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Editor's Note

We are ecstatic to share the news that Carmelight Journal of Science (Biological, Chemical and Physical Sciences) Volume 17 (October 2022) is now available both digitally and in print. I would like to take this opportunity to express my gratitude to the Editorial Board Members, Reviewers and Authors who have worked so diligently to ensure that this issue will be ready for you on time. We are very happy to see such a strong commitment to making sure we get high-quality publications.

It is very interesting to note that the present issue includes diverse topics ranging from the synthesis of nanoparticles and nano-polymers to the use of zinc oxide and stannic oxide nanoparticles in water purification and oxygen gas sensors, respectively. The emphasis on agricultural sustainability has been highlighted in research work on the application of vermicompost in enhancing bioconversion of lignocellulosic wastes, in chicken manure biochar for the alleviation of cadmium stress in pearl millet and in moringa powder as a bio-coagulant for purifying water bodies. Studies on food-based interventions for combating anaemia in children and the attitudes and practises of college students on anaemia, highlights the importance of bringing nutritional studies to the forefront. With the associated side-effects of indiscriminate use of allopathy, it becomes imperative to dig deeper into alternatives for treating paraquat-induced neurotoxicity and formulating herbal medicines for their ability to sanitize.

This issue also carries three review papers on diverse subjects that bring to light the critical social issue of female genital mutilation and its effect on the mental and gynaecological health of women. Linking cause to effect, the role of mitochondria in pancreatic cancer has been emphasized. Amid the COVID-19 pandemic only strengthens our resolve to understand reverse vaccination and its impact on the development of novel vaccines against SARS-CoV.

It clearly states that there is scope for all those who are working in different areas to share their knowledge through our publications. Now, Carmelight is an open-access journal that provides readership to one and all.

With sincere appreciation and best wishes,

Dr Kavitha G Singh

Patron

Dr. Sr. Arpana
Principal
Mount Carmel College
Autonomous

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INDEX

Sl. No.	Title with Author Name	Page No.
1	**Microbial Synthesis of Silver Nanoparticles under Different Physical conditions and their Characterization** *V S Viswamalya, Deeksha Chugh, Bannhi Das*	9
2	**Evaluation of Antibacterial Activity of Herbal Products for Potential Applications as Sanitizing Agents** *Supriya Yalpu, Rakshitha Nagaraja, Rajeswara Babu Mythri*	33
3	**Effect of vermicompost conditioning in enhancing lignocellulosic waste bioconversion to lactic acid by selected microorganisms.** *Sanhita Chatterjee, Meenambigai J, Gayathri L N, Preetha Nair*	39
4	**Study of antibacterial activity of camellia sinensis and its phytochemical analysis** *Parvathy M, Shalini S*	55
5	**Alleviation of Cadmium Stress in *Pennisetum glaucum* (Pearl millet) by Supplementation with Chicken Manure Biochar** *Myrene R. D, Harshitha P, Divya F*	67
6	**Synthesis and Characterization of Chitosan Linked Plant Nanopolymer using *Allium Fistulosum* Extracts** *Shlini P, Teena Magline Immaculate V, Mary Clare H*	85

7	**Isolation of pectin from *Artemisia vulgaris* and *in vitro* studies of residue after extraction of pectin** *Adikeshavan Thilagavathy, Riya Pradhan, Tripraggya Rai*	97
8	**A Comparative Study on Efficiency of *Moringa Oleifera* Leaf and Seed Powder and their Combinatorial Effect as Biocoagulants for Treating Water Bodies** *Vyshali P, Suwathi S, Kavya C S*	109
9	**Nutritional Properties and Antioxidant Activity of Roselle (*Hibiscus Sabdariffa*)** *Shireen Feroz, Mitravinda Aneesh*	125
10	**Knowledge, Attitude and Practice of College Going Students on Iron Deficiency Anemia** *V. Padma, Mahima Lalwani*	137
11	**Perceptions Among Doctors and Nutritionists Regarding Food-Based Interventions for Combating Anaemia in The First 1000 Days Of Life** *Sangeeta Pandey, Vaishnavi Arun*	151
12	**Effect different drying techniques on the physical and morphological characteristics of multi cereal and legume mix** *Vasantha Kumari P, Narayanasamy Sangeetha*	167
13	**Marital Adjustment in Working Couples** *Sujata Gupta Kedar, Asmita Naidu*	177

14	**Synthesis of Zinc oxide nanoparticle and its photo catalytic activity for the application of water purification** *Trisha Mitra, Chaitra V, Pradeep N*	185
15	**Stannic oxide thin film prepared by spin coating technique and its uses in oxygen gas sensor** *Kauser Zehra, Pradeep N, Chaitra V*	199
16	**Neuroprotective effect of aqueous extract of Acorus *calamus* rhizome against paraquat-induced neurotoxicity in *Drosophila melanogaster*.** *Athira Venugopal, Tasha Suzana Brooks, M Sangeetha, Sowmya Kumar*	215
17	**Mitochondria: The Cause of Pancreatic Cancer** *Oishee Sinha*	233
18	**Female Genital Mutilation: How Are Women at The Receiving End of Mental and Gynaecologic Ill-Health?** *Bhoomika Sharma*	243
19	**Reverse Vaccinology: A Promising Method to Develop Covid-19 Vaccine** *Anushka R*	253

Microbial Synthesis of Silver Nanoparticles under Different Physical conditions and their Characterization

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Abstract : *Nanoparticles (NP) are minute particles having a diameter ranging between 1nm to 100nm.Several different methods can be used for the production of silver nanoparticles (AgNPs) including chemical, physical, and biological methods.*

This study is targeted towards the synthesis of AgNPs from bacterial cultures of Pseudomonas aeruginosa and Staphylococcus aureus under different physical parameters ($AgNO_3$ concentration, pH, and incubation time) and characterization of the synthesized particles by UV-Visible Spectrophotometric analysis.

The AgNPs that were synthesized under the different physical parameters were ofa similar size range ie., 20-40 nm. In the case of Pseudomonas aeruginosa, an increase in $AgNO_3$ concentration, pH, and incubation time favored AgNP synthesis but that wasn't the case for Staphylococcus aureus.

Keywords: *Silver Nanoparticles*, *Green Synthesis*, *Silver Nitrate*, *UV- Visible Spectroscopy, Pseudomonas sp., Staphylococcus sp.*

INTRODUCTION

Nanotechnology is a very interesting field of research that investigates materials on a nanoscale level, i.e. materials having dimensions of fewer than 100 nm in diameter. Nanoscience and nanotechnology have attracted global attention in recent years due to

its potential impact on a wide variety of scientific fields, including energy, medicine, diagnosis, pharmaceuticals, space industry, food industries, etc. [1, 2, 3, 4]. However, their importance in the medical field is increasing sharply with time, in fact, their capability to kill microbes has made them a hopeful target for researchers to treat antibiotic-resistant microbes. Antibiotic resistance in pathogenic bacteria is a critical concern in biomedical and pharmaceutical research [5,6,7]. Metallic NPs are the most promising because of their enormous surface area to volume ratio, which gives them good antibacterial characteristics [8,9,10,11].

Silver has long been recognized as a threat to a variety of microorganisms, with almost 116 silver-sensitive microbes being identified till now [12]. Though the Silver ions have awell-known bactericidal action the exact mode of their action is still partially understood. It's been hypothesized that ionic silver interacts significantly with the thiol groups of essential enzymes, rendering them inactive [13,14,15]. Other research has shown indications of structural alterations in cellular membranes [16].

Initially, silver nanoparticles (AgNP) were synthesized by either physical or chemical methods. Some of the widely used methods include chemical reduction, thermal synthesis; electrochemical reduction, vaporization, and photochemical reduction [17,18,19]. However, even though these procedures produce pure and defined NPs, they have significant drawbacks, such as being costly and having environmental hazards. So, there is an increasing demand for efficient, safe, cheap and, eco-friendly methods for the production of NPs. As a result, researchers have directed their attention towards the biological synthesis of AgNPs. These methods are expected to offer an alternate approach for resolving the problems associated with physical or chemical synthesis[20,21,22,23,24]. To date, several reports are depicting the synthesis of AgNPs by bacteria, plants, fungi, algae, and yeasts [2,25,26,27]. Microorganisms may produce inorganic NP via either extracellular or intracellular processes [28,29,30,31,32]. However, if the synthesis of nanoparticles is intracellular it requires additional steps to be recovered from the cells for commercial purposes. Some of these methods may involve the use of appropriate

detergents or ultrasonic treatment. However, extracellular synthesis does not involve any cell lysis method and hence is more cost-effective than intracellular biosynthesis [33,34].

Based on the information available regarding nanoparticle synthesis by microbes, wehave directed our attention towards the synthesis of AgNPs by soil isolated strains of *Pseudomonas* sp. and *Staphylococcus* sp. Though there are reports of *Pseudomonas* sp. being involved in the synthesis of AgNPs [35,36,37] but there are few reports related to *Staphylococcus* sp. mediated AgNP synthesis [38]. Hence, the present study attempts to check the capability of the isolated strains to synthesize AgNPs under different physical parameters. Physical parameters play a crucial role in the size and properties of nanoparticles, we have tried to focus on the effect of different concentrations of $AgNO_3$, different pH, and duration of incubation period on the synthesis of AgNPs. Though synthesis is a crucial aspect of the study, determining the effectiveness of the produced nanoparticles based on their antibacterial activity is equally important.

MATERIALS AND METHODS

Sub culturing and Maintenance of Bacterial Culture:

Pseudomonas sp. and *Staphylococcus* sp. cultures were obtained from the Department of Biotechnology repository. The cultures were sub-cultured in Nutrient broth and then on LBAgar media. They were incubated at 37 °C for 24 hrs. These cultures were further subjected to biochemical tests and staining to reconfirm their genus.

Biochemical Characterization of Bacterial Culture:

For confirmation of *Pseudomonas* sp. Kings Agar media was used as a selective and differential media. The combination of *Pseudomonas* agar F (King's media B) and *Pseudomonas* agar P allows for the traditional phenotypic test to distinguish *Pseudomonas aeruginosa* from other *Pseudomonas* species [39]. The *Pseudomonas* culture was streaked on this media and incubated for 24 hrs to observe if there is a yellow to greenish-yellow region around the colonies which is due to the synthesis

of fluorescein, which is a signature of *Pseudomonas aeruginosa*. MSA is a selective and differential medium for *Staphylococcus* sp. The high salt concentration in the medium allows the selective growth of *Staphylococcus* sp. only. The *Staphylococcus* culturewas streaked on Mannitol Salt Agar (MSA) media.

Simmons Citrate Agar was used to distinguish between enteric species bacteria based on their ability to ferment citrate as a primary carbon source [40]. The change in color of bromophenol blue from green to blue indicates the positive result for citrate utilization test. Simmons citrate agar slants were made and both the cultures were streaked using aseptic techniques over these slants. The change in the colour of the medium was observed after 24 hrs of incubation at 37 °C [41,42].

Acclimatization and synthesis of Silver Nanoparticles:

Before using the organisms for AgNP synthesis they were grown in increasing concentrations of $AgNO_3$ (0.005 mM, 0.01 mM, 0.05 mM, 0.1 mM, 0.5 mM, 1 mM) containing medium. *Pseudomonas aeruginosa* and *Staphylococcus aureus* were inoculated in 10 ml of Nutrient broth containing 0.01 mM, 0.05 mM, 0.1 mM $AgNO_3$ and grown for 24 hrs. From the 0.1 mM culture, further inoculation was done in a broth containing a higher concentration of $AgNO_3$ (0.5, and 1 mM). Nutrient agar plates with the same concentrations of $AgNO_3$ were also maintained and stored as master plates.

Synthesis of NP was carried out with cell-free extract of *Pseudomonas* sp. and *Staphylococcus* sp. Briefly, the bacterial cultures were grown in 100 ml nutrient broth and incubated overnight in a shaker incubator at 37 ^{0}C and 150 rpm. The cultures were then transferred to centrifuge tubes and centrifuged at 5000 rpm for 10 minutes. The cell-free extract (CFE) was transferred to a fresh 250 ml Erlenmeyer flask and $AgNO_3$ solution was added from a stock of 100 mM solutionto make up the final concentration to 1 mM. This was then incubated in a shaker incubator

for72 hours at 37 ^{0}C and 150 rpm [43]. The synthesized NP were characterized by UV-Vis Spectrophotometric study.

Effect of two physical parameters on the synthesis of silver nanoparticles

In this study, the physical parameters considered were concentration of $AgNO_3$ and effect of pH.

Effect of $AgNO_3$ concentration

To study the effect of $AgNO_3$ concentration on the silver nanoparticle synthesis, different concentrations of $AgNO_3$ (0.1 mM, 0.5 mM, 1.0 mM, and 2.0 mM) were added to cell-free extract of 24 hrs old cultures of *Pseudomonas* sp. and *Staphylococcus* sp. These tubes were thenincubated at 37 ^{0}C for 72 hrs under dark conditions.

Effect of pH

To evaluate the effect of pH on the synthesis of AgNPs, the optimum concentration of $AgNO_3$ obtained from the previous set of experiments (1 mM) was added to the cell-free extract of both the cultures at different pH conditions (6,7 and 9) and incubated at 37 ^{0}C for 72 hrs under dark conditions. After synthesis, the AgNPs were analyzed by UV-Vis Spectrophotometer.

Effect of time

To evaluate the effect of time on the synthesis of AgNPs, 1 mM $AgNO_3$ was added to the cell- free extract of both the cultures and were incubated for different time periods (3 days, 4 days and 5 days). The tubes were kept at 37 ^{0}C under dark conditions for the synthesis. After the incubation periods change in colour of the tubes were observed and UV-Vis spectrophotometric analysis was carried out.

Characterization of silver nanoparticles using UV-Visible Spectrophotometer

Color shift showed the reduction of Ag^+ ions to AgNPs, which was later confirmed by UV- Visible spectroscopy (Systronics UV-Visible Spectrophotometer 117). The nanoparticle suspension was taken in a quartz cuvette. The blank used for this experiment was fresh uninoculated broth. The absorbance of the suspension was checked under a wavelength range between 350 nm – 450 nm.

The effect of each operational parameter was investigated while keeping the other two parameters constant.

RESULTS AND DISCUSSION

The *Pseudomonas* sp. and *Staphylococcus* sp. that were streaked onto LB agar plates, from their glycerol stocks, showed growth within 24 hours of incubation. The *Pseudomonas* sp. colonies were large, smooth, opaque, and light tan in color on clear amber-colored LB agar. The *Staphylococcus* sp. colonies were white-golden, opaque, and smooth on clear amber-colored LB agar.

The gram staining of the pure culture of *Staphylococcus* sp. *showed* gram-positive coccus and *Pseudomonas* sp. *exhibited* gram-negative bacilli and under 100X magnification. (Figure 1A & B).

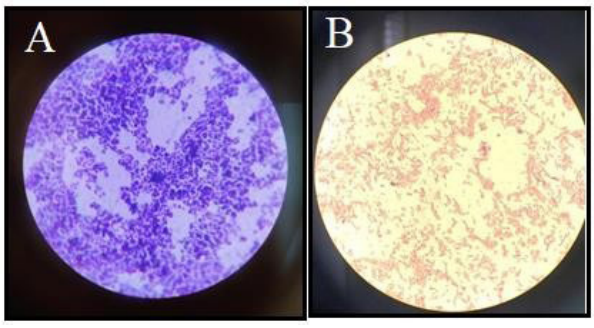

Fig. 1: Gram staining of bacterial cultures. A) *Staphylococcus* **sp. B)** *Pseudomonas* **sp.**

King's Agar for *Pseudomonas aeruginosa* confirmation

The *Pseudomonas* culture on King's Agar media showed yellow-coloured colonies. This is a biochemical confirmation of the strain to be *Pseudomonas aeruginosa.*

MSA Agar for *Staphylococcus aureus* confirmation:

The *Staphylococcus* culture on MSA media produced yellow-coloured colonies, confirming theculture to be *Staphylococcus aureus.*

Citrate utilization test:

The Simmon citrate test for both the organisms confirmed that they were capable of utilizing citrate as a carbon source. Both *Pseudomonas aeruginosa* and *Staphylococcus aureus* exhibited citrate positive character. The change in colour of the medium from green to deep Prussian blue is a confirmation of the citrate utilization by the organisms. Citrate catabolism produces alkaline carbonates and bicarbonates, which increase the pH of the medium above 7.6, causing the bromothymol blue to change from the original green colour to blue.

Acclimatization of organisms:

Initially, growth was observed for both *Pseudomonas aeruginosa* and *Staphylococcus aureus* in nutrient broth containing 0.01 mM, 0.05 mM, 0.1 mM $AgNO_3$. Upon subculturing from the 0.1 mM $AgNO_3$ to a higher concentration of $AgNO_3$ (0.5 and 1 mM), growth was observed at these concentrations also (Figure 2A & B). These acclimatized cultures were further used for the synthesis of AgNP.

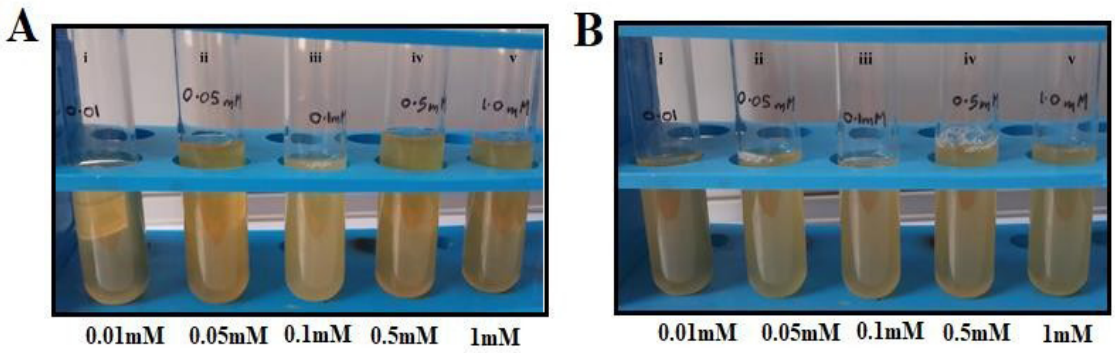

Fig. 2. Acclimatization of microbial cultures in increasing $AgNO_3$concentration.
A. Acclimatization of *Pseudomonas aeruginosa* in increasing concentration of silver nitrate 0.01 mM, 0.05 mM, 0.1 mM, 0.5 mM and 1.0 mM.
B. Acclimatization of *Staphylococcus aureus* in increasing concentration of silver nitrate 0.01 mM, 0.05 mM, 0.1 mM,0.5 mM and 1.0 mM.

Synthesis of Silver Nanoparticles:

Synthesis of AgNPs was carried out using cell-free extract of 24 hours old culture. After the incubation of the cell-free extract with the AgNO3 solution for 72 hours, there was a significant change in colour of the media for both the microorganisms (Figure 3). The control in the figure shows the 24 hrs old culture before removing the cells from the media. The appearance of the brown color after indicates the biotransformation of silver ions to silver atom indicating synthesis of AgNP. The change to brown colour is due to the excitation of surface plasmon vibrations (SPV) in the particles. This is a preliminary indication that the bacterial strains are able to synthesize AgNPs under the experimental conditions provided. By the acclimatization process, the organisms were adapted to an environment containing silver nitrate prior to the synthesis process.

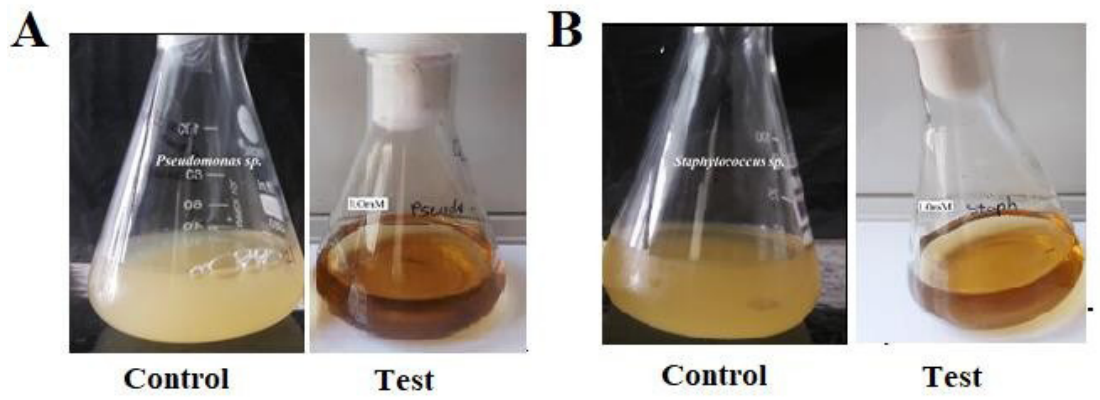

Fig. 3. Synthesis of Silver Nanoparticles.
A) Synthesis with *Pseudomonas aeruginosa.*
B) Synthesis with Staphylococcus aureus

EFFECT OF THREE OPERATIONAL PARAMETERS ON THE SYNTHESIS OF SILVER NANOPARTICLES

Effect of different $AgNO_3$ concentrations:

With the increase in concentration of $AgNO_3$ (0.1 mM, 0.5 mM, 1.0 mM, and 2.0 mM) after 72 hrs of incubation there was a clear increase in the intensity of the brown colour in the tubes containing cell-free extract of *Pseudomonas aeruginosa* (Figure 4A). However, *Staphylococcus aureus* under the same synthesis condition exhibits a different pattern (Figure 4B). The pictures were taken on Day 0 (immediatelyafter the mixing of CFE and $AgNO_3$ solution) and Day 3 after 72 hrs of incubation to depict the visual change in the colour. This observation was further assessed by UV- Visible spectrophotometric analysis.

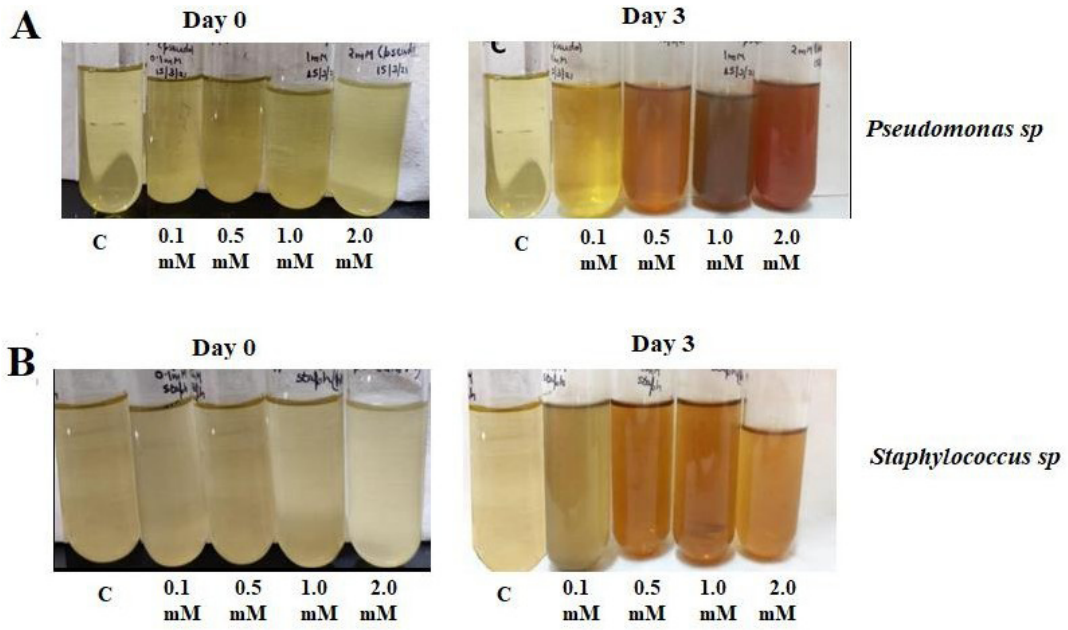

Fig. 4. Effect of different $AgNO_3$ concentrations on NP synthesis.

A) *Pseudomonas aeruginosa* cell-free extract incubated with different concentrations of AgNO3 at Day 0 and Day 3.

B) *Staphylococcus aureus* cell-free extract incubated with different concentrations of AgNO3 at Day 0 and Day 3

The appearance of brown colour is an indication of the formation of AgNP. The colour change was due to the surface plasmon resonance and reduction of silver ions. The wavelength, at which the solution exhibits absorbance, helps in determining the size of the NP too. Hence, the formation of AgNP was confirmed by using a UV-Visible Spectrophotometer. The UV- Visible spectrophotometric analysis of the NP synthesized by *Pseudomonas aeruginosa showed* peaks around 410 nm, 410 nm, 420 nm, and 410 nm for 0.1 mM, 0.5 mM, 1 mM, and 2 mM of $AgNO_3$ concentration respectively (Table – 1; Figure 5). This indicates that the particles synthesized are in the range of 10-30 nm. The data shown is an average of three independent experiments.

Table 1: Absorbance of NP synthesized at different concentrations of $AgNO_3$ (0.1 mM, 0.5 mM, 1 mM and 2 mM) with *Pseudomonas aeruginosa* after 72 hours incubation.

Wavelength (in nm)	0.1 mM	0.5 mM	1 mM	2 mM
360	0.481	1.290	1.838	1.927
370	0.532	1.339	1.895	2.050
380	0.587	1.393	1.965	2.150
390	0.640	1.475	2.097	2.330
400	0.683	1.570	2.187	2.504
410	0.720	1.654	2.310	2.663
420	0.640	1.586	2.223	2.595
430	0.555	1.450	2.077	2.462
440	0.483	1.319	1.936	2.336
450	0.393	1.186	1.790	2.143
460	0.283	1.028	1.612	1.890
470	0.213	0.872	1.420	1.630
480	0.172	0.724	1.218	1.373

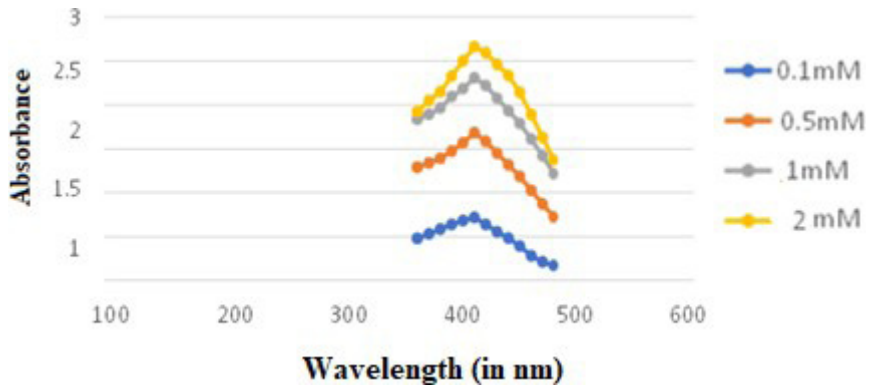

Fig. 5: Absorbance of AgNPs synthesized at different concentrations of $AgNO_3$ by *Pseudomonas aeruginosa*

Whereas for *Staphylococcus aureus* the AgNP synthesized at different $AgNO_3$ concentration (0.1 mM, 0.5 mM, 1 mM and 2 mM) showed peaks at 410 nm, 420 nm, 420 nm and 420 nm respectively (Table 2; Figure6). Hence, the size of the nanoparticle in this case also ranges between 20- 40 nm. The data shown is an average of three independent experiments.

Table 2: Absorbance of NP synthesized at different concentrations of $AgNO_3$ (0.1 mM, 0.5 mM, 1 mMand 2 mM) with *Staphylococcus aureus* after 72 hours incubation.

Wavelength (in nm)	0.1 mM	0.5 mM	1 mM	2 mM
360	1.280	0.599	1.140	0.830
370	1.327	0.657	1.198	0.914
380	1.390	0.709	1.275	0.994
390	1.437	0.768	1.341	1.086
400	1.492	0.830	1.407	1.179
410	1.577	0.904	1.459	1.294
420	1.532	0.975	1.550	1.394
430	1.474	0.921	1.440	1.304
440	1.378	0.875	1.381	1.201
450	1.321	0.832	1.280	1.129
460	1.278	0.810	1.200	1.057
470	1.214	0.791	1.140	0.978
480	1.175	0.769	1.084	0.901

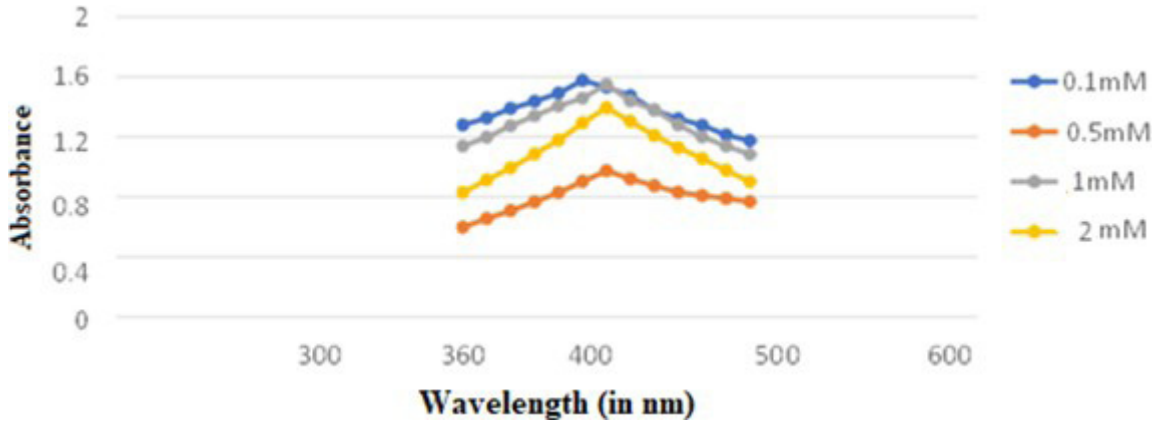

Fig. 6: Absorbance of AgNPs synthesized at different concentrations of $AgNO_3$ by *Staphylococcus aureus*

The absorbance value of AgNP synthesis with 2 mM $AgNO_3$ was found to be higher at 410 nm, in the case of *Pseudomonas aeruginosa.* Thus, an increase in $AgNO_3$ concentration from 1 mM to 2 mM, (Table 1 & Figure 5) led to the increase in the amount of silver nanoparticle synthesis. When a higher concentration solution is used, the plasmon resonance absorbance increases, meaning that there is more silver ion available for reduction. However, for *Staphylococcus aureus i*ncrease in the concentration of $AgNO_3$ is not leading to an increased synthesis of NP as the absorbance for 0.1 mM is highest (Table 2 & Figure 6). This indicates that increasing the concentration of $AgNO_3$ is causing aggregation of AgNP synthesized by *Staphylococcus aureus.*

Effect of different pH

The effect of pH on the synthesis of AgNP was investigated for different pH conditions 6, 7, and 9. There was a visual change in the colour of the media for all the pH conditions. For *P. aeruginosa* there was an increase in the intensity of the brown colour with an increase in pH (Figure 7A). However, for *S. aureus* there was no noticeable increase in intensity of the brown colour. For all the three pH conditions the color appeared to be almost the same (Figure 7B). The colour change was further analyzed by UV- Spectrophotometer.

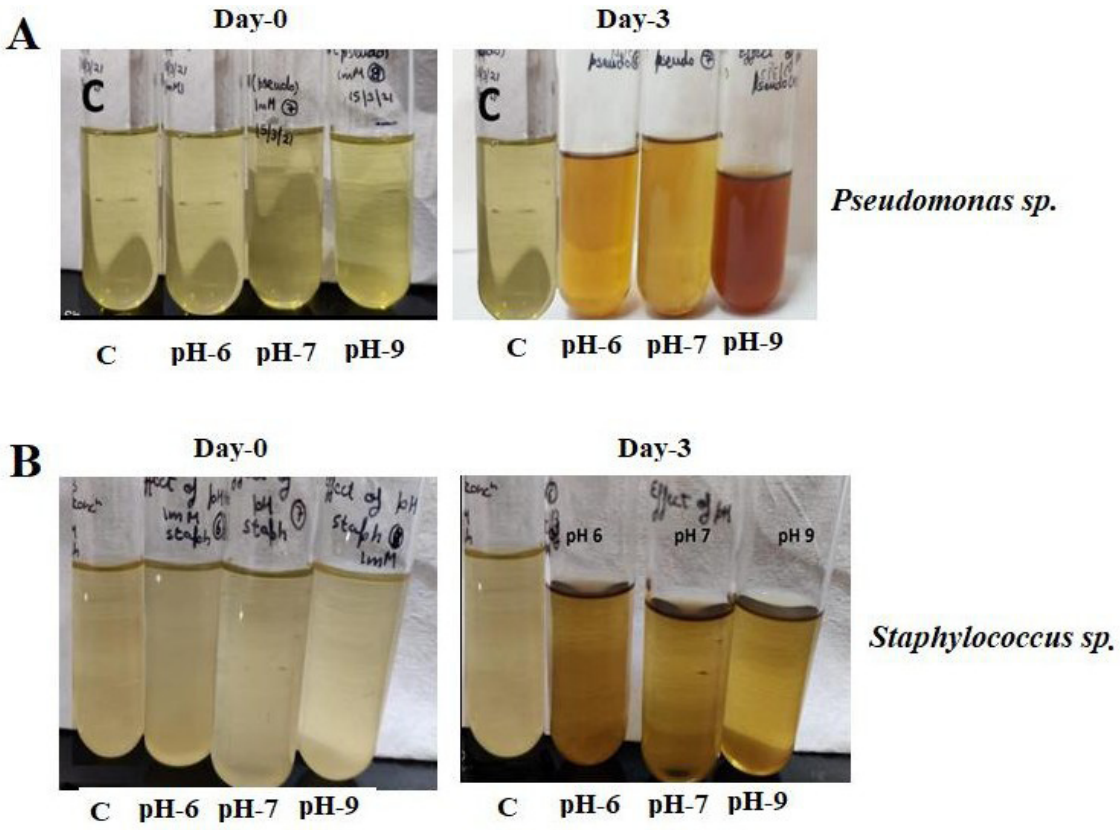

Fig. 7. Effect of different pH on NP synthesis.
A) *Pseudomonas aeruginosa* cell-free extract incubated with $AgNO_3$ at pH 6, 7 and 9 at Day 0 and Day 3. **B)** *Staphylococcus aureus* cell-free extract incubated with $AgNO_3$ at pH6, 7 and 9 at Day 0 and Day 3

AgNP synthesized by *Pseudomonas aeruginosa* under different pH (6, 7, and 9) showed peaks at 410 nm, 410 nm, and 420 nm respectively (Table 3; Figure 8A). This indicates that the particles synthesized are of mostly a 20-40 nm size range [44]. The data shown is an average of three independent experiments. Though there is an increase in absorbance with the change in pH, there is no shift in the wavelength range, this indicates that the NP synthesized at pH 6, 7, and 9 are almost of a similar size range. However, at pH 9, the absorbance was higher at 420 nm compared to pH 6 & 7 (Table 3; Figure 8A). The absorbance indicatesthat the amount of AgNPs formed at pH 9

was higher than pH 6 & 7. Hence for *Pseudomonas aeruginosa,* pH 9 seems to be a better choice for NP synthesis with 1 mM $AgNO_3$ than pH 6.

Table 3: Absorbance of NP synthesized under different pH conditions (pH-6, 7 & 9) with *Pseudomonasaeruginosa* cell-free extract and *Staphylococcus aureus* cell-free extract.

Wavelength (in nm)	*Pseudomonas aeruginosa* cell-free extract.			*Staphylococcus aureus* cell-free extract.		
	pH 6	pH 7	pH 9	pH6	pH 7	pH 9
360	0.810	0.817	1.915	1.346	0.854	0.488
370	0.842	0.852	2.109	1.410	0.894	0.542
380	0.897	0.892	2.230	1.496	0.941	0.597
390	0.943	0.957	2.471	1.582	0.994	0.638
400	0.994	1.004	2.612	1.652	1.031	0.682
410	1.101	1.125	2.752	1.709	1.067	0.708
420	0.966	0.983	2.814	1.642	0.993	0.721
430	0.814	0.828	2.712	1.559	0.925	0.652
440	0.700	0.707	2.543	1.467	0.843	0.583
450	0.625	0.634	2.276	1.376	0.786	0.526
460	0.561	0.574	1.942	1.278	0.730	0.480
470	0.510	0.525	1.643	1.187	0.648	0.439
480	0.461	0.481	1.371	1.094	0.587	0.403

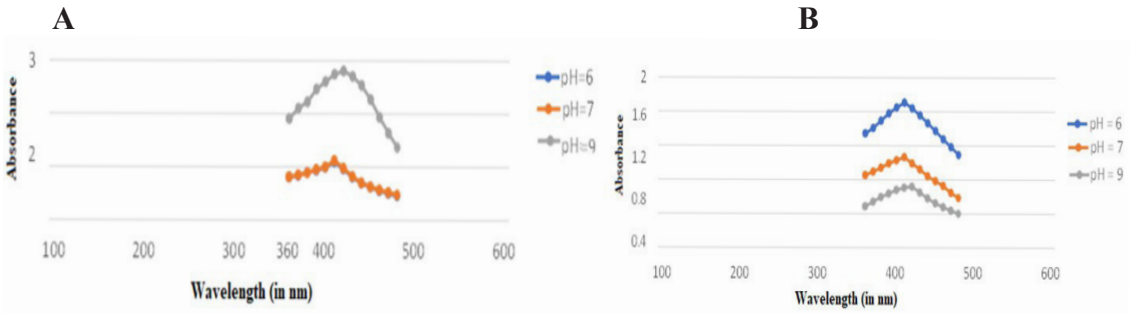

Fig. 8: Absorbance of NP synthesized under different pH conditions (pH-6,7 & 9) with A) *Pseudomonas aeruginosa* cell-free extract **B)** *Staphylococcus aureus* cell-free extract

For NP synthesized by *Staphylococcus aureus,* CFE peak was observed at 410-420 nm (Table 3; Figure8B) very similar to that of NP synthesized by *Pseudomonas aeruginosa.* Hence, the size of the particle, in this case, is also mostly 20-40 nm. But for *Staphylococcus aureus,* the highest absorbance was observed at pH 6 compared to pH 9 for *P.aeruginosa.* Though *Pseudomonas aeruginosa* exhibits almost similar absorbance pattern at pH 6 & 7, for *Staphylococcus aureus* the absorbance pattern for all three pH conditions is different (Figure 8B).

Hence, the results indicate that the two microorganisms, even though they synthesis NP of similar size, theyshow different preferences of pH for nanoparticle synthesis.

Effect of different incubation periods on NP synthesis

In the initial part of the experiment, a colour change was observed in the cell-free extract after incubation of72 hrs, which indicates the synthesis of NP. Further investigation was carried out to study the effect of increased incubation period on the synthesis of NP.

For *P. aeruginosa* the spectrophotometric data exhibited a peak at 410 nm for all three days. However, there is hardly any remarkable change between days 3 and 4, which indicates that there is no change in the synthesized NP size or amount. But then there is an increase in absorbance at Day 5 (Figure 9A; Table 4)

Table 4: Absorbance of NP synthesized under different incubation period (Day- 3, 4 &5) with *Pseudomonas aeruginosa and Staphylococcus aureus* cell-free extract.

Wavelength (in nm)	*P. aeruginosa*			*Staphylococcus aureus*		
	Day 3	Day 4	Day 5	Day 3	Day 4	Day 5
360	1.818	1.796	1.862	1.128	0.542	0.337
370	1.895	1.846	2.023	1.164	0.579	0.368
380	1.965	1.927	2.169	1.194	0.611	0.399
390	2.097	2.059	2.325	1.237	0.638	0.439
400	2.187	2.194	2.514	1.284	0.684	0.495
410	2.310	2.318	2.682	1.308	0.734	0.543
420	2.223	2.219	2.662	1.336	0.807	0.589
430	2.077	2.091	2.572	1.259	0.730	0.629
440	1.936	1.960	2.398	1.184	0.658	0.562
450	1.790	1.819	2.156	1.120	0.593	0.508
460	1.612	1.638	1.837	1.063	0.533	0.459
470	1.420	1.436	1.535	1.014	0.481	0.419
480	1.218	1.220	1.250	0.969	0.434	0.383

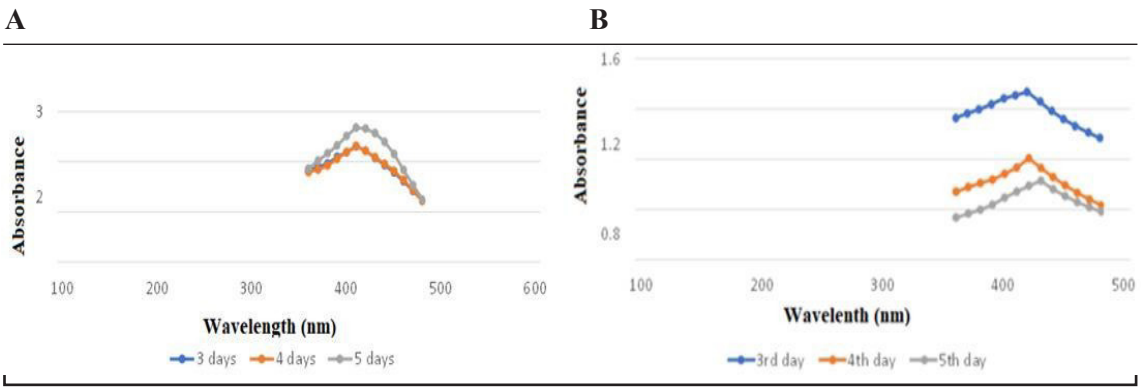

Fig. 9: Absorbance of AgNPs synthesized under different incubation periods (Day- 3, 4 &5) with A) *Pseudomonas aeruginosa* cell-free extract B) *Staphylococcus aureus* cell-free extract.

For *Staphylococcus aureus,* the spectrophotometric data exhibited a peak at 420 nm. But compared to *P. aeruginosa it* exhibited a decrease in absorbance with an increase in the incubation period, which indicates that increasing the incubation for *Staphylococcus aureus* is not suitable for the synthesis of the NPs (Table4; Figure 9B).

The AgNPs synthesized from *Pseudomonas aeruginosa* and *Staphylococcus aureus* gave a peak at 410 nm and 420 nm which corresponds to the silver NPs in the size range of 20-40 nm, approximately. For *P.aeruginosa* increasing the concentration of $AgNO_3$ and increase in pH favored the synthesis of AgNPs. Even with the increase in the incubation period, there was an increase in the NP synthesis. However, *Staphylococcusaureus* exhibits a different pattern. The data indicates that for *Staphylococcus aureus* ideal concentration of $AgNO_3$ is 0.1 mM. Higher pH conditions or incubation time are also not favorable.

CONCLUSION

There has been a growing interest in the field of nanoparticle synthesis, especially green synthesis. Green synthesis can be performed from various biological sources like plant extracts, fungi, bacteria, and viruses. Though there are several papers on Silver nanoparticle synthesis from *Pseudomonas aeruginosa,* there are very few papers on Silver

nanoparticle synthesis from *Staphylococcus aureus*. The standard synthesis condition with microbial CFE is suitable for both the organisms, but both of them shows different pattern ofsynthesis with alteration of physical conditions like concentration of $AgNO_3$, pH, and incubation period, The UV- visible spectrophotometric analysis depicts that the synthesized NPs are in the size range of 20-40 nm, which needs to be further verified by experiments like DLS (Dynamic Light Scattering) and FTIR (Fourier Transform Infrared Spectroscopy) for more convincing results. The present study illustrates that synthesis of NPs is possible from both *Pseudomonas aeruginosa* and *Staphylococcus aureus* under proper experimental conditions. Another important aspect of the study can be related to the antimicrobial activity of these NPs. However, the data needs to be validated on a large scale to confirm the economic feasibility of the synthesis process and the quality of NPs.

