K. and Gupta, P.C. (2006). General and applied technology (Fish and Fisheries) S. Chand and Company, New Delhi.
10. Grafton, R. Q., Williams, J., & Jiang, Q. (2015). Food and water gaps to 2050: preliminary results from the global food and water system (GFWS) platform. Food Security, 7(2), 209–220.
11. Huang, F., Liu, Z., Ridoutt, B. G., Huang, J., & Li, B. (2015). China's water for food under growing water scarcity. Food Security, 7(5), 933–949.
12. Jhingram, V.G. (1985). Fish and fisheries of India. Hindustan Publishing corporation, Delhi, India.
13. Mahananda, M.R., B.P. Mohanty & N.R. Behera. (2010). Physico-chemical analysis of surface and ground water of Bargarh District, Orissa, India. Int. J. Res. Rev. Appl. Sci., 2(3): 23-29
14. Nagabhatla, N., Beveridge, M., Haque, A. B. M. M., Sophie Nguyen-Khoa, S.,& Brakel, M. V. (2012).Multiple water use as an approach for increased basin productivity and improved adaptation: a case study from Bangladesh. International Journal of River Basin Management, 10(1), 121–136.
15. Nikolosky, G.V. (1963). The ecology of fishes. Academic Press, London, U.K.
16. Olopade, daniyi. (2013). Lakes reservoirs and ponds, 7 (1), 9-19.
17. Padhi, S.N., Das, S.K.,Panda, A. and Panda, Sasmita. (2015). In Employment through aquaculture. Nanda Kishore Publication, Bhubaneswar.

18. Patil, A.A. (2013). Status of water quality of Bhambarde and Lengre reservoir of Sangli district, Maharashtra (India). Flora and Fauna, 19(1): 35-40
19. Philips, M.J., Beveridge, M.C.M. and Clark R.M. (1991). Impact of aquaculture on water resourses. In D.E. Brune and J.R. Tomasso (eds), Advance in aquaculture, 3:568-591.
20. Sharma, K., K. Shvetambri, P. Verma & S. P. Sharma. (2009). Physico-chemical assessment of three freshwater ponds of Jammu (J&K), Curr. World Environ, 4(2): 367-373.
21. Zweig, R.D., Morton, J.D. and Stewart, M.M. (1999). Source water quality for aquaculture. A guide for assessment World Bank Report, 74.

Table 1. Temperature variations observed across the three ponds (P1, P2 and P3) during November, 2018 to December, 2019

Month-Year	P1	P2	P3
Nov-18	20.08	20.10	20.31
Dec-18	18.04	18.09	18.12
Jan-19	19.02	19.23	19.42
Feb-19	20.1	20.24	20.4
Mar-19	26.3	26.4	26.2
Apr-19	30.3	30.2	30.4
May-19	34.2	34.2	34.3
Jun-19	33.1	33.3	33.4
Jul-19	32.4	32.3	32.6
Aug-19	30.4	30.3	30.4
Sep-19	26.7	26.8	26.7
Oct-19	26.6	26.1	26.1
Nov-19	22.06	22.04	22.01
Dec-19	19.4	19.5	19.4

Table 2. pH variations observed across the three ponds (P1, P2 and P3) during November, 2018 to December, 2019

Month-Year	P1	P2	P3
Nov-18	7.2	7.4	7.3
Dec-18	7.4	7.5	7.4
Jan-19	7.5	7.5	7.3
Feb-19	7.6	7.5	7.3
Mar-19	7.8	7.7	7.7
Apr-19	7.9	7.9	7.8
May-19	7.9	7.8	7.8
Jun-19	7.8	7.7	7.9
Jul-19	7.8	7.8	7.9
Aug-19	7.9	7.8	7.8
Sep-19	7.9	7.9	7.7
Oct-19	7.8	7.8	7.6
Nov-19	7.7	7.8	7.4
Dec-19	7.4	7.6	7.7

Table 3. Variations in total alkalinity (mg $CaCO_3.l^{-1}$) observed across the three ponds (P1, P2 and P3) during November, 2018 to December, 2019.

Month-Year	P1	P2	P3
Nov-18	80.45	105.85	112.25
Dec-18	110.24	109.95	120.16
Jan-19	120.65	134.62	120.6
Feb-19	132.58	148.7	128.3
Mar-19	142.36	147.25	156.15
Apr-19	143.61	152.85	178.34
May-19	174.2	196.15	184.12
Jun-19	98.36	110.62	132.4
Jul-19	84.26	105.2	96.2
Aug-19	102.39	108.15	89.1
Sep-19	89.1	96.38	92.49
Oct-19	95.36	94.02	96.37
Nov-19	89.61	91.05	102.37
Dec-19	90.76	91.09	102.79

Table 4. Variations in dissolved oxygen observed across the three ponds (P1, P2 and P3) during November, 2018 to December, 2019

Month-Year	P1	P2	P3
Nov-18	6.40	6.30	6.50
Dec-18	6.60	6.40	6.60
Jan-19	7.40	7.10	7.30
Feb-19	7.60	7.80	7.50
Mar-19	7.50	7.60	7.40
Apr-19	7.60	7.80	7.50
May-19	6.60	6.40	6.50
Jun-19	6.20	6.30	6.40
Jul-19	6.00	6.10	6.00
Aug-19	6.40	5.90	6.70
Sep-19	7.10	7.20	7.10
Oct-19	6.90	6.80	7.10
Nov-19	6.50	6.80	6.20
Dec-19	6.50	6.30	6.40

Table 5. Variations in nitrate-nitrogen ($\mu g.l^{-1}$) observed across the three ponds (P1, P2 and P3) during November, 2018 to December, 2019

Month-Year	P1	P2	P3
Nov-18	2.75	4.8	3.6
Dec-18	2.98	4.9	3.9
Jan-19	2.1	5.4	4.8
Feb-19	1.8	4.26	5.1
Mar-19	1.32	3.6	2.7

Apr-19	1.18	3.2	3.81
May-19	2.12	2.7	2.9
Jun-19	1.9	2.9	3.6
Jul-19	3.12	4.01	3.94
Aug-19	3.4	4.5	3.7
Sep-19	3.51	4.8	3.9
Oct-19	4.1	4.21	3.8
Nov-19	3.52	4.8	3.89
Dec-19	3.9	4.6	3.9

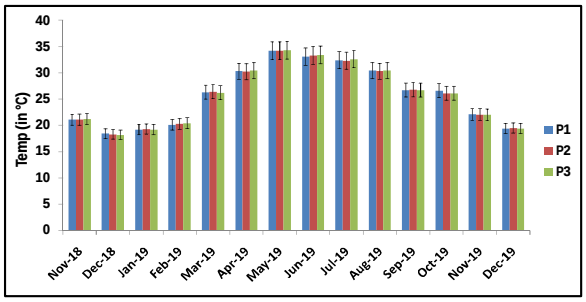

Fig 1. Temp. (⁰C) in three ponds (Average value) (Nov, 2018-Dec, 2019)

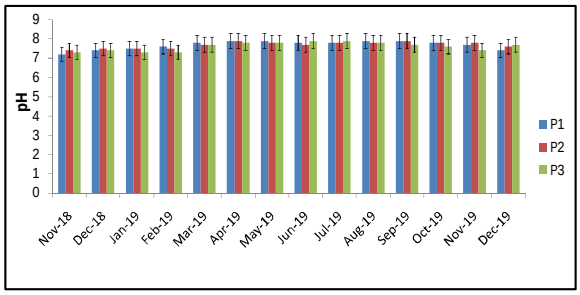

Fig 2. pH in three ponds (Average value) (Nov 2018-Dec-2019)

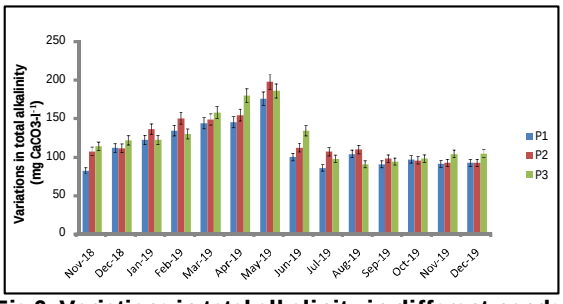

Fig 3. Variations in total alkalinity in different ponds (Nov, 2018-Dec, 2019)

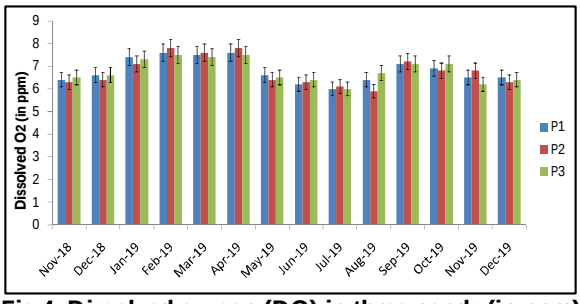

Fig 4. Dissolved oxygen (DO) in three ponds (in ppm) (Nov 2018-Dec-2019)

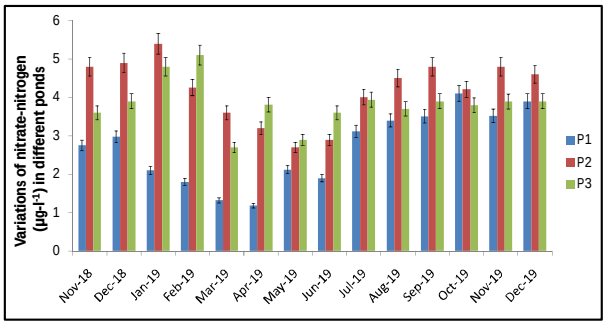

Fig 5. Variations of nitrate-nitrogen in different ponds (Nov, 2018-Dec, 2019)

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RESEARCH ARTICLE

The Generalized ISI Index of Some Carbon Nanotubes

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ABSTRACT

Chemical graph theory is an indispensable branch of mathematical chemistry which has broad range of applications. In chemical sciences, topological index is a real number that is used to study the physico-chemical properties of chemical compounds theoretically.Recently,Buragohain et al.[2] propose and study a novel generalized topological index,called generalized ISI index, in connection with some chemical structures. Many standard topological indices can be obtained as special cases of this index. In this communication, we compute the generalization of ISI index of some Carbon nanotubes.

Keywords: Topological index, Inverse sum indeg (ISI) index, Generalized ISI index, Nanotubes.

INTRODUCTION

In chemistry, a topological index or a molecular descriptor is a numerical quantity associated with a graph structure or a molecular graph which is potentially used to study the various physicochemical properties such as boiling point, accentric factor, total surface area, isomer discrimination, structural-activity relationships(SAR), structural-property relationships(SPR), chemical docu-mentation and pharmaceutical drug designing etc[10]. A nanotube is an object of intermediate size between microscopic and molecular structure.Carbon nanotubes (CNTs) are carbon's allotropes that have cylindrical structures. CNTs areof numerous shapes that differ in their length, width and the number of layers. Nanotubes are broadly classified as single-walled and multi-walled nanotubes. CNTs have various physical and chemical properties such as its has high thermal conductivity, high electrical conductivity, highly flexible, and electrically polarizable etc. As individual molecules, nanotubes are hundreds times stronger than steel. Carbon nanotubes are widely used in the fields of Nanotechnologies, electronics, optical communication and other fields of material sciences. These are also potentially useful in Infection therapy, Gene therapy, Cancer therapy etc.[5, 6, 22, 23]. In 1991, Iijima [15] discovered carbon nanotubes as multi-walled structures. For more information on computing topological indices of nanostructures may be found in [3, 6, 13, 14, 16, 20, 21, 26]

In 2020, Buragohain et al.[2] propose and study a novel generalized topological index forsome chemical structures and is defined as

$$ISI_{\alpha,\beta}(G) = \sum_{uv\in E(G)}[d(u)d(v)]^{\alpha}[d(u)+d(v)]^{\beta},$$

where, α and β are some real numbers. Clearly, for α = 1 &β = -1, this index is nothing but the ISI index [25]. Some standard topological indices such as Zagreb indices [7, 12], Randić index [9, 17, 19], Sum connectivity index [28], Harmonic index [4, 18, 27], Geometric-Arithmetic index[9], Hyper Zagreb index [11, 24], Generalized Randić index[1, 8], and General sum connectivity index[29] are the special cases of this generalized index. . Table1 shows that the relationships between $ISI_{(\alpha,\beta)}$-index with some other topological indices.

RESULTS AND DISCUSSION

In this section we compute the generalized ISI index of some well-known carbon nanotubes such as $TUAC_6$, $TUZC_6$, $TUC_4C_8(R)$, $TUC_4C_8(S)$,$TUHC_5C_7$,$TUSC_5C_7$, $TUHAC_5C_7$,and $TUHAC_5C_6C_7$. Let G be one of the above mentioned nanotube. It is easy to see that the degree of each vertex in G either two or three. So we can classify the edge set of G intofollowing ways

$$E_1(G) = \{uv \in E(G): d(u) = 2 \text{ and } d(v) = 2\},$$
$$E_2(G) = \{uv \in E(G): d(u) = 2 \text{ and } d(v) = 3\},$$
$$E_3(G) = \{uv \in E(G): d(u) = 3 \text{ and } d(v) = 3\}.$$

$TUAC_6$nanotubes

Let $G = TUAC_6(p,q)$ be an armchair polyhex nanotube, where p is the number of hexagons in each row and q is the number of rows in the molecular graph of G as shown in Figure1, from the molecular graph we have $|E_1(G)| = 2p, |E_2(G)| = 4p, |E_3(G)| = 6pq - 8p$.

Theorem 1. The (α,β)-ISI index of $G = TUAC_6(p,q)$ nanotubes is given by

$$2p[4]^{\alpha+\beta} + 4p\left[6^{\alpha}5^{\beta}\right] + (6pq-8p)\left[9^{\alpha}6^{\beta}\right].$$

Proof:

$$ISI_{\alpha,\beta}(G) = \sum_{uv\in E(G)}[d(u)d(v)]^{\alpha}[d(u)+d(v)]^{\beta}$$
$$= \sum_{uv\in E_1(G)}[4]^{\alpha}[4]^{\beta} + \sum_{uv\in E_2(G)}[6]^{\alpha}[5]^{\beta} + \sum_{uv\in E_1(G)}[9]^{\alpha}[6]^{\beta}$$
$$= |E_1(G)|[4]^{\alpha+\beta} + |E_2(G)|[6]^{\alpha}[5]^{\beta} + |E_3(G)|[9]^{\alpha}[6]^{\beta}$$
$$= 2p[4]^{\alpha+\beta} + 4p[6]^{\alpha}[5]^{\beta} + (6pq-8p)[9]^{\alpha}[6]^{\beta}.$$

For different values of α, we compute some other standard topological indices and the results are found as shown in the following corollary

Corollary 1. p is the number of hexagons in each row and q is the number of rows in the molecular graph of G.Then

I. $ISI_{0,1}(G) = M_1(G) = 36pq - 20p.$

II. $ISI_{1,0}(G) = M_2(G) = 54pq - 40p.$

III. $ISI_{-\frac{1}{2},0}(G) = R(G) = 2pq + (1 + \frac{4}{\sqrt{6}} - \frac{8}{3})p.$

IV. $ISI_{0,-\frac{1}{2}}(G) = SCI(G) = \sqrt{2}pq + (1 + \frac{4}{\sqrt{5}} - \frac{4\sqrt{2}}{3})p.$

V. $ISI_{1,-1}(G) = ISI(G) = 9pq - \frac{26}{5}p.$

VI. $2ISI_{0,-1}(G) = H(G) = 2pq - \frac{1}{15}p.$

VII. $ISI_{0,2}(G) = HM(G) = 216pq - 156p.$

VIII. $ISI_{\alpha,o}(G) = R_\alpha(G) = 2^{\alpha+1}p(1 + 2 \times 3^\alpha) + (6pq - 8p)6^\alpha$.

$TUZC_6$ nanotubes

Let $G = TUZC_6(p, q)$ be an a zigzagpolyhex nanotube, where p is the number of hexagons in each row and q is the number of rows in the molecular graph of G as shown in Figure 2, from the molecular graph we have $|E_1(G)| = 0, |E_2(G)| = 4p, |E_3(G)| = 3pq - 5p.$

Theorem 2. The (α, β)-ISI index of $G = TUZC_6(p, q)$ nanotubes is given by

$$4p[6^\alpha 5^\beta] + (3pq - 5p)[9^\alpha 6^\beta].$$

Proof:

$$ISI_{\alpha,\beta}(G) = \sum_{uv \in E(G)} [d(u)d(v)]^\alpha [d(u) + d(v)]^\beta$$

$$= \sum_{uv \in E_1(G)} [4]^\alpha [4]^\beta + \sum_{uv \in E_2(G)} [6]^\alpha [5]^\beta + \sum_{uv \in E_1(G)} [9]^\alpha [6]^\beta$$

$$= |E_1(G)|[4]^{\alpha+\beta} + |E_2(G)|[6]^\alpha [5]^\beta + |E_3(G)|[9]^\alpha [6]^\beta$$

$$= 0 \times [4]^{\alpha+\beta} + 4p[6]^\alpha [5]^\beta + (3pq - 5p)[9]^\alpha [6]^\beta.$$

$$= 4p[6]^\alpha [5]^\beta + (3pq - 5p)[9]^\alpha [6]^\beta$$

$$=4p[6^\alpha 5^\beta] + (3pq - 5p)[9^\alpha 6^\beta].$$

TUC_4C_8 Nanotubes

A C_4C_8net is a trivalent decoration constructed from alternating squares $C_4$and octagons C_8: two classes of these nanotubes are $TUC_4C_8(R)$ and $TUC_4C_8(S)$nanotubes.

$TUC_4C_8(R)$ Nanotubes

Let $G - TUC_4C_8(R)$ be nanotube which molecular graph is constructed from alternating squares and octagons as shown in Figure 3. In the molecular graph p is the number of squares in each row and q is the number of squares in each column. From the molecular graph we have $|E_1(G)| = 0, |E_2(G)| = 4p, |E_3(G)| = 6pq - 5p$.

Theorem 3. The (α, β)-ISI index of $G = TUC_4C_8(R)$ nanotubes is given by

$$4p[6^\alpha 5^\beta] + (6pq - 5p)[9^\alpha 6^\beta].$$

Proof:

$$ISI_{\alpha,\beta}(G) = \sum_{uv \in E(G)} [d(u)d(v)]^\alpha [d(u) + d(v)]^\beta$$

$$= \sum_{uv \in E_1(G)} [4]^\alpha [4]^\beta + \sum_{uv \in E_2(G)} [6]^\alpha [5]^\beta + \sum_{uv \in E_1(G)} [9]^\alpha [6]^\beta$$

$$= |E_1(G)|[4]^{\alpha+\beta} + |E_2(G)|[6]^\alpha [5]^\beta + |E_3(G)|[9]^\alpha [6]^\beta$$

$$= 0 \times [4]^{\alpha+\beta} + 4p[6]^\alpha [5]^\beta + (6pq - 5p)[9]^\alpha [6]^\beta.$$

$$= 4p[6]^\alpha [5]^\beta + (6pq - 5p)[9]^\alpha [6]^\beta$$

$$= 4p[6^\alpha 5^\beta] + (6pq - 5p)[9^\alpha 6^\beta].$$

$TUC_4C_8(S)$ Nanotubes

Let $G = TUC_4C_8(S)$ be nanotube which molecular graph is constructed from alternating squares and octagons as shown in Figure 4. In the molecular graph p is the number of squares in each row and q is the number of squares in each column. From the molecular graph we have $|E_1(G)| = 2p, |E_2(G)| = 4p, |E_3(G)| = 6pq - 8p$.

Theorem 4. The (α, β)-ISI index of $G = TUC_4C_8(S)$ nanotubes is given by

$$2p[4]^{\alpha+\beta} + 4p[6^\alpha 5^\beta] + (6pq - 5p)[9^\alpha 6^\beta].$$

Proof:

$$ISI_{\alpha,\beta}(G) = \sum_{uv \in E(G)} [d(u)d(v)]^\alpha [d(u) + d(v)]^\beta$$

$$= \sum_{uv \in E_1(G)} [4]^\alpha [4]^\beta + \sum_{uv \in E_2(G)} [6]^\alpha [5]^\beta + \sum_{uv \in E_3(G)} [9]^\alpha [6]^\beta$$

$$= |E_1(G)|[4]^{\alpha+\beta} + |E_2(G)|[6]^\alpha [5]^\beta + |E_3(G)|[9]^\alpha [6]^\beta$$

$$= 2p[4]^{\alpha+\beta} + 4p[6]^\alpha [5]^\beta + (6pq - 8p)[9]^\alpha [6]^\beta$$

$$= 2p[4]^{\alpha+\beta} + 4p[6^\alpha 5^\beta] + (6pq - 5p)[9^\alpha 6^\beta].$$

TUC_5C_7 Nanotubes

A C_5C_7 net is a trivalent decoration constructed from alternating pentagons (C_5) and heptagons (C_7). Three classes of this type of nanotubes are $TUHC_5C_7$, $TUSC_5C_7$ & $TUHAC_5C_7$.

$TUHC_5C_7$ Nanotubes

The molecular graph of the nanotubes $TUHC_5C_7(p,q)$ as shown in Figure 5 consists of pentagons and heptagons. We denote p is the number of pentagons in each row. In this nanotube, the four first rows of vertices and edges are repeated, alternatively. We denote the number of this repetition by q. The cardinality of the edge sets of the graph are $|E_1(G)| = 0, |E_2(G)| = 4p, |E_3(G)| = 12pq - 5p$.

Theorem 5. The (α,β)-ISI index of $G = \mathrm{TUHC_5C_7}(p,q)$ nanotubes is given by

$$4p\left[6^{\alpha}5^{\beta}\right] + (12pq - 5p)\left[9^{\alpha}6^{\beta}\right].$$

Proof:

$$ISI_{\alpha,\beta}(G) = \sum_{uv\in E(G)} [d(u)d(v)]^{\alpha}[d(u)+d(v)]^{\beta}$$

$$= \sum_{uv\in E_1(G)} [4]^{\alpha}[4]^{\beta} + \sum_{uv\in E_2(G)} [6]^{\alpha}[5]^{\beta} + \sum_{uv\in E_3(G)} [9]^{\alpha}[6]^{\beta}$$

$$= |E_1(G)|[4]^{\alpha+\beta} + |E_2(G)|[6]^{\alpha}[5]^{\beta} + |E_3(G)|[9]^{\alpha}[6]^{\beta}$$

$$= 0 \times [4]^{\alpha+\beta} + 4p[6]^{\alpha}[5]^{\beta} + (12pq - 5p)[9]^{\alpha}[6]^{\beta}$$

$$= 4p\left[6^{\alpha}5^{\beta}\right] + (12pq - 5p)\left[9^{\alpha}6^{\beta}\right].$$

$TUSC_5C_7$ Nanotubes

The molecular graph of the nanotubes $TUSC_5C_7(p,q)$ as shown in Figure 6 consists of pentagons and heptagons. We denote p is the number of pentagons in each row. In this nanotube, the two first rows of vertices and edges are repeated, alternatively. We denote the number of this repetition by q. The cardinality of the edge sets of the graph are $|E_1(G)| = p, |E_2(G)| = 6p, |E_3(G)| = 12pq - 12p$.

Theorem 6. The (α,β)-ISI index of $G = \mathrm{TUHC_5C_7}(p,q)$ nanotubes is given by

$$p[4]^{\alpha+\beta} + 6p\left[6^{\alpha}5^{\beta}\right] + (12pq - 12p)\left[9^{\alpha}6^{\beta}\right].$$

Proof:

$$ISI_{\alpha,\beta}(G) = \sum_{uv\in E(G)} [d(u)d(v)]^{\alpha}[d(u)+d(v)]^{\beta}$$

$$= \sum_{uv\in E_1(G)} [4]^{\alpha}[4]^{\beta} + \sum_{uv\in E_2(G)} [6]^{\alpha}[5]^{\beta} + \sum_{uv\in E_3(G)} [9]^{\alpha}[6]^{\beta}$$

$$= |E_1(G)|[4]^{\alpha+\beta} + |E_2(G)|[6]^{\alpha}[5]^{\beta} + |E_3(G)|[9]^{\alpha}[6]^{\beta}$$

$$= p[4]^{\alpha+\beta} + 6p[6]^{\alpha}[5]^{\beta} + (12pq - 12p)[9]^{\alpha}[6]^{\beta}.$$

$$= p[4]^{\alpha+\beta} + 6p\left[6^{\alpha}5^{\beta}\right] + (12pq - 12p)\left[9^{\alpha}6^{\beta}\right].$$

$TUHAC_5C_7$ Nanotubes

The molecular graph of the nanotubes $TUHAC_5C_7(p,q)$ as shown in Figure 7 consists of pentagons and heptagons. We denote p is the number of pentagons in each row. In this nanotube, the three first rows of vertices and edges are repeated, alternatively. We denote the number of this repetition by q. The cardinality of the edge sets of the graph are $|E_1(G)| = 0, |E_2(G)| = 4p, |E_3(G)| = 12pq - 5p$

Theorem 7. The (α,β)-ISI index of $G = \mathrm{TUHAC_5C_7}(p,q)$ nanotubes is given by

$$4p\left[6^\alpha 5^\beta\right] + (12pq - 5p)\left[9^\alpha 6^\beta\right].$$

Proof:

$$ISI_{\alpha,\beta}(G) = \sum_{uv\in E(G)} [d(u)d(v)]^\alpha [d(u)+d(v)]^\beta$$

$$= \sum_{uv\in E_1(G)} [4]^\alpha [4]^\beta + \sum_{uv\in E_2(G)} [6]^\alpha [5]^\beta + \sum_{uv\in E_3(G)} [9]^\alpha [6]^\beta$$

$$= |E_1(G)|[4]^{\alpha+\beta} + |E_2(G)|[6]^\alpha [5]^\beta + |E_3(G)|[9]^\alpha [6]^\beta$$

$$= 0 \times [4]^{\alpha+\beta} + 4p[6]^\alpha [5]^\beta + (12pq - 5p)[9]^\alpha [6]^\beta$$

$$= 4p\left[6^\alpha 5^\beta\right] + (12pq - 5p)\left[9^\alpha 6^\beta\right].$$

$TUHAC_5C_6C_7$ Nanotubes

The molecular graph of the nanotubes $TUHAC_5C_6C_7(p,q)$ as shown in Figure 8 consists of pentagons, hexagons, and heptagons. We denote p is the number of pentagons in each row. In this nanotube, the three first rows of vertices and edges are repeated, alternatively. We denote the number of this repetition by q. The cardinality of the edge sets of the graph are $|E_1(G)| = 0, |E_2(G)| = 8p, |E_3(G)| = 24pq - 10p$.

Theorem 8. The (α,β)-ISI index of $G = \mathrm{TUHAC_5C_6C_7}(p,q)$ nanotubes is given by

$$8p\left[6^\alpha 5^\beta\right] + (24pq - 10p)\left[9^\alpha 6^\beta\right].$$

Proof:

$$ISI_{\alpha,\beta}(G) = \sum_{uv\in E(G)} [d(u)d(v)]^\alpha [d(u)+d(v)]^\beta$$

$$= \sum_{uv\in E_1(G)} [4]^\alpha [4]^\beta + \sum_{uv\in E_2(G)} [6]^\alpha [5]^\beta + \sum_{uv\in E_3(G)} [9]^\alpha [6]^\beta$$

$$= |E_1(G)|[4]^{\alpha+\beta} + |E_2(G)|[6]^\alpha [5]^\beta + |E_3(G)|[9]^\alpha [6]^\beta$$

$$= 0 \times [4]^{\alpha+\beta} + 8p[6]^\alpha [5]^\beta + (24pq - 10p)[9]^\alpha [6]^\beta$$

$$= 8p\left[6^\alpha 5^\beta\right] + (24pq - 10p)\left[9^\alpha 6^\beta\right].$$

CONCLUSION

In this study, we obtain some closed expressions of the Generalized ISI index and some popular degree based topological indices as special cases of this index for some Carbon nanotubes. The computation of this generalized index for some other chemical compounds can be a challenging topic for further study.

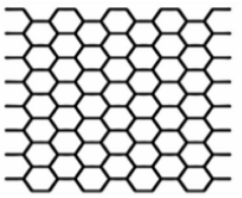
REFERENCES

1. B. Bollobas, & P. Erd□s, Graphs of extremal weights, ArsCombin., 50 (1998), pp. 225-233.
2. J. Buragohain, B. Deka, & A. Bharali, A Generalized ISI index of some chemicalstructures, Journal of Molecular Structure, 1208 (2020), p. 127843.
3. M.V. Diudea, & C.L. Nagy, Periodic nanostructures, Springer Dordrecht, Netherlands,2007.
4. S. Fajtlowicz, On conjectures of Graffiti, Annals of Discrete Mathematics, 38 (1988),pp. 113-118.
5. F. Falaha-Nezhad, & M. Azari, The Inverse sum indeg index of some nanotubes,Studia UBB Chemica, LXI, 1 (2016), pp. 63-70.
6. Y. J. Ge, J. B. Liu, M. Younas, M. Yousaf, & W. Nazeer, Analysis of $SC_5C_7[p,q]$ and $NPHX[p,q]$ nanotubes via topological indices, Hindawi Journal of Nanomaterials, (2019), Article ID 2072789, 10 pages.
7. I. Gutman, & N. Trinajstić, Graph theory and molecular orbitals, total π-electronenergy of alternant hydrocarbons, Chem. Phys. Lett., 17 (1972), pp. 535-538.
8. I. Gutman, & M. Lepović, Choosing the exponent in the definition of connectivityindex, J. Serb. Chem. Soc., 66(9) (2001), pp. 605-611.
9. I. Gutman, & B. Furtula (Eds.), Recentresults in the theory of Randić index, Univ.Kragujevac, Kragujevac, 2008.
10. I. Gutman, Degree based topological indices, Croat. Chem. Acta, 86 (2013), pp. 351-361.
11. I. Gutman, On Hyper Zagreb index and coindex, Bull. Acad. Serbe Sci. Arts Cl. Sci.Math.Natur., 150 (2017), pp. 1-8.
12. I. Gutman, E.Milovanović, & I. Milovanović, Beyond the Zagreb indices,AKCE International Journal of Graphs and Combinatorics, (2018). DOI:https://doi.org/10.1016/j.akcej.2018.05.002
13. Y. Hou, J. B. Liu, S. Ahmad, N. Farah, U. Ahmad, M. R. Farahani, & M. Imran,On certain topological indices of TUC_5C_8nanotubes, Journal of Computational andTheoretical Nanoscience, 16 (2016), pp. 9158-9161.
14. N. Idrees, M. J. Saif, A. Sadiq, A. Rauf, & F. Hussain, Topological indices of H- Naphtalenicnanosheet, Open chemistry, 16 (2018),pp. 1184-1188.
15. S. Iijima, Helical microtubes of graphitic carbon, Nature, (1991), 354, 56.
16. A. Iranmanesh, & M. Azari, The first and second Zagreb indices of several interestingclasses of chemical graphs and nanostructures, in M. V. Putz, & O. Ori (Eds.), Exoticproperties of carbon nanomatter, Springer Dordrecht,Netherlands, 8 (2015), p. 153.
17. X. Li, & I. Gutman, Mathematical aspects of Randić type molecular structuredescriptors, Univ. Kragujevac, Kragujevac, 2006.
18. M. Matejić, I. Milovanović, & E. Milovanović, On bounds of harmonic topologicalindex, Filomat, 32 (2018), pp. 311-317.
19. M. Randić, On characterization of molecular branching, J. Am. Chem. Soc., 97 (1975),
pp. 6609-6615.
20. N. K. Raut, & S. N. Ipper, Computing some topological indices of nanotubes,International Journal of Scientific and Research Publications, 5 (2015), pp. 2250-3153.
21. H. Saati, & J. Aghakazemi, Relationship between structures of C_5C_7carbon nanotubes and geometry of ornamental, J. Math. Stat., 1 (2018), p. 102.
22. N. Saifuddin, A. Z. Raziah, & A. R. Junizah, Carbon Nanotubes: A review on structure and their interaction with Proteins, Journal of chemistry, (2013), 676815, 18 pages.
23. Md. ShahzadAnjum, & Md. U. Safdar, K banhatti and K hyper-banhatti indices of nanotubes,Eng. Appl. Sci. Lett., 2(1) (2019), pp. 19-37.

24. G. H. Shirdel, H. Rezapour, &A. M. Sayadi, The Hyper-Zagreb index of graphoperations, Iran. J. Math. Chem., 4 (2013), pp. 213-220.
25. D. Vukičević, & M. Gašparov, Bond additive modeling1. Adriatic indices, Croat.Chem. Acta, 83 (2010), pp. 243-260.
26. A. Ye, M. I. Qureshi, A. Fahad, A. Aslam, M. K. Jamil, A. Zafar, & R. Irfan, Zagrebconnection number index of nanotubes and regular hexagonal lattice, Open Chem., 17 (2019), pp. 75-80.
27. L. Zhang, The Harmonic index for graphs, Appl. Math. Lett., 25 (2012), pp. 561-566.
28. B. Zhou, & N. Trinajstić, On a Novel Connectivity Index, J. Math. Chem., 46 (2009),pp. 1252-1270.
29. B. Zhou, & N. Trinajstić, Ongeneral sum-connectivity index, J. Math. Chem., 47 (2010), pp. 210-218.

Table 1. Relationships between Generalized ISI index and some other Topological indices.

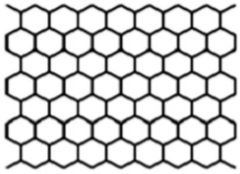
Topological index	Corresponding $ISI_{\alpha,\beta}$-index
First Zagreb index, $M_1(G)$	$ISI_{0,1}(G)$
Second Zagreb index, $M_2(G)$	$ISI_{1,0}(G)$
Randić index, $R(G)$	$ISI_{-\frac{1}{2},0}(G)$
Sum connectivity index, $SCI(G)$	$ISI_{0,-\frac{1}{2}}(G)$
Inverse sum indeg index, $ISI(G)$	$ISI_{1,-1}(G)$
Harmonic index, $H(G)$	$2ISI_{0,-1}(G)$
Geometric-Arithmetric Mean index, $GA(G)$	$2ISI_{\frac{1}{2},-1}(G)$
Hyper-Zagreb index, $HM(G)$	$ISI_{0,2}(G)$
First Generalized Randić index, $R_\alpha(G)$	$ISI_{\alpha,0}(G)$
General sum-connectivity index$\chi_\alpha(G)$	$ISI_{0,\alpha}(G)$



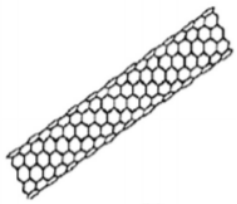
(a)

(b)

Figure 1 (a) Two dimensional lattice of $TUAC_6(4,8)$ nanotube, (b) $TUAC_6$nanotubes

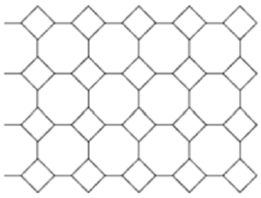


(a)

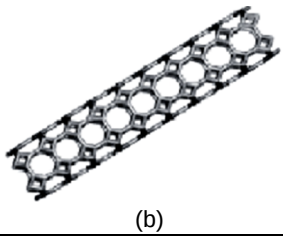


(b)

Figure 2: (a) Two dimensional lattice of $TUZC_6(8,8)$ nanotube, (b) $TUZC_6$nanotubes.

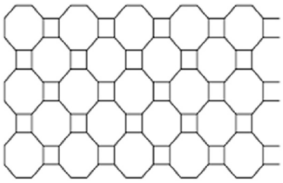


(a)

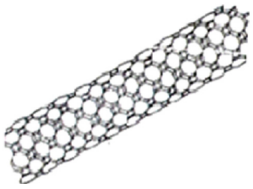


(b)

Figure 3. (a) Two dimensional lattice of $TUC_4C_8(R)$ nanotube with p=5 and q=4, (b)$TUC_4C_8(R)$ nanotubes.

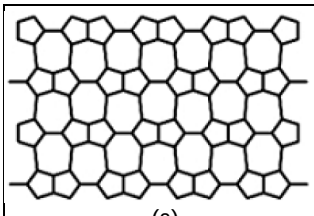


(a)



(b)

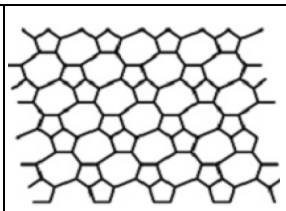
Figure 4. (a) Two dimensional lattice of $TUC_4C_6(S)$nanotube with p=5 and q=6, (b) $TUC_4C_8(S)$ nanotubes



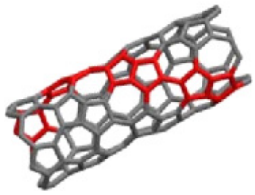
(a)

(b)

Figure 5: (a) Twodimensional lattice of $TUHC_5C_7(8,2)$nanotubes, (b) $TUHC_5C_7$nanotubes.

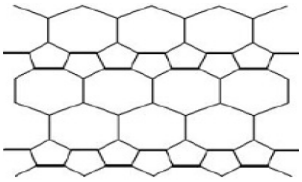


(a)

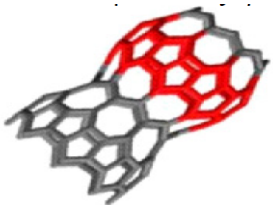


(b)

Figure 6: (a) Two dimensional lattice of $TUSC_5C_7(4,4)$ nanotube, (b) $TUSC_5C_7$nanotubes

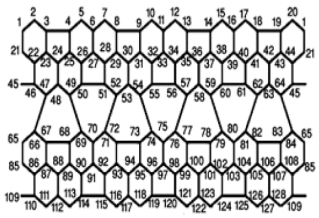


(a)



(b)

Figure 7: (a) Two dimensional lattice of $TUHAC_5C_7(4,2)$ nanotube, (b) $TUHAC_5C_7$nanotube



(a)

(b)

Figure 8: (a) Two dimensional lattice of $TUHAC_5C_6C_7(4,2)$ nanotube, (b) $TUHAC_5C_6C_7$nanotube.

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RESEARCH ARTICLE

A Hybrid Model of Artificial Neural Network and Particle Swarm Optimization for Forecasting of Stock Price of Tata Motors

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ABSTRACT

The forecasting of the financial time series has always attracted much interest from investors and researchers. Stock market movements are extremely complex and are influenced by different factors. Hence it is very important to find the most important factors for the stock market. But the high level of noise and complexity of the financial data makes this job very difficult. Many authors already used the comparatively overcome this challenge traditional statistical and machine learning techniques. The dormant high noises data mess up the performance, so to reducing the noise would be competent while constructing the forecasting model. To achieve this task, we employ the hybridization of ANN with PSO in this proposed paper. This paper analyzes a set of seven technical metrics used in common stock market studies, and performs ANN and PSO algorithms. The efficiency of the proposed method is measured by Bombay Stock Exchange (BSE) with 3950 number of daily transactional data from Tata Motors stock price. Empirical prediction analysis shows that the proposed model enhances the performance in comparison to traditional regression model.

Keywords: Artificial Neural Network; Financial Time Series Forecasting; Particle Swarm Optimization; Stock Market.

INTRODUCTION

Analysis of the stock markets has always been an essential part of any country's financial sector. Many investors currently rely on smart trading systems to forecast stock market price, based on different conditions. Precision of

these forecast systems with minimum risk factors is important for better investment decisions. The stock price prediction has been useful to individual as well as institutional investors. The stock data is most difficult as it is complex, non-liner and not every parameter. Investors therefore find it very difficult to predict financial stock market without studying their patterns. The selection and execution of an appropriate forecasting method plays a vital role to most of the financial traders. The organizational and monetary permanence of an organization depends on the accurateness of the prediction as such information determines to make key decisions in the areas of possessions, acquisition, advertising, planning and development of any organization and firm.

Technological analysis is an admired approach to study the stock market analysis. To forecast future trends or prices, Researchers use a range of machine learning and intelligent artificial approaches. Many artificial neural network (ANN), support vector machine (SVM), and logistic regression (LR) used for this type of forecasting tasks. Among all these ANN is considered to be one of the best performing techniques given that its regularization parameter is properly initialised. We used the artificial neural network and particle swarm optimization technique for forecasting the stock price of Tata Motors. Technical metrics are derived from the historical trading data used in this study. Lagged data in the time series domain have always affected forecast accuracy. Availability of lagged data for our proposed model ANN-PSO leads to better performance than standard support vector regression. The rest of the paper is set out as follows. Section 2 highlights a review of the literature and section 3 gives a brief description of ANN and PSO. Section-4, i.e., ANN-PSO, then clarifies the approach and process involved in the hybrid model under review. Section 5 presents the experimental analysis, and finally the paper concludes in Section 6.

Literature Review

The literature review on the topic ANN has extended significantly. Initially the authors Reid (1968), Bates (1969) and Clemen (1989) were broadly discussed about ANN and interpreted the source of information in this field. Wedding (1996) proposed a hybrid model of ANN with the radial basis function. Luxhoj, Riis and Stensballe (1996) proposed an econometric model of ANN to forecast the sales price. Pelikan (1992) and Ginzburg (1994) were developed a feed forward neural networks to forecast the power consumption and enhanced the prediction precision of time series data. Tsaih (1998) developed a novel ANN model by integrating the concept of artificial intelligence (AI) to predict the index of American stock market. Medeiros (2000) constructed a hybrid model of ANN with smooth transition auto regressive model to forecast time series data. In current years, many hybrid prediction models have been proposed by incorporating ARIMA with ANN to archive the better performance. Pai and Lin (2005) incorporated SVR along with ARIMA for forecasting stock price. Armano (2005) proposed a novel hybrid model by incorporating GA with ANN and applied to predict stock index. Lai (2005) presented a nonlinear forecasting model by assembling ANN with Generalized Linear Auto Regression (GLAR) to predict accuracy in stock market. Chen and Wang (2007) proposed a hybrid forecasting model by the combination SARIMA with SVR. Khashei, M., Hejazi, S. R., & Bijari, M. (2008) are developed a hybrid model using the core principles of artificial neural networks to address nerve limitations in case of incomplete data sets. Khashei and Bijari (2010) proposed hybrid model of ANN-ARIMA and got more accurate prediction than the artificial neural networks. This proposed model has been incorporated PSO to optimize the network of ANN to forecast the stock price of Yahoo and Microsoft. Finally our model gives higher prediction accuracy as compared with existing models.