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Evaluation of Antibacterial Activity of Herbal Products for Potential Applications as Sanitizing Agents

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Abstract : *Maintenance of personal and environmental hygiene is of utmost importance. While there are several synthetic products that are commercially available, they are not free from undesired side effects. In light of this there is increased emphasis on the use of safe and eco-friendly herbal products as sanitizing agents. The aim of this study was therefore to assess the antibacterial activity of herbal products viz. dish wash, hand sanitizer, hand wash, toilet cleaner, and floor cleaner. We used two bacterial strains namely, Escherichia coli and Staphylococcus aureus for the assessment of the antimicrobial properties of the above-mentioned herbal products. The antimicrobial properties of the samples were compared with standard antibiotics: ampicillin, tetracycline, streptomycin, and penicillin. All herbal products failed to inhibit the growth of Escherichia coli; however, floor cleaner, hand wash, dish washer, and toilet cleaner showed good activity against Staphylococcus aureus.*

Keywords: *Antimicrobial susceptibility, herbal products, personal hygiene, environmental-friendly agents*

INTRODUCTION

Maintenance of good hygienic practice is essential to protect oneself from contagious diseases. Communicable pathogens can spread through physical contact with affected individuals or contaminated surfaces or food. While our body is mostly equipped to fight most disease-causing agents, occasionally they could be a cause of concern with adverse consequences including death. Yeasts, molds, *Escherichia coli*,

and *Staphylococcus aureus* are a few prominent pathogens which can spread through contact with affected individuals or contaminated surfaces, such as knobs, handles, switches, drawers, countertops, drains, faucets, laundry area, and floor around the toilet, living room, personal items and also through pets [1]. It is therefore very important to employ various sanitizing agents to control the spread.

Chemicals used for cleaning and in cleaning products, such as laundry detergents, bleaches, dishwashing solutions and other household cleaners help improve cleaning efficiency [2]. Unfortunately, many traditional store-bought products for cleaning contain 2-butoxyethanol, quaternary ammonium compounds, glutaraldehyde, ethanolamines, alkylphenol ethoxylates, and volatile organic compounds and harmful toxins are released leading to environmental hazards. Long-term use of such products could result in respiratory disturbances, sensory irritation, digestive system dysfunction, and other chronic illnesses [3]. Additionally, the concentrated forms of some commercial cleaning products are classified as hazardous and create potential handling, storage, and disposal issues [2].

Ethanopharmacologists, botanists, microbiologists, and therapists are therefore investigating phytochemicals as potential antimicrobial agents. These compounds can be used in disinfectants, antiseptics, dentifrices, and chemotherapeutic agents. The utilization of such natural products in cleansing agents could help reduce risk to human and environmental health. Plants are reservoirs of many secondary metabolites which play a crucial role in their defence mechanisms against pathogens. Such phytochemicals show high structural and functional diversity and therefore could target a large repertoire of pathogenic organisms [4]. The scientific community is currently emphasizing the need to investigate plant and microbial extracts, essential oils, pure secondary metabolites, and other natural products as potential antimicrobial agents.

In the current study we investigated herbal sanitizing products such as Hand Wash (HW), Nano Floor Cleaner (FC), Dish Wash (DW), Hand Sanitizer (HS), and Toilet Cleaner (TC) for their antimicrobial activity using common opportunistic pathogenic

bacteria. The bacteria employed in this study were *Escherichia coli* and *Staphylococcus aureus. E. coli* is a common inhabitant of the environment, food, and the gut. Typically, most strains of *E. coli* are non-pathogenic, however, some serotypes cause serious digestive tract infections and food poisoning. The feco-oral transmission of the pathogenic form is the major cause of infections. Likewise, *S. aureus* is a common inhabitant of the anterior nares but can transform into an opportunistic pathogen. It can cause skin infections, respiratory tract infections, and infections of the digestive tract when ingested. We therefore investigated the antimicrobial activity of the herbal products against these bacterial forms.

MATERIALS AND METHODS

Herbal Sanitizing products- HW, FC, DW, HS, and TC were kindly provided by Pharexcel Consulting, Bangalore and Vayuvah Ecoscience Pvt. Ltd.

E. coli MG1655 (MTCC No. 1586) and *S. aureus* (MTCC 3160) were maintained in agar slants and a loopful of each masterstock was used as inoculum for all experiments. The inoculum was introduced into 50 ml Luria Bertani (LB) broth (10 g of tryptone, 5 g of yeast extract, and 10 g of NaCl in 1 L distilled water; pH - 7.0) and incubated at 37°C for 24 h. The bacterial suspension was used for further experiments.

Antimicrobial susceptibility test

Hundred microliters of the bacterial inoculum (corresponding to an OD of 0.4 at 600 nm) was uniformly spread on LB agar. Serial dilution of each herbal product was prepared in LB broth. Seven mm diameter wells were cut into the agar medium and 10 µL of each dilution was added to each of them (5). Alternatively, Whatman filter paper discs were infused with 10 µL of the diluted herbal products and placed on the surface of the agar plates inoculated with the respective bacteria. Ten µL of standard antibiotics solution / commercially available discs was used as control to compare the susceptibility of *E. coli* and *S. aureus* against the herbal products. The plates were incubated at 37 ºC for

24 h. The zone of inhibition was measured around each of the wells/discs.

RESULTS AND DISCUSSION

Here, we investigated the susceptibility of *E. coli* and *S. aureus* to various dilutions of the herbal products in comparison to standard antibiotics. The zone of inhibition of bacterial growth around the discs/wells containing the various herbal products was measured in cm. Results indicated that none of the herbal products inhibited growth of *E. coli*, suggesting their inefficiency in killing this organism. However, when tested with *S. aureus*, except HS, all other products showed a dose dependent inhibition of bacterial growth. Table 1 depicts the zones of inhibition for all herbal products at the given concentrations and the standard antibiotics. As evident, HS was ineffective against *S. aureus*, but all other products showed a dose dependent increase in inhibitory activity (Fig. 1). Results indicated that all products except HS were comparablein efficiency to the standard antibiotics at 80-100% concentrations.

Table 1: A. Table depicts the zone of inhibition (ZOI) of *S. aureus* in cm for each of the products at the specified concentrations. NC – Negative control. B. S1, S2, S3, and S4 indicate ZOI for commercially available standard Streptomycin (1 mg/well), Ampicillin (10 mcg/disc), Tetracycline 30 mcg/disc), and Penicillin (10 units/disc) discs respectively.

A

Conc (%)	FC	HW	DW	TC	HS	NC
0	0.00	0.00	0.00	0.00	0.00	0.00
20	1.09	0.73	0.58	0.63	0.00	0.00
40	1.24	0.88	0.66	0.83	0.00	0.00
60	1.36	1.07	0.83	0.93	0.00	0.00
80	1.32	1.04	0.94	1.05	0.00	0.00
100	1.48	1.08	1.06	1.22	0.00	0.00

B

Standard antibiotics	S1	S2	S3	S4
ZOI (cm)	1.59	1.11	2.29	0

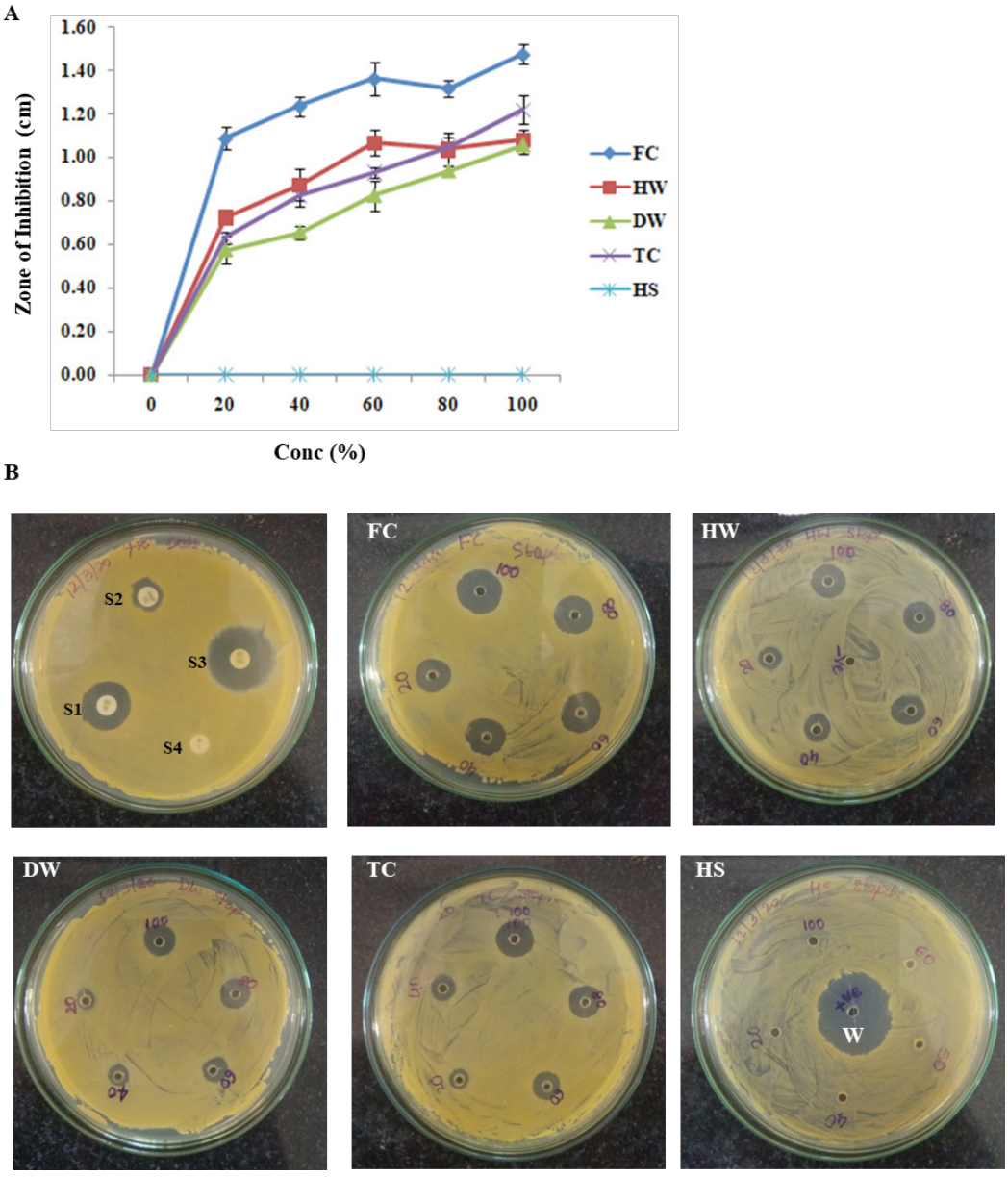

Fig. 1: A – Graph indicates a dose dependent increase in antimicrobial activity of FC, HW, DW, and TC, but not HS. **B –** LB agar plated with *S. aureus* showing the zones of inhibition in a concentration dependent manner as indicated. S1, S2, S3, and S4 indicate zones of inhibition for commercially available standard Streptomycin, Ampicillin, Tetracycline, and Penicillin discs respectively. W – Positive control of 10 µl Streptomycin (100 mg/ml).

CONCLUSION

Our data therefore suggests that all herbal products tested, except the hand sanitizer were effective against *S. aureus* and could be used as promising antimicrobial agents. However, commercial application of these products requires further investigations in additional bacterial strains, particularly those that are pathogenic.

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Effect of vermicompost conditioning in enhancing lignocellulosic waste bioconversion to lactic acid by selected microorganisms.

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Abstract : *Agricultural waste accumulation and usage of undegradable plastic can be minimized to a considerable extent by bioplastic production from cellulosic agro waste. Poly L Lactic acid (PLA) is considered as a very good alternative bioplastic in various fields. This study is focusing on bioconversion of lignocellulosic waste(LCW) in the form of hay/straw from agricultural field to fermentable sugars like glucose and further fermentation to Lactic acid. Lactic acid is the monomer which can get polymerized to PLA. For this the microbial systems selected were Bacillus and Saccharomyces species. Their efficiency in this bioconversion and fermentation were enhanced by vermicompost (VC) preconditioning. The bioconversion potential was assessed by estimating cellulose, glucose and lactic acid at specific incubation intervals (Day 7 and 14). The lactic acid extracted at the end of 14 days was polymerized in the presence of $SnCl_2$ to PLA. Since the final objective is zero waste, the fermentation residue was used for soil augmentation which was assessed by lab scale germination studies with raggi seeds.*

Keywords: *Lignocellulosic Waste(LCW), Vermicompost (VC), Poly Lactic acid (PLA)*

INTRODUCTION

In today's world plastic is ubiquitous, in day to day life as an unavoidable article in all aspects of life. Population increase had aggravated plastic accumulation. The recalcitrant nature of plastic causes lot of environmental problems. Thus the earth is covered with non-biodegradable plastics all over. They are harmful to the environment causing soil infertility, threatening wildlife, poisoning our food chain, clogging water bodies etc [1].

The recycling options which are available can also be harmful to the environment. Some plastics cannot be incinerated or recycled, as they release toxic products.

Even though various plastic alternatives are in practice like steel, glass and clothe; still the problem of nonbiodegradable accumulation on earth persists. This has led scientists to explore Bioplastics, which are derived from natural substances in place of petroleum and preferably biodegradable or compostable. Bioplastic can be made from agricultural by-products and also from used plastics by using microorganisms [2]. Bioplastics offer several advantages apart from preserving the scarcely available fossil energy reserves. The merits include low energy costs, improved compostability leading to less waste accumulation, prevention of toxic gas generation, and much low carbon foot print.

The monomers and oligomers of bioplastic polymers can be produced from plant biomass by microbial fermentation methods. This approach also is a solution to avoid accumulation of agricultural waste products. High content of cellulose and its biodegradation to Glucose and subsequent fermentation to Lactic acid can be achieved by employing suitable microorganisms[3].

In this work, an attempt has been made to analyse the bioconversion effect of microorganisms, which are easily available (*Bacillus* and *Saccharomyces*) and can be selected by incubating with and without vermicompost conditioning. Vermicomposting is an effectivemethod of biodegrading LCW by the combined action of earthworm intestinal enzymes and gut microflora along with soil microbial systems [4]. Our attempt is to find outwhether conditioning with vermicompostingenhances the fermentation capacity of *Saccharomyces* and *Bacillus* species. The undegraded cellulose, product of cellulose degradation- glucose and glucose fermented- lactic acid were estimated in thesamples. Further, the lactic acid extract was subjected to polymerization. The bio-augmentation activity of the fermentation residue by seed germination studies were also assessed.

The study of production of lactic acid from lignocellulosic wastes has considered to

be a promising field in the current biological research, since it (a)helps in the conservation of fossil fuels, (b)eliminates the accumulation of agricultural wastes, (c)helps to avoid undegradable plastic accumulation and toxic gas production due to incineration of both waste product.

The main objective of the work is the production of lactic acid from lignocellulosic waste (dried hay). Previous studies in our lab showed the efficiency of each of the microbial system in either cellulose to glucose or glucose to lactic acid conversion. Second objective of this study was the comparison of biodegradable capacity of same microorganisms with or without vermicompost treatment. Furthermore, this work also checks the polymerization into polylactic acid and the soil bio-augmentation parameters.

In both systems – Microbial culture with vermicompost conditioning and without vermicompost conditioning; Cellulose to glucose, and glucose to lactic acid conversion were analysed. It is done by estimating total cellulose, glucose and lactic acid concentration. Undigested cellulose, converted glucose and fermented product lactic acid gives us the efficiency of biodegradation capacity of microbial systems chosen.

The relevance of this work is not only bioplastics (PLA) from lactic acid; but also efficient management and treatment of the lignocellulosic wastes. It can be used as an industrially important and useful process in wide variety of industries.

METHODOLOGY

Preparation of substrate

Dried Straw were obtained from fruit stall and washed with deionized water. After drying at 70°C in hot air oven, the straw was subjected to pretreatment.

Pretreatment of substrate

Straw was cleaned and washed repeatedly for 3 to 4 times to reduce the contamination, then dried and subjected to pretreatment steps. At first mechanical pretreatment of the

hay was done by grinding and the powdered form is subjected to chemical pretreatment steps.

i) 20g of straw was weighed to which 250ml of sulfuric acid was added.

ii) the mixture was incubated for 1 hour at 21°C.

iii) after the incubation the solution was filtered, and the filtrate was neutralized with NaOH and subsequently washed with PBS (0.1M, pH-7.4)

iv) the filtrate was air dried and used for further process.

Inoculation of the samples without vermicompost conditioning

Co-culture of isolated bacteria(*Bacillus*) and fungi (*Saccharomyces*) starter culture (obtainedfrom department repository) was inoculated in 0.1g of dried pretreated lignocellulosic filtrate, 50ml of PBS (0.1M, p H-7.4) and incubated at 37˚C. Controls were also maintained simultaneously without microbial inoculation. Cellulose, Glucose and Lactic acid were quantified at different intervals of time (0, 7th, 14th day)

Inoculation of the samples with vermicompost conditioning

10g of vermicompost(VC) was autoclaved. The VC was divided into two conical flasks each of 5g and one was inoculated with yeast culture and other with bacterial culture; and left for incubation for one week. The microorganisms were isolated by Serial dilution. Then the sample was spread plated on to the selective agar plates (Nutrient agar for bacteria & Sabouraud agar for yeast). Selected colonies were picked from both plates and they were cultured on liquid media (Nutrientbroth & Glucose peptone broth), after biochemical characterisation. The *Bacillus* and *Saccharomyces* species were selected. After this step, coculture of the vermicompost conditioned *Bacillus* and *Saccharomyces* species were inoculated in 0.1g of pretreated lignocellulosic filtrate, with 50ml of PBS (0.1 M, pH 7.4) and incubated at 37°C. Controls were also maintained simultaneously without microbial inoculation. Cellulose, Glucose and Lactic acid were quantified at

different intervals of time (0,7th, 14th day)

Sampling at day 0, 7 and 14

2ml of the solution from each sample was pipetted out into sterile Eppendorf tubes using sterile pipettes under sterile conditions.These tubes were stored at 4˚C for further tests. This was done at the time of inoculation(day 0) and repeated on 7th and14th day also.

Estimation of cellulose concentration by UpDeGraff method

Briefly, 0.1ml of the sample made upto 1 mL and made to react with 3ml of acetic/nitric reagent was added to the diluted sample and mixed in a vortex mixture at 100°C for 30min. The contents were cooled and centrifuged and to the supernatant, 10ml of 67% sulphuric acid was added and allowed to stand for 1hr. 1ml of the above solution was diluted to 100ml. To 1mL of this diluted solution, 10ml of anthrone reagent was added and mixed well. The tubes were heated in boiling water bath for 10 minutes. The tubes were cooled and absorbance was read at 630 nm (5). The concentration of cellulose in the sample was estimated using standard values.

Estimation of glucose concentration using DNS method

To 0.1ml of sample diluted to 1ml , 3ml of DNS reagent was added and incubated in a boiling water bath for 10 minutes. After 10 minutes, 6ml of water was added. The absorbance was checked at 540 nm (6). Using a standard curve with different concentrations of glucose, the amount glucose not subjected to fermentation was calculated.

Estimation of Lactic Acid

To 0.1ml sample made up to 1ml, added 0.05ml of 4% CuSO4. Add 6ml concentrated sulphuric acid and kept it in boiling water bath for 5 minutes. 0.1ml of p-hydroxy diphenyl reagent added drop by drop and mixed contents incubated at 30°C for 30 minutes with periodic shaking. The tubes were kept subsequently in boiling water for 90 sec. Take the readings at 560 nm (7). Standard lactic acid curve with known

concentrations was used to calculate the amount of lactic acid formed.

Solvent-Solvent Extraction

Lactic Acid was extracted by solvent-solvent extraction method. First, the sample was filtered and debris was removed. The pH was adjusted 7.4 by diluted H_2SO_4 (0.1N). NaCl was added to the sample in a ratio of 3:1 and shaken for 5 min. The top phase was then extracted and this procedure was repeated for 5 times (8). The extracted Lactic acid was pooled together.

Polymerization of Lactic Acid

Lactic acid extracted was subjected to polymerization trial with stannous chloride ($SnCl_2.2H_2O$) (9). Briefly, to the extracted lactic acid sample, 0.5g of stannous chloride was added, in two separate petridishes (with and without vermicompost conditioned samples). They were left aside for 2 days to get completely polymerized. The results were recorded.

Bioaugmentation Studies- Seed Germination

1g of the fermentation residue was mixed with 1g of garden soil and 10ml H_2O in 3 separate petridishes. Control was taken as 1g of garden soil alone with water. Raggi seeds were sowed into this moist soil samples. The petridishes were kept in sun for a week, and water was sprinkled at specified time intervals. The observations were recorded and compared [10].

RESULTS AND DISCUSSION

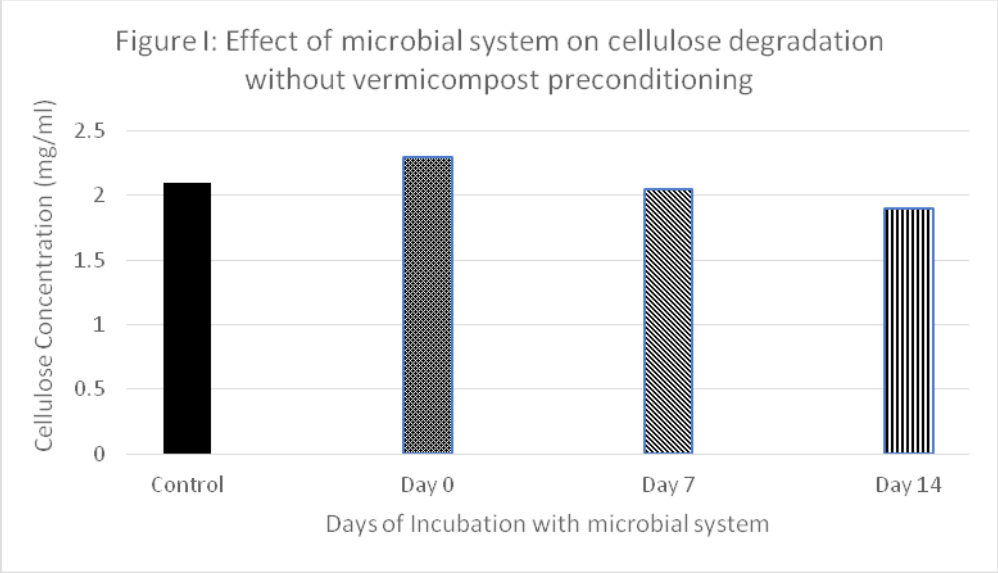
Effect of microorganisms on lactic acid production from lignocellulosic waste by microbial system with and without vermicompost conditioning was assessed by estimating cellulose degradation and the resulting glucose getting converted to lactic acid.

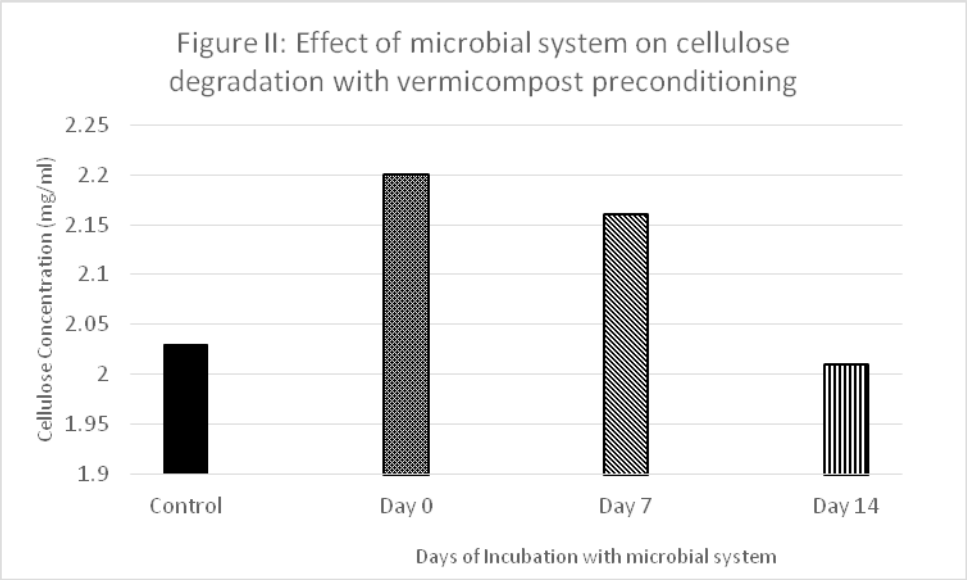
Estimation of Cellulose degradation

The cellulose degradation estimated at specific intervals will be an indicator of the efficiency of microbial system employed in converting cellulose to glucose or any other products. Here Control is the LCW material untreated with microbial culture. And immediately after inoculation the sample withdrawn for analysis (Day 0). And after 7th and 14th day also same procedures were done.

The effect of microbial system in the bioconversion of pretreated LCW material was analysed with and without vermicompost preconditioning [4]. Our assumption is vermicompost as an additive for preconditioning and will improve the biofermentation capacity of selected organisms.

The figures I and II shows whether such a preconditioning step improves microbial efficiency in the biodegradation of cellulose.

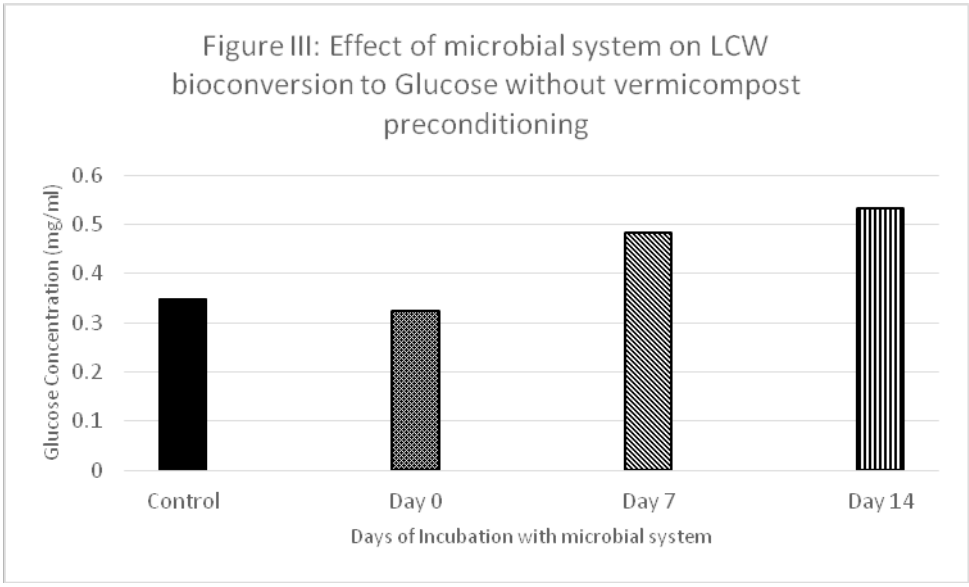


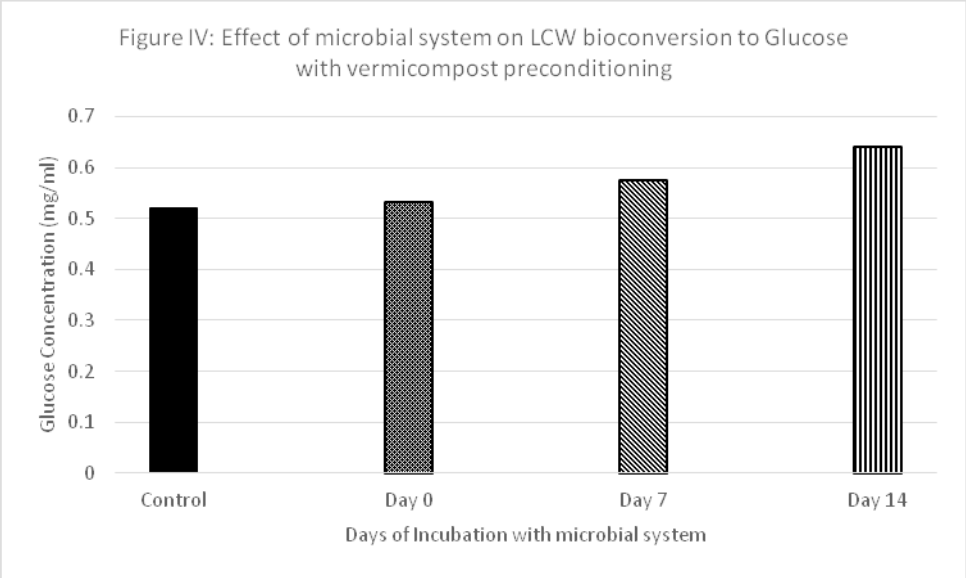


The results show similar results of cellulose level during the incubation period irrespective of vermicompost preconditioning. There is an increase in cellulose degradation depending on the incubation period. The improvement of microbial system (*Bacillus* and *Saccharomyces* sps) in bioconversion is suspected to be due to increase in inherent biodegradative enzymes. Further biodegradation by the microbial system may be improved by the factors in Vermicompost [11]

Estimation of Glucose release.

The next agenda in this study is to find out whether the biodegraded cellulose is converted to fermentable glucose or not. The following figures III and IV provides the comparative analysis of glucose level and whether it follows same pattern as in cellulose degradation. It also provides an insight to the processing of glucose to further fermentation products.



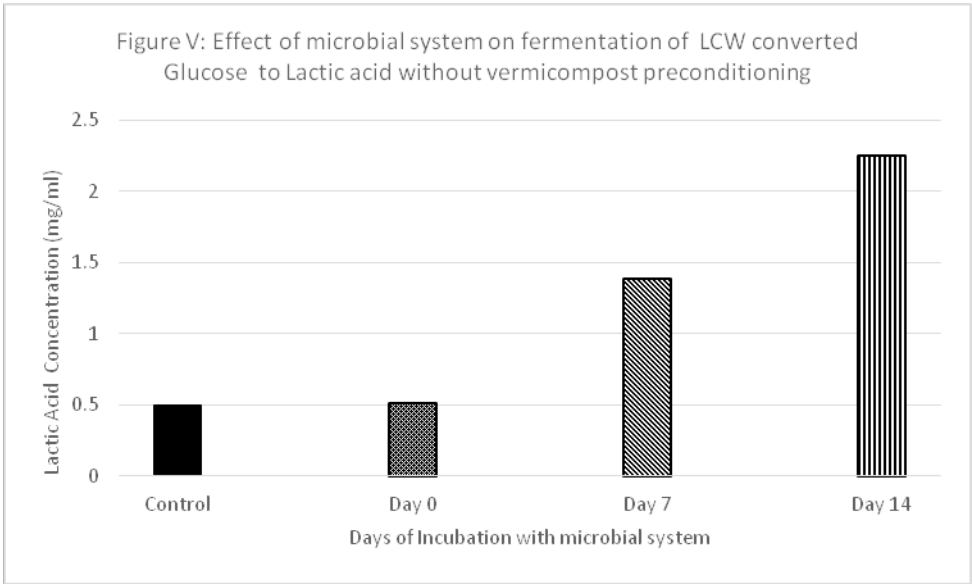


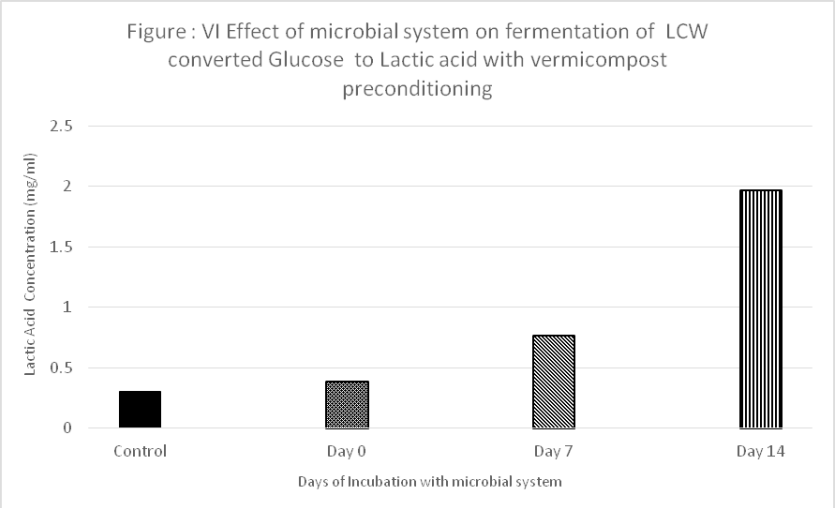
The incubation time dependent efficiency of vermicompost preconditioned microbial system in converting cellulose to glucose is again reflected in figures III and IV. No significant change in Glucose concentration was observed depending on the time of incubation. Here the objective was to find the combined effect of bacteria (*Bacillus* sps)

and fungi(*Saccharomyces* sps) in converting complex polysaccharide (cellulose) to bio fermentable sugar(glucose). The natural efficiency of these two species in the biomass degradation of cellulose was expected to be improved by vermicompost preconditioning which can be due to increased production of hydrolytic enzymes as stated by Lopes *et al* in 2018 [12]. Since these microbial systems were also responsible for fermentation, the extent lactic acid produced has to be analysed to explain the efficiency of microorganisms.

Estimation of Lactic Acid Conversion

As the main objective of the study is Lactic acid production capacity of selected microbial system (*Bacillus* and *Saccharomyces* sps) and influence of preconditioning with vermicompost. The following figures V and VI throws light on lactic acid production.





The figures V and VI showed incubation time dependent increase in lactic acid production irrespective of preconditioning methods. Consolidated bioprocessing with different microbial species was reported to be effective in earlier studies also [13]. Our study also proves that bacterial and fungal co culture can compartmentalize the necessary metabolic activities efficiently, in cellulose to glucose and further to lactic acid conversion.

Polymerization of Lactic Acid

Lactic acid polymerization at the lab scale was observed in presence of stannous chloride as catalyst in both the samples (with and withoutvermicompost conditioning).

a) **b)**

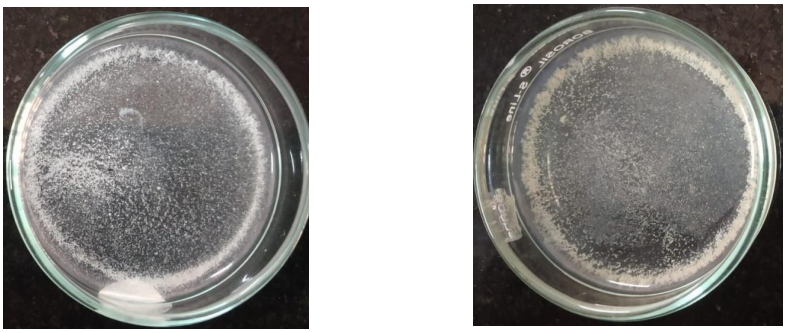


Fig. VII a and b): Polymerisation of Lactic acid (with and without VC preconditioning, a and b respectively) to polylactic acid (PLA)

The ring opening of lactide and subsequent polymerization to poly L lactic acid with $SnCl_2$ done in this study, can be compared to similar work done by Fang *et al* [14]. By this preliminary study it was confirmed Lactic acid produced can be polymerized to PLA.

Germination studies

The bioaugmentation potential of fermentation residue in supporting plant growth, was analysed by seed germination studies (Raggi seeds). The seeds were germinated in control fermentation residue compared to fermentation residue without VC preconditioning microbial culture (Figure: VIII a and b). But VC preconditioned microbial culture used fermentation residue showed significant result with more seeds germinated (Figure: VIII c).

a) **b)**



c)



Fig. VIII a, b and C: Raggi seed germination studies with fermentation residue

Fermentation residue has stimulating factors to induce seed germination, which may increase biological properties of soil. This effect gets enhanced when microbial system was preconditioned with vermicompost before employing for fermentation with LCW. Soil amendment and growth promoting effect of fermentation residue observed can be corelated to the observations made by Ievins *et al*., [15] in which vermicompost was used for similar studies.

CONCLUSION

The microbial system with *Bacillus* and *Saccharomyces* subjected to vermicompost treatment found to be more efficient in degrading cellulose in lignocellulosic waste to glucose. Further Lactic acid production also found to be enhanced by this treatment. Prolonging the incubation period beyond 14 days and assessment of other fermentable sugars and if they are present, and their efficient conversion to lactic acid also can be investigated for increase in lactic acid production. The seed germination studies also showed bioaugmentation capacity of fermentation residue. The negative results for seed germination with VC free treatment may be due to absence of proper enzyme systems to release nutrient factors in the residue for germination. Since this study is an attempt to use ligno cellulosic agrowaste for useful product formation (monomer lactic acid for PLA the bioplastic constituent) along with utilization of fermentation residue for soil quality enhancement, technically it is possible to have 'zero waste' in agricultural field.

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STUDY OF ANTIBACTERIAL ACTIVITY OF *CAMELLIA SINENSIS* AND ITS PHYTOCHEMICAL ANALYSIS

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Abstract : *Camellia sinensis, commonly known as tea, is a recreational drink used all over the world. It is well known for its stimulatory effects on human brain. It has numerous therapeutic properties like antibacterial, antioxidant, antifungal, anti-tumour, anti-inflammatory effects. The present study is mainly focused on antibacterial activity of commercially available green and black tea leaves and to analyse the phytochemicals present in them. Antibacterial activities of ethanol extracts of green tea and black tea were done using agar well diffusion method against four different bacterial strains- Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia and Pseudomonas aeruginosa. Qualitative phytochemical analysis was done on the ethanol extracts of both the tea varieties. Since polyphenols are largely present in tea, tannins and flavonoids were quantitatively analysed. Antibacterial activity results showed that all the bacteria were susceptible for both the teas but P. aeruginosa was highly susceptible when compared to other bacteria. The least susceptible was E. coli. Qualitative analysis of phytochemicals of green tea showed the presence of tannins, flavonoids, quinone, phenols, saponins and cardiac glycosides whereas black tea showed tannins, saponins, flavonoids, phenols, cardiac glycosides and terpenoids. Quantitative estimation of tannins and flavonoids showed that green tea had 50,744µg/ml and 183.846µg/ml of tannins and flavonoids respectively and black tea extract had 34,116µg/ml and 237.942µg/ml of tannins and flavonoids respectively. This study confirms that Camellia sinensis can act as natural antimicrobial agent with additional benefits exhibited by their phytochemicals especially tannins and flavonoids. It can also act synergistically with antibiotics to enhance its activity.*

Keywords: *Camellia sinensis, Antibacterial, Phytochemical analysis, Tannins, Flavonoids.*

INTRODUCTION

Since ancient times, plants have been used to cure many diseases for its therapeutic properties present. Today, people all over the world consume tea as a recreational drink for its stimulating effect on brain. *Camellia sinensis* which is commonly called as tea was originated in North-Eastern states of India, South-West of China and Tibet. The different types of tea consumed are Green tea, White tea, Black tea, Yellow tea, Fermented tea, Oolong tea, etc. Tea has numerous health benefits like protection of lungs, esophagus, pancreas, liver, boosting immune system, lowering cholesterol levels, gives mental alertness, skin cancer etc. [1]. Many studies have been done to study the phytochemical aspects of tea plants and showed the presence of numerous phytochemicals like alkaloids, flavonoids, tannins, caffeine, anthocyanins etc which attribute for various other properties like antibacterial property, antifungal property and antioxidant property.

Antibacterial activity is the process of inhibiting or killing various disease-causing bacteria. Use of conventional antibiotics to control disease may have numerous side effects and bacteria can become resistant to that particular antibiotic after few days [2]. Hence, plant-based antibiotics might overcome those problems.

The objectives of the present study were to study the antibacterial activity of commercially available black and green tea using ethanol as solvent against four different bacteria, namely *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia and Pseudomonas aeruginosa,* qualitatively analyze the phytochemicals in green tea and black tea and also to evaluate the quantity of tannins and flavonoids present in both the types of teas. The results of our study, clearly indicates that *Camellia sinensis* can act as natural antimicrobial agent with additional benefits exhibited by their phytochemicals especially tannins and flavonoids. It also has the potential to act synergistically with antibiotics to enhance its activity.

MATERIALS AND METHODS

Samples used

Tetley green tea and black tea which is available commercially was purchased. Clinical isolates of bacteria strains- *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia* and *Pseudomonas aeruginosa* were obtained from Bhagwan Mahaveer Jain Hospital (January, 2021). Nutrient agar (NA), which is a general-purpose medium, was used for the growth of bacteria. The composition of Nutrient agar is as mentioned in [3].

Inoculation of bacteria

5 ml of nutrient broth was added into each test tube. Loopful of bacteria was taken from pure culture of clinical isolates and was inoculated into it. The inoculated test tubes were incubated for 24hrs.

Preparation of plant extracts

Absolute ethanol was used to extract the phytochemicals from green tea and black tea using Soxhlet apparatus. 3 grams of green tea and black tea leaves were weighed and its phytochemicals were extracted using 160 ml of 100% ethanol. After the extraction, ethanol containing phytochemicals was transferred to rotary evaporator to evaporate the ethanol solvent. The tea extract which is obtained after evaporation was made into four different concentrations like 0.2%, 0.3%, 0.4%, 0.5% using the same solvent [4].

Agar well diffusion assay

Antibacterial activity of the ethaol extract of tea samples was done by agar-well diffusion method. Nutrient agar was poured onto the Petri plate and made to solidify. Wells were made using 8mm autoclaved cork borer. Bacterial cultures were smeared uniformily on the surface of media. 10µl of leaf extract was added into the well using a micro pipette. These inoculated plates were incubated at 37°C and checked for zone of clearance after 24 hrs and diameter of zone of inhibition was measured. Ethanol was taken as negative control and antibiotic streptomycin was taken as positive control [1].

Qualitative estimation of phytochemicals

Quantitative extimations of Tannins, Saponins, Alkaloids, Flavonoids, Glycosides, Quinones, Phenols, Terpenoids and Cardiac Glycosides were done as per the methodology mentioned by Roghini *et al*, 2018 [5].

Procedure for quantitative estimation of tannins

100mg of tannic acid was dissolved in 100ml of distilled water was used as the standard solution of tannic acid. Using this standard solution, different dilutions of tannic acid were made up to 1ml with varying amount of distilled water. To this, added 0.5ml of Folin-Denis reagent and 1ml of sodium carbonate solution and made up to 10ml with distilled water. After shaking well, the absorbance reading was taken at 760nm after 30minutes.

Extraction of tannins from tea:

Weighed about 0.5g of tea and transferred it into conical flask containing 75ml of water. Heat the flask gently for 30 minutes and centrifuged the boiled extract at 2000rpm for 20 minutes. Collected the supernatant and added it to 100 ml conical flask and made up the volume to 100ml. Transferred 1ml of sample extract to 100ml flask containing 75ml water. Add about 5ml of Folin-Denis reagent, 10ml sodium carbonate solution and dilute to 100ml with water and shaken well. Read the absorbance at 700nm after 30 minutes of incubation. Reading of blank was taken by adding water instead of sample [6].

Procedure for quantitative estimation of flavonoids.

A stock of quercetin of 100µg/ml concentration was prepared by adding 10mg of quercetin to 100ml of methanol in a standard flask. This was used as the standard solution of quercitin. From this stock solution of quercetin, different concentrations were prepared using methanol. To each of the sample dilutions made, added 1.5ml methanol, 0.1ml of aluminium chloride solution, 0.1ml of potassium acetate solution and 2.8ml of distilled water. This solution was mixed well and absorbance was taken at 415nm after 30 minutes of incubation [7].

Extraction of flavonoids from tea:

Sample was ground with mortar and pestle in 80% ethanol. The extract was filtered using Whatman filter paper. The volume was made up to 5ml. Aliquots of 0.5ml extract was taken and made up the volume to 3ml using methanol. To this 0.1ml of aluminium chloride (10%), 0.1ml of potassium acetate solution and 2.8ml of distilled water was added and shaken gently. Absorbance was taken at 415nm after the incubation for 30 minutes.

RESULTS

Antibacterial activity

Antibacterial activity of the tea extracts were studied by agar well diffusion method. It was observed that the zone of inhibition increased as the concentration of ethanol extract of green tea was increasing. Maximum zone of inhibition was shown at the concentration of 0.5% against all 4 bacterial strains (**Figure 1**). The maximum zone of inhibition was exhibited against *P. aeruginosa* in all concentrations. The least inhibition was exhibited against *E.coli. E.coli* and *K. pneumoniae* almost showed the same inhibition of about (1.3cm, 1.35cm) at 0.5%. The measurements of the diameter of zone of inhibition are given in **Table 1.** These values are represented in the form of a graph as shown in **Figures 2 and 3**.

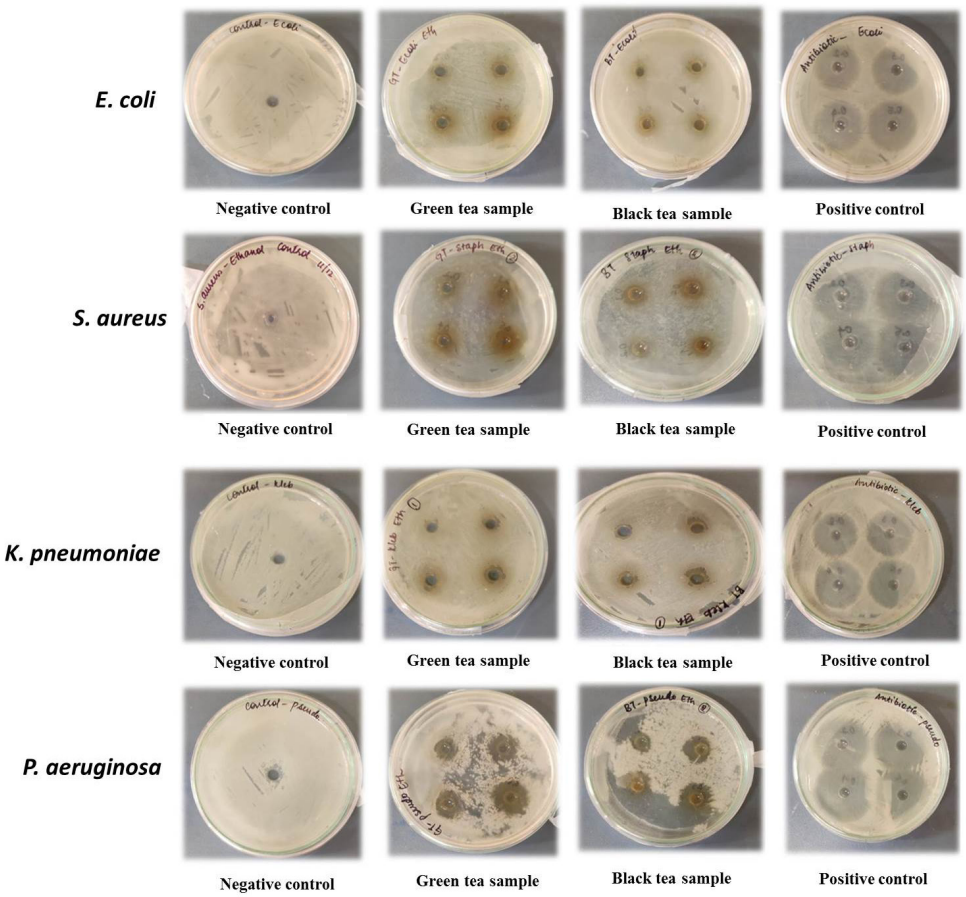

Fig. 1: Antibacterial activity of ethanolic extracts of green tea and black tea against four different bacterial strains

Table 1: Values for zone of inhibition of Green tea and black tea against the four bacterial strains. (GT= Green Tea, BT= Black Tea)

Sl. No.	Concentrations (%)	*E. coli*		*S. aureus*		*K. pneumoniae*		*P. aeruginosa*	
		GT	BT	GT	BT	GT	BT	GT	BT
1	0.2	1.05	1.1	0.9	1	0.95	1.1	1.15	1.4
2	0.3	1.1	1.1	1.05	1.1	1.1	1.1	1.6	1.65
3	0.4	1.15	1.2	1.25	1.15	1.2	1.15	1.7	1.8
4	0.5	1.3	1.25	1.45	1.3	1.35	1.4	2	2

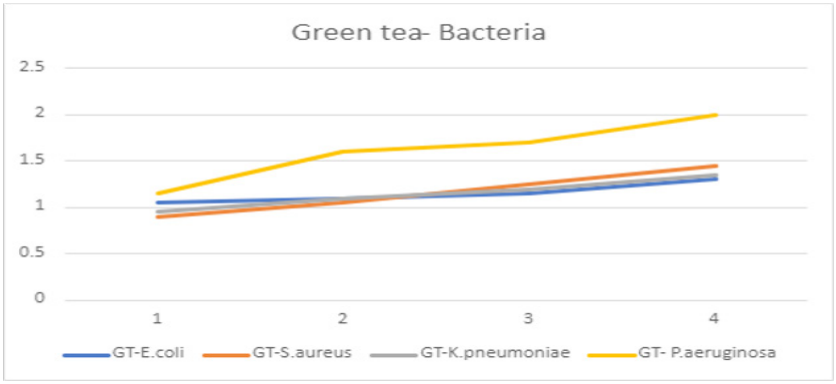

Fig. 2: Antibacterial activity of green tea against all the four bacterial strains.

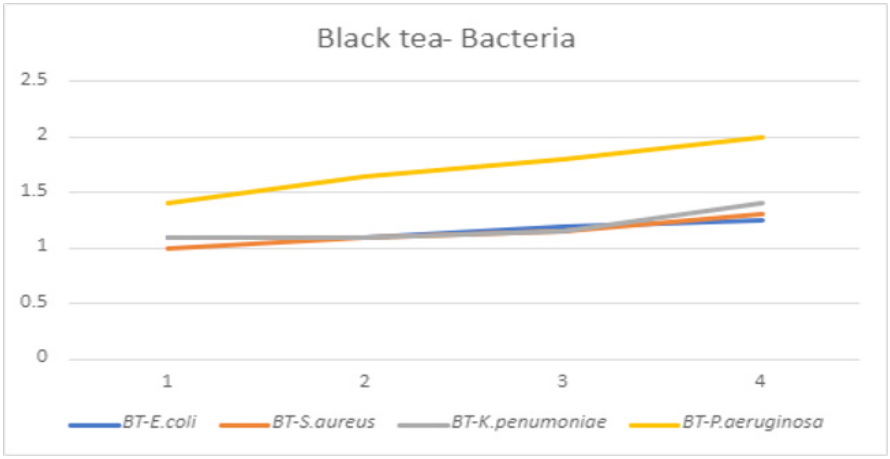

Fig. 3: Antibacterial activity of black tea against all the four bacterial strains

Qualitative phytochemical analysis

Ethanol extracts of Green tea exhibited positive results for tannins, saponins, Flavonoids, Quinone and Phenols whereas black tea revealed positive results for tannins, saponins, flavonoids, quinonone, phenols and terpenoids. The results are given in **Table 2**.

Table 2: Qualitative analysis of phytochemicals in green tea and black tea extracts.

Green tea (Ethanol extract) **Black tea (Ethanol extract)** extract)

Phytochemicals	Results	Phytochemicals	Results
Tannins	+	**Tannins**	+
Saponins	+	**Saponins**	+
Alkaloids	-	**Alkaloids**	-
Flavonoids	+	**Flavonoids**	+
Glycosides	-	**Glycosides**	-
Quinone	+	**Quinone**	+
Phenols	+	**Phenols**	+
Terpenoids	-	**Terpenoids**	+
Coumarins	-	**Coumarins**	-
Anthraquinone	-	**Anthraquinone**	-
Cardiac glycosides	+	**Cardiac glycosides**	+

Quantitative phytochemical analysis

Quantitative phytochemical analysis was done for tannins and flavonoids. The standard graph of tannic acid and quercitin were plotted with the OD obtained for the different concentrations of the two compounds. From this standard graph, it was observed that 0.5g of green tea contains 50,744µg/ml of tannins and 183.846µg/ml of flavonoids. 0.5g of black tea contains 34,116µg/ml of tannins and 237.942µg/ml of flavonoids.

DISCUSSION

The conventionally available antibiotics might have numerous side effects and bacteria can become drug resistant after few days of usage. Hence, there is a need to produce antibiotics from naturally available plants. In the present study, *P. aeruginosa* was more susceptible to green tea and black tea. *E. coli* was least susceptible. In previous studies [8] done by M. Radji *et al.*, 2013, *P. aeruginosa* which is implicated in various nosocomial infections including bacteremia and urinary tract infection is multi drug resistant bacteria, showed the highest inhibition of about 18mm which is almost same as this study. *Staphylococcus aureus* showed zone of inhibition of about 1.45cm and 1.3cm in green tea and black tea. This bacterium causes skin infection, food intoxications and is found on the epidermis of skin and upper respiratory tract. Hence tea leaves can be used for many formulations to treat skin problems, pimples, rashes etc. Green tea and black tea also showed inhibition against shiga toxin producing *E. coli* and many respiratory diseases causing *K. pneumoniae*. Therefore, green tea and black tea can be used as antibacterial agent and also be used synergistically to enhance the antibiotics.

Phytochemicals are the secondary metabolites which are produced when the plants are in stress and they serve as a protection mechanism against different pathogenic microbes, animals and pests that are attacking them. They might have numerous health benefits. Ethanol extract of green tea and black tea revealed the presence of tannins, saponins, flavonoids, quinone and phenols and terpenoids was only present in black tea. Polyphenols which are the effective phytochemical found in tea is found to have antibacterial activity [8]. According to previous study [9]**,** Epigallocatechin which is proanthocyanidin and an oligomeric flavonoid present in tea was shown to interact with the outer membrane of the bacteria and prevented the attachment to mammalian cells. It is also reported to have anticarcinogenic property, cardioprotective activity but higher doses of it might act as mutagens, very good prooxidants that produce free radicals and also as inhibitors of enzymes involved in different hormone metabolism. Hence, drinking more of tea might be harmful to health. A previous study [10] had emphasised on the importance of phytochemicals especially polyphenols like phenols and flavonoids and

that they prevent ailments like cardiovascular diseases, cancers and kidney stones. Tannins which are found abundantly in tea and it can bind to proteins which are proline rich and interfere with synthesis of protein. Another phytochemical catechins found in tea are found to prevent tooth decay due to their anti-bacterial activity and hence, has the potential to be be used as a mouthwash also[11]. A similar result was obtained in another study [12] which demonstrated that green tea-derived epigallocatechin gallate (EGCG) decreased the growth and acid production of oral *S. mutans*. Hence, it can be inferred that phytochemicals in *C.sinensis* are protective against oral bacteria. Flavonoids are the phenolic compounds which are normally synthesized in plants in response to microbial infection. They are also having the capacity to form complex with extracellular and soluble proteins and also with bacterial cell walls. Saponins are also having the property of antibacterial activity for its ability to cause leakage of proteins and certain enzymes from the cell [2]. Therefore, it could attribute for effective antibacterial activity against diverse microorganisms.

CONCLUSION

This study confirms that *Camellia sinensis* can act as natural antimicrobial agent. The presence of various phytochemicals, especially tannins and flavonoids, helps in its beneficial activities and also increases its potential to act synergistically with antibiotics to enhance its activity.

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Alleviation of Cadmium Stress in *Pennisetum glaucum* (Pearl millet) by Supplementation with Chicken Manure Biochar

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Abstract **:** *Soil that is contaminated by Cadmium (Cd), a non-essential heavy metal will have negative impacts on grain quality, crop productivity and human health. Biochar has been traditionally used as a modification to reduce the toxicity of heavy metals. The objective of this study was to evaluate the role of Chicken Manure Biochar (CMB) on alleviation of Cd toxicity in Pennisetum glaucum. The experiment was conducted on 7-day old P. glaucum. The plant was subjected to stress for 5 days with different concentrations of $CdCl_2$ (50, 100, 150 mg/kg of soil) and Cd-treated plants supplemented with CMB (15g/kg). The uptake of Cd in plant tissue was determined by atomic absorption spectroscopy. Biomarkers of stress such as hydrogen peroxide, ascorbic acid, proline, reduced glutathione, total soluble sugars and enzymes like catalase, guaiacol peroxidase and glutathione reductase were evaluated. Cd stressed plants showed stunted growth, chlorosis, and necrosis whereas, on supplementation with CMB, growth was not hindered. The Cd treatment increased the concentration of proline, glutathione, and phenols in stressed seedlings. However, supplementation with CMB increased the concentration of catalase and guaiacol peroxidase.*

Keywords*: ascorbic acid, biochar, cadmium, glutathione, proline, Pennisetum glaucum.*

ABBREVIATIONS

ASC-ascorbate; APX-ascorbate peroxidase; CAT-catalase; EDTA-ethylenediaminetetra-acetic acid; GR-glutathione reductase; GSH-reduced glutathione; POX-peroxidase;

PRO-proline; ROS-reactive oxygen species; RWC-relative water content; SOD-superoxide dismutase

INTRODUCTION

Cadmium (Cd) is a non-essential element that has an adverse effect on plant growth and development. It is released into the environment by nuclear power plants, metallurgical industries, heating systems, and vehicular traffic. It is used in a variety of applications, including electroplating, pigments, plastic stabilisers, and nickel-cadmium batteries [1]. Cd is a highly toxic inhibiting shoot and root growth and yield. It is often accumulated by agriculturally crucial crops before entering the food chain, posing a substantial health risk to animals and humans [2]. It affects absorption of nutrients and disrupts homeostasis. Cd toxicity can develop when plant metabolism is disrupted by mineral nutrient absorption and translocation issues [3]. It affects plant mineral uptake by reducing the population of soil microbes or affecting the availability of minerals from the soil [4]. It impacts stomatal opening, transpiration, and photosynthesis, but the metal is more readily absorbed into plants in nutrient solutions than in soil [5]. Cd-accumulating plants may experience a variety of physiological, biochemical, and structural changes [6].

Chemical additives and organic composts have been shown to be effective metal immobilisers, and as a result, plant metal absorption is reduced [7]. CMB is a carbonaceous material made by pyrolyzing a variety of feedstocks in low-oxygen conditions at high temperatures (600 °C). It is regarded as a green pollutant sorbent due to its cost-effectiveness and environmental friendliness. It is a valuable soil additive due to the presence of critical nutrient elements such as sulphur and nitrogen. Improved composting reduced drought and salinity stress, and increased crop yield are only a few of the applications for CMB in agricultural production. Several reports have documented enhanced nutrient uptake and plant growth by CMB in drought-stressed maize plants [8]. Due to the existence of comparatively more negative charges on the surface of CMB, arsenic sorption is limited [9]. It greatly increased the nitrogen, phosphorus, and magnesium absorption in corn and kiwi plants. Under both natural and stressful conditions, CMB established significant

growth characteristics and yield. Poultry farming is one of the world's largest and fastest-growing agri-food industries, creating vast amounts of waste, much of which is difficult to recycle. Converting these materials into biochar is a different process that makes good use of the nutrients they contain [10]. This process has the potential to offer not only tangible environmental benefits, but also a solution to the frequently contentious issue of how to handle these types of materials [11].

MATERIALS AND METHODS

Plant material and chemicals

The seeds of Pearl millet (*Pennisetum glaucum*) were procured from the National Seed Project, University of Agricultural Science, Bengaluru, India. Fine chemicals and reagents used in this study were purchased from Hi-Media and Sigma-Aldrich, Bengaluru, India. All other chemicals were of analytical grade.

Relative Water Content (RWC)

The relative water content was estimated according to the method of Turner and Kramer [12] using the equation: RWC = (FW-DW) X 100 / (TW-DW). Leaf discs of 10 mm diameter were weighed to determine the fresh weight (FW), soaked in distilled water at 25 ^{o}C for 4 h to determine the turgid weight (TW), then oven dried at 80 °C for 24 h to determine the dry weight (DW).

Determination of germination indices under Cd stress

About 10 g of seeds imbibed for 24 h in distilled water were transferred to petriplates lined with a layer of cotton wool and wetted with 5.0 ml $CdCl_2$ (5, 10 and 50 µM), in half-strength Hoagland medium. Control seedlings added to the same volume of half-strength Hoagland medium. After the stipulated period of 5 days, the percentage germination was determined, and the mean length of the shoot and root was recorded in centimeter from 100 seedlings.

The dry mass of shoot and root was recorded from 5-day-old seedlings after keeping them in an oven at 80 °C for 48 h. Tolerance Index (TI) of 5-day-old seedlings and vigour index after 48 h of treatment was calculated respectively [13,14] as follows:

$$Tolerance\ index\ (TI) = \frac{Length\ of\ the\ longest\ root\ in\ the\ test\ sample}{Length\ of\ the\ longest\ root\ in\ control}\ x\ 100$$

$$Vigour\ index\ (VI) = \%\ germination\ at\ 48\ h\ x\ Length\ of\ embryonic\ axis\ at\ 48\ h$$

Metal uptake

Total leaf accumulation of heavy metal in *Pennisetum glaucum* was determined after 72 h of treatment to estimate the accumulation of metal in roots and translocation into leaves. Leaves were harvested, washed in distilled water for 2 min, air dried and then oven dried at 80 ºC for 2 days. The tissue was ground into a fine powder using a pestle and mortar. A known amount of this powder (1 g) was dissolved in binary mixture containing 3 parts of 1M HNO_3 and 1 part of 1M HCl (3:1 ratio), and the metal concentration was analyzed by flame photometer (Systronics India Limited, Model No. 128). Different concentrations of metal salts were used as standards.

Stress treatment and experimental design

Seeds of *P. glaucum*were surface sterilized with 0.1% (w/v) $HgCl_2$ for 1 min followed by three rinses in sterile distilled water andimbibed overnight in distilled water. The sprouts were sown in trays containing cocopith and soil (1:1 w/w) and irrigated daily with distilled water. The germination was carried out under natural greenhouse conditions; day/night temperature and relative humidity were 30/25°C and 75/70 % respectively. The average photoperiod was 12 h light/12 h dark.

Seven-day old seedlings of uniform size were treated as follows: (1) For $CdCl_2$ treatment, seedlings were fed with half-strength Hoagland medium [15] containing 50, 100, 150 mg $CdCl_2$/kg of soil. (2) For $CdCl_2$ treatment in the presence of biochar (alleviation studies), seedlings were fed with half-strength Hoagland medium containing

50, 100, 150 mg/kg of $CdCl_2$ in the presence of biochar (15g/kg). Shoot samples were collected 5 days after stress (DAS), and frozen until further analysis. Plants irrigated with half-strength Hoagland media without $CdCl_2$ (C) and media with CMBserved as control. Each experiment was done in triplicate. The experimental design used was carried out at random factorial scheme with 8 media regimes: controls (C and CMB), Cd (50, 100, 150 mg $CdCl_2$/kg of soil) and Cd + CMB and 1 evaluation point (5 DAS).

Determination of stress response factors

Hydrogen peroxide (H_2O_2): Hydrogen peroxide content in control and stressed seedlings were determined according to Velikova [16]. Shoot tissue was homogenized in an ice bath with 5 ml of 0.1% (w/v) trichloroacetic acid. The homogenate was centrifuged at 10,000 rpm for 15 min and 0.5 ml of the supernatant was added to 0.5 ml of 0.1M potassium phosphate buffer (pH 7.0) and 1 ml of KI. The absorbance of the supernatant was measured at 390 nm.

Proline (Pro): The estimation of proline was carried out according to the method of Bates et al., (1973). Proline was extracted from 0.1 g of fresh tissue in 1.5 ml sulphosalicylic acid (3%), and the homogenate was centrifuged at 10000 rpm for 10 min. 0.2 ml of the supernatant obtained was used for assay. This was mixed with 2.0 ml of glacial acetic acid and 2.0 ml of ninhydrin reagent. The contents were boiled for 30 min and cooled rapidly on ice. The colour was extracted in 4 ml of toluene by vigorous shaking and the organic phase recorded at 520 nm against toluene as blank. Standard curve was prepared for different concentrations of proline.

Total soluble sugars (TSS): The estimation of total soluble sugars was carried out according to the method of Roe [18]. 0.1g of sample was homogenized in 5 ml of 2.5 N HCl and the homogenate was hydrolyzed in a boiling water bath for 3 h and cooled. The hydrolyzed sample was centrifuged. The supernatant was used for analysis. Total soluble sugars were estimated using anthrone reagent and glucose as standard. The green colour obtained was read at 630 nm against a suitable blank.

Total phenolics: Total phenols was estimated by the method of Slinkard and Singleton [19]. 0.1 g of sample was homogenized in 70% ethanol. To 0.1 ml of sample, 0.5 ml Folin-Cioalteau reagent and 2.0 ml sodium carbonate was mixed and boiled exactly for 1 min. Absorbance was determined at 650 nm against a suitable blank. Catechol was used for the standard curve.

Determination of antioxidant and antioxidant enzymes

Reduced glutathione **(GSH):** GSH was estimated according to Beutler [20]. The tissue was homogenized with 3% metaphosphoric acid. DTNB [5,5'- dithiobis(2-nitrobenzoic acid)] was added to supernatants cleared by centrifugation. The formation 5-thio-2-nitrobenzoic acid, which is proportional to total reduced glutathione concentration, was monitored at 412 nm at 25°C against reagent controls.

Ascorbic acid (AsA): Ascorbic acid was estimated volumetrically following the titrimetric method using 2,6-dichlorophenolindophenol (DCPIP) as dye [21]. Following standardization of indophenol solution, 1.5ml of the test sample was pipetted and to it 5 ml of oxalic acid-acetic acid mixture was added and titrated against indophenol till a permanent pale pink appeared.

Extraction of enzymes

The frozen samples were homogenized with pre-chilled 50 mM sodium phosphate buffer (pH 7.0) containing 5 mM β-mercaptoethanol and 1mM EDTA using pestle and mortar. L-ascorbate was raised to a final concentration of 2 mM for extraction of APX. The homogenate was centrifuged at 12,000 g for 15 min at 4°C. The supernatant was used as a source of enzymes. Soluble protein content was determined using BSA as the standard [22].

Guaiacol peroxidase: Guaiacol peroxidase activity was measured in reaction mixture of 3.0 ml consisting of 50 mM phosphate buffer (pH 7.0) containing 20 mM guaiacol, 10 mM H_2O_2 and 10% enzyme extract [23]. The formation of tetraguaiacol was

followed by an increase in absorbance at 470 nm. The amount of enzyme which catalyses the conversion of one micromole of H_2O_2 per minute at25ºC.

Catalase: Catalase activity was assayed by following the decline in optical density at 240 nm ($\varepsilon = 39.4M^{-1}\ cm^{-1}$) [24]. The reaction mixture consisted of 0.05 ml of enzyme extract and 50mM sodium phosphate buffer (pH 7.0). The reaction was started by addition of H_2O_2 to a final concentration of 10mM, and its consumption was measured for 2 min. One unit of activity is defined as the amount of enzyme that catalyzes the oxidation of 1 µmol of H_2O_2 min^{-1} under the assay conditions.

Glutathione Reductase: GR activity was determined by monitoring the oxidation of NADPH at 340 nm ($\varepsilon = 6220\ M^{-1}\ cm^{-1}$) [25]. The reaction mixture contained 50 mM Tris-HCl buffer (pH 7.5), 3 mM $MgCl_2$, 0.5 mM GSSG, 0.2 mM NADPH and 250 µl of enzyme extract in a total volume of 1.5 ml. One unit of NADPH causes the oxidation of 1.0 µmol NADPH at 25°C at pH7.0.

Statistical analysis

The experiment was performed for eight regimes and expressed as means of triplicate (n=3) experiments unless mentioned otherwise. Comparisons of means were performed using Microsoft Excel ± standard error. The data were subjected to one-way analysis of variance (ANOVA), and the mean differences were compared by lowest standard deviation (LSD) test. Comparisons with $P \leq 0.05$ were considered significantly different.

RESULTS AND DISCUSSION

Due to the sessile nature of plants, plants are subjected to series of environmental stress both abiotic and biotic. Stress isan unfavourable condition or substance that affects or blocks a plant's metabolism, growth, or development. It can also be defined as one which alters an equilibrium.Stresses that effectgermination may affect plant propagation and yield which in turn influences the biodiversity and agriculture.The results of the

investigation in terms of stress markers, antioxidants and antioxidant enzymes which determined the overall response of the plant *Pennisetum glaucum* (Pearl millet) to applied cadmium stress and the possible alleviation of this stress by supplementation with Biochar are discussed in this paper.

Chlorosis in the leaf laminae was visible in Cd treated plants (Fig 1). These results are similar to those seen in chickpea plants that have been exposed to Cd [26]. Chlorosis is thought to be caused by an increase in the production and accumulation of reactive oxygen species (ROS), which leads to a decrease in photosynthetic activity as shown by a decrease in chloroplastic pigments. Chlorotic leaf necrosis could be caused by Programmed Cell Death (PCD) arising due to Cd toxicity [27]. Similar morphological changes have been observed in *Brassica juncea*and Indian mustard grown in Cd and Hg respectively [28,29].

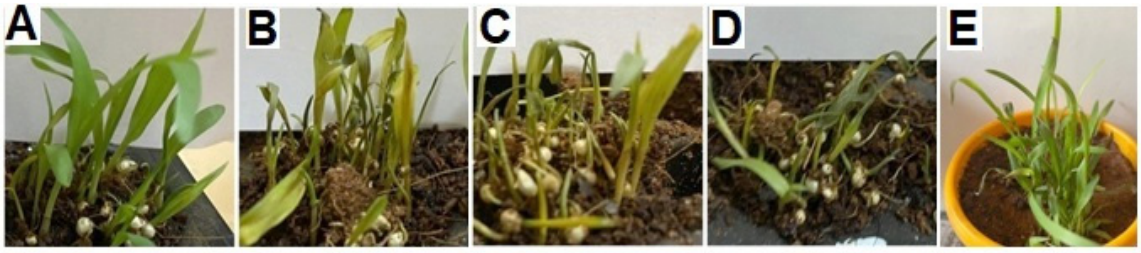

Fig 1: Cadmium stress treatment: Pearl millet seedlings were treated with differing $CdCl_2$ concentrations. A) Control; B)50mg/kg; C) 100mg/kg; D)150mg/kg; E) Cd150mg/kg + CMB supplementation. A decrease in the surface area and chlorosis of leaves in stressed seedling can be seen.

Exposure of Pearl millet seedlings to Cd resulted in a noticeable decrease in the growth parameters such as length and weight of shoot, tolerance index and vigor index, and RWC (Table 1) due to inhibition of water absorption and translocation in the plant. Similar results were seen in bush bean [30] and hyacinth bean [31] seedlings and was due to the effect of the metal on phytohormones resulting in a decrease in the number and size of xylem vessels [32]. Increasing Cd levels in the shoots of Pearl millet seedlings corresponded to the concentration of $CdCl_2$ in the growth medium (Table 1). Similar

concentration-dependent uptake of Cd was also reported in *Brassica juncea* [28], faba bean [33], maize [34], and *Lablab purpureus* [31].

Table 1: Effect of Cd stress on metal uptake and germination indices of Pearl millet.

[Metal]	Metal uptake	Length (cm)	Tolerance Index (TI)	Vigor Index (VI)	RWC (%)	Weight (g)	
						Fresh	Dry
0	0.00	6.2 ± 0.3	-	98.61	93.6 ± 0.7	1.27 ± 0.09	0.31± 0.01
5	0.14	5.6 ± 0.9	72.16	87.12	87.9 ± 3.2	1.06 ± 0.05	0.18 ± 0.05
10	0.36	5.0 ± 0.3	68.31	79.01	79.3 ± 1.7	0.70 ± 0.06	0.10 ± 0.00
50	0.71	4.1 ± 0.6	52.65	65.33	69.7 ± 2.4	0.63 ± 0.04	0.04 ± 0.02

Accumulation of the osmolyte proline is a response to metal toxicity is associated with signal transduction through which it prevents membrane distortion [35]. Proline as a compatible solute will help plants to adjust to a variety of environmental factors and also serves as an adaptive mechanism to stabilize cellular and sub-cellular structure and function, scavenging surplus ROS, and controls redox environment.Proline was found to increase when stressed with $CdCl_2$in a concentration-dependent manner, stressed seedlings supplemented with CMB exhibited lower levels of proline (Fig 2).Increase in proline content under Cd stress may be due to heightened activity of proline biosynthetic enzymes as well as over expression of as pyrroline-5-carboxylate reductase and gamma-glutamyl kinase genes [36].

The total soluble sugars (TSS) serve to vitrify the cytoplasm by which maintenance of various cellular membranes and structures are protected from harmful influences of stress. By increasing TSS under Cd stress conditions, the excess carbohydrate storedenables the maintenance of basal metabolism at the desired level.TSS levels increased with an increase in $CdCl_2$ concentration of the treatment media (Fig 2). Supplementation with CMB caused only minor increase in TSS levels. The enhanced sugar levels are also implicatedin decreased RWC (Table 1) of cells, contributing to a reduction in plant growth [37].

Abiotic and biotic stress conditions modulate polyphenolsynthesis. A rise in total phenol levels has an important role in scavenging toxic radicals, thereby preserving cellular structure. Total phenol (TP) levels in the leaves of Pearl millet were found to increase in a concentration-dependent manner (Fig 2). On supplementation with CMB, the levels of total phenolics declined when compared to that of Cd stress. Under Cd stress, total phenols accumulate, implying that phenolic chemicals are stress-induced. Excess ROS is created in stressed plants, which is extremely reactive and toxic, and destroys carbohydrates and proteins. By modifying peroxidation kinetics, plants improve reactive oxygen scavenging systems to avoid severe effects. Therefore, increasing phenolic content is a protective strategy with a potential of relieving ROS [37].

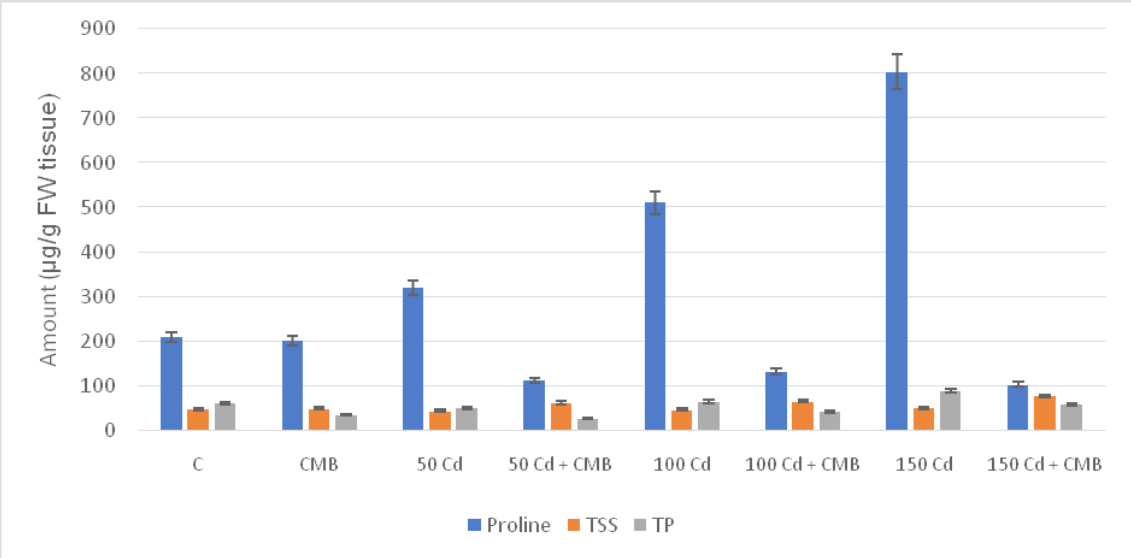

Fig 2: Effect of $CdCl_2$ on the levels of proline, total soluble sugars (TSS)and total phenols (TP) in Pearl millet. Pearl millet treated with different concentrations of $CdCl_2$ (50,100,150 mg/kg) and supplemented with chicken manure biochar (CMB).

Cadmium stress increased H_2O_2levels in the leaf tissue of Pearl milletwhile the application of CMB significantly reduced it (Fig 3). The high levels of H_2O_2 in Cd stressed plants are attributed to an increase in SOD activity and decrease in CAT activity (Fig 4). There was a significant increase in H_2O_2content in plants supplemented with

CMB, possibly due to lipid peroxidation on account of Cd-mediated membrane damage [9]. Glutathione is a water-soluble antioxidant with a non-protein thiol group that is widely distributed throughout the cytosol, chloroplast, mitochondria, apoplast, and peroxisome. It scavenges a range of ROS such as H_2O_2 and OH^- [38]. Reduced GSH is found to increase as the concentration of Cd increases whereas there is a slight decrease in the levels of GSH when supplemented with CMB(Fig 3). Ascorbate is a powerful ROS scavenger in the cell, reducing H_2O_2 to water through the activity of APX [38]. The amounts of ascorbate were found to be the same in all the Cd stress treatments (Fig 3). Guaiacol peroxidase, an enzyme that oxidises phenols and flavonoids, uses ascorbate to receive an electron from phenoxyl or other flavonoid radicals as a substrate. Ascorbate is transformed into the monodehydroascorbate radical, which can then be converted to dehydroascorbate [39]. On application of CMB there was a significant and identical decline in the levels of ascorbic acid (Fig 3).

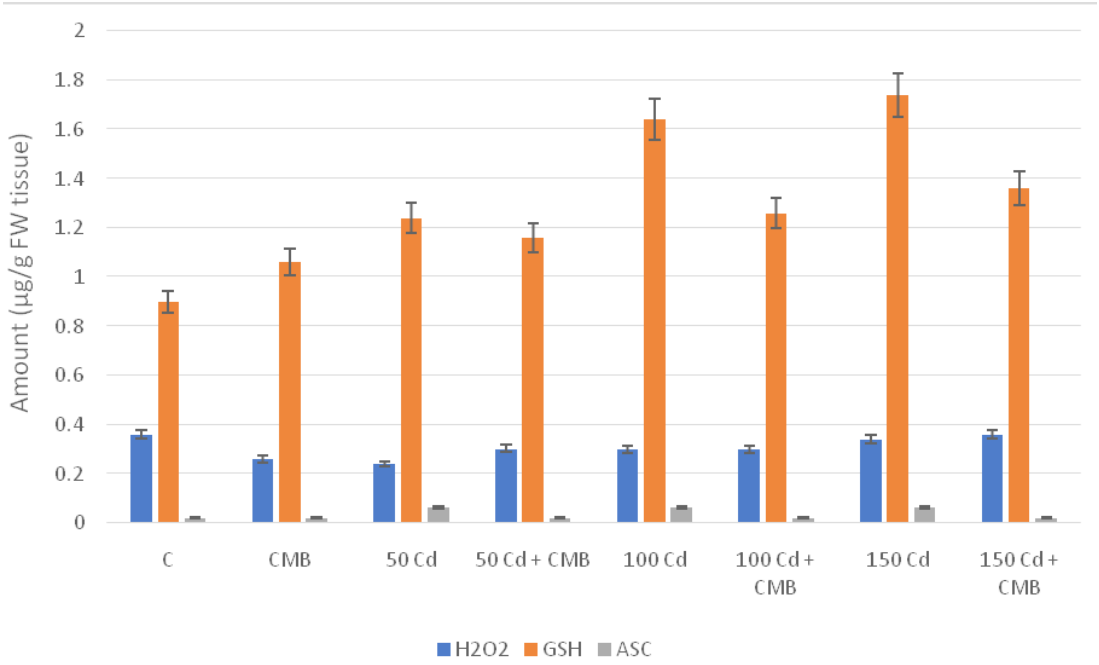

Fig 3: Effect of $CdCl_2$ on the levels of H_2O_2, reduced glutathione (GSH) and ascorbate (ASC) in Pearl millet. Pearl millet treated with different concentrations of $CdCl_2$ (50,100,150 mg/kg) and supplemented with chicken manure biochar (CMB).

In aerobic organisms, catalase (CAT) is vital forcontrolling O_2- and H_2O_2 levels through cellular detoxification. There was no activity of catalase (CAT) in the cadmium stressed plants whereas when supplemented with biochar increased activity was found. The decreased activity of CAT in Pearl millet (Fig 4) subjected to elevated levels of Cd arises due to inhibition of the enzyme and its synthesis [40]. The activity of POX was found to decrease as the concentration of Cd increases but is elevated on the application of CMB (Fig 4). Glutathione reductase (GR)is involved in the breakdown of H_2O_2 via the ascorbate-glutathione cycle. The activity of glutathione reductase (GR) is increased in a concentration -dependent manner with Cd concentration, whereas application of biochar causes a slight decrease (Fig 4). GR Elevated levels of GR are attributed to an increase in its substrate GSSG [41]. NADPH is used as a reductant by the enzyme. GR is an antioxidantenzyme that scavenges ROS such as hydrogen peroxide and superoxide and hence plays a vital role in the response to oxidative stress by keeping the intracellular glutathione pool primarily in the reduced form. Due to its effective ROS scavenging capabilities, overexpression of GR resulted in abiotic stress tolerance in a variety of agricultural plants [42].

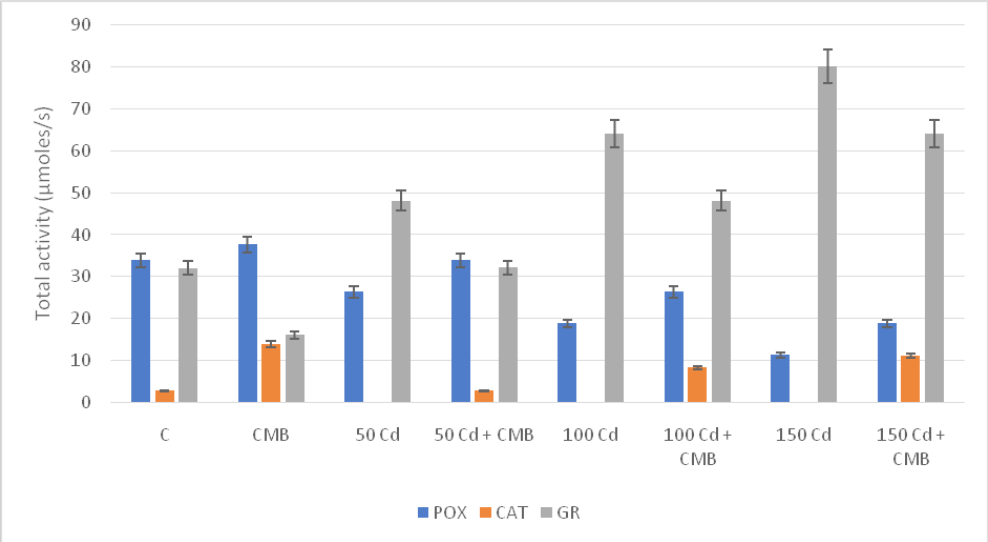

Fig 4: Effect of $CdCl_2$ on the activities of guaiacol peroxidase (POX), catalase (CAT) and glutathione reductase (GR) in Pearl millet. Pearl millet treated with different concentrations of $CdCl_2$ (50,100,150 mg/kg) and supplemented with chicken manure biochar (CMB).

CONCLUSION

According to this study, introduction of realistic levels of $CdCl_2$ to the growing medium appears to disrupt cellular redox state of Pearl millet. The plant has a well-coordinated antioxidative defence system that works by coordinating activities of antioxidative enzymes and metabolites in a timely and sequential manner. The capabilities of some enzymes rose even before evident adverse consequences could be seen. Furthermore, the ascorbate-glutathione cycle plays a major role in dissipating oxidative stress caused by heavy metals. The oxidative stress induced is most likely indirect because Cd is not a redox-active metal. To determine the underlying molecular and metabolic mechanisms, further study on the indirect mechanism of Cd-induced oxidative stress is required.

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Synthesis and Characterization of Chitosan Linked Plant Nanopolymer using *Allium Fistulosum* Extracts

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Abstract : *The present investigation was aimed to synthesize and characterize chitosan nanopolymer from the methanolic leaf extracts of Allium fistulosum. In this study, the plant sample was extracted using methanol as a solvent. Phytochemical screening and TLC was performed to analyse the phytochemical constituents present in the extract. The synthesis of chitosan plant nanopolymer was performed using ion-gelation technique and the formed nanopolymers were characterized by UV-Visible spectrophotometer and Fourier-transform infrared spectroscopy. The phytochemical screening indicated the presence of various constituents like terpenoids, tannins, flavonoids, reducing sugars, phenolics, cardiac glycosides and amino acids. The UV-Vis investigation showed the increase in the optical density of chitosan plant nanopolymer when compared to chitosan standard which was due to the bonding between the functional groups of chitosan and the phytochemical compounds like flavonoids. FTIR results revealed the strong peaks at 3319.53 cm^{-1}, 2084.10 cm^{-1}, 1636.25 cm^{-1}, 1277.59 cm^{-1}, 1016.17 cm^{-1}, 566.25 cm^{-1} demonstrating the presence of O-H bonds and C=C stretch at 1636 cm^{-1} confirms synthesized chitosan plant nanoparticle. The C-N stretch for aliphatic amines is indicated with peaks at 1017 cm^{-1} and the aromatic groups at 530 cm^{-1}. The results obtained suggests that the synthesized chitosan plant nanopolymer could be considered as a promising substance for varied bio-medical applications.*

Keywords: *chitosan, nanopolymer, Allium fistulosum, spring onion, chitosan plant nanopolymer, FTIR.*

INTRODUCTION

The nanoscience and technology industry is presently aiding nanoscience as "green technology" which improves the environmental conditions of the prevailing industries and allow achieving environmentally benign economic expansion. The breakdown of organic compounds by the green technology approach using plants can prominently be due to the presence of polyphenols present in the degradable material [1]. They are polymeric biomolecules for example long chain biomolecules involved covalently connected rehashing monomeric units [2]. Biopolymers do have endless number of indispensable capacities, for example, acting as energy reservoir, protection and transmittance of hereditary data, and cell development, in vivo.

Chitosan a polysaccharide of chitin has a molecular weight between 300-1000 kDa based on its origin or source. Cellulose is considered as the major and abundant polymer on the planet followed by chitin. It is found in crustaceans. The chitosan has deacetylation degree with 60% on glucosamine residue. Chitin can be deacetylated by chemical hydrolysis under strong alkaline conditions or by the action of enzymes such as chitin deacetylases [3][4]. Chitosan is excellent in biocompatibility; it can form gel and thin film; it can chelate and has absorptive capacity. These unique properties will provide a wider range of applications to Chitosan, such as: drug carrier for controlled release [5]. Chitin or chitosan derivatives when conjugated with specific anticancer agents can exhibit a very good anticancer effects with gradual release of free drug in cancer tissues. Chitosan promotes phagocytosis and production of ostepontin and leukotriene B by polymorphonuclear leukocytes, production of interleukin-1and production of interleukin-8 by fibroblasts, enhancing immune responses. Chitosan and hydroxyapatite are the well-known bioactive biomaterials in the field of bone tissue engineering. Chitosan or Hydroxyapatite composite materials results in better mimicking the organic portion and in mimicking the inorganic portion of natural bone [5].