METHODOLOGY

Artificial Neural Network (ANN):

ANN is a parallel processing computational model, motivated by the efficient work process of genetic neurons in which mammalian brain progress information represent to it. ANN is a compilation of mathematical models and imitates some of the empirical phenomenon in a genetic nervous system, most prominently adaptive natural

learning. One distinctive and significant property of ANN model is the excellent configuration for inter-connected input neurons with assigned weight and hidden layers for processing among themselves. Each neuron in the network mapping is associated with some weight. Every input neuron is multiplied by the corresponding assigned weights. Bias is added to improve the network performance. It adjusted the output neurons with the weighted sum input. After that sum of above products are passes through an activation function to compute the result. sigmoid function is used for activation function. Our aim is to compute the output neurons for every set of input neurons.

Particle Swarm Optimization (PSO)

PSO is one of the leading meta-heuristic optimization methods which is motivated by birds and fishes co-ordinated, collective social behaviour. The basic conception of PSO was developed by Kennedy and Eberhart (1995) Inspired by the social behaviour of flocking and of bird and fish schooling. When searching for food, the basic concept that the model was based on is that the birds are either dispersed randomly (without a priori knowledge of the field) or go together before they could find the place where good quality food can be found. During this search process there are always few birds transmits the information of better quality food to the other birds. They arrive at the place where good quality food can be found because of this knowledge update or sharing between them. This behaviour is delineated by a population of particles (or individuals) called swarm, which develops the iteration by moving to the optimal solution in their area. The particle has connected to current position and velocity, by which they have the knowledge of the best local position (lbestj) that each has found and the best global position (gbest) that all the particles have found. In each iterative step the particles travel by applying their corresponding velocity from the current position. In the previous iteration, the direction and magnitude of the velocity is determined by the velocity, simulating momentum and relative position to (lbestj) and (gbest). The motion mechanism that dynamically adapts the velocity and position of the particles in each generation's evolution is formulated as

$$v_j^{p+1} = w \times v_j^p + c_1 \times rand(0,1) \times \left(lbest_j^p - x_j^p\right) + c_2 \times rand(0,1) \times (gbest^p - x_j^p)$$

$$x_j^{p+1} = x_j^p + v_j^{p+1}$$

where v_j^{p+1} and v_j^p denote the velocity of the j^{th} particle in $(p+1)^{th}$ and p^{th} iteration, respectively. w represents the initial weight coefficient; c_1 represents the personal factor and c_2 represents the social learning factor. "rand" is a random number in (0, 1). x_j^{p+1} & x_j^p denotes the position of the j^{th} particle in $(p+1)^{th}$ and p^{th} iteration respectively; α is the controlling weight factor of the velocity.

Proposed ANN-PSO Hybrid Model

Problem Formulation

Present day's prediction of financial market leads a vital role for the development of economy of a country. Prediction of stock index is very complicated due to unstable and depending on various socio-economic. In the present study we implemented the architecture of ANN by considering the historical time series data of Tata Motors as the input neurons and predict the corresponding closing price. The PSO optimized the assigned weights to minimize the prediction error. The time series data sets of TaTa Motors were collected from BSE, which consists of 3950 trading days' data from which 3160 data were used for training and 790 data for testing. The mentioned features were considered to perform the proposed forecasting prediction model which is specified below.

Pre-processing

As the data values are very large, we normalized the data first. Data standardization was introduced to reduce the range and to counteract the superiority of features with greater numerical ranges over smaller numerical ranges. Data standardisation reduces the data's gross effects. It maximizes the integrity as helps to achieve the algorithm's improved accuracy. The range of original data was moved to another scale in this phase of normalization, which reduces its range and continues to transmit the data more rapidly. In this hybrid model the set of data is structured using the equation

$$NV_k = \frac{A_k - A_{min}}{A_{max} - A_{min}}, for\ k = 1,\ 2,\ 3,\ \ldots\ldots, l$$

Where A_k denotes the original cost of the k^{th} features, *l* represents the total number of processed data, A max is the highest value, and A min is the lowest value in the entire data set. The corresponding normalized value is referred to by NV_k. After normalization the data is in the closed [0.0, 1.0] range.

Design and Implementation

Artificial neural networks (ANN) can estimate a huge data set with high prediction accuracy since the input informations are processed parallel. The network model does not require any prior assumption where as it is mainly build up by the characteristics of the data. We design the architecture of ANN which involves of three layers, which are input layer, hidden layer and output layer. The output layer processed from the hidden layer. The activation function helps to convert input signal into output signal. Here we used the sigmoid activation function to process the hidden layer. This model was characterized by the above three network layers connected through finite directed graph.

The relationship among the output (y_n) and the input nodes $(y_{n\ 1},\ y_{n\ 2}\ldots\ldots,y_{n\ p}$ is representing mathematically as follows $y_n = w_0 + \sum_{j=1}^{Q} w_j\, g\left(w_{0j} + \sum_{i=1}^{\mu} w_{i,j}\, y_{n-1}\right)$……(1) Where w_0 is the bias node used to adjust the output along with the weighted sum of the inputs to the neuron and g(.) is the activation function, which processed the received information. $w_{i,j}\ (i = 0,\ 1,\ 2,\ 3\ \ldots\ldots P,\ j = 1,\ 2,\ 3,\ \ldots\ldots Q)$ and $w_j\ (j = 1,\ 2,\ 3,\ \ldots\ldots Q)$ are the weight assigned to the input nodes. P and Q denote the number of input nodes and hidden nodes respectively of the designed network. The sigmoidal function $Sig(x) = \frac{1}{1 + e^{\ x}}$ is used to determine the output of the neural network. Sigmoid function is smooth and bounded function which gives real valued output between 0 and 1. The ANN model is designed by the equation (1) mapped from the precedent observation to the upcoming target value $y_k = f(y_{k\ 1},\ y_{k\ 2},\ y_{k\ 3}\ldots\ldots,y_{k\ p}, W)$, where W is the vector representation of all the weights and $f(y_{k-1},\ y_{k-2},\ y_{k-3}\ldots\ldots,y_{k-p}, W)$ is the function of connective weights of network structure. Hidden layer Q depends upon the input data and there is no specified rule is to assign the parameters weight.

Neural network architecture is governed by input, output and one hidden layer. The process of setting the parameters for the neural network to mimic a particular behaviour is called the learning algorithm. It can be defined as a set of rules for finding optimum weight and bias values which optimize the performance of the neural network. There are different techniques that are used to find suitable values of ANN weights and biases depending on the form of learning the weights of the neural network are adjusted to maximize the output according to specific criteria. The Sigmoid activation function is used to approximate continuous functions as it takes any real valued input and returns a bounded output from 0 to 1. The function is used as a transfer function in this analysis. To build an ANN, we need to determine how many inputs, how many hidden layers and how many hidden neurons there are. Weights

and bias are initially set at random and are modified by a learning algorithm during the training phase. After defining the number of input neurons, we need to optimize the weight and bias of both hidden and output layer of ANN using the optimization technique Particle Swarm Optimization (PSO). PSO being a metaheuristic information sharing technique in which particles update themselves with the internal velocity. PSO requires few parameters to adjust and gives the optimal output with less number of iteration.PSO update the weights and bias until to get the optimum criteria.

The proposed hybrid model (ANN-PSO) has been implemented in Mat Lab r2013a with the help of library file FANN (Fast Artificial Neural Network) This hybrid model was designed with ANN at the core along with PSO to optimize its parameters. The system that was used for Intel ® Corei3-4005U 1.7GHz 4 GB RAM implementation.

Results Analysis

Estimation of Errors

We have used three common statistical metrics for output assessment of the proposed hybrid model. They were described in Table 2. Our main objective is to minimize the predictive errors in order to obtain high precision in the proposed model.

RESULTS AND DISCUSSION

The performance of our projected hybrid model i.e., ANN-PSO is compared with standard ANN model is designed with the core concept of ANN with single hidden layer and PSO optimized the assigned weight and bias to minimize the prediction error. In this analysis, the errors measured in the training phase with MAE, RMSE, and MAPE in the Tata Motors data sets are 3.11, 4.72 and 0.73 percent (approx) respectively and the errors in the test phase are 3.16, 5.34 and 0.78 percent (approx.) respectively. The Table-3 displays the measurements of error observed for both data sets. This empirical study shows a better accuracy of ANN-PSO than the previous ANN model (MAPE 2.15). Figures-2 to 4 provides a comparison of the actual stock value and the stock value forecast using ANN-PSO. It also shows the comparisons of ANN and ANN-PSO models in terms of MAE, RMSE and MAPE and presented model show minimal error so it is very effective for Tata Motors dataset stock value forecasting.

CONCLUSION

In this chapter, an efficient hybrid model using ANN and PSO for forecasting stock price has been proposed. T ANN-PSO hybrid model, which consists of two leading techniques, is applied to the forecasting issue of the next day closing stock price and the results suggest that the model is not only suitable for analysis but also from an implementation point of view. The dataset consisted of 35 attributes that included seven critical features (mentioned in Table-1) over the last five days lagging data for the closing price forecast. The test results from the empirical study showed an mean absolute percentage error (MAPE) of 0.78 per cent (approx.). The experiment result is tested and verified in Mat Lab r2013a in normal system configuration. Therefore we recommend this model will work well for above data set.

REFERENCES

1. Armano, G., Marchesi, M., & Murru, A. (2005). A hybrid genetic-neural architecture for stock indexes forecasting. Information Sciences, Vol. 170: 3–33.
2. Bates, J. M., & Granger, W. J. (1969). The combination of forecasts. Operation Research, Vol. 20: 451–468.

3. Chen, K. Y., & Wang, C. H. (2007). A hybrid SARIMA and support vector machines in forecasting the production values of the machinery industry in Taiwan. Expert Systems with Applications, Vol. 32: 54–264.
4. Clemen, R. (1989). Combining forecasts: A review and annotated bibliography with discussion. International Journal of Forecasting, Vol. 5: 559–608.
5. Ginzburg, I., & Horn, D. (1994). Combined neural networks for time series analysis. Advance Neural Information Processing Systems, Vol. 6: 224–231.
6. Khashei, M., Hejazi, S. R., & Bijari, M. (2008). A new hybrid artificial neural networks and fuzzy regression model for time series forecasting. Fuzzy Sets and Systems, Vol. 159: 769–786.
7. Khashei, M. and Bijari, M. (2010). An artificial neural network (p, d, q) model for time series forecasting, Expert Systems with Applications, Elsevier, Vol. 37: 479–489.
8. Lai, L. and Liu, J. (2014). Support vector machine and least square support vector machine stock forecasting models, Computer Science and Information Technology, Vol. 2 (1): 30-39.
9. Luxhoj, J. T., Riis, J. O., & Stensballe, B. (1996). A hybrid econometric-neural network modelling approach for sales forecasting. International Journal of Production Economics, Vol. 43: 175–192.
10. Medeiros, M. C., & Veiga, A. (2000). A hybrid linear-neural model for time series forecasting. IEEE Transaction on Neural Networks, Vol. 11(6): 1402–1412.
11. Siddique, M., Mohanty, S. and Panda, D. (2018), A hybrid forecasting model for prediction of stock value of Tata steel using support vector regression and particle swarm optimization, International Journal of Pure and Applied Mathematics, Vol.119 (14): 1719-1727.
12. Pai, P. F., & Lin, C. S. (2005). A hybrid ARIMA and support vector machines model in stock price forecasting. Omega, Vol. 33: 505–597.
13. Pelikan, E., de Groot, C., & Wurtz, D. (1992). Power consumption in West-Bohemia: Improved forecasts with decorrelating connectionist networks. Neural Network World, Vol. 2: 701–712.
14. Reid, M. J. (1968). Combining three estimates of gross domestic product. Economica, Vol. 35: 31–444.
15. Tsaih, R., Hsu, Y., & Lai, C. C. (1998). Forecasting S&P 500 stock index futures with a hybrid AI system. Decision Support Systems, Vol. 23: 161–174.
16. Wedding, D. K., & Cios, K. J. (1996). Time series forecasting by combining networks, certainty factors, RBF and the Box–Jenkins model. Neurocomputing, Vol. 10: 149–168.

Table 1 – Features selected of Tata Motors under the study of ANN and PSO

Sl. No.	1	2	3	4	5	6	7
Variable	Open Price	Highest Price	Lowest Price	Closing Price	Number of Shares	Number of Trades	Turnover

Table 2 – Performance measure principle for the hybrid model

Sl	Metric	Definition
1	MAE	$\frac{1}{l}\sum_{i=1}^{l}\lvert y_i - d_i\rvert$
2	RMSE	$\sqrt{\frac{1}{l}\sum_{i=1}^{l}(y_i - d_i)^2}$
3	MAPE	$\frac{1}{l}\left(\sum_{i=1}^{l}\left\lvert\frac{y_i - d_i}{d_i}\right\rvert\right)100$
Where, l is the complete evaluation information, di is the original value and yi is the value obtained by the forecasting process.		

Table 3 – Performance of ANN-PSO Models on Tata Motors data sets

		Models	
		ANN	ANN-PSO
Training	**MAE**	6.21	3.11
	RMSE	8.24	4.72
	MAPE	1.98 %	0.73%
Testing	**MAE**	8.56	3.16
	RMSE	13.51	5.34
	MAPE	2.15 %	0.78%

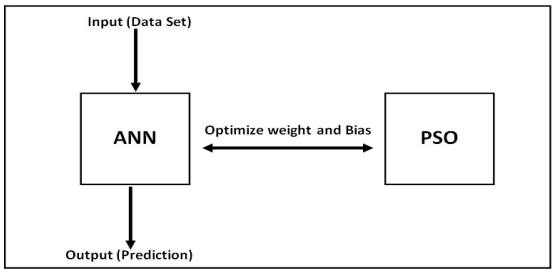

Figure 1 : Flow chart of ANN-PSO Mechanism

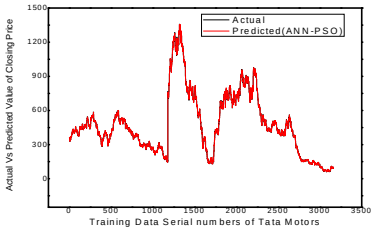

Figure 2: Actual Vs Predicted value of Tata Motors in training phase

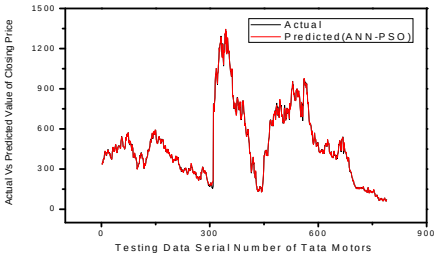

Figure 3 : Actual Vs Predicted value of Tata Motors in testing phase

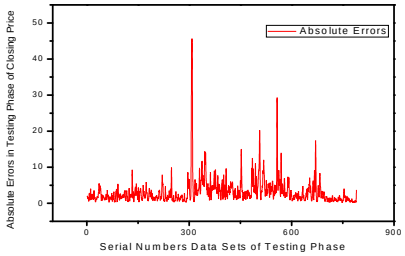

Figure 4: Absolute Errors of Tata Motors in testing phase

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RESEARCH ARTICLE

Diversity and Distribution of Aquatic, Marshy and Amphibious Flora in Chromite Mining Sites of Sukinda in Odisha

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ABSTRACT

Vegetation is an important part of the environment but is subjected to disturbance in areas close to mines resulting in a slow rate of biomass growth, leading to fading of vegetation. Simultaneously heavy metals stored in vegetation being constantly released weaken vegetation ability to act as carbon sink. An exhaustive survey of selected mining area of Sukinda chrome zone was undertaken during different seasons of the academic year 2013-2015 to explore, identify and document the distribution and abundance of diverse plant groups especially hydrophytic, marshy and amphibious forms. During the present investigation a total of 151 hydrophytic, marshy and amphibious plant species were identified belonging to 104 genera and 49 families. Amongst the genera, *Cyperus* was dominant representing 13 species followed by *Polygonum* with 5 and *Brachiaria, Fimbristylis* and *Commelina* with 4 species each. Among the angiospermic families Poaceae was observed to be the most dominant having 41 species followed by Cyperaceae with 22, Commelinaceae and Scrophulariaceae having 7 species each. As regards to the hydrophytic types 13 species were rooted floating, 7 free floating, 5 floating submerged, 2 rooted submerged and rest 124 species were found to be of amphibious. The results also revealed that *Salvinia cucullata* Roxb. ex Bory, *Salvinia molesta* D.Mitch., *Pistia stratiotes* L. and *Spirodella polyrhiza* (L.) Schleiden were the dominant floating species along with a wild grass namely *Brachiaria mutica* (Forssk.) Stapf having large population in the area under study.

Key words: Hydrophytes, Amphibious plants, Sukinda mining sites, Odisha**.**

INTRODUCTION

Mining is any activity that involves excavating the earth surface for the purpose of exploiting its mineral wealth. This could be for local economic and industrial development or for export purpose (David, 2002). The presence of mine has led to the economic development and also has adverse environmental impacts on soil, water and vegetation (Arzoo and Satapathy, 2016). Wetlands are potentially rich in aquatic resources, which play a significant role in maintaining biodiversity. They offer habitats suitable for supporting growth of variety of aquatic vegetations called hydrophytes which are adapted to live in aquatic environment (Hazarika et. al., 2012). Wetlands are also transitional lands between terrestrial and aquatic ecosystem where the water table is usually at or near the surface of the land and is covered by shallow water (Mitsch, 1986). In India it occupied an area of 58.2 million hectares (Sukumaran and Jeeva, 2011). Aquatic plants play a vital role in the form of trapping solar energy and in determining the primary productivity of aquatic system (Mohapatra et. al., 2007).

Odisha is a state of India lying between the latitudes 17.780° N & 22.730° N and between longitudes 81.37° E & 87.53° E. The state has an area of 1, 55,707 km^2, which is 4.87% of total area of India. The major mining sites which were studied in this experiment are Sukinda mining sites of Jajpur district of Odisha. As Sukinda chromite mining sites were observed to be contaminated with nickel, a detailed study with special reference to the survey of amphibious and hydrophytic flora of the area had been undertaken.

MATERIALS AND METHODOLOGY

The investigation was carried out in different seasons of the academic year 2013-2015 to explore, identify and document the distribution and abundance of diverse plant groups especially hydrophytic, marshy and amphibious forms in the study site, involving extensive field visit to the mining area at frequent intervals in different seasons. For the convenience of plant specimen collection, the study area was artificially divided into different geographical zones and each zone was visited several times in different seasons. Plant specimens were mostly collected while the representative mother plants were experiencing the state of flowering and fruiting as it became easy to ascertain the exact identification of the species bearing reproductive features. The specimens were collected in sets of 3 with a field number to choose the best specimen for making herbarium. Customary procedures were followed for the preparation of herbarium for long term preservation as well as for further study and reference. The specimens were identified following 'The Botany of Bihar and Orissa (Haines, 1922-25)' and 'The Flora of Orissa (Saxena & Brahmam, 1994-96)' as well as most of recent monographs and reviews. Finally the identified plant specimens were preserved at the herbarium of Department of Botany, Utkal University, Vani Vihar, Bhubaneswar, Odisha.

RESULTS AND DISCUSSION

During the floristic exploration, a total of 151 hydrophytic, marshy and amphibious plant species were identified which belong to 104 genera and 49 families (Table 1- 4). Amongst the genera, *Cyperus* was dominant having 13 species followed by *Polygonum* with 5 and *Brachiaria, Fimbristylis* and *Commelina* with 4 species each. The angiospermic family Poaceae was observed to be the most dominant having 41 species followed by Cyperaceae with 22, Commelinaceae and Scrophulariaceae having 7 species each. So far as the hydrophytic forms are concerned 13 species were rooted floating, 7 free floating, 5 floating submerged, 2 rooted submerged and rest 124 species were found to be of amphibious. The results also revealed that *Salvinia cucullata* Roxb. ex Bory, *Salvinia molesta* D. Mitch., *Pistia stratiotes* L. and *Spirodella polyrhiza* (L.) Schleiden were the dominant floating species along with a wild grass namely *Brachiaria mutica* (Forssk.) Stapf with large population in the area under study. Similar type of vegetations were also found from a survey report of eastern region of Odisha carried out earlier and reported a good number of aquatic and marshy plants (Chand et. al., 2009; Satapathy et. al., 2012; Satapathy, 2015; Nayak and Satapathy, 2015).

It is evident from the present investigation that a wide range of hydrophytic species documented are not only economically important but these also thrive well in the polluted water bodies in the vicinity of mining sites. Further studies on these species can identify their potential in phytoremediation related investigations so that they can be recommended for reduction of heavy metal toxicity for abating pollution.

ACKNOWLEDGMENTS

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REFERENCES

1. David KK. A report of the civil society Review of implementation of agenda 21 in Kenya, Kenya NGO Earth Summit 2002 Forum, Journal for natural resources and minerals, 2002.
2. Arzoo A, Satapathy KB. Socio-economic and environmental impacts of mining in Odisha, India. Scholars Academic Journal of Biosciences 2016; 4(7): 560-564.
3. Hazarika S, Borthakur SK. Hydrophytic flora of Assam-I: five new records. Pleione 2012; 6(2).
4. Mitsch WI, Gosselink IG. Wetlands. New York: Van Nostrand Reinhold. 1986; 539.
5. Sukumaran S, Jeeva S. Angiosperm flora from wetlands of Kanyakumari district, Tamilnadu, India. Journal of species lists and distribution 2011; 7(4).
6. Mohapatra A, Reddy CS, Biswal AK. Aquatic plants diversity of Similipal Biosphere reserve, Orissa, India. P.G. Department of Biotechnology, North Orissa University. 2007.
7. Chand PK, Satapathy KB, Patra SN. Floristic analysis of medicinal plant diversity of Thakurani Hill forest at Barbil of Keonjhar district of Orissa. Proc. of 12th Orissa Bigyan Congress, ISCA, Bhubaneswar Chapter, Bhubaneswar 2009; 51-57.
8. Satapathy KB, Sahu BB, Jena GS. Crop weeds diversity and their ethnomedicinal uses in the treatment of common ailments in Jajpur district of Odisha, India. IJMA 2012; 2(1): 80-89.
9. Satapathy KB. Dwindling medicinal plant diversity in Sukinda valley of Jajpur district of Odisha, India: utilization and conservation. International journal of Current research 2015; 7(1): 11274-11279.
10. Nayak SK, Satapathy KB. Diversity, uses and origin of Invasive Alien plants in Dhenkanal district of Odisha, India. International Research journal of Biological Sciences 2015; 4(3): 6-12.

Table 1: List of amphibious flora of the Study sites

Sl. No.	Botanical name	Vernacular name(s)
Family: Elatinaceae		
1	*Bergia ammannioides* Roxb.	Rakta-biprusa (O); Ammania, Waterwort (E).
Family: Oxalidaceae		
2	*Oxalis corniculata* L.	Ambiliti, Kumari (O); Changeri, Chukrika (San); Amrul sak, Chukutri ati (H); Creeping wood sorrel (E).
Family: Balsaminaceae		
3	*Hydrocera trifolia* (L.)Wt. & Arn.	Jala-shrungi (O); Domuti (Beng); Marsh henna, Floating balsam (E).
Family: Lythraceae		
4	*Ammania baccifera* L.	Mula-kurandica (O); Kurandica (San); Dadamari (H); Blistering ammannia (E).
5	*Ammania multiflora* Roxb.	Bahu-kurandica (O); Many-flowered ammannia (E).

6	*Rotala fimbriata* Wt.	Danti-chakrabala (O); Fringed-flower rotala (E).
7	*Rotala indica* (Willd.) Koehne	Deshee-chakrabala (O); Indian tooth-cup (E).
8	*Rotala rotundifolia* (Buch.-Ham. ex Roxb.) Koehne	Chakri, Chakrabala (O); Round-leaf tooth-cup (E); Sim-sindur (S).
Family: Onagraceae		
9	*Ludwigia adscendens* (L.) Hara	Jala bila-labanga (O); Water primrose (E).
10	*Ludwigia perennis* L.	Kshudra bila-labanga (O); Perrenial water primrose (E).
Family: Molluginaceae		
11	*Glinus oppositifolius* (L.) DC.	Sweta-pitagahama (O); Jima (Beng, H); Lonely traveler (E).
12	*Mollugo pentaphylla* L.	Pita-saga (O); Khet-papara, Jul-papara (Beng); Five- leaved carpet-weed (E).
Family: Apiaceae		
13	*Centella asiatica* (L.) Urban	Thalkudi, Hatee-khojia patra (O); Coinwort, Indian Pennywort, Spade leaf (E).
14	*Hydrocotyle sibthorpioides* Lam.	Hastee-pada (O); Lawn-marsh pennywort (E).
Family: Rubiaceae		
15	*Dentella repens* (L.) J.R. & G. Forst.	Shubhra-taraka (O); Creeping dentella (E).
16	*Mitracarpus villosus* (Sw.) DC.	Abruta-phala (O); Girdle pod (E).
Family: Asteraceae		
17	*Eclipta prostrata* (L.) L.	Bhrungaraj, Kesharaj, Keshadura (O); False daisy (E).
18	*Enydra fluctuans* Lour.	Hidmichi, Madhurango (O); Water cress (E).
19	*Spilanthes paniculata* Wall. ex DC.	Mundi, Prachira bandha (O).
20	*Synedrella nodiflora* (L.) Gaertn.	Hemagrapuspi (O); Node weed (E).
Family: Campanullaceae		
21	*Sphenoclea zeylanica* Gaertn.	Manginee (O); Chicken spike (E).
Family: Gentianaceae		
22	*Exacum pedunculatum* L.	Shyama-indu (O); Stalked persian-violet (E).
Family: Hydrophyllaceae		
23	*Hydrolea zeylanica* (L.) Vahl	Shyama-taraka (O); Koliary (H); Ceylon hydrolea (E).
Family: Scrophulariaceae		
24	*Limnophila heterophylla* (Roxb.) Benth.	Bisami- jalalovi (O).
25	*Limnophila indica* (L.) Druce	Desi-jalalovi (O); Ambujah (San); Indian marshwood (E).
26	*Lindernia anagallis* (Burm.f.) Pennell.	Bhramapadee (O); Brittle false pimpernel (E).
27	*Lindernia ciliata* (Colsm.) Pennell	Khetkura (O); Fringed false pimpernel (E).
28	*Lindernia crustacea* (L.) F.v. Muell.	Khetakurei (O); Malayasian false pimpernel (E).
29	*Mazus pumilus* (Burm.f.) Steenis	Ankurika (O); Asian mazus (E).
30	*Mecardonia procumbens* (Mill.) Small.	Pinjala-puspa (O); Baby jump-up (E).
Family: Acanthaceae		
31	*Hygrophila auriculata* (Schum.) Heine	Baripriya, Koilikhia (O); Bhankari (H); Marsh barbel (E).
32	*Hygrophila heini* Sreemadh	Anupa-baripriya (O).
Family: Verbenaceae		
33	*Lippia javanica* (Burm.f.) Spreng.	Gandha-patali (O); Lemon bush (E).
34	*Phyla nodiflora* (L.) Greene	Jala-pippali (O); Bhui-okra (H); Frog fruit (E).
Family: Amaranthaceae		
35	*Alternanthera philoxeroides* (C.Martius) Griseb.	Agra-meenakshi, Bada-madaranga (O); Alligator weed (E).
36	*Alternanthera sessilis* (L.) R.Br. ex DC.	Desi-madaranga saga (O); Sessile joy-weed (E).

Family: Polygonaceae		
37	*Polygonum barbatum* L.	Romi bahukoni (O); Bekh-unjubaz (Beng); Knot grass (E).
38	*Polygonum glabrum* Willd.	Jwala bahukoni (O); Dense-flowered knotweed (E).
39	*Polygonum lapathifolium* L.	Patri bahukoni (O); Curly-top knot weed (E).
40	*Polygonum minus* Huds.	Krushi bahukoni (O); Pygmy smart weed (E).
41	*Polygonum pulchrum* Bl.	Tari bahukoni (O).
Family: Euphorbiaceae		
42	*Phyllanthus fraternus* Webster	Badi aenla (O); Bhumyaamlaki (San); Gulf leaf flower (E).
Family: Urticaceae		
43	*Pilea microphylla* (L.) Liebm.	Baruda gachha (O); Gun-powder plant (E).
Family: Commelinaceae		
44	*Commelina attenuta* Koenig ex Vahl	Sthuli-kansiri (O).
45	*Commelina benghalensis* L.	Kanisiri (O); Kosapuspi (San); Bengal day flower (E).
46	*Commelina diffusa* Burm.f.	Nagna kanisiri (O); Spreading day flower (E).
47	*Commelina erecta* L.	Sidha kansiri (O); White mouth day flower (E).
48	*Cyanotis cristata* (L.) D. Don	Chuli neelima (O).
49	*Murdannia spirata* (L.) Brueck.	Kundali, Shyamashova (O); Asiatic dew flower (E).
50	*Tonningia axillaris* (L.) Kuntze	Godhuli (O); Creeping cradle plant (E).
Family: Juncaceae		
51	*Juncus prismatocarpus* R.Br.	Poornakuta (O).
Family: Typhaceae		
52	*Typha angustata* Bory & Chaub.	Marjara-puchha (O); Lesser Indian reed mace (E).
Family: Araceae		
53	*Alocasia macrorrhizos* (L.) G.Don	Manasaru (O); Manna (H); Giant taro (E).
54	*Colocasia esculenta* L.	Saru (O); Green taro, Cocoyam, Taro (E).
Family: Eriocaulaceae		
55	*Eriocaulon quinquangulare* L.	Sweta-bitanica (O); Phurki (Beng).
Family: Cyperaceae		
56	*Cyperus alopecuroides* Rottb.	Berua (O); Foxtail flat sedge (E).
57	*Cyperus articulatus* L.	Sandhi mutha (O).
58	*Cyperus brevifolius* (Rottb.) Hassk.	Harit mutha (O).
59	*Cyperus cuspidatus* Kunth	Ankusha mutha (O).
60	*Cyperus difformis* L.	Dweebhagi mutha (O).
61	*Cyperus diffusus* Vahl	Prasari mutha (O).
62	*Cyperus distans* L.f.	Durika mutha (O).
63	*Cyperus halpan* L.	Nagamastaki mutha (O).
64	*Cyperus iria* L.	Sanku mutha (O).
65	*Cyperus kyllingia* Endl.	Shubhra mutha (O).
66	*Cyperus paniceus* (Rottb.) Boeck.	Manjari mutha (O).
67	*Cyperus pygmaeus* Rottb.	Kharba mutha (O).
68	*Cyperus rotundus* L.	Mutha (O); Coco grass, Nut grass, Purple nut-sedge (E).
69	*Eleocharis acutangula* (Roxb.) Schult. & Schult.f.	Randhri anupashova (O).
70	*Eleocharis dulcis* (Burm.f.) Hensch.	Harit Anupashova (O); Chinese water chestnut (E).
71	*Fimbristylis aestivalis* (Retz.) Vahl	Barsidantasalaka (O); Summer fimbry (E).
72	*Fimbristylis argentea* (Rottb.) Vahl	Rajat dantashalaka (O).
73	*Fimbristylis dichotoma* (L.) Vahl,	Kani dantashalaka (O); Bara nirbishi (Beng); Summer

		fimbry (E).
74	*Fimbristylis miliacea* (L.) Vahl	Shata dantashalaka (O); Bara javani (Beng).
75	*Fuirena ciliaris* (L.) Roxb.	Pakshya lomasira (O).
76	*Lipocarpha chinensis* (Osbeck) Kern.	Prasari snehaphala (O).
77	*Scirpus articulatus* L.	Gaichira (O); Pappati chikta (Beng).
78	*Alloteropsis cimicina* (L.) Stapf	Keepa bhinnapakshak (O).
79	*Alloteropsis semialata* (R.Br.) Hitchc.	Pakshya bhinnapakhyak (O); Cockatoo grass (E).
80	*Bothriochloa pertusa* (L.) A.Camus	Basana (O); Kada chandi ghas (S); Sandhur (H).
81	*Brachiaria distachya* (L.) Stapf	Pakshya bahubalaya (O); Korama gaddi (Beng).
82	*Brachiaria miliformis* (J.S.Presl.) Chase	Bahu bahubalaya (O).
83	*Brachiaria mutica* (Forssk.) Stapf	Abindu bahubalaya (O).
84	*Brachiaria ramosa* (L.) Stapf	Sakha bahubalaya (O).
85	*Chloris barbata* Sw.	Harit smashruki (O); Jargi gandi (H).
86	*Coix lacryma-jobi* L.	Lotaka kambhu (O).
87	*Cynodon dactylon* (L.) Pers.	Duba (O); Durva, Shambhabee (San).
88	*Dactyloctenium aegypticum* (L.) P.Beauv.	Krusamanjari (O); Makra, Makri (H).
89	*Desmostachya bipinnata* (L.) Stapf	Kusa (O); Darbha (San).
90	*Digitaria abludens* (Roem. & Schult.) Veldek.	Brunti-angusthika (O).
91	*Digitaria ciliaris* (Retz.) Koeler	Rakta-angusthika (O); Takri (H); Makur-jali (Beng).
92	*Digitaria longiflora* (Retz.) Pers.	Lamba angusthika (O).
93	*Echinochloa colona* (L.) Link	Suan-ghasa (O); Shama (Beng).
94	*Echinochola stagnina* (Retz.) P.Beauv.	Bada-suan (O).
95	*Eleusine indica* (L.) Gaertn.	Ana-mandia (O).
96	*Eragrostis ciliata* (Roxb.) Nees.	Pakhayabha Pritimanjari (O).
97	*Eragrostis unioloides* (Retz.) Nees. ex Steud.	Kambhu Preetimajari (O); Koni (Beng).
98	*Heteropogon contortus* (L.) P. Beauv ex Roem. & Schult.	Shankolika (O); Sauri (M); Saiya (Ho); Saurighas (H).
99	*Hygroryza aristata* (Retz.) Nees ex Wt & Arn.	Jalamuli (O); Neevara (San).
100	*Oplismenus burmanii* (Retz.) P.Beauv.	Nali Kakapadi (O); Nini (H).
101	*Oplismenus compositus* (L.) P.Beauv.	Jugma, Kakapadi (O)
102	*Oryza rufipogon* Griff.	Balunga (O).
103	*Panicum brevifolium* L.	Harit kurchika (O).
104	*Paspalidium flavidum* (Retz.) A. Camus	Romi-bileilangi (O); Sanka (H).
105	*Paspalidium geminatum* (Forssk.) Stapf	Kuji-bileilangi (O)
106	*Paspalum distichum* L.	Dweebahu kodua (O).
107	*Paspalum scrobiculatum*(L.)Mant.	Kodua dhana (O); Kodaka (H).
108	*Pennisetum pedicellatum* Trin.	Bruti pakshyasanku (O); Swati (Beng).
109	*Pennisetum purpureum* Schumach	Hastee pakshyasanku (O); Elephant grass (E).
110	*Pseudoraphis brunoniana* Griff.	Kutasutrika (O); Pseudoraphis (E).
111	*Rottboellia cochinchinensis* (Lour.) Clayton	Agrabhangura (O); Bara swati (Beng); Barsali (H).
112	*Saccharum spontaneum* L.	Kashatandi (O); Kans (H); Payal (Beng).
113	*Sacciolepsis indica* (L.) Chase	Desi syutashalka (O); Nardula (Beng).

114	*Sacciolepsis interrupta* (Willd.) Stapf	Bandha-syutashalka (O); Nardula (Beng).
115	*Setaria pumila* (Poir.) Roem. & Schult.	Chhota-shankuja (O); Bandra (H); Pinginatchi (Beng).
116	*Setaria verticillata* (L.) P. Beauv.	Bada-shankuja (O); Silnaja (Beng).
117	*Sporobolus indicus* (L.) R.Br. var. *diander* (Retz.) Jovet & Guedes	Renuprava (O); Tandelan (H); Bena joni (Beng).
118	*Vetiveria zizanioides* (L.) Nash	Bena (O); Khas-kas (H); Siron (Beng); Vetiver (E).
Family: Pteridaceae		
119	*Pteris vittata* L.	Mrudheeka-angu (O); Feather fern (O).
Family: Adiantaceae		
120	*Adiantum philippense* L.	Shailachhanda (O); Kali jhat (H).
Family: Ceratopteridaceae		
121	*Ceratopteris thalictroides* (L.) Brongn.	Shrunga pakshi (O); Water ferns, Floating ferns (E).
Family: Thelypteridaceae		
122	*Ampelopteris prolifera* (Retz.) Copel.	Latapakshi (O).
Family: Dryopteridaceae		
123	*Dryopteris cochleata* (D. Don.) C. Chr.	Drumila pakshee (O); Common male fern; Wood fern (E).

Table 2: List of floating submersed and rooted submerged hydrophytic flora of the Study sites

Sl. No.	Botanical name	Vernacular name(s)
Family: Haloragaceae		
1	*Myriophyllum tetrandrum* Roxb.	Shahashra patri (O); Water-mifoils (E).
Family: Lentibulariaceae		
2	*Utricularia stellaris* Lour.	Syutika dala (O); Jhangi (Beng).
Family: Ceratophyllaceae		
3	*Ceratophyllum demersum* L.	Shrunjaparnee dala (O); Coon's tail (E).
Family: Hydrocharitaceae		
4	*Blyxa echinosperma* (C.B. Cl.) Hook.	Jalankura (O); Blyxa (E).
5	*Hydrilla verticillata* (L.f.) Royle	Chingudia dala (O); Indian Star grass (E).
6	*Ottelia alismoides* (L.) Pers.	Pani kundri (O); Duck lettuce (E).
Family: Najadaceae		
7	*Najas graminea* Del.	Shesanaga dala (O); Rice field water nymph (E).

Table 3: List of rooted floating hydrophytic flora of the Study sites

Sl. No	Botanical name	Vernacular name(s)
Family: Ranunculaceae		
1	*Ranunculus scleratus* L.	Pani dhania (O); Celery leafed crow foot, Cursed buttercup, Poisonous buttercup, Blister buttercup (E).
Family: Nymphaeaceae		
2	*Nelumbo nucifera* Gaertn.	Padma (O); Lotus, Sacred lotus, East Indian lotus (E).
3	*Nymphaea pubescens* Willd.	Nalikain, Dhalakain (O); Indian waterlily (E).
Family: Mimosaceae		
4	*Neptunia oleracea* Lour.	Pani-lajakuli (O); Alambusa (San); Lajalu (H); Pani najak (Beng); Sensitive water plant (E).
Family: Trapaceae		
5	*Trapa natans* L.var *bispinosa* (Roxb.) Makino	Pani singada (O); Shringataka (San); Pani-phal (H); Water chest nut (E).
Family: Menyanthaceae		
6	*Nymphoides hydrophylla* (L.) Kuntze	Purna chandramala (O); Tagarmul (H); Chandmalla (Beng);

		Crested floating heart (E).
7	*Nymphoides indica* (L.) Kuntze	Chira chandramala (O); Kumudini (San); Barachuli (H); Panchuli (Beng); Water snowflake (E).
Family: Pontederiaceae		
8	*Monochoria hastata* (L.) Solms	Kajalapati (O); Nukha (Beng); Leaf pond weed (E).
9	*Monochoria vaginalis* (Burm.f.) Presl	Bhaga kajalapati (O); Indivarah (San); Nanka (H, Beng); Oval leaf pond weed (E).
Family: Alismataceae		
10	*Sagittaria trifolia* L.	Anku dhanushira (O); Muya muya, Chhoto-kut (Beng); Three leaf arrow head (E).
Family: Potamogetonaceae		
11	*Potamogeton nodosus* Poir.	Ugragandha (O); Long-leaved pond weed (E).
Family: Aponogetonaceae		
12	*Aponogeton natans* (L.) Engl. & Krause	Teebragandhi (O); Floating lace plant (E).
Family: Marsileaceae		
13	*Marsilea minuta* L.	Chhota sunsunia (O); Water-clover, Pepperwort (E).
14	*Marsilea quadrifolia* L.	Sunsunia (O); Water-clover, Pepperwort (E).

Table 4: List of free floating hydrophytic flora of the Study sites

Sl. No.	Botanical name	Vernacular name(s)
Family: Pontederiaceae		
1	*Eichhornia crassipes* (Mart.) Solms	Bilatidala (O); Jalakumbhi (San); Jal kumbhi (H); Kachuripana (Beng); Common water hyacinth (E).
Family: Araceae		
2	*Pistia stratiotes* L.	Borajhanji (O); Water lettuce, Tropical duck-weed (E).
Family: Lemnaceae		
3	*Lemna perpusilla* Torrey	Bataka dala (O); Duck weeds (E).
4	*Spirodella polyrhiza* (L.) Schleiden	Kundalidala (O); Greater duck weed (E).
Family: Azollaceae		
5	*Azolla pinnata* R.Br.	Chunidala (O); Azolla (E).
Family: Salviniaceae		
6	*Salvinia cucullata* Roxb. ex Bory	Khudra Indurakarnee, Musakani (O); Indurkanni (Beng); Kariba weed salvinia (E).
7	*Salvinia molesta* D. Mitch.	Bruhat Indurkarnee, Musakani (O); Indurkanni (Beng); Kariba weed, Salvinia (E).

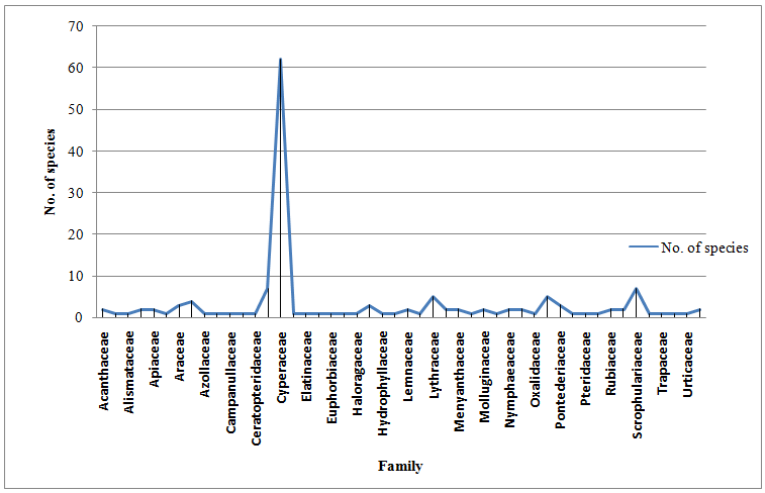

Fig. 1. Family wise distribution of plants

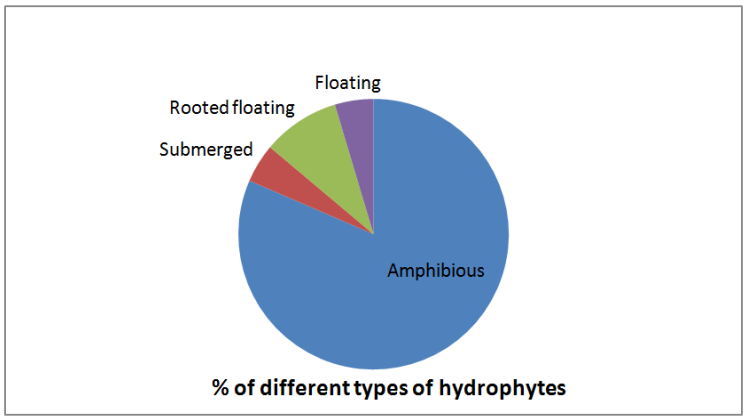

Fig. 2. Habitat wise distribution of plants

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REVIEW ARTICLE

A Review on Azolla Cultivation as a Potential Feed for Live Stock

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ABSTRACT

India has the biggest animals populace on this earth. To meet the present and future requests of the developing human population certain new systems are to be adjusted to meet the information necessities for production of domesticated animals and their results. In spite of the fact that India stands first on the planet regarding milk production and bovine populace, normal production despite everything should be improved; this might be because of low sustenance because of lacking accessibility of good quality feed/feed. This has prompted discover exchange wellsprings of good quality unusual feed/feed for proficient animals production. Now a days the demand for milk and meat is increasing as a profitable occupation. It is also expanding. The availability of fodder from different crops is decreased more due to introduction of high yielding dwarf varieties. Requirement for milk and the lack of fodder is along these lines being remunerated with business feed, Resulting in expanded expense of production of meat and milk .increasingly over business feed is mixed with urea and other artificial milk supporters. It has impact on nature of milk delivered which thusly prompts degenerative ailments like tumour and coronary diseases. The people look for an option took us to an awesome plant " Azolla" which holds the guarantee as a potential feed substitute for live stock.

Key Words: Azolla cultivation, potential livestock feed, composition.

INTRODUCTION

Domesticated animals production is the foundation of the Indian economy and has been a wellspring of work in provincial regions for a considerable length of time. Ruminants assume a significant job in giving wholesome and work security to a large number of country families in India. Customary enhancement dependent on cottonseed cake is costly and can't satisfy the necessary supplements to creatures. Numerous researchers (Khutan andAli, 1999; Satish and Ustuge,2009 and Tamang and Samanta, 1993) have recognized numerous off beat feed and feed to keep up the milk production especially in off season. Azolla holds the guarantee of giving a supportable feed to domesticated animals.India has the biggest domesticated animals populace on the planet. To meet the present and future requests of the developing human populace certain new procedures are to be adjusted to meet the information necessities for production of domesticated animals and their results. Despite the fact that India stands first on the planet as far as milk production and bovine populace, normal production despite everything should be improved; this might be because of low plane of nutrition because of inadequate accessibility of good quality feed/feed. This has prompted discover interchange sources of good quality unpredictable feed/feed for effective domesticated animals production. The search for options in contrast to concentrates/grain/feed to various types of animals, a magnificent plant called Azolla, which holds the guarantee of giving a feasible feed to live stock animals.

Since Azolla contains a large portion of the supplements which are required for all classes of animals including poultry and fish. The Azolla can be taken care of to these creatures with no antagonistic impacts. Different examinations uncovered that taking care of Azolla, in dairy cows expands milk production by 15 to 20 %. Taking care of Azolla in poultry feathered animals improves the heaviness of oven chicken and builds the egg production layers. Thus the Azolla can be utilized as off beat high potential feed asset for non-ruminants. Most importantly, for the best execution diets of pullet chicks can be detailed with incorporation of Azolla up to 10 percent. The Azolla and Salvinia are acceptable sources of minerals and basic amino acids; their use is constrained in pig production because of their low digestible energy. Data acquired from various examinations revealed that Azolla has high supplement and it is very much acknowledged by sheep and goats. Azolla can be utilized as a perfect wellspring of feed for cattle, sheep, goats, pigs, hares and fish as a substitute source to a concentrate/feed/fodder to improve the yield status of the animals.

The Azolla anabaena advantageous interaction is extraordinary because of its high profitability joined with its capacity to fix nitrogen at high rates. Along these lines, in late decades, innumerable examinations have been directed on this affiliation, however with lacking synthesis and coordination.*Azolla pinnata* attempted as a feed for broiler chicken (Alalade and Iyayi, 2006; Balaji et al., 2009; Dhumal et al., 2009; Bolka, 2011), goats (Samanta and Tamang, 1993) and buffalo calves (Indira et al., 2009). *Azolla filiculoides* was additionally utilized in for dairy animals (Leterme et al., 2010) and as partial repacement of protein source for developing fatty pigs (Duran, 1994; Becerra et al., 1995). Moreover, it was likewise attempted as a protein supplement for Rabbits (Gualtieri et al., 1988; Wittouk et al., 1992, Sreemannaryana et al., 1993; Abdella et al.,1998; Sadek et al., 2010). Azolla has huge potential as a live stock feed because of its high substance of proteins,essentials amino acids, vitamins,(vitamin A,vitamin B12, beta carotene) and minerals .It has capacity to multiply without inorganic nitrogen fertilization, It has been utilized for a long time all through Asia and Africa to take care of pigs ,ducks, chickens, cattle, fish,sheep,goats and rabbits. Azolla is plentiful in proteins , amino acids, nutrients. The minerals including Ca, Phosphorous, potassium, ferrous ,copper ,magnesium, On a dry weight premise Azolla has 25-35% protein content , 10-15% mineral substance and 7-10% mix of amino acids ,bioactive substances and biopolymers (Kamalasanana et al., 2002).

Azolla has been utilized effectively as a protein supplement to dairy animals. As indicated by Ambade et al., (2010), milk yield was expanded by 15 to 20% in the wake of taking care of Azolla in the eating regimen of dairy cows. Sanginga and Van Hove (1989) revealed that the important character affecting the estimation of Azolla as its feed is its amino acid composition. Azolla contains 25.78% crude protein , 15.71% fiber, 3.47% ether extract , 15.76% ash

content and 30.08% nitrogen free concentrate. Azolla is the most economic and feed substitute for live stock .It is effortlessly processed by animals because of its high protein and low lignin content. Azolla is also called as duck weed and it belongs to the family Salvinaceae , class pteridophyte ,order- salvinales and genus Azolla .

MORPHOLOGY

The free buoyant macrophyte *Azolla pinnata* ranges from 1cm to 2.5 cm long and 15cm or more in the species like *A.nilotica* (Raja et al . 2012). the leaves are little and alternate. Numerous unbranched adventitious roots which hang down inside the water, these roots ingest the supplements from the water. Each leaf is having two projections airborne dorsal lobe and submerged ventral lobe. The dorsal lobe is chlorophyllous and ventral lobe is dull and cup shaped it helps for buoyancy. Climate: Azolla develop in a spots with direct and sufficient daylight. A study habitat ought to be prefered, where daylight is extraordinary. Azolla like to develop in acidic soil at 5.3-5.8 pH in shade. Azolla require phosphorus for their development and appropriate supplements, for example, dairy animals manure slurry, micronutrients must to be enhanced at frequent intervals of time.

Cultivation of Azolla

Select low land field with small ponds of 320 meters size. Add water & 10-15 cm standing water should be there in the ponds. To culture Azolla with 50- 200gm/sqm add super phosphate at the rate of 20kg/ha as a phosphorous source into the pond containing water level of 15 cm . Rapid development of Azolla forms a green colour mat just like carpet in the ponds, within 14 to 21 days. The Azolla can be harvested and released in the rice field or can be used after washing & drying as an animal feed.

Types of Azolla

Azolla has used successfully as a protein diet for dairy animals. There are at least 8 species of azolla globally they are: *Azolla caroliniana, Azolla circinata, Azolla japonica, Azolla mexicana, Azolla microphylla, Azolla nilotica, Azolla pinnata* and *Azolla rubra*. The widespread species of Azolla in India is *Azolla pinnata*. It grows naturally in lentic and lotic water bodies (Senthilkumar and Manivannam, 2016 ; Bacchu Singh et al.,2017).

Uses of Azolla

- Used as bio fertilizer for agriculture because it fixes atmospheric nitrogen and store in leaves and it also used as green manure.
- Nutritional supplement for Livestock.
- In the Reclamation of saline soils.
- Used in bioremediation of toxicants.
- As a mosquito repellent.
- As a human food.
- Production of biogas and bioenergy.
- As a Component of space diet.
- It is important for animal health and milk production.
- It forms a thick layer on water surfaces and so it control the weed in paddy field.

Azolla As A Nutritional Supplement For Live Stock

Azolla is utilized as nourishment supplement for variety of faunas like cows, goat, pigs, rabbits, chickens, ducks and fish. Seultrope (1967) led an analysis and announced that Azolla can be utilized as fodder for cattles and pigs. It was

likewise discovered that broilers feed with Azolla came about in growth and body weight esteems like those subsequent from the utilization of maize-soya bean. Das et al.(1994) found that processed Azolla slurry staying after biogas production was reasonable as fish pond compost. Murthy, et al.(2013) fed 2 kg new Azolla for each day to the cows repacing half of concentrate for 3 months and revealed that Azolla kept up great dairy execution while, declining feed and work costs by 16.5% and milk production costs by 18.5%. If there should arise an occurrence of 'BlackBengal' goat, substitution of the concentrate up to20% with sun-dried Azolla resulted better development with no adverse impact on its wellbeing (Tamang, etal., 1993).

Parthasarathy et al.(2002) reported that 5 % replacement broiler ratio with dried Azolla was very beneficial and safe for chicken production. Ali,et al(1995) led a trial with taking care of feeding chicken with maize and soybean meal 10% replaced by dried *A. pinnata* and announced that feed cost altogether diminished without influencing them eat production resulting higher net return. Rai et al(2012) led a test and revealed that layer fowls fed with fresh Azolla had a higher body weight at about two months or higher egg production at 40 and 72 days than control.

CONCLUSION

Anitha et al (2016) have reported that dried Azolla showed rich in crude protein, trace minerals and vitamins and hence it can be used as livestock feed as a unconventional feed. Azolla is an ideal feed for domesic animals. One can take good care of Azolla in a culture pond and can harvest good quality duckweed every day, and it absolutely reduce the cost on food and manure.

REFERENCES

1. Abdella MM, El-Sayaad GAE, Ghazal FM, El-Baz TA (1998) Sundried Azolla as new feedstuff in growing rabbit diets Egyptian Journal of Rabbit Science 8: 81-93.
2. Alalade OA, Iyayi EA (2006) Chemical composition and feeding value of azolla (*Azolla pinnata*) meal for egg type chicks. International Journal Poultry Science 5: 137-141.
3. Ali MA, Leeson S et al (1995) Nutritional value and aquatic weeds in the diet of poultry. World's Poultry Science Journal 50: 239- 251.
4. Ambade R B, Jadhav S N and Phalke NB (2010). Impact of azolla as a protein supplement and its Influence on feed utilization in livestock. Livestock line. 4(4): 2
5. Anitha KC , Rajeshwari YB, Prasanna SB and Shilpa Shree J.2016. Nutritive evaluation of Azolla as livestock feed. Journal of Experimental Biology and Agricultural Sciences, November - 2016; Volume 4(Issue-6):670-674.
6. Balaji K, Jalaludeen A, Richard CR, Peethambaran PA,Senthilkumar S (2009) Effect of dietary inclusion of azolla (*Azolla pinnata*) on production performance of Broiler chicken. Indian Journal of Poultry Science 44: 195-198
7. Becerra M, Preston TR, Ogle B (1995) Effect of replacing whole boiled soya beans with azolla in the diets of growing ducks. Livestock Research for Rural Development Volume7,Article #26. Retrieved February 18, 2016, fromhttp://www.lrrd.org/lrrd7/3/7.htm
8. Bolka PC (2011) Nutritional evaluation of Azolla (Azollapinnata) in broiler layers. Ph.D.Thesis submitted to Karnataka Veterinary, Animal and Fisher Science University,Bidar.
9. Bacchu Singh, G.S. Meena, K.C. Meena, R.K. Meena, Bachchu Singh and Deepa Indoria.2017. Effect of a Wonder Herb Azolla on Buffaloes Milk Yield. Int.J.Curr.Microbiol.App.Sci.6(11):1059-1066.doi: https://doi.org/10.20546/ijcmas.2017.611.124
10. Das, D.; Sikdar, K. and Chetterjee, A. K. 1994. Potential of *Azolla pinnata* as biogas generator and as a fish feed, Indian J. Environ. Health., 36: 186-191.
11. Duran AO (1994) raw palm oil as the energy source in pig fattening diets and *Azolla filiculoides* as a substitute for soyabean meal. Livestock Research for Rural Development.Volume 6, Article#2.Retrieved February 18,2016, from http://www.lrrd.org/lrrd6/1/ocampo2.htm.

12. Dhumal MV, Siddiqui MF, Siddiqui MBA, Avari PE (2009) Performance of broilers fed on different levels of Azolla meal. Indian Journal of Poultry Science 44 : 65-68.
13. Gualtieri M,Rapaccini BalloniW(1988) Effects of the inclusion of *Azolla filiculoides* meal in feeds for growing rabbits. Rivista di Coniglicoltura 25: 55
14. Indira D, Sarjan RK, Suresh J, Venugopal NK, Ravi A (2009)Azolla (Azolla pinnata)a feed supplement in Buffalo calveson Growth performance. Indian Journal of Animal Nutrition26: 345-348.
15. Khutan A, and Ali MA (1999). Comparisionof nutritive value for laying hens of diets containing azolla (*Azolla pinnata*)based on formulation using digestible protein and digestible amino acid versus total protein and total amino acid. Animal Feed Sci Tech 81(1-2): 43-56.
16. Kamalasanana P, Premalatha S, and Rajamony S, (2002). Azolla – A sustainable feed substitute for livestock. Leisa India. March 2002, pp 15-17.
17. Leterme P, Londono AM, Ordonez DC, Rosales A, Estrada F,Bindelle J, Buldegen(2010)Nutritive value and intake of aquatic ferns (*Azolla fillicoides* Lam. and *Salvinia molesta*Mitchell) in sows. Animal Feed Science and Technology 155:5564.DOI:10.1016/j.anifeedsci.2009.10.002
18. Meena, G.S., B.L. Dhaka, Bacchu Singh, R.K. Meena and Meena, K.C. 2017.Effect of Azolal as feed Supplement on Milk Yield in Buffaloes. Int.J.Curr.Microbiol.App.Sci.6(12):3490-3494
19. Murthy, T. N. K.; Ashok, M.; Thirumalesh, T.; Umesh, B.U. and Nataraju, O. R., 2013. Effect of partial replacement of Azolla for concentrate supplement on lactating crossbreed cows. Environment and Ecology, 31(2): 415-417
20. Parthasarathy, R.; Kadirvel, R. and Kathaperumal, V. 2002. Azolla as a partial replacement for fish meal in broiler rations. Indian Vet. J., 79 (2): 144-146
21. Peters, G. A. 1978. Blue-green algae and algal associations. BioScience 28: 580-585.
22. Rai, R. B.; Dhama, K.; Damodaran, T.; Ali, H.; Rai S.; Singh, B. and Bhatt, P. 2012. Evaluation of Azolla (*Azolla pinnata*) as a poultry feed and its role in poverty alleviation among landless people in northern plains of India. Vet. Pract., 13 (2): 250254
23. Raja W.; Rathaur P.; John, S. A. and Ramteke, R.W. 2012. Azolla: an aquatic Pteridophyte with great potential International Journal of Research in Biological Sciences 2(2): 68-72.
24. Roy,D.C.,M.C.Pakhira and S.Bera.2016.A Review on Biology, Cultivation and Utilization of *Azolla*. Advances in Life Sciences 5(1):11-15.
25. Sadek MF, Fatma, Ahmed G, Hanan AM, Hassanein, MarvatM, Arafa. and ElhamM(2010)Using of azolla silage ingrowing rabbits feeding. Egyptian Journal of Rabbit Science20: 67-8
26. Samantha G, Tamang Y (1993) Feeding value of azolla (Azolla pinnata) an aquatic fern in BlackBengal goats. IndiaJournal Animal sciences 63:188190.
27. Sreemannarayana O, Ramachamdraiah K, Sudarshan KM,Ramanaiah NV, Ramaprasad J (1993) Utilization of azolla as rabbit feed. Indian Veterinary Journal 70:285-286.
28. Sanginga N, Van Hove C(1989) Amino acid composition of azolla as affected by strain and population. Plant & Soil 117:263-267. DOI: 10.1007/BF02220720.
29. Satish B and Ustuge SM (2009) Azolla production and livestock feeding. National symposium on Organix Livestock Farming- Globle issue, trends and challenges, 26-28 Feb, Kotkata, pp21.
30. Seulthrope, C.D. 1967. The biology of aquatic vascular plants, Edward Arnold (Pub) Ltd. London., pp. 610- 615.
31. Senthilkumar,S and Manivannam C (2016) .Adoption of azolla cultivation technology in the framers field: An analysis. Inter J Sci Env Tech 5(5):3081-3087.
32. Tamang Y, Samanta G, Chakraborty N, Mondal L (1993) Nutritive value of Azolla (Azolla pinnata) and its potentiality of feeding in goats. Environment and Ecology 10: 755-756.
33. Wittouck P, Detimmerman F, Petry M, Hove C, Van (1992)Azolla as a food for rabbit in Africa. Journal of Applied RabbitResearch 5: 1058-1062.
34. https://agriculturegurujji.com/azolla-cultivation/

Table 1: Nutritional qualities of fresh Azolla (Source: FAO,2015; Roy et al.,2016)

1. Main analysis	Unit	Avg	Min	Max
Dry matter	% as fed	6.7	5.1	8.7
Crude protein	% DM	20.6	13.9	28.1
Crude fibre	% DM	15.0	11.3	22.8
NDF	% DM	43.8	35.4	52.3
ADF	% DM	31.8	24.0	38.9
Lignin	% DM	11.4	9.3	13.5
Ether extract	% DM	3.8	1.9	5.1
Ash	% DM	15.9	9.8	21.6
Starch (polarimetry)	% DM	4.1	2.7	5.5
Gross energy	MJ/kg DM	17.0		
2. Minerals	**Unit**	**Avg**	**Min**	**Max**
Calcium	g/kg DM	11.0	5.8	17.0
Phosphorus	g/kg DM	6.1	0.3	15.5
Potassium	g/kg DM	17.4	10.9	22.5
Sodium	g/kg DM	9.0	2.8	12.5
Magnesium	g/kg DM	5.0	3.9	6.1
Manganese	mg/kg DM	762	208	1429
Zinc	mg/kg DM	38	11	77
Copper	mg/kg DM	16	10	28
Iron	mg/kg DM	3900	711	8200
3. Amino acids	**Unit**	**Avg**	**Min**	**Max**
Alanine	% protein	6.4	5.3	7.4
Arginine	% protein	5.9	5.1	6.6
Aspartic acid	% protein	9.3	8.2	10.3
Cystine	% protein	1.6	0.7	2.3
Glutamic acid	% protein	12.6	11.6	13.5
Glycine	% protein	5.6	4.5	6.6
Histidine	% protein	2.1	1.6	2.4
Isoleucine	% protein	4.5	3.7	5.4
Leucine	% protein	8.4	7.0	9.2
Lysine	% protein	4.7	3.5	6.5
Methionine	% protein	1.4	1.2	1.9
Phenylalanine	% protein	5.4	5.2	5.6
Proline	% protein	4.9	3.5	6.8
Serine	% protein	4.5	3.9	5.6
Threonine	% protein	4.7	4.0	5.3
Tryptophan	% protein	1.8	1.5	2.0

SS

Figure 1. Azolla as a feed for live stock (Source: theazollafoundation.org; indiancattle.com)

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RESEARCH ARTICLE

Study the Importance of *Citrus limetta* (Sweet Lime) and Analyzing its Phytochemical and Bioactive Behavior

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ABSTRACT

Citrus limetta (Sweet Lime) is one of the high nutrients fruit and phytochemicals that were beneficial for health. The research paper have briefing on nutrient content in lime-mosambi and pictorial representation of important benefits of the citrus for human being along with deep literature review. The research work made different experiment to find the Phytochemical behavior of *Citrus limetta* and confirm the availability of tannins, saponins, phenolics, starch, flavonoids, terpenoids, steroids, glycosides, anthraquinone and phlobatannins. The paper also have experimental result to investigate the Bioactive Compounds such ascarbohydrates, phenolics, flavonoids, vitamin C, tannins, protein and lipid, etc. Finally paper concluded by finding of Percentage Stabilization for Protein Denaturation on the solution of concentration 50, 100, 200, 400, 800 µg/ml. by different extract method in which the Standard Diclofenac Sodium method have highest value in % of 19.07 , 46.25, 58.55, 77. 11. 92.82 respectively. The paper has good analysis by graphical representation of the finding on Phytochemical and Bioactive Behavior of *Citrus limetta* (Sweet lime). This research successfully attempted to open the door of exploring fruit wastes as strong clinically biotherapeutic agents.

Key Words: *Citrus limetta*, Phytochemical Screening; Bioactive Compound; Protein Denaturation; Health benefit nutrition.

INTRODUCTION

History is witnesses, that human illness were primarily treated using traditional medicine and consumption of fruits and vegetables taken as major role to for the same and maintaining the human health. There is lot of research made

to investigate the nutritional values, the focus has shifted from the traditional study of vitamin and mineral deficiencies to the study of naturally occurring bioactive compounds having important effects on human health [1]. Several recent reviews have evaluated benefits of consumption of fruits and vegetables and prevention of the onset of cardiovascular diseases and cancer [2.3].

In this research paper, the *Citrus* taken as naturally occurring bioactive compound. *Citrus* contained nutrients and phytochemicals that were beneficial for health. *Citrus* fruits and juices contain a wide range of substances including carbohydrates, fiber, vitamin C, potassium, folate, calcium, thiamine, niacin, vitamin B6, vitamin A, phosphorus, magnesium, copper, riboflavin, pantothenic acid and a variety of phytochemicals. These substances are necessary for proper functioning of the body but some confer additional protection against chronic disease over and basic nutrition. *Citrus* fruits and juices are a great source of bioactive compounds including antioxidants such as ascorbic acid, flavonoids, phenolic compounds and pectins that are important to human nutrition [4-6]. The peel which represents almost one half of the fruit mass has the highest concentrations of flavonoids in the *Citrus* fruit [7-8]. It also has a relatively low glycaemic index which helps in maintaining a more stable blood glucose level and generally healthier carbohydrate metabolism. Citrus fruits also contained many phytochemicals including essential oils, alkaloids, flavonoids, coumarins, psoralens and carotenoids.

Study on citrus fruit peel wastes of *Citrus limon* and *Citrus sinensis*. Authors demonstrated the occurrence of significant amount of secondary metabolites such as tannin, steroids, reducing sugar, proteins and high content of carbohydrates from the aqueous and chloroform extracts and other secondary metabolites. Researchers work out, the fruit peel ethanolic extract of C.limon and C.sinensis exhibited potent anti-oxidant activity and antibacterial activity against Gram positive and Gram negative organisms. Paper help to understand the efficient extraction, processing and utilization of these citrus fruit peel wastes and also to characterize the phytochemicals, antioxidant property and antibacterial activities [9]. Therapeutic properties of citrus fruits, like anticancer, antiviral, anti-tumor, anti-inflammatory activities, and effects on capillary fragility as well as an ability to inhabit platelet aggregation. Photochemical and bioactive compounds such as flavonoids, carotenoids, vitamins and minerals available in citrus fruits mentioned its application against cancer, tumor growth, cardio diseases and macular degene [10]. Investigate on flavor components of sweet lime obtained using cold-press and hydro distillation. and analyzed using GC and GC-MS conclude oxygenated compounds present in the oil which also important in pharmaceutical, and cosmetics industries [13,14,17].

The mathematical model for drying characteristics of kinnow and sweet lime peels as a function of drying method and temperature [18]. Flavonoid content, ascorbic acid content and assessment of Vitamin C activity, mineral content and antioxidant potential [20,21,23]. Phytochemicals present in the fruit wastes (flavedo, albedo and seeds). Proximate analysis was carried out on the dried and ground wastes while phytochemical screening was carried out on ethanolic and hydroethanolic extracts. The results showed that value-added products of varied industrial application may be available in the seeds and peels of sweet orange [24,25]

About *Citrus limetta* and Benefits

Citrus plants belong to the family Rutaceae are one of the fruit crop grown throughout world. India produces 8.70 million tones of citrus fruits annually and rank third in the production. In food manufacturing, citrus is mainly used for producing fresh juice or citrus-based drinks, in the last decade, several studies suggested that *citrus* waste could be used as natural sources of antioxidants. In this regard the *Citrus limetta* (Sweet lime)- Mosambi is one of the species of the citrus which is look for low calorie or low fat diet antioxidant fruit. Due to its nutritional value, sweet lime is considered beneficial for an overall good health. The inclusion of sweet lime or mosambi in your regular diet is an ideal option for those who are fitness freaks and aspire to get rid of extra flab. If we take a 100 gm mosambi, it will provide 43 calories with some amount of Flavonoids and other phenolics, carotenoids and limonoids. The nutrient content of sweet lime-mosambi is as below for 100 gm weight.

MATERIALS AND METHODS

Stage-1

Collection and preparation of sample

Citrus limetta (Sweet lime) was collected from local market. The peel was washed thoroughly to remove dust particles and dried in hot air oven at 25° – 30° C. The dried peel was powered using mixer grinder. The dried finely ground powder was subjected to soxhlet extraction with acetone, ethanol and methanol. The residual extracts were evaporated to dryness and stored in refrigerator for further analysis.

Phytochemical screening

The extracts was analyzed for the presence of tannins, saponins, phenolics, starch, flavonoids, terpenoids, steroids, glycosides, anthraquinone and phlobatannins.

Estimation of Bioactive Compounds

Bioactive compounds include primary and secondary metabolites such ascarbohydrates, phenolics, flavonoids, vitamin C, tannins, protein and lipid, etc.

Stage-2

Sample Collection

The peels of *Citrus limetta* were obtained from the local market.

Preparation of the extract

Fresh peels dried in room temperature & press mechanically and sieved by No-25 mesh sieve, Approx. 4 g of powder soaked with 100ml of ethanol, water, ether, and methanol in different flasks for 24 h and then macerated at room temperature for 2 hours.

Finding of Protein Denaturation

Bovine serum albumin (0.2ml) + phosphate buffered saline (PBS, pH 6.4) for 2.8ml + 2 ml of varying concentrations of extract => convert final solution of concentration 50, 100, 200, 400, 800 µg/ml. The mixtures were incubated at 40ºC in a BOD incubator for 20 minutes and then heated at 70ºC for 5 minutes. After cooling, absorbance was measured at 340 nm (SHIMADZU, UV 1800) by using vehicle as blank. Percentage inhibition = (Abs Control –Abs Sample) X100

RESULTS AND DISCUSSION

Phytochemical Screening

The invigorating properties of remedial plants are perhaps due to the presence of discrete secondary metabolites such as tannins, saponins, phenolics, starch, flavonoids, terpenoids, steroids, Glycosides, anthraquinone, phlobatannins etc.

Estimation of Bioactive Compounds

Bioactive compounds are extra nutritional constituents that typically occur in small quantities in foods. The quantitative analysis of bioactive compounds in the extracts is given in Table 2. From the table it was evident that bioactive compounds such as phenols, flavonoids, vitamin C, tannins, proteins and carbohydrates were found in maximum quantityin methanolic extract of *Citrus reticulate* peel. Inflammation is a reaction of living tissue towards injury. Phytochemical screening of *Citrus limetta* indicates the presence of polyphenols, flavonoids, tannins, saponins, glycosides etc. Hence, from the obtained results, it can be concluded that *Citrus limetta* peel extracts possesses anti-inflammatory and anti arthritic activity from the obtained results, it can be concluded that *Citrus limetta* peel extracts possesses anti-inflammatory activity. However, the methanolic and ethanolic extracts showed significant activity when compared with the standard drug.

Present analysis confesses that *Citrus reticulata* peel will be a perfect effective source of various bioactive compounds. Since the peel extracts shows the presence of important phytochemicals such as flavonoids, phenols, tannins, terpenoids, glycosides and steroids. Extracts of such an important bioactive compounds from the peel itself instead of fruit is notable worthy from the economic point of view as well as eco-friendly manner. The research paper have briefing on nutrient content in lime-mosambi and pictorial representation of important benefits of the citrus for human being along with deep literature review. The research work made different experiment to find the Phytochemical behavior of *Citrus limetta* and Bioactive Compounds. This attempt is a step to open the door of exploring fruit wastes as strong clinically biotherapeutic agents. It also reveals fruit peel could be used as alternative useful agents in food industries.

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Conflict of Interest

The author declares that there is no conflict of Interest. Author has seen and agreed with the contents of the manuscript and there is no financial interest to report. We certify that the submission is original unpublished work, is not being submitted for publication elsewhere at the same time and is not under review at any other publication. Author agree to transfer the copyright of the submitted article to the publisher upon accepting the material for publication and deemed void if the manuscript is rejected or not accepted by the Editorial Board.

REFERENCES

1. Sohail Ejaz, Ahmed Ejaz , Kiku Matsuda & Chae Woong Lim, Limonoids as cancer chemopreventive agents. *Journal of Science and Agriculture*, 2006; 86(3) pp 339-345
2. Steinmetz, K.A. and Potter, J.D, Vegetables, Fruit, and Cancer Prevention. A Review. *Journal of the American Dietetic Association*, 1996; 96, 1027-1039
3. Veer,Jansen, Klerk, & Kok,. Fruits and vegetables in the prevention of cancer and cardiovascular disease. *Public Health Nutrition* 2000; 3(1):103-7
4. Ebrahimzadeh et al., Antioxidant activity, phenol and flavonoid contents of 13 Citrus species peels and tissues. *Pakistan journal of pharmaceutical sciences* 2004;22(3):277-81
5. Fernandez-Lopez et al., Antioxidant and antibacterial activities of natural extracts: application in beef meatballs. *Meat Sci*. 2005; 69(3):371-80.

6. Jayaprakasha and Patil,. In vitro evaluation of the antioxidant activities in fruit extracts from Citron and Blood Orange. *Food Chemistry* 2007; 101(1):410-41.
7. Manthley and Grohmann, Phenols in Citrus Peel Byproducts. Concentrations of Hydroxycinnamates and Polymethoxylated Flavones in Citrus Peel Molasses. *Journal of Agricultural and Food Chemistry*, 1996; 49(7):3268-73
8. Anagnostopoulou et al., Radical scavenging activity of various extracts and fractions of sweet orange peel (Citrus sinensis), *Food Chemistry* 2006; 94(1):19-25
9. Suja D, Bupesh G, Nivya Rajendiran1, Mohan V, Ramasamy P, Muthiah NS, Arul Amutha Elizabeth, Meenakumari K and Prabu K, Phytochemical Screening, Antioxidant, Antibacterial Activities of Citrus Limon and Citrus Sinensis Peel Extracts. *International Journal of Pharmacognosy and Chinese Medicine*, 2017;1(2).
10. Donatus Ebere Okwu, Citrus Fruits: A Rich Source Of Phytochemicals And Their Roles In Human Health. Int. *J. Chem. Sci*., 2008; 6(2), 451-471.
11. Ahmed Abdullah Khan , Tarique Mahmood , Hefazat H. Siddiqui and Juber Akhtar. Phytochemical and pharmacological properties on Citrus limetta (Mosambi), *Journal of Chemical and Pharmaceutical Research*, 2016; 8(3):555-563.
12. D.C. Sikdar, Vikas Gunaki, Rakesh Rao, Extraction Of Citrus Oil From Sweet Lime (Citrus limetta) Peels By Steam Distillation And Its Characterizations. *International Journal of Technical Research and Applications*, 2016; 4(6), PP.141-145.
13. Behzad Babazadeh Darjazi, Comparison of Peel Components of Sweet lime (Citrus limetta Risso) Obtained using Cold-press and Hydrodistillation Method. *Bulletin of Environment, Pharmacology and Life Sciences Bull. Env. Pharmacol. Life Sci*., 2014; 4(1), 178-184.
14. María C. Colecio-Juárez , Rubria E. Rubio-Núñez , José E. Botello-Álvarez , Gloria M. Martínez-González , José L. Navarrete-Bolaños , and Hugo Jiménez-Islas, Characterization Of Volatile Compounds In The Essential Oil Of Sweet Lime (Citrus limetta Risso), *Chilean Journal Of Agricultural Research* 2012; 72(2).
15. Dr Ali Esmail Al-Snafi, Nutritional value and pharmacological importance of citrus species grown in Iraq, *IOSR Journal Of Pharmacy*, 2016; 6(8), PP. 76-108.
16. Pathogens Shalu Hasija, Geeta Ibrahim, Ashok Wadia, Antimicrobial Activity of Citrus Sinensis (Orange), Citrus limetta (Sweet Lime) and Citrus Limon (Lemon) Peel Oil on Selected Food Borne. *International Journal of Life Sciences Research* 2015; 3(3), 35-39.
17. Megha Mahendera and Mumtaj Shah, Extraction and Characterization of Essential oil of Sweet Lime (Citrus limetta Risso) peel using Microwave-assisted Hydrodistillation, *Research Journal of Chemical Sciences* , 2014; 4(11), 51-55.
18. Manoj Kumar Mahawar, Kirti Jalgaonkar, Bhushan Bibwe, Bhupendra M Ghodki, Bharat Bhushan, Mathematical modelling and drying kinetics of kinnow and sweet lime peels, *International Journal of Chemical Studies*; 2017; 5(6): 885-888
19. Nelofer Jamil, Rukhsana Jabeen, Muzaffar Khan, Musarat Riaz, Tayyiba Naeem, Aqsa Khan, Noor Us Sabah, Sadaf Aslam Ghori, Uzma Jabeen, Zahoor Ahmed Bazai, Ayesha Mushtaq, Sabeena Rizwan, Shagufta Fahmid, Quantitative Assessment of Juice Content, Citric Acid and Sugar Content in Oranges, Sweet Lime, Lemon and Grapes Available in Fresh Fruit Market of Quetta City. *International Journal of Basic & Applied Sciences* , 2015; 15(1).
20. Gattuso, Davide Barreca, Claudia Gargiulli, Ugo Leuzzi and Corrado Caristi,. Flavonoid Composition of Citrus Juices Giuseppe. *Molecules*, 2007; 12, 1641-1673
21. Deekshika B, Praveena Lakshmi B, Hemanth Singuluri and M.K. Sukumaran, Estimation of ascorbic acid content in fruits & vegetables from Hyderabad, India – A theoretical assessment of Vitamin C activity. *Int.J.Curr.Microbiol.App.Sci*, 2015; 4(1): 96-99.
22. Riddhi Rajesh Gada1 and Unnati Padalia, Biosynthesis Of Silver Nanoparticles Using Citrus Fruits, *International Journal of Pharmacy and Biological Sciences* 2018; 8(1), 146-149.
23. Pooja Singh and Preeti Verma,. Nutritional Evaluation Of Sweet Lime Peel Powder And Formulation Of Apple Jam By Incorporating It. *International Journal of Recent Scientific Research*, 2016; 7(5):10938-10940.
24. Oikeh1 E. I., Oriakhi K. and Omoregie E. S, Proximate Analysis and Phytochemical Screening of Citrus sinensis Fruit Wastes. *The Bioscientist*, 2013; 1(2):164-170.

25. Chede P.S, Phytochemical Analysis of Citrus sinensis Pulp. *International Journal of Pharmacognosy and Phytochemical Research*; 2013, 4(4); 221-223.
26. Emad Mohamed Abdallah, Preliminary Phytochemical and Antibacterial Screening of Methanolic Leaf Extract of Citrus aurantifolia, *Pharmaceutical Biotechnology: Current Research*, 2016; 1(2) ,1: 2.
27. Kumar KA, Narayani M, Subanthini A, Jayakumar M, Antimicrobial activity & phytochemical analysis of citrus fruit peels-utilization of fruit waste. *IJEST,* 2011; 3(6): 0975-5462.
28. Balasundram N, Sundaram K, Samman S, Phenolic compounds inplants and agri-industrial byproducts: Antioxidant activity, occurrence, and potential uses. *Food Chem* 2006; 99: 191-203.
29. Adams LS, Seeram NP, Agarwal BB, Takada Y, Sand D, et al., Pomegranate juice, total pomegranate ellagitannins, and punicalagin suppress inflammatory cell signaling in colon cancer cells. *J Agri Food Chem* 2006; 54(3): 980-985.
30. Mathur A, Verma S, Purohit R, Gupta V, Prasad VK, et al., Evaluation of in vitro antimicrobial & antioxidant activity of peels & pulp of some citrus species. *Int J Biotech Bio therap* 2011; 2: 2229-2278.
31. Saadi MAK, Salih HAM, Abbas AM,. Study of the fruit peels of Citrus sinesis & Punica granatum. *J Babylon Uni* 2003; 3(9): 243-342.
32. Subhashini R, Mahadeva-Rao US, Sumathi P, Gunalan G, A comparative phytochemical analysis if cocoa and green tea. *Indian J Sci Tech* 2010; 3(2): 188-192.
33. Mathur J, Khatri P, Samanta KC, Sharma A, Mandal S, Pharmacognostic and Preliminary phytochemical investigation of Amaranthus Spinosus (Linn.) Leaves. *Int J Pharm Pharmaceu Sci* 2010; 4(2): 121- 124.
34. Perumal S, Klaus B, Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (Moringa oleifera Lam.) leaves. *J Agri Food Chem* 2003; 51(8): 2144-2155.
35. Omoregie ES, Osagie AU, Antioxidant Properties of Methanolic Extracts of some Nigerian Plants on Nutritionally-Stressed Rats. *Nigerian J Basic Appl Sci* 2012; 20(1): 7-20.
36. Devasagayam TPA, Tilak JC, Boloor KK, Sane KS, Ghaskadbi SS, et al., Free Radicals and Antioxidants in Human Health: Current Status and Future Prospects. *JAPI* 2004; 52: 794-804.
37. Mathew B, Jatawa SK, Tiwaari A, Phytochemical analysis of Citrus limonum pulp and peel. *Int J Pharm Pharmaceu Sci* 2012; 4(2): 269-371.

Table. 1: Brief of Experiments to test the Phytochemical behavior of *Citrus limetta*

SN	Type of Test	Experiment Details	Test Observation	Test Result
1	Test for tannins (Ferric chloride test)	Ferric Chloride : 1 drop Extract : 1 ml	Blueish black colour on solution	Confirm the tannins
2	Test for saponins (Foam test)	Distil Water : 5 to 8 ml Extract : 0.2 to 0.4 ml Heat till boil	Mess type cream colour bubbles	Saponins confirm
3	Test for phenolics (Ferric chloride test)	Small amount of Ferric Acid approx 1 drop in 5ml extract	Greeneish colour on solution	Presence of Phenolics
4	Test for starch (Iodine test)	Iodne Solution : 2 to 3 drop Extract : 1 ml	Blue colour on solution	presence of starch
5	Test for flavonoids (Shimoda test)	Amonia Solution : 2 to 3 drop Extract : 2 ml	Yellow colour on solution	presence of flavonoids.
6	Test for terpenoids (Terpenoids test)	Chloroform :2.5ml H2SO4 (Concentrated): 2.5ml Extract : 5 ml	Red brown precipitate	presence of terpenoids
7	Test for steroid (Salkowski test)	acetic acid anhydride :2.5ml H2SO4 : 2.5ml Extract : 5 ml	Brown ring at interface	presence of glycosides

		Solution underplayed with 1ml of concentrated sulphuric acid		
8	Test for anthraquinone (Borntragers test)	HCl :1ml Extract: 2.5 ml Boil in water bath for few min., thenfilter and make cool. Same amount of CHCl3 was added to the filtrate and add drops of ammonia → mix and heated	Pink colour appear	Presence of n-hexane, chloroform, ethyl acetate and methanol which confirm anthroquinone .
9	Test for phlobatannins (Phlobatannins test)	One drop of aqueous HCl in 5 ml of aqueous extract	Deposition of a red precipitation	evidence for the phlobatannins

Table. 2: Brief of Experiments to test the Bioactive Compound of *Citrus limetta*

SN	Checking	Experiment	Output	Result
1	Estimation of phenols	Extract 1ml in triplicate and volume made upto 1ml with methanol. Folin's phenol 5ml added. sodium carbonate (7.5%) : 4ml added. Leave at Room Temp. for 2 hrs	Intensity 765 nm. Graph of gallic acid plotted	√
2	Estimation of flavonoids	To 1 ml of extract, 0.5 ml of aluminium chloride (1.2%) and 0.5 ml of potassium acetate (120 mM) was added and incubated for 30 min at room temperature.	absorbance was measured	√
3	Estimation of vitamin C	One ml of extract was taken and made up to 3 ml with methanol: water (1:1). To this 0.2 ml DTC reagent was added, mixed well and incubated at 37°C for 3 hours. 1.5 ml of 85% H2SO4 was added and kept at room temperature for 30 min	Colour intensity was read at 520 nm. A standard graph of ascorbic acid was plotted, from which the vitamin C content of the extract was determined	√
4	Estimation of tannins	One ml of the extract was taken in triplicates and the volume was made up to 1 ml with methanol. One ml of water served as blank. To this, 5 ml of Folin's phenol reagent was added followed by 5 ml of 3.5% sodium carbonate and kept at room temperature for 5 min. The intensity was read at 640 nm.	A standard graph of tannin was plotted, from which the tannin content of the extract was determined	√
5	Estimation of carbohydrates	One ml of each extract was pipetted out into 25 ml standard flask, 2 ml of freshly prepared anthrone reagent was added to each extract and finally the volume was made upto25 ml with distilled water. Blank was maintained without adding extract. A standard calibration curve was plotted using Glucose as standard.	Absorbance recorded at 750 nm against reagent blank. From the standard curve, concentrations of carbohydrates were calculated	√
6	Estimation of proteins	Five ml of alkaline copper sulphate reagent was added to 1 ml of each extract and mixed	After incubating for 30 min the absorbance	

		thoroughly. After 10 min of incubation 0.5 ml of Folin's reagent was added.	was recorded at 660 nm against the blank. Bovineserum albumin was used as standard	√

Table-3: Experimental value of Protein Denaturation of *Citrus limetta*

SN	Concentration (µg/ml)	Percentage Stabilization				
		Water Extract	Ether Extract	Methanolic extract	Ethanolic extract	Standard (Diclofenac Sodium)
1	50	4.7	2.38	8.95	6.82	19.07
2	100	26	19.25	25.88	25.85	46.25
3	200	40.42	35.03	48.05	41.5	58.55
4	400	60.12	51.20	65.08	62.11	77.11
5	800	79.21	69.22	91.51	84.22	92.82

Table. 4: Qualitative analysis of bioactive compounds in the different extract.