Allium fistulosum, the Welsh onion, also commonly called bunching onion. Usually known as spring onion, is a type of evergreen plant developing consistently. This has

characteristic hollow leaves and scapes. A typical vegetable in Asia, where there are a wide range of cultivars, it is generally developed more on a nursery scale in Europe and America, essentially for its edible leaves [6]. *Allium fistulosum* usage has proved in reducing the risk of developing the cardiovascular disease and can act as anti-cancer due to its antiorostanoid and anti-inflammatory responses and they also reduces the rate of DNA damage [8][9]. A tea made from the root extracts of *Allium fistulosum* is used as sedative for children [10]. The purpose of this study was to synthesize chitosan linked plant nanopolymer using *Allium fistulosum* extract and characterizing it using UV-Visible spectrophotmeter and FTIR.

MATERIALS AND METHODS

Chemicals: Methanol, Fehling's A and Fehling's B solution, α - naphthol, Benedict's reagent, sodium hydroxide, copper sulphate, Nitric acid, Ninhydrin reagent, Dragendroff's reagent, ferric chloride, chloroform, sulphuric acid, glacial acetic acid, ammonia, isopropanol, distilled water and benzene was obtained from Fischer Scientific. TLC silica gel plate was of silica gel GF_{254} Merck. Chitosan was analytical grade chemicals form HiMedia, Karnataka.

Plant source: The leaves of Spring Onion (*Allium fistulosum*) was collected from Ulsoor market, Bangalore, Karnataka. India.

Extraction: The plant extract was prepared from the leaves of *Allium fistulosum*. The residue particles were expelled by washing under running tap water. The leaves of *Allium fistulosum* was dried at 60°C in hot air oven and made into fine powder utilizing the blender. The coarse powder of *Allium fistulosum* [2.5g (w/v)] was extracted with 80% methanol using magnetic stirrer for 1hr and extract was filtered using a muslin cloth and the filtrate obtained was utilized for further examination.

Phytochemical test: The fresh methanol extract was subjected to various phytochemical screening for the detection of various plant constituents, characterized for their possible bioactive compounds [13] - [15].

Analysis by thin layer chromatography: TLC was performed to identify the major flavonoids present in the methanolic concentrate of *Allium fistulosum.* TLC was performed on a silica gel plate (silica gel GF254, Merck) 2-5 µL of different plant extract was deposited to the origins of TLC plates from the 1.5cm of the origin with the help of capillary tubes. After the application of the sample on the plates, the plates were kept in TLC glass chamber (solvent saturated) then mobile phase was allowed to move through adsorbent phase up to 3/4th of the plate. The solvent system used was - Benzene/Acetic acid/water (125:72:3). TLC plates were visualized under ultraviolet light (λ 254 and 366 nm). Retention values of the different bands were then calculated using the equation:

$$R_f = \frac{\text{Distance travelled by component}}{\text{Distance travelled by solvent}}$$

Synthesis of chitosan nanopolymers: The chitosan nanopolymer is synthesized by ionic-gelation method. About 1% (w/v) of the plant extract was added drop wise into the chitosan solution containing 0.1% (w/v) chitosan and 1% (v/v) acetic acid under gentle magnetic stirring on REMI 2-MLH. The solution was incubated for 20 minutes and used for characterization.

CHARACTERISATION OF NANOPOLYMERS

UV-Vis spectrophotometer: UV-Visible spectrum was used to observe the interactions between chitosan and the target compound present in the plant extract. The absorbance spectrum of the nanopolymer suspension was recorded immediately after the incubation period of synthesis was completed and deionized water was used as a reference. To determine the stability of nanopolymer, the absorption spectrum was recorded for pure chitosan extract and chitosan-*Allium fistulosum* extract. The spectrophotometer used was a Chemito UV Scan 2600 (Thermo Fisher) and the software was Spectrum™ Version 6.87. Absorbance spectra were recorded over the range of 220-400 nm. Wavelength of peak absorbance and λmax was calculated.

FTIR spectral analysis: After the synthesis, the chitosan nanopolymer suspension was used for FTIR spectroscopy studies. First, background measurement was taken without placing the sample. Then, FTIR analysis of chitosan nanopolymer sample was performed with Burker Fourier transform infrared spectrometer, model Alpha II. Sample spectra were recorded in the range from 4000cm $^{-1}$ to 500cm $^{-1}$ with the resolution of 4cm $^{-1}$ in the absorbance mode for 16 scans at room temperature. FTIR spectrum of chitosan-plant nanopolymer sample was compared with the spectrum of Chitosan standard.

RESULTS AND DISCUSSION

In this study, chitosan nanopolymer was synthesized and to characterized from the methanolic leaf extracts of *Allium fistulosum. Allium fistulosum* is easily available in the market and it possesses wide range of phytochemicals. These possesses enormous applications in ethnobotany, pharmacology and various other applications. Apigenin in particular has enormous benefits in the field of medicine.

Phytochemical analysis: Methanolic extract of *Allium fistulosum* was subjected to phytochemical and pharmacological activities. The phytochemical screening showed the presence of various constituents like terpenoids, tannins, flavonoids, reducing sugars, phenolics, cardiac glycosides and amino acids. The phytochemicals present in *A. fistulosum* which revealed that the crude extract of *A. fistulosum* with ethanol indicates the existence of alkaloids, phenols, tannins, terpenoids, flavonoids and saponins whereas aqueous extract indicated the existence of tannins, saponins, terpenoids, phenols but not flavonoids [14]. This revealed that the organic solvents could be more effective than the inorganic solvents for the secondary metabolite extraction.

Table 1: Phytochemical screening of the *Allium fistulosum*

Phytochemical test	*Allium fistulosum*
Fehling's test	++
Molisch test	-
Benedict's test	+
Biuret test	-
Xanthoproteic test	-
Ninhydrin test	++
Dragendroff's test	-
Test for Tannins	+
Teat for Flavonoids	+
Test for Terpenoids	-
Libemann-Burchard's test	-
Keller Killani's test	+
Borntrager's test	-
Test for Phenols	+

Analysis of the plant extracts by TLC: Thin layer chromatography for the methanolic extract of *A. fistulosum* was performed. TLC profiling of this plant extract indicated the existence of phytochemicals present in the sample. Different type of solvent systems was used to confirm the presence of diverse potent biomolecules in these plants. TLC investigation delivers the information on polarity of a chemical constituent such that a compound displaying high R_f value in the less polar solvent system possess lesser polarity and the compound with less R_f value possess higher polarity. TLC results revealed the presence of flavonoid in the *A. fistulosum* plant extract.

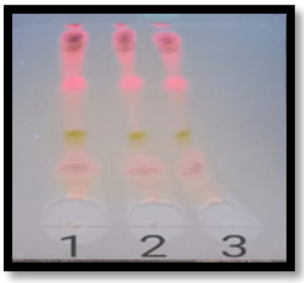

Fig 1: TLC profiling of *A. fistulosum* extract under long UV (365 nm). Lane 1, lane 2 and lane 3 are crude extracts of *A. fistulosum*

Characterization of nanopolymer: Chitosan is a natural compound as stated previously and has a wide scope for applications due to the prevalence of varied functional properties. Nowadays nanopolymers play a major role in the drug delivery. So, the bioactive component (phytochemical components) loaded chitosan could be a very effective compound.

UV Spectrophotometer analysis: The validity of the prepared chitosan *A. fistulosum* nanopolymer was determined spectrophotometrically. A strong peak of chitosan *A. fistulosum* nanopolymer was observed at 0.736 O.D. As revealed, at 220-322nm chitosan nanoparticles showed broad absorption bands in UV Vis spectrophotometer analysis [17]. The optical density of both pure chitosan and chitosan *A. fistulosum* nanopolymer were compared and the increase in the optical density of chitosan *A. fistulosum* nanopolymer confirmed the synthesis of nanopolymer. In our investigation, the increase in the absorbance of chitosan plant nanopolymer was due to the bonding between the amino and hydroxyl groups of chitosan in the presence of phytochemical compound such as flavonoids. This indicated that chitosan was linked to the flavonoid molecules.

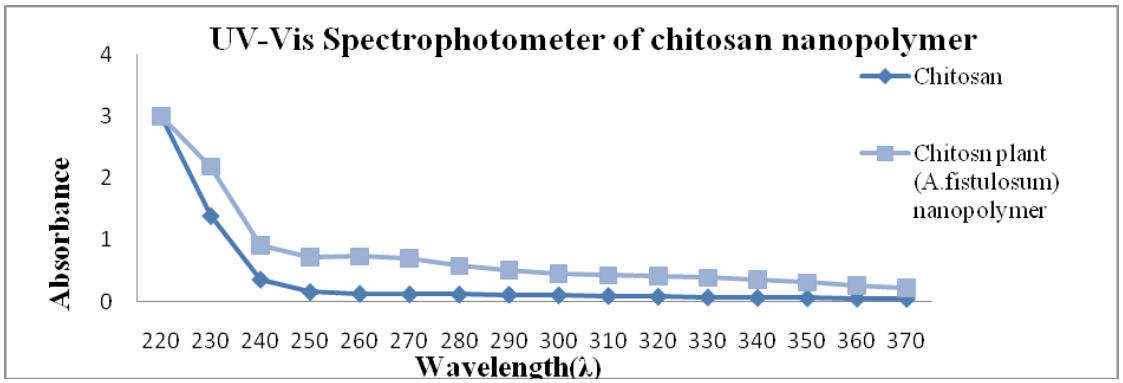

Fig 2: UV-Visible spectrum of pure 0.1% chitosan and solution of 0.1% pure chitosan + 1% *Allium Fistulosum* plant extract.

FTIR analysis: FTIR analysis was employed for the determination of plant chitosan interactions. FTIR analysis was conducted to recognize the possible interactions which led to the formation of chitosan nanopolymers. The analysis of chitosan nanopolymer and chitosan-plant nanopolymer have been depicted in the figure (3) and (4). The IR studies of chitosan nanopolymer indicated peaks at 3326.83 cm^{-1}, 2125.08 cm^{-1}, 1635.99cm^{-1}, 538.28 cm^{-1} while the FTIR spectra of *A. Fistulosum* chitosan nanopolymer indicated peaks at 3319.53 cm^{-1}, 2084.10 cm^{-1}, 1636.25 cm^{-1}, 1277.59 cm^{-1}, 1016.17 cm^{-1}, 566.25 cm^{-1}. The peak obtained at 3320 cm^{-1} for the synthesized nanoparticle confirms the O-H bonds presence. The peak at 1636 cm^{-1} indicated the interaction of ammonium group of chitosan with that of the phosphorous groups. The isolated alkene groups of the C=C stretch was found at 1636 cm^{-1} for the biomolecules loaded chitosan nanoparticle. The C-N stretching of aliphatic amines was observed at the peaks of 1017 cm^{-1} and the aromatic groups presented with an intense peak at 530 cm^{-1} for the synthesized nanoparticles. The results obtained indicate that there is an enhanced inter and intra-molecular actions in chitosan nanoparticles due to the presence of several functional groups present that was present both in plant and the chitosan.

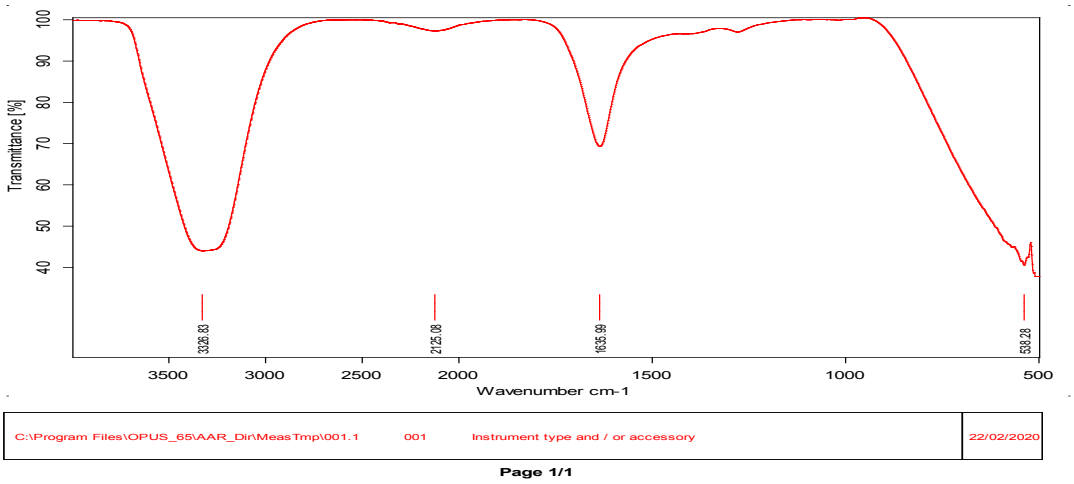

Fig 3: FTIR Spectral analysis of chitosan nanoparticles

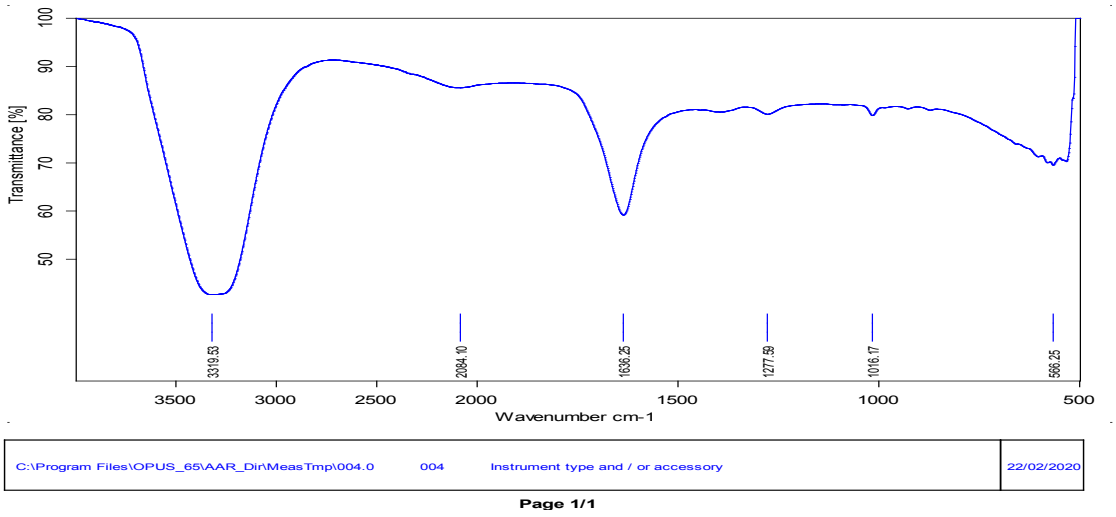

Fig 4: FTIR Spectrum analysis of chitosan *A.Fistulosum* nanopolymer

CONCLUSION

The present study was aimed at the synthesis of effective chitosan plant nanoploymers using *Allium fistulosum* leaves. This study indicated that their leaves contained good amounts of secondary metabolites like flavonoids, tannins, terpenoids,

phenols, glycosides and amino acids. Chitosan linked plant nanopolymer was synthesised using 5% methanolic extract of Allium fistulosum leaves and was characterised by UV-Vis spectrophotometer and FTIR. The investigation using UV-Vis confirmed the formation of chitosan linked plant nanopolymer FTIR results indicated the presence of O-H, C=C stretch, C-N stretch of aliphatic amines and the aromatic groups were present in the synthesized nanoparticles. It is very evident that the chitosan linked plant nanopolymer using *Allium fistulosum* possess promising applications in the field of nanomedicine, industrial and pharmaceuticals and biomedical engineering, fields. This could be more effective than the chitosan itself. Later on, nanotechnology may gain more progress and they will be utilized progressively in the fields of tumor immunizations, gene and drug carriers.

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Isolation of pectin from *Artemisia vulgaris* and *in vitro* studies of residue after extraction of pectin

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Abstract : *Artemisia vulgaris commonly known as mugwort is an aromatic plant used as folk medicines. This study on A. vulgaris was conducted by isolating the pectin from the plant, whereas the residue obtained after the separation of the pectin was tested for antioxidant, anti-inflammatory and antihelminthic properties. The pectin obtained from the plant was tested for its methoxyl content which was present in a low quantity of 8.525% which can be used for commercial purposes. The antioxidant property of the plant was analyzed where the maximum scavenging percentage was obtained from 1% of stem and leaf residue. Anti-inflammatory activity was investigated by measuring inhibition of protein denaturation activity, where maximum inhibition was seen in leaf residue (10 mg/mL) showing 87.39% inhibition. The aqueous residue extract of the leaf showed a positive antihelminthic property in 100 mg/ mL concentration.*

Key words: *Low methoxyl pectin, anti-inflammatory activity, antihelminthic activity, antioxidant property.*

INTRODUCTION

Artemisia vulgaris belongs to the family Compositae is native to Asia, Europe, and Northern Africa. It is grown in semiarid or arid areas of the Mediterranean basin and Himalayas [1]. Phytochemical studies on these plants indicate the presence of several classes of bioactive compounds, such as terpenoids, coumarin derivatives, caffeoylquinic acids, sterols and flavonoids. The presence of significant amounts of essential oils and

other terpenoids in this species can be attributed to their potential uses as flavouring agents in foods and as ingredients of pharmaceutical products [2].

Pectin is a linear 1, 4- α linked D-Galacturonic acid chain with varying degrees of methylation. Pectins are structural polysaccharides found in most plant cell walls holding the cellulose fibrils and linked covalently to other polymers [3]. Pectins are extensively used as food additives as emulsifying, gelling and stabilizing agents in jam, jellies, milks and confectionery products [4]. Recently pectin polymers are commercially used as edible films or edible coatings that act as a natural barrier to protect fruits and vegetables from microbial contamination, yet they allow the exchange of gases, moisture between the environment and food [5]. Commercial pectins are generally viewed as gelling polysaccharides isolated from miscellaneous plant byproducts and are extracted at low pH and high temperature. Short hydrolysis times produces high-methoxyl pectins (HMP), while extended acid treatment de-esterifies the methyl esters to pectic acids and generates low-methoxyl pectins (LMP) [6]. HMP is used as gelling agents in presence of sugar and acid, whereas LMP does not require sugar for gelation, instead they form gels in presence of divalent cations like calcium ions (Ca^{2+}), through ionic interactions [7]. LMP is used as gelling agent in dietetic jellies and jams. Also, LMP gels can be repetitively remelted and reformed. Therefore, LMC pectins are a better choice as commercial gelling agents in the baking industry [8].

From ancient times natural products have been considered an important source of medicines as they are rich in a vast array of bioactive molecules. Natural products are the basis of many commercially available drugs in modern medicine [9]. Most of the researchers focus on obtaining eco-friendly pharmaceutical agents with good solubility, stability, reduced toxicity to humans and other animals and also high biological activity [10]. The use of medicinal plants for treatment has been followed traditionally, but not with much scientific validation. *In-vitro* studies of the antioxidant properties of Indian medicinal plants can lead to discover a superior and safer way of relieving oxidative stress [11]. Anti-inflammatory drugs interfere in the pathophysiology of inflammation, minimizing

tissue damage. The major classes of anti-inflammatory agents are the glucocorticoids and non-steroidal anti-inflammatory drugs (NSAIDs) which demonstrate possible side effects [12]. Moreover, synthetic drugs are very expensive to develop. Therefore, anti-inflammatory drugs from natural sources with minimal side effects are being explored all over the world as an alternative to available drugs [13]. On the contrary, countless medicines of plant origin have been used for long time without any adverse effects. The research into plants with potential folk use as pain relievers, anti-inflammatory agents, should therefore be viewed as a rational research strategy in the search for novel anti-inflammatory drugs [14]**.** Parasitic helminthes of humans and other animals cause diseases of major socio-economic importance globally. In particular, parasitic nematodes have immense, long-term impact on human health particularly in children. The World Health Organization (WHO) estimates that ~ 1.5 billion people were infected with intestinal worms in 2018, predominantly in disadvantaged communities [15]. Parasitic worms also infect livestock, with a resultant economic impact [16]. Medicinal plants have been used traditionally for centuries and are mostly safe, therefore can act as a source of effective treatments in antihelminthic therapy [17].

This article describes the isolation, identification, and analysis of pectin for commercial application and also pharmacological studies of the extracts of the *Artemisia vulgaris* such as antioxidant, anti-inflammatory and antihelminthic properties. The studies were carried out based on the folklore claims to give scientific evidence and to support the usage of these plants.

MATERIALS AND METHODS

Extraction and identification of pectin from *Artemisia vulgaris*

Isolation of pectin was carried out from the leaves and stem of the plant separately. The samples were homogenized using de-ionized water (1:1.5 w/v). Lemon juice was added to the homogenate to adjust the pH to 2-2.5. The autoclaved homogenate was filtered, and the pectin was precipitated from the filtrate by using an equal volume of ethanol. The procedures from FCC-Food Chemical Codex were used to identify and

characterize the extracted pectin [18, 19]. The tests given below were carried out to identify the formation of pectin.

(a) To the sample in water, an equal volume of alcohol was added. A gelatinous precipitate confirms the presence of pectin.

(b) To 5 mL of the sample in water, 1 mL of sodium hydroxide was added and allowed to stand at room temperature for 15 min. A gel was formed which indicated the presence of pectin.

(c) To the gel formed in the previous step 1 mL of HCl was added, a colourless, gelatinous precipitate formed, which on boiling, became white and flocculent. This indicates the presence of pectinic acid.

Determination of methoxyl content of pectin

Methoxyl content of pectin was analyzed according to Ranganna, [20]. Briefly, 0.5 g of Pectin substance was weighed in a conical flask and moistened with 5 mL of ethanol. 1 g of sodium chloride was added to sharpen the end point. 100 mL of carbon dioxide free distilled water was added followed by 6 drops of phenol red indicator. All the pectin substances were dissolved without any lumps and titrated against 0.1 N NaOH until the colour of the indicator changes (pH 7.5) and the colour change should persist for at least 27-30 seconds. The neutralized solution is used for methoxyl determination. 25 mL of 0.25 N sodium hydroxide was added and shaken thoroughly and then allowed to stand in room temperature in a stoppered flask. 25 mL of 0.25 N HCl was added and titrated against 0.1 N NaOH to the same end point as before.

Evaluation of in-vitro antioxidant activity

The residue obtained after pectin extraction was sun-dried and utilized for preparing extracts. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) method was used for estimating Free Radical Scavenging Activity of the methanolic extracts as suggested by Braca et al., [11]

2 mL of methanolic solution of DPPH (0.1 mmol) was mixed with various two different doses of leaf and stem residue methanolic extract (5 mg/mL and 10 mg/mL) in test tube and final volume of 3 mL was made with methanol. The absorbance of the mixture was measured after 40 min at 517 nm against methanol as blank. Ascorbic acid was used as standard. FRSA (%) of test samples was evaluated by comparing with control (2 mL DPPH and 1 mL of methanol).

Evaluation of *in-vitro* anti-inflammatory activity

Anti-inflammatory assay by measuring inhibition of protein denaturation was performed according to the procedure given by Mizushima et.al [21] and Sakat et.al [13]. 1% BSA was used as a control. Aspirin was used as standard drug that inhibit the protein denaturation process.1 mL of BSA was treated with 0.2 mL of different samples of plant and with 0.2 mL of Aspirin. It was incubated at 51°C for 20 minutes. The solution was cooled, and the absorbance was read at 660nm and the percentage of inhibition of protein denaturation was calculated.

Evaluation of antihelminthic activity

Antihelminthic activity of the given plant extract was evaluated by the procedure given by Ajaiyeoba et al [22] and Ashok Kumar et.al [23]. The earthworm models were separated in two groups. One group of earthworm models were used for the antihelminthic property using stem residue extract and other group using the leaf residue extract. The above groups were further divided into 5 each, where the concentration of the plant residue varied from 100 mg/5 mL to 500 mg/5 mL. The time of paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. The time of death of the worms were recorded after ascertaining that the worms neither moved when shaken vigorously or even when dipped in warm water. Albendazole (200 mg/5mL) was used as reference standard while water served a control.

RESULTS AND DISCUSSION

Extraction and identification of pectin:

Pectin a naturally occurring polysaccharide found in many plants that have long been used as the mechanism by which jams and jellies are thickened was isolated and the presence of pectin was tested which gave a positive test for the presence of pectin. The methoxyl content of pectin was tested and was found to have considerable percentage of methoxyl which contributes to the pectin quality and structure.

Pectin from the leaf and stem sample obtained from the ethanolic extraction of test plant was identified by using FCC-Food Chemical Codex.

Test 1: a gelatinous precipitate was obtained.

Test 2: formation of gel was observed.

Test 3: a colourless gelatinous precipitate was formed

Hence all the three tests confirm the presence of pectin in the sample.

Determination of methoxyl content of pectin:

From the calculated methoxyl content of the pectin obtained from leaf and stem both was observed to contain equal percentage of methoxyl content (8.525 %) which is not too high hence can be used as a good gelling agent. High methoxyl pectin requires a large amount of sugar for the process of gelling, but low methoxyl pectin requires sugar in less quantity, therefore low methoxyl pectin has been excessively used for baking and recipes designed low calorie diet and diabetic diets. Low methoxyl pectin can also be used as fat substitute in baked goods and to stabilize acidic protein drinks such as yogurt.

Evaluation of *in-vitro* antioxidant activity:

The DPPH has been widely used for estimating the free radical-scavenging activities of plant extracts which shows their antioxidant property. Antioxidant capacity of the extracts was calculated using FRSA in percentage. Increase in the scavenging percentage

inhibition means that the alcoholic extract of the plant at higher concentration captured more free radicals formed by DPPH. In the performed experiment 1% leaf residue extract i.e., 10 mg/mL showed 81.48% ± 0.87 scavenging inhibition followed by the 85.18% ± 2.64 inhibition by the stem residue extract (10 mg/mL). 5 mg/mL of the leaf and the stem residue extract showed 74.07% ± 1.28 and 77.7% ± 1.27 respectively. The maximum scavenging percentage was obtained from 1% of stem and leaf residue which corresponds to 10 mg/mL. This range of scavenging activity for standard ascorbic acid was obtained with a concentration of 40 µg - 80 µg/mL.

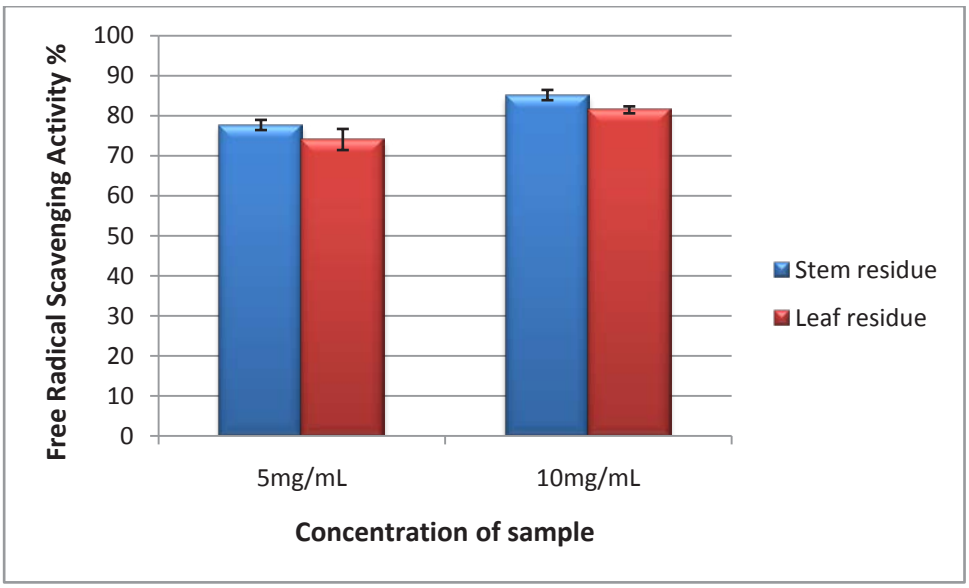

Fig 1: Graphical representation of the scavenging activity of the plant samples.

Evaluation of in vitro anti-inflammatory activity:

Screening of plant extracts for anti-inflammatory activity has shown that plants represent a potential source of new anti-inflammatory drugs. Protein denaturation is a well documented cause of inflammation. The leaf and stem extracts were effective in inhibiting protein denaturation. The maximum inhibition was seen in 1% (10 mg/mL) leaf residue showing 89.39% ± 0.62 inhibition followed by 1% (10 mg/mL) of stem residue which gave 83.51% ± 1.41 inhibition. The decrease in the concentration of the plant

residue to 0.5% i.e., 5 mg/mL showed a decrease in the inhibition of protein denaturation. In case of the stem residue, 0.5% showed 62.18% ± 0.537 inhibition and the leaf extract showed 68.06% ± 1.21 inhibition. Overall, maximum inhibition of protein denaturation was found to be in leaf residue. Hence it can be deduced that the plant sample has anti-inflammatory properties as compared to the standard aspirin of 1mg/mL exhibited 91.34% inhibition.

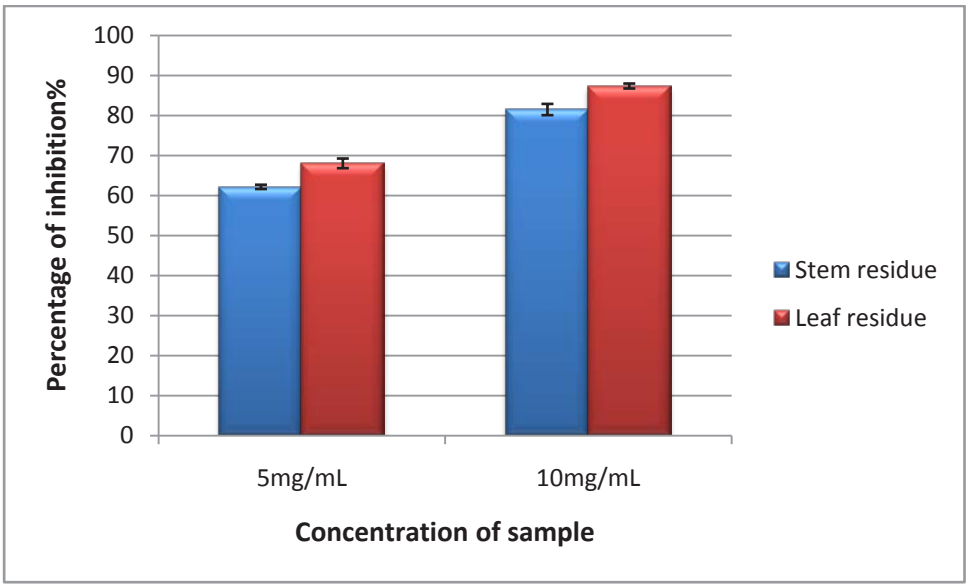

Fig 2: Graphical representation of the percentage of inhibition of protein denaturation obtained by aspirin and plant sample.

Evaluation of antihelminthic activity:

The antihelminthic property of the test sample was studied by using the plant sample and the standard de-worming drugs on the earthworms as earthworms resemble the parasitic worms living in our body. The time taken for the drug and the plant sample to paralyze and kill the worms was noted down to check how effective is the plant sample compared to the standard drug. It was observed that the stem residue extract had no effect on the worms and hence did not lead to paralysis or death. The leaf residue had

considerable effect on the worms with time. Leaf residue extract of 500 mg/ 5 mL was able to kill the organism in 57 minutes, whereas the reference standard Albendazole was able to kill the organism within 5 minutes. There was no paralysis observed in petri dish containing 100 mg/5 mL, 200 mg/5 mL 300 mg/5 mL. The movement of the organism was less in the petri dish containing 400 mg/ 5 mL concentration of the leaf extract. Therefore, it was observed that the plant leaf residue extract has antihelminthic property at a higher concentration.

Table 1: Results of antihelminthic activity, showing the time of paralysis and death of the earthworm when treated with standard drug and leaf residue

Sample	Concentration (mg/ 5mL)	Time of paralysis (minutes)	Time of death (minutes)
Control (water)		No paralysis	No death
Standard drug (Albendazole)	200	1 minute	5 minutes
Leaf residue	100	No paralysis	No death
	200	125 minutes	No death
	300	70 minutes	No death
	400	35 minutes	80 minutes
	500	21 minutes	57 minutes

CONCLUSION

This pectin extracted from *Artemisia vulgaris* had low methoxyl content which can be potentially used as a stabilizer and thickening agent in food processing. The in vitro studies using residue after pectin extraction showed promising antioxidant, anti-inflammatory and antihelminthic activities. Further scientific studies to prove the effective ingredient of the plants with the pharmacological activities would expand their scope for further use.

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A Comparative Study on Efficiency of *Moringa Oleifera* Leaf and Seed Powder and their Combinatorial Effect as Biocoagulants for Treating Water Bodies

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Abstract: *Moringa oleifera is a small or medium-sized tree that has several purposes. Its seeds are reported to have a strong water purification potential as a biocoagulant in treating waste or contaminated water. As it is economical when compared to other chemicals like alum and also with no side effects it is commonly used as a natural method. The present study investigates the physicochemical and biological properties of both M. oleifera leaf powder, seed powder and its combinatorial effect as a dose in treating lake water. Physicochemical properties include the pH, hardness, total dissolved solids (TDS), biological oxygen demand (BOD), dissolved oxygen (DO) and chemical oxygen demand (COD). Biological parameters such as Most Probable Number (MPN), Jar test (flocculator) were also conducted to ensure the quality of water and also to identify the purification potential of M. oleifera seed and leaf powder.*

Keywords: *Moringa oleifera, water treatment, biocoagulant, combinatorial effect, lake water.*

INTRODUCTION

Water pollution occurs when some harmful substances like chemicals, microorganisms are present in them that render the water quality. When these waters are untreated and consumed it causes adverse effects on human health by spreading

water-borne diseases like polio, typhoid, diarrhoea, cholera, poliomyelitis, hepatitis, encephalitis and related secondary disease conditions [1]. Thus, treatment involving improving the physical and chemical quality is required to convert polluted water into potable. Treatment for physical quality includes clarification, discoloration etc. and for chemical quality includes coagulation and disinfections. Coagulation is clumping together of very fine colloidal and dispersed particles into larger and visible miscellany of the particles by using chemicals called coagulants. There are several chemical coagulants in use among them aluminium sulphate or commonly called alum is most widely used. But studies have indicated that use of alum in treating water has some drawbacks such as human health hazards related to Alzheimer's disease which is associated with high aluminium residues in treated water.

Some of the natural coagulants used for the water treatment are *Cicer arietinum* (chickpea), *Moringa oleifera* (drumstick tree), *Lablab purpureus* (Dolichos lablab). *M. oleifera* has been confirmed to contain water soluble substances, proteins, and antibacterial activity in water treatment. Recently, many researchers have confirmed that *M. oleifera* seeds acts as a biocoagulant, a good alternative for alum in water treatment [2].

Moringa oleifera Lam., is one of the species belonging to family Moringaceae, commonly known as "Drumstick". The trees are found mainly in tropical and subtropical areas, native to the Southern foothills of the Himalayas in Northwestern India. It is a fast growing, drought resistant tree which can tolerate a wide range of soil conditions but prefer a neutral to slightly acidic (pH 6.3 to 7.0), well drained sandy or loamy soil [3]. Compared to other natural chemical coagulants, *M. oleifera* has several advantages such as low cost, production of low sludge and also it has no effect on pH of the water. *M. oleifera* seeds also possess turbidity removal and antimicrobial properties. They contain natural polyelectrolyte which clarifies turbid waters. After the treatment with seed powder pH, electrical conductivity, TDS, TSS, hardness, chlorides, COD, BOD was reduced with increasing dose [4-8]. Various parts of *M. oleifera* have been analysed for secondary

compounds such as glucosinolates and phenolics, anthocyanins, proanthocyanidins and cinnamates. These functional groups that contain sulphur and nitrogen are responsible for good metal sequestration from aqueous solution [9].

Recent work has also explored the uses of *M. oleifera* leaves in pharmaceutical and water treatment process. It is also used as a protein supplement for human being and animals due to the presence of high protein content and it can be considered as safe supplement due to its non-toxic effect. Also used as a bio-sorbent or coagulant for heavy metals in water treatment [10].

MATERIALS AND METHODS

Study area: Bengaluru city. Sampling Sites: Kudlu Lake Park near VGP layout Bengaluru.

Preparation of seed powder: *M. oleifera* seed powder was purchased from the market and stored in an airtight container.

Preparation of leaf powder: The leaves of *M. oleifera* were collected from the market. The leaves were separated, washed and allowed it to oven-dry at 60ºC for 3 hours. After proper drying, the leaves were pulverized under aseptic conditions using a grinder into a fine powder, sieved and stored in a dry airtight glass jar [11].

Jar Test: The powders were directly used as coagulants to treat the lake water. Treatment to water was given by directly mixing seed and leaf powder. The water quality parameters were checked before and after treatment. Doses of seed and leaf powder i.e., 50, 100 and 200 mg/l were selected for treatment [12]. The best results were chosen for the synergistic effect and checked the results keeping alum as a control. The powders were mixed in a jar containing lake water and were mixed continuously for 4-5 min then left overnight for settling. After settling the water sample was filtered and the filtrate was further carried out to analyse the water quality parameters for understanding physicochemical characters as per standard methods [13].

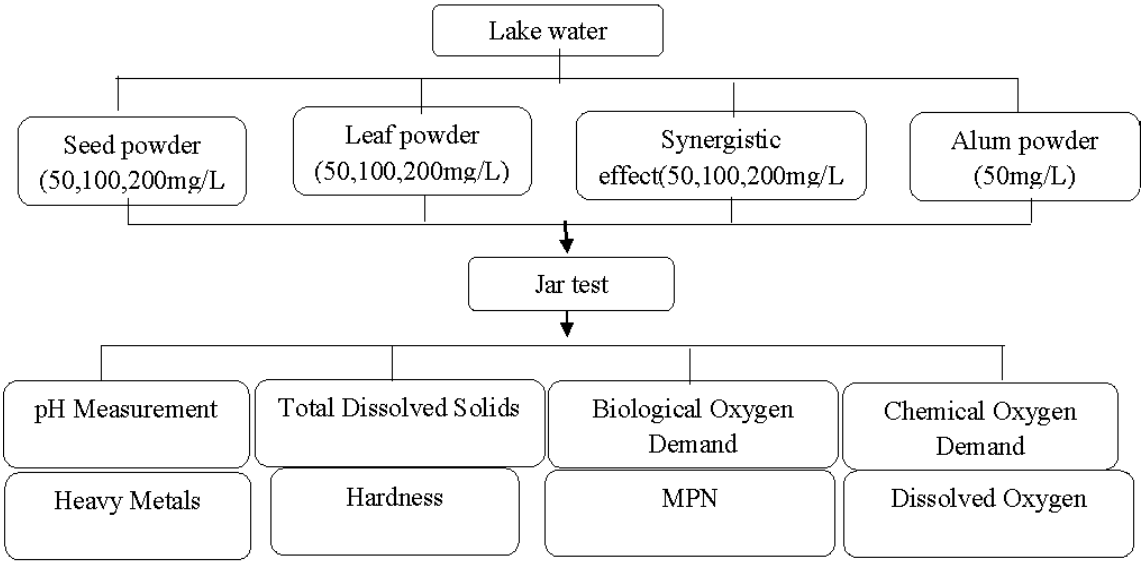

Fig 1: General flowchart of the plant sample analysis

PHYSICAL AND CHEMICAL PARAMETERS

Effect of *M. oleifera* seed, leaf and synergistic effect on pH: 100 ml of the sample was taken in a beaker and measured using electrodes. pH is analyzed within 5min of uncapping the sample bottle. The sample was stirred very gently, preferably with a magnetic stirrer. The pH values were recorded and tabulated for both water samples under untreated and treated conditions [14].

Effect of *M. oleifera* seed, leaf and synergistic effect on Total Dissolved Solids (TDS): TDS of water was determined by gravimetry method. The initial weight of the crucible and final weight of the crucible after drying was noted. TDS is calculated by the formula [15].

Initial weight of the crucible = W_1 g

Final weight of the crucible = W_2 g

$$\text{Amount of TDS in the sample} = \frac{(W2-W1)\times 1000}{volume\ of\ sample\ taken\ (ml)} \times 1000$$

Effect of *M. oleifera* seed, leaf and synergistic effect on Hardness: Titrimetric method was used to determine the total hardness of the water. Water samples were titrated against EDTA using EBT as an indicator. The concordant values were noted [14].

Effect of *M. oleifera* seed, leaf and synergistic effect on Dissolved Oxygen (DO): DO of water was determined using the titrimetric method. The water sample was titrated against $Na_2S_2O_3$ by using starch as an indicator. The concordant values obtained for DO are calculated using the formula [15].

$$\text{DO in mg/L} = \frac{0.2\times 1000\times ml of thiosulphate}{volume of sample}$$

Effect of *M. oleifera* seed, leaf and synergistic effect on Biological Oxygen Demand (BOD): BOD for the water sample was estimated using the titrimetric method. BOD bottles were kept in dark for 5 days. The water sample was titrated against $Na_2S_2O_3$ by using starch as an indicator. The concordant values obtained for BOD are calculated using the formula [15].

1ml of 0.025N $Na_2S_2O_3$ = 0.2 mg of O_2

$$\text{DO in Mg/L} = \frac{0.2\times 1000\times ml\ of\ thiosulphate}{volume\ of\ sample}$$

$$BOD = DO_{initial} - DO_{final}$$

Effect of synergistic effect on COD: Determination of COD was done for the untreated water and the synergistic effect by Robust laboratory, Bangalore [16].

BIOLOGICAL PARAMETERS

Most Probable Number (MPN) Method: Standard multiple tube fermentation method was used to determine the presence of coliforms in the water. Aseptically sample

was transferred to lauryl tryptose fermentation tubes. Sample volume and number of tubes to be inoculated will depend on the quality of water samples [15].

Heavy metal analysis: Heavy metals such as Cd, Pb, Hg and As analysis were analysed for synergistic effect and untreated water. The analysis was done by Robust laboratory, Bangalore (IS 3025 (Part - 48): 1994) (IS 3025 (Part - 48): 1988) [16].

RESULTS:

Jar Test: Jar test is conducted in the laboratory to confirm the coagulation property present in the sample. They are the basic test conducted. Different concentrations of seed (Fig 2) and leaf powder (Fig 3) were added to lake water kept overnight and observed for coagulation activity.

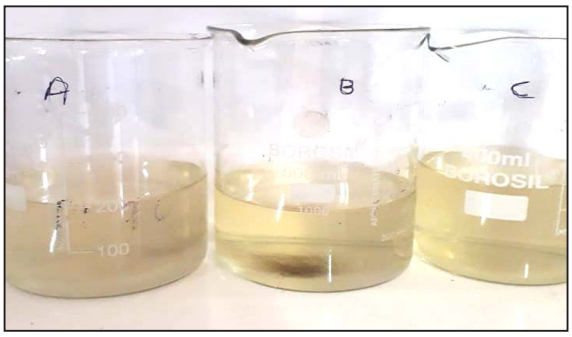

Fig 2: Jar test conducted for different concentrations of seed powder. A (50 mg/L), B (100 mg/L) and C (200 mg/L).



Fig 3: Jar test conducted for different concentrations of leaf powder A (50 mg/L), B (100 mg/L) and C (200 mg/L).

Physical and chemical parameters: The physico-chemical and microbiological properties of the samples are presented in Table I.

Table 1: Treatment of lake water by *M. oleifera* seed and leaf powder at different concentration and combinatorial effect. Data represented as ± SD.