CHEMICAL TESTS	ACETONE	ETHNOL	METHANOL
Tannins	√	√√	√√
Saponins	x	x	x
Phenolics	√	√√	√√
Starch	x	x	x
Favonoids	√	√√	√√
Terpenoids	√	√	√√
Steriods	√√	√	√√
Glycosides	x	√√	√√
Anthraquinone	x	x	x
Phlobatannins	x	x	x

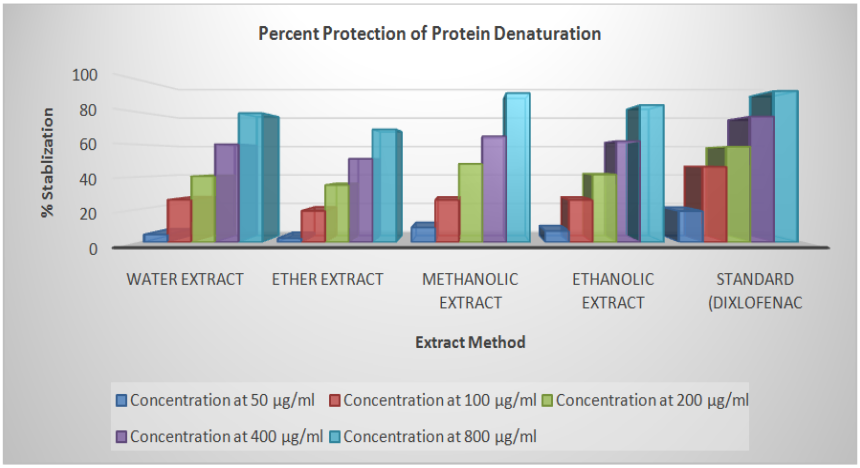

Fig.1: Bar Chart Representation of Protein Denaturation of *Citrus limetta*

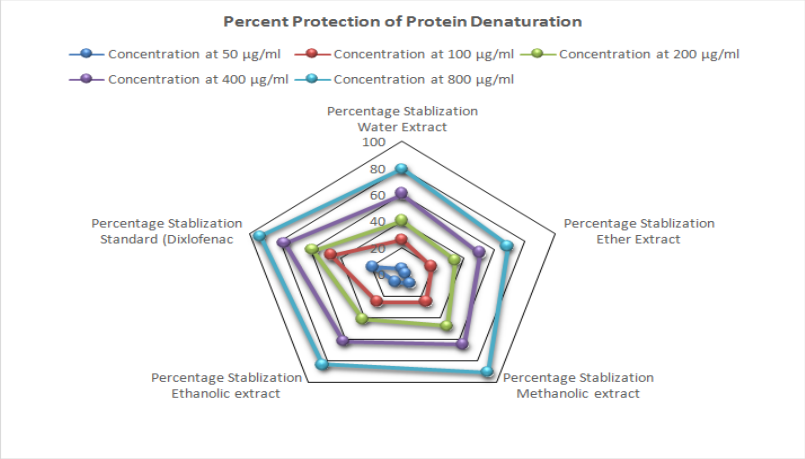

Fig-2: Graphical Representation of Protein Denaturation of *Citrus limetta*

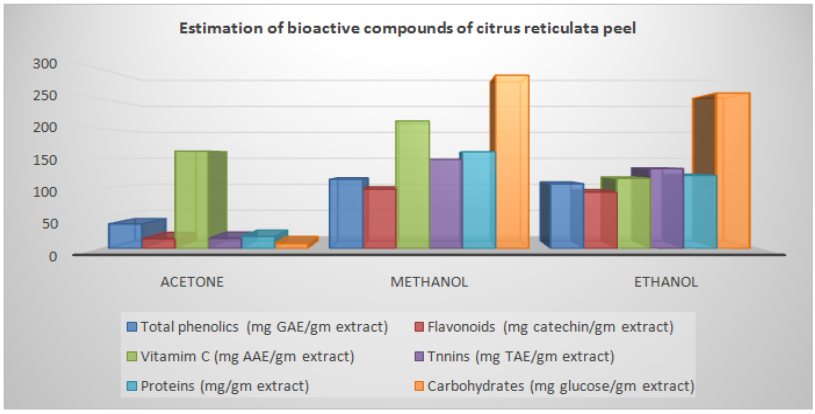

Fig-3: Bar Chart Representation of Estimated Bioactive Compound in *Citrus limetta*

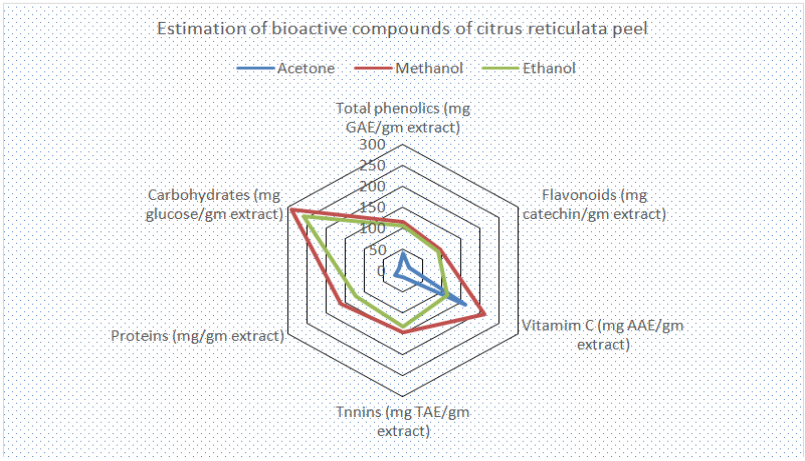

Fig-4: Graphical Representation of Estimated Bioactive Compound in *Citrus limetta*

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RESEARCH ARTICLE

Development of Vermicompost from Acacia (*Acacia auriculiformis*) Leaf Litter by using Earthworm *Eisenia fetida*

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ABSTRACT

Phytowastes are one of major cause of environmental pollution thatdegrades slowly by natural decomposition. Vermicomposting is a special eco-friendly technique for safe disposal of solid organic wastes. Earthworms are playing major role along with macro and microorganism in vermicomposting where solid wastes converted to high nutrient rich humus product for sustainable plant growth and effective clearance of environment. The objective of present work has been carried out to reveal the activity of *Eisenia fetida* on leaf litter as a phytowaste that cover large area obstructing soil reformation process for steady degradation that significantly converted to enriched soil. The effectiveness of vermicomposting for the treatment of leaf litter was analyzed by different physical parameters. The different proportions of leaf litter of *Acacia auriculiform is* of 10%, 20%,30% and 40% were treated along with control and experimental as whole by introducing *Eisenia fetida.* The obtained vermicompost was significantly increased with physical parameters of pH, electro conductivity and moisture that found in low range previously before treatment. It also aided ingrowth and increase population rate of earthworm and also enhanced seed germination and growth of plant.The results of the experiment were indicated that the high enriched vermicompost formed due to the subsequently degradation of leaf litter in the treating pot.

Keywords: Phytowaste, vermicomposting, *Eisenia fetida*, *Acacia auriculiformis*, physical parameter.

INTRODUCTION

Organic wastes are biodegradable and produce from either plant or animals usually broken down by decomposers may be microorganism or macro organism over time. The biodegradable wastes are mostly vegetable scraps, fruit debris, grass, paper, bones, and leaf litter etc. eventually rot.During the natural process of composting it may produce

fouling smell causes greenhouse effect by adding of methane gas. Leaf litter (dry leaves) is one type of organic waste that causes environmental pollution undergoes a steady process of composting and application of vermicomposting can tackle this problem. The environmental risk due to leaf waste material reduces into a safer and more stable product suitable for application to soil can be achieved by the process of vermicomposting (Lazcano et al., 2008). Vermicomposting is a special type of technique for decomposition & biodegradation applying worms to convert organic waste including both domestic as well industrial into nutrient rich organic manure compare to regular compost which can be returned to the soil for sustainable plant growth. It is a faster degradeable process allowing breeding & production of worms and harvesting worm cast is the production of worm fertilizer increase soil fertility similar to regular compost (Singh and pillai, 1973; Edward and Lofty, 1977). "Vermi" means-Worms, Compost means-degradation.Vermicompost is anend product containing active organic material generated from the coaction of earthworms and different type of microorganisms during vermicomposting microbiological process (Dominguez, 2004).

Vermicomposting is simply a bioconversion method of organic waste into a nutrient-rich fertilizer due to earthworms' activity. Earthworms are utilized as biological agents in the deprivation of organic wastes that plays an important role in the process of decomposition of organic matter and soil metabolism (Syres et al.,1979;Albanella et al.,1988;Jambakar,1992). It is found in the soil ,feeding on live and dead organic matter. They are mentioned as indicator of soil health. The organic waste passes throughtheir digestive system of earthworm after feeding and givesout a granular form as excretaknown as vermicast. (Bhoyar and Bhide, 1996). It helps to make the soil more nutritious and used as an organic fertilizer are called as best partner for the soil and the farmer. They are normally found in the soil,feeding on live and dead organic matter and are referred as indicator of soil health.

The production of vermicompost have been reported from various works using leaf of different plant such asTeak leaf litter (Nagalakshmi and prakash 2016),Leaf litter ofMango and Guava (Vasanti et.al.,2013),Leaf litter of Rubber (Nath and Caudhuri,2014), Tendu leaf litter (Mushan and Rao,2012), *Eucalyptus* leaf litter (Kumar H.D. and Sahoo L. 2011), Sugarcane leaf (Alagesan and Dheeba,2010) Paddy straw and wheat straw (Indrajeet and singh 2010), Ashoka tree leaf litter(*Polyalthia longifolia*),Teak tree leaves litter(*Tectona grandis*) and Neem tree leaf litter (*Azadirachta indica*) (Jayanthi et.al.,2010),etc.The present investigation focus on thevermicomposting of Acacia leaf litter by using one of the species belongs to Oligochaeta i.e.*Eisenia fetida*.

MATERIALS AND METHODS

Acacia auriculiformis

It is widely known asAuri, ear leaf Acacia, Ear pod wattle, Papuan wattle, and Tan wattle. It is a fast- growing tree is placed in the familyof Fabaceae. It grows up to 30metres tall with a crooked bole that is up to12 meters long and 50cm in diameter. The wood is a source of rich fuel and is widely utilized for that purpose. Leaves are simple, alternative, reduced to flattened leaf stalks. The color of the leaf seen as dark green and sometimes slightly curved, 5-8c.m in long , with 3-7 main parallel. At the end of the dry season some of the tropical species lose their leaves. When leaf litters undergoes decomposition, it produces nutrients that nurtures the soil and alsoprovide shelter and food to the terrestrial life. In present investigation dried leaflitter of ***Acacia auriculiformis*** allowed for vermicomposting by mixed with cow dung in various proportions.

Earthworm

Earthworms are found all over the world and it's role in case of soil formation and soil fertility proposed by (Darwin,1881;Edwards et al.,1995;Kale,1998;Lalitha et al,2000). Various species of worms, usually red wigglers, white worms ,and other earthworms are used in vermicompost for creating a mixture of degraded organic

wastes , bedding material and vermicast. Earthworm sexcreted breakdown of organic matter and the end-product is Vermicast.The vermicast containing nitrogen (N), phosphorous (P) and potassium (K) along with trace elements depending on the feedstock type [2;4-14;17-19]. Vermicast also contains hormones and enzymes which stimulate plant growth and discourage plant pathogens. This is a veryessential part of organic farming today. Different earthworms' species are used in vermicomposting. One of the most commonly species used frequently for vermicomposting is *Eisenia fetida* (red worm), which has high potentialforbio-converting organic waste into vermicast These worms act on rotting vegetation, organic wastes and obtained manure. The earthworms are hermaphrodite and they reproduce quickly within 60 days pertaining to the environment situation in which they live.

Collection of Samples

The samples such as leaf litter waste,soils, cow dung are collectedfrom centurion university of technology and management (CUTM) campus, Bhubaneswar, Odisha,India.

(i) Collection of leaf litters waste
The dry leaf litter of*Acacia auriculiformis*(Common name: Acacia) was collected randomly from the CUTM campus used as a major substrate for experimental set up.

(ii) Collection of cowdung
Urine free cowdung was collected in large sized rectangular plastic container from cattle-shed of CUTM campus.

(iii) Collection of soil
Soil was collected from garden area of CUTM campus, Bhubaneswar

(iv) Collection of Earthworm Species
The exotic species *Eisenia fetida* was used for vermicomposting. Earthworms were obtained from vermiculture center of CUTM campus

(v) Collection of Plastic Pots
Plastic pots with 18c.m heights and about 9c.m-14c.m in diameter from bottom to top were collected for composting and vermicomposting processes.

Preparation of Composting Mixtures

The first phase was conducted to dry the collected samples(cow dung,soil,leaf litters) for moisture free, exposed in bright sunlight with chopping the large particles into smaller and turned the materials in regular interval up & down for 3-4 days. In the second phase the dried samples of soil,cow dung and leaf litter were chopped finely and sieved by a diameter of 3.35mm sieve. The sieving materials were collected in large plastic containers and were used for weighing through a weighing machine. The collectedleaf litter wastes were sliced into small pieces(app.3 to4 cm length). The chopped waste was mixed with organic matters (cowdung and soil) to make various proportions(1:9,2:8, 3:7, 4:6) up to 1kg and kept in plastic pots.The soil proportion is constant for all experimental pots and were labeled with C-Control (contained soil and cow dung in 1:1),P-I(1;9),P-2(2;8),P-3(3:4),P-4(4:6),E-(Experimental of 100% leaf litter)in proportions.These composting mixture weights of all proportions were taken in two sets for present study.

Experimental set up for vermicomposting

The mixtures for composting were placed into 6 pots and were made for 2 sets. Water was sprinkled in regular interval of 7-8 days by turning the mixture uniformly up and down till these started to compost. Within 15-20 days of

composting the mixture of each proportion was placed in stack by adding a layer of slurry (cowdung:water@2:1) in the pots and was introduced with earthworms.

Treated by Earthworm

After partial decomposition of the composting materials i.e. after 25days about 6 earthworms of Juvenile and adult stage of specimens *E. fetida*, having an average length 3-4c.m & 10-12cm are introduced into composting .

Method of Vermicomposting

Vermicomposting process was initiated after adding the earthworms *E.Fetida* in plastic pots containing the mixture. These pots were placed in a shaded area for observing decomposition. It can be done indoors because worms are very sensitive to extremes of hot and cold temperature. In present study the windrow compost method was used in which composting material was not provided with pipes for ventilation and remains uncovered. The treated pots were sprinkled with water after to maintain moisturequantity and added slurry for the nutrition of earthworm in regular interval. Process was monitored until the surface of plastic pots appear almost completed on its upper surface (vermicast) suggested vermicomposting process, black granular coated humus material. Watering was stopped at this point before 4-5 days of harvest. Prepared vermicompost was arranged in such a way that the earthworms settled down and the vermicompost was collected from the top without upsetting the layers below. To get uniform vermicompost the collected vermicompost was filtered through a fine sieve. The vermicompost pH and electrical conductivity (EC) were monitored using laboratory instruments.

Vermicompost Harvesting

To enable the separation of worms from vermicomposting, the moisture content in the compost is reduced by discontinuing the addition of water for 4-5 days prior to maturation, which guarantees the drying of compost by migrating worms into plastic pot. This forces around 80 percent of the worms to the bottom of the plastic pot for vermicomposting. The rest of the worms are removable by hand. The mature compost, a substance rich in black, fine loose, granular humus, is drained, dried and packed out of the plastic pot. Afterwards the vermicompost is ready for use. Vermicompost's nutritional content varies with the action of different earthworm species and raw materials utilized for the process. The final product is therefore not a single standard product. Vermicompost's average nutrient content is: 0.6-12% N, 0.13-0.22% P,0.4-0.7% k_2O_2, 0.4% CaO and 0.15% MgO. Vermicompost's unique feature is its rapid composting process which takes around 2-3 months depending on the ecological conditions.The excess worms gathered from the pot may be transferred and used in other pots, sold for compost inoculation to other farmers, and may be used as feed or fish food for animals and poultry.

RESULTS AND DISCUSSION

From the present investigation like other solid wastes the leaf litter of plant Acacia was also used as organic substratein various ratio of 1:9, 2:8,3:7,4:6 with combination of cowdung+soil. During vermicomposting physico-chemical variables were also measured as follows in tabulated form:

Tabulation-1

(TABULATION-I It was shown the measurement of physical parameters with variation during before and after vermicomposting of leaf litter *Acacia)*

During laboratory experiment the tabulated data-I for the measurement of pH was found slightly increased with alkaline range, where the initial pH values are also in alkaline range. The observed differences of pH values between

initial and final stage of vermicomposting for various ratio ranges from 0.2 to0.7, except the 100% leaf litter it was slightly acidic at initial stage 6.64 then converted to alkaline 7.08 with a difference 0.4. This pH analysis for Acacia concordance with the result of (Nagar,Titov and Bhati,2017)in green *Eucalyptus* leaf litter.Vermicompost's final pH content was toward the alkaline side. During the vermicompost process lower pH was observed due to the abundance of carbohydrate due to the production of organic acid and CO_2 by microorganisms (Elvira et.al. 1998; Haimi and Huhta 1986; Haimi and Huhta 1900). As the source of carbohydrates is reduced, microbial metabolism changes into organic nitrogen compound resulting in an increase in the pH of ammonia (Ndegwa et.al. 2000). The Electro conductivity test was observed that the capacity of conductivity varies from initial to final reading with a difference of 0.17-0.52.It was analyzed that the electro conductivity reduced from control to all respective experimental proportions including the 100% leaf litter. Due to adding of dry leaf litter it was took time to keep moisture.The temperature of materials before composting in each proportion was found more or less equal to room temperature while aftercomposting there was a slight increase in temperature due to assimilation of waste.

The survival of earthworm was found during vermicomposting in control,P-1,P-2 while increasing of dryleaves in P-3,P-4,and in 100% dry leaf litter was effected with reducing in number of earthworms due to unviability of proper aeration and nutrition during their physiological activity. The germination of seed in the humus soil was observed in P-1,P-2,P-3 including control with the duration of 60 days of vermicomposting and in the P-4,100% dry leaf litter was not observed with proper germination which is carried out in further studies.

Tabulation-II

Tabulation-II (It was shown with the different variables during vermicomposting of *Acacia* leaf litter)

The level of moisture content as a 100 per cent leaf litter comparable was maximum than other experimental pots. Vermicomposting during. Moisture plays a vital role for the survival of earthworm speciesif it is suitable to consume oxygen as a maximum, but about 70 percent of the moisture content is suitable for its physiological activities, growth and movement, and for higher microbial activity because of which food is easy to feed on. The humidity level was maintained at regular intervals of 4th day by adding water. The level of humidity was initially lower and then rising day by day. During the process of vermicomposting due to microbial decomposition of organic matter, heat is generated that initially turns water into vapour, and the content of moisture decreases. As organic matter consumed, no additional heat is produced, and the amount of moisture remains high in that state.

The estimation of biomass was carried out through the height of the plastic pots contain material which level were decreased than initial contents in all the respective control,experimental pots and 100 % dry leaf litter after decomposing wastes through earthworms. The maximum depletion was found in control and in others it decreased with increasing of proportions in experimental pots.In 100% dry leaf litter took more time due to slow decomposition level within 60 days.The biological support between the earthworms and related microbes is primarily responsible for the loss of C from organic waste. Kaviraj and Sharma (2003) recorded decreases in organic carbon content of vermicomposted municipal solid waste mixed with Cow dung by 20–45 per cent. Prakash and Karmegam (2010) reported similar observations during vermicomposting of sugar industry press-mud. The growth rate of earthworms was increased in control,EP-1,EP-2,EP-3 whereas in EP-4,100% Dry leaf litter the rate of growth was observed as slow in comparison due to reduce in nutrition and oxygen during the process of vermicomposting .

CONCLUSION

In the present investigation it was studied that within 80-90days of vermicomposting process most of the organic wastes as leaf litter when subjected to the feeding by earthworms including microorganisms converted into blackish powdery, odour less of humus called vermicompost. It was observed that for each physico-chemical variables, the collected data after laboratory work converted to fine vermicast having an increase level than before

vermicomposting .The result is showing almost in all proportions with humus soil except in 100% dry leaf litter which turned humus after 150 days for the reason of high quantity of leaf litter. It is observed that the rate of formation of humus soil is varies dependupon the proportion of leaf litter and it is in reciprocal quantity as per the gradation of leaf litter in each pot. The sustainability of plant was also observed after seed germination contributing by each pot besides 100% of leaf litter which was shown result after adding of with extra soil. However it was observed that the leaf litter was obtained with enriched vermicomposting that suitable for plant growth and sustainability.

REFERENCES

1. Albanell E., Plaixats J., and Cabero T. (1988) Chemical changes during vermicomposting (Eiseniafetida) of sheep manure mixed with cotton industrial wastes. Biology and Fertility of Soil 6: 266-69
2. Alagesan, P. and R. Dheeba (2010) Utilization of earthworms in organic waste management.
3. Proceedings of the 15th International Forestry and Environment Symposium. Department of Forestry and Environmental Science, University of Sri Jayewardenepura, Sri Lanka.1-9.
4. Bansal,S. and Kapoor, K. K.(2000).Vermicomposting of crop residues and cattle dung with*Eiseniafetida*, Bioresource Technology,73:95-98.
5. Bhiday, M.R., (1994). Earthworms inagriculture. Indian Farming 43(12): 31-34.
6. Bhoyar, R.V., Bhide, A.D. (1996). Vermicomposting of organic (vegetable) solid wastes. International conference on Environmental planning and Management,VRCE,Nagpur,India.
7. Chaudhuri, P.S., Pal, T.K., Bhattacharjee, G. and Dey, S.K.(2000).Chemical changes during vermicomposting (Perionyxexcavatus) of kitchen wastes. Tropical Ecology. 41(1):pp.107-110.
8. Domínguez J. (2004). State of the art and new perspectives on vermicomposting research. In: C.A.
9. Edwards (Ed.). Earthworm Ecology (2nd edition). CRC Press LLC. 401-424.
10. Darwin, C. (1881). The formation of vegetable mould through the action of worms, with observations on their habitats. Murray, London. 326 pp.
11. Edwards, C.A., Bohlen, P.J., Linden, D.R. and Subler, S. (1995). Earthworms in agroecosystems. In: Earthworm Ecology and Biogeography in North America. (Hendrix, P. F. eds.), Lewis Publisher, Boca Raton, FL, pp: 185-213.
12. Edwards C A and Lofty J R 1978 The influence of arthropods and earthworms upon root growth of direct drilled cereals: J. Atrpl. Ecol. 15789-795
13. Elvira, C.; Sampedro, L.; Benitez, E.; Nogales, R., (1998). Vermicomposting of sludges from paper mill and dairy industries with Eiseniaandrei: A pilot scale study. Bioresource Tech., 63, 205-211
14. GargVK,GuptaA,SatyaS.Vermicomposting of different types of waste using Eiseniafetida a comparative study.Bioresource Technology 206;97:391-395.
15. Haimi, J.; Huhta, V. (1986). Capacity of various organic residues to support adequate earthworm biomass for vermicomposting. Biol. Fert. Soils, 2, 23-27.
16. .Haimi, J. and Huhta, V. 1900. Effects of earthworms on decomposition processes in raw humus forest soil: A microcosm study. Biol. Fert. Soils, 10: 178-183.
17. Indrajeet and Singh,J.(2010).preparation of recipe for quality production of vermicompost.Journal of recent advances in applied sciences(JRAAS)25,12-14.
18. Janakiram, T,Sridevi,K. (2010).Conversion of waste into wealth:A Study in Solid waste Management ,E-Journal of Chemistry,7(4):1340-1345.
19. Jayanthi,B., Ambiga, G. and Neelanarayanan P. (2010). Utilization of mixed leaves litter for converting into vermicompost by using an epigeic earthworm Eudriluseugeniae.Nature Environment and Pollution Technology. 9(4),763-766.
20. Jonczak, J., Parzychand, A. and Sobisz, Z. (2015). Decomposition of four tree species leaf litters in headwater Riparian Forest. Baltic Forestry. 21(1):p.40.
21. .Jambakar, H.A. (1992) Proceedings of National Seminar on Organic Farming, MPVK, Pune, 53-54.

22. Kaviraj, and Sharma S.(2003). Municipal solid wastes management through vermicomposting employing exotic and local species of earthworm. Biores. Technol., 90: 169-173
23. Kaplan,D.L.,Hartenstein, R.D. and Nouhauser, E.F.(1980).Physico chemical requirements in the environment of the earthworm *Eiseniafetida* ,Soil Biology and Biochemistry ,12:347-352.
24. Kumar, H.D. and Sahoo, L. (2011).A review on Phytochemical and pharmacological of Eucalyptus globulus:A multipurpose tree.International Journal of Research in Ayurveda & Pharmacy.2 (5),1527-1530.
25. Lazcano-Ponce,E., Herrero,R., Muñoz,N., et al. (2001)Epidemiology of HPV infection among Mexican women with normal cervical cytology, *Int J Cancer*, vol. 91
26. Ndegwa Henry Maina, LeenaPitkänen, Sami Heikkinen, PäiviTuomainen, LiisaVirkki, MaijaTenkanen (2014). Challenges in analysis of high-molar mass dextrans: Comparison of HPSEC, AsFlFFF and DOSY NMR spectroscopy. Carbohydrate Polymers,99,199-207.DOI: 10.1016/j.carbpol.2013.08.021.
27. Mushan, L.C. and Rao, K. R. (2012). Physicochemical analysis of tendu leaf litter vermicompost processed By Eudriluseugeniae. DAV International Journal of Science. 1(2):pp.100-102.
28. Mitchell, M.J., Hornor, S.G., Abrams, B.I. (1980). Decomposition of sewage sludge in drying beds and the potential role of the earthworms, Eiseniafetida. Journal of Environmental Quality 9:37-378.+
29. Nagar, R., Titov, A. and Bhati, P. (2017). Vermicomposting of green Eucalyptus leaf litter by Eiseniafetida and Eudrilus Eugenia. International Journal of Environment, Agriculture and Biotechnology (IJEAB). 2(6):2811-2818.
30. Nagalakshmi, P. K. and Prakash, M.(2016). A Microcosm Study of Cast and Gut of an epigeic earthworm Perioynxceylanensisreared on different substrates. Int. J. Curr. Trend. Pharmacobiol. Med. Sci.1(2),45-51.
31. Nath, S. and Chaudhuri, P. S.(2014).Growth and reproduction of Pontoscolexcorethrurus (Muller) with different experimental diets. Tropical Ecology .55(3),305-312.
32. Prakash, M. and Karmegam, N. (2010). Vermistabilization of pressmud using Perionyxceylanensis Mich. Bioresour. Technol., 101: 8464-8468
33. Sandeep, Singh D., Yadav,J.andUrmila (2017). Assessment of nutrient status of vermicompost of leaf litter using Eiseniafetida. Journal of Entomology and Zoology Studies 5(2): 1135- 1137.
34. Shouche, S., Bhati, P. and Pandey, P. (2011). Study about the changes in physical parameters during vermicomposting of floral wastes. Journal of Environmental Research and Development. 6: 63-68.
35. Shuster, W.D., Subler, S. and McCoy, E.L. (2000). Foraging by deep-burrowing earthworms degrades surface soil structure of a fluventicHapludoll in Ohio. Soil & Tillage Research., 54: 179-189.
36. Syres J.K., Sharpley A.N., and Keeney D.R. (1979) Cycling of nitrogen by surface casting earthworms in a pasture ecosystem. Soil Biology and Biochemistry 11: 181-85.
37. Vasanthi K., Chairman K., Ranjit Singh A.J.A.(2013). Vermicomposting of leaf litter ensuing from the trees of Mango (Mangiferaindica) and Guava (Psidiumguujuvu) leaves. International Journal of Advanced Research 1(3): 33-38.

Table1: Variation during before and after vermicomposting of leaf litter *Acacia* of tabulation-1 (Part-1)

Sl. No.	Observations	Experimental set up	Electroconductivity (before(initial) & after (final) vermicomposting)			pH (before & after vermicomposting)		
			Initial reading	Final reading	Difference	Initial reading	Final Reading	Difference
1	C	S+C(1:1)	0.485	0.986	0.501	7.5	8.15	0.65
2	P-1	S+C+LL(10:9:1)	0.385	0.691	0.306	7.46	7.67	0.21
3	P-2	S+C+LL(5:4:1)	0.257	0.767	0.51	7.48	7.86	0.38
4	P-3	S+C+LL leaves(5:3:2)	0.466	0.774	0.308	7.53	7.87	0.34
5	P-4	S+ C + LL leaves(10:7:3)	0.321	0.491	0.17	7.37	7.55	0.18
6	E-100%	LL(1k.g)	0.123	0.353	0.23	6.64	7.08	0.44

Table 2: Variation during before and after vermicomposting of leaf litter *Acacia* of tabulation-1 (part-2)

Sl. No.	Observation	Experimental set up	Temperature in °C			Survival of earthworms in each treating pot	Seed germination
			Initial reading	Final Reading	Difference	All pots treated by 6 no.of EW	
1	C	S+C(1:1)	28.8	29.8	1	(all)	Y
2	P-1	S+C+LL(10:9:1)	29	29.5	0.5	(all)	Y
3	P-2	S+C+LL(5:4:1)	28.6	29.4	0.8	(all)	Y
4	P-3	S+C+LL leaves(5:3:2)	29.2	29.6	0.4	-4	Y
5	P-4	S+ C + LL leaves(10:7:3)	28.7	29.3	0.6	-5	Y
6	E-100%	LL(1k.g)	28.2	28.7	0.5	-2	Y

Note: (C=Control, P=Proportion, E=Experimental)

Table 3. Variables during vermicomposting of *Acacia* leaf litter of tabulation-11(part-1)

Sl. No.	Observation	Experimental set up	Moisture content of leaf litter		
			Initial reading of composts	Final reading of dried materials	Difference
			(gm)	(gm)	(gm)
1	C	S+C(1:1)	100	120.85	20.85
2	P-1	S+C+LL(10:9:1)	50	22.838	27.016
3	P-2	S+C+LL(5:4:1)	100	33.5	66.451
4	P-3	S+C+LL leaves(5:3:2)	150	77.052	72.948
5	P-4	S+ C + LL leaves(10:7:3)	200	97.941	102.05
6	E-100%	LL(1k.g)	1000	501.65	498.35

Table 4.Variables during vermicomposting of *Acacia* leaf litter of tabulation-11(part-2)

Sl. No.	Observation	Experimental set up	Biomass measurement			Growth rate of earthworm		
			Initial reading	Final Reading	Total Decomposition	Initial reading	Final Reading	Total Growth at the end of expt.
			(cm)	(cm)	(cm)	(cm)	(cm)	(cm)
1	C	S+C(1:1)	14	9.3	3.7	12-Apr	8-17.5	4-5.5
2	P-1	S+C+LL(10:9:1)	14.7	11.9	2.8	4.7-11	9.2-16.3	4.5-5.3
3	P-2	S+C+LL(5:4:1)	15.8	12.7	3.1	5.3-12.4	9.8-19.3	4.5-6.9
4	P-3	S+C+LL leaves(5:3:2)	16.6	14.4	2.2	4.7-11.3	9.2-18.7	4.5-7.4
5	P-4	S+ C + LL leaves(10:7:3)	17.5	15.2	2.3	5.3-11.2	8.6-13.9	3.3-2.7
6	E-100%	LL(1k.g)	<18	16.6	1.4	4-12.3	7.1-13.5	3.1-1.2

Fig.1.Soil Sample

Fig.2 Cowdung Sample

Fig. 3 Before Chopping

Fig. 4 After Chopping

Fig. 5 Sieve of 3.35mm

Fig.6 Experimental labelled pots

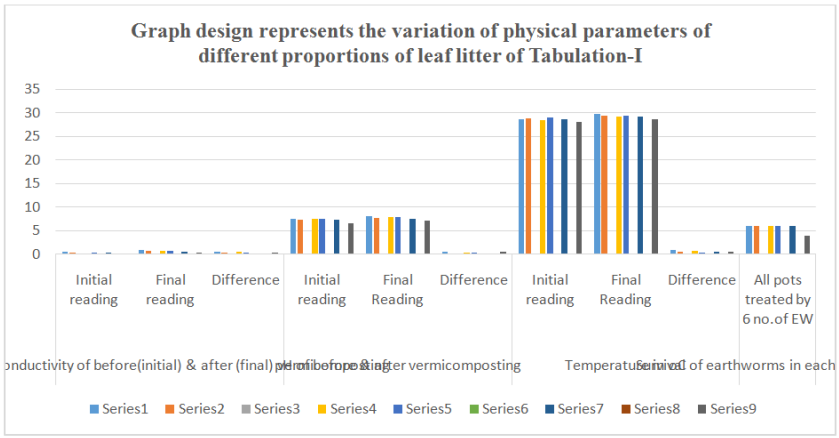

Fig. 7 Variation of physical parameters of different proportions of leaf litter of Tabulation-I

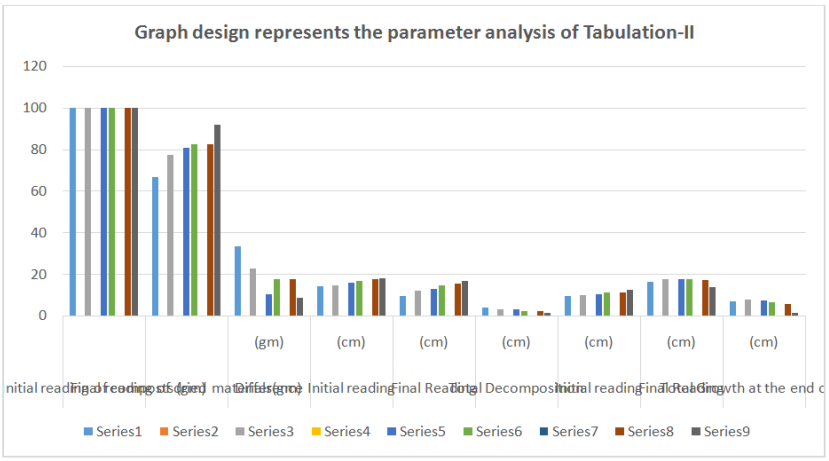

Fig.8 Parameter analysis of Tabulation-II

Fig.9 Formation of vermicast

Fig.10 Obtained with vermicompost in various proportions

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RESEARCH ARTICLE

Molecular Interaction of Dextran with (1N) NaOH through Ultrasonic Technique

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ABSTRACT

Ultrasonic techniques for interpretation of liquid is relatively simple as it involves less number of parameters. The Acoustic parameters are helpful to understand the impact of frequency on the interactions of solute dextran in solvent (NaOH–H_2O).The density (ϱ) viscosity (η) and ultrasonic velocity (U) at 313 K have been estimated in the aqueous medium of polymer dextran and sodium hydroxide using pycnometer, Ostwald's viscometer, and multi-frequency ultrasonic interferometer rangingfrequency from 1 to 12 MHz respectively. The acoustical parameters such as Acoustic impedance (Z), Adiabatic Compressibility (β), Intermolecular free length (L_f), Relaxation time (τ) and Gibb's free energy (ΔG) are determined. The outcomes are interpreted in terms of molecular interaction between the partsof the solutions.

Key Words: Acoustic impedance, adiabatic compressibility, intermolecular free length, relaxation time.

INTRODUCTION

The solution structures of polymer have been carried out in past by various researchers [1-2] from different angles using ultrasonic techniques. The study of acoustic properties presents a convenient method for investigation of liquid structures particularly of dilute solution of the polymers. It gives a firm insight into the possible structure of the polymer and the solvent molecules at different concentration and also some information about the extent of association using simple methods. Earlier some authors [3-5] have used ultrasonic techniques for interpretation of liquid structure, due to the fact that the ultrasonic technique is relatively simple and this method of investigation

involves less number of parameters. Polymer molecules are giant molecule as compared to the solvent molecules in which they are dissolved and are comprised of several chain fragments. These long molecules, moreover, are not as an all-inclusive straight-chain however as firmly collapsed irregular coils (fig-1). Individuals molecules coils are likewise not discrete and isolated but rather are interpenetrating and entrapped with each other. There are likewise shifting degrees of cohesive and attractive forces between various portions of a similar molecular coil just as neighbouring coils. Forces such as dispersion, induction, dipole-dipole cooperation and hydrogen bonding (both intramolecular and intermolecular) hold the molecular coils and their portions together firmly. Because of large size and coiled nature of the polymer molecules and also because of strong force of attraction between them, solvent molecules take time to establish interaction with polymer molecules.

In order to get a reasonably correct picture of the size and shape of polymer particles, various thermoacoustic parameters like Acoustic impedance (Z), Adiabatic Compressibility (β), Intermolecular free length (Lf), Relaxation time (τ) and Gibb's free energy (ΔG)are determined. To calculate these parameters the ultrasonic technique is applied as it is simple and quite accurate. The ultrasonic procedureuncovers frail intermolecular interactions because of its valuable wavelength extend.Numerous workers have examined the acoustical parameters by the estimation of density, viscosity and ultrasonic speed of various polymer frameworks at various temperatures and frequencies, various concentrations of solute and in various solvents [6-8]. In this paper, values of η, ϱ, U have been measured of the polymer dextran with sodium hydroxide solutions. The related thermodynamic and acoustic parameters have been determined and the solute-solvent interactions for the aqueous solution of dextran of various concentrations have been examined with four distinct frequencies 1, 5, 9 & 12MHz at 313K temperature. Dextran, a water-solvent polymer, is a α-D-1, 6-glucose connectedglucan with side chains-3 connectedto the spine units of the polymer. We also determine the thermo dynamical and acoustical parameters for characterizing the physics and chemistry properties of dextran in solution in different temperature and frequency

Experimental Section

Materials and techniques embraced are equivalent to in my past paper [9].

Theoretical Aspect

The basic parameters U, η, ρ were measured at various concentration and temperatures. The various acoustical parameters like Z,β,Lf, τ and ΔG were calculated from U, η, ρ value using standard formulae [9].

RESULTS AND DISCUSSION

The experimental data relating to ρ, η, and U at 313 K for frequencies 1, 5, 9, and 12 MHz for the given solution have been exhibited in table 1 and table 2. Calculated values of Z,β,Lf, τ and ΔG are exhibited in tables.2-4. The ultrasonic speed in aqueous sodium hydroxide increases with dextran concentration as fig.-1, suggest that the quantity of atoms in the solution expands, making the solution denser hence sound velocity increases that signify the rise of the toughness of the solution and hence association occurs. The association occurs because ofhydrogen bonding or dipole-dipole interaction among NaOH and water molecules. An increase in frequencies declines the interaction, which may be due to a rise in excitement among molecules causing a decrease in ultrasonic speed at higher frequencies fig.-2, and hence dissociation. This is because the quantity of dextran atomsdiminishes and thusmore particles are not accessiblefor strong ion-dipole interaction between Na+ of NaOH and aqueous dextran. This process leads to weak interaction force [10]

Addition of the solute increases the mass of the solution in the medium leads to rise of acoustic impedance. In most cases, the size will build up that expands the attachment impacts thus the segments will in general move quickly.This expansion in molecule development is confined by the surrounding molecule that builds the net acoustic impedance.

This shows the presence of interaction between solute–solute or solute–solvent. With increase in frequency, acoustic impedance decreases. This factor gives inertial and elastic properties of the medium and subsequentlysupports the chance of atomic interactions [11].

Analyzing the respective figures (5, 6, 7 and8), it is seen that the L_f reflects a comparative pattern as that of β. It is seen that the pattern appeared by β and L_f decreases with increasing solute in a solution [12]. Showingthat the medium is getting denser. This leads to significant interaction between constituent atoms, suggesting a structure advancing behavior on the addition of solute and these outcomes are likewise supported by the viscosity information. The increase in β (Figure 6), and Lf (Figure 8), suggests minimum interaction between unlike molecules with frequency increases [13]. Relaxation time depends on the viscosity of liquid. It gives the orientation of the molecules in a particular direction. Relaxation time increases at a slower rate as frequency increases. The increment in the relaxation time suggest that the interaction between the molecules of components is stronger than the attractive forces between the atomsof every segment.An expanding estimation of ΔG suggests that the nearest approach of unlike atoms is because of hydrogen bonding and the mobility of the molecule is low. Whenfrequency increases, the energy imparted to the atomsclearly expedites the rearrangement procedure [14].

CONCLUSION

It is inferred that ultrasonic examinations give a far reaching investigation of molecular association in the liquid solution. Summarizing the trends and variation of thermodynamic parameters with the frequency of the ultrasonic wave has been studied in detail which will give us a thought regarding the idea of molecular interactions in the aqueous dextran solution.

ACKNOWLEDGEMENTS

The principalauthor is sincerely thanks to the HOD and staffs members of Department of Physics Centurion University of Technology and management, Odisha and ABIT management, Cuttack for their strategic help and support.

REFERENCES

1. Langemann, R.T., Dunbar, W.S., 1945. Relationships between the velocity of sound and other physical properties of liquids. J. Phys.Chem. 49 (5), 428–436.
2. Aswar AS. Interaction studies on biomolecules in aqueous medium. J. Pure Appl. Ultrasonic. 1998; 20: 82.
3. Praharaj MK, Satapathy A, Mishra P and Mishra S.Ultrasonic studies of ternary liquid mixtures of N-N-dimethylformamide, nitrobenzene, and cyclohexane at different frequencies at 318 K Journal of Theoretical and Applied Physics 2013; 7:23-6
4. Das JK, Dash SK, Swain N, Swain BB. Ultrasonic investigation in a polar-polar system-Metyl isobutyl ketone (MIBK) and aliphatic alcohols. J of Molecular Liq. 1999; 81(2):163–9.
5. Paikaray R. and Mohanty N., Journal *of Acoustical Society of India,* 37(2010)70-73.
6. Singh R P., *Acoust. Soc. Ind.* 21 (3) (1993) 159–170.
7. Rajagopalan S. and Sharma S J., *J. Pure Appl. Ultrason*. 27 (2005) 105- 109.
8. Nath G., Sahu S. and Paikaray R., *Indian Journal of Physics*, 83(11) (2009) 1567-1574.
9. Subhraraj Panda and Achyuta Prasad Mahapatra, Ultrasonic study of acoustical parameters of dextran solution with 1(N) NaOH at different temperatures and concentrations, J. P*ure Appl. Ultrason. 40 (2018),100-105*
10. QUANTITATIVE STUDY OF INTERACTION OF VINYL POLYMERS BYULTRASONIC METHOD – *V. Seetharaman, S. Kalyanasundaram, A. Gopalan,* Journal ofMolecular Liquids, Vol. 121, (2005), pp. 156-159.

11. EVALUATION OF ULTRASONIC VELOCITIES IN SOME BINARY LIQUID MIXTURES BY"RAO SPECIFIC SOUND VELOCITY" – *V.D.Gokhale and N.N.Bhagvat*, Journal of Pure and AppliedUltrasonics, Vol.11, (1989), pp.21- 25.
12. ULTRASONIC SPEEDS AND ISENTROPIC COMPRESSIBILITIES OF {DIFURYLMETHANE +(C1-C6) N-ALKANOL} BINARY MIXTURES AT T=298.15K , B.T. Mmereki, I.Oathotse ,W.A.A.Ddamba, Department Of Chemistry,University Of Botswana, Private Bag 0022 Gaborona,Botswana, J.Chem Thermodynamics 42(2010)1346-1351
13. ULTRASONIC VELOCITY AND APPARENT MOLAR COMPRESSIBILITY OFTRICHLOROACETIC ACID IN AQUEOUS ETHANOL - *P.S.Nikam and Mehdi Hasan,* Asian Jouranlof Chemistry Vol. 5, No.2 (1993), 319-321
14. Subhraraj Panda and Achyuta Prasad Mahapatra, International Journal of Innovative Technology and Exploring Engineering (IJITEE) ISSN: 2278-3075, Volume-8 Issue-11, September 2019742-748.

Table:1. Values of ρ and η of solution

Temp. K	Concentration									
	0.10%		0.25%		0.50%		0.75%		1%	
	ρ Kg.m^{-3}	η 10^{-3} N.s.m^{-2}	ρ Kg.m^{-3}	η 10^{-3} N.s.m^{-2}	ρ Kg.m^{-3}	η 10^{-3} N.s.m^{-2}	ρ Kg.m^{-3}	η 10^{-3} N.s.m^{-2}	ρ Kg.m^{-3}	η 10^{-3} N.s.m^{-2}
313	1031.842	0.767	1032.634	0.800	1033.822	0.810	1034.218	0.835	1036.781	0.865

Table: 2. Values of Uand Zofsolution

Conc.	U m/s				Z(10^6 kg·m^2·s^{-1})			
	1MHz	5MHz	9MHz	12MHz	1MHz	5MHz	9MHz	12MHz
0.10%	1600.000	1592.000	1585.000	1580.000	1.651	1.643	1.635	1.630
0.25%	1601.000	1598.000	1589.000	1585.000	1.653	1.650	1.641	1.637
0.50%	1603.000	1600.000	1595.000	1591.800	1.657	1.654	1.649	1.646
0.75%	1604.000	1600.750	1597.050	1596.000	1.659	1.656	1.652	1.651
1%	1606.000	1602.000	1600.000	1599.000	1.665	1.661	1.659	1.658

Table:3. Values of βand L_f of solution

Conc.	β(10^{-10}N^{-1}.m^2)				L_f(10^{-10} m)			
	1MHz	5MHz	9MHz	12MHz	1MHz	5MHz	9MHz	12MHz
0.10%	3.786	3.824	3.858	3.882	3.928	3.947	3.965	3.977
0.25%	3.778	3.792	3.835	3.855	3.924	3.931	3.953	3.963
0.50%	3.764	3.778	3.802	3.817	3.916	3.924	3.936	3.944
0.75%	3.758	3.773	3.791	3.796	3.913	3.921	3.930	3.933
1%	3.740	3.758	3.768	3.772	3.904	3.913	3.918	3.921

Table- 4 Values of τand ΔG of solution

Conc.	τ (10^{-13}Sec.)				ΔG (10^{-20}kJ·mol^{-1})			
	1MHz	5MHz	9MHz	12MHz	1MHz	5MHz	9MHz	12MHz
0.10%	3.871	3.910	3.945	3.970	173.80	175.68	177.34	178.52
0.25%	4.032	4.047	4.093	4.114	181.47	182.18	184.30	185.24
0.50%	4.064	4.079	4.105	4.121	182.93	183.63	184.81	185.56
0.75%	4.183	4.200	4.219	4.225	188.35	189.11	189.98	190.23
1%	4.313	4.335	4.345	4.351	194.10	195.03	195.50	195.74

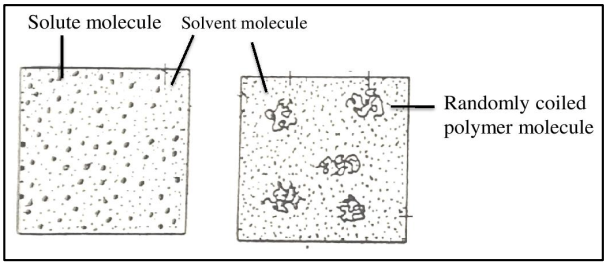

Fig.1 Schematic representation of solution and solvent molecules present in dilute solution compounds and polymers

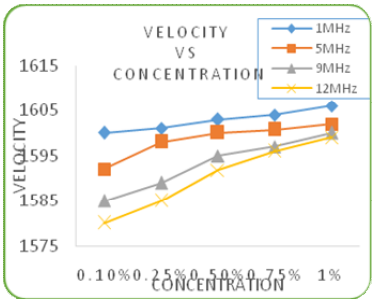

Figure 2.Plot of U with concentration

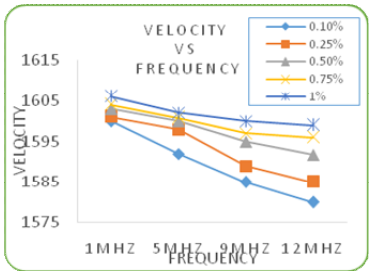

Figure 3.Plot of U with frequency

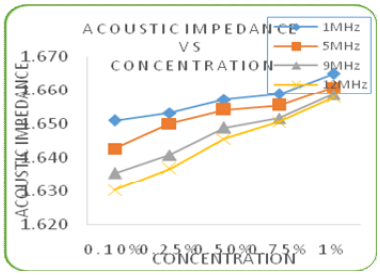

Figure 4.Plot of Z with concentration

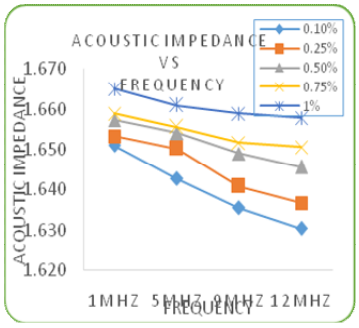

Figure 5.Plot of Z with frequency

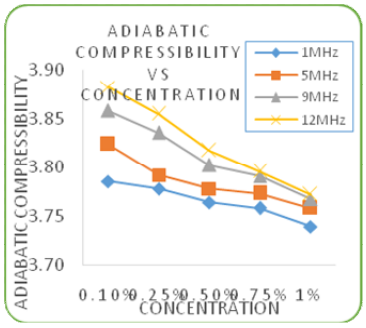

Figure 6. Plot of β with concentration

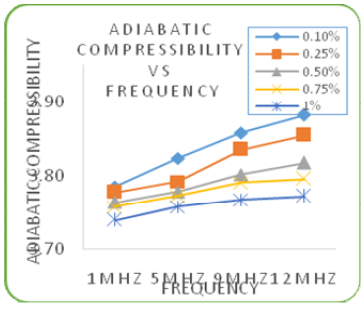

Figure 7.Plot of β with frequency

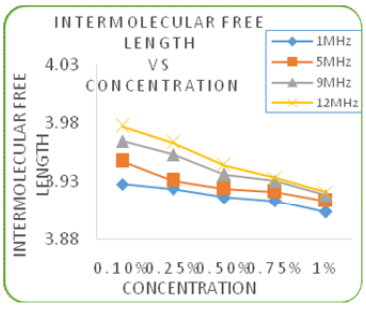

Figure 8. Plot of L_f with concentration

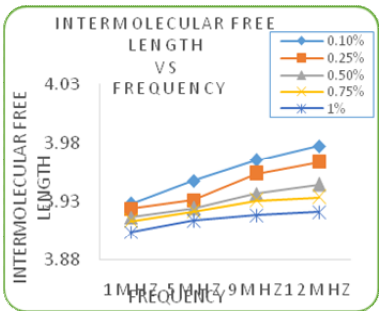

Figure 9. Plot of L_f with frequency

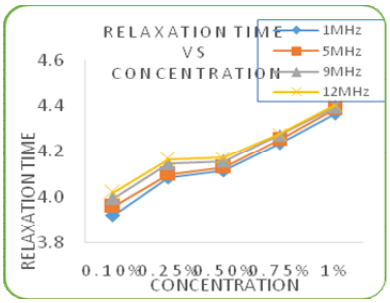

Figure 10. Plot of τ with concentration

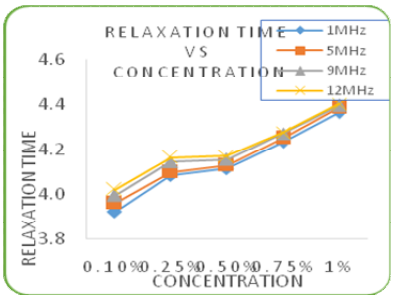

Figure 11. Plot of τ with frequency

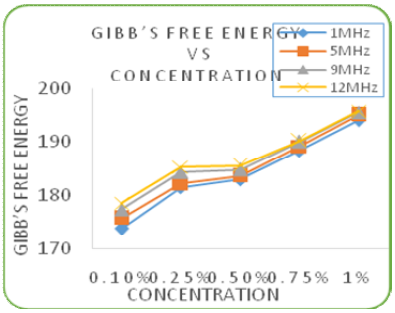

Figure 12. Plot of ΔG with concentration

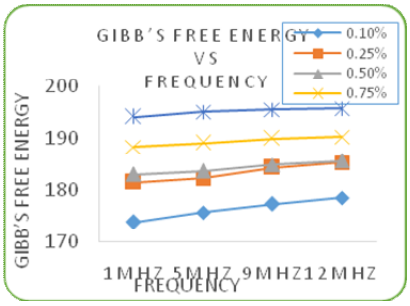

Figure 13. Plot of ΔG with frequency

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RESEARCH ARTICLE

Frequency of Hypocalcemia in Patients with Chronic Liver Disease

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ABSTRACT

Hypocalcemia may occur primarily or secondary to other associated disease conditions, which increases the complications, symptomatic burden and the intensity of physiological alterations associated with each etiological factor.The aim of the current study was to determine the frequency of hypocalcemia in patients with chronic liver disease (CLD) due to chronic hepatitis. This cross-sectional, single center study was conducted from July 2018to June 2019. A total of 166 CLD patients presented to Department of Medicine, Nishtar Hospital, Multan, between the age group of 18-60 year irrespective of gender were included in the study. After attaining consent from the patients, data regarding demographic details and clinical characteristics were recorded. The serum Ca^{+2} concentration was assessed using the blood sample (10 ml) drawn from each patient. Based on the serum Ca^{+2} concentration the patients were then categorized as hypocalcemic and normocalcemic. The recorded data was analyzed using SPSS version 22. Results Of these 166 study enrolled cases, there were 92(55.4%) male patients and 74(44.6%) female patients with the mean age 49.95 ± 7.53 years. Around 54.8% patients had CLD for more than 18 months. The mean treatment duration was 9.54 ± 2.78 months, only 28(16.9%) cases were receiving treatment. Moreover, 28 CLD cases were observed with co-existing hepatitis B while 138 cases with hepatitis C. Mean serum calcium level was 7.54 ± 0.67 mg/dl, where hypocalcemia was present in 147 (88.6%) cases. It can be concluded from the study results that hypocalcemia is significantly associated with CLD as high frequency of calcium deficiency was observed among the studied population suffering from both CLD and viral hepatitis.

Key Words: Vitamin D, Chronic Liver Disease, Cirrhosis, Vitamin D deficiency, Hepatitis B virus infection, Hepatitis C virus infection.

INTRODUCTION

Hypocalcemia is a common electrolytic abnormality that can range in severity from being asymptomatically mild to an acute life-threatening crisis [1]. It has multifactorial etiologies i.e. Vitamin D deficiency, inadequate Vitamin D metabolism, hypoparathyroidism, hypoalbuminemia and hormonal resistivity [2,3]. In mild cases, if the etiological factors are timely managed and addressed appropriately such imbalances can be reversed but under chronic conditions with persistent and life-long stimulators for e.g. genetic abnormality, autoimmune destruction and post-surgery irreversible damages to the parathyroid gland, it can be fatal4.Therefore, diagnosis must be made on the basis of symptomatology, clinical presentation, associated laboratory assessments, previous medical history, ongoing treatment, and also genetic makeup of the suspect [4].

Calcium deficiency is highly prevalent in critically ill patients and occurs secondary to several other disorders that affect vitamin D-parathyroid-calcium axis which is responsible for maintaining homeostatic balance of calcium [5]. Hypocalcemia associated with Vitamin D deficiency is most commonly observed among the patients with CLDas liver plays an important role in Vitamin D hydroxylation. Diseased liver interrupts the synthesis of vitamin D and resultsin decreased calcium metabolism [6].Also evident through the literature, a study reported decreased calcium concentration in more than half of thedecompensated CLD patients enrolled in his study [7].

Liver cirrhosis (LC) is a fatal late stage liver disease, causes prolonged and progressive impairment of liver functioningand eventually leads to liver failure. This deteriorating condition occurs in response to malabsorption, malnutrition, loop diuretics utilization, magnesium deficiency, concurrent renal disease, andhypoparathyroidism etc8. One of the leading causesfor LC are Hepatitis B and C virus (HBV and HCV) [8]. Henceall these life-threatening conditions are interconnected with calcium metabolism which in turn depends upon Vitamin D regulation [9].Only a few reports have evaluated the correlation of hypovitaminosis and hypocalcemia among CLD patients [9,10]. Globally much literature highlights the interconnection of vitamin D deficiency and associated illnessesbut very limited data directly relates hypocalcemia and CLD due to chronic viral hepatitis. No local studies had been conducted in order to determine the frequency of hypocalcemia among patients with decompensated liver disease in our general population. Therefore, the current study was conducted to pave way for decreasing disease morbidity, mortalityand for improving quality of life of these patients.

METHODOLOGY

A cross-sectional study was conducted from July 2018to June 2019at Department of Medicine, Nishtar Hospital, Multan. The study continued for a duration of 6 months including 166 patients with CLD, aged between 18-60 years of both genders while patients with history of autoimmune disorder (autoimmune hepatitis), essential hypertension and pregnant females were excluded from the study sample. After attaining the ethical approval from the ethics committee and research department of Nishtar Hospital, written informed consent was taken from each patient prior to the enrollment in the study. Demographic details regarding age, gender, body mass index (BMI), disease duration, treatment duration and frequency of hypocalcemia was recorded. Blood sample (10ml) was collected from each CLD patient and using this the serum Ca^{+2}concentration was assessed, based on which the patients were then classified as hypocalcemic or normocalcemic. Data was analyzed using SPSS version 22.0 and qualitative variables were presented through frequency and percentage while mean and standard deviation wasused for quantitative variables.Effect modifiers like age, gender, duration of disease, treatment status, residential status, socio-economic status and obesity were controlled by stratification. Chi square test was utilized for evaluating the relationship significance where p-value<0.05 was considered statistically significant.

RESULTS

Total 166 CLD patients were enrolled in the study with the mean age of 49.95±7.53 years of which 92(55.4%) were males.The mean serum calcium of the population was 7.54±0.67 mg/dl with 147(88.6%) patients presented with hypocalcemia. Moreover, disease duration, treatment status and hepatitis incidences were also observed and are given in table 1. CLD patients with hypocalcemia were mostly above 40 years of age. Moreover, the hypercalcemic incidences were more common among patients with more than 18 years of disease duration and untreated CLD cases ($p<0.05$) (Table 2). HCV was more common among hypocalcemic patients i.e. 132/147 while 15/147 had HBV. Moreover, a significant association was observed between the two variables ($p<0.05$) (Figure 1).

DISCUSSION

Pakistan due to its compromised economy and healthcare crisis, has provided an easy gateway to many acute and chronic diseases. Although during the past few years, the mortality and morbidity rate associated with the liver disease is controlled, through the universal vaccine implementation and antiviral therapies. But the disease often remains silent and undiagnosed unless the signs of decompensation develop and condition becomes chronic. For improved and controlled disease outcomes, it is essential to develop understanding of cirrhosis pathophysiology and management. The increasing prevalence of hypocalcemia is mainly due to low socio-economic status, nutritional insufficiency and multiple pregnancies etc [11]. Based on our findings, majority of the studied CLD patients were hypocalcemic i.e. 147 (88.6%) out of 166 patients (Table 1). Supported by a study conducted by Xing Z in 2014, 87.7% CLD patients enrolled in his study were also having hypocalcemia [5]. This imbalance may be triggered by several other disorders like chronic liver disease, chronic renal failure, protein deficiency and pancreatitis, nephrotic syndrome etc. moreover, it may be secondary to drug intake, lactation and other mineral deficiencies (phosphate and magnesium) [11].

Universally, it is a well-known fact that presently viral hepatitis is the leading silent killer. According to the WHO report (2015), globally HBV and HCV resulted in around 1.34 million deaths [12]. Moreover, it increases the risk of LC and hepatocellular cancer (HCC) by damaging the liver, hence the ratio of liver cancer triggered through these viruses attained prominence among the developing countries [12]. Among all the CLD patients enrolled in our study 138(83.1) had HCV and 28(16.9%) were suffering from HCV (Table 1). Similarly, a study conducted in Ethiopia also indicated the high prevalence of Hepatitis B and C among the CLD patients [13]. Moreover, the calcium deficiency was also monitored among the patients suffering from viral hepatitis among the studied population (Figure 1). The results were highly significant and indicated that the co-existing viral hepatitis and CLD are interdependent and are also involved in the endorsement of hypocalcemia among the sufferers ($p<0.05$).

The study aimed to determine the co-existing triggers involved in precipitation of chronic liver disease in order to understand the symptoms and systematic pathophysiology of all the multiple complications occurring parallel. The study holds several limitations, it was a single center study with limited sample size and only two leading causes for promotion of CLD were focused including hepatitis and hypocalcemia. No data regarding these individual conditions was recorded, no follow-ups were taken and no durations were marked, as when these conditions first appeared in the patient and when diagnosed.

CONCLUSION

It can be concluded from the study results that hypocalcemia is significantly associated with liver cirrhosis due to chronic viral hepatitis. Although calcium deficiency itself is multifactorial but the frequency of hypocalcemia was observed among the enrolled subjects keeping other etiologies aside. Considerable progress has been made

worldwide in the management of CLD and development of treatment regimens for controlling the primary disease together with all secondary promoting etiological factors. The physicians and healthcare providers must monitor calcium concentration in the routine practice to decrease the CLD associated morbidity and mortality rate. Moreover, the clinicians must also be equipped with latest knowledge and treatment modalities introduced for early and appropriate diagnosis, as these conditions grow silently.

Conflicts of Interest

The authors declare no conflicts of interest.

ACKNOWLDGEMENTS

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Nil

REFERENCES

1. Fong J, Khan A. Hypocalcemia:updates in diagnosis and management for primary care. Can Fam Physician. 2012;58:158-162.
2. Schafer AL, Shoback D. Hypocalcemia. In Primer on the Metabolic Bone Diseases, Eighth edition, Rosen, C. J. (ed), John P Wiley, 2013; 572-578
3. Schafer AL, Shoback DM. Hypocalcemia: Diagnosis and treatment. InEndotext [Internet] 2016 Jan 3. MDText. com, Inc.
4. Nair P, Lee P, Reynolds C, Nguyen ND, Myburgh J, Eisman JA, Center JR. Significant perturbation of vitamin D–parathyroid–calcium axis and adverse clinical outcomes in critically ill patients. Intensive care medicine. 2013;39(2):267-274.
5. Xing Z. The clinical aspect of serum calcium changes in chronic viral hepatitis[internet]. Acta Academiae Medicinae Xuzhou; [cited 2014 Nov 30]. Available from: http://en.cnki.com.cn/Article_en/CJFDTotal-XZYX199303015.htm.
6. Wiegand J, Berg T. The etiology, diagnosis and prevention of liver cirrhosis: part 1 of a series on liver cirrhosis. DeutschesÄrzteblatt International. 2013;110(6):85.
7. Cirrhosis of the Liver Information Sheet. Ver 3.0 – July 2013; 1-7. Available at: https://www.mirecc.va.gov/cih-visn2/Documents/Provider_Education_Handouts/Cirrhosis_Information_Sheet_for_BHPs_Version_3.pdf
8. Manco M, Ciampalini P, Nobili V. Low levels of 25-hydroxyvitamin D(3) in children with biopsy-proven nonalcoholic fatty liver disease. Hepatology. 2010;51:2229.
9. Miyashita K, Yasuda T, Kaneto H, Kuroda A, Kitamura T, Otsuki M, Okamoto Y, Hamada N, Matsuhisa M, Shimomura I. A Case of Hypocalcemia with Severe Vitamin D Deficiency following Treatment for Graves' Disease with Methimazole. Case reports in endocrinology. 2013;2013.
10. Narayanasamy K, Karthick R, Raj AK. High Prevalent Hypovitaminosis D Is Associated with Dysregulation of Calcium-parathyroid Hormone-vitamin D Axis in Patients with Chronic Liver Diseases. Journal of clinical and translational hepatology. 2019;7(1):15.
11. Fong J, Khan A. Hypocalcemia: updates in diagnosis and management for primary care. Canadian family physician. 2012;58(2):158-162.
12. World Health Organization. Viral hepatitis: a hidden killer gains visibility. 2017. Available from: https://www.who.int/publications/10-year-review/hepatitis/en/

13. Ayele AG, Gebre-Selassie S. Prevalence and risk factors of hepatitis B and hepatitis C virus infections among patients with chronic liver diseases in public hospitals in Addis Ababa, Ethiopia. ISRN Tropical Medicine. 2013 Jan 3;2013.

Table 1. Demographic & clinical characteristics of the study population

Variables	Sub-categories	(n=166)
Mean age (Years)		49.95±7.53
Age groups (Years)	*18 – 40*	37(22.3)
	41 – 60	129(77.7)
Gender	*Male*	92(55.4)
	Female	74(44.6)
Residential status	*Rural*	65(39.2)
	Urban	101(60.8)
Socioeconomic status	*Poor*	119(71.7)
	Middle Income	47(28.3)
Obesity	*Yes*	46(27.7)
	No	120(72.3)
Mean Serum Calcium (mg/dl)		7.54±0.67
Hypocalcemia	*Yes*	147(88.6)
	No	19(11.4)
Disease duration	*Up to 18 months*	75 (45.2)
	More than 18 months	91 (54.8)
Treatment status	*Treated*	28 (16.9)
	Un-treated	138 (83.1)
Hepatitis	*Hepatitis B*	28(16.9)
	Hepatitis C	138(83.1)

**Values are given as n(%) and Mean ± SD*

Table 2: Clinical and demographic characteristics of the study patients based on their serum calcium levels

Variables	Sub-categories	CLD patients		P-value
		Hypocalcemia	Normocalcemia	
Gender	*Male*	73	19	0.000
	Female	74	00	
Age Groups (Years)	*18 – 40*	30	07	0.129
	41 – 60	117	12	
Residential status	*Rural*	55	10	0.220
	Urban	92	09	
Socioeconomic status	*Poor*	110	09	0.019
	Middle Income	37	10	
Obesity	*Yes*	46	00	0.000
	No	101	19	
Disease duration	*Up to 18 months*	61	14	0.009
	More than 18 months	86	05	
Treatment status	*Treated*	20	08	0.006
	Untreated	127	11	

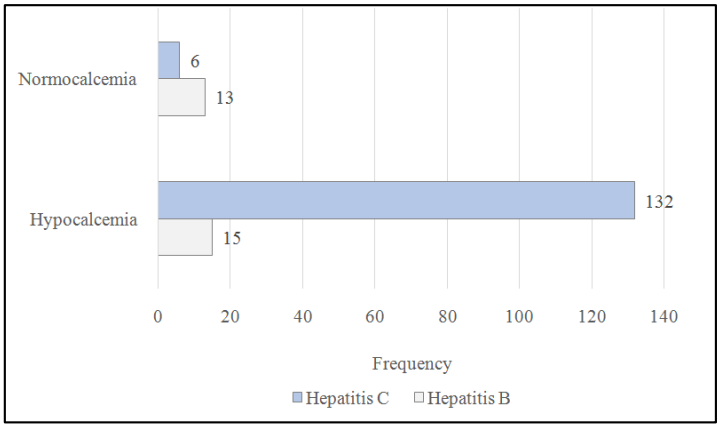

Figure 1: Indicates the distribution of CLD patients based on their calcium profile in association with the frequency of hepatitis B & C in the study population.

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RESEARCH ARTICLE

Compact Frequency Reconfigurable Metasurface Antenna

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ABSTRACT

The proposed frequency reconfigurable metasurface (FRMS) antenna is composed of a planar rectangular patch antenna consisting of circular ground plane of radius 20 mm, and a circular metasurface of radius 20 mm also. The MS is placed directly on top of the patch antenna to make the FRMS antenna compact and low profile with a total thickness of 3.048 mm. The MS consists of electric-LC (ELC) structure unit cells arranged uniformly along the vertical and horizontal directions.The antenna can be reconfigured by rotating the MS around the centre of the patch antenna. The rotation of the MS causes a change in the relative permittivity and permeability of the structure, and also the resonant frequency of the FRMS antenna. The proposed design has been simulated with the 3D full wave electromagnetic solver of HFSS software. By using Taconic RF-35(tm) substrate, the proposed FRMS antenna resonates from 5.5 GHz to 6 GHz. The proposed antenna is suitable for various wireless communication applications such as the wireless local area network (WLAN) band (5.180-5.825 GHz), industrial scientific and medical (ISM) band (5.725-5.875 GHz) and so on.The antenna parameters such as return loss, gain, radiation pattern and efficiency are analysed using HFSS software.

Keywords: Frequency reconfigurable antenna, unit cells, metasurface, source antenna.

INTRODUCTION

With the rapid growth of wireless communications, and the high demand for integration of multiple wireless standards into a single platform, it is highly desirable that the operating frequency of antennas can be reconfigured. Substantial research work has been reported in the literature to make the operating frequency of antennas reconfigurable. Frequency reconfigurable antennas can be achieved by two mechanisms: electrical or mechanical.

The electrical mechanism employs discrete tuning and continuous tuning methods. These can be achieved by using p-i-n (PIN) diodes [2], varactor diodes [3], and field-effect-transistors (FETs) [4]. For operating these electronic components in the antenna circuit, direct-current (DC) source and biasing circuits are needed. An electrical reconfigurable antenna thus relies on a DC electrical source and electronic switching components which have an adverse effect on the operation and performance of the antenna. The mechanical mechanism employs movable parts to achieve frequency reconfiguration. This can be achieved by using radio-frequency microelectromechanical systems (RF-MEMS) [1]. Mechanically reconfigurable antennas have good performance in dynamic environments as they don't need biasing circuits [5]. They are, however, bulky and expensive. Changing shape and size is a crucial problem in mechanically reconfigurable antennas. To overcome the drawbacks of the electrical and mechanical mechanisms, this work employs an electromagneticmetasurface to design a reconfigurable antenna.

Metamaterials can be extended three-dimensionally by arranging small electrical scatterersor holes in a two-dimensional pattern on a surface [6]. Metamaterials are known as double negative (DNG) materials because their permeability and permittivity are simultaneously negative in a given frequency band. Metamaterials possess a negative index because of their negative permittivity and permeability, and have near zero refractive index. The two-dimensional equivalent of metamaterials is called metasurface (MS). A metasurface has the advantages of taking up a small physical space and being a low-loss structure [6]. With its concise planar configuration and low price, a MS can be used to develop planar antennas. Placing a MS on top of a patch antenna can improve the performance of the antenna. The amalgamation of a MS and a patch antenna is called a MS antenna. A MS, which consists of electric-LC (ELC) structure unit cells, is used to tune the frequency and polarization of the antenna [8]. This paper presents design and simulation of a frequency reconfigurable metasurface (FRMS) antenna in electromagnetic simulation tool of HFSS software. In the FRMSantenna, the MS is mounted on top of the patch antenna by eliminating the air gap with them which leads to have a compact size, low cost, easy-to-fabricate antenna. The ELC structures are applied to form a multiband antenna, and achieve miniaturization of the antenna size by enabling a quasi-static resonant frequency at wavelengths that are much smaller than the guided wavelength [11]. The proposed FRMS antenna is designed to operate within the frequency range of 5.5 GHz-6GHz.

Design of Frequency Reconfigurable Metasurface Antenna

The proposed FRMS antenna consists of a patch antenna and a metasurface as shown in Figure 1. The metasurface is designed by a number of unit cells placed monotonously in the vertical and horizontal directions on a substrate. The ELC unit cell structure is shown in Figure 2. In the designed FRMS antenna, both the patch antenna and the metasurface have a circular shape of the same size to achieve easy reconfigurable operation. The patch antenna is constructed over a double-sided substrate whereas the metasurface is built on a single sided substrate. The metasurface consists of a combination of uniformly placed unit cells brought together in both horizontal and vertical directions. By rotating the metasurface around the centre of the patch antenna, frequency reconfigurability can be achieved. As the metasurfaceis symmetric in both horizontal and vertical directions, the maximum rotation angle without repetition is 90^0. To attain a low profile structure with compact size, the no-copper side of the metasurface is placed in direct contact with a radiator. The feed line of the antenna is characterized by 50Ω where a SubMiniature version-A (SMA) coaxial probe is connected to the feed line through the ground plane and substrate material. The dielectric substrate used here is Taconic RF-35(tm), having a thickness of 1.524mm and the relative permittivity of ε_r=3.5. The detailed geometrical dimensions of the proposed antenna are listed in Table 1.

Analysis of Metasurface

The shape of the ECL unit cells is symmetrical in both horizontal and vertical axes. The metasurface behaves the same for equal angle of rotation either in clockwise or anticlockwise direction and the response repeats after every 90^0.The aim is to achieve a change in FRMS by revolving the metasurface with respect to the patch antenna. In case of the FRMS antenna, the patch antenna radiates linearly polarized signals and has fixed fundamental resonant

frequency. Throughout the antenna analysis, a metasurface of infinite size is considered whereas the polarization direction of the linearly polarized plane waves makes an angle θ with the unit cell along y direction. Instead of modelling the entire metasurface, only a unit cell is modelled using HFSS software by providing master-slave boundary conditions. Twofloquent ports i.e. floquet port-1 and floquent port-1 are used for incident wave excitation and termination of transmitted and reflected fields. A single ELC unit cell is used to calculate S_{11} and S_{21} when the plane wave is incident normally on the metasurface though the floquet port-1. If there is a rotation of electric field with respect to the y-axis, then the reflection coefficient and transmission coefficient will also change with the rotation of unit cell. Here the E-field is making an angle 'a' with the y-axis of unit cell. The allowable frequency range to calculate the S-parameters starts from 5-7 GHz. By providing appropriate boundary conditions to the metasurface in HFSS, both S_{11} and S_{21} of a single unit cell are obtained at angles in complex form [7]. Since the MS has infinite size, periodic boundary is considered in HFSS simulation to evaluate S_{11} and S_{21} for the evaluation of the equivalent impedance 'Z' and the refractive index 'n' of the MS by using the following two equations:

$$z = \pm\sqrt{\frac{(1+S_{11})^2 - S_{21}^{\ 2}}{(1-S_{11})^2 - S_{21}^{\ 2}}} \quad \text{.....(1)}$$

$$e^{ink_0 d} = X \pm i\sqrt{1-X^2} \quad \text{.....(2)}$$

where $X = \frac{1-S_{11}^{\ 2}+S_{21}^{\ 2}}{2S_{21}}$, k_0 is the wave number, d is the equivalent thickness of the metasurface.

We have: $\Rightarrow t = e^{ink_0 d} = X \pm i\sqrt{1-X^2}$ .....(3)

Taking natural logarithm (ln) on both sides of Equation (3):

$$\ln(t) = ink_0 d$$

$$\Rightarrow n = \frac{\ln(t)}{ik_0 d} \quad \text{.....(4)}$$

The permeability of the metasurface is $\mu_r = nz$ and its permittivity is $\varepsilon_r = \frac{n}{z}$ which can be obtained using Equations (1) and (4).

Analysis of the S-parameters of Unit Cell

The simulation model of unit cell design in HFSS is shown in Figure 3.

S-parameters Calculation

1**.** Draw the structure with exact/required specifications.
2. Draw the boundary box with Z+ = 5 units and Z- =5 units.
3. Assign boundary to each face (Master-1, Master-2, Slave-1 and Slave-2).
4. Assign excitation (Floquet port 1 & port-2).
5. Analysis set up.
6. Add frequency sweep.

The simulated magnitude and phase of S_{11} and S_{21} with different a=25^0,45^0,65^0,80^0 and 85^0 for the frequency band from 5 to & 7 GHz is shown in Figure 4. Using Equations (1)-(3) and the results shown in Figure 4, the calculated ε_r and μ_r are shown in Figure 5. It can be seen from Figures 4 and 5 that the angle 'a' affects S_{11} and S_{21}; hence, the equivalent ε_r and μ_r change due to te rotation angle 'a' as shown in Figure 5. The value of ε_r and μ_r depend on the polarization direction of the plane wave. The metasurface could be considered as a special dielectric slab which has variable permittivity $\varepsilon_r$and permeability μ_r when targeted by a linearly polarized plane wave. In the proposed FRMS antenna, the rotation angle theta1 is equivalent to 'a'. Rotating the metasurface by the rotation angle theta1 with respect to the patch antenna will change the relative permittivity and relative permeability of the metasurface, hence changing the equivalent signal wavelength and increasing the resonant frequency of the antenna.

FRMS Antenna Simulation Results and Discussions

Using the reflection coefficient $S_{11,}$ the frequency reconfigurability is studied. The simulated S_{11} of the antenna with different rotation angles 'theta1' is shown in Figure 6. As the angle of the rotation increases from 25^0 to 45^0, 65^0, 80^0, 85^0, the resonant frequency increases from 5.75 to 5.79, 5.95, 5.98 and 6.0 GHz, respectively, as shown in Table 2. Theoretically, the array of the unit cells becomes densely packed with an increase in the angle of incidence because of the highest periodicity. Thus, the array is loosely packed at lower incidence angle due to its lowest periodicity. Hence, the permittivity changes as the periodicity changes.From Figure 6 return loss is observed to be decreasing from -21 dB to -10.5 dB as the rotation angle is increased. The radiation pattern of the FRMS antenna is considered in far-field region. It is observed from Figure 7 that the radiation pattern of the antenna is directional and same as the source patch antenna.

Figure 7 shows the simulated radiation patterns and realized gain of the FRMS antenna at two resonant frequencies which are 5.75 GHz and 6 GHz. The radiation pattern of the patch antenna is the same as that of the FRMS antenna. Co-polarization (linear polarization along the y-axis) put more impact (stronger) than the cross polarization (linear polarization along the x-axis) as the polarization isolation is more than 10 dB, which results in shifting of metasurface with the resonant frequency without affecting the shape of the radiation pattern or polarization. Results show that the simulated efficiencies are above 88% at resonant frequencies as shown in Figure 9. The feed efficiency of metasurface antenna can reach more than 95% by using planar or quasi-planar circularly symmetric sources [10]. The simulation results provide the 3D-Polar plot which is shown in Figure 8. Figure 9 shows the simulated efficiencies which is defined as the ratio of the radiated power to the input power to the antenna.

It is obvious that the reported antenna can operate with different resonant frequencies from 5.75 GHz to 6 GHz as per the rotation of the metasurface. It is observed that the realized gain of the antenna varies from 1.1 dBi to 2.1 dBi for the resonant frequency range which meets the design requirements of the wireless local area network (WLAN) band (5.180-5.825 GHz), and industrial scientific and medical (ISM) band (5.725-5.875 GHz).The radiation patterns and polarizations are not changed at different resonant frequencies, same as the source patch antenna and hence it is a frequency reconfigurable antenna.

CONCLUSION

A compact frequency reconfigurable metasurface antenna for wireless applications was presented in this paper. The antenna performance is analyzed in HFSS in terms of return loss, gain, radiation pattern, and efficiency. The antenna can be tuned at different frequencies by rotating the metasurface around the center patch antenna. The advantage of the frequency reconfigurable antenna is that it can be tuned continuously from 5.5 GHz to 6.0 GHz. The antennais suitable for the WLAN and ISM bands to satisfy the diverse requirements of the end applications without any volume increase. Results have shown that the resonant frequency of FRMS antenna can be tuned continuously over a range of 500 MHz at around 5.50 GHz to 6.0 GHz.

Author contributions

Harish Chandra Mohanta has designed and simulated the low profile frequency reconfigurable metasurface antenna using HFSS. The simulated results are reviewed by Prof. Abbas Z. kauzani and Prof. S.K. mandal.

REFERENCES

1. H. Rajagopalan, J.M. Kovitz, and Y. Rahmat-Samii, "MEMS reconfigurable optimized E-shaped patch antenna design for congnitive radio," *IEEE Trans. Antennas Propag*., vol. 62, no. 3, pp. 1056-1064, Mar. 2014.
2. C. Y. D Sim, T. Y. Han, and Y. J. Liao, "A frequency reconfigurable half annular ring slot antenna design," *IEEE Trans. Antennas Propag.,* vol. 62, no. 6, pp. 3428-3431, Jun. 2014.
3. L. Ge and K. M. Luk," Frequency-reconfigurable low-profile circular monopolar patch antenna," *IEEE Trans. Antennas Propag.,* vol.62, no.7, pp. 3443-3449, Jul. 2014.
4. T. Aboufoul, A. Alomainy, and C. Parini, "Reconfiguring UWB monopole antenna for cognitive radio applications using GaAs FET switches," *IEEE Antennas Wireless Propag. Lett*., vol. 11, pp.392-394, 2012.
5. J. Costatine, Y. Tawk, S.E. Barbin, and C.G. Christodoulou, "Reconfigurable antenna: design and applications," *IEEE Proc., vol*. 103, no. 3, pp. 424-437, 2015.
6. C. L. Holloway, E. F. Kuester, J. A. Gordon, J. O'Hara, J. Booth, and D. R. Smith, "An overview of theory and applications of metasurfaces: The two-dimensional equivalents of metamatrials," *IEEE Antennas propag. Mag.,* vol.54, pp. 10-35, 2012.
7. X.Chen, T. M. Grzegorczyk, B.-I.Wu, J. Pacheco, Jr., and J.A.Kong,"Robust method to retrieve the constitutive effective parameters of metamaterials," *Phys. Rev. E*, vol. 70, p. 016608(1-7), Jul. 2004.
8. H. L. Zhu, X. H. Liu, S. W. Cheung, and T.I. Yuk, "Frequency-reconfigurable antenna using metasurface," *IEEE Trans. Antennas Propag*., vol. 62, pp. 80-85, Jan. 2014.
9. L. Han, C. Wang, X. Chen and W. Zhang, "Compact frequency-reconfigurable slot antenna for wireless applications," *IEEE Antennas Wireless Propag.Lett.,* vol. 15, pp. 1795-1798, 2016.
10. G. Minatti, E. Martini and S. Maci, "Efficiency of Metasurface Antennas", *IEEE Trans. Antennas Propag.,* vol. 65, no. 4, Apr. 2017.
11. K. Li, C. Zhu, L. Li, Y. Cai and C. Liang, "Design of electrically metamaterial antenna with ELC and EBG loading,"*IEEE Antennas Wireless Propag. Lett*., vol. 12, pp. 678-681, 2013.