Sl. no	Parameters	Before treatment (mg/L)	After treatment with Alum (mg/L)	After treatment with Seed powder (mg/L)			After treatment with Leaf powder (mg/L)			Synergistic effects (mg/L)	WHO Standards [17]
		0	50	A (50)	B (100)	C (200)	A (50)	B (100)	C (200)	50+50	-
1	pH	8.91 ± 0.02	8.2 ±0.02	8.17 ±0.02	8.13 ±0.02	7.83 ±0.02	7.725 ±0.03	7.745 ±0.03	7.78 ±0.04	7.81 ±0.01	6.5-8.5
2	DO (mg/L)	9.6	5.6	4.8	4	3.5 ±0.42	-	-	-	3.6 ±0.56	6.5-8
3	BOD (mg/L)	2.4	0.8	1.6	2 ±0.56	1.6	-	-	-	1.2 ±0.56	<5
4	Hardness (ppm)	177.75 ±3.18	159.75 ±3.18	144	137.25 ±3.18	132.75 ±3.18	128.25 ±3.18	135	150.5 ±3.53	123.75 ±3.18	<500
5	Total Dissolved Solids (mg/L)	112.25 ±3.18	84.75 ±3.18	96.5 ±2.12	93 ±0.7	91 ±1.41	91.75 ±2.47	97.75 ±0.35	104.5 ±0.7	96.5 ±2.12	<500
6	MPN	-	-	-	-	-	-	-	-	-	NIL
7	Jar Test	NA	+	+	+	+	+	+	+	+	NIL

Effect of ***M. oleifera*** leaf powder and seed powder on pH: pH is a significant parameter for testing the quality of drinking water. Treated lake water samples with different concentrations of seed and leaf powder showed decreased pH levels when compared to untreated lake water (pH 8.91). After treating with the seed powder, the pH was observed to decrease while the pH increased as the leaf powder concentration increased. The range of pH was 7.0-8.0, within the standard limit specified by WHO [17]. Based on the results obtained from seed and leaf powder treatment the minimum dosage of seed and leaf was taken to study the synergistic effect. The pH of the synergistic dose was 7.8 which is within the limit (Table 1; Figure 4). Alum treated water sample was considered as the positive control. The pH of alum was 8.2 which are slightly higher than the treated water samples.

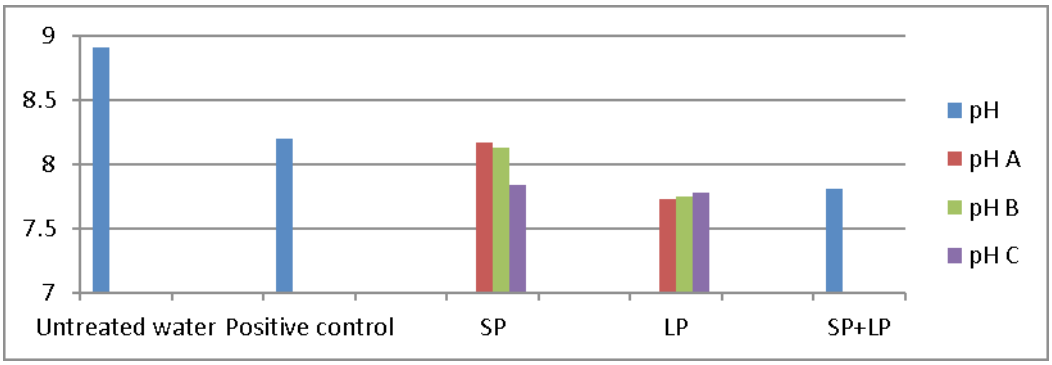

Fig 4: Effect *M. oleifera* seed and leaf powder and combined concentration on pH of water sample. Untreated water- lake water. Positive control- lake water treated with alum. SP-Seed powder; LP-Leaf Powder, SP+LP- Seed Powder+ Leaf Powder of concentration (50+ 50 mg/L); pH A (50 mg/L), pH B (100 mg/L), pH C (200 mg/L).

Effect of *M. oleifera* seed and leaf powder and combined concentration on TDS of water sample: Initial TDS of lake water was 112.25 mg/L. The treated water samples showed a range between 95-105 mg/L. As the concentration of seed increased the rate of TDS decreased whereas TDS levels were raised as the leaf concentration increased. The combinatorial effect of TDS was 96.5 mg/L. The TDS of positive control were within the standard limit (Table 1; Fig 5).



Fig 5: Effect of *M. oleifera* seed and leaf powder and combined concentration on TDS of water sample. Untreated water- lake water. Positive control- lake water treated with alum. SP- Seed powder; LP-Leaf Powder, SP+LP- Seed Powder+ Leaf Powder of concentration (50+ 50 mg/L); pH A (50 mg/L), pH B (100 mg/L), pH C (200 mg/L).

Effect of *M. oleifera* seed and leaf powder and combined concentration on Hardness of water sample: Initial readings of the hardness of lake water was 177.75 ppm. After treatment with seed and leaf powder, the hardness value was between 130-150 ppm. The hardness of synergistically treated water was 123.75ppm. The standard hardness value is <500 ppm (Table I: Figure VI).

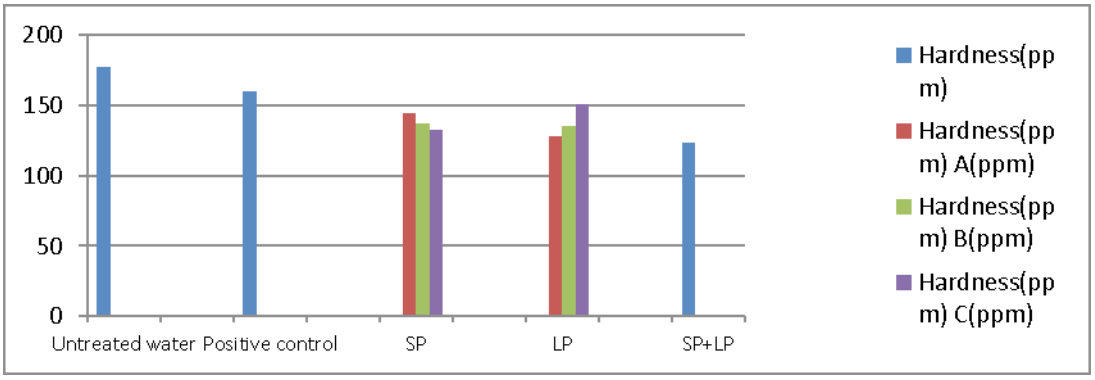

Fig 6: Effect of *M. oleifera* seed and leaf powder and combined concentration on Hardness of water sample. Untreated water- lake water. Positive control- lake water treated with alum. SP- Seed powder; LP-Leaf Powder, SP+LP- Seed Powder+ Leaf Powder of concentration (50+ 50 mg/L); pH A (50 mg/L), pH B (100 mg/L), pH C (200 mg/L).

Effect of *M. oleifera* seed and leaf powder and combined concentration on DO of water sample: The DO of untreated lake water was 9.6 mg/L. After treatment with different seed powder concentrations, DO ranged between 3.5 to 5 mg/L. The obtained result was below the standard limit. The treatment of lake water with *M. oleifera* leaf powder had negligible effect. The DO of positive control was 5.6 mg/L which is slightly closer to WHO standards [17]. The DO of the combined dosage of seed and leaf powder was 3.6 mg/L i.e., lower than the limit (Table I; Fig 7). The aeration of water was required to increase the DO to reach the standards.

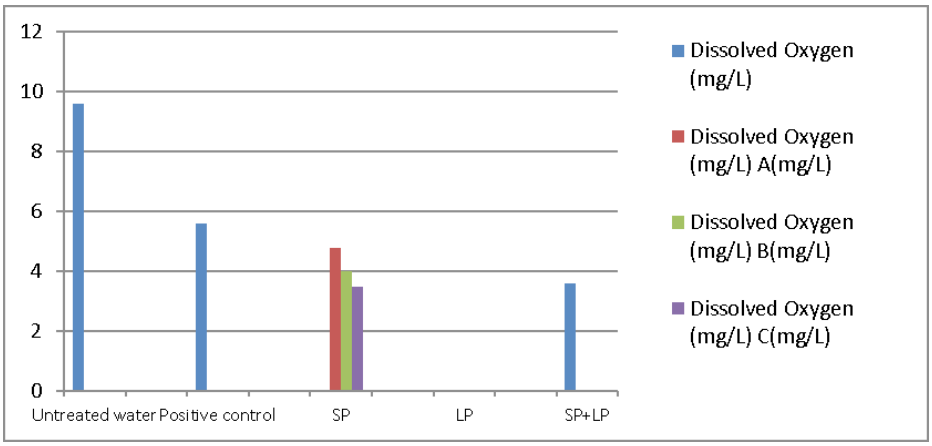

Fig 7: Effect of *M. oleifera* seed and leaf powder and combined concentration on DO of water sample. Untreated water- lake water. Positive control- lake water treated with alum. SP- Seed powder; LP-Leaf Powder, SP+LP- Seed Powder+ Leaf Powder of concentration (50+ 50 mg/L); pH A (50 mg/L), pH B (100 mg/L), pH C (200 mg/L).

Effect of *M. oleifera* seed and leaf powder and combined concentration on BOD of water sample: Before treatment the BOD of lake water was 2.4 mg/L. After treatment with seed and combined dosage powder, the BOD values obtained were within the limit. The BOD of alum treated sample was 0.8 mg/L (Table 1, Fig 8).

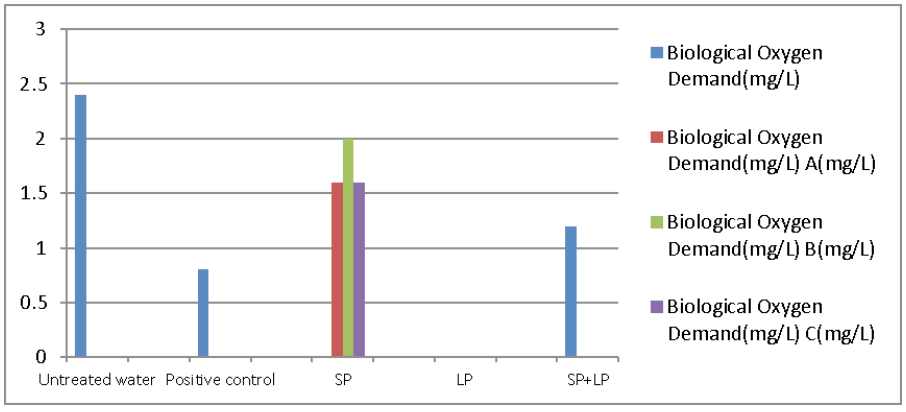

Fig 8: Effect of *M. oleifera* seed and leaf powder and combined concentration on BOD of water sample. Untreated water- lake water. Positive control- lake water treated with alum. SP- Seed powder; LP-Leaf Powder, SP+LP- Seed Powder+ Leaf Powder of concentration (50+ 50 mg/L); pH A (50 mg/L), pH B (100 mg/L), pH C (200 mg/L).

Effect of *M. oleifera* seed and leaf powder and combined concentration on COD of water sample: Determination of COD was done for the untreated water and the synergistic effect by Robust laboratory, Bangalore. The untreated water had COD of 104.7 mg/L. the combinatorial dosage treated water had COD of 62.8 mg/L.

BIOLOGICAL PARAMETER

Most Probable Number (MPN) method: The MPN test is conducted to determine the total coliforms quantitatively. There was no fermentation due to the absence of coliforms hence no gas production was seen. Thus, MPN test had no positive results.

Heavy Metal Test: Heavy metals such as Cd, Pb, Hg and As analysis were analysed for synergistic effect and untreated water (Table 2).

Table 2 : Heavy metal content in water before and after treatment.

Heavy metal	Before treatment (mg/L)	After treatment(mg/L)
Cd	< 0.01	< 0.01
Pb	<0.01	<0.01
Hg	<0.001	<0.001
As	<0.005	<0.005

Since there was very little concentration of heavy metals seen in sample water, the exact mechanism of *M. oleifera* seed and leaf powder in reducing them was not clear.

DISCUSSION

Jar Test: The jar test conducted on small scale to predict the functioning of a large-scale treatment operation demonstrated that the different concentrations of seed and leaf increased the coagulation rate.

Effect *M.oleifera* seed and leaf powder and combined concentration on pH of water sample: pH of water treated with different concentrations of seed powder showed

a decrease in pH level as the seed concentrations were increased whereas pH levels increased as the concentration of leaf powder were increased. The combinatorial effect shows that the pH decreases to a certain extent when compared to the individual values. This could be due to the action of water-soluble cationic proteins in the seeds. The basic amino acid seen in the protein of *M. oleifera* powder acts as an acceptor of a proton from water causing the release of hydroxyl group resulting in basic solution [18].

Effect of *M. oleifera* seed and leaf powder and combined concentration on TDS of water sample: TDS of untreated water was 112.25 mg/L and the water treated with seed and leaf powder of different concentrations had TDS value ranging from 91-104 mg/L, whereas the combinatorial dose had TDS of 96.4 mg/L all of which come under the standard limits. Thus, this test supports the fact that *M. oleifera* decreases the TDS levels seen in the water and is known to be a natural cationic polyelectrolyte and flocculent [18].

Effect of *M. oleifera* seed and leaf powder and combined concentration on Hardness of water sample: The test showed that the positive control sample has a value higher than the combinatorial value (123 ppm) in accordance with the individual value of range 130-145 ppm and 128-150 ppm for seed treated and leaf treated samples respectively.

Effect of *M. oleifera* seed and leaf powder and combined concentration on DO of water sample: According to the results, the seed powder has lowered the DO of water to a much greater level than the initial level of the sample. DO of water treated with leaf powder had no oxygen content, thus, DO values for water treated with leaf powder were neglected. Combinatorial effects also showed DO content of 3.6 mg/L which is below the standard limit hence aeration is required (Table 1).

Effect of *M. oleifera* seed and leaf powder and combined concentration on BOD of water sample: BOD of water treated with *M. oleifera* seed powder at different concentrations shows a range of 1-2 mg/L which is within the standard limit. BOD of

the leaf was neglected as the DO was not seen. Positive control had a value of 0.8 and untreated lake water had a BOD of 2.4 mg/L.

Effect of *M. oleifera* seed and leaf powder and combined concentration on COD of water sample: COD of untreated water was 104.7 mg/L. The combinatorial dosage treated water had COD of 62.8 mg/L. This can be supported with the fact that *M. oleifera* has water-soluble proteins which acts as a good coagulant, which helps to decrease the maximum COD load of water [19].

BIOLOGICAL PARAMETER

Most Probable Number (MPN) method: Consecutive triplicate trials of MPN test were performed with seed and leaf samples and the results obtained were negative.

Heavy Metal Test: The water sample was tested for the presence of important heavy metals i.e., cadmium, lead, mercury and arsenic. The untreated water had a minute amount of these metals present in them. After treatment with the combinatorial dose of seed and leaf powder, there were traces of these metals seen. These studies can be accorded to the earlier work that, *M. oleifera* has some effects in reducing the levels of metals present in wastewater [20].

Conclusion: The present study infers that *M. oleifera* has some coagulant properties that can be a natural alternate coagulant for chemical such as alum. The seed and leaf powder rapidly reduce the pH, BOD, DO, COD, hardness, TDS of water. The combinatorial effects leaf and seed powder also showed great results in the reduction of pH, BOD, DO, COD, hardness, TDS of water thus, can be used as an alternate mode of water treatment. In future perspectives, the combinatorial dose can be converted into the nanoparticle and impregnated into a natural, cost-effective bio-sorbent, which can be used as a filter to treat water bodies. Thus, the use of natural coagulant from a plant source shows a vital development in sustainable environmental technology to improve the quality of life with the environment.

Conflict of interest: The Author(s) declare(s) that there is no conflict of interest.

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Nutritional Properties and Antioxidant Activity of Roselle (*Hibiscus Sabdariffa*)

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Abstract : *Roselle or Hibiscus sabdariffa is rich in nutrients and may be helpful in combating non-communicable diseases. The objectives of the study were to (i) analyze and compare the physicochemical, nutritional and functional properties of Roselle seeds, petal and calyces grown in two regions of India and (ii) to assess the acceptability of the product developed with Roselle seeds, petals and calyces. We procured fresh Roselle from Kolkata (K), West Bengal and dried seeds, petals and calyces from Gadag (G), Karnataka. We developed a standard snack bar and incorporated the Roselle seeds, petals and calyces in to four variations of the bar. Sensory evaluation of the bar was carried out with 25 semi-trained panelists using a nine-point hedonic scale. The physicochemical parameters such as moisture, macronutrients, calcium and iron content of the seeds, petals and calyces were analyzed. Further, the antioxidant activity of the petals and calyces was studied. There were no differences in the mean carbohydrate (K = 24.66±4.31g/100g v G =21.85±5.11g/100g) and fat content (K = 25.33±16.87/100g v G = 41.33±10.98g/100g) of the two seed samples (p > 0.05). The Kolkata seeds had significantly higher content of protein (22.77±0.72g/100g) than the Gadag seeds (17.21 ± 0.76g/100g, p < 0.05). The petals and calyces had a good antioxidant activity (IC50 - K: 33.73 µg/ml, G: 38.88 µg/ml). The snack bar developed with 15% Roselle seeds and 2% petal and calyces was most accepted. Roselle has an impressive nutritional and antioxidant profile that needs to be promoted for inclusion in our regular diets.*

Keywords: *Roselle, Hibiscus sabdariffa, protein, antioxidant property, Roselle, snack bar*

INTRODUCTION

Forests have blessed mankind with not only water and oxygen but with a wide range of medicinal plants and herbs. These medicinal plants and herbs are being used from a very long time as a part of traditional treatment for various diseases. There are around 17,000 medicinal plants species out of which 2000 medicinal plants are recognized and used by Ayurveda, Unani and Siddha medicine systems. A sizable number of medicinal plants are found in Uttaranchal, followed by Sikkim and North Bengal [1]. Medicinal herbs like basil, turmeric, curry leaves and many more have been growing in forests as well as on our backyards and balconies. These herbs possess a lot of health benefits and can be used in our daily diet. They come with zero toxicity and are very much safe for human consumption [2].

Roselle or *Hibiscus sabdariffa* is a genus of the Malvacae family. Roselle is a shrubby plant with green to reddish stem. It requires tropical and sub-tropical temperatures to grow. It is widely grown across many regions of the world but is native to Africa. In India, it is grown in Ahmednagar district, Maharashtra, Gadag, Karnataka and West Bengal. It is a seasonal vegetable mostly found in winter months [3].

In the Indian market, Roselle and its different species are used in fabric industry, cosmetic industry. The petals and calyces of Roselle are used for making tea [4]. It is used for making a popular African beverage called Soborodo. It is also used in making jams, jellies, salad dressings, ice cream, cookies and many more in other countries [5].

The Roselle seeds are a good source of carbohydrates, protein, fat, fiber and minerals like calcium and iron. The petals and calyces are rich in flavonoids, polyphenols and antioxidants [6]. Owing to this, Roselle possesses several health benefits. In the ancient times, it was used for treatment against infections like cough, fever, respiratory distress in Africa. Animal studies indicated that Roselle could help in regulating non-communicable diseases like cancer, diabetes, hypercholesterolemia, hypertension [7,8]. It has shown antifungal, antibacterial, diuretic and laxative properties [3, 4, 5].

MATERIALS AND METHODS

Procurement of Roselle seeds, petals and calyces

Roselle flowers were procured from two regions in India- Gadag, Karnataka and Kolkata, West Bengal. In Kolkata, Roselle flowers were procured from a local market in Manicktala area. Around 2.5kg of fresh Roselle flowers were procured. The second set of Roselle flowers were procured from Herbs and Health company in Gadag, Karnataka. The Roselle petals, calyces and seeds were already dehydrated so it was after ground into a fine powder.

Preparation of Roselle Samples

Preparation of Roselle seed, calyx and petal powder (Kolkata) **-** The fruit coat was allowed to dry for 6-8 hours so that the seeds could be easily taken out. After drying the fruit coat, the seeds were taken out and put in a tray and were kept for shadow drying for four hours. The dried seeds tray was put under the sun for drying for 3-4 hours. Dried seeds were further dehydrated in a dehydrator at 60 °C for 4-6 hours. The dehydrated seeds were allowed to cool down. They were milled in a flour mill. The seed flour was further ground into a fine powder in a mixer grinder. The powder was further stored in a zip lock pouch until used.

Fresh Roselle flowers were spread on a clean table for shadow drying for 3 hours. After 3 hours, the petals and calyces were separated and washed. The washed petals and calyces were spread on the table and were shadow dried for 8 hours. Thereafter, the petals and calyces were placed under the sun for drying for 2-3 hours. The dried calyces and petals were ground in a grinder and made into a fine powder. The powdered petals and calyces were stored in a zip lock pouch until use. Thus, from 2.5 kg fresh Roselle, we obtained 90 g of dried seeds powder and 100 g of petals and calyces powder.

Preparation of Roselle seed powder (Gadag) **-** Dried Roselle seeds were taken out from a zip lock pouch and kept on a plate. Around 150g of the whole seeds were weighed and milled using a flour mill. The seeds were milled twice to get a fine powder.

The powder was transferred to a zip lock pouch until further use. Dehydrated petals and calyces (200 g) were ground in a mixer and made into a fine powder. The powder was stored in a zip lock pouch until further use.

Nutrient analysis

We analyzed carbohydrate by Anthrone method, protein by Kjel Dahl method, and fat by Soxhlet method [8,11,13]. We also analyzed moisture and ash [8,11,13]. We calculated the energy using factorial method [8,11,13]. The micronutrients analyzed were calcium (Titration method) and iron [8,11,13].

Physicochemical analysis

Seed length and breadth of the Roselle seeds were measured using vernier caliper [9]. Particle density of Roselle seeds and calyces' powder were measured using a measuring cylinder [10]. Loose and packed bulk density of Roselle seeds powder was measured using a measuring cylinder [10]. pH of Roselle petal and calyces' powder was measured by preparing a homogenized solution with 5gm of Roselle petal and calyx powder with water and digital pH meter was used for determining the acidity of the powder, estimation of color was done by using Lovibond colorimeter where the paleness of the Roselle petal and calyx powder was analyzed.

Analysis of functional properties of Roselle petals and calyces' powder

Antioxidant activity was estimated using DPPH radical scavenging assay [8,11,13]. Total polyphenol content was measured using Folin Ciocalteau Assay and total flavonoid content was determined using Aluminium Chloride Colorimetric Assay [8,11,13].

Development of Standard Snack bar

All the dry and wet ingredients were weighed and kept aside. In a pan instant oat (10%), rolled oats (10%) and barley powder (5%) were added and sautéd until aromatic. In a mixing bowl dried coconut powder (7%), rice crispy (10%) and chopped dried fruits (15%) were added. The roasted cereal mixture was added to the mixing bowl. In another

pan peanut butter (10%), jaggery powder (10%) and dates puree (10%) were added and sauted. Then the mixture was added to the mixing bowl. Acacia gum powder (3%) and honey (10%) were added. All the ingredients were mixed together. In a butter paper the mixture was spread and shaped. The bar was kept in the refrigerator for 12 hours. It was taken out 3-4 hours prior to serving.

To this standard snack bar different proportions of Roselle seeds, petal and calyces' powder were added and the proportion of the cereals such as oats was reduced. In variation 1 (V1) 5% Roselle seed powder followed by (1%) petal and calyx powder were added. In variation 2 (V2), 10% of Roselle seed powder and 1.5% of petal and calyx powder were added. In variation 3 (V3), 15% (15g) of Roselle seed powder and 2% of petal and calyx powder were added. In variation 4 (V4), 20% of Roselle seed powder and 2.5% of petal and calyx powder were added. The total quantity of the dry ingredients was maintained by reducing the content of rolled oats and instant oats. The other ingredients remained constant throughout the variations.

Sensory evaluation of Snack bar

The sensory evaluation of the standard snack bar was done using nine-point hedonic scale. Twenty-five semi-trained panelists were a part of the panel. Aspects like appearance, color, flavor, texture and taste were evaluated.

RESULTS AND DISCUSSION:

Physicochemical analysis

The physicochemical parameters –pH, particle density, loose bulk and packed bulk density for Kolkata seeds, petal and calyces and Gadag seeds, petal and calyces were studied (**Table 1**). We found no differences in the physicochemical parameters between seeds, calyces and petals from Gadag and Kolkata.

Table 1: Physicochemical properties of Roselle Seeds, Calyces and Petals

Physicochemical properties of Roselle Seeds				
Seed Parameters	**Kolkata Mean ± S.D**	**Gadag Mean± S.D**	**t**	**p**
Length (mm)	166.3 ± 6.16	170.7 ± 1.06	1.02	0.410
Breadth (mm)	163.7 ± 5.49	171.2 ± 0.63	1.70	0.230
Particle density (kg/m^3)	160 ±0.01	180±0.01	0.20	0.860
Loose bulk density (kg/m^3)	470 ± 0.03	550± 0.02	2.10	0.160
Packed bulk density (kg/m^3)	590± 0.02	660 ± 0.02	1.90	0.190
Physicochemical properties of Petals and Calyces				
Petals and Calyces	**Kolkata Mean ± S.D**	**Gadag Mean± S.D**	**t**	**p**
Particle density (kg/m^3)	170±0.01	170±0.01	0.14	0.890
pH	2.1 ± 0.07	1.8 ± 0.07	0.25	0.120

Nutrient analysis for Roselle seeds, calyces and petals

We did not find any differences in the moisture, fat, carbohydrate and calcium content of the seeds, calyces and petals obtained from Gadag and Kolkata (Table 2). However, the protein and iron content of the seeds procured from Kolkata were significantly higher than those from Gadag ($p < 0.05$). Likewise, the protein content of the calyces and petals procured from Kolkata was significantly higher than those from Gadag ($p < 0.001$).

Table 2: Nutrient composition of the Kolkata and Gadag seed, petals and calyces powder

Nutrient composition of the seeds powder				
Nutrient per 100gm	**Kolkata Mean ± S.D**	**Gadag Mean± S.D**	**t**	**p**
Moisture (%)	5.7±1.71	4.9±0.89	0.41	0.710
Energy (kcal)	417.42±89.45	544.85±93.73	0.80	0.500
Protein (g)	22.77 ± 0.72	17.21 ± 0.76	4.59	0.040*
Fat (g)	25.33±16.87	41.33±10.98	0.97	0.431
Carbohydrates (g)	24.66±4.31	21.85±5.11	2.39	0.750
Calcium (mg)	263.33 ± 26.5	152 ± 24.86	2.65	0.110
Iron (mg)	26.33±2.49	9.66±0.81	7.60	0.010*
Nutrient composition of the petal and calyx powder				
Nutrient per 100gm	**Kolkata Mean ± S.D**	**Gadag Mean± S.D**	**t**	**p**
Moisture (%)	7.9 ±0.89	8.5±0.40	0.10	0.500
Energy (kcal)	120.23±6.96	107.84±22.92	0.93	0.440
Protein (g)	8.49±0.41	1.99±0.50	10.92	<0.001**
Fat (g)	5.0±0.89	7.0±2.44	0.10	0.500
Carbohydrates (g)	10.31±0.55	9.15±1.54	1.17	0.300
Calcium (mg)	66.0 ± 5.7	56.3 ±7.25	3.71	0.060
Iron (mg)	4.0±0.70	10.6±3.6	2.34	0.140

*Significance at 5% level; *Significant value of Iron 0.01

The protein and carbohydrate content of the seeds from Gadag and Kolkata was lower than that grown in Maharashtra [9, 11]. The mean protein content of seeds grown in Maharashtra was found to be 38.06±0.21g/100g. The mean carbohydrate content of the seeds grown in Maharashtra 13.87±0.11g/100g. This difference would be due to several reasons such as growth conditions, maturity of seeds, seasonal variation. The mean fat content of Kolkata seeds was comparable with that of the seeds grown in Maharashtra [11]. Seeds grown in Egypt had a favorable fatty acid profile with 73.13% of total

polyunsaturated fatty acid (PUFA). Among the unsaturated fatty acid, seeds had 41.18% of linoleic acid and 31.22% of oleic acid [12].

The petals and calyces grown in Kolkata and Gadag had much lower protein and carbohydrate content than those grown in Assam. The mean protein content of sundried petals and calyces grown in Assam was 14.22±0.02g/100g and oven-dried was 15.52±0.04/100g [13]. The mean carbohydrate content of sundried petals and calyces were 44.67±0.03/100g and oven dried petals and calyces was 43.68±0.02/100g [13]. The fat content of the samples in the present study were higher than the samples from Assam. For the sundried values were 1.22±0.02/100g and oven dried value was 1.44±0.03/100g [13]. These differences could be attributed to factors such as maturity of seed, seasonal variant and geographical difference.

The mean calcium content of seeds grown in Maharashtra (320.45±1.58 mg/100g) was higher than that noted in the present study [9,11]. On the other hand, the Kolkata seeds have a higher iron content than Gadag and Maharashtra (11.45±1.89mg/100g) seeds [9]. The mean calcium content of the Roselle petals and calyces from Kolkata and Gadag was similar to that grown in Assam [13]. However, in the sundried and oven-dried calyces of Assam, the iron content was 28.98mg per 100g, and 35.68mg per 100g, respectively, which was higher than that of Gadag and Kolkata petals and calyces [13]. The mineral content of the seeds depends on the soil. This may explain the difference found in the samples from different regions of the country.

Antioxidant activity of Roselle petal and calyces' powder:

The Roselle petals and calyces extract showed free radical scavenging activity. At a concentration of 100 **µg**/ml, the samples from Kolkata and Gadag were able to inhibit 96.2% and 90.78% free radicals respectively. The antioxidant activity expressed as IC 50 value was 33.73 µg/ml for Kolkata petals and calyces and 38.88 µg/ml for Gadag petals and calyces.

Table 3: DPPH radical scavenging activity of Roselle petals and calyces

Concentration (ug/ml)	Ascorbic acid	Percentage inhibition (%)	
		Kolkata	Gadag
20	31.14	39.21	32.99
40	50.19	58.45	56.34
60	69.89	65.25	66.02
80	74.86	79.99	75.33
100	95.21	96.20	90.78

Sensory evaluation for Snack bar

Sensory evaluation of the snack bar was studied in terms color, texture, appearance, flavor, taste and overall acceptability using a nine-point hedonic scale. The evaluation was conducted among 25 trained and semi-trained panel members. The scores of the sensory evaluation of the snack bar are given in Table 4. The appearance, colour, taste, flavor and overall acceptability of V3 (15% seeds, 2% petals and calyces) was significantly greater than that of the standard bar as well as the other variations. However, the texture score of the V1 was significantly higher than other variations.

Table 4: Sensory Scores of the Standard and Variations of the Snack Bar

Parameters	Sensory scores					p
	Standard	V1	V2	V3	V4	
Appearance	9.0 ^{a}	7.64±0.87 ^{ab}	7.24 ± 0.74 ^{c}	8.08±0.78 ^{d}	7.24±1.04 ^{e}	0.020
Color	7.96±0.89 ^{a}	7.8 ±0.73 ^{b}	7 ± 0.83 ^{ac}	8.2±0.39 ^{ad}	7.36 ± 0.99^{e}	< 0.001
Texture	9.0^{a}	9.0 ^{b}	7.52 ±0.83^{c}	8.16 ±0.35^{d}	7.2 ±1.07^{e}	< 0.001
Taste	9.0^{a}	8 ± 0.83^{b}	7.08 ±0.95^{c}	8.16 ±0.35^{d}	8.0^{e}	< 0.001
Flavor	9.0^{a}	7.9 ± 0.87^{b}	7.16 ± 1.13^{c}	8.16 ± 0.35^{d}	6.76 ± 1.44^{e}	< 0.001
Overall acceptability	8.79 ±0.17^{a}	8.07± 0.57^{b}	7.2 ± 0.73^{c}	8.15± 0.38^{d}	7.32 ± 0.74^{e}	< 0.001

The same superscript in a row indicates same to each other and different subscripts in a row indicate different to each other and are significantly different (p value <0.05) Standard- Control, V1- 5% Roselle seed powder and 1.5% Roselle petal and calyces'

powder, V2- 10% Roselle seed powder and 2% of Roselle petal and calyces' powder, V3- 15% Roselle seed powder and 2.5% Roselle petal and calyx powder, V4- 20% Roselle seed powder and 3% Roselle petal and calyx powder.

We calculated the nutrient composition of the standard and the most accepted variation of the snack bar (V3). Inclusion of 15% Roselle seed powder and 2.5% petal and calyx powder resulted in increase in protein (7.5%), fat (32.4%), calcium (169%) and iron (74%). The carbohydrate content of the bar was reduced by 11.4%.

Hibiscus sabdariffa or Roselle seeds obtained from Kolkata were noted to be a good source of protein (22% energy), fat (54% energy), calcium (263 mg%) and iron (26 mg%). Further, these seeds were noted to have lower content of carbohydrate (25% energy). Likewise, the free radical scavenging activity of the petals and calyces' powder from Kolkata was greater than that of the Gadag ones. The seeds from Kolkata were therefore superior to those from Gadag. These properties of Roselle enable its inclusion in different food products for nutrient enhancement. Along with nutrient enrichment, the antioxidant property of the calyces and petals appear to be responsible to prevention and management of infections and non-communicable diseases.

CONCLUSION

Roselle seeds appear to be a good source of protein, fat, calcium and iron. Further, the carbohydrate content is lower as compared to the other grains. The petal and calyces have a high antioxidant activity. These nutritional attributes can be utilized by incorporating Roselle seeds, petal and calyces in to a wide range of food products in our daily diet. People are largely unaware about its uses in daily diet. Those who were aware, used the calyces and petals for food preparation and discarded the seeds. Literature suggests the preventive and therapeutic benefits of the plant in combating diseases like metabolic syndrome, cancer, inflammation, and infections. Hence, more awareness should be created regarding this plant so that it can be used to regularly in our diet.

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Knowledge, Attitude and Practice of College Going Students on Iron Deficiency Anemia

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Abstract : *Iron Deficiency Anemia is a prominent public health problem predominantly affecting women of reproductive age. The study aims to assess the knowledge, attitude, and existing practice of college students regarding iron consumption and IDA in a private college in Bengaluru. Cross-sectional study was carried out to assess knowledge, attitude, and existing practices of 100 college students of reproductive age group (20-23 years). Data collection was done through a structured KAP questionnaire which was statistically analyzed. Overall prevalence of anemic individuals was 10%. Majority of the student's showed inadequate knowledge (35%), favorable attitude (74%) and low practice (66%). The findings of the study show that even after demonstrating a positive attitude, the knowledge and practice levels were towards an undesirable direction. Food based approaches along with greater awareness can help reduce the prevalence of anemia.*

Keywords: *Iron deficiency anemia, reproductive age, knowledge, attitude, practice, food-based approach, awareness.*

INTRODUCTION

Iron is one of the most essential and abundant trace elements of the Earth's crust [1]. It plays a critical role in transporting oxygen to all the parts of the body and ensures optimal health and performance. Despite having abundant sources of iron on Earth, iron deficiency is very common in human beings. It is the most widespread cause of anemia globally and affects huge portions of the population in underdeveloped countries [2].

According to the World Health Organization, a long-term negative balance in the iron status results in iron deficiency. It constitutes almost 50% of all anemia, and almost a million deaths a year; three-quarters of the deaths occur in Africa and South-East Asia. Iron Deficiency Anemia (IDA) is among the "top ten" risk factors contributing to global burden of disease and therefore, was made a part of the Global Burden of Disease (GBD) 2000 project [3].

Iron Deficiency Anemia (IDA) is therefore a nutritional deficiency disorder in which the body has too little iron in the bloodstream. Every age group is vulnerable. However, IDA is a significant public health problem among women of reproductive age (15-49 years) as stated in the situation analysis done by Mawani et al. [4]. This may be due to multiple determinants including genetic and environmental factors. Some other factors include age, parity, obesity, socio-economic status, family size, education level, low dietary iron intake, and the additional demand for iron imposed by iron loss during menstruation, pregnancy, lactation, blood loss, infections, worms, inflammatory bowel diseases or blood donations [5].

IDA especially in the developing countries has been recognized as one of the most common forms of malnutrition in the world. Anemia affects a whopping 614 million women globally in the ages of 15 and 49 years with India facing a serious nutrition burden with 51.5% women suffering from anemia in the same age group [6]. According to the National Family Health Survey, 2015-16, prevalence of anemia among women of reproductive age (15-49 years) in Urban Bangalore was seen as high as 40.7% in 2016 [7].

A knowledge, attitude and practices (KAP) survey help in understanding what people know regarding a certain notion or crisis and how they look and deal with it. These results assist in filling the gaps in turn helping in the successful execution of a program [8]. Hence, the present study aims at evaluating the knowledge, attitude and practice among college going students about iron consumption and anemia.

MATERIALS AND METHODS

Study site and population: A cross-sectional study was carried out among college going students. The survey was done in a private women's college in Bengaluru, India. The samples were selected from the Mount Carmel College, Autonomous, Bengaluru since they were in the reproductive age group and were conveniently available as the researcher belonged to the same college and were easily approachable taking cost and time in consideration.

Sample size and sampling: The sample population was chosen based on non-probability convenient sampling. A total of 100 students were selected pursuing Masters in different courses i.e., Commerce, Science and Humanities. 10 students were randomly selected from each of the following departments of: Science (Botany, Food science and Nutrition, Life Science, Biochemistry and Computer Applications), Commerce (M. Com International Business, M. Com Financial Analysis and M. Com Regular) and Humanities (Economics and Psychology).

Study tool: A KAP questionnaire was designed to gather data. The questionnaire comprised 5 components: socio-demographic information, anthropometric measurement, knowledge about iron deficiency and anemia, attitude towards iron rich foods and practice of consuming iron rich foods. The data was analyzed using a scoring system by classifying:

- Knowledge level as inadequate (≤50 % score), moderate (51-75% score) and adequate (>75 % score).
- Attitude level as unfavorable (≤ 50 % score), moderate (51-75 % score) and favorable (> 75 % score).
- Practice level as low (≤ 50% Score), moderate (51-75% score) and high (> 75% score)

Data collection: The final questionnaires were distributed as Google forms among the study population in different departments. Primary data was collected through the questionnaire by the researcher herself. The questionnaire was framed in English. The responses gathered by the data collection were quantified in an excel sheet for further statistical analysis.

Statistical analysis: The collected data was entered into the Microsoft excel sheet. Data analysis was analyzed with suitable statistical tests using SPSS 23. Mean, standard deviation, ANOVA test and chi-square test were used for the data analysis.

RESULTS AND DISCUSSION

Socio demographic details of the study subjects revealed majority were within the age group of 22- 23 years. Hindu was observed to be followed as preferred religion. The trend of nuclear family was also noticed in the study and most of the samples belonged to urban locality (Table 1).

Non – Vegetarian type of food habit was more commonly practiced with frequency of outside food consumption being commonly observed. Hemoglobin levels below 9mg/100 ml indicating anemia was seen in 10 per cent population. General pain symptoms experienced during menstrual cycles among the subjects were vomiting, backpain, bloating, headache, and mood swings (Table 2).

Table 1: Socio-demographic characteristics of subjects

Socio-demographic variables	Category	Respondents	
		Number	Percent
Age group (years)	20-21	24	24.0
	22-23	76	76.0
Religion	Hindu	67	67.0
	Muslim	11	11.0
	Christian	18	18.0
	Jain	4	4.0
Place of Residence	Rural	11	11.0
	Urban	89	89.0
Type of family	Nuclear	83	83.0
	Joint	17	17.0
Family size	3-4 members	59	59.0
	5-6 members	28	28.0
	7-10 members	13	13.0
Family income/month (Rs)	Rs. 20,000-40,000	29	29.0
	Rs.41,000-60,000	25	25.0
	Rs.61,000-99,000	22	22.0
	> Rs.1,00,000	24	24.0
Educational level	Master's degree	100	100.0
Course studying	Nutrition	14	14.0
	Science	51	51.0
	Commerce	18	18.0
	Arts	17	17.0

Table 2: General information of subjects

Variables	Category	Respondents	
		Number	Percent
Food habits	Vegetarian	30	30.0
	Non-vegetarian	66	66.0
	Ovo- vegetarian	4	4.0
Food allergy	None	87	87.0
	Egg	1	1.0
	Seafood	1	1.0
	Soy	1	1.0
	Others	10	10.0
Consumption pattern	Always	16	16.0
	Sometimes	84	84.0
Frequency of consuming outside food	Daily	7	7.0
	Weekly thrice	22	22.0
	Weekly twice	21	21.0
	Weekly once	27	27.0
	Fortnightly	4	4.0
	Monthly	7	7.0
	Occasionally	12	12.0
Hemoglobin levels	<12mg/dl	10	10.0
	>12mg/dl	90	90.0
Age at menarche (years)	10-11	24	24.0
	12-13	64	64.0
	14-15	12	12.0
Occurrence of menstrual cycle	Regular	88	88.0
	Irregular	12	12.0
Duration of menstrual bleeding	3-4 days	43	43.0
	5-7 days	57	57.0
Symptoms during menstruation (Multiple response)	Vomiting	13	13.0
	Back ache	64	64.0
	Stomachache	47	47.0
	Others	21	21.0
Deworming status	Yes	29	29.0
	No	71	71.0

Table 3 portrays the anthropometric measurements (height, weight, waist, and hip circumference) of the subjects.

Among the 100 samples evaluated for anthropometric measurements, nearly one fourth were underweight and little more than one fourth were overweight as per WHO (9). Majority the subjects had normal waist/hip ratio and only 12 % belonged to obese category

Table 3: Anthropometric details of subjects

Anthropometric variables	Category	Respondents	
		Number	Percent
Height (cm)	146-155	23	23.0
	156-165	51	51.0
	>165	26	26.0
Weight (kg)	40-52	39	39.0
	53-65	34	34.0
	>65	27	27.0
Body Mass Index (BMI)	Under weight (<18.5)	23	23.0
	Normal (18.5-24.9)	51	51.0
	Overweight (25.0-29.9)	26	26.0
Waist circumference (cm)	26-28	35	35.0
	29-31	38	38.0
	> 31	27	27.0
Hip circumference (cm)	25-37	28	28.0
	38-39	43	43.0
	>40	29	29.0
Waist/hip ratio (WHR)	Normal (≤0.85)	88	88.0
	Obese (>0.85)	12	12.0

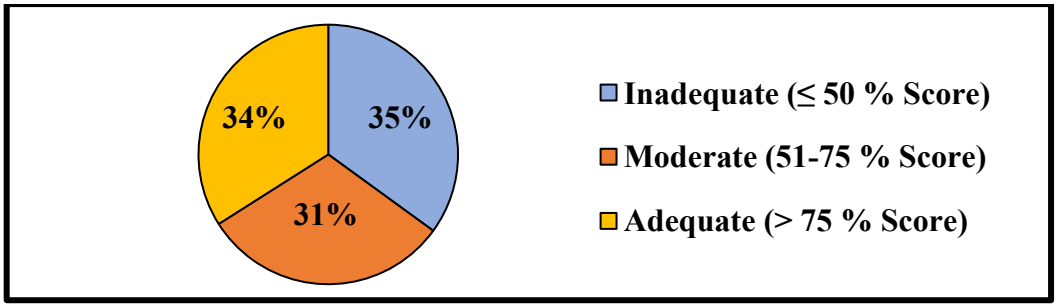

Fig 1: Knowledge level on iron consumption

Figure 1 depicts the knowledge level of subjects on iron consumption. Results indicate that 35% subjects had inadequate knowledge about iron consumption.

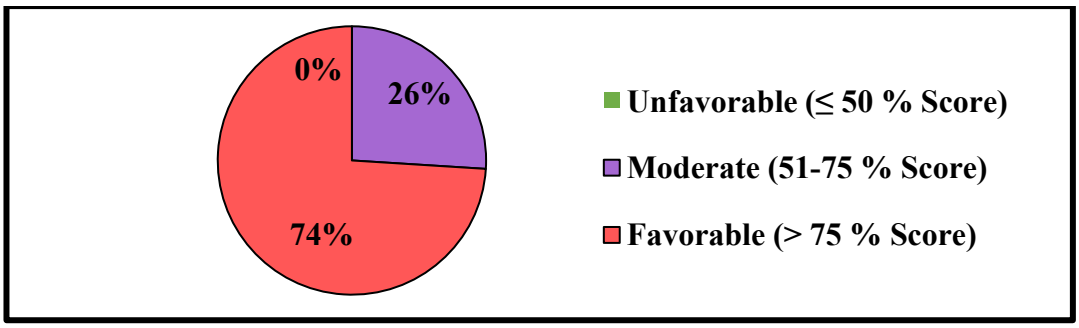

Fig 2: Attitude level on iron consumption

The attitude level of subjects on iron consumption is portrayed in Figure 2. Favourable attitude towards iron consumption was noticed while one fourth of the subjects showed moderate attitude towards iron consumption.