Table 1. Dimensions of the FRMS antenna in mm

P_a	P_b	a	b	w	x	L_p	W_p	W_f	P_f	P_e	D
12.4	5.4	10	3	0.5	0.3	16	12	2	11	2	40

Table 2.Simulated resonant frequencies at different MS rotation angles.

Resonant Frequency(GHz)	5.75	5.79	5.95	5.98	6.0
Rotation Angle of Metasurface(MS)	25^0	45^0	65^0	80^0	85^0

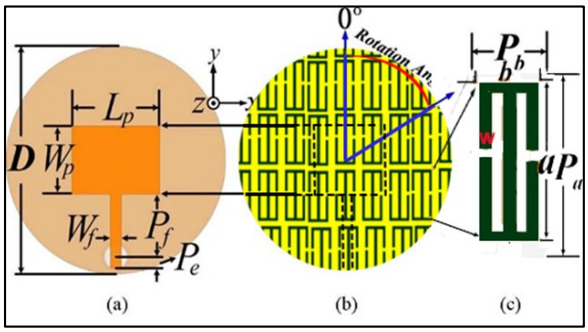

Figure 1. Geometries of (a) patch antenna (source antenna), (b) metasurface, and (c) unit cell of the FRMS antenna

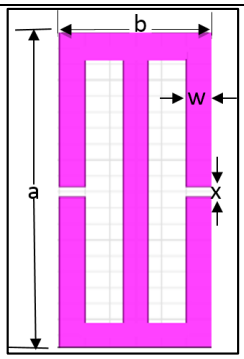

Figure 2.ELC unit cellstructure

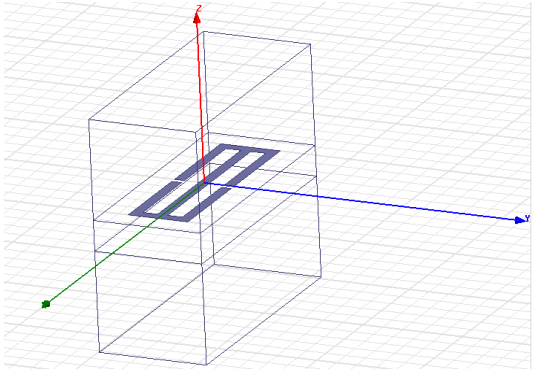

Figure 3. Unit cell design in HFSS.

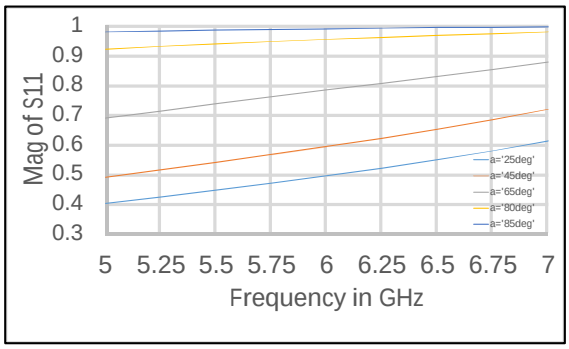

Figure 4 (a) Simulated S_{11} magnitude w.r.t frequency for different a=25⁰,45⁰,65⁰,80⁰, 85⁰

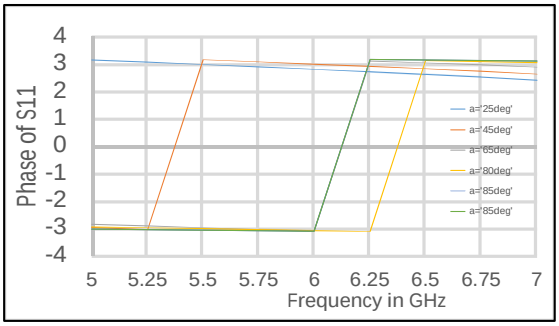

Figure 4 (b) Simulated S_{11} phase in radian w.r.t frequency for different a=25⁰,45⁰,65⁰,80⁰ and 85⁰

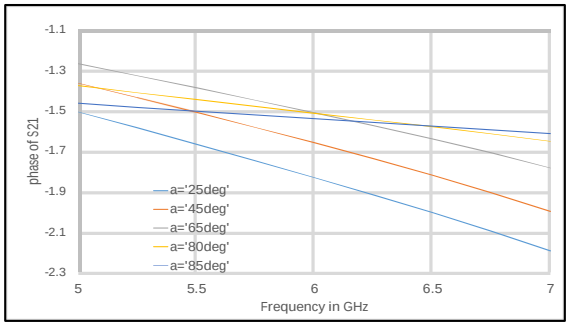

Figure 4 (c) Simulated S_{21} phase w.r.t frequency for different a=25⁰,45⁰,65⁰,80⁰, and 85⁰

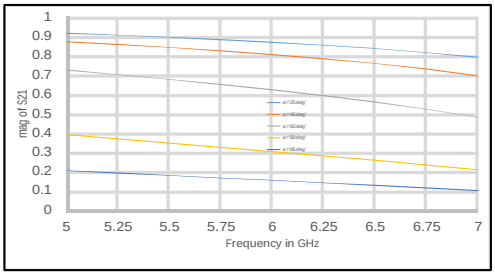

Figure 4 (d) Simulated S_{21} magnitude w.r.t frequency for different a=25⁰,45⁰,65⁰,80⁰, and 85⁰.

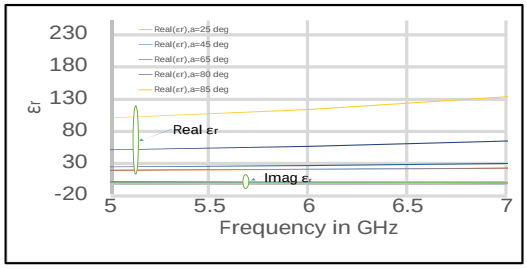

Figure 5. (a) Calculated □ᵣ w.r.t frequency for different a=25⁰,45⁰,65⁰,80⁰, and 85⁰

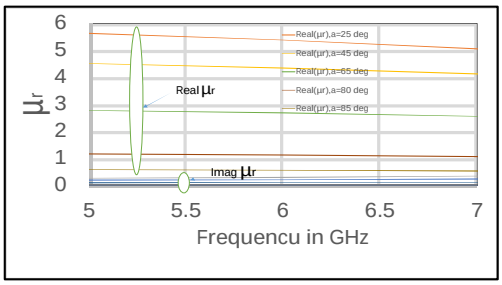

Figure 5. (b) Calculated μ_r w.r.t frequency for different A=25⁰,45⁰,65⁰,80⁰, And 85⁰

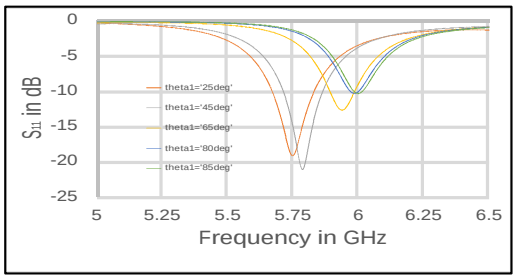

Figure 6. Simulated S_{11} of the FRMS antenna at different rotation angles

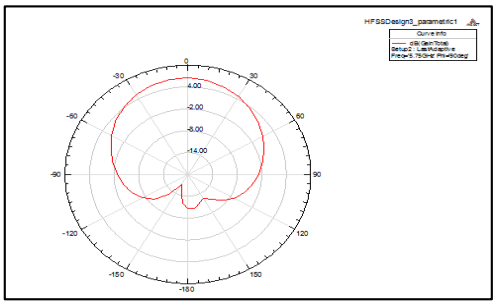

Figure 7. (a) Radiation pattern at theta1=25⁰ rotation of MS of FRMS antenna at resonant frequency at 5.75 GHz i.e. in yz-plane

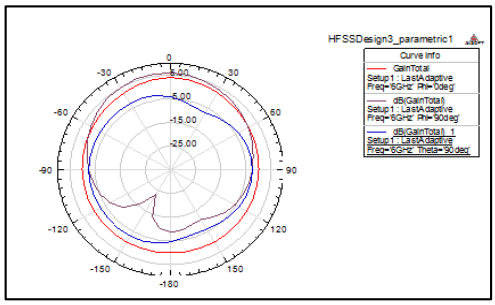

Figure 7. (b) Radiation pattern at 6 GHz for theta1=45⁰ in xy, yz and xz-plane.

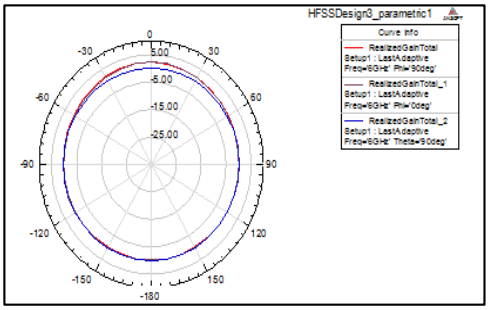

Figure 7. (c) Realized gain at 6GHz for theta1=45⁰ in yz, xz and xy-plane.

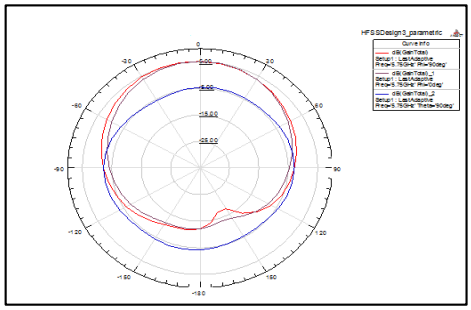

Figure 7. (d) Radiation pattern at 5.75GHz for theta1=65⁰

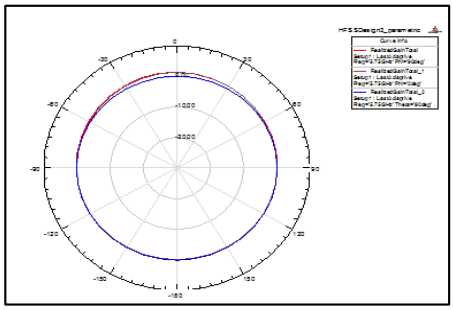

Figure 7.(e) Realized gain at 5.75 GHz for theta1=65^0

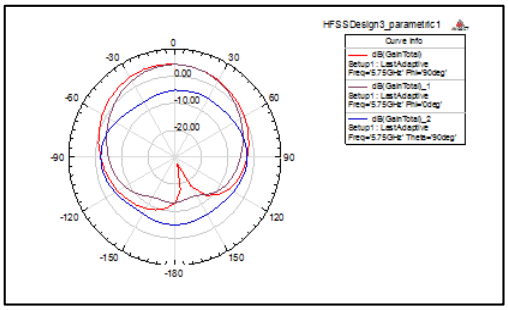

Figure 7.(f) Radiation Pattern at 5.75 GHz for theta1=85^0.

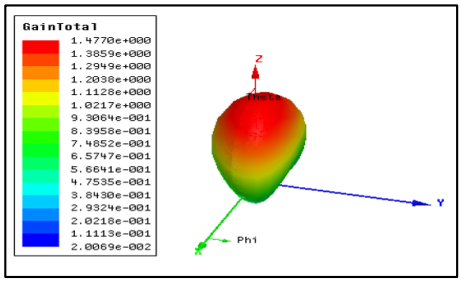

Figure 8. 3D Polar Plot of FRMS antenna.

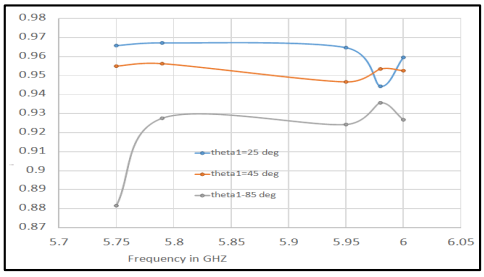

Figure 9. Simulated efficiencies at different rotation angles i.e. theta1=25^0,45^0,85^0.

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RESEARCH ARTICLE

Biological Activity of *Gloriosa superba* against Pathogenic Fungus of *Candida albicans, Trichoderma viride* and *Aspergillus niger*

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ABSTRACT

In the present investigation the flower of *Gloriosasuperba*was selected to evaluate its antifungal potential against *Candida albicans, Trichoderma viride* and *Aspergillus niger*. The collected plant material was extracted using chloroform and methanol solvents and stored at 4°C for future studies. The antifungal activity of chloroform and methanolic extracts of *Gloriosa superba* flower was tested against *C. albicans, T.viride* and *A.niger* by disc diffusion method using Sabouraud Dextrose Agar (SDA) medium. The plates were incubated at 28□ for 24 hrs. The maximum zone of inhibition 46mm was observed at 1mg/ml concentration of methanolic flower extract of *Gloriosa superba* followed by 44mm zone of inhibition was observed at 0.75mg/ml against *Trichoderma viride.*The minimum zone of inhibition 6mm and 9mm at 0.75mg/ml and 0.50mg/ml concentrations on chloroform and methanolic extracts of *G.superba* against *A. niger*. It concluded that methanolic extract of flowers from *G.superba* proved very strong antifungal activity against selected pathogenic fungus.

Key words: Antifungal, *Gloriosa superba*, *Candida albicans, Trichoderma viride* and*Aspergillus niger*

INTRODUCTION

Fungus belongs to microbes that cause a wide range of diseases, from skin irritation to life threatening diseases. It is generally understood that various classes of human pathogenic and phytopathogenic fungi can cause several diseases to humans, livestock's and plants.The phytopathogenic fungi can cause the cereal grains at storage period, resulting them unfit for consumption of humans by losing the safety and quality of food products (1). In the past few

decades, fungal diseases incidence has been increased in worldwide and developing resistance of several species to different fungicides were used in medicinal practice. Hence, isolation of new prototype fungicidal molecules are essential to control their situation (2).Kacaniova reported that more than 1,00,000 fungal strains are important natural contaminants of food and agricultural products (3). In general, effective, safe and potent new antifungal molecules are expected by clinicians, agrochemical and pharmaceutical companies, researchers (4).

A huge number of chemical fungicides such as socking treatment, dusting and slurry are being used. However some of the pesticides are cannot be used on grains due to the toxicity (5). But nowadays people were realised that chemically synthesised fungicides cause severe environmental toxicity, toxic to human and non-target organisms (6). Moreover inappropriate use of agrochemical especially chemical fungicides which are found to cause more carcinogenic risk that herbicidal and insecticidal agents (7) and it give rise to unwanted side effects (8). The developing resistance in microbial agents against antifungal molecules has severe implications of infections. These antifungal molecules produce toxicity to target mammalian cells and some adverse effects (9). An antifungal drug Ketoconazole used against both deep seated and superficial infections.However, its unpleasant adverse effects like slow therapeutic response, abdominal pain, nausea, itching (10).Hence an urgent need to develop a new microbial management system to minimise the usage of chemically synthesised pesticides.

Gloriosa superba L. is one of deciduous perennial herb that scrambles of clime over the plants with the help of tendrils present on the leaves end. It reaches about 3 to 4 meters in height. The plant parts contain colchicine and associated alkaloids especially tuberous rhizome. Many people from India, Africa and other parts of the world using traditionally for various diseases such as ulcers, snake bite, open wounds, kidney problems (11), nocturnal emission (12), and leprosy (13), many types of internal parasites (14), insecticidal (15) and antimicrobial (16). The methanoland chloroform extract of the rhizomes of *Gloriosa superba* extracttested against selected bacteria and fungus. They reported thatremarkable antifungal sensitivity was observedin fraction of n-butanol against *Trichophytonlongifusus* (78%), *C. albicans* and *C. glaberata* (90%) and fraction of chloroform showed 80% against *Microsporum canis*(16).

The leaves decoction of *G. superba* is applied to kill head lice and some tribal people were used one of the important ingredient in arrow poisons, and also exhibited cytogenetic properties against antimosquito (15). Duke examined the phytochemicals in *G. superba* and noticed that the Chelidonic acid, salicylic acid and alkaloids like 3-desmethyl colchicine, β-Lumicocolchicine, 2-desmethyl colchicine, N-Formyldesacetyl-colchicine including gloriosine and colchicines (17).

MATERIALS AND METHODS

Plant material

The *Gloriosa superba* Linn.flower was collected in and around Kanchipuram District of Tamil Nadu, India and brought to the laboratory. The plant material was authenticated, preserved and deposited in the herbarium at Post Graduate and Research Department of Botany, Arignar Anna Govt Arts College, Cheyyar, Thiruvannamalai District, Tamil Nadu, India for future references.

Extraction

The *G. superba* flower materials were washed and shade dried under room temperature and pulverized in to fine powder. The powdered plant material was soaked in chloroform and methanol (3:1 ratio) and shake at 12 hrs time interval. After 15 days shocked plant material were filtered using Whatman no. 1 filter papers. The filtrate was concentrated using rotary evaporator and stored at 4□ to evaluate antifungal activity. Number of sample used in this study was two (Chloroform and Methanol)

Number of micro organism used in this study was three (*Candida albicans, Trichoderma viride* and *Aspergillus niger*) Standard: Amphotericin – B (20µl/disc) was used.

Preparation of Inoculum

Stock cultures were maintained at 4□ on Sabouraud Dextrose Agar Slant. Active culture for experiments were prepared by transferring the stock cultures into the test tube containing Sabouraud Dextrose broth that were incubated at 48 hrs at room temperature. The assay was performed by agar disc diffusion method.

Agar Disc Diffusion Method

Antifungal activity of the extracts was determined by disc diffusion method on Sabouraud Dextrose Agar (SDA) medium is poured in to the petriplate. After the medium was solidified, the inoculums were spread on the fungal suspension. Amphotericin – B is taken of positive control. Samples and positive control of 20 µl each were added in sterile discs and placed in SDA plates. The plates were incubated at 28□ for 24 hrs. Then antifungal activity was determined by measuring the diameter of zone of inhibitions.

RESULTS

The chloroform extract of *Gloriosa superba* flower against *Candida albicans, Trichoderma viride* and *Aspergillus niger* was given in Table 1. The chloroform extract of *Gloriosa superba* flower showed moderate antifungal activity against selected human pathogenic fungal strains of *C. albicans* inhibited 24mm, 18mm and 12mm at 1mg/ml, 0.75mg/ml and 0.50µg/ml concentrations (Fig.A).Where as, *T. viride* potential inhibited 42mm, 36mm and 21mm (Fig. B) and *A. niger* minimally inhibited 7mm, 6mm and 6mm at 1mg/ml, 0.75mg/ml and 0.50µg/ml concentrations (Fig. C), respectively. The highest zone of inhibition 42mm was observed at 1mg/ml chloroform extract against *T.viride.* The anti fungal activity of methanol extract of *Gloriosa superba* flower was given in Table 2. The methanolic extract of *Gloriosa superba* flower showed potential antifungal activity against human pathogenic fungal strains. The *Candida albicans* was affected 23mm, 19mm and 15mm at the concentration of 1mg/ml, 0.75mg/ml and 0.50mg/ml (Fig. 2A).Where as, *Trichoderma viride* showed 46mm, 44mm and 32mm and *Aspergillus niger* caused 10mm, at 1mg/ml, and 9mm zone of inhibition in both 0.75mg/ml and 0.50mg/ml concentrations (Fig. 2B and 2C), respectively. The highest zone of inhibition 46mm at 1mg/ml and 44mm at 0.75mg/ml in *Trichoderma viride* was noticed.

DISCUSSION

Antimicrobial properties of plant materials such as stems, roots, fruits, leaves and flowers from different species spices and herbs have been reported (18). Appropriate isolationand identification of active molecules from plants is basically dependent on the different types of organic solvent used for extraction. Medicinal plants were using traditional health care system and it can be recognized as a starting point for the development of novel drugs for various ailments. Hence, flower of *Gloriosa superba* was extracted using chloroform and methanol and screened their toxicity against selected pathogenic fungal strains.Antifungal activity of chloroform and methanol extract of *Gloriosasuperba*flower was evaluated against pathogenic fungisuch as *Candida albicans, Trichoderma viride* and *Aspergillus niger* compared with Antibiotic standard. The chloroform extract showed good to excellent activity 42mm of zone of inhibition at 1mg/ml against *Trichoderma viride* which caused dermatophytosis that affects hair, skin and nails of humans and animals. Our results were coincides with earlier reports (19-20). They noticed that *Gloriosa superba* highly affects the growth of *Candida albicans* and *Candida glaberata* which are main causative agents of invasive candidiasis including vaginal candidiasis. The *Aspergillus niger* showed no respond to chloroform extract of *Gloriosa superba* flower at all concentration. The methanol extract of G. superb flower showed very high antifungal activity

against *Trichoderma viride* 38mm and 37mm at 1mg/ml and 0.75mg/ml concentration of flower extract. The results are more consistent with many authors they were found methanolic extract of *G. superba* more antifungal activity against pathogenic fungus. The *Candida albicans* showed highly sensitive to *Gloriosa superb* flower which is the main causative agent of candidiasis and corroborates with earlier findings (21-23). Jagtap and Satpute evaluated acetone, chloroform, methanol and water extracts of *G. superba* tuber against *Salmonella typhi, Staphylococcus aureus E. coli A. niger.* After 24 hrs the highest zone of inhibition was observed in water extract on *S. aureus* and *S. typhi*. The *S. aureus* caused upto 0.2cm zone of inhibition on methanolic extract and *S. typhi* caused 0.6 cm zone of inhibition on acetone extract of *G. superba.* The *E. coli* bacteria and fungal strain of *Aspergillus niger* didn't show any zone of inhibition on methanolic extract. Similarly all the micro organisms didn't respond when treated with chloroform of *G. superba* (24).Lakshmi et al., reported that several plant materials extracted using methanol which showed potential activity compared with aqueous extract (25).

The antifungal activity of *Vinca rosea, Phyllanthus niruri, Lawsonia inermis, Tephrosia purpurea, Mimosa pudica* against *Pythium debaryanum*. They noticed that aqueous extract of all the plants dint show any zone of inhibition. When treated with methanolic extract inflicted 20mm inhibition on *Mimosa pudica* and 10mm inhibition on *Vinca rosea*. Where as, n-butanol and methanol extract of *Lawsonia inermis* showed highest activity of 15 to 20mm zone of inhibition and followed by *Phyllahtus niruri* reflected 15 to 20 mm and *Tephrosia purpurea* inhibited 10mm to 15mm against *Pythium debaryanum* (26).

The antifungal activity of methanolic extracts from 21 plant species was tested against anthracnose disease pathogen of *Colletotrichum musae* using paper disc method (27). They reported that potential antifungal activity (30.7 mm) was observed in *Prosopis juliflora* followed by (19mm) in *Acacia albida* as compared with control. The remaining plant extracts produce no inhibitory activity against *C. musae.*The antifungal activity of aqueous, chloroform and methanol extracts of *Annona squamosa* were analysed against 5 different pathogenic fungi strains such as *Aspergillus niger, Fusarium solani, Alternaria alternata, Microsporum canis,* and *Candida albicans* by the agar well diffusion method (28). They noticed that both methanol and chloroform extract exhibited similar activity against *Alternaria alternata* (79.10 % and 83.58 %), *Candida albicans* (27.69 % and 64.62 %), and *Fusarium solani* (91.67 % at 1 mg/mL and 108.33 %2 mg/mL of chloroform extract, respectively. When evaluated with methanol extract 34.33 % and 74.63 %; 43.08 % and 70.77 %; 60.42% and 97.92% at 1mg/ml and 2 mg/mL concentrations, respectively. The lowestzone of inhibition 20.90% and 43.28% was recorded against *A. alternata* and 9.23 % and 33.85% was recorded against *C. albicans* at 1 mg/mL and 2 mg/mL concentrations, respectively. When treated against *A. niger* chloroform, methanol and aqueous extracts caused 25.86%, 31.08%, and 22.41% at 1 mg/mL and 77.59%, 81.03%, and 84.84% at 2 mg/mL concentrations, respectively.

The antifungal activity hydro alcohol (50%) and hexane extracts of *Tinospora cordifolia*, *Valeriana jatamansi*, *Andrographis paniculata*, *Plantago depressa, Asparagus racemosus*, *Coleus barbatus*, *Berberis aristata*, *Achyranthes aspera* was tested against opportunistic pathogens of *Candida albicans* and *Aspergillu sniger*(29). All the hydro alcoholic extract of plant species exhibited maximum antifungal activity compared with hexane extracts. Especially hydro alcoholic extract of *A. paniculata* showed highest activity against *A. niger* with 20 mm zone of inhibition. When treated with hexane extract of *A. paniculate* showed 10mm zone of inhibitory activity where in the case of candida albicans no activity was recorded. Antifungal activity of *Abrus precatorius*, *Aegle marmelos, Aporosa lindleyana*, *Areca catechu*, *Brassica juncea* against *A. niger.* They reported that minimum antifungal activity was observed in *A. lindleyana* and maximum antifungal activity was observed in *Abrus precatorius*, *Aegle marmelos,Areca catechu*, *Brassica juncea* (30). The methanolic extracts of different parts of plants exhibited notable antibacterial activity against *Fusarium verticillioides, Dreschlera turcica, Aspergillus flavus*(31).

The results observed in the present investigation revealed that *Gloriosa superba* has excellent antifungal activity against *Candida albicans, Trichoderma viride* and less sensitive activity against *Aspergillus niger.* Hence it can be used to formulate antifungal drugs, cosmetic and health care products.

REFERENCES

1. Salhi N, Saghir SAM, Terzi V,Brahmi I, Ghedairi N, Bissati S. Antifungal activity of aqueous extracts of some dominant Algerian medicinal plants. BioMed Res Int 2017;1-6.
2. Sati SC, Joshi S. Antibacterial potential of leaf extracts of *Juniperuscommunis* L. from Kumaun Himalaya. Afr J Microbiol Res 2010;4:1291-1294.
3. Kacaniova M. Feeding soybean colonization by microscopic fungi.TrakyaUniv J Sci2003;4:165-168.
4. Selitrennikoff CP. Screening for antifungal Drugs. In: Biotechnology for Filamebtous Fungi, Firkeftein, D. and C. Ball (Eds.). Dutterworth, London, 1992; pp: 189-217.
5. Harris CA, Renfrew MJ, Woolridge MW.Assessing the risk of pesticide residues to consumers: recent and future developments.Food AdditivContam2001;18(12):1124–1129.
6. Anon, Pest control background. Int J Pest Cont2005;45(2):232–233.
7. Research council board of Agriculture, Regulating Pesticides in Food. The Delaney Paradox.National Academy Press. Washington, DC. 1987.
8. Osman KA, Al-RehiayaniS. Risk assessment of pesticide to human and the environment. Saudi J BiolSci2003;10:81-106.
9. Lucca AJD, Walsh TJ. Antifungal peptides: novel therapeutic compounds against emerging pathogens. Antimicrob.Agents Chemother 1999;43:1-11.
10. Pyun MS, Shin S. Antifungal effects of the volatile oils from Allium plants against Trichophyton species and synergism of the oils with ketoconazole. Phytomed 2005;13:394-400.
11. *Gloriosasuperba*. Archived 2013-06-06 at the Wayback Machine World Checklist of Selected Plant Families.2011. Royal Botanic Gardens, Kew.
12. Lal HS, Mishra PK. *Gloriosasuperba* – an endangered plant spotted for the first time from forest of Tpchanchi, Hazaribag (Jharkhand) India. Sci Res Report2011;1(2):61-64.
13. Oudhia P. *GloriosaSuperba*. New Crop Resource Online Program.Center for New Crops & Plant Products. Purdue University. 2002.
14. Dounias E. *Gloriosasuperba* L. Archived 2014-07-14 at the Wayback Machine Protabase Record Display. Plant Resources of Tropical Africa (PROTA).
15. Choochote W, Rongsriyam K, Pitasawat B, Jitpakdi A, Rattanachanpichai E, Junkum A, Tuetun B, Chaiwong P. Evaluation of the colchicine-like activity of *Gloriosasuperba* extracted fractions for mosquito (Diptera: Culicidae) cytogenetic study. J Med Entomol 2004;41:672–676.
16. Khan H, Murad Ali Khan, TahiraMahmood and Muhammad Iqbal Choudhary. Antimicrobial activities of *Gloriosasuperba*Linn (Colchicaceae) extracts. J Enzyme Inhib Med Chem2008:23(6):855–859.
17. Duke JA. Handbook of medicinal herbs. USA: CRC Press. 1985.
18. Mau J, Chen CP, Hsieh PC. Antimicrobial effect of extracts from Chinese chive, cinnamon, and Cornifructus. J Agric Food Chem 2001;49:183-188.
19. AlyR, Hay RJ, Del Palacio A, Galimberti R. Epidemiology of tinea capitis. MedMycol2000;38:183–188.
20. Atman S, Haroon TS, Hussain I, Bokhari MA,Khurshid K. *Tineaunguium* in Lahore, Pakistan. Med Mycol2001;39:177–180.
21. Saville SP, Lazzell AL, Bryant AP, Fretzen A, MonrealA, Solberg EO, MonteagudoC, Lopez-Ribot JL,Milne GT. Inhibition of filamentation can be used to treat disseminated candidiasis. Antimicrob Agents Chemother.2006;50:3312–3316.
22. Viudes A, Peman J, Canton E, Ubeda P, Lopez-Ribot JL,Gobernado M. Candidemia at a tertiary-care hospital: Epidemiology, treatment, clinical outcome and risk factors for death. Eur J ClinMicrobiol Infect Dis.2002;21:767–774.
23. Tanju K, Birsay G, Banu U. Phospholipase activity of Candida albicans isolates from patients with denture stomatitis: The influence of chlorhexidine gluconate on phospholipase production. Arch Oral Biol2007;52:691–696.

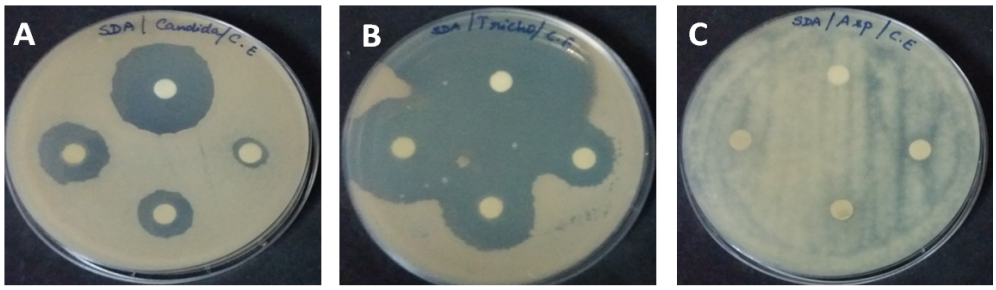
24. Jagtap S, Satpute R, Phytochemical Screening, Antioxidant, Antimicrobial and Flavonoid Analysis of *Gloriosasuperba*Linn. Rhizome Extracts. J AcadIndust Res 2014;3(6):247-254
25. Lakshmi BS, Naidu KC, Murthy YLN, Bobbarala V, Pandit N. Bio-efficacy of some medicinal plants against pathogens of cereal crops and phytochemical examination of *Prosopisjuliflora* (Sw) Dc. J Pharm Res 2010;3(2):356-360.
26. Ambikapathy V, Gomathi S, Panneerselvam A. Effect of antifungal activity of some medicinal plants against *Pythiumdebaryanum*(Hesse). Asian J Plant Sci Res 2011;1(3):131-134
27. SetuBazie, AmareAyalew and KebedeWoldetsadik.Antifungal Activity of Some Plant Extracts against (*Colletotrichummusae*) the Cause of Postharvest Banana Anthracnose. J Plant PatholMicrob 2014,5:1-4.
28. NarasimharajuKalidindi, NagarasanakoteVenkataswamyThimmaiah, NagepallyVenkataramareddyJagadeesh, RamaiahNandeep, SathyanarayanaSwetha, Bharat Kalidindi. Antifungal and antioxidant activities of organic and aqueous extracts of *Annona squamosa* Linn.Leaves.J.Food Drug Anal2015;23(4):795-802.
29. AbhishekMathur, Ravinder Singh, SajadYousuf, AprajitaBhardwaj, Satish K. Verma, PradeepBabu, Vinay Gupta, GBKS Prasad and V.K. Dua. Antifungal activity of some plant extracts against Clinical Pathogens. AdvApplSci Res 2011;2(2):260-264
30. Raji R,Raveendran K. Antifungal activity of selected plant extracts against phytopathogenic fungi *Aspergillusniger.* Asian J Plant SciRes 2013;3(1):13-15
31. Mahesh B,Satish S. Antimicrobial activity of some important medicinal plants against *A. nilotica* and *S. cardifolia*. World J AgriSci2008;4:839 – 843.

Table 1: Chloroform extract of *Gloriosa superb* flower against selected fungal strains.

Fungal strains	Zone of Inhibition (mm)			Antibiotic 1mg/ml
	1mg/ml	0.75 mg/ml	0.50 mg/ml	
Candida albicans	24	18	12	32
Trichoderma viride	42	36	21	50
Aspergillus niger	7	6	6	6

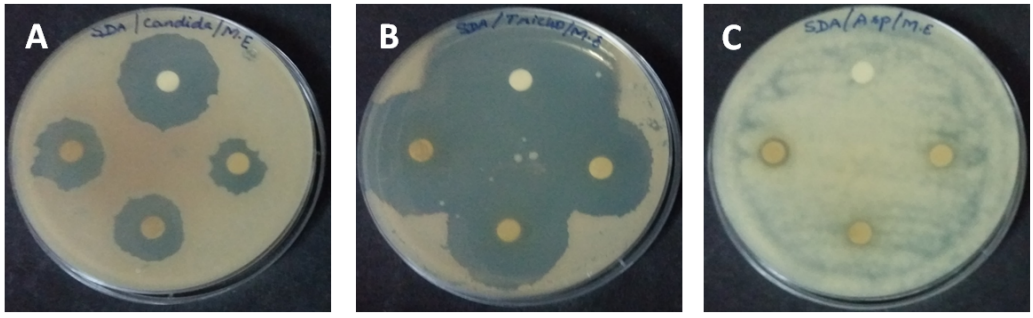
Table 2: Methanolic extract of *Gloriosa superba* flower against selected fungal strains.

Fungal strains	Zone of Inhibition (mm)			Antibiotic 1mg/ml
	1mg/ml	0.75 mg/ml	0.50 mg/ml	
Candida albicans	23	19	15	30
Trichodermaviride	46	44	32	50
Aspergillusniger	10	9	9	9



Candida albicans ***Trichoderma viride*** ***Aspergillus niger***

Figure 1. The antifungal activity of chloroform extract of *Gloriosa superba* flower *Candida albicans, Trichoderma viride, Aspergillus niger*



Candida albicans ***Trichoderma viride*** ***Aspergillus niger***

Figure 2. Methanolic extract of *Gloriosa superba* flower against *Candida albicans, Trichoderma viride, Aspergillus niger.*

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RESEARCH ARTICLE

Fabrication and Characterization of Chitosan/ PCL-G-NH_2PAMAM Polyamidoamine Dendrimer Complex for Controlled Release of Anticancer Drug Cyclophosphamide

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ABSTRACT

Chitosan/PCL-G-NH_2PAMAM based dendrimer complex has been developed andthe extended release of Cyclophosphamide (CYC) was investigated by changing formulation variables. The dendrimer hydrogels so formed were characterized by FTIR, Thermogrametry Analysis (TGA) and Scanning Electron Microscopy (SEM).The equilibrium swelling indicated the distinct sensitiveness of the matrix to pH value and temperature.Within 10 h the cumulative release amount of CYC- loaded in the matrix was about 84% and 86% at pH 7.4 and 3.4 respectively. The Controlled Drug delivery of CS/PCL-G-NH_2PAMAM was studied using broad spectrum anticancer drug Cycloposphamide. The most conspicuous part of the present study lies in the population of various kinetic models for drug delivery systems. A simple kinetic method has been used to monitor drug delivery kinetics. The various kinetic parameters like k and n value have been computed and the mechanism of invitro drug release of the model drugs have been postulated. The complex developed so far are useful as carriers in drug delivery systems. These complexes can be used as antibacterial agents in pharmaceutical and other medical applications.

Key Words: Chitosan, Cyclophosphamide, dendrimerhydrogels, phosphate buffer solution (PBS), Drug delivery systems,invitro drug release

INTRODUCTION

Dendrimers are a unique polymeric material which is a support to drug delivery system. The highly branched monodisperse macromolecular structure is suitable for biomedical and industrial applications like gene transfection, drug delivery system, diagnostics in tumour therapy etc. The today nanoparticles drug delivery system is more

reliable and suitable to increase the stability of therapeutic agents. Dendrimeric polymer materials are suitable for controlled release of any drug due to its nontoxic, hydrophilic and biocompatibility nature. It also conjugated with the anticancer drug, antibody, peptides molecules by its hyper branched structure. However, the dendrimer is providing new architecture polymer designing structure which is attached to back bone of the drug molecules, i.e., prospective relevant to drug delivery applications [1-4].Chitosan is a most popular carbohydrate polysaccharide polymer which is easily available in Indian market. Chitosan is widely used in drug delivery due to itsnon-toxicity, biocompatibility, hydrophilicity, low molecular weight, cost effectiveness, environmentally friendly characteristics. Now days the enhancement of chitosan properties in micro and nano form is a new ambition for future research [5].

Also, interesting properties of chitosan and its derivatives with mucoadhesion nature is showing good potentiality of drug delivery system [6]. The controlled release of drugs is a very important aspect in health care. Today cancer is a great challenge for medical and pharmaceutical arena due to which the discovery of new drug and implementation of drug in the human body is a matter of concern. Morden organic chemistry creates a new hope in curing and successful release of the anticancer drug to the targeted cancer cells. Sashiwa H et al.reported preparation and characterization of chitosan with PAMAM dendrimer G (1-3) by reductive N-alkylation. Sialic acid residue bound to PAMAM dendrimer of each generation were successfully attached to chitosan [7]. Additionally, he has also reported that chitosan – dendrimer hybrids having various functional groups like carboxyl, poly (ethylene glycol) and ester were prepared successfully using dendrimer acetal with biodegradation nature [8]**.**

Rita B et al. Pulmonary administration offers excellent advantages over conventional drug delivery routes, including improving therapeutics bioavailability, and avoiding long-term safety problems. Formulations of nano-in-micro dry powders for lung delivery are conducted using *(S)*-ibuprofen as a model drug. These biodegradable formulations comprise of nanoparticles in drug-loaded POxylatedpolyurea dendrimers coated with chitosan using supercritical-fluid-assisted spray drying. The formulations are identified regarding morphology, particle-size distribution, in vitro aerodynamic particle pulmonary distribution, and glutathione-*S*-transferase assay. It is shown that ibuprofen-loaded nanoparticles can be actively incorporated into microspheres with adequate aerodynamic properties, mass median aerodynamic diameter (1.86–3.83 μm), and fine particle fractionation (28%–45%), for deposition into the deep lung. The *(S)*-ibuprofen dry powder formulations exhibit enhanced solubility, high swelling behaviour and a sustained drug release at physiologic pH. Also, pixelated polyureas decrease the *(S)*-ibuprofen toxic effect on cancer cellular growth. The 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assays show no significant cytotoxicity on the metabolic activity of human lung adenocarcinoma epithelial (A549) cell line for the lowest concentration (1×10^{-3}m), even for longer periods of contact with the cells (up to 120 h), and in the normal human dermal fibroblasts cell line the toxic effect is also reduced [9].

Aryabadie, S, et al. introduced chitosan-PAMAM dendrimer hybrid by the pad-dry-cure method, with an average particle sizesof 265 to 278 nm, respectively. They also reported that chitosan-PAMAM dendrimer possess antibacterial activities against gram-negative bacteria (*Escherichia coli*)in comparison to chitosan [10].Qingxing Xu et al. reported that chitosan and PAMAM dendrimer are used for gene delivery. However, chitosan, has excellent biocompatible profile and biodegradability. It also eases to modification. PAMAM dendrimer has controlled structure and size, minimal cytotoxicity, high transfection efficiencies and biodegradability [11].Sarkar K et al. described that dendronized chitosan was prepared by grafting 'dendrimer-like' Polyamidoamine (PAMAM) using Michael addition reaction followed by amidation reaction. The outcome of this experiment introduced that dendronized chitosan might be an effective tool in removing anionic dyes from the wastewater [12].Jing Ji et al. reported chitosan is considered as a promising material in the pharmaceutical and biomedical fields based on its unique biological properties [13].Pereira V.H. et al. reported that Carboxymethyl chitosan/poly(amidoamine) dendrimer nanoparticles have recently been proposed for intracellular drug delivery. They have been introduced that neither particles were observed in the brain parenchyma, nor any apparent deleterious histological changes. The Carboxymethyl chitosan/poly (amidoamine) dendrimer was stable in circulation for a period, targeting the main organs/systems through internalization by the cells present in their parenchyma [14].Polycaprolactone (PCL) is a

biodegradable and semicrystalline polymer which is generally used for several purposes such as the cross-liking polymer, drug delivery carrier, packaging, recycling of polymer, tissue engineering, blending polymer etc. Currently, FDA has been approved to PCL as a biomaterial for drug delivery purpose [15]. PCL has unique properties such as bio-regulatory activity, hydrophobicity, low malting point, slow rate of degradation and neutral charge distribution [16]. Also, it has been reported that PCL can be blended with other natural biopolymers like chitosan, starch, cellulose, and zein [17,18]. PCL has some disadvantages like the slow rate of drug release due to slow degradation rate, hydrophobicity nature, and high crystallinity. Besides, it has also been explained that the PCL can be enhanced biodegradability by cross-link or copolymerizing with another polymer [19-23]. Blending two polymers is an effective way to develop new material with combinations of properties not possessed by individual polymers [24-27].In this research we have designing chitosan-based dendrimeric polymeric matrix which is suitable for drug release in cancer treatment.