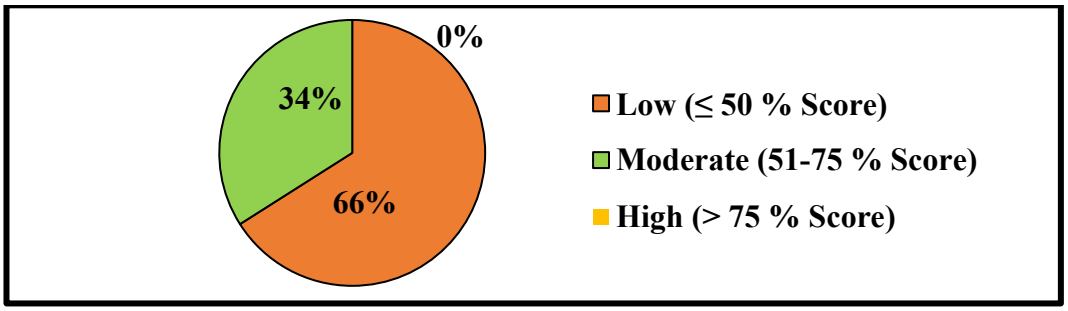

Fig 3: Practice level on iron consumption

Figure 3 illustrates the practice level of subjects on iron consumption. Majority of the subjects had a low practice level of consuming iron rich foods, while none of them showed a high practice level of iron consumption.

The mean KAP scores of subjects on iron consumption is tabulated below.

Table 4: Mean KAP scores of subjects on iron consumption

No.	Aspects	Statements	Max. Score	Scores			
				Mean	SD	Mean (%)	SD (%)
I	Knowledge	10	10	6.42	2.00	64.2	20.0
II	Attitude	10	20	17.28	3.02	86.4	15.1
III	Practice	10	10	4.63	1.54	46.3	15.4

Tables 4 give a picture of the mean knowledge, attitude, and practice scores of respondents on iron consumption. It was clearly observed that the respondents have a greater positive attitude as compared to the knowledge and practice levels. Low KAP scores necessitate nutrition education interventions

DISCUSSION

Demographic information is crucial as it establishes whether the individual participants in a study are a representative sample of the target population for generalized purpose [10]. Majority of the subjects (76%) were within 22-23 years, belonged to Hindu religion (67%) and from an urban background (89%). Most of them were from nuclear families (83%) with a family income between Rs 20,000-40-000 (29%). Education constitutes an elemental social health determinant that helps in improving health by increasing the efficiency and enhancing self-control thus encouraging healthy standard of living [11]. All the subjects were pursuing master's degree with majority studying Science (51%). Religion, cultural and economic status leads to iron-poor vegetarian diets in the Indian population. Vegetarianism (30%) was predominant in the study population

and a minor portion of the population (13%) revealed food allergy/intolerance to egg, soy, seafood, milk, and milk products. A comparative study by Mahajani and Bhatnagar showed that non-vegetarian showed higher mean iron intake and anemia was more prevalent in the vegetarian group [12].

Consumption of outside foods lacks nutrients in general. Eating a poor-quality diet high in junk food is linked to unhealthy lifestyle and a higher risk of metabolic disorder and anemia. The present study highlighted that 16% consumed outside food always whereas 84% consumed it sometimes. Weekly consumption of outside food was mostly observed. Inadequate iron intake is associated with vegetarian diet, malnutrition, and lack of iron in diet. Foods with high fat and sugar content, junk food and low-calorie diet can cause anemia [13]. One-tenth of the sample population (10%) had hemoglobin levels below the normal range as revealed by their biochemical reports.

Higher incidence of anemia is often associated with girls who attain menarche early and have irregular menstrual periods [14]. Majority (64%) of the subjects attained their menarche at the age of 12-13 years and had regular menstrual cycle (88%). It was seen that the duration of menstrual bleeding for 43% was 3-4 days, while for 57% it was 5-7 days. The subjects reported various symptoms during menstruation- 13% reported vomiting, 64% reported back ache, 47% reported stomachache and 21% reported other symptoms such as headache, bloating, bowel issues and mood swings. A study conducted by Moschonis et al. [15] stated that on multivariate logistic regression analysis girls with menses were 2.57 times more likely to be iron depleted compared to girls with no menses.

Regular deworming along with iron-folic acid supplementation is an effective strategy to combat anemia and reduce soil transmitted helminth infections in women of reproductive age. Nearly one-third (29%) of the population had received deworming in the past 6 months. Casey et al. [16] carried out a study that demonstrated that regular intake of IFA supplement and deworming treatment led to an increase in mean Hb levels and decrease in anemia prevalence.

Most of the subjects had a height between 156-165 cm (51%) and weight between 40-52 kg (39%). Half of the population (51%) had normal BMI. Thus, a significant number of respondents were facing the dual burden of malnutrition. A cross sectional study was conducted by Agarwal et al. [17] where BMI exhibits higher prevalence of anemia among normal weight, followed by underweight, pre-obese and obese. Nearly one-tenth of the sample population had higher waist hip ratio indicating abdominal obesity. This was in par with a study done by Hemamalini [18] where it was seen that women with overweight/ obesity or central obesity were less likely to be anemic as compared to normal weight women.

Majority of the subjects (69%) did not have adequate knowledge about iron consumption. Hence, there is a need to increase the awareness through nutrition education on iron consumption and anemia among the subjects to enhance their knowledge level. A similar study was done by Geethanjali where majority women of reproductive age (18-45years) showed inadequate knowledge regarding anemia [19]. The present study showed that the majority (74%) had a favorable attitude towards iron consumption. It was seen that the majority (66%) of the subjects had a low practice level of consuming iron rich foods mainly because most of them were staying in paying guest or college hostels attributing to low iron consumption. A study done by Joglekar, *et al.* stated that anemia was a major health problem among the college going girls in government hostels [20].

The mean KAP scores of subjects on iron consumption clearly showed that they demonstrated good attitude however, their knowledge and practice levels were unfavorable. This was in par with the study by Anees and Rajeshwari [21] where the college girls exhibited a good attitude toward anemia but poor knowledge and practice.

CONCLUSION

Anemia remains a widespread health problem among women of reproductive age due to lack the appropriate knowledge, attitude, and practices. The study highlights that one-tenth of the population were anemic., three-fourth had a favorable attitude,

and only one-third of the population had adequate knowledge and showed moderate practice levels on iron consumption. College students showed a greater positive attitude towards iron consumption. Food based approaches are the need of the hour. Short-term supplementation; medium-term food fortification; and a long-term focus on balanced nutrition can be deployed individually or in combination to help combat anemia. There is a need to improve the knowledge about anemia and its prevention at the grassroots level. Continuous monitoring programs and nutritional health education sessions by health experts can help in improving their nutritional status thereby reduce the prevalence of anemia. Good knowledge, attitude and practice levels towards iron consumption can help to reduce the prevalence of IDA

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CONFLICT OF INTEREST

No conflict of interest

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Perceptions among Doctors and Nutritionists Regarding Food-Based Interventions for Combating Anaemia in the First 1000 Days of Life

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Abstract : *Anaemia is described as a 'silent killer among women in India. The Indian scenario of first 1000 days is a crucial period of shaping life and has a positive impact on both the mother and the child. Thus, controlling anaemia, during the first 1000 days of life, starting from conception becomes critical. Doctors diagnose anaemia and provide the necessary therapeutic treatment. Ideally, they need to refer patients to nutritionists who can help in providing long term, sustainable approaches. Similarly, nutritionists need to perceive value of their role in mitigating anaemia. The aim of the study was to understand the perceptions of both doctors and nutritionists regarding the role of food – based interventions in anaemia prevention during the first 1000 days of life. The study was conducted in Bangalore and data was collected from doctors and nutritionists, working in multispecialty hospitals and private clinics. Purposive random sampling technique was used. Maximum (52%) number of doctors and nutritionists considered therapeutic interventions to be most effective, while only 27% were aware regarding its negative effects. All the doctors agreed regarding the importance of counselling for iron deficiency anaemia, however only 24% referred their patients to a nutritionist. Only 18% of nutritionists reported that patients did not turn up for follow up due to preference of therapeutic interventions and socio – economic reasons. The study concluded that it is important that doctors refer their patients to nutritionists for effective counselling on food – based interventions.*

Keywords: *Food based interventions, Therapeutic interventions, Iron Deficiency Anaemia, counselling*

INTRODUCTION

India is a developing country where the productivity of the nation is largely dependent on the people working towards it. As a result, any health condition that affects people can in turn affect their role at work. One of the major causes of poor productivity is anaemia. Thus, anemia is a matter of national concern. Anaemia affects the productivity of an individual in various ways. It causes fatigue, tiredness, headache, light-headedness, muscle cramps, insomnia and also inability to work. Anaemia affects various age groups of an individual such as pre - school children, pregnant and non-pregnant women of childbearing age. It is described as a silent killer among women in India. 'Adolescence' is a shaping period of an individual where the highest amount of physical, psychological and behavioral changes takes place. Adolescent girls experience blood loss as a result of menstruation and poor sanitary practices. Due to these causes, their demands for hemoglobin are high. The most vulnerable age groups are the pregnant women and infants in whom anemia causes high death rates. In pregnant women, anaemia related death could be due to post - partum hemorrhage and eclampsia. In infants, it is due to prematurity and low birth weight. Anaemia, which begins during childhood, can worsen during adolescence and can get aggravated during pregnancy and the cycle follows with succeeding generations. Anemia caused during infancy can also lead to further health consequences, which last till adulthood. Some of these consequences are poor brain development, failure in language capabilities and body balance coordination skills. Hence controlling anaemia during the first 1000 days of life, starting from conception becomes important.

The Indian scenario of the first thousand days of life as a crucial period of shaping life was summarized. [1] This period has a positive impact on a child's cognitive and physical development. The right nutrition provided to the mother and for the child can have a profound impact on the child's growth and development. It can also reduce the risk of disease and protect the mother's health. Focusing on multi – sectorial nutrition efforts on evidence – informed interventions targeting this critical window can have lasting

implications across life cycle. The combination of good health and reduced risk of disease on mother and children can have powerful, lasting effect on the country's prosperity.

Food - based interventions are considered to be the most desirable and sustainable method for preventing micronutrient malnutrition. The strategies undertaken to enhance these approaches include, improving the availability of micronutrient rich foods, ensuring their access to households and changing feeding practices with regard to these foods. Food fortification approach to improve the diet of target populations shows less immediate impact but is sustainable and cost effective over a longer period of time.

Doctors diagnose anaemia and provide the necessary treatment. This will help only on a short - term basis. Ideally they need to refer the patients to nutritionists who in turn can help in providing long term, sustainable benefits. Nutritionists need to perceive the value of their role in mitigating anaemia just as much as the role of doctors. Studies showed that women perceived anaemia as normal during pregnancy and did not recognize the symptoms that were associated with it. Therefore the clinicians felt that the physiological processes were beyond parent's understanding. As a result, they felt that the nutritionists were well suited to collaborate with the health care team for addressing this issue.[2]

This problem can be solved to an extent by educating the parents regarding the seriousness of anaemia during pregnancy and its devastating consequences on the foetus. During the period of first 1000 days, the role of doctors is of key importance, due to their constant interactions with the parents of the child. As the hectic work schedule of most doctors, permits their interactions with parents only regarding therapeutic and other passive means of targeting anaemia. The role of the nutritionist now is important in describing the importance of food - based strategies as more effective and sustainable over a prolonged period of time. Hence this study is undertaken to obtain perceptions among doctors and nutritionists regarding therapeutic and food - based interventions as means of controlling anaemia in the first 1000 days of life.

MATERIALS AND METHODS

Research Design: A pre-structured questionnaire was developed for data collection. A phenomenological approach was used to collect data as it was felt that this would most suit the research goals and provide important early insights. The data was collected during the period of 2019-20. Purposive random sampling technique was employed to collect data from 100 health care professionals (Nutritionists, Paediatricians and Gynaecologists); of which 50 samples comprised of paediatricians, gynaecologists and the other 50 samples were of nutritionists. All samples were selected from private hospitals / private clinics. The subjects were selected as per defined inclusion criteria as willingness to participate in the study.

Selection of study subjects: Since doctors and nutritionists were involved in diagnosis and counselling process, these study subjects were selected to determine their perceptions regarding therapeutic and food - based interventions to control anaemia. The prevention and control of anaemia during the first thousand days is crucial and combines the involvement of both doctors and nutritionists in preventing and controlling it. Hence these study subjects were specifically selected to be a part of this study.

Development of tool: The developed tool was self - administered questionnaire. A combination of multiple choice, dichotomous and scaling questions were used. This design was created based on the assumption that doctors might or might not know about food – based approaches. Also, nutritionists might have a thorough knowledge regarding food – based approaches but might have issues with its implementation. The questionnaires were circulated through google forms via Whats app and email or through direct links with institutions.

The components of questionnaire of therapeutic interventions included oral iron supplementation and intravenous iron supplementation. Similarly, the components of food - based interventions included food fortification, complementary foods, foods with high iron – bioavailability and importance of nutrition counselling among women. The

type of questions that were measured in this section included, significance of anaemia in the first 1000 days, number of patients suffering from anaemia, causes and factors that contribute to anaemia in a pregnant / lactating woman and an infant, consequences of anaemia and questions based on therapeutic and food-based approaches towards anaemia. The part of the questionnaire that was developed for doctors consisted of questions related to necessity of counselling sessions, format of counselling sessions and referring to the right person in charge for carrying out the counselling sessions.

The part of the questionnaire that was developed for nutritionists consisted of questions related to the effectiveness of counselling sessions and whether doctors referred patients to them at the right time for such sessions. Perceptions regarding effectiveness of implementation of food - based – interventions were also incorporated. Participants have given their consent via email to participate in the study.

Statistical analysis: Statistical analysis was followed by data collection and tabulation of data. The statistical tests used in the study were: Chi – square test and Microsoft Excel and Microsoft Word were used to design tables.

RESULTS

Table 1 illustrates the demographic characteristics of the respondents wherein the age of doctors ranged from 27 to 64 years and nutritionists belonged to 23 to 56 years. Out of total, 42% of doctors belonged to the age group of 27 to 49 years, whereas 58% belonged to 50 to 65 years of age. Out of total nutritionists, 62% belonged to less than 27 years of age and 38% belonged to 27 to 49 years of age. Whereas, in contrast with the doctor's data, none of the nutritionists belonged to 50 to 65 years. Majority of doctors belonged to the age group of 50 to 65 years. Maximum female dominance was observed among nutritionists (50%). A significant difference ($p<0.05$) was observed between the age group and gender status of males and females in this study. Among doctors, maximum (92%) was married and only whereas, among nutritionists maximum (68%) were unmarried.

Table 1: Demographic characteristics of respondents

Character-istics	Category	Respondents						χ^2 Test
		Doctors		Nutritionists		Total		
		N	%	N	%	N	%	
Age group (years)	Below 27	0	0.0	31	62.0	31	31.0	60.10*
	27-49	21	42.0	19	38.0	40	40.0	
	50-65	29	58.0	0	0.0	29	29.0	χ^2 (0.05,2df) = 5.991
	Total	**50**	**100.0**	**50**	**100.0**	**100**	**100.0**	
Sex	Male	14	28.0	0	0.0	14	14.0	16.28*
	Female	36	74.0	50	100.0	86	86.0	
	Total	**50**	**100.0**	**50**	**100.0**	**100**	**100.0**	χ^2 (0.05,1df) = 3.841
Marital status	Unmarried	4	8.0	34	68.0	38	38.0	38.20*
	Married	46	92.0	16	32.0	62	62.0	
	Total	**50**	**100.0**	**50**	**100.0**	**100**	**100.0**	χ^2 (0.05,1df) = 3.841

*Significant at 5% level

Table 2 reveals that maximum (58%) number of doctors had 20 years and above experience whereas, maximum number of nutritionists (70%) had up to 5 years of experience. With respect to the employment status, maximum (72%) number of doctors worked full time and on the contrary, among nutritionists, maximum (92%) number worked as full time.

Table 2: Classification of respondents according to their employment and professional experience

Characteristics	Category	Respondents						χ^2 Test
		Doctors		Nutritionists		Total		
		N	%	N	%	N	%	
Employment	Full time	36	72.0	46	92.0	82	82.0	6.78*
	Part time	14	28.0	4	8.0	18	18.0	χ^2 (0.05,1df) = 3.841
	Total	50	100.0	50	100.0	100	100.0	
Professional Experience	Up to 5	4	8.0	35	70.0	39	39.0	46.63*
	6-19	17	34.0	12	24.0	29	29.0	χ^2 (0.05,2df) = 5.991
	20 & above	29	58.0	3	6.0	32	32.0	
	Total	50	100.0	50	100.0	100	100.0	

*Significant at 5% level

It was observed (table 3) that, maximum (52%) number of doctors found therapeutic interventions to be the most effective, while only 14% found community based interventions to be most effective. In contrast, among nutritionists, maximum (52%) number of nutritionists found therapeutic interventions to be most effective, while only 22% found community-based interventions to be most effective. However, a significant difference was observed at 5% level ($p<0.05$) with respect to community - based interventions between doctors and nutritionists.

Table 3: Suggestions on type of effective intervention for anaemia treatment

Effective Intervention	Effective Intervention (%)						χ^2 Test
	Doctors			Nutritionists			
	Most effective	Effective	Least effective	Most effective	Effective	Least effective	
Therapeutic	52.0	38.0	42.0	52.0	42.0	6.0	0.60 ^{NS}
Food - based	46.0	52.0	2.0	36.0	56.0	8.0	2.48 ^{NS}
Community based	14.0	52.0	34.0	22.0	24.0	54.0	8.32*

*Significant at 5% level, NS : Non-significant, χ^2 (0.05,2df) = 5.991

Table 4 depicts multiple responses regarding major reasons to prescribe therapeutic interventions shows that maximum (70%) number of doctors suggested ease in achieving the recommended dietary intake as a major reason. While nutritionists data shows that maximum (80%) number suggested ease in achieving the recommended dietary intake as the major reason, while only 44% suggested helps in achieving faster results as the major reason for prescribing therapeutic interventions.

Table 4: Knowledge regarding major reasons for prescribing therapeutic interventions

No.	Major reasons for therapeutic intervention	Respondents					
		Doctors (n=50)		Nutritionists (n=50)		Total (n=100)	
		N	%	N	%	N	%
1	Ease of consumption	29	58.0	24	48.0	53	53.0
2	Ease in achieving the recommended dietary intake	35	70.0	40	80.0	75	75.0
3	Easier to explain to parents	15	30.0	24	48.0	39	39.0
4	Helps in achieving faster results	24	48.0	22	44.0	46	46.0
	$\chi 2$ Test	2.74^{NS}					

NS : Non-significant, χ^2 (0.05,3df) = 7.815, @ Multiple Response

Table 5 depicts the knowledge regarding negative and positive effects of therapeutic interventions among both doctors and nutritionists. Among doctors, maximum (40%) number of doctors, agreed regarding the existence of negative effects of therapeutic interventions, while only 22% were unsure regarding its existence. Whereas, maximum (52%) number of nutritionists were unaware regarding the existence of negative effects of therapeutic interventions while only 14% were aware of it. Statistical significance ($p<0.05$) was observed with respect to knowledge of negative effects of therapeutic interventions between doctors and nutritionists. Knowledge regarding positive benefits of food - based interventions for anaemia in the first 1000 days of life recorded in multiple responses.

Table 5: Knowledge regarding negative effects of therapeutic interventions

Negative effects for Therapeutic interventions	Respondents						$\chi 2$ Test
	Doctors		Nutritionists		Total		
	N	%	N	%	N	%	
Yes	20	40.0	7	14.0	27	27.0	8.63* *Significant at 5% level, $\chi 2$ (0.05,2df) = 5.991
No	19	38.0	26	52.0	45	45.0	
Maybe	11	22.0	17	34.0	28	28.0	
Positive benefits of food - based interventions							
Sustainable over a longer period	37	74.0	34	78.0	71	71.0	NS : Non-significant, $\chi 2$ (0.05,3df) = 7.815, @ Multiple Response
Cost effective	38	76.0	31	62.0	69	69.0	
No administration by health care professionals	33	66.0	24	48.0	57	57.0	
No negative side effects	18	36.0	25	50.0	43	43.0	
$\chi 2$ Test	2.80^{NS}						

Maximum (76%) number of doctors suggested cost effectiveness as the reason for suggesting food - based interventions for anemia, while only 36% suggested non–visibility of negative side effects as the reason for suggesting food - based interventions. Furthermore, maximum (78%) number of nutritionists suggested sustainability over a prolonged period as the reason for suggesting food - based interventions, while only 48% suggested food - based interventions, since it does not require administration by health care professionals.

Table 6 shows maximum (96%) number of doctors suggested green leafy vegetables to the patients. On the other hand, only 42% suggested fortified foods with iron to the

patients. Likewise, maximum (90%) number of nutritionists, suggested green leafy vegetables and meat and poultry to the patients while, only 52% suggested fortified foods high in iron as a food - based intervention to the patients.

Other reasons that were suggested include ragi, dates, nuts with jaggery and association of vitamin C rich foods along with foods high in iron. Multiple responses were obtained from both doctors and nutritionists.

Table 6: Types of food - based interventions suggested to the patients.

No.	Types of foods based interventions	Respondents					
		Doctors (n=50)		Nutritionists (n=50)		Total (n=100)	
		N	%	N	%	N	%
1	Green leafy vegetables	48	96.0	45	90.0	93	93.0
2	Meat and Poultry	42	94.0	45	90.0	87	87.0
3	Fortified foods with iron	21	42.0	26	52.0	47	47.0
4	Fruits and vegetables high in iron	36	72.0	33	66.0	69	69.0
5	Others	4	8.0	5	10.0	9	9.0
	$\chi 2$ Test	0.94^{NS}					

NS : Non-significant, $\chi 2$ (0.05,4df) = 9.488, @ Multiple Response

From table 7 it can be observed that all the 50 doctors (100%) agreed to the fact that counselling is required by all patients encountered suffering from Iron Deficiency Anemia (IDA). Table also showed the referral by doctors to the right professional in charge for carrying out the counselling process. The table also determines the rate of patient turn up for follow up counselling sessions. Maximum (40%) number of nutritionists determined that patients do turn up for counselling sessions, while only 18% disagreed with the fact that patients did turn up for follow – up counselling sessions. Of the respondents, who disagreed with the fact, 6% was due to preference of therapeutic interventions and the remaining 12% was due to lack of implementation due to socio – economic reasons. Among the respondents who agreed with the fact, 22% agreed that patients turned up

once for a follow up session and 18% of the respondents suggested that patients turned up more than once for a follow up counselling session.

Table 7: Necessity and referral to Nutritionist for counselling regarding Iron Deficiency Anaemia (IDA)

Necessity of counselling	**Response**	**Respondents (N=50)**	
		N	%
Necessity of counselling regarding Iron Deficiency Anaemia (IDA)	Yes	50	100
	No	0	0.0
Referral to the right – professional in charge			
Refer someone who carry out the counselling process	Yes	12	24.0
	No	38	76.0
Refer to a Nutritionist / Dietician	Yes	12	24.0
	No	0	0.0
Carry out the counselling process by yourself	Yes	38	76.0
	No	0	0.0
Patients turn up for follow-up counselling sessions	Yes	20	40.0
	No	9	18.0
	Sometimes	21	42.0
Yes-How often	Once	11	22.0
	More than Once	9	18.0
No-Why	Preference of therapeutic interventions	3	6.0
	Lack of implementation due to socio economic reasons	6	12.0

DISCUSSION

Iron deficiency anaemia, a significant public health problem in India is caused due to various reasons such as inadequate intake of iron, low bio – availability of dietary iron from plant foods and presence of inhibitory factors in the diet. The focus must be made towards food-based approaches instead of relying on iron tablets, syrups and other forms of medical supplementation. Therefore, this study was undertaken to determine the perceptions among doctors (Gynecologists, Pediatricians) and Nutritionists regarding food-based interventions for anaemia and to determine its awareness and effectiveness of implementation in practice among both doctors and nutritionists.

A study that was conducted to determine anaemia among infants and toddlers showed peak nutritional Iron Deficiency Anaemia (IDA) in infants aged 9 – 12 months, thus describing similarity with this particular study that nutritional anaemia was most frequently encountered anaemia.[3] Homogenous results were obtained from another cross – sectional survey that was conducted on anaemic women. The prevalence of anaemia was found to (27.1%). Of the anemic women, (34.5%) were deficient in Vitamin B_{12} and (71.7%) were deficient in folate, thus indicating nutritional Iron Deficiency Anaemia (IDA). [4]

The type of diet consumption is one of the important factors contributing to iron deficiency anemia. A study that was conducted to assess iron deficiency in Chilean infants, depicted that most families followed pediatric society recommendations. The diet that was suggested provided = 5 mg Fe/d/ mostly of vegetable origin with poor bioavailability, thus indicating that low intake of bio – available iron was one of the major causes of Iron Deficiency Anaemia (IDA) in infancy. [5] Another study conducted described the high prevalence of anaemia in India low dietary intake, poor iron (less than 20 mg /day) and folic acid intake (less than 70 micrograms/day); poor bioavailability of iron (3-4 percent only). The low dietary intake of iron and folic acid coupled with poor bioavailability of iron is the major factor responsible for very high prevalence of anaemia in the country [6]. As reported in a study that the nutrient intake per day was lower than the recommended

intake and the iron requirements were less than 50% of the RDA in school going children. This could be due to consumption of low bioavailable foods sources or low intake of Vitamin C which hinders the absorption of iron [7].

In a healthy woman, attention must first be paid to dietary habits. A study that was conducted among 269 pregnant women attending antenatal clinic described that dietary factors was a major contributor to iron deficiency and thus showed that the women on a vegetarian diet suffered with high prevalence of anaemia (40.14%) when compared to a mixed diet [8].

In a study to assess the association between Iron Deficiency Anaemia (IDA) and cognitive functions in infancy, it was observed that infants with Iron Deficiency Anaemia (IDA) and those with hemoglobin levels <105g/L showed poorer recognition memory than infants without Iron Deficiency Anaemia (IDA) [9].

Nutritionists must understand Iron Deficiency Anaemia (IDA) and doctors including (physicians, general practitioners and others) should be aware of the dietary requirements that must be prescribed to the patients. Henceforth both health care professionals have to come together to briefly explain, with examples, the type of diet that must be recommended to patients suffering from Iron Deficiency Anaemia (IDA) [10].

Factors associated with Hb and anaemia may inform anaemia reduction interventions among school-going adolescents and suggest the need to tailor them uniquely for boys and girls. Age was positively associated with anaemia among girls, but negatively associated among boys. [11] Such data suggests that treatment (therapeutic interventions) is both desirable and effective. Similar results were obtained in another study conducted on pregnant women in Northern Tanzania, where it was observed that women who reported having received iron supplementation during pregnancy had lower prevalence (20.2%) than those who did not receive supplementation (29.5%) [12].

Results observed were similar to a questionnaire based cross – sectional study of eligible pregnant women receiving antenatal care, one of the major barriers to compliance to iron supplements included gastrointestinal side effects of iron supplements (41.7) thus indicating that gastrointestinal side effects were the most commonly observed negative effect of therapeutic interventions [13].

Majority of the respondents in this study showed positive results in comparison to a qualitative study that was conducted to determine the adherence to nutritional counselling by patients. It was observed that participants indicated that a doctor's role in referring patients to dietitians is a strong factor in the patient's decision to continue the counselling. Lack of attendance for counselling session may indicate a lack of adherence to treatment [14].

In order to overcome Iron Deficiency Anaemia (IDA), among the adolescent girls there is need to conduct T-3 camps at regular intervals in schools. Vegetarian diet, underweight deworming was the associated factors with occurrence of anaemia. [15] In conclusion doctors and nutritionists must suggest improved dietary intake and improved knowledge on anemia as a common behavioral change needed to combat nutritional anaemia. Uttarakhand study shows prevalence that anaemia is a major public health problem and requires effort to reduce the prevalence [16].

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Effect different drying techniques on the physical and morphological characteristics of multi cereal and legume mix

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Abstract : *The food physical properties define the application and use of food materials for the formulation of food products. The present research study emphasis on the preparation of multicereal and legume composite mix with the application of processing and drying techniques. The whole good and mature cereals grains and legumes with good nutritional potency were chosen for the preparation of multicereals and legume composite mix. The selected raw grains were processed by washing to remove the unwanted dust particles, weighed, and soaked in plain water for 12 hours, after draining the excess water it was allowed to sprout for 24 hours, and then subjected to drying methods namely Forced Convection Tray Drying at 60°C for 8 hours and Fluidized Bed Drying 60°C for 6 hours. The processed raw grain materials were milled to a fine flour. The findings of the processed multi cereals and legume composite mix were studied in terms of physical and morphological characteristics. The drying techniques had affected significantly ($p<0.05$) the water holding capacity, bulk density, foam capacity, and stability, irrespective of different drying methods adopted. The morphology of the flour possesses the starch granules that are captured in the protein network. Other fat globule bodies and protein bodies were found above the starch granules. Hence this multi cereal and legume composite mix will serve as multipurpose flour for the development of functional foods.*

Keywords: *Cereals, Legumes, Drying, Morphology, Composite Mix*

INTRODUCTION

Many research studies have highlighted on the health-promoting and promising perceptions of cereals and legumes which can decrease the risk of metabolic as well as degenerative diseases [1-3]. Processing performs an important role in improving the sensorial properties of the food in addition it increases the bioavailability of nutrients by inactivating anti-nutritional factors, growth inhibitors, and heam-agglutinins. Amongst different processing techniques, germination and dehydration are classic types of processing techniques which alter the physical and chemical characteristics of the raw material for a food formulation. In addition, germination of cereals and legumes will improve the nutritional potency and thereby provide good nutritional status. Dehydration is most important procedures in the production of food to extend the shelf life, accelerate transport convince and produce value-added products [4]. Ideally, drying processes can be structured to decrease drying time and reduce energy costs while sustaining high product quality [5]. The prime objective of the current study is to study the impact of different drying techniques in the physical properties of multi cereal and legume composite mix.

MATERIALS AND METHODS

There is a shred of growing evidence base research that whole cereals and legumes have important roles in the prevention of diseases. The whole cereals and legumes with massive nutritional potentiality namely Parboiled rice *(Oryza punctata)* Kodo millet *(Paspalum scrobiculatum)* Sorghum *(Sorghum bicolor)* Foxtail millet *(Setaria italica)* Green gram *(Vigna radiata)* Bengal gram *(cicer arietinum)* Black gram *(Vigna mungo)* Cow pea *(Vigna unguiculata)* Soyabean *(Glycine max)* were selected for the formulation of processed multicereals and legume composite mix. The selected raw materials were acquired from the supermarket in a wholesale quantity and stored in airtight containers to ensure safety and uniformity in the raw materials throughout the processing. Some minor millets like sorgum, foxtail millet, and kodo millet were obtained from country medicine shop because of its reduced utility among the people. Raw material was processed by soaking for overnight, sprouting for 24 hours, dehydration for 6 hours, and milling in

a proper environment for the formulation of multi cereal composite mix [6]. The raw material was washed and soaked overnight and allowed for germination for 12 hr in normal room temperature after which they were subjected to a Forced Convection Tray Dryer (FCTD) at 60 ^{o}C for 8 hours and Fluidized Bed Drier (FBD) at 60 ^{o}C for 6 hours. The dehydrated multi cereals were milled to a fine flour. The fine flour obtained was stored in proper packaging material and stored in airtight container for further use. The multi cereal and legume composite mix is evaluated for the physical and morphological characteristics. The steps carried out in the development of multi cereal and legume composite in presented in Figure 1.

Quality Characteristics of Multi Cereal and Legume Composite Mix: The most important physical parameters for multicereal and legume composite mix was studied in terms of swelling power (g/g) [7], water holding capacity (%) [8], bulk density (g/cm^3) [9], foam stability and foam capacity (%) [10] and moisture content (%) [11]. The morphology of multi cereal and legume composite mix was assessed by scanning electron microscope (SEM) (QUANTA FEG 250 ESEM).

Statistical Analysis: The data were statistically analysed by using SPSS and the results were represented as the mean and standard deviation.

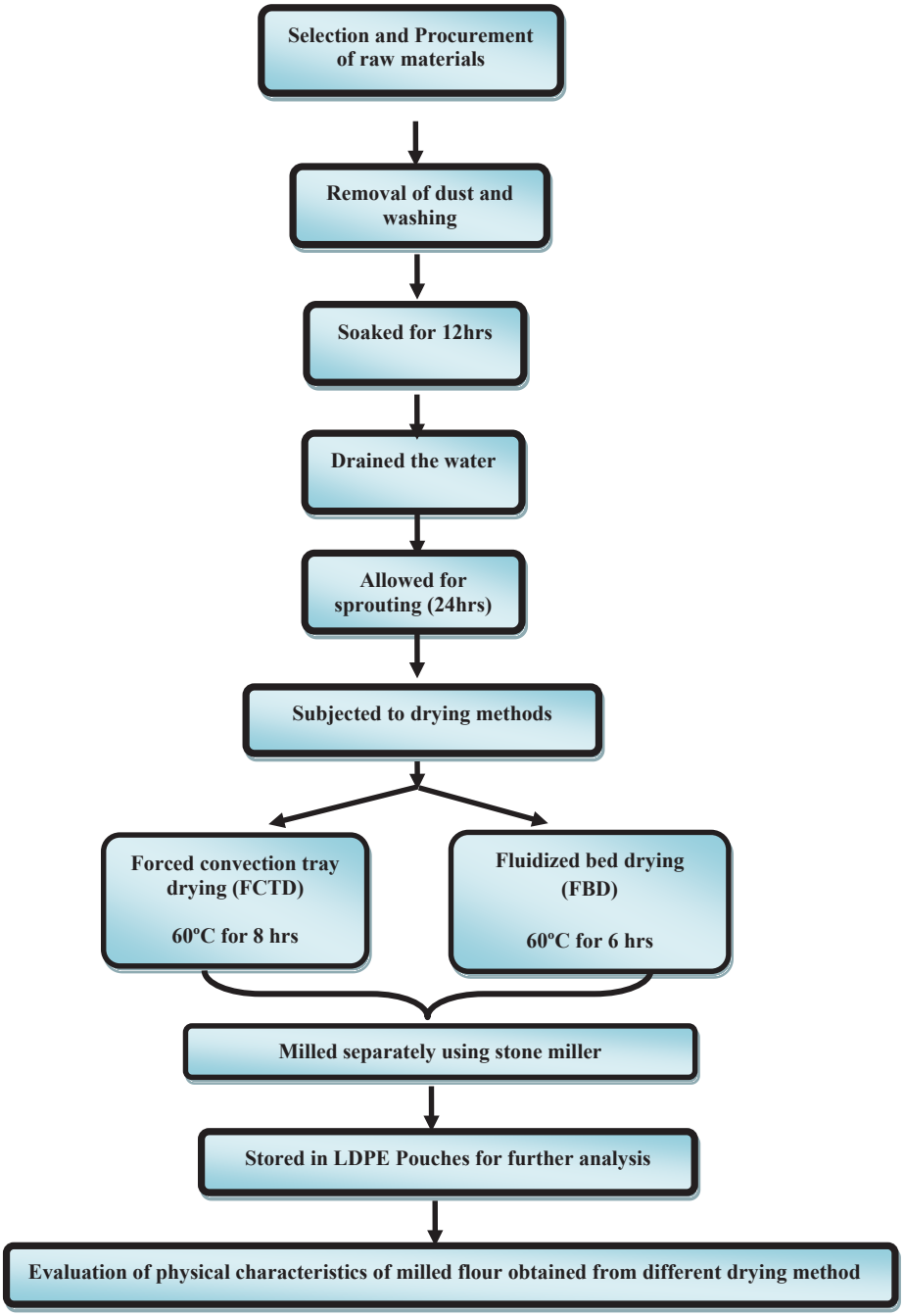


Fig. 1 : Steps involved in the formulation of processed multi cereals and legume composite mix

RESULTS AND DISCUSSION

The physical characteristics of the composite mix are very vital to define the final attributes of the product. The physical characteristics of the multicereal and legume composite mix shown in Table 1.

Table 1. Physical Characteristics of Multi Cereal and Legume Composite Mix

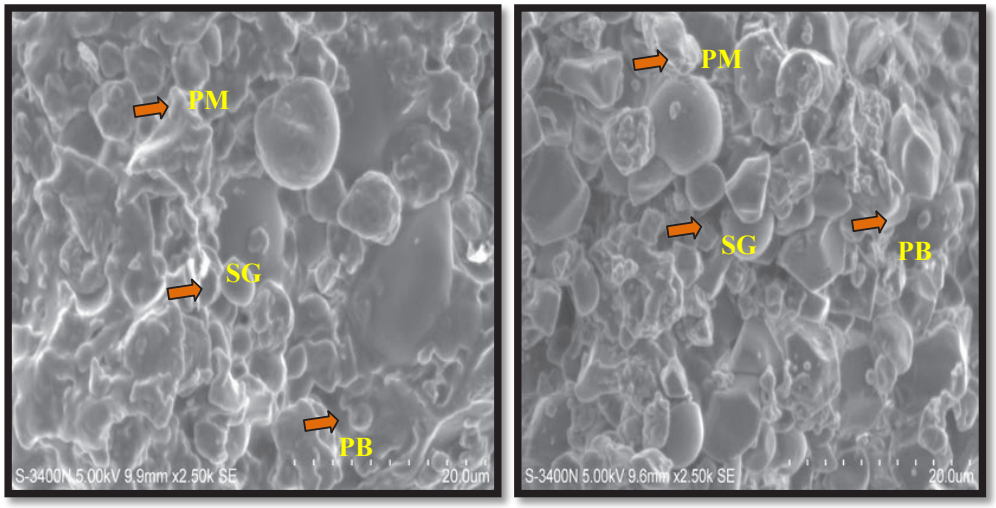
Parameters	FBD	FCTD	pValue
Moisture (g/100g)	6.63 ± 0.30	4.96 ± 0.15	0.00*
Bulk Density (g/cm^3)	0.78 ± 0.02	0.85 ± 0.02	0.002*
Swelling Power (%)	2.16 ± 0.15	2.13 ± 0.15	0.013
Foam Capacity (ml)	33.33 ± 1.52	37.00 ± 1.00	0.00*
Foam Stability (ml)	27.70 ± 0.10	29.70 ± 0.10	0.00*
Water Holding Capacity	4.10 ± 0.10	4.30 ± 0.10	0.018*

All values are means of triplicate determinations ± standard deviation (S.D); FBD– Fluidized Bed Drying, FCTD– Forced Convection Tray Drying, * Significantly different ($p \leq 0.05$) by ANOVA

Wetness is a most essential part of the grains, the findings of research studies shows that moisture content lesser percentage in the food samples is an acceptable parameter, since the microbial activity is reduced [12]. The shelf life of the food products can be extended if the moisture percentage is less [13] in tern increased moisture percent in the foods lead to microbial load leading to food spoilage [14]. In the current study, the findings show ($p \leq 0.05$) significant difference in the moisture percent of the multicereal and legume composite mix which is obtained from the raw material which is obtained from different drying techniques. On comparing the different drying systems, forced convection tray drying retained less moisture content when compared to other FBD drying techniques. The normal moisture level of the grains is 8%, which is relatively reduced after dehydration. Bulk density is an imperative parameter that indicate the porosity of a product which affects package desig. It is also important in infant feeding where less bulk density is desirable, and the finding of the present study is on equality with Iwe (1998)

[14]. Significant ($p \leq 0.05$) was observed between the two drying techniques FBD shows less bulk density compared to FCTD. Swelling power shows the hydration capacity of starch, regardless of different drying methods adopted, the swelling index of the composite mix exposed to FBD possess slightly high level ($p \leq 0.05$) of swelling when compared to other drying technique. The retention of the water is majorly depending on the proportion of amylose content and amylopectin which helps in the gel structure to retain water [15]. If the allocation of amylose in starch granules was not uniform, then it will affect the swelling power of starch [16]. The formation of the foam in the raw material varies on the presence of flexible protein molecules which may decrease the surface tension of water [17]. The formation of form in the food raw material varies with the type of protein, solubility, and other factors [18]. The results disclosed that maximum foaming capacity and foaming stability was observed for the composite mix exposed to FCTD is significantly ($p \leq 0.05$) high when compared with the FBD. The low foamability of flour indicates the presence of highly ordered globular protein molecules which increase the surface tension. Water holding capacity of food can be defined as the ability to hold its own and added water during application of force, pressing, centrifugation or heating. The water holding capacity was significantly higher ($p \leq 0.05$) in FCTD drying techniques. The difference might be due to the quality of the damaged starch and undamaged starch present in flour before and after sprouting.

Morphological characteristics of multi cereal and legume composite mix: The scanning electron micrograph of the multi cereal and legume composite mix obtained from FCTD and FBD is shown in Figure 2.



FBD FCTD

Fig 2. SG- starch granules, PM -Protein Matrix, PB- Protein Bodies

Figure 2 show off the morphological presence of granulated starch with varying size and shape in the form of small to large and oval to elliptical, respectively. The outer surface of the particles. The exterior surface of most of the granules was rather smooth and rough. The starch granulates are observed to be encircled by small particles which is like protein, fibers providing the exterior surface like 'raising dust'. From the figure it depicts different granular sizes in the composite mix subjected to FCTD and FBD. These particles have been attached owing to the existence of protein residuals [19] or due to the drying methods the production of heat which form the gel on the top layer of granulates and project the granules to form adhered appearance which together to form aggregates [20]. The particle of the composite mix forms a steady amorphous layer which is giving a network structure [21].

CONCLUSION

Utilization of multi cereal and legume composite mix possesses great functional properties in terms of swelling index, bulk density, and foaming properties. The product

developed using will be excellent to the products formulated using refined flours and extend a wider spectrum for people who wish to improve the nutritional quality of their diet. Successful utilization of cereals and legumes with added potential functionality in the snack food sector will open new dimensions to the food industries.

Acknowledgments: Financial support from the Pondicherry University for smooth conduct of research.

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MARITAL ADJUSTMENT IN WORKING COUPLES

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Abstract : *This paper presents the marital adjustment of working married couples focusing on other factors like the gender, age groups and the years of marriage of that couple. A sample of 60 working couples (total of 120 subjects) were selected residing in Bangalore city. To carry out this study "Marital Adjustment Questionnaire" by Pramod Kumar and Kanchana Rohatgi was used. This paper results in finding out the marital adjustment of working men and women, individually and as couples.*

Keywords- *Marital adjustment, relationships, sexual adjustment, social adjustment,*

INTRODUCTION

Marital adjustment is the state in which there is an overall feeling in husband and wife of happiness and satisfaction with their marriage and with each other. Working females have to play dual roles as they are employed outside house. They shoulder more responsibilities than males, adjust their time and energy for children, spouse, and household activities, religious and social activities. In a study on inter role conflict and marital functioning found that conflict regarding work and family roles was associated with lower levels of marital adjustment among dual earner couples.

Lasswell (1982) pointed out, "understanding the individual trait of the spouse is an on- going process in marriage; because even if two people know each other before of at

the time of marriage, there is a possibility that people change during the life cycle. Sinha and Mukerjee (1990) defines marital adjustment as, "the state in which there is an overall feeling between husband and wife, of happiness and satisfaction with their marriage and with each other."

A working woman has to perform two roles. Firstly, a mother and housewife, and secondly of an employee. Both the roles demand on her time and energy. Her employment not only affects her entire personality but also her family relationship and is also liable to face crisis of adjustment which can result in depression.

Most couple's marriage are filled up with expectations. Some of the expectations will be realistic while others unrealistic. This is due to the complex nature of marriage and each individual is as complex as a universe. Therefore, in marriage two universes close together. Marital adjustment calls for maturity that accepts and understands growth and development in the spouse. If this growth is not experienced and realized fully, death in marital relationship is inevitable. The attempt has been made through this paper to study the marital adjustment in working couples.

METHODS & MATERIALS

The sample included of 120 subjects, out of which 60 were working males and 60 were working females. Each group of working couples were divided according to the age in equal numbers i.e., 20 couples in between 20 years to 35 years, 20 couples in between 35 years to 50 years, 20 couples in between 50 years to 65 years both in working each. Random sampling was done in the Bangalore city. Descriptive research design was used for this research.

"Marital Adjustment Questionnaire" by Pramod Kumar and Kanchana Rohatgi was used. This test was divided into 3 areas of adjustment- sexual adjustment, social adjustment and emotional adjustment. Statistical analysis was done using t-test and f-test

RESULT AND DISCUSSION:

The present study was investigated with broad objective of studying the marital adjustment of working couples residing in Bangalore city.

The results obtained are evaluated and presented below:

Table 1 : Comparison of mean scores between age groups under various dimensions of marital adjustment of wife

Dimensions of Marital adjustment	Age groups (in Yrs)			Significance of F value
	20 - 35	35 - 50	50 - 65	
Sexual	3.40 ± 0.88	2.95 ± 0.99	1.30 ± 0.22	34.8848**
Social	7.95 ± 1.20	7.30 ± 1.48	6.85 ± 0.76	5.3066**
Emotional	10.30 ± 0.74	10.40 ± 1.62	10.40 ± 0.77	0.0635^{NS}
Overall	21.65 ± 3.71	20.65 ± 3.60	18.55 ± 2.26	15.6759**

**** Significant at 1% level NS Not significant**

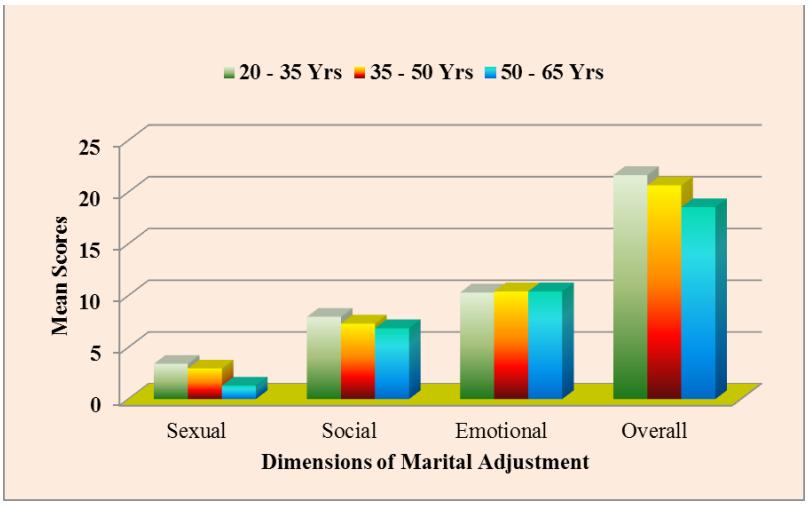

Fig. 1 : Comparison of mean scores between age groups under various dimensions of marital adjustment of wife

Table 1 and Fig 1 shows the mean score of marital adjustment of wives (working married women). It was found that there is a significant difference in the sexual and social dimensions of marital adjustment. The overall marital adjustment also shows the significant difference. Only in one dimension i.e., Emotional dimension has no effect of age on it. It has no significant difference because of age.

Table 2 : Comparison of mean scores between age groups under various dimensions of marital adjustment of husband

Dimensions of Marital adjustment	Age groups (in Yrs)			Significance of F value
	20 - 35	35 - 50	50 - 65	
Sexual	3.55 ± 0.47	2.95 ± 0.68	1.30 ± 0.22	59.2931**
Social	8.05 ± 0.68	6.65 ± 1.60	6.90 ± 0.62	11.4927**
Emotional	10.35 ± 1.85	9.85 ± 1.81	10.25 ± 1.14	1.2305NS
Overall	21.95 ± 1.20	19.45 ± 3.52	18.45 ± 2.36	27.4749**

**** Significant at 1% level NS Not significant**

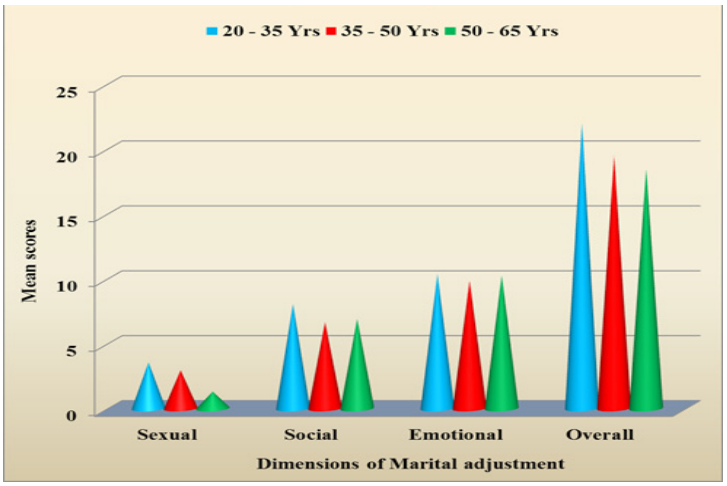

Fig. 2 : Comparison of mean scores between age groups under various dimensions of marital adjustment of husband

Table 2 states that the mean score of marital adjustment of husbands. It was found that there is a significant difference in the sexual and social dimensions of marital adjustment. The overall marital adjustment also shows the significant difference. Only in one dimension i.e., Emotional dimension has no effect of age on it. It has no significant difference because of age.

Table 3 : Comparison of mean scores between age groups under various dimensions of marital adjustment of couples

Dimensions of Marital adjustment	Age groups (in Yrs)			Significance of F value
	20 - 35	35 - 50	50 - 65	
Sexual	3.20 ± 1.01	2.50 ± 1.31	1.25 ± 0.19	23.2002**
Social	7.30 ±1.90	6.30 ± 1.58	6.35 ± 0.45	4.8292**
Emotional	9.45 ± 0.68	9.75 ± 1.67	9.75 ± 0.93	0.5476NS
Overall	19.95 ± 3.83	18.55 ± 3.94	17.35 ± 1.81	10.5804**

**** Significant at 1% level NS Not significant**

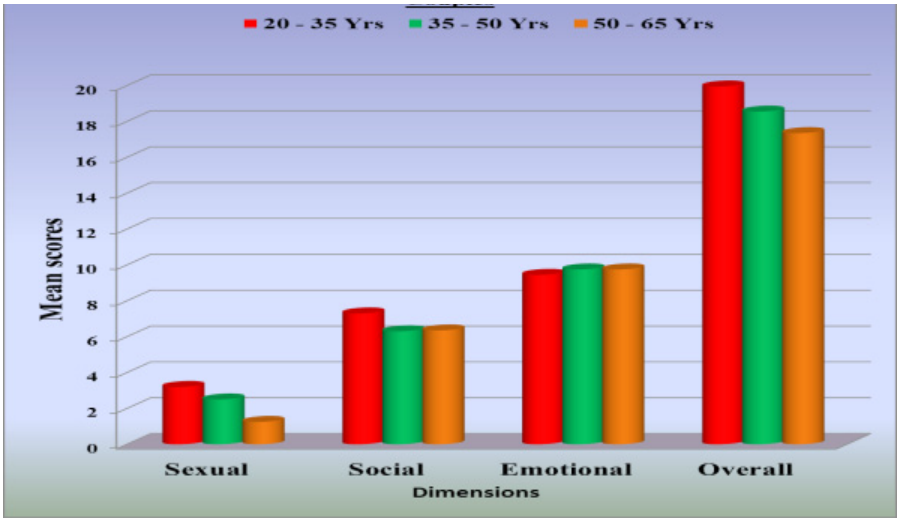

Fig. 3 : Comparison of mean scores between age groups under various dimensions of marital adjustment of couples

Table 3 states that the mean score of marital adjustment of couples. It was found that there is a significant difference in the sexual and social dimensions of marital adjustment. The overall marital adjustment also shows the significant difference. Only in one dimension i.e., Emotional dimension has no effect of age on it. It has no significant difference because of age.

Table 4 : Significance of Correlation coefficient marriage age & wife's marital adjustment

Correlation coefficients	Age groups (in Yrs)		
	20 - 35	**35 - 50**	**50 - 65**
Sexual	- 0.7118**	- 0.6213**	- 0.1527^{NS}
Social	0.1858^{NS}	- 0.2976^{NS}	0.2654^{NS}
Emotional	- 0.4768*	0.3991^{NS}	- 0.4857*
Overall	- 0.4553^{NS}	- 0.2500^{NS}	- 0.1784^{NS}

**** Significant at 1% level * Significant at 5% level NS Not significant**

Above table 4 indicates that married women in the age group of 20 -35 shows significant difference in sexual and emotional aspects of marital adjustments. Whereas at 50 – 65 only emotional dimension of marital adjustment shows a difference which means age does affect the various dimensions of marital adjustments.

Table 5 : Significance of Correlation coefficient age of marriage and husband's marital adjustment

Correlation coefficients	Age groups (in Yrs)		
	20 - 35	**35 - 50**	**50 - 65**
Sexual	- 0.5455**	- 0.3031^{NS}	- 0.3676^{NS}
Social	0.0961^{NS}	- 0.1027^{NS}	0.0968^{NS}
Emotional	- 0.1578^{NS}	0.7026**	- 0.2675^{NS}
Overall	- 0.3647^{NS}	0.3020^{NS}	- 0.2488^{NS}

** Significant at 1% level NS Not significant

As far as husbands are concerned the above table 5 shows age does has an effect on the sexual adjustment and the emotional adjustment . there is a significant difference in sexual aspect in males at the age of 20 – 35 and in the emotional aspect at 35- 50 years.

CONCLUSION:

This study has shown that there is no significant difference found in the marital adjustment of married couples and age does shows a effect on the adjustments between husband and wife. The study resulted in the difference between the married male and female marital adjustments. The study also showed a significant of correlation of the three areas of marital adjustment – sexual, social and emotional dimension and are correlated to each other.

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Synthesis of Zinc oxide nanoparticle and its photo catalytic activity for the application of water purification

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Abstract : *This report is based on the effect of different molarities of ZnO nanoparticles on the photodegradation of the Methylene Blue dye. ZnO was synthesized using the sol gel method with different molarities such as 0.1M, 0.3M, and 0.5M. The prepared samples were characterizations by X-Ray Diffraction (XRD) results has shown that the samples have a hexagonal wurtzite structure Scanning Electron Microscopy (SEM) has confirmed the sizes of the nanoparticles were spherical and flak structure, Energy Dispersive X-Rays (EDX) has proved the presence of Zinc and Oxygen in the samples and Ultraviolet-Visible (UV-Vis) Spectroscopy has helped to find out the absorption spectrum of the synthesized samples. The prepared material was mixed with methylene blue solutions and the degradation study was performed. Methylene Blue dye has been used to study the photocatalytic effect of the synthesized samples. On observation, it is found that the amount of dye degraded has subsequently decreased in concentration.*

Keywords: *photo catalytic, water purification, Zinc oxide nanoparticles.*

INTRODUCTION

According to IUPAC, photocatalysis is defined as "Change in the rate of a chemical reaction or initiation under the action of ultraviolet, visible infrared radiations in the presence of the substance-the photocatalyst- that absorbs light and is involved in the chemical transformation of the reaction partners." [1]. Photocatalysis occurs when there

is simultaneous oxidation and reduction reaction taking place on the surface of the catalyst and the reacting material. The reaction takes place due to the oxidation occurring on the photogenerated holes and reduction occurring on the photogenerated electrons [2].

Every day enormous amounts of dyes are being produced on demand by industries. These ultimately end up in the water bodies as pollutants and discharges. To address these issues of such impurities, photocatalyst efficacious answer. Making use of the help of abundant sunlight bestowed upon earth which is a combination of both visible and ultraviolet ranges of the electromagnetic spectrum, enables us to experiment with a variety of photocatalytic active elements [3].

Kalpesh et al., [4] studied the degradation of Methylene Blue after synthesizing ZnO nanoparticles under UV irradiation. Different concentration of Methylene blue was prepared namely 50 ppm, 100 ppm and 150 ppm. Sol gel technique was used to synthesize nanoparticles and it is the ability to control precisely, the particle size and morphology through systemic monitoring of the reaction parameters. And as compared to the other synthesis methods it provides a higher yield percentage.[5] Zinc oxide nanoparticles were synthesized using various chemical synthesis methods using different precursors. Different measures were taken to control the size of the particle along with its uniformity.

Li, D., Huang, et al., reports the synthesized potassium doped ZnO photocatalysts for the degradation of Rhodamine Blue Dye solution by using starting materials. In this report, ZnO nanoparticle preparation was done using sol gel technique for different molarity concentrations. The prepared material was used to study the photocatalytic effect for Methylene blue dye.

MATERIALS AND METHODS

Zinc oxide synthesis using sol gel method: Firstly 0.1 M, 0.3 M and 0.5 M Zinc acetate solution were prepared and mixed in 20 ml DMSO, thereby the solution was stirred for 30 mins. Following that, another solution containing 1.2M KOH in 20 ml

ethanol was prepared. Then this solution was added to the first solution dropwise and stirred for another 5 minutes. After that 0.12 ml (2-3 drops) of thioglycerol is added to the solution and it was stirred for 1 hour until the solution turned milky. Finally, it is centrifuged using distilled water and the ethanol supernatant was thrown away and the UV-Vis spectra taken. [1,2]

Synthesis of methylene blue solution: Initially, 100 mg of methylene blue is taken and dissolved in 100 ml water to make a 1000 ppm stock solution. Then it was diluted with 50 ml water and stirred for 10 minutes and then again it is diluted with 50 ml water to make it 100 ml and stirred for another 30 minutes to give an even solution. Thereby 5 ml, 10 ml and 15 ml solutions were taken out from the stock solution and dissolved in 100ml water to make solutions of 50, 100 and 150 ppm respectively. Following that, the different molarities of Zinc oxide was dissolved in the methylene blue dye.

Photo degradation of the dye solutions: The catalyst 10 mg is added to the prepared concentrations of the methylene blue solutions and stirred in the dark for 30mins to achieve the adsorption-desorption equilibrium. The dye peak is observed and noted. This solution is then transferred to the UV chamber for 2 hours to observe the degradation under UV light (365 nm). After every 30 minutes, 3 ml of the dye solution is taken, centrifuged to get rid of any catalyst particles, and UV peak intensity is observed. The same is repeated for all three concentrations of the dye solution for the three types of the sample prepared.

RESULTS AND DISCUSSIONS

X-Ray Diffraction (XRD) analysis of ZnO nanoparticles: XRD spectrum of the synthesized samples was show in figure 2. The samples have a hexagonal/wurtzite structure (JCPDS00-036-1451) [9]. The maximum (2thetavalue) peak intensities forth samples are attained at (101),the 2 theta value being 36.02°.Other peaks for(100), (002), (102) and (110) were obtained at 31.72°,32.99°,47.31°and56.4° respectively. From the

figure it's found that pure phases for all the three samples have formed with little or no impurities at all.

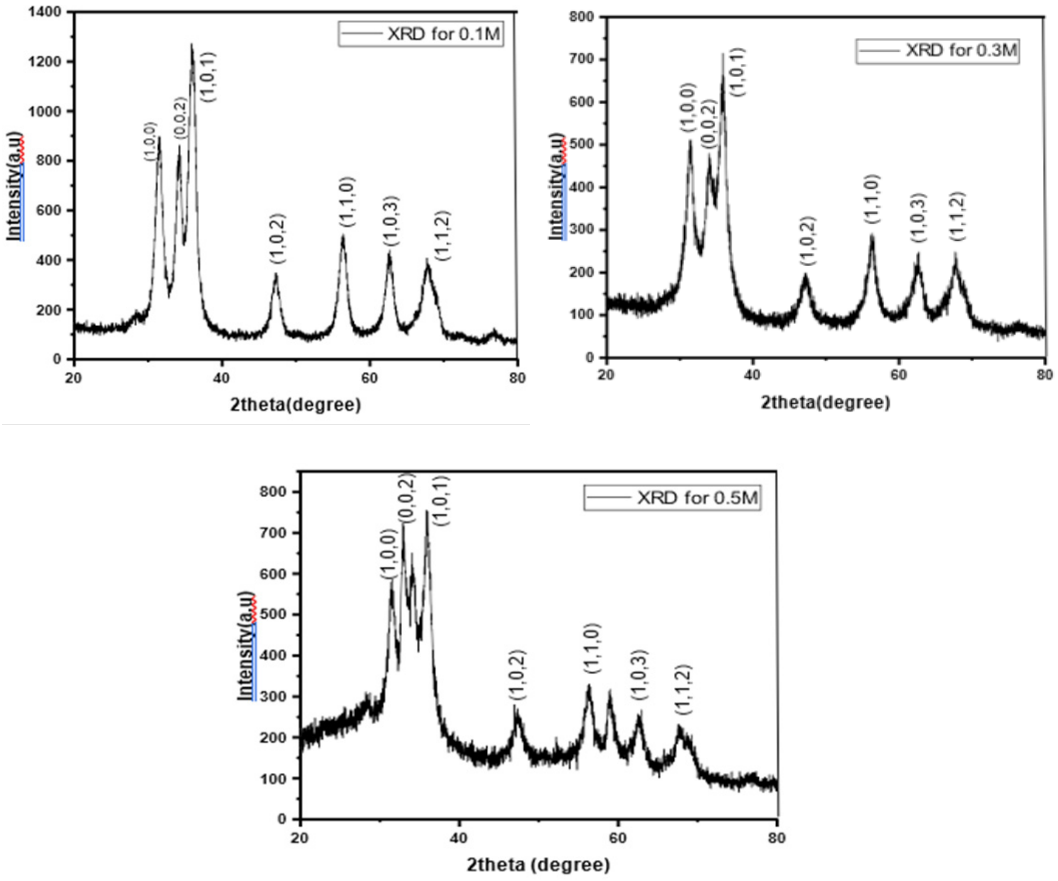

Fig 2: XRD for 0.1, 0.3, and 0.5 M of prepared Zinc oxide nanomaterials

Table 1: XRD data calculated from the graph

Sample	Lattice Parameter a	Lattice Parameter c	Crystallite size (nm)
0.1M	3.13	5.13	3.29
0.3M	3.13	5.11	4.4
0.5M	3.12	5.13	2.43

The crystallite size is found out using the Debye Scherrer's formula:

$$\mathbf{D} = \frac{k\lambda}{\beta \cos\theta}$$

Where K= shape constant = 0.9

λ = wavelength of the x-ray source = 0.154 nm

β= FWHM (full width at half maximum)

θ= Bragg's angle of diffraction angle.

It is observed that the crystallite size has first increased from 0.1M to 0.3M and then it has decreased to 2.43M due to agglomeration of the particles at a higher concentration. [6]

Scanning Electron Microscope analysis of ZnO nanoparticles

Fig 2 (a,b,C) shows the Scanning Electron microscope image for surface analysis of Zinc oxide nanoparticle . The size of the nanoparticles ranges from 19.65 nm to 49.11 nm. It shows that the particle size varies within the range from 14.65 nm to 35.28 nm. Figure 2a and 2b depicts the porous structure ZnO while figure 2c depicts the flake like structure of Zinc Oxide. This is a flake like structure in contrast with the porous structures. This structure formation may be due to the agglomeration of the nanoparticles at a higher concentration.[7].

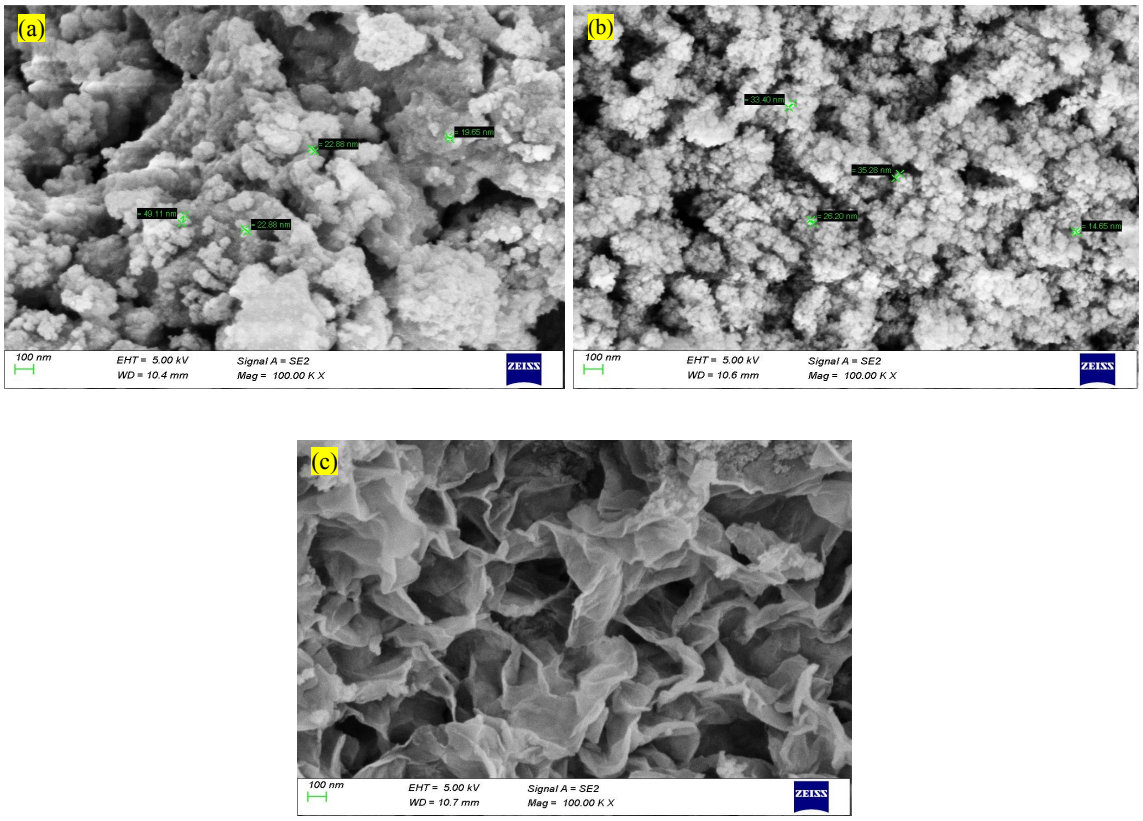

Fig 2: SEM image (a) 0.1M sample, (b) 0.3M sample, and (c) 0.5M sample

Energy Dispersive X-Ray Analysis of ZnO nanoparticle: The EDX characterization proved the presence of Zinc and Oxygen in the synthesized nanoparticles of 0.1M, 0.2M, and 0.3M. The presence of oxygen and zinc and other materials were in bulk as indicated in the table.

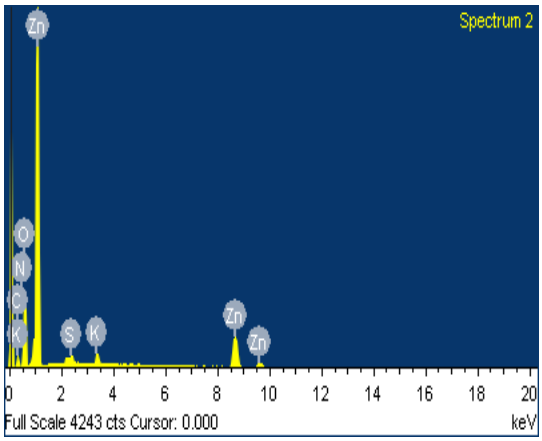

Fig 3a: EDX of 0.1M sample

Element	Weight%	Atomic%
C	9.49	24.98
O	20.19	39.88
S	0.91	0.90
K	2.17	1.75
Zn	67.24	32.50
TOTAL	100	

Table 2a: EDX data of 0.1M sample

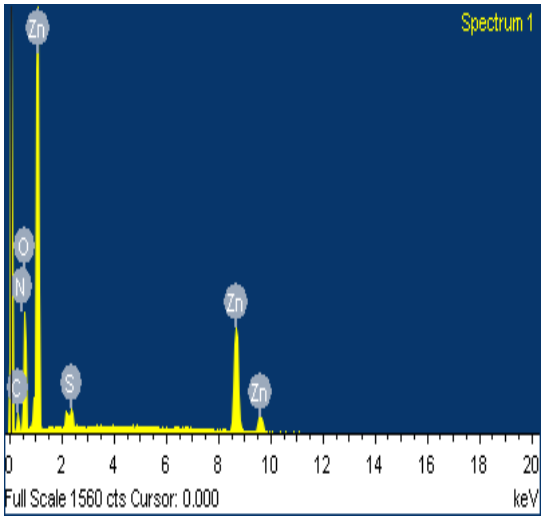

Fig 3b: EDX of 0.3M sample

Element	Weight%	Atomic%
C	11.60	28.05
O	23.07	41.90
S	2.22	2.01
Zn	63.11	28.05
TOTAL	100	

Table 2b: EDX data for 0.3M sample

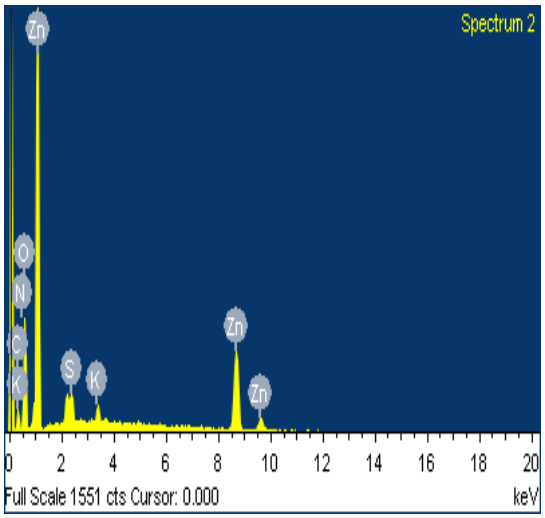

Fig 3c: EDX for 0.5M sample

Element	Weight%	Atomic%
C	13.90	32.21
O	22.26	38.70
S	2.51	2.18
K	2.82	2.01
Zn	58.50	24.90
TOTAL	100	

Table 2c: EDX data for 0.5 M sample

The impurities of sulphur (S), potassium (K) peaks were observed because of the synthesis technique. The carbon peak arises due to the carbon tape which was used in the Scanning electron microscope analysis.

UV-VIS Spectroscopy analysis for ZnO

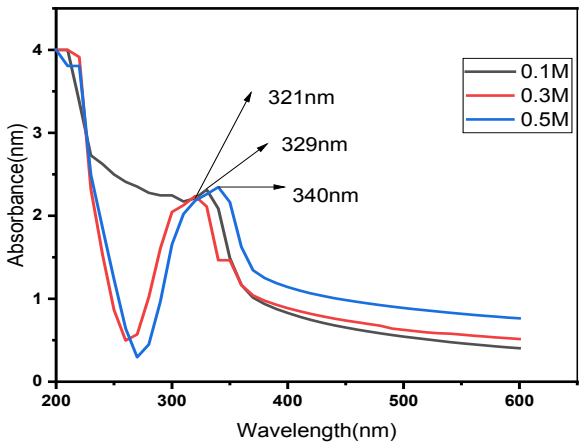

Fig 4a: UV spectrum of the samples.

It is observed that the absorption peaks of the 0.1M, 0.3M and 0.5M samples are 329 nm, 321 nm and 340 nm respectively.

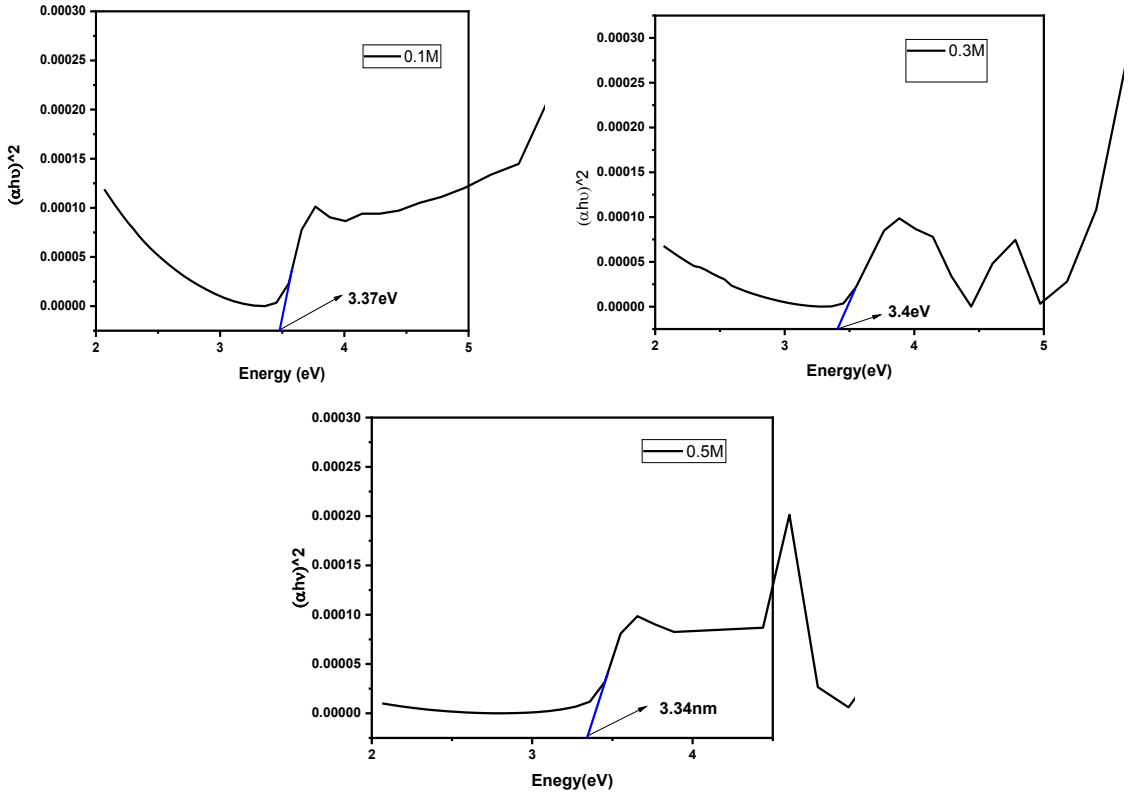

Fig 5: Energy Gap graph for (a) 0.1M, (b) 0.3M, and (c) 0.5M

From 5a, the energy band gap of 0.1M sample of Zinc Oxide found to be 3.37eV. From 5b, the energy band gap of the 0.3M sample of Zinc Oxide was found out to be 3.4eV and from figure 5c, the energy band gap of 0.5M Zinc Oxide was found to be 3.34eV.

Photocatalytic activity: Here it is observed that the degradation percentage decreases with the increase in concentration for all three molarities. This is because as the concentration of Methylene blue increases the number of molecules also increases and the number of available sites for the photocatalyst to react decreases subsequently and hence the efficiency decreases and it takes more time to degrade the dye. The degradation

efficiency of each sample was calculated for their respective ppm using the formula:

$$D = \frac{C_o - C_t}{C_o} \text{X100}$$

Table 3: Comparison of Degradation efficiency of different molarities at different concentrations.

PPM	0.1M	0.3M	0.5M
50 ppm	74.67%	73.70%	75.3%
100 ppm	40.48%	41.8%	41.17%
150 ppm	30.83%	34.63%	32.9%

Degradation study for 0.1M ZnO

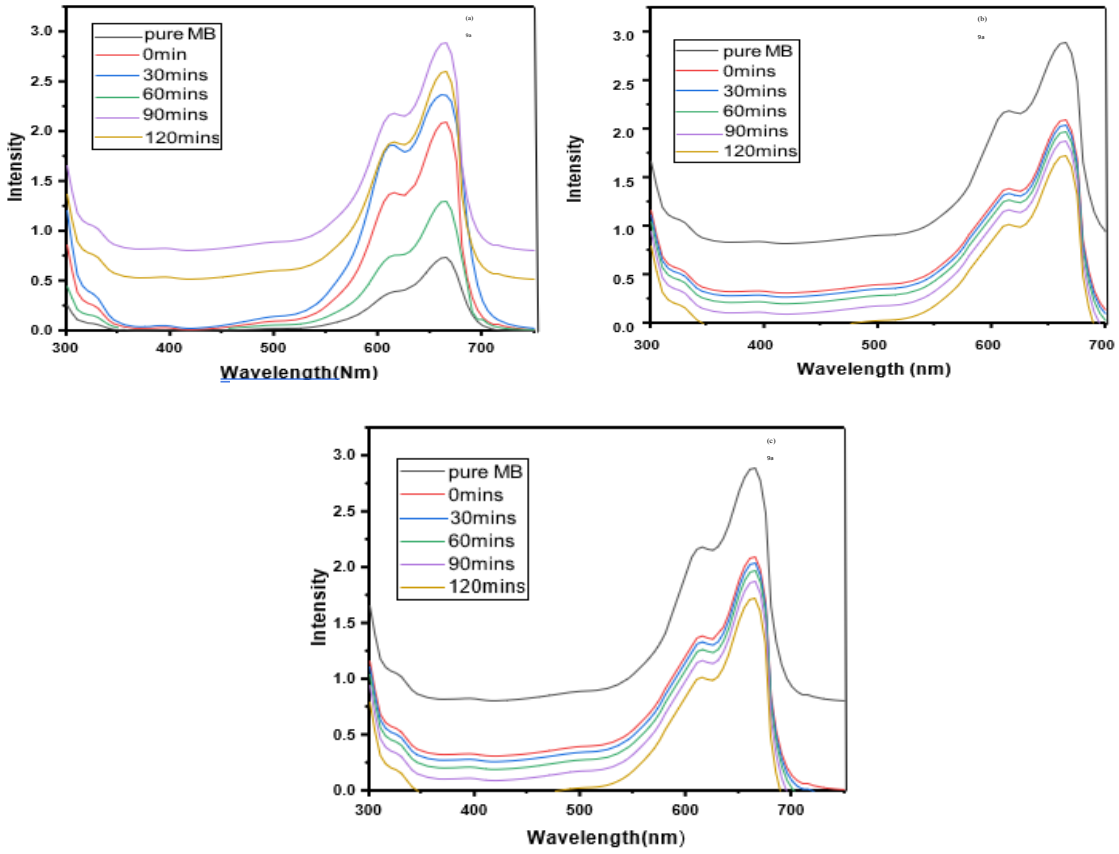

Fig 6: (a) 50 ppm, (b) 100ppm, and (c) 150ppm

Degradation studies for 0.3M:

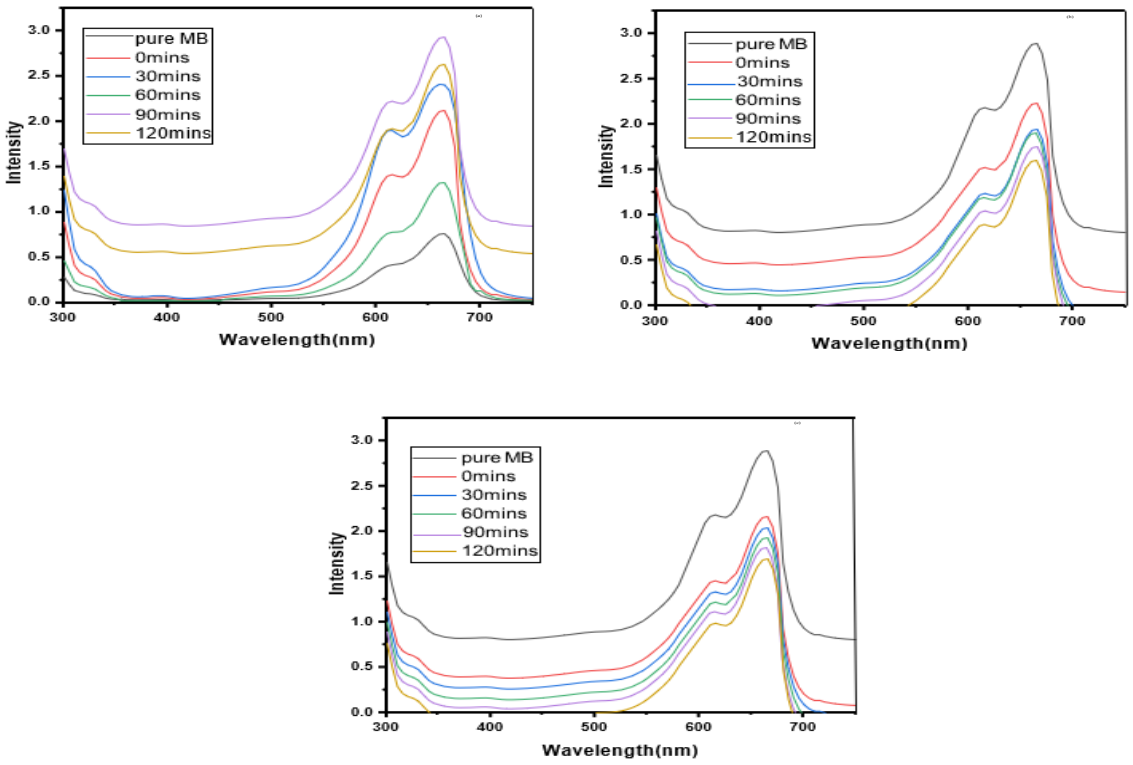

Fig 7: (a) 50ppm, (b) 100ppm, (c) 150ppm

Degaradation Studies for 0.5M sample:

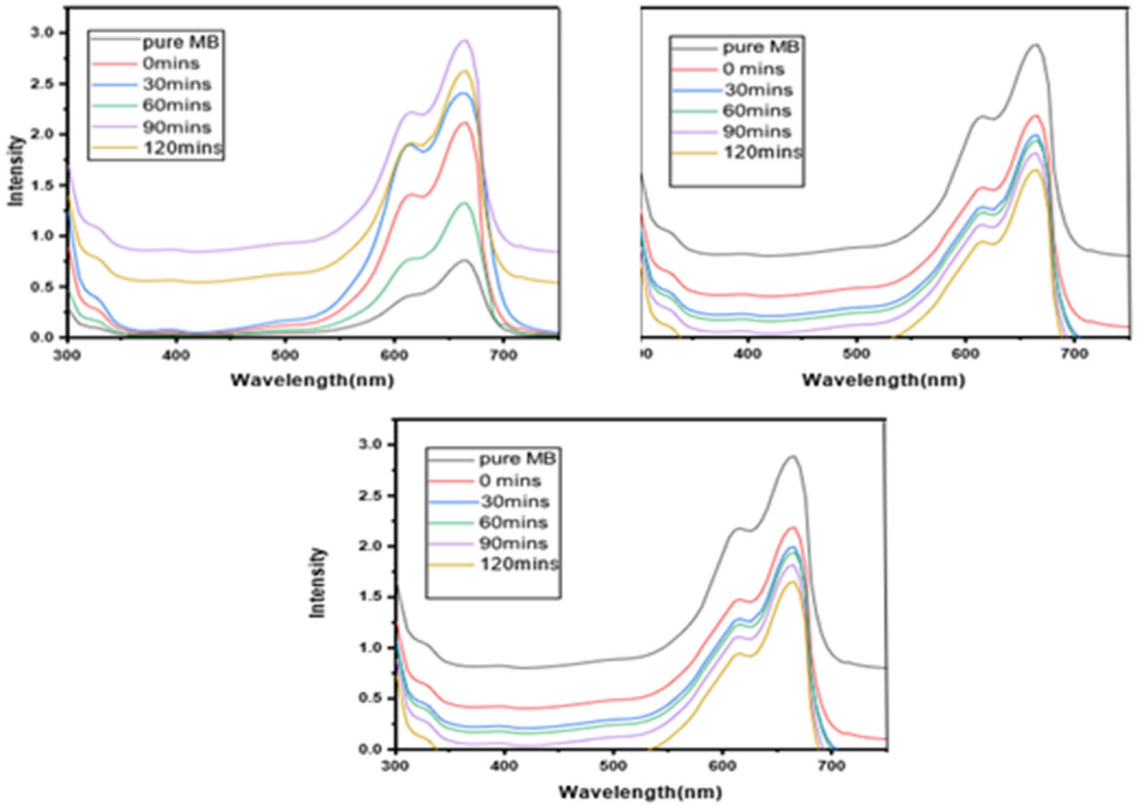

Fig 8: (a) 50ppm, (b) 100ppm, and (c) 150ppm

CONCLUSION

The ZnO nanoparticles have been successfully synthesized using sol gel method. The prepared materials were characterized using the SEM. The SEM results display that the 0.5M sample shows a flake-like structure due to the agglomeration of the samples. XRD results have analyzed the lattice parameter and the crystallite size of the different samples. EDAX is shown its indeed ZnO material. The energy band gaps obtained from UV analysis for the0.1M, 0.3M and 0.5M samples are 3.37 eV, 3.4eV and 3.34 eV. From the photocatalytic study, it is observed that the degradation percentage decreases with the

increasing concentration of Methylene Blue. Hence the ZnO nanoparticle showed good performance towards the degradation of Methylene blue dye.

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Stannic oxide thin film prepared by spin coating technique and its uses in oxygen gas sensor

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Abstract *: The work was a study of stannic oxide (SnO_2) based thin film for oxygen detection at a constant temperature. Different molarity of stannic oxide solution was prepared by chemical method. The prepared stannic oxide solution is deposited on glass substrates by using Spin Coating technique. The prepared film was characterized using X-ray diffraction (XRD), scanning electron microscopy (SEM) and Energy Dispersive X-ray Energy Dispersive X-ray (EDAX) respectively. XRD revealed the presence of pure SnO_2 crystalline thin film with a tetragonal rutile structure with decreasing grain size with an increase in molarity. The SEM observations confirmed the granular morphology with the presence of pores in the film surface. Films optical characteristics were studied using a UV-VIS spectrophotometer which showed an energy band gap of 3.5 eV for all the samples. EDAX analysis confirms the presence of Sn and O in the thin films. Oxygen gas is used to study the gas sensing property of SnO_2 thin films. The temperature and voltage were kept constant and the change in resistance was measured. 0.25M thin film sample showed better response compared to other molar samples.*

Keywords: *Gas sensor, SnO_2 thin film, oxygen, glass substrate, response*

INTRODUCTION

SnO_2 is naturally non-stoichiometric having a rutile phase that eases the adsorption of oxygen on its surface and thus is highly sensitive towards many toxic and harmful gases. However, the requirement of a higher operating temperature (>200°) is a major

issue. It is reported that the quality of SnO_2 film with desired surface morphology plays an important role in reducing the operating temperature and enhancing the sensing response characteristics towards a target gas which in turn depends on the growth kinetics [3,6]. In recent years, semiconductor metal oxide has received considerable attention because of its possible application in gas sensors, solar cells, photochemical and photoconductive devices, liquid crystal display, gas discharge display, lithium-ion batteries, etc. [1-3]. SnO_2 is an n- type semiconductor that has strong gas sensing properties. In particular, the size of the crystalline forming the gas response layer determines both the absolute gas sensitivity and the signal stability. To achieve the maximum gas response, the grain size must be smaller than two times of depletion layer [4]. In general, the semiconductor metal oxide gas sensors like SnO_2, ZnO, and WO_3 [5-7] have been widely studied due to their range of conducting variability and their response towards both the oxidizing and reducing gases. They are used in the form of bulk material. Thick or thin films gas sensing is the surface phenomenon and hence thin films sensor have an advantage due to their higher surface to volume ratio.