EXPERIMENTAL

Materials

Caprolactone (PCL), Chitosan (CH), Gelatin (G), polyvinyl alcohol (PVA), methanol and Poly acetic acid were purchased from Himedia. Doxorubicin (DOX) and Cyclophosphamide (CPA) were purchased from Biogenix India, Polyamide-amine dendrimer G (5)-NH_2 PAMAM was purchased from Sigma-Aldrich, USA. All other reagents and chemicals were used as the analytical grade. Millipore water was used in the entire experimental work.

Preparation of CH-PCL-PAMAM

1% (w/v) chitosan solutions with 0.5M acetic acid and PCL in distilled water were prepared then the solution was autoclaved at 120^o C in a wet cycle for 20 min. The mixture of chitosan and PCL solution were prepared with different proportion such as (Chitosan: PCL) 100/0, 80/20, 70/30, 60/40, 0/100(w/w) respectively [28]. The blends were stirred at room temperature for 2 hours to obtain homogeneous solutions. Additionally, G (5)-NH_2 PAMAM dendrimers were added in the blended mixture as 1%, 3%, 5% and 6%.The mixture was stirred at 37^{0}C for 10h up to the formation of the homogenized mixture.

Drug loading

The calculated amount of CS/PCL-G (5)-NH_2 PAMAMsample was stirring at 200 rpm at room temperature. Then the prepared CYC drugs with different percentages like 10 wt %, 20 wt %, 30 wt %, 40 wt %, 50 wt %, 60% and 70% were placed into CS/PCL-G (5)-NH_2 PAMAM solutions and constantly stirring for one hour. After preparation homogenized drug loaded dendritic polymer matrix were kept at room temperature for drying [29]. A schematically representation was drawn for CS/PCL-G (5)-NH_2 PAMAM dendrimer matrix for controlling drug release of CYC as in figure 1. The CYC loaded CS/PCL-G (5)-NH_2 PAMAMdendritic matrixwas calculated using a UV-VIS spectrophotometer. The drug loading efficiency and drug loading content of CYC was calculated as

Loading efficiency

$$LE = \frac{W_a}{W_d} X\ 100\%$$

Loading content

$$LC = \frac{W_a}{W_{np}} X\ 100\%$$

Where LC: Loading content; LE: Loading efficiency, W_a: quantity of drug found in the drug-loaded dendrimer, W_d: quantity of drug being added into the system and W_{np}: quantity of drug-loaded dendrimer

Dissolution Experiments

The dissolution observation was done using the dissolution tester equipped with six paddles at a paddle speed of 100 rpm. Phosphate buffer solution (pH 3.4 and 7.4) was used as the dissolution buffer medium similar to stimulate gastrointestinal tract (GIT) medium at room temperature. The fresh new stock solution was prepared for dissolution media, the mass of CYC released was studied apply UV-visible spectrophotometer at the λ_{max} value of 424nm [30].

RESULTS AND DISCUSSION

FTIR

Figure 2 shows the IR absorption spectra of PCL, CS/PCL and G (5)-NH_2 PAMAM/. The PCL peak was located at 2997 (-CH_2 stretching vibration) and 1749 cm^{-1} (-C=O) respectively. Furthermore, the CS/PCL introduced band in between 3200 and 3700 cm^{-1}, which was much high intense than the stretching absorbance at 3000-3600 cm^{-1}. Additionally, the spectrum of CS/PCL has identified absorbance intensity differ peak at 1650cm^{-1} (primary amide, secondary amide) and 1590 cm^{-1} (non-acylated primary amide). The IR spectra of the CS/PCL/G (5)-NH_2 PAMAM/CYC dendrimeric matrix was shown the peak at 3205 cm^{-1} (N-H amide), 2962 and 2531 cm^{-1}(C-H stretching), 1748 cm^{-1} (C=O), 1183 cm^{-1} (C-O bending). Compare to G (5)-NH_2 PAMAM and CYC individually

XRD

Figure 3 shows the XRD pattern of PCL and CS/PCL/G-(60:40)-5% G (5)-NH_2 PAMAM,PCL shows an intense reflection peak at 2θ = 26,28 .The appearance of sharp reflections and diffuse scattering is characteristic of crystalline and amorphous phases of conventional semi-crystalline polymers. In theXRD pattern for CH/PCL/G (5)-NH_2 AMAM/CYC exhibited diffraction peaks at 2θ = 13.5, 19.6, 24, 41, 44. The reported XRD data suggested the crystal nature of the hydrogel.

SEM

SEM has been employed for the observation of the surface morphology of the different CS/PCL and CS/PCL/G (5)-NH_2 PAMAM dendritic polymer matrix. The microstructure of CS/PCL matrix was found to have homogenous plane formation and relatively well dispersed in the CS matrix compared with pure CS in figure 4. In CS/PCL/G (5)-NH_2 PAMAM it is clear that rough like surface increases at 5% G (5)-NH_2 PAMAM. As the concentration of the G (5)-NH_2 PAMAM increases from 1% to 5% surface roughness increases because of the intercalation of the G (5)-NH_2 PAMAM along the polymer matrix, indicating the surface modification.

Tensile strengths

The tensile strengths of the cross-linked CS with PCL polymer matrix films are shown in figure 5. The result indicates the development in mechanical properties with the increase in CS concentration ratio up to 60:40 where the tensile strength at failure was 28 ± 62 Mpa as evidenced from figure 5a. After that, there was a decrease in the mechanical properties with the subsequent increase in the CH concentration. Similarly (CS/PCL/G (5)-NH_2 PAMAM) composites films are shown in figure 5b. The results indicatean improvement in mechanical properties with the increase in G (5)-NH_2 PAMAM concentration up to 5% where the tensile strength at failure was 35 ± 42MPa. This could be explained due to swelling behavior of matrix where it was found that with the increase in G (5)-NH_2 PAMAM concentration,

there was a subsequent increase in intermolecular hydrogen bonding and then decreases subsequently. In figure 5cit is demonstrated that theCS/PCL/G (5)-NH_2 PAMAM has lower percentages of elongation than the corresponding pure CS. This is confirmed that the blended polymer matrix were more brittle and less flexible than the pure components. Similar behaviour was observed in the case of CS/PCL/G (5)-NH_2 PAMAM with different percentages of G (5)-NH_2 PAMAM. A negligible increase in E% wasreported at 1 to 6% of G (5)-NH_2 PAMAM in figure 5d.

TGA

The TGA results of CS, CS/PCL and CS/PCL/ G (5)-NH2 PAMAM with different proportion were summarized in Table 3 and 4. It can be introduced that the blended polymer matrix having CS/PCL/ G (5)-NH_2 PAMAM ratio of 60:40 shows the smallest quantity of water content unlike blended polymer matrix containing other ratios which show higher water content compared to CS matrix. A closer observation of figure disclosed that all blends have only one onset due to the degradation starts through temperature. This result indicated that the two components might be interacted with each other due to the hydrogen bonding formation in between the functional groups of the blended polymer matrix.

Swelling Index

Figure 7 shows the swelling behaviour of CS, CS/PCL, CS/PCL- G (5)-NH_2 PAMAM PAMAMwith different percentages of the drug, the swelling response of the polymer influences its bioadhesive characteristics. The rate of adhesion increases with the degree of hydration until a point where over hydration influences a direct drop in adhesive strength due to disentanglement at the polymer tissue interface. The rate and the extent of polymer matrix hydration and swelling also affect matrix adhesion and consequently the drug release from the polymer matrix. The formulations CS/PCL have reported a slower rate of swelling. The presence of dendrimer, a hydrophilic polymer, increases the extent of swelling; therefore, maximum swelling among the chitosan films is obtained for the formulation CS/PCL/G (5)-NH_2 PAMAM, which contains higher amounts of the dendrimer. The poor solubility of chitosan limits the swelling of the films; hence the swelling index measured is the least for CS/PCL, in which dendrimer is absent, with an SI value of 2.1.

In vitro drug released

To study the outcome of pH on the swelling of composite like CS/PCL/G (5)-NH_2 PAMAM (60:40 /5%), the % cumulative release in both pH 3.4 and 7.4 media was measured. The result of percentages of cumulative release was introduced at pH from 3.4 to 7.4 in figure 8. A significant increase in percentages of cumulative release was recognized for all formulated drug loaded polymer matrix. From Figure 8(a) and 8(b), it can be seen that the 70% drug loaded polymer matrix have determined longer drug release rates than the other polymer matrix. Thus, drug release depends upon the nature of the dendrimeric polymer matrix as well as pH medium. It has been reported that percentage of drug loading was at pH 7.4 = 84% and pH 3.4= 86% Cf. This result was suggested that the CYC drugs in the blend dendritic polymer matrix may be used to be suitable for the basic and acid medium environment of the large intestine, rectal mucosa, and colon.

Release Kinetics Parameters

From the result, the kinetics of drug release was evaluated by plotting percentages of c.f data verses time at selected to an exponential equation-1. The results have evaluated the value of k for all formulations which are presented in Table 5. The value of n and k were dependence on the % drug loading into the polymer matrix. Values of n was calculated by varying the quantity of drug containing 10, 20, 30,40,50,60 and 70 wt% and keeping constant CS/PCL/G/G (5)-NH_2 PAMAM (60:40/5%) matrix, ranged from 0.37-1.72 proposing shifting of drug molecular

transportation from non-fickian to anomalous. The value of n introduced in between 0.37 and 1.72 which was indicated swelling and diffusion controlled release by anomalous transport. The value 1 indicated case-II transport due to polymer matrix relation during the swelling of the polymer matrix. The value near about 0.37 signifies that the drug release from polymer matrix is due to fickian diffusion as evidenced from Table 5. However, the value of n more than 1 may be in the regions of low micro viscosity inside of the polymer matrix of micro cavities during the swollen state of the polymer matrix. Furthermoresimilar result has also been reported due to the effect of different polymer matrix ratios and other factors on dissolution kinetics.

CONCLUSION

A novel pH/temperature sensitive CS/PCL/G (5)-NH_2 PAMAM based dendrimer matrix has been developedand investigated for the extended-release of Cyclophosphamide (CYC) drugs by changing formulation variables. The structure and morphology of dendrimeric matrix were studied by FTIR, XRD, TGA, SEM and tensile strength. The equilibrium swelling indicates the distinct sensitivities of the matrix to pH value and temperature. In pH = 7.4 phosphate buffer solution (PBS), the cumulative release amount of CYC-loaded in the matrix was about 84% within 10h, whereas this value only reached 86% in pH = 3.4 PBS. The performance of chitosan-based dendrimeric polymer matrix is judged by its drug delivery properties. This study reveals that selection of a perfect polymer matrix like CS/PCL/G (5)-NH_2 PAMAM can be used as an efficient drug carrier in different targeted drug delivery systems.

REFERENCES

1. Tomalia, D.A., Baker.H., Dewald, J.R., Hall ,M., Kallos, G., Martin, S., Roeck, J., Ryder, J., and Smith, P., "*Dendrimers II: Architecture, nanostructure and supramolecular chemistry"*, Macromolecules, 19,2466 ,1986.
2. Malik, A., Chaudhury, S., Garg, G., Tomar, A., "*Dendrimers: A tool for Drug Delivery*". Advances in Biological Research, 6(4),165-169, 2012.
3. Liu, M., Kono, K., Frechet, J.M.J., "*Water-soluble dendritic unimolecular micelles: Their potential as drug delivery agents*". J. Controlled Release, 65, 121–131,2000.
4. Liu, M., Kono, K ., and Frechet, J.M.J., "*Water soluble dendrimer poly (ethylene glycol) starlike conjugates as potential drug carrier", Journal of Polymer Sciences*, Part A, Polymer Chemistry, 37(17), 3492-3503,1999
5. Jayakumar, R., Menon, Deepthy., Manzoor. K., Nair.S.V., Tamura, H., "*Biomedical applications of chitin and chitosan based nanomaterials—A short review*", Carbohydrate Polymers, 82(2), 227-232, 2010.
6. Karen, C., Liu, and Yoon, Yeo., "*Zwitterionic Chitosan–Polyamidoamine Dendrimer Complex Nanoparticles as a pH-Sensitive Drug Carrier"*, *Mol. Pharmaceutics*, *10* (5), 1695–1704, 2013.
7. Sashiwa, H., Yoshihiro, Shigemasa., Rene Roy, "*Chemical modification of chitosan 11: chitosan–dendrimer hybrid as a tree like molecule"*,Carbohydrate Polymers , 49(2), 195-205, 2002.
8. Sashiwa, H., Yajima, H., Aiba, S., "*Synthesis of a chitosan-dendrimer hybrid and its biodegradation"*. Biomacromolecules. 4(5),1244-92003.
9. Rita, B., Restani, A., Sofia, Silva., Rita, F., Pires, Renato., Cabral, Ilídio J., Correia,Teresa., "*Aguiar-Ricardo,Nano-in-Micro POxylatedPolyurea Dendrimers and Chitosan Dry Powder Formulations for Pulmonary Delivery*" , 33(11), 851–858,2016.
10. Aryabadie, S., Sadeghi-Kiakhani, M., "*Antimicrobial and Dyeing studies of treated cotton fabrics by prepared Chitosan-PAMAM Dendrimer/Ag Nano-emulsion"*, M. FibersPolym, 16: 2529, 2015.
11. Qingxing, Xu., Chi-Hwa, Wang., and Daniel, W.P., "*Polymeric Carriers for Gene Delivery: Chitosan and Poly(amidoamine) Dendrimers*", Curr Pharm Des. 16(21), 2350–2368,2010.
12. Sarkar, K., Banerjee, SL., Kundu, P.P. , "*Removal of Anionic Dye in Acid Solution by Self Crosslinked Insoluble Dendronized Chitosan"*. Hydrol Current Res 3,133,2012.

13. Jing, J.i., Li, W., Haojie Yu. , Yongsheng, Chen. , Yulai, Zhao. , Hongtao, Zhang. , Wael, A., Amer, Y. Sun., "*Chemical Modifications of Chitosan and Its Applications*", Polymer-Plastics Technology and Engineering , 53(14), 2014.
14. Pereira, V.H., Oliveira,J.M., Salgado, A.J.. "*In vivobiodistribution of carboxymethylchitosan/poly(amidoamine) dendrimer nanoparticles in rats*", 26,6, 2011
15. Wise, D. L., "*Encyclopedic Handbook of Biomaterials and Bioengineering Part A: Materials",* Marcel Dekker: New York, 22, 1995.
16. Ha, J. H., Kim, S., Han, S. Y., Lee, Y.M., Kang, I. K., Cho, C. S., "*Assessment of nutritional status and analytical methods*", J Controlled Release, 49, 2531, 231997.
17. Koenig, J. L., "*Spectroscopy of Polymers*", Elsevier, Amsterdam, 2nd Edition, 1999.
18. Olabarrieta, D., Forsstrom, U., Gedde, M., "*Transport properties of chitosan and whey blended with poly (ε-caprolactone) assessed by standard permeability measurements and microcalorimetry*"Polymer, 42, 4401,2001.
19. Allen, C., Han, J., Maysiger, D., "*Eisenberg A*", J Controlled Release, 63, 275, 2000.
20. Cai, Q., Bei, J., Wang, S. J., "*Bone tissue engineering using polycaprolactone scaffolds fabricated via selective laser sintering*", BiomaterSci, 11, 273,2000.
21. Paul, D. R., Newman, S., "*Polym Blends"*. Academic Press: New York, 2, 35–89,1987.
22. Hubbell, D. S., Cooper, S. L., "*Studies of polyester/chlorinated poly (vinyl chloride) blends*", J ApplPolymSci 1978, 21, 3035.
23. Zhu, K., Xiangzhon, J., Shilin, L., "*Preparation, characterization, and properties of polylactide (PLA)–poly (ethylene glycol)(PEG) copolymers: a potential drug carrier*", J ApplPolymSci 39, 30,1990.
24. Cohn, D., Younes, H., "", J Biomed Mater Res, 22, 993, 1988.
25. Zhang, Y., Zhang, M., "*Research progress in the heat resistance, toughening and filling modification of PLA*", J Biomed Mater Res, 55, 304-432,2001.
26. Khor, E., Lim, L., Y."*Implantable applications of chitin and chitosan*", Biomaterials , 24, 2339,33,2003.
27. Liu , X., Liu ,C., Catapano, C.V., Peng, L., Zhou, J., Rocchi, P., "*Structurally flexible triethanolamine-core poly(amidoamine) dendrimers as effective nanovectors to deliver RNAi-based therapeutics*", *BiotechnolAdv*, 32(4),844–852, 2014.
28. Sarasam, A. , Madihally, S. V., "*Characterization of chitosan–polycaprolactone blends for tissue engineering applications*"Biomaterials , 26, 55,2005.
29. Yabbarov, NG., Posypanova, G.A., Vorontsov, E.A., Popova, O.N., "*Targeted delivery of doxorubicin: drug delivery system based on PAMAM dendrimers*", Biochemistry (Mosc). 78(8), 884-94, 2013
30. Vignaduzzo, S. E. , Castellano P. M. , and Kaufman T. S. , "*Development and Validation of a Dissolution Test for Meloxicam and PridinolMesylate from Combined Tablet Formulation"*,Indian J Pharm Sci. 72(2), 197–203, 2010.

Table.1. Sample preparation of CS/PCL-G (5)-NH_2 PAMAM dendrimeric polymer composite

Sample	CS	PCL	G(5)-NH_2 AMAM
CS/PCL (100/0)	100	0	-
CS/PCL (80/20)	80	20	-
CS/PCL (70/30)	70	30	-
CS/PCL (60/40)	60	40	-
CS/PCL (100/0)	50	50	-
CS/PCL (60/40)	60	40	1%
CS/PCL (60/40)	60	40	3%
CS/PCL (60/40)	60	40	5%
CS/PCL (60/40)	60	40	6%

Table: 2. Sample preparation of CS/PCL-G (5)-NH_2 PAMAM dendrimeric polymer composite with different % of drug loading (n)

Sample	CS	PCL	G(5)-NH_2 AMAM	% of Drug	Loading Content of CYC (%)	Loading Efficiency (%)
CS/PCL (60/40)	60	40	5%	10	6.12± 0.19	61.2± 0.56
CS/PCL (60/40)	60	40	5%	20	17.39± 0.41	86.95± 0.76
CS/PCL (60/40)	60	40	5%	30	27..82± 0.31	92.73± 1.39
CS/PCL (60/40)	60	40	5%	40	35.27± 0.86	88.17± 0.62
CS/PCL (60/40)	60	40	5%	50	45.21± 1.43	90.42± 1.74
CS/PCL (60/40)	60	40	5%	60	56.31± 0.74	93.85± 2.43
CS/PCL (60/40)	60	40	5%	70	66.49± 0.32	94.98± 1.63

Table-3. The weight loss (%) of the pure CS and PVA components and their blends at different temperatures

Temperature	Pure CS	PCL	90/10	80/20	70/30	60/40	50/50
100	5.7	4.2	7.1	7.2	8.56	8.89	8.02
200	7.8	5.6	10.23	14.34	15.21	16.42	16.01
300	31.2	16.23	35.62	37.23	38.39	36.81	36.12
400	51.34	36.67	57.71	61.91	63.14	67.38	66.21
500	57.34	63.89	63.81	65.98	68.37	72.45	71.68

Table - 4. The weight loss (%) of the pure CS/PVA/ G (5)-NH_2 PAMAM components and their blends at different temperatures

Temperature	CS/PCL/G (5)-NH_2 PAMAM 1%	CS/PCL/G (5)-NH_2 PAMAM 3%	CS/PCL/G (5)-NH_2 PAMAM 5%	CS/PCL/G (5)-NH_2 PAMAM 6%
100	8.98	9.12	9.45	9.12
200	16.83	17.23	17.88	17.78
300	37.23	37.86	38.11	38.01
400	68.21	68.97	69.59	62.73
500	72.89	73.28	75.35	75.31

Table. 5. Release kinetics Parameters of different Formulations at pH 7.4 and pH 3.4

Sample Code	K		n		Co-ordination-coefficient , R^2	
	pH 7.4	pH 3.4	pH 7.4	pH 3.4	pH 7.4	pH 3.4
10 wt%	0.17	0.18	1.19	0.37	0.9825	0.9758
20 wt%	0.19	0.21	1.24	1.42	0.9864	0.9736
30 wt%	0.23	0.25	1.29	1.55	0.9882	0.9744
40 wt%	0.27	0.29	1.31	1.66	0.9892	0.9716
50 wt%	0.29	0.31	1.33	1.72	0.9911	0.9762

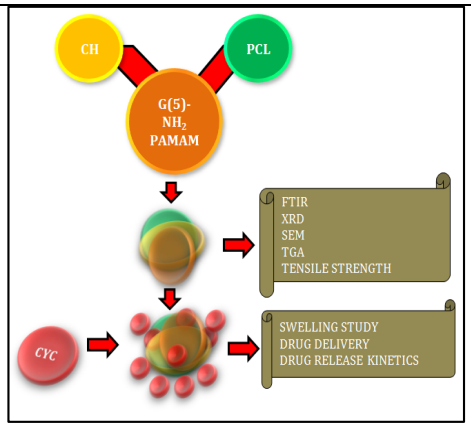


Figure.1.Schematically CS/PCL-G (5)-NH_2 PAMAM dendrimer matrix for controlling drug release of CYC

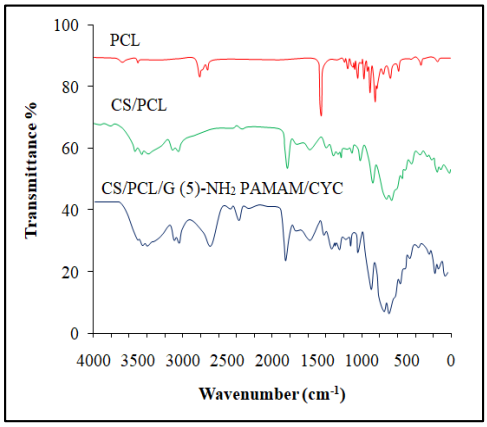


Fig.2. FTIR of PCL, CS/PCL (60:40), CS/PVA (60:40)-5% G (5)-NH_2 PAMAM

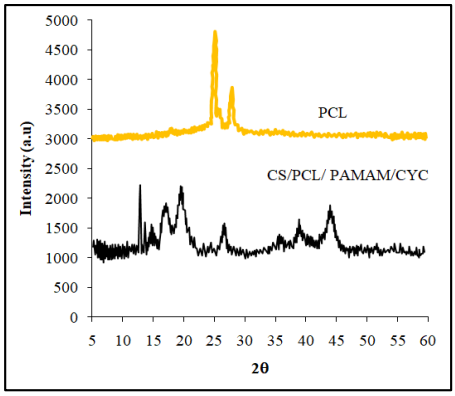


Fig.3. XRD PCL and CS/PCL/G-(60:40)-5% G (5)-NH_2 PAMAM

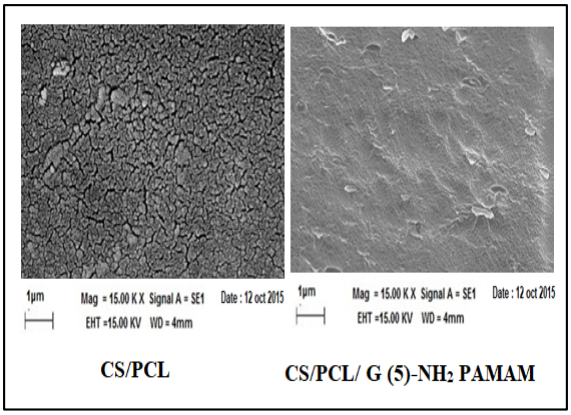


Fig.4. SEM of CS/PCL and CS/PCL/ G (5)-NH_2 PAMAM /CYC

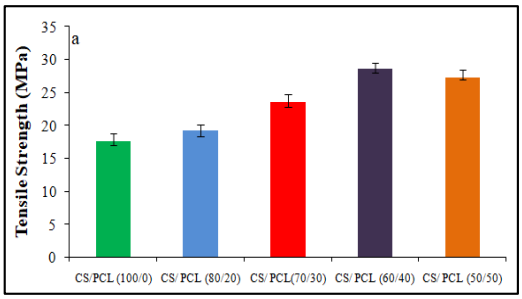


Figure.5a. Tensile strength of CS and CS/PCL

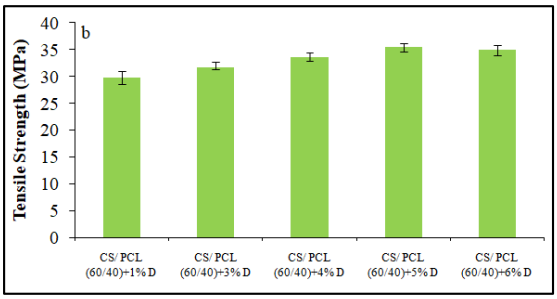


Figure.5b. Tensile strength of CS/PCL/ G (5)-NH_2 PAMAM

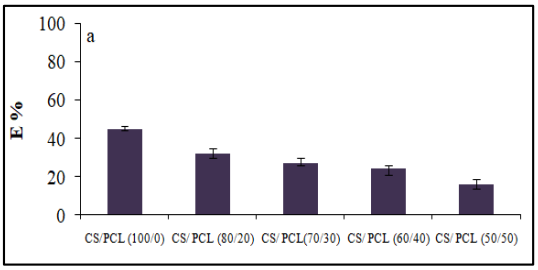


Figure 5c. Elongation % of CS/PCL

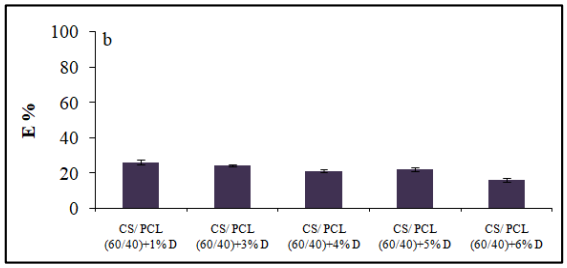


Figure 5d. Elongation % of CS/PCL- G (5)-NH_2 PAMAM (b) blended films

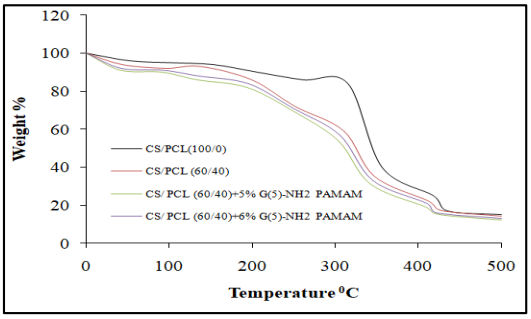


Figure 6.TGA of CS/PCL/ G (5)-NH_2 PAMAM

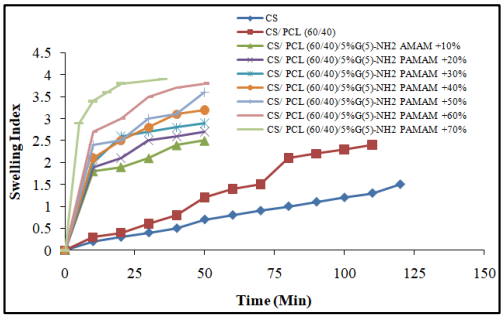
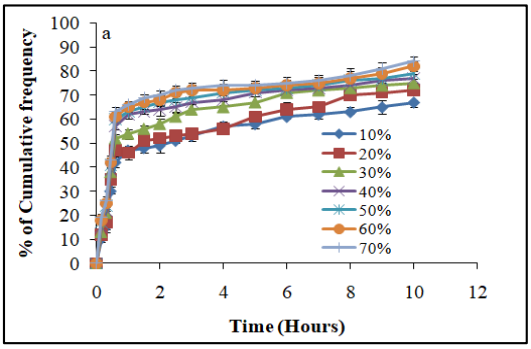


Figure -7. Swelling index of CS, PCL, and CS/PCL/ PAMAM



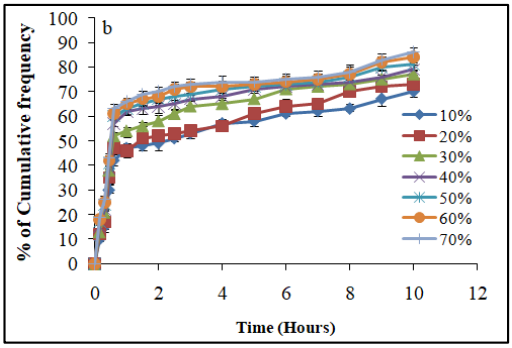


Figure- 8. % of drug released in different pH value a: 7.4 and b: 3.4

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REVIEW ARTICLE

A Review on Diabetic Foods and UTI

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ABSTRACT

Urinary Tract Infections (UTIs) are second most common bacterial infection after the respiratory tract infections. It is well noted that women are majorly affected by the infection because of the factors such as shorter urethra and location of bladder close to both vagina and anus. The incidence of UTIs increase with age as reducing levels of hormone estrogen result in atrophic changes in urogenital tract. The risk of UTIs is higher in diabetes patients which is a key health concern worldwide. In this review article, the author focusses on factors associating UTI with diabetes in women.

Keywords: Diabetes, food safety, infection, microorganism,Prevention, Urinary Tract Infections.

INTRODUCTION

Diabetes is a common metabolic, chronic disorder affecting about 422 million people worldwide, according to WHO. Diabetes is a condition which impairs the body's ability to process glucose in the blood. It results from inefficiency of pancreas to either produce insulin at all or to produce functional insulin. Both these conditions result in increasing blood glucose level or hyperglycemia. According to the Centers for Disease Control and Prevention (CDC), Type 2 Diabetes (non-insulin dependent or adult-onset diabetes) affects 90 to 95 percent of people in the United States. Diabetes is related with various complications which are responsible for significant mortality and morbidity such as, microvascular complications including neuropathy, nephropathy, and retinopathy, while macrovascular complications consist of cardiovascular disease, stroke, and peripheral artery disease (PAD) (Papatheodorou et. al. 2017).

The presence of nervous system damage (neuropathy) and renal system damage (nephropathy) are the major factors associated with increased risk of contracting urinary tract infection (UTI). A Urinary Tract Infection, or UTI, is an infection in any part of the urinary system, which includes your kidneys, bladder, ureters, and urethra. These are the organs involved in production of urine and carrying it out of the body. Increasing blood pressure and hypertension also are associated with an increased risk of progression of diabetic renal disease (Deshpande et. al. 2008). Likewise, neuropathy results in weakened nerves and sensory loss which results in symptoms such as frequent urination, incomplete bladder emptying. These UTI symptoms contribute in harbouring of bacteria and their proliferative growth. The Urinary Tract Infections in association with diabetes is said to be complicated UTI.

The most common causative agent for complicated UTIs is *E. coli* followed in prevalence by*Enterococcus spp., K. pneumoniae, Candida spp., S. aureus, P. mirabilis, P. aeruginosa* and GBS (Flores-Mireles et. al. 2015). Complicated UTI incidence is associated with specific risk factors. For example, there is a 10% daily risk of developing bacteriuria with indwelling bladder catheters, and up to a 25% risk that bacteriuria will progress to a UTI. Bacteriuria (presence of bacteria in urine) occurs in up to 14% of diabetic females but does not tend to occur with a higher frequency in diabetic males (Sabih et. al. 2019). This anomaly is seen to be connected with hormonal changes in post-menopausal women. Oestrogen, female steroid sex hormone is known to have an important role in functioning of lower urinary tract. Hence, oestrogen deficiency occurring following the menopause is known to cause atrophic change and may be associated with lower urinary tract symptoms such as frequency, urgency, nocturia, urgency incontinence and recurrent infection. These may also co-exist with symptoms of urogenital atrophy such as dyspareunia, itching, vaginal burning and dryness (Robinson et. al. 2013). Mostly asymptomatic UTI is observed in diabetic patients leading to severe kidney damage and renal failure (Aswani et. al. 2014). Emphysematous pyelonephritis, emphysematous cystitis, renal and perinephric abscesses, urosepsis, and bacteraemia are the complications of diabetes-associated UTI. Longer hospitalization, recurrence of UTI, relapse and re-infection, bacteraemia, azotaemia, and septic shock are the outcomes of diabetes-associated UTI (Prajapati 2018). Symptoms of UTI vary according to the anatomical position of the infection.

Clinically depending on the location of infection, UTIs has been categorized into three types: Urethritis (the infection present in the urethra, the hollow tube draining urine from bladder to outside of the body), Cystitis (the bacterial infection in the bladder which has often moved up from the urethra) and Pyelonephritis (kidneys get infected as the bacteria inhabits the whole urinary tract). Prostatitis, urethritis and cystitis mostly caused by ascending of faecal coliforms are broadly grouped under Lower UTI while acute pyelitis and acute pyelonephritis fall under Upper UTIs. Lower UTI infections include frequency, urgency, dysuria and suprapubic pain whereas upper tract infections include symptoms like costovertebral angle pain/tenderness fever and chills, with or without lower UTI symptoms.
Diabetes can slow down the body's ability to fight against pathogens by weakening the immune system, which may lead to a greater frequency and severity of certain infections, especially foot infections, yeast infections, surgical site infections and urinary tract infections (Casqueiro et. al. 2012). Poor circulation in diabetics, reduced ability of white blood cells to fight infection, dysfunctional bladders that contract poorly may contribute to the increased prevalence of urinary tract infections among diabetic individuals (Nitzan et. al. 2015).

UTI also contributes in severe diabetes as bacteriuric women were significantly more likely than non-bacteriuric women to have non-insulin-dependent diabetes mellitus, longer duration of diabetes, neuropathy, and heart disease (Zhanel et. al. 1995). Disturbances in humoral innate immunity have been reported in diabetic patients. Most studies show decreased function in diabetic polymorphonuclear cells and monocytes/macrophages (Aswani et. al. 2014). These impairments ultimately help the pathogens to thrive well. And also, some microorganisms become more virulent in a high glucose environment (Aswani et. al. 2014). Increased adherence of microorganisms to diabetic compared to diabetic cells could be another mechanism leading to the increased prevalence of infections in diabetic women (Geerlings et. al. 1999). Susceptibility to UTI increases with longer duration and greater severity of diabetes (Chen et. al. 2009). Patients with type 2 diabetes and UTI might present with hypo- or hyperglycaemia, non-ketotic

hyperosmolar state, or even ketoacidosis, all of which prompt a rapid exclusion of infectious precipitating factors, including UTI(Fünfstück et. al. 2012, Carton et. al. 1992).

The presence of symptoms should immediately lead to diagnostic steps. These include collection of midstream urine which must be examined for leukocytes as pyuria is present in almost all cases of UTI (Nitzan et. al. 2015).Further, culturing of urine sample for which voided, clean-catch, midstream urine should be collected(Bennett et. al. 2015). In patients with long-term indwelling catheters, the preferred method of obtaining a urine specimen for culture is replacing the catheter and collecting a specimen from the freshly placed catheter, due to formation of biofilm on the catheter(Hooton et. al. 2009, Kunin et. al. 1987).Lab analysis is sometimes followed by urine culture to ensure which bacteria are causing the infection and which medication will be effective. Cystoscopy is also done in some cases using a long thin tube with a lens (cystoscope) to see inside urethra and bladder. The cystoscope is inserted in the urethra and passed through the bladder. Other diagnostic tests include pyuria detection: pyuria can be detected either by microscopic examination (defined as >10 leukocytes/mm^3) or by dipstick leukocyte esterase test (sensitivity of 75-96% and specificity of 94-98%); colonization: an absence of pyuria on microscopic assessment can suggest colonization, instead of infection, when there is bacteriuria; microscopic examination: allows for visualizing bacteria in urine; dipstick tests for the presence of urinary nitrite, positive test indicates the presence of bacteria in urine and negative test indicates low count bacteria that lack the ability to reduce nitrate into nitrite.

METHODOLOGY

Several studies were conducted throughout India and the world. In one of them, 100 diabetic patients admitted between November 2012 and 2014 at NRI Medical College and General Hospital, Guntur, India were selected irrespective of their sex, duration of diabetes, treatment, and adherence to treatment. Inclusion criteria were: (a) age >60 years; (b) diagnosis of type 2 diabetes; (c) fasting blood glucose levels ≥ 126 mg/dl and post-prandial blood glucose level ≥ 180 mg/dl; and (d) clinical or microbiological features of urinary tract infections. A detailed history was taken with special reference to age, gender, duration of diabetes, type of anti-diabetic treatment, adherence to treatment, and complications of diabetes. Common symptoms related to urinary tract infection like frequent urination, urinary urgency, burning micturition, dysuria, haematuria, pyuria, suprapubic pain, flank pain, and fever were noted. Blood samples were collected for the estimation of haemoglobin levels, leukocytes level, fasting blood sugar levels, and HbA1c levels. Based on the findings of urine culture analysis, patients were divided into two groups: (a) patients with bacteriuria; and (b) patients without bacteriuria. Subsequently, the clinical and laboratory profiles were compared between these two groups (Sharma et. al. 2017).

Another prospective study was conducted at the Department of Medicine at a tertiary care hospital, Karnataka. A total of 476 patients were screened, of which 305 patients were included in the study carried out from October 2010 to June 2012. The study included 181 diabetics (83 males and 98 females) and 124 non-diabetics (52 males and 72 females) with culture positive UTIs. The following data including age, sex, occupation and symptomatology were taken and clinical examination was done. All proven diabetics with fasting venous glucose > 126mg/dl and postprandial (2h) venous glucose > 200mg/dl were included in the study irrespective of reason for admission. The laboratory tests included complete blood picture, renal and liver function test and urine microscopy including culture. For urine microscopy, 5ml of clean catch midstream urine was centrifuged at 3000 rpm for five minutes and centrifuge was viewed under microscope and more than five WBC per high power field was considered significant. A fasting sugar, postprandial sugar and HbA1c were done for all diabetics (Aswani et. al. 2014).

The study was conducted in the medicine department of a tertiary care hospital. Both inpatients and outpatients were included in the study which was carried out over a period of three months. All patients more than 60 years of age with type 2 DM diagnosed with UTIs (urine showing significant bacteriuria ≥105 CFU/ml of urine) were included in the study. A total of 60 subjects were included in the study. After an informed consent from the patients, they were

subjected to a detailed history, physical examination and relevant clinical investigations. Selection criteria for type 2 DM were: a) Fasting blood sugar more than 126 mg/dl OR b) Post-prandial blood sugar more than 200 mg/dl OR c) Patients on drug treatment for diabetes. Patients having age less than 60 years, hypertension, chronic kidney disease, Type 1 DM or known anatomical or surgical defects in the genitourinary tract were not included in the study. Diagnosis of urinary tract infection was made on the basis of clinical history, symptoms and detailed clinical examination. Biochemical investigations of blood and urine were done to confirm the diagnosis. The study subjects, after giving proper instructions, were asked to submit a midstream urine sample which was transported to the microbiology laboratory of the hospital immediately. After ensuring that the urine sample was uncontaminated (by normal vaginal/urethral flora), it was centrifuged and examined under a microscope(×400 magnification). Presence of pyuria (≥ 10 leukocyte/hpf) with a positive leukocyte esterase and/or nitrite tests was considered as a positive urinalysis. The collected samples were then subjected to gram staining and culture testing to identify the species of the pathogens. Significant bacteriuria (SB) was defined as presence of ≥ 105 colony forming units (CFUs) of isolated organisms per millilitre of urine sample in urine culture. Presence of significant bacteriuria with urinary symptoms was diagnosed as UTI. Presence of SB in two consecutive urine samples collected at a seven-day interval but without classic urinary symptoms was diagnosed as asymptomatic bacteriuria (AB). Positive history of UTI within past six months was diagnosed as recurrent UTI.

Pyelonephritis was diagnosed by presence ofsymptoms like fever, abdominal pain, leucocytosis and associated with SB. The following investigations were carried out: a)Complete Hemogram - Haemoglobin (Hb in gm/dl); total and differential leukocyte count (TLC, DLC in lac/cumm); erythrocyte sedimentation rate (ESR) (by auto-analyser); b) Urine routine including pH, specific gravity, proteins & albumin, sugar. In Microscopic examination pus cells, epithelial cells, red blood cells, bile salts, bile pigments. c) Urine culture & Blood culture; d) Renal function tests including blood urea (mg/dl), serum creatinine (mg/dl); e)Blood sugar level profile including fasting blood sugar (mg/dl), post prandial blood sugar (mg/dl) (by Glucose Oxidase – Peroxidase method); f) Glycosylated haemoglobin (HbA1C) (by Resin method); g) Ultrasound of abdomen and pelvis and X-ray kidney, ureter, bladder (KUB) as and when required. Data was analysed using statistical package SPSS version 16. The mean was the primary statistical measure used. The chi-square statistical test was used and a p-value < 0.05 was considered statistically significant(Vaishnav et. al. 2015).

A prospective cross-sectional study with a total number of 180 patients (90 with DM and 90 without DM) with clinically diagnosed UTI, attending both outpatients and inpatients of Dhulikhel HospitalKathmandu University Hospital (DH-KUH) were studied. Study was conducted during period of February 2013 to July 2013 in the Department of Microbiology and Department of Clinical Biochemistry,Dhulikhel HospitalKathmandu University Hospital(DH-KUH). The diagnosis of diabetes was based on WHO-2003 glucose-based criteria. Clean voided midstream urine samples were collected in sterile containers and samples were processed in the laboratory within 2 hours of collection. Urine cultures were done by inoculating urine samples on Blood agar and MacConkey agar plates using a calibrated loop (0.001ml) and incubated at 37 °C for 18-24 hours. Those culture reports were considered positive who had colony forming units more than 105/mL of voided urine. The presence of yeast in any number was considered to be significant. The pathogens were isolated and specific biochemical tests were done for identifying the species of the pathogens. Antimicrobial sensitivity was done by Kirby-Bauer disc diffusion method according to CLSI guidelines(Acharya et. al. 2015).

RESULTS

In all the above studies, it was revealed that Urinary Tract Infections are more common in females suffering from diabetes. In the first study, bacteriuria was found in 43% of type 2 diabetic patients over the age of 60 years. When a comparison was made between the two groups of with and without bacteriuria, it was found that the female patients with >15 years of diabetes and complications like neuropathy and diabetic foot had bacteriuria commonly. Increased

frequency was observed in 76.7% while urgency was found in 67.4% and dysuria in 65.1% bacteriuric patients. It was seen that *E. coli* was the most common causative organism, which was followed by *Klebsiella*, *Enterococci, Pseudomonas* and *Candida.* Antibiotic sensitivity of most of isolated organisms was seen towards nitrofurantoin, imipenem and amikacin; *Enterococcus* cultures were sensitive to imipenem and nitrofurantoin; while *Klebsiella spp.* was sensitive to piperacillin.

Second study carried out at the Department of Medicine at a tertiary care hospital, Karnataka between diabetic and non-diabetic patients presented results similar to other studies. Fever was reported as the most common symptom but 30% of both the groups could not be diagnosed with urinary tract infections. Another result observed was higher prevalence of pyelonephritis in diabetics as compared to non-diabetic patients. Same as all other studies, *E. coli* was the most common bacteria isolated from the urine culture in this case too. *Enterococcus* showed maximum susceptibility to linidazole, teicoplanin and vancomycin. It was seen that the most common predisposing condition for Urinary tract infections in females was the presence of indwelling catheter. Although, the prevalence of recurrent UTI was seen to be higher in diabetics as compared to those who don't have diabetes.

The third study too showed *E. coli* as the most frequently isolated pathogen from urine culture. It was followed by *Klebsiella spp., Enterococcus spp., Enterobacter spp., Citrobacter spp., Proteus spp.* and *C. albicans*. E. coli was highly sensitive to Gentamycin and nitrofurantoin followed by cotrimoxazole, norfloxacin and ciprofloxacin. Least sensitive rates were found in ampicillin and cephalexin.

DISCUSSION

These studies showed that elderly aged people are more prone to urinary tract infections along with the ones with a history of diabetes. It is also understood that women with diabetes are about two to three times more likely to have urinary tract infections than women without diabetes [Nitzan et. al. 2015]. A significant correlation was found between duration of diabetes and the prevalence of bacteriuria [Bahl et. al. 1970]. The risk of bacteriuria increased 1.9-fold every 10 years of diabetes. This is thought to be because of autonomic neuropathy and subsequent incomplete bladder emptying in longer duration of diabetes [Keane et. al. 1998]. As fever was the most common symptom associated, its presence should indicate a diagnosis for infection in urinary tract. It was concluded that there was an independent higher risk of recurrent UTI in women with diabetes compared with women without diabetes [Gorter et. al. 2010]. It was also understood that fungal urinary tract infections among diabetic population were more common in patients with prolonged hospital stay, catheterisation and prolonged parenteral antibiotic use [Joshi et. al. 1999]. It was also concluded that the practices of self-medications, the drugabuse and indiscriminate misuse of antibiotics among the general population favoured the emergence of resistance strains [Acharya et. al. 2015].

A well understood management of diabetes is required in order to reduce the risk of contracting a urinary tract infection. It depends on a number of factors such as distinction between symptomatic and asymptomatic UTI. As evident from the studies, bacteriuria increases with advancing age. Antibiotic therapy is an important part of the therapeutic strategy for UTI. The increased antibiotic resistance seen in recent years suggests that the choice of antibiotic should be guided by culture and sensitivity assays [Minardi et. al. 2011]. First line treatment is done with Nitrofurantoin: 100 mg three times daily for 5 days or Fosfomycin trometamol 3 g single dose, or trimethoprim-sulfamethoxazole 960 mg twice daily for 3 days and Quinolones, beta lactams are used as second line of antibiotic treatment.

Other than these treatments, prevention of urinary tract infections should be given much importance. UTIs can be prevented by practicing certain habits in daily lives such as: Drinking plenty of water and urinating often as it flushes the bacteria, wiping from front to back since bacteria tend to hang around the anus and this practice reduces the chances of getting an infection, washing up before sex and urinating after, minimizing the use of irritating feminine

products, avoiding the use of diaphragms or spermicides as a birth control agent as they increase the likelihood of major bacterial growth. However, drinking cranberry juice is not recommended to the diabetes patients due to presence of high sugar content in the juice.

CONCLUSION

Diabetes is a chronic disorder which offers many complications to the sufferers. Urinary tract infections are very common among the diabetics and even more prevalent in females due to anatomical differences in urogenital tract as compared to males. It also increases with growing age. Hence, it becomes necessary to manage the diabetes as to reduce the chances of UTI. The key of reducing the risk of an infection lies in the proper treatment of diabetes through disciplined lifestyle and food habits.

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REFERENCES

1. AcharyaD, BogatiB, Shrestha G, GyawaliP. Diabetes mellitus and Urinary Tract Infection: Spectrum of Uropathogens and their Antibiotic Sensitivity. Journal of Manmohan Memorial Institute of Health Sciences. 2015;*1*(4), 24-28.
2. Aswani SM, Chandrashekar U, Shivashankara K, Pruthvi B. Clinical profile of urinary tract infections in diabetics and non-diabetics. Australasian Medical Journal.2014;7(1):29-34
3. Bahl A L, Chugh R N, Sharma K B. Asymptomatic bacteremia in diabetics attending a diabetic clinic. Indian Journal of Medical Sciences. 1970; 24:1-6
4. Bennett JE, Doli R, Blaser MJ. Mandell, Douglas, and Bennetts Principles and Practice of Infectious Diseases. 8th ed. Elsevier Inc.2015
5. Carton JA, Maradona JA, Nuño FJ, Fernandez-Alvarez R, PérezGonzalez F, Asensi V. Diabetes mellitus and bacteraemia: a comparative study between diabetic and non-diabetic patients. European Journal of Medicine.1992;1(5):281–287
6. Casqueiro J, Casqueiro J, Alves C. Infections in patients with diabetes mellitus: A review of pathogenesis. Indian Journal of Endocrinology Metabolism.2012;16 Suppl 1:S27-36
7. Chen SL, Jackson SL, Boyko EJ. Diabetes mellitus and urinary tract infection: epidemiology, pathogenesis and proposed studies in animal models. The Journal of Urology.2009;182:S51-6
8. Deshpande AD, Harris-Hayes M, Schootman M. Epidemiology of Diabetes and Diabetes-Related Complications. Physical Therapy.2008;88(11):1254-1264
9. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nature Reviews Microbiology.2015;13(5):269-284
10. Fünfstück R, Nicolle LE, Hanefeld M, Naber KG. Urinary tract infection in patients with diabetes mellitus. Journal of Clinical Nephrology.2012;77(1):40-48
11. Geerlings SE, Hoepelman AI. Immune dysfunction in patients with diabetes mellitus (DM). FEMS Immunology and Medical Microbiology.1999;26(3-4):259-265

12. Gorter K J, Hak E, Zuithoff N P, Hoepelman A I, Rutten G E. Risk of recurrent acute lower urinary tract infections and prescription pattern of antibiotics in women with and without diabetes in primary care. Family Practice. 2010;27(4):379-385
13. Hooton TM, Bradley SF, Cardenas DD, et al; Infectious Diseases Society of America. Diagnosis, prevention, and treatment of catheter-associated urinary tract infection in adults: 2009 International Clinical Practice Guidelines from the Infectious Diseases Society of America. Clinical Infectious Diseases.2010;50(5):625–663.
14. Joshi N, Caputo G M, Weitekamp M R, Karchmer A W. Infections in patients with diabetes mellitus. The New England Journal of Medicine. 1999;341(25):1906-1912
15. Keane E M, Boyko E J, Reller L B, Hamman R F. Prevalence of asymptomatic bacteriuria in subjects with NIDDM in San Luis Valley of Colorado. Diabetes Care. 1998; 11:708-12
16. Kunin CM, Chin QF, Chambers S. Indwelling urinary catheters in the elderly: relation of “catheter life” to formation of encrustations in patients with and without blocked catheters. American Journal of Medicine.1987;82:405–411.
17. Minardi D, d’Anzeo G, Cantoro D, Conti A, Muzzonigro G. Urinary tract infections in women: etiology and treatment options. International Journal of General Medicine.2011;4:333-343
18. Nitzan O, Elias M, Chazan B, Saliba W. Urinary tract infections in patients with type 2 diabetes mellitus: review of prevalence, diagnosis, and management. Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy.2015;8:129-136
19. Papatheodorou K, Banach M, Bekiari E, Rizzo M, Edmonds M. Complications of Diabetes 2017. Journal of Diabetes Research.2018;2018:3086167
20. Prajapati AK. Urinary tract infection in Diabetics. Microbiology of Urinary Tract Infections -Microbial Agents and Predisposing Factors.2018.DOI:10.5772/intechopen.79575
21. Robinson D, Toozs-Hobson P, Cardozo L. The effect of hormones on the lower urinary tract. Menopause International.2013;19(4):155-162
22. Sabih A, Leslie SW. Complicated Urinary Tract Infections. [Updated 2019 Mar 5]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2019 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK436013/
23. Sharma S, Govind B, Naidu SK, Kinjarapu S, Rasool M. Clinical and laboratory profile of urinary tract infections in type 2 diabetics aged over 60 years. Journal of Clinical and Diagnostic Research.2017;11(4):OC25-OC28
24. Vaishnav B, Bamanikar A, Maske P, Rathore VS, Khemka V, Sharma D. Study of clinico-pathological and bacteriological profile of urinary tract infections in geriatric patients with type 2 diabetes mellitus. International Journal of Current Research and Review. 2015;7(21):13.
25. World Health Organization: Definition, Diagnosis and Classification of diabetes mellitus and its complications; Part 1: Diagnosis and Classification of diabetes mellitus, Geneva. Department of non-communicable Disease Surveillance. WHO;2003
26. Zhanel GG, Nicolle LE, Harding GK. Prevalence of asymptomatic bacteriuria and associated host factors in women with diabetes mellitus. Clinical Infectious Diseases.1995;21(2):316-322
27. https://www.medicalnewstoday.com/articles/318472.php

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REVIEW ARTICLE

Plants for Fertility Regulation: A Review

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ABSTRACT

Menstrual disorders and reproductive health issues related to women is a natural, physiological process and part of the active life of young women around the world. This problem occurs during the reproductive age in majority of women. Medicinal plants are recognized as a prolific source of secondary metabolites which include important function both *in vivo* and *in vitro* during the ovarian folliculogenesis and steroidogenesis in many animal species. Some secondary metabolites can act as antioxidants normally through their ability to scavenge reactive oxygen species (ROS) or can regulate ovarian hormonal production. In broad-spectrum, these properties are responsible for the medicinal functions to treat woman infertility disorder. Some plants contain biologically active substances which comprise to be used in the treatment of reproductive dysfunction. The present review highlights some medicinal plants used in the therapy of woman disorders related to infertility.

Keywords: Infertility, Women-related disorders, menstrual disorders, Medicinal herbs, Fertilization

INTRODUCTION

Infertility can be defined as inability of a couple to achieve a pregnancy after one year of unprotected intercourse. Impaired fertility, variously described as infertility or sub-fertility, might be owing to a relative or absolute inability to conceive. It affects both men and women in around equal proportions, causing extensive personal suffering and interruption of family life. The finest approach for commerce with the problem of infertility is its prevention.

Although various cases of impaired fertility can be corrected by simple procedures, other cases involve complicated diagnostic procedures and treatment. An empathic approach to individuals and couples who have infertility problems is required. This includes an positive reception of cultural and social customs, the individual's awareness of sexuality, an understanding of the reproductive function and an knowledge of the aetiology and occurrence of infertility in the community.

Prevelance of infertility are not incredibly correct and vary from region to region, it is expected that 10-15% of couples experience various form of infertility problem for the period of their reproductive lives. Infertility can be owing to male factors and female factors or a combination of these. Male factor accounts for 40% of the infertility problems. The causes of male infertility can be classified as:

- Abnormal spermatogenesis
- Disorders of secretory function of accessory organs
- Obstruction of the genital tract and
- Abnormal sperm function.

The causes of female infertility are:

- Ovulatory disorders
- Tubal occlusion
- Peritoneal factors

Eg: pelvic inflammatory disease (PID).and endometriosis
Cervical factors and Luteal phase defect in which ovulation occurs but progesterone formation is insufficient for implantation.

Conception is a complicated process that depends upon various factors:

1. On the production of healthy sperm by the man and healthy eggs by the woman.
2. Unblocked fallopian tubes that allow the sperm to reach the egg ; the sperms ability to fertilize the egg.
3. The ability of the fertilized egg (embryo) to become implanted in the uterus.
4. Sufficient embryo quality.

When just one of these factors is impaired, infertility can result.
The public should be ready to aware, from side to side education programmes, of factors which affect fertility. It must be widely revealed that infection caused by STDs is a common cause of infertility. In addition to that the contribution of infection caused by poor obstetric care and unsafe abortion should be stressed.
These could be fulfilled by promoting programmes such as :

- The control of sexually transmitted disease, including the use of STD diagnostic kits, the promotion of safe sex, and the use of condoms ;
- Better obstetric care at the primary health care level, including adequate training of traditional birth attendants;
- The prevention of dangerous abortion via improving right of entry to effective contraception and safe abortion services ;
- Improving accessibility of reproductive health services (including information and education) for adolescents.

Medicinal plants have been playing an essential role in the development of human traditions. As a basis of medicine, medicinal plants have always been at forefront virtually all cultures of civilizations. Many of the modern medicines are produced from Medicinal plants which are regarded as a rich resources of traditional medicines. Medicinal

plants have been used to treat health disorders, to add flavour and conserve food and to prevent diseases epidemics. The biological characteristics of plant species used throughout the world is due to the secondary metabolites produced by the plants. The microbial growth in diverse situations is controlled by plant derived products. Traditional systems of medicine continue to be widely practised on many accounts. The use of plant materials as a source of medicines for a wide variety of human ailments is emphasised due to the rise in population, inadequate supply of drugs, prohibitive cost of treatments, side effects of several synthetic drugs and development of resistance to currently used drugs for infectious diseases. Recently, WHO (World Health Organization) estimated that 80 percent of people worldwide rely on herbal medicines for some aspect of their primary health care needs. According to WHO, around 21,000 plant species have the potential for being used as medicinal plants. Treatment with medicinal plants is considered very safe as there is no or minimal side effects. These remedies are in sync with nature, which is the biggest advantage. The golden fact is that, use of herbal treatments is independent of any age groups and the sexes.

Herbs are believed to be the only solutions to cure a number of health related problems and diseases by ancient scholars. Several studies were conducted thoroughly to study about the same and experimented to arrive at accurate conclusions about the efficacy of different herbs that have medicinal value. They found most of the drugs, thus formulated, are free of side effects or reactions. This is the reason why herbal treatment is growing in popularity across the globe. Many internal diseases, which are otherwise considered difficult to cure can be treated with these herbs that have medicinal quality. Medicinal plants are considered as a rich resources of ingredients which can be used in drug development either pharmacopoeial, non- pharmacopoeial or synthetic drugs. Apart from that, these plants play a critical role in the development of human cultures around the whole world. Moreover, some plants are considered as important source of nutrition. There are natural and herbal treatments that also can help fight the root cause of infertility and increase chances of getting pregnant.

Ashwagandha (*Withania somnifera*)

Ashwagandha (*Withania somnifera*) has been described in traditional Indian Ayurvedic medicine as an aphrodisiac that can be used to treat male sexual dysfunction and infertility. Ashwagandha (*Withania somnifera*), also documented as “Indian ginseng” due to its rejuvenating effects. And it is also described as an aphrodisiac and geriatric tonic in folk medicine. Different investigators comprise report that Ashwagandha is beneficial in the treatment of male infertility. Experimental studies indicates that treatment with Ashwagandha induced testicular development and spermatogenesis in immature Wistar rats by directly affecting the seminiferous tubules improved prosexual behaviour of sexually lethargic mice, and increased testicular daily sperm production and serum testosterone level. There was a significantly improved semen parameters in concert with improved and regulated sexual hormone levels in oligospermic males when treated with a high-concentration, full-spectrum root extract of Ashwagandha. Ashwagandha root extract as evidenced by an increase in sperm concentration, ejaculate volume, and motile sperm count and an increase in the serum levels of testosterone. In women it enhances the endocrine system, thus regulating the adrenal and thyroid glands, which may help balance the reproductive hormones. Ashwagandha is also believed to strengthen the ovaries, uterus, and the immune system.

Dong Quai (*Angelica sinensis*)

Official name: *Angelica sinensis*, Pharmaceutical name: Radix *Angelica sinensis*, Dang Gui is Chinese name. The main constituents of *Angelica sinensis* are Ferulic acid and ligustilide. Angelica sinensis, has been used in endometriosis. The endometriosis is an condition of blood stasis is believed to be a occurrence whereby small vessels are incapable of carrying normal blood flow, therefore causing clotting and bleeding, resulting in lower abdominal pain and infertility. The major mechanism of action of *A. Sinensis* is enhancing the production of IL-2 and inhibiting formation of thromboxane-A2. Animal studies have reported that A. sinensis can increase proliferation of splenic lymphocytes and upregulate interferon (IFN)-γ expression.

False unicorn (*Chamalerium luteum*)

Chamaelirium is a genus of flowering plants containing the single species *Chamaelirium luteum*, commonly known as false unicorn. The tradition use of this herb is to prevent miscarriages and it has the reputation of improving fertility. Nowadays it is used to treat different problems such as menstrual problems, pregnancy complaints, fertility issues, ovarian cysts and diuretic.

Wild yam (*Dioscorea villosa*)

The traditional use of Wild yam is for uterine and ovarian spasm, including dysmenorrhea. When used for infertility it optimizes estrogen levels and improves the quality and quantity of cervical mucus, if the cervical mucus is too viscous or too sparse. The steroidal saponins dioscin and gracilin and quinuclidine are the active constituents of wild yam and alkaloids such as dioscorine and tannins. It is useful as an antispasmodic to sooth oviductal and fallopian tube spasm, which can interfere with conception and implantation. The herb relies on adequate gut flora to enable to conversion of the steroidal saponin aglycone diosin to diosgenin for optimal bioavailability. It is currently speculated that the bowel flora consumes a glycoside molecule from diosin, liberating diosgenin. The mucous membranes of the bowel absorb Diogenin into the bloodstream. Diosgenin may exert its effect by interaction at the hypothalamic estrogen receptor sites, regulating the production of estrogen by cheering an increased production of FSH from the pituitary. Wild yam has demonstrated an estrogenic activity in vitro, and was shown to enhance estradiol by binding to estrogen receptor cites. Ther is no known side effects, interactions, or contraindications for Wild yam.

Vitex (*Chaste tree*)

Premenstrual syndrome (PMS) and its derivative PMDD are medical disorders. PMS possesses a variety of symptoms including anxiety moodiness, melancholia, breasts sensitivity and food desire. The premenstrual syndrome is linked with hormonal and neuronal dysbalance, diet and lifestyle. An important cause considered to be part of the endocrine disorder hyperprolactinaemia. PMDD is one of the major complications of the premenstrual phase.

Vitex agnus-castus

Vitex agnus-castus is widely used to treat PMS and PMDD. In addition, it was shown to be beneficial in post-menstrual cases and it also contributes in anti-infertility treatment. Dopaminergic compounds available in *Vitex agnus-castus* contribute to improve premenstrual mastodynia as well as other symptoms of the premenstrual syndrome. Some of the chemical compounds present in the plant influence the pituitary gland explaining its effects on hormonal levels. A decrease of prolactin will affect the levels of follicle-stimulating hormones (FSH) and estrogen in women and testosterone in men. In conclusion, *Vitex agnus-castus* (AC) is a phytopharmaceutical compound shown to be widely used to treat PMS and PMDD. Furthermore, it was shown to be beneficial in post- menstrual cases. In addition, it contributes to treatment of infertility cases in both men and women. Dopaminergic compounds found in the plant are helpful in improving premenstrual mastodynia as well as other symptoms of the premenstrual syndrome.

Chaste berry has a long history of use for regulating menstrual cycles, which may result from its ability to regulate prolactin levels, enhance corpus luteum development, and correct relative progesterone deficiency. Vitex is beneficial for ovulatory factors associated with infertility, in particular, modulating the anterior pituitary's production of luteinizing hormone (LH), while mildly inhibiting follicle stimulating hormone (FSH). Vitex has been shown to downregulate the production of excess prolactin in hyperprolactinemia via dopaminergic activity and improved symptoms of a variety of menstrual disorders including secondary amenorrhea, cystic hyperplasia of the endometrium, deficient corpus luteum function, metrorrhagia, polymenorrhea, and oligomenorrhea. Chaste berry reduces thyroxin-releasing hormone (TRH)–induced prolactin release (essentially a pituitary– thyroid axis problem), normalizes shortened luteal phases, corrects luteal phase progesterone deficiencies, and reduces PMS symptoms in women with luteal phase defects caused by latent hyperprolactinemia. Chaste berry should be considered a first-line botanical therapy for infertility associated with secondary amenorrhea, hyperprolactinemia, and luteal insufficiency.

Black cohosh (*Cimicifuga spp*)

Black cohosh (*Cimicifuga spp*) demonstrate positive fertility effects in women with PCOS. It binds with α oestrogen receptors in the pituitary and reduces LH secretion. Which results in the increase of luteal progesterone concentration, improves endometrial thickness for infertile women with PCOS. It also lowers LH in women with PCOS. FSH:LH ratio in women with PCOS is improved. The mechanism occurred through competitive inhibition of oestrogen following the selective binding of oestrogen receptors (ERα) on the hypothalamus and pituitary.

Ficus platyphylla

Ficus platyphylla Delile (family- Moracea) commonly called gutta percha tree is a deciduous plant found in savannah areas. It grows widely in the Northern part of Nigeria, up to 60 ft. high and is known as 'gamji' by the Hausas. The stem bark, leaves and seeds of F. platyphylla seems to promote fertility by maintaining uterine integrity and increasing the number of pups in female Rattus norvegicus Wistar strain without any noticeable teratogenic effect. Fertility is promoted with combined use of seeds, bark and leaves of this plant. The reduced post implantation loss and increased litter size promotes fertility with the use of *F. platyphylla*.

Cadaba fruticosa

Cadaba fruticosa, commonly known as Indian Cadaba is used traditionally to treat various female reproductive health problems such as amenorrhea, dysmenorrhea and uterine obstructions without scientific core. Various parts of plant *Cadaba fruticosa* are used for female reproductive disorders.

Ficus asperifolia

Ficus asperifolia, a medicinal plant with ethnopharmacological reputation in the treatment of some cases of sterility/ infertility in women. Real pro implantation, pro-development and uterotrophic-like activities are possessed by *Ficus asperifolia*. The presence of sterols in the aqueous extract of *Ficus asperifolia* are revealed by Preliminary phytochemical which may account for the reported activity. Indeed, many studies have suggested that phytosterols may have effects on the reproductive system and in particular that they possess estrogenic activity.

Pimpinella anisum L

Pimpinella anisum L., a plant belonging to the Umbelliferae family, is one of the oldest medicinal plants. It is beneficial in the treatment of impotence. It can also reduce morphine dependence and has beneficial effects on dysmenorrhea and menopausal hot flashes in women.

Amburana cearensis

The Chemical compound Protocatechuic acid, epicatechin, p-coumaric acid, gallic acid, kaempferolin is present in the *Amburana cearensis*. So it maintained the follicular survival and promoted the development of isolated secondary follicles.

***Eremomastax speciosa* (Acanthaceae)**

The plant is a robust, polymorphous shrub growing to a height of 2 m. The stem is quadrangular, and the leaves are purple on the underside. Several constituents already have been approved, such as flavonoids, alkaloids, triterpenoids, and sterol. E. speciosa is cited for its various beneficial effects, which include stomach complaints, dysentery, hemorrhoids, urinary tract infection, painful menstruation, diarrhea, and male and female infertility and is commonly referred to as "blood plant" since it is also widely used to treat cases of anemia. The aphrodisiac activity of these plants is attributed to the presence of one or more phytoconstituents such as sterols, phenols, alkaloids, amino acid, and saponin responsible for improving sexual function through the regulation of neurotransmitters and relaxation smooth muscle of the corpora cavernosa.

***Coccinia cordifolia* L**

In the West Bengal state of India, *Coccinia cordifolia* L. (Family: Cucurbitaceae), commonly known as Ivy gourd is used to infertility. The aerial parts of *Coccinia cordifolia* L. are used to treat female infertility in West Bengal state of India.

***Justicia insularis* T. Anders**

Justicia insularis T. Anders (Acanthaceae) is a medicinal plant whose leaves are used to improve fertility and to reduce labour pains in women of the Western Region of Cameroon. Follicular cells growth generally culminates up to the ovulation of well differentiated ones which are then transformed into corpus luteum. Thus, the number of corpus luteum in the ovary is an efficient and excellent parameter of ovarian folliculogenesis induction. The significant increase in the number of corpus luteum recorded in scientific research.

Asparagus racemosus

Asparagus racemosus can serve as a powerful male tonic. With a bittersweet taste, this herb renders cooling and purifying effect to the liver and blood, and targets its main site in the small intestine. Its cooling properties balance the heating herbs which are used to improve sperm count, such as, garlic, onion, ashwagandha, etc. This prevents depletion of sperm caused by burning. Owing to its heavy and nourishing properties and it can be used for fatigue, low sexual energy, anger, stress, irritability, inflammation, hyperacidity, urogenital infections, burning sensations etc.

Pomegranate

Pomegranate also boosts fertility in women. It helps increase blood flow to the uterus and thickens the uterine lining to reduce the chance of miscarriage and it promotes healthy development of the fetus.

Cinnamon

Cinnamon can help with proper ovarian functioning and thus be effective in fighting infertility. It even helps in the treatment of PCOS, one of the main causes of infertility. It is also used to treat problems such as endometriosis, uterine fibroids and amenorrhea (absence of menstrual periods) that can affect a woman's fertility and cinnamon may help prevent yeast infections.

Maca Root

Maca root is an effective herb that can treat infertility in both women and men. This herb boosts normal hormone production and is particularly beneficial for women with hypothyroidism as it supports thyroid function. It is also a good source of different nutrients that boost fertility.

***Inula viscosa* (Asteraceae)**

Inula viscosa (Asteraceae) is a herbaceous perennial Mediterranean plant. Its very common use in folk medicine used to increase the sperms count, total serum testosterone level. It is very effective in the treatment of infertility.

Gauriphala

Gauriphala (Eng-Red raspberry, latin Rubus idaeus) - It is considered as a fertility promoting herb. It contains fibres which regulate blood vessels and prevent insulin. This increases ovulation and fertility. This herb tones the reproductive organs. It is best to use this herb in combination with peppermint.

Green tea

Green tea (Latin-Camellia sinensis) - It is another effective herbal remedy to induce ovulation. It is a powerful antioxidant which improves reproductive health. This herb stimulates the sex hormone called binding globulin. This helps to lower estrogen and androgen in body and also induces ovulation.

Evening Promrose Oil

Evening Promrose Oil (latin- Uenothera biennis) - It contains essential fatty acids such as linoleic acid and gamma-linoleic acid which balances female reproductive hormones and also helps to lubricate the mucous membrane. It increases the quality of cervical fluid.which improves the longevity of sperm in the female reproductive tract. Take evening primrose oil from inception of menstruation to ovulation to increase fertility.

Atasi

Atasi (Eng-Flaxseed, Latin- Linum usitatussimum) - It is another powerful herbal remedy for ovulation. It contains lignans which balances hormones within the body and regulates the reproductive cycle. Vrishchikali (Eng-Nettle leaf, Latin- Urtica dioica) - It is one amongst the most effective herbs for ovulation. It is rich in antioxidants and calcium which affects woman's ability to conceive. This herb is also rich in vitamins A, C, D and K, potassium, phosphorous, iron and sulphur. Consuming nettle leaf helps to ensure good reproductive health.

Dalbergia sisoo Roxb

(Family: Fabaceae) part used: Leaf 4-5 crushed leaves were poured for overnight in a glass of water. Next morning the decoction is mixed with sugar and was given to the women in empty stomach for 15 days. It produces ovulation.

Alternative remedies

Nutritional supplements

According to American Dietetic Associations, cell damage can be prevented and repaired with the help of antioxidants. N-acetylcysteine, vit C, vit E and selenium are used as antioxidants which help to improve sperm function. The amino acid called L-carnitine turns fat into suitable energy. L-carnitine is very important agent for sperm metabolism. The university of Maryland medical centre says that sperm cells are one of the locations where the body stores carnitine and that low levels of amino acid may negatively impact sperm count and motility. CoQ10 is a fat soluble antioxidant made by human body. Researchers from Italy concluded that oral CoQ10 supplement increase the amount of the substances in semen and improves sperm cell movement. Folic acid is a micronutrient which helps in DNA and RNA synthesis. Supplementation with zinc is known to improve male infertility as it improves spermatogenesis and also the motility of sperm. Low levels of folate have been linked with birth defects.

Zinc is a cofactor in some enzyme which plays an important role in conversion of testosterone into its more active form, 5α-dihydro testosterone which improves male fertility. Zinc was found to be a key to the final development of egg cells. It is important for male sexual health, since very high levels are found in the seminal fluid. Inadequate zinc may lower male fertility; a recent study found that men who consumed 1.4mg daily produced fewer sperm and had lower levels of testosterone than men whose daily zinc intake was 10.4mg – the zinc Recommended Dietary Allowance (RDA) for adult men is 11mg.

Healthy diet plays an important role in boosting fertility. Fresh and organic juicy fruits such as mangoes, peaches, plums and dried fruits dates, figs, raisins, green leafy vegetables such as asparagus racemosus, legumes, broccoli, garlic, dairy proteins including milk, butter milk, paneer, curd, cheese, nuts like soaked almonds, walnuts sprouts, beans and whole grains, and spices like clove, cumin seed, cinnamon, cardamom are the sources of zinc. Nutritional supplement containing chaste berry, green tea extracts, L-arginine, vitamins (including folate) and minerals that could be an alternative option for the optimization of reproductive health in some women. Good nerve function, healthy hormone levels and an unobstructed blood flow to the pelvic area are essential to sexual performance. To keep these systems in working order, a diet should be based on legumes, grain products and plenty of fruits and vegetables.

Drink Plenty of Water

Every aspect of fertility including aiding in vitamin absorption, cleansing the body from fertility-inhibiting toxins, and warding off dehydration can be achieved by keeping your body hydrated at all times. It's also necessary for optimizing cervical mucus which is what protects and nourishes sperm until it reaches the egg.

Avoid

Avoid drinking coffee, tea, soft drinks, fried and spicy foods. Also stay away from smoking and drinking alcohol.

RESULTS AND DISCUSSION

Infertility is a relatively common problem, which can be estimated at anytime, approximately 10% of those who wish to have children can be considered infertile and about 30 to 40% of women are presented with ovarian dysfunction. The mechanisms of these plants on this problem are not clear. Women-related disorders are very diverse, such as dysmenorrhoea, amenorrhea, an increased amount or timing of menstrual bleeding or a decreased menstrual cycle length, etc. These may occur at any age and for any reason and cause anxiety among women and young girls because they set the alarm for the existence of a problem in the body. There are various reasons which may lead to these kind of problems, such as infection, IUD, stress, ovarian cysts, malnutrition, cancer, hormonal disorders, drug use and pregnancy and a number of other causes. Medicinal plants usually alleviate the estrogens level which seems to control such disorders. Medicinal plants may improve one or some symptoms or increase the possibility of controlling the disorders. Women-related disorders are also related with increase in oxidative stress.

Most of the plants mentioned in this article have antioxidant activities. Hence these plants might have beneficial effects on women-related disorders by decreasing the oxidative stress. There are a lot of plants which have phenolic compound. These plants, especially the ones with flavonoid compounds usually have antioxidant activities. These plants can scavenge free radicals and reduce oxidative stress. Therefore, the plants with these properties may also be effective in menstruation and reduction of menopausal syndrome. These plants have also other advantageous effects which patient may benefit. The modern research reveals that the normal hormonal regulation is disturbed by stress and it decreases the LH secretion, which ultimately leads to anovulation. Patients with weak mental stamina are more susceptible to stress which results in hormonal disturbances that leads to anovulation. Number of medicines mentioned in Ayurvedic classics for the management of infertility is found to be an efficient therapy for modality in anovulation.

CONCLUSION

Humans are a social being, where family and childbearing are considered as a right of every human being. Infertility is a health disorder that requires appropriate treatment strategy. Advanced therapies to assist reproduction are developed by modern medical science. The major causes of infertility are the problems of fallopian tubes, hormonal imbalance, ovarian failure and impotence. Many reasons are sorted out for female infertility, but through proper diagnosis and counseling for treatment of infertility can be the only ray of hope. Review reveals extensively all the major causes, diagnosis and infertility treatments. This review will be helpful to all the scientific, medical researchers who can put efforts to put an end to infertility.

REFERENCES

1. Benjamin.F.Miller M.D., Claire Brackman Keane R.N B.S.M.Ed., Encyclopedia & Dictionary of Medicine, Nursing & Allied Health. 3rd Edition W.B.Saunders company 1983; 971.
2. Douglas.M.Considine Van nostrand's Scientific Encyclopedia. 8th Edition International Thomson Publishing Inc 1994, 1698.

3. Chaudhary.S,Chaudhary.A, Human Anatomy & Physiology, S.Vikas & Co,2010, 259.
4. Vijay R. Ambiye, Deepak Langade, Swati Dongre, Pradnya Aptikar,Madhura Kulkarni, and Atul Dongre. Clinical Evaluation of the Spermatogenic Activity of the Root Extract of Ashwagandha (Withania somnifera) in Oligospermic Males: A Pilot Study. Hindawi Publishing Corporation Evidence-Based Complementary and Alternative Medicine Volume 2013, Article ID 571420, 6 pages.
5. Carla Burks-Wicks, Misha Cohen, Josef Fallbacher, Robert N Taylor & Fritz Wieser. A Western primer of Chinese herbal therapy in endometriosis and infertility. Women's Health (2005) 1(3), 447-463.
6. Wikipedia. https://en.m.wwikipedia.org/wiki/
7. Angela j. hywood, chapter 12 - fertility challenges, botanical medicine for women's health, 2010, pages 334-346.
8. Mahmoud Rafieian-Kopaei, Mino Movahedi, Systematic Review of Premenstrual, Postmenstrual and Infertility Disorders of Vitex Agnus Castus, Electronic Physician (ISSN: 2008-5842), January 2017, Volume: 9, Issue: 1, Pages: 3685-3689.
9. Susan Arentz, Jason Anthony Abbott, Caroline Anne Smith and Alan Bensoussan. Herbal medicine for the management of polycystic ovary syndrome (PCOS) and associated oligo/ amenorrhoea and hyperandrogenism; a review of the laboratory evidence for effects with corroborative clinical findings. BMC Complementary and Alternative Medicine 2014, 14:511 pg 1- 19.
10. Chinenye J Ugwah-Oguejiofor, Shaibu O Bello, Raymond U Okolo, Emmanuel U Etuk, Michael O Ugwah and Vincent U Igbokwe. Ficus platyphylla promotes fertility in female Rattus norvegicus Wistar strain: a preliminary study.
11. Murugesan amudha, Shanmugam rani. Fertility inducing effect of Cadaba fruticosa (l.) druce in female albino rats, International Journal of Biology, Pharmacy and Allied sciences IJBPAS. 2016; 5(4): 850-863.
12. Pierre Watchoa, Esther Ngadjuia , Nkeng-Efouet P. Alangob, Nguelefack T. Benoîta, Albert Kamanyi. Reproductive effects of Ficus asperifolia (Moraceae) in female rats. African Health Sciences. 2009; 9 (1): 249-53.
13. Asie Shojaii and Mehri Abdollahi Fard; Review of Pharmacological Properties and Chemical Constituents of Pimpinella anisum; International Scholarly Research Network ISRN Pharmaceutics Volume 2012, Article ID 510795, 8 pages.
14. World Health Organization. Infertility. 2013.
15. WHO. Sexual and reproductive health. 2013.
16. Taylor A. Extent of the problem. ABC of subfertility. 2003; 327(7412): 434-436.
17. Kakarla N, Bradshaw K. Evaluation and Management of the Infertile. Glowm. 2008.
18. Kamel RM. Management of the infertile couple: an evidence based protocol. Reproductive Biology and Endocrinology. 2010;8:21.
19. Tayebi N, Ardakani SMY. Incidence and prevalence of the sexual dysfunctions in infertile women. European Journal of General Medicine. 2009; 6(2):74-77.
20. Fraser DM, Cooper MA. Myles text book for midwives, 15th ed. Churchill Livingstone Elsevier. 2009.
21. Centers for Disease Control and Prevention. Infertility FAQs. 2013
22. B. Nchegang, C. Mezui, F. Longo, Z. E. Nkwengoua, A. P. Amang, and P. V. Tan. Effects of the Aqueous Extract of Eremomastax speciosa (Acanthaceae) on Sexual Behavior in Normal Male Rats BioMed Research International Volume 2016, Article ID 9706429, 10 pages.
23. Urmilesh Jhaa, Mohammed Asada, Syed Mohammed , Basheeruddin Asdaqa, Amit Kumar Das a, V. Satya Prasad. Fertility inducing effect of aerial parts of Coccinia cordifolia L. in female rats. Journal of Ethnopharmacology; 127 (2010): 561–564.
24. Jansirani Ponnaiah , Karthikeyan S and Johny Kumar Tagore. Medicinal Plants Used for Fertility and Menstrual Disorders by the Women Belonging to the Nilgiris Tribe Community of Southern India. International Journal of Scientific Research and Reviews, 2018; 7(4): 601-608.
25. http://www.livestrong.com/article/439944-dietary supplements-to-increase-fertility.
26. Joe Schwarcz Ph.D., and Fran Berkoff R.D., Reader's DigestFoods That Harm Foods That Heal, An A-Z Guide to safe and healthy eating, The Readers Digest Association Inc, NY218-9, 2007; 324.

27. Manalisha Deka, Jogen Ch. Kalitha,Gajen C.Sarma, Traditional use of fertility inducing plants used by the herbal practitioners in some parts of the state Assam, N E India, a Survey report, Int. J of Science and Advanced Technology. 1(6), Aug 2011.
28. P.C. Trivedi, Herbal Medicine-Traditional Practices, Aavishkar Publishers, Jaipur, 2006; 126,136-8,215-20, 243.
29. http://www.onlymyhealth.com/acupuncture-infertility in women-1298540272.
30. http://www.onlymyhealth.com/ayurvedic- cure- infertility1310122405.
31. Hari Kaur Khalsa R.Y.T.Sexuality, YOGA: an adjunct to infertility treatment, Reproduction and Menopause, vol.1, Issue 1, Oct-2003, 46-51.
32. Tajallaie-Asl F, Mardani M, Shahsavari S, Abbaszadeh S. Menstruation Phytotherapy According To Iran Ethnobotanical Sources. Journal of Pharmaceutical Sciences and Research, 2017; 9(6):986–990. 27. Mehrotra S, Mehrotra BN. Role of traditional and folklore herbals in the development of new drugs. Ethnobotany, 2005; 17: 104–111.
33. Pandikumar P, Chellappandian M, Mutheeswaran S, Ignacimuthu S. Consensus of local knowledge on medicinal plants among traditional healers in Mayiladumparai block of Theni District, Tamil Nadu, India. Journal of Ethno pharmacology, 2011; 134(2): 354–62.
34. Pius OL, Sujanapal P, Udayan PS. Observations on the medicinal plant diversity of Malappuram district of Kerala with special reference to Folk and Indian systems of medicines. International Journal of Advanced Research, 2015; 3(2): 314–325.
35. Lams C, Turner N, Brauer G, Lourenco G, George K. Ethnoveterinary medicines used for horses in Trinidad and in British Columbia, Canada. Journal of Ethnobiology and Ethnomedicine, 2006; 2: 31.
36. John D. One hundred useful drugs of the Kani tribes of Trivandrum forest division, Kerala, India. Journal of Crude Drug Research, 1984; 22: 17–39.
37. Muthu C, Ayyanar M, Raja N, Ignacimuthu S. Medicinal plants used in traditional healers in Kancheepuram District of Tamil Nadu, India. Journal of Ethno biology and Ethno medicine, 2006; 2:43.
38. Nadanakunjidham S. Ethnomedicinal studies of Attapadi hills, Western Ghats: traditional herbal remedies for gynecological diseases. Journal of Swamy Botanical Club, 2003; 20: 33– 38.
39. Van Andel T, de Boer HJ, Barnes J, Vandebroek I. Medicinal plants used for menstrual disorders in Latin America, the Caribbean, sub-Saharan Africa, South and Southeast Asia and their uterine properties: A review. Journal of Ethnopharmacology, 2014; 155: 992–1000.
40. Mukherjee PK, Wahil A. Integrated approaches towards drug development from Ayurveda and other systems of medicine. Journal of Ethnopharmacology, 2006; 103: 25–35.
41. De Boer HJ, Cotingting C. Medicinal plants for women's health care in south east Asia: a meta-analysis of their traditional use, chemical constituents, and pharmacology. Journal of Ethnopharmacology, 2014; 151:747–767.
42. Angela Hywood. Phytotherapy for polycystic ovarian syndrome, Australian Journal of Herbal Medicine, 2012 ,24(3), page no.81.
43. Gaware. V. M., Parjane. S. K. , Merekar Abhijit N., Pattan S. R. , Dighe N. S Kuchekar.B. S., Godge Rahul K. Female infertility and its treatment by alternative medicine: A review,Jourrnal of chemical and pharmaceutical research,2009,1(1),Pg 148-162.
44. Bhaskaruni Subbalakshmi. Managementof Vandhyatwawith Anovulatory Cycles (Artava Dosha) By Ayurvedic Medicines, Ayurpharm International Journal Of Ayurveda And Allied Sciences., Vol.3, No.6 (2014) Pages 177 - 185 ,ISSN: 2278-4772 .