MATERIALS AND METHODS

Preparation of SnO_2 solution: SnO_2 solutions were prepared by chemical synthesis technique. Different molar concentrations of the precursor solutions were prepared (0.1M, 0.15M, 0.2M, 0.25 and 0.3M). Add a weighed amount of $SnCl_2$ in 100ml of ethanol. Then, the solution was mixed by stirring for 3 hours at 80˚C. It turns to pale yellowish colour. Then, the prepared SnO_2 solution was kept for 3 days to synergise.

Deposition of SnO_2 thin films and fabrication as gas sensor: Deposition of SnO_2 thin film on glass substrate was prepared using spin coating technique**.** Initially the glass slides were first cleaned by using hydrochloric acid diluted in water and then washed off by labolene. Then the slides are cleaned by acetone before keeping it for ultra-sonication for a time period of 15 minutes. Then the process is followed by drying of the glass substrates.

A few drops of prepared SnO_2 solution were applied to glass substrate and kept in the holders of spin coating instrument. The substrate is rotated at 2,500 rpm in order to spread the fluid by centrifugal force. The solution was coated 25 times with 30 seconds of spin time. Then the thin film substrate was kept in the hot air oven at 250˚C for 60 min for drying. Silver contacts were made for the prepared SnO_2 thin film samples using silver paste for gas sensor characteristics.

RESULT AND DISCUSSIONS

X-Ray Diffraction (XRD) Studies of SnO_2 thin film: The structural analysis of prepared samples is determined from the X-Ray Diffraction XRD pattern which was taken in the range 20˚ to 80˚. The peaks were obtained at 26.90˚, 33.88˚, 51.93˚ and 63.1˚ values of 2θ corresponding to the planes (1 1 0), (1 0 1), (2 1 1) and (3 1 0) respectively. From the graph it is understood the samples have rutile/tetragonal structure (cassiterite) of SnO_2 (JCPDS card – 41-1445).

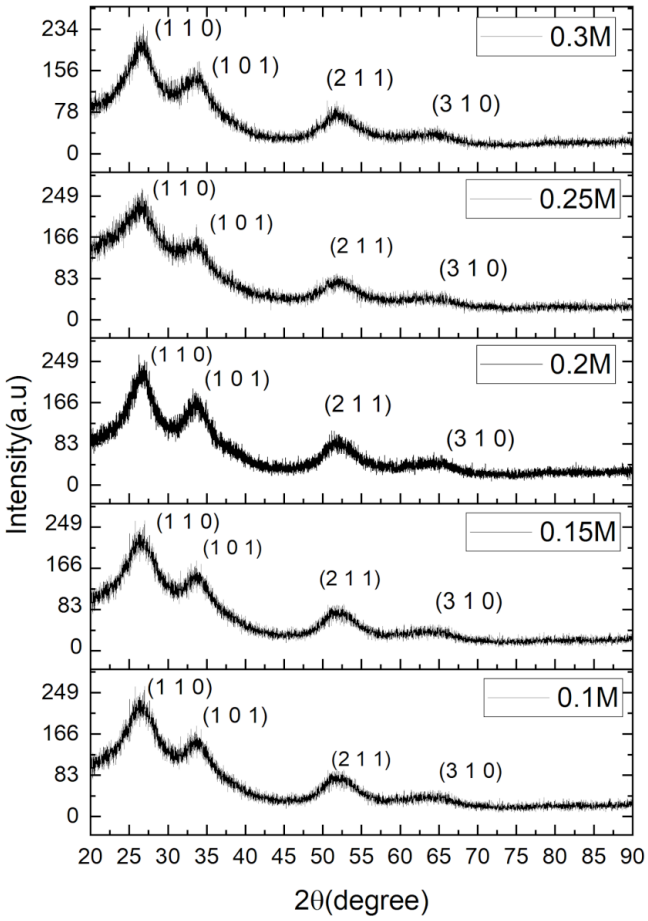


Fig. 1: X-ray diffractogram of SnO_2 thin film

The prominent peaks that were taken to find the average crystallite size D of the thin films was found using Debye Scherer formula

$$\mathbf{D} = \frac{k\lambda}{\beta cos\theta} \mathbf{D} = \frac{k\lambda}{\beta cos\theta} \quad \text{----------(1)}$$

Where, K= shape constant = 0.9

λ = wavelength of the x-ray source = 0.154 nm

β= FWHM (full width at half maximum)

θ= Bragg's angle of diffraction angle.

By using the Scherer formula, the average grain sizes of the SnO_2 thin films were calculated.

The formula for rutile crystal systems was used to calculate the lattice parameters of the obtained films:

$$d^2 = \frac{h^2+k^2}{a^2} + \frac{l^2}{c^2} d^2 = \frac{h^2+k^2}{a^2} + \frac{l^2}{c^2} \text{----------(2)}$$

Where, d = inter-planar distance
h, k, l = miller indices
a=b=c = lattice parameter

Table 1: Calculated values of the lattice parameters and grain size.

Sample Molarity	Grain Size (nm)	Lattice parameter (a=b=c) (Å)	Interplanar distance (d)
0.1	1.20	5.468	3.152
0.2	1.27	5.546	3.2024
0.3	1.12	5.523	3.1312
0.4	0.61	5.417	3.2167
0.5	0.63	5.472	3.1278

Ultraviolet Visible Analysis for SnO_2 thin film: Optical properties of the prepared thin films were studied using by UV-Vis spectroscopy. The transmittance percentages of the stannic oxide thin films were taken in the range of 200 nm to 750 nm wavelength. The percentage of transmission was found to decrease with increase in molarity of the material. 0.1M SnO_2 thin film has the highest transmittance value of 86.2% and 0. 3M has the lowest transmittance valve of 49.7%. While those of 0.15M, 0.2M and 0.25M SnO2 thin films have transmittance values of 81.2%, 80.4% and 82.6% respectively.

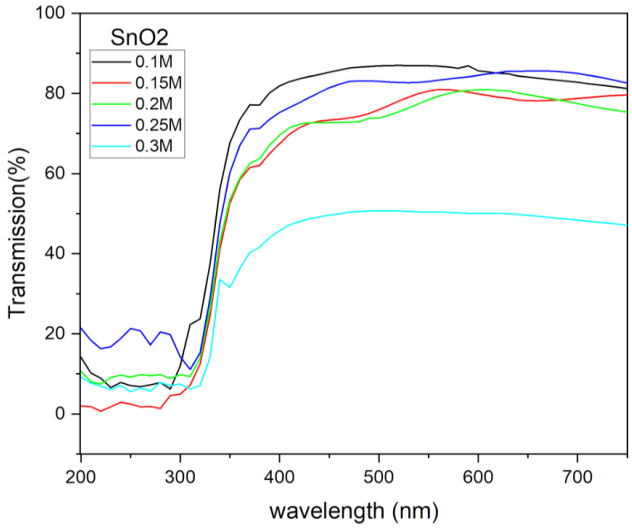


Fig. 2: Transmittance versus wavelength plot

The thickness of each sample was calculated by using the formula: $t = \lambda/2n$

Where λ= the wavelength of the transmittance peak in nm and

n= refractive index of stannic oxide.

Table 2: The thicknesses of all the thin films obtained SnO_2

Sample Molarity (M)	Thickness (nm)	Band Gaps (eV)
0.1	101.70	3.5
0.15	108.80	3.5
0.2	109.68	3.5
0.25	106.45	3.5
0.3	120.59	3.5

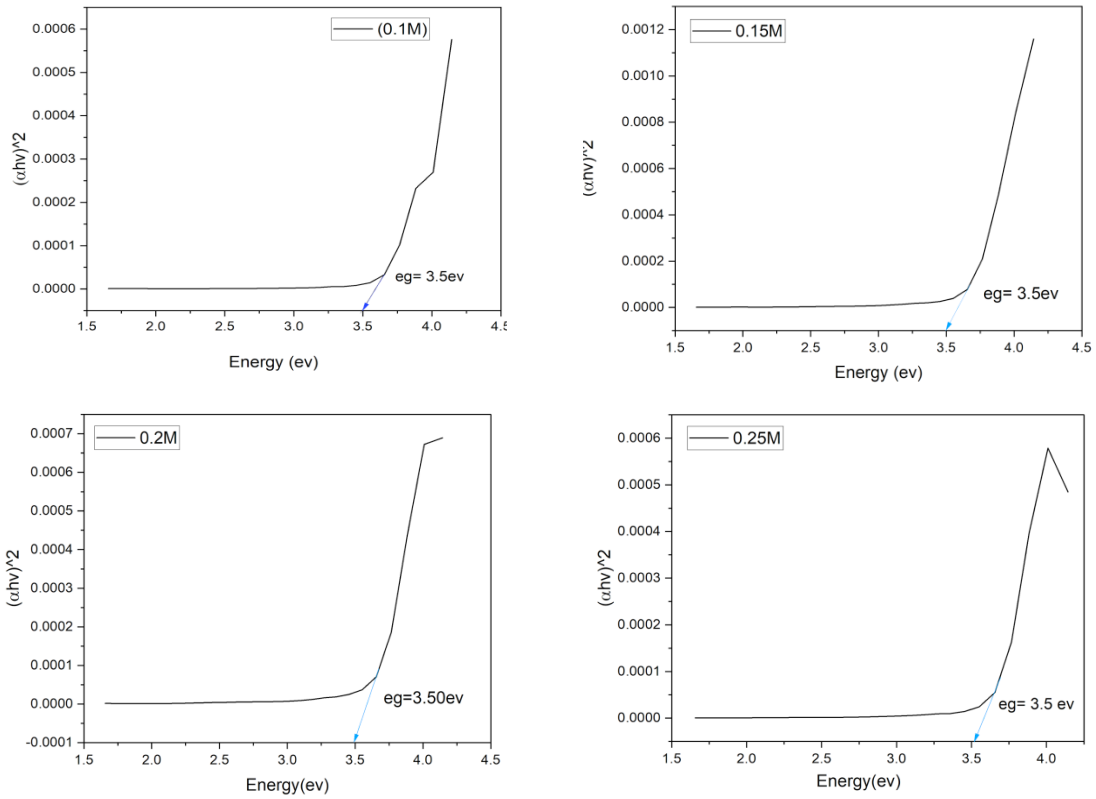


Fig. 3: Plots of $(\alpha h\nu)^{\wedge 2}$ versus Energy for the 0.1M 0.15M, 0.2M, and 0.25M Stannic oxide films.

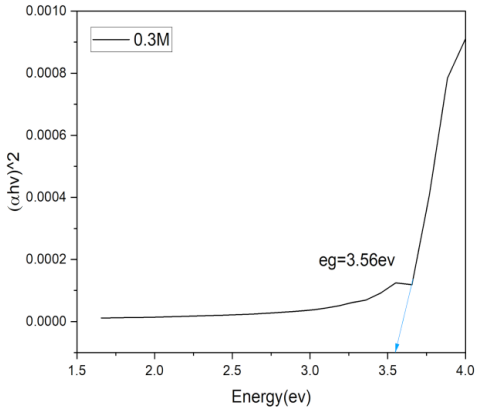


Fig. 4: Plots of $(\alpha h\nu)^{\wedge 2}$ versus Energy for the 0.3M Stannic oxide films.

The calculated band gaps of the obtained thin films are found to be smaller than the theoretical band gap value of stannic oxide. Estimated value of SnO_2 was 3.6eV. The obtained band gaps of the prepared stannic oxide thin films are tabulated. Table 2 shows the thickness and band gaps of different molarity of the thin film. This shows that the band gaps of the prepared Stannic oxide thin films are similar even with the changes in molarity of the materials. This indicates that the prepared material is indeed SnO_2. The bandgaps were similar due to, the obtained thickness values were nearly same, the changes were minimal in nanometre range, and this may be the reason for the similar bandgaps.

SEM analysis explored the surface morphology of the synthesized thin films. Prepared films display agglomeration that can be seen from SEM images with irregular morphology. In Fig 5, SnO_2 film of 0.15M showed that the surface is uneven and irregularly shaped with less roughness. Moreover, it also observes the good dispersion of all samples. All samples are structurally almost the same. To increase the molarity concentration, the surface morphology of all the films has small grains and is equally covered with more roughness [10]. The small grains with roughness, therefore, enhances the sensing capabilities of the film [11]. It is also seen from the SEM images that all molarity thin film has uniformly distributed porous structure formation. In Fig 5, the SnO_2 film of 0.25 showed that particles size on the surface of the thin film are 41.12 nm, 27.79 nm, 18.76 nm and 19.65 nm. The agglomeration could be due to the smaller sizes of the particles hence provide a better response to gas sensing. It is seen from the SEM images that the particle size in the least molarity thin film i.e., 0. 3M has some agglomeration. In the 0.025M thin film, a clean smooth surface was obtained. The agglomeration could be due to the smaller sizes of the particles.

Scanning electron microscopy (SEM)

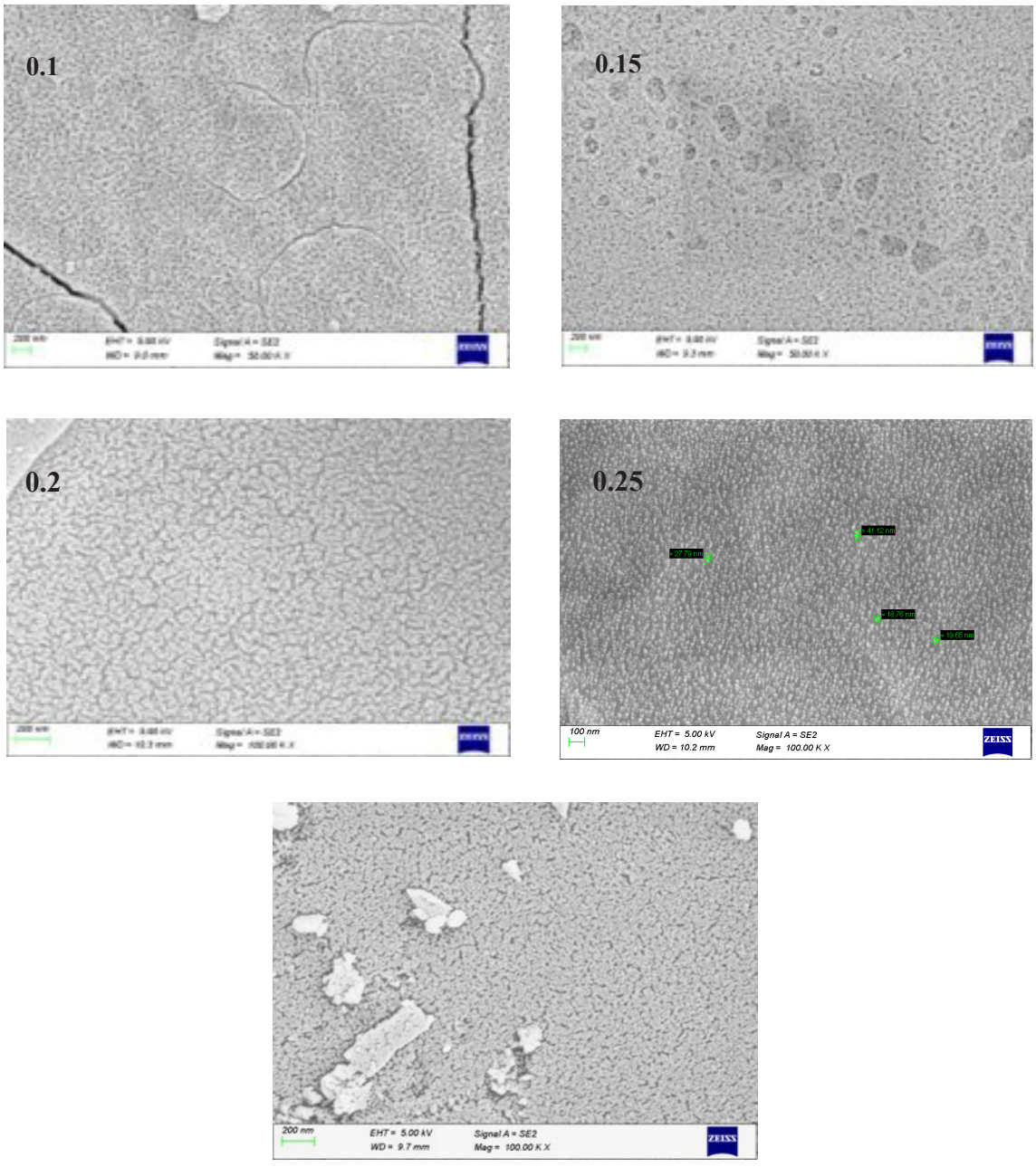


Fig 5: SEM images shows the different molarities of thin film

Energy Dispersive X-ray (EDAX)

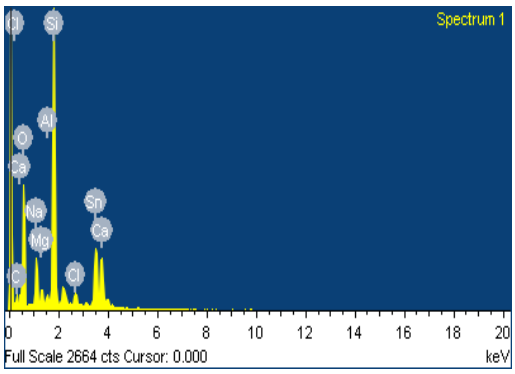


Fig. 6(a): EDAX of 0.1M SnO_2 thin film

Element	Weight%	Atomic%
O	40.26	9.17
Sn	36.53	61.48
Total	100	100

Table 3: EDAX values for 0.1M SnO_2 thin film

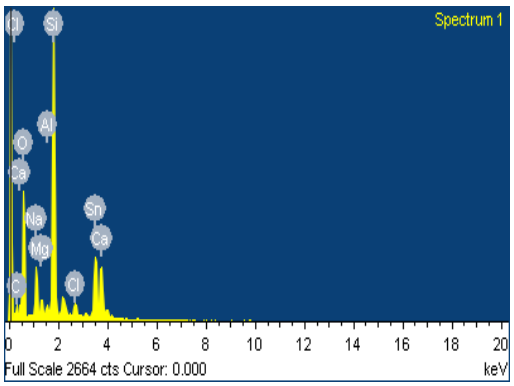


Fig. 6(b): EDAX of 0.1M SnO2 thin film

Element	Weight%	Atomic%
O	42.48	10.17
Sn	29.47	52.36
otal	100	100

Table 4: EDAX values for 0.15M SnO_2 thin film

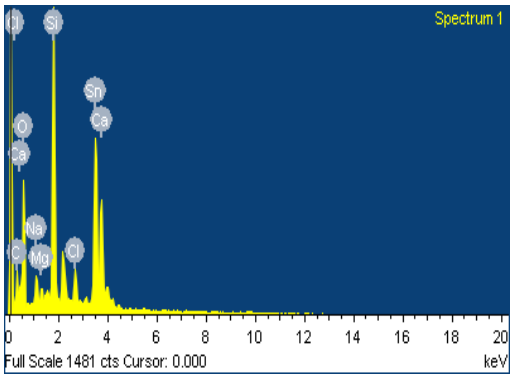
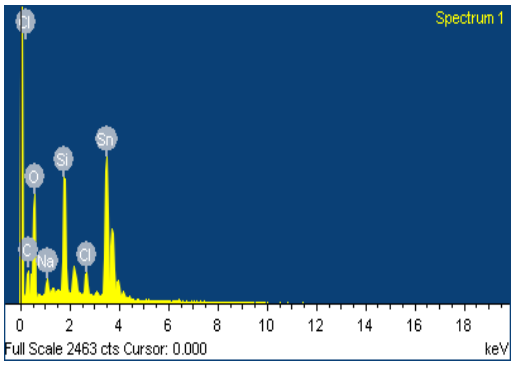


Fig. 6(c): EDAX of 0.2M SnO_2 thin film

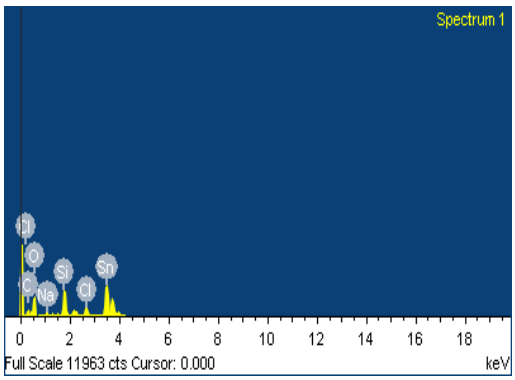
Element	Weight%	Atomic%
O	26.19	5.44
Sn	37.00	57.05
Total	100	100

Table 5: EDAX values for 0.2M SnO_2 thin film



Element	Weight%	Atomic%
O	49.00	12.11
Sn	33.53	61.48
Total	100	100

Fig 6(d): EDAX of 0.25M SnO2 thin film Table 6: EDAX values for 0.25M SnO2 thin film



Element	Weight%	Atomic%
O	51.55	13.31
Sn	29.76	57.01
Total	100	100

Fig 6(e): EDAX of 0.3M SnO2 thin film Table 7: EDAX values for 0.3M SnO_2 thin film

Energy dispersive x-ray analysis is used to analysis elemental composition which is present in a material shows in all the Fig 6 (a), (b), (c), (d) and (e) and respective Tables 3 - 7. It is observed from the graph, there is a prominent major peak seen for Sn and hence from the EDAX analysis, it is confirmed that tin (Sn) and oxygen (O) are present in all the prepared samples and occupy the major composition. The element like calcium (Ca), chlorine (Cl), sodium (Na) was observed due to the starting precursors. Magnesium (Mg), silicon (Si), calcium (Ca) peak was higher because glass was used as substrate.

Gas response characteristic of SnO2 thin film: Oxygen gas sensor study of the prepared samples for a temperature of 150°.

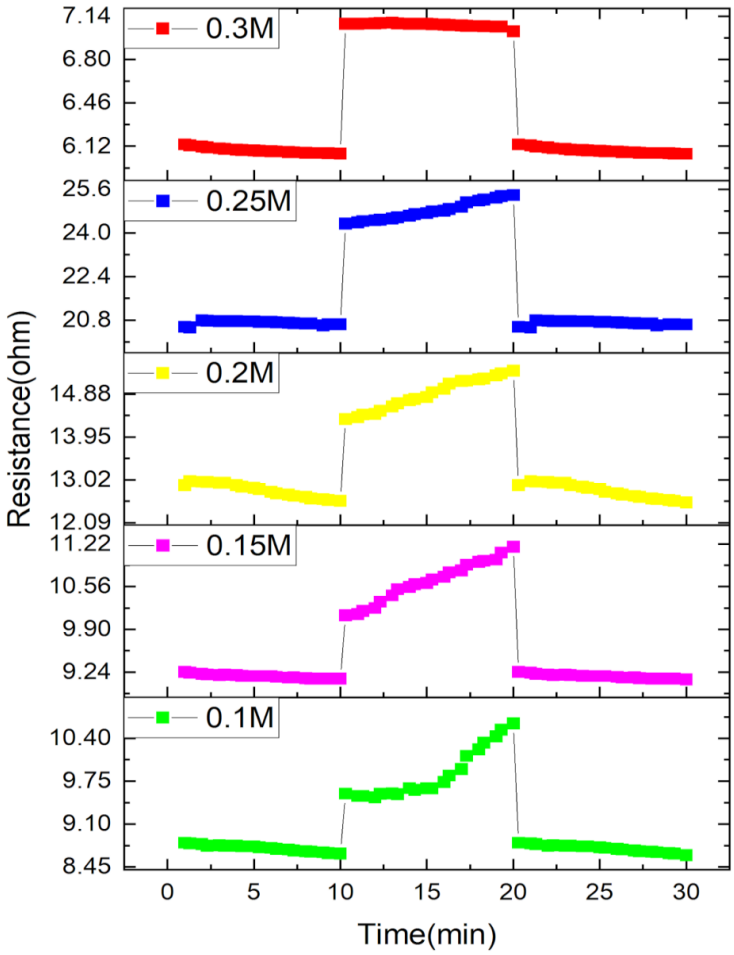


Fig. 7: Plot of resistance versus time

The above Fig 7 gives the variation of resistance of the 0.1M, 0.15M, 0.2M, 0.25M and 0.3M SnO_2 thin films with respect to change in time. The sample was exposed to atmospheric gas to oxygen gas for the dynamic response. Oxygen was used as sensing gas; the temperature was maintained 150°C and 5000 ppm of gas concentration was maintained throughout the experiment. Graph shows an increase in the resistance values from atmospheric gas to oxygen gas for all the molarity samples. This is because of

stannic oxide is an n-type semiconductor when it is exposed to oxygen gas, it oxidizes the sample i.e., oxygen (electron acceptor), so the values of resistance increase with the exposure of oxygen gas. Once atmosphere is introduced again, the sample gain back the electron and it reaches the initial resistance. The response and recovery time was almost same for all the samples.

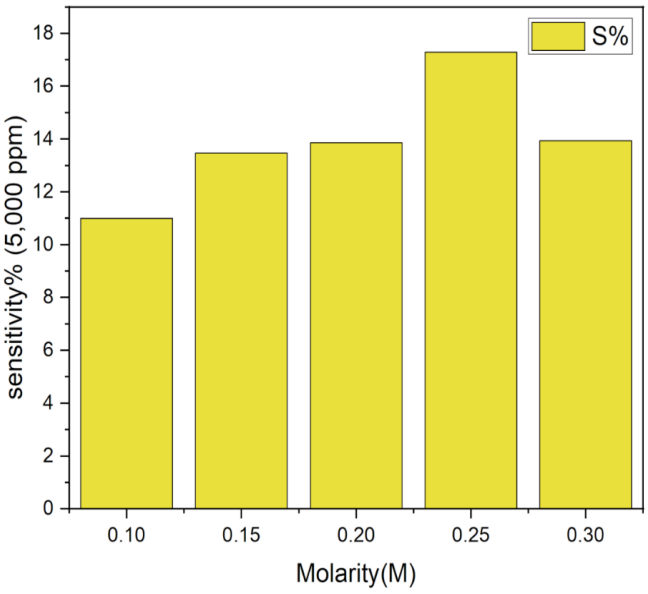


Fig. 8: Response variation of the prepared samples

Fig. 8 shows the sensitivity SnO_2 thin films samples against oxygen gas. The sensitivity (%) was calculated by sum of resistance of the thin films in air, Ra, and in the presence of a gas, Rg, it has been observed from the histogram that 0.25M SnO_2 thin film contributes to the highest response when compared to the other SnO_2 thin films. The 0.25 molarity SnO_2 thin film shows high sensitivity than 3Molarity sample, this is due to, the prepared thin film of 0.25M SEM images in Figure 4 shows the distinct, uniform nanoparticle structure on the surface than the 3M sample. The distinct and uniform nanoparticle favour for the gas sensing mechanism. The 3M sample shows in Fig 4 few cracks and micro particles along with the uniform thin film this may reduce the sensitivity of 3M sample

CONCLUSION

SnO_2 thin films with different molarity concentration (0.1M, 0.15M, 0.2M, 0.25M and 0.3M) were successfully deposited on the glass substrates by a spin coating method for oxygen gas sensing applications. The crystalline structures, morphologies and elemental analysis of the prepared thin films have been characterized by XRD, SEM and EDAX. The X-ray diffraction results indicate that the grain size is reduced depending on the molarity concentration of SnO_2 thin film. SnO_2 thin film sample is observed to be crystalline with a tetragonal rutile structure. The EDS and elemental mapping verified the Sn and O is present in the prepared thin films. The optical band gap values are in the range of 3.5eV. The gas sensing characteristics of among SnO_2 thin film 0.25M shows the high sensitivity.

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Neuroprotective effect of aqueous extract of Acorus *calamus* rhizome against paraquat-induced neurotoxicity in *Drosophila melanogaster*.

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Abstract : *Neurodegeneration is the progressive drop of the structural integrity and functions of the neurons leading to various neurodegenerative disorders. Various environmental factors lead to neuronal damage one such agent is paraquat a herbicide, that can cross the blood-brain barrier and alter neuronal structure and function. The Acorus calamus is a traditional herb known for its neuroprotective and neuro-stimulatory effects. The rhizome of this plant is a powerhouse of phytochemicals which are potent antioxidants. These phytochemicals act as antioxidants and have free-radical scavenging activity. Neurotoxins lead to the build-up of free radicals in the body which generates oxidative stress; this oxidative stress is countered by the antioxidants as they neutralize the free radicals. In the current study, the aqueous solution of the rhizome is tested for the presence of various phytochemicals which are potent antioxidants. The safety of the rhizome aqueous extract was assessed by feeding different concentrations of extract to Drosophila melanogaster and we could observe that 0.01% was found to be the safe dose. Paraquat is the neurotoxin used to induce neurotoxicity in the flies the lower concentration of 10mM was found to be the lowest safe dose to induce toxicity in flies. The group of flies treated with paraquat showed increased SOD activity suggesting the protective action of A calamus extract. The neuroprotective action of A. calamus extract can be accredited to the antioxidant constituents while further assays and mechanisms of action are needed to be further investigated.*

Keywords: *Neuro-degeneracy, Neuroprotection, Neurotoxicity, Paraquat, Oxidative stress, Acorus calamus, Antioxidants.*

1. INTRODUCTION

Neurodegenerative disorders have been on a rise since the time the human mind has been clogged up with the worries of the future world. Neurodegeneration is considered a progressive loss of the structure and function of the neurons. These degenerated neurons are generally not replaced, which leads to loss of cognitive function and results in neurodegenerative diseases. Parkinsonism, Alzheimer's, Dementia and Schizophrenia are some of the most threatening neurodegenerative diseases [1]. Neurotoxins are the substances that lead to brain damage or deterioration peripheral nervous system on exposure, causing neurotoxicity. These neurotoxins can alter the activity of the nervous system which can disrupt or kill nerve cells. Herbicides are known to cause oxidative stress that may cause the cellular and molecular changes associated with Parkinsonism [2]. A longer duration of exposure to such toxicants can cause serious behavioural and changes in motor activity which are the symptoms of Parkinson's disease [3]. These neurotoxins lead to the build-up of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the body, which in turn leads to protein oxidation and lipid peroxidation [4]. This oxidation of protein leads to the build-up of toxic proteins that lead to secondary brain damage. Our present study deals with the herbicide Paraquat which can induce Parkinson's disease-associated phenotypes including dopaminergic neuron loss. N, N1-dimethyl 4-4'- bipyridium dichloride is a bipyridyl non-selective contact herbicide. The reason why the studies on Parkinson's concerning paraquat (PQ) exposure was because of its similarity in the molecular structure to the neurotoxin 1-methyl-4 phenyl pyridinium ion (MPP+), the active metabolite of 1-methyl-4 phenyl-1,2,3,6 tetrahydropyridine (MPTP) [5].

Neurodegeneration takes place over some time and so neuroprotection can be achieved before the onset of any neuro-disease or during the progression of the disease [6]. In the first instance, it protects the neurons from future harm and in the second instance, it inhibits or retards the spreading of diseases to protect the neighbouring healthy neurons. Neuroprotection can be achieved by pharmacological interventions, non-pharmacological interventions, and cellular and genetic approaches. The rhizome of the plant is of prime

medicinal importance and is used for the extraction of its phytochemicals and preparation of herbal medicines [7]. The rhizome is sub-cylindrical bearing transverse leaf scars and is light brown in colour. It is the powerhouse of phytochemicals. The active phytochemicals with potent medicinal properties are concentrated in the rhizome part of the plant [8]. Vacha has been used traditionally to enhance memory, treat amnesia and strengthen the nervous system. These health benefits are conferred by the phytochemicals present in the rhizome of the plant. Steroids, phenols, tannins, vitamin c and flavonoids are some of the phytochemicals that confer this neuro-stimulatory effect. Out of these phytochemicals, α and β - asarone particularly stand out due to their vast pharmacological activities [9]. These two compounds possess a wide range of pharmacological activities and are used as a sedative, CNS depressant, behaviour modifier, anti-convulsant, acetylcholinesterase inhibitor, and memory enhancer. Apart from the asarones, the rhizome is also rich in volatile oils. The principal constituents of the volatile oil are amyl alcohol, eugenol, asarone and a glucoside called Acorn, which gives this plant its characteristic taste [10]. Drosophila melanogaster is a versatile model system for investigating the parameters like nervous system disorders, ageing and age-related functional changes, locomotory disorders, etc. The advantage of using an invertebrate living system, such as Drosophila melanogaster, is that it has a shorter lifespan, and it is easy to identify elements to particularly target them during diagnosis, drug design, and therapy. The Oregon K strain is a wild strain of Drosophila melanogaster that was identified for the first time in the state of Oregon, USA [11].

Thus, the purpose of this study is to test the neuroprotective functions of the *Acorus calamus* in *Drosophila melanogaster* flies against paraquat-induced neurotoxicity and neuro-degeneracy.

2. MATERIALS AND METHODS

2.1 Procurement of plant material and processing

Fresh rhizomes of the *Acorus calamus* were collected from the natural population growth in Bengaluru, Karnataka, India in November 2019. The rhizomes were washed

thoroughly 2-3 times with running water and once with sterile distilled water. After washing the rhizomes were air-dried initially and then placed in a hot air oven at 42°C for a week.

2.2 Preparation of the rhizome powder and the extract

The dried roots of the plant were obtained from the market, and they were ground to get a fine powder. The aqueous extract of the root was prepared by homogenizing 20 g of the powdered rhizome in 1000 ml of distilled water. And was allowed to stand for 24 hours at room temperature. The homogenate was then filtered using Whatman filter paper No 1 and the filtrate was stored in the refrigerator. From this 20% stock filtrate, 5% solution of the plant extract was made to use for further analysis [12].

2.3 Drosophila melanogaster culture and maintenance

The wild-type Drosophila melanogaster Oregon K strain collected from the experimental laboratory was used for the study. The population were cultured on standard media containing agar, jaggery, Rava and propionic acid and were maintained at room temperature in a humid place. The gene expression levels and the activity in Drosophila melanogaster are related to that of humans and hence the objective of this study will be satisfied [13]

2.4 Phytochemical analysis of the plant extract

Qualitative analysis of the plant extract was performed to understand the components in the plant that contributes to the antioxidant activity. The standard test for carbohydrate, protein, steroids, flavonoids, alkaloids, phenols, flavanones and anthocyanin was performed using selective reagents and the results were noted [14].

Carbohydrates

Fehling's test: 1ml each of Fehling's solutions A and B was taken in a test tube and boiled for 1 minute, after which 1ml of plant extract was added to the

test tube. This mixture was heated in a boiling water bath for 5-10 minutes. Benedict's test: equal volumes of the plant extract and benedict's reagent were taken in a test tube and heated in a boiling water bath for 5 minutes.

Proteins

Biuret test: To 3 ml of the plant extract 4% NaOH and 1% $CuSo_4$ was added.

Steroids

Salkowski reaction: 2ml of plant extract was mixed with 2ml of chloroform and 2ml of concentrated H_2SO_4

Flavonoids

Alkaline reagent test: Few drops of NaOH solution were added to2ml of plant extract

Alkaloids

Dragendroff test: To 2ml of plant extract, a few drops of Dragendroff reagent were added.

Phenols

To 2ml of plant extract, a few drops of 5% $FeCl_3$ was added.

Flavanones

To 2ml of plant extract, a few drops of 10% NaOH was added.

Anthocyanins

To 2ml of plant extract, a few drops of concentrated H_2SO_4.

2.5 Quantitative estimation of phenols

The quantitative estimation of phenol was done by the FC method. Different concentrations of Gallic acid were taken into the plot of the standard graph at 750nm. The phenol content of the 5% plant extract was calculated from the standard graph [15].

2.6 Quantitative estimation of flavonoids

The quantitative estimation of flavonoids was done using the Aluminium Chloride method. Different concentrations of quercetin were taken to plot the standard graph at 420nm. The flavonoid content of the 5% plant extract was calculated from the standard graph [15].

2.7 Plant extract toxicity test – Paraquat Resistance assay

A plant extract toxicity test was done to confirm the lethal dose concentration of the plant extract to be given to the Drosophila melanogaster to induce the neuroprotective function in them. The flies were treated separately with 0.1%, 0.05%, 0.025%, 0.0125% and 0.00625% dilutions of the plant extract mixed with the culture medium and were left undisturbed for 2 hours after which the mortality was observed and noted.

In the preliminary study, the flies were treated with different concentrations of the PQ. From the 100mM stock solution, dilutions of 10mM, 20mM and 40mM were prepared to check for the lethal concentration of the paraquat required to induce the neurotoxicity in Drosophila melanogaster. Around 20 flies were transferred to each bottle containing the standard culture medium with 5% sucrose solution and the respective PQ-soaked filter paper and after every 24 hours, the mortality rate was noted for the next 3 consecutive days [16].

2.8 Grouping of flies

Paraquat resistance assay

To perform the assays that confirm the neuroprotective action of the plant extract, the flies were grouped accordingly.

Group 1: Control

The flies were fed and grown on a normal culture medium containing 5% sucrose for 7 days.

Group 2: Pre-treatment

The flies were fed and grown on a medium containing 0.0125% plant extract for 7 days and on the 8th day the flies were transferred to a medium containing 10mM paraquat for 24 hours.

Group 3: Co-treatment

The flies were fed and grown on a medium containing both 0.0125% plant extract and 10mM paraquat for 7 days. Fresh medium was provided after every 2 days.

Group4: Paraquat

The flies were fed and grown on a medium containing 10mM paraquat for 24 hours.

2.9 Assays

2.9.1. Longevity assay

According to the above-mentioned groups, 4 culture bottles were prepared and 20 flies were added to each bottle. The mortality rate of the flies after the end of the treatment period for each group was noted [17].

2.9.2. Protein estimation

The flies from each group at the end of the treatment period were homogenised separately using a lysis buffer of pH 7.4. The homogenate was centrifuged at 10,000rpm for 15 minutes at 4°C. The supernatant was collected and 10µL of the supernatant was mixed with water and used for protein estimation by the Folin-Ciocalteau method. Bovine serum albumin was used as the standard and the standard graph was plotted. From this standard graph, the protein concentration of the plant extract was determined [15].

2.9.3. SOD activity

SOD activity was determined by monitoring the inhibition of quercetin auto-oxidation. A total volume of 3mL reaction mixture containing 30µL sample and 2970µL SOD buffer, pH 7.8; and the reaction was started by adding 0.15% quercetin dissolved in dimethylformamide. The reaction was monitored after every 10 seconds for 5 minutes at 406nm using a UV- spectrophotometer and expressed as the amount of protein required to inhibit 50% of the quercetin auto-oxidation [17].

The SOD activity was calculated using the formula,

$$\% \text{ oxidation} = \frac{\Delta \text{ test}}{\Delta \text{ control}} \times 100\%$$

$$\text{SOD activity at } 50\% = \frac{\% \text{ oxidation}}{50\%}$$

3. RESULTS

3.1 Phytochemical analysis

The phytochemical analysis of the rhizome part of the A. calamus showed the following results.

3.1.1 Carbohydrates

Fehling's test: Red colouration indicated the presence of carbohydrates Benedict's test: Red colouration indicated the presence of carbohydrates.

3.1.2 Proteins

Biuret Test: No change in the colour indicated the absence of proteins.

3.1.3 Steroids

Salkowski reaction: No change in the colour indicated the absence of steroids

3.1.4 Flavonoids

Alkaline reagent test: Light yellow colouration indicates the presence of flavonoids.

3.1.5 Alkaloids

Dragendroff test: No change in colour indicated the absence of alkaloids.

3.1.6 Phenols

The presence of deep bluish-black colouration indicated the presence of phenols

3.1.7 Flavanones

The change of colour from yellow to orange indicated the presence of flavanones.

3.1.8 Anthocyanins

The appearance of yellowish-orange colour indicated the presence of anthocyanins.

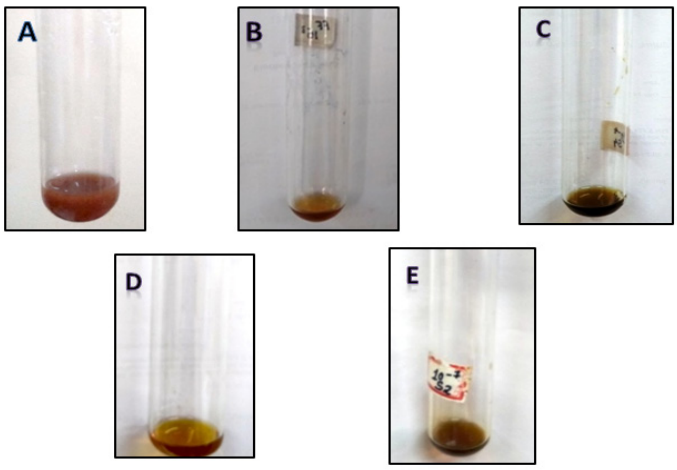

Fig 1- Positive results for phytochemical analysis

Table 1: Phytochemical analysis of an aqueous extract of *A. Calamus*

PHYTOCHEMICAL TESTS	AQUEOUS EXTRACT
Carbohydrates	+
Proteins	-
Steroids	-
Flavonoids	+
Alkaloids	-
Flavanones	+
Phenols	+
Anthocyanins	+

3.2 Estimation of Phenols by F.C method

The amount of phenol that is present in the plant extract was estimated to be 236µg/ml from the standard graph plotted. This indicates the presence of a high amount of phenol in the *A. calamus* which acts as a potent antioxidant. Quantitative estimation of Flavonoids by Aluminium chloride method

3.3 Plant extract toxicity test

Rhizome extract of 0.0125% concentration showed 50% mortality of flies. This suggests that the plant extract shows a protective effect at 0.0125% concentrations whereas the higher concentrations were toxic to the flies. Hence the LC_{50} was considered to be 0.0125%.

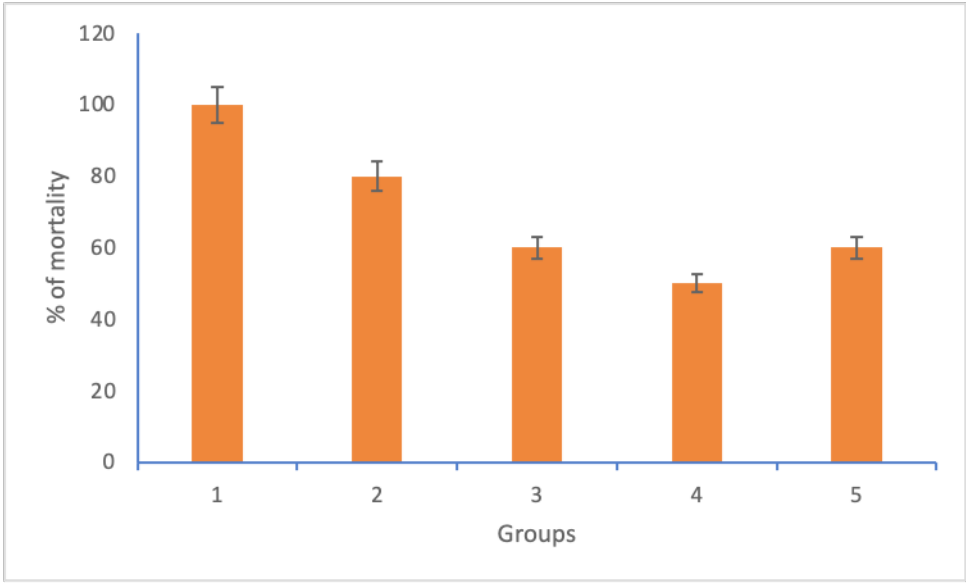

Fig. 2: Graph showing effect of different concentrations rhizome extract on flies Group 1-1%, Group 2-0.5%, Group 3-0.025%, Group 4-0.0125% and Group 5 - 0.00625%.

3.4 Paraquat resistance assay

As there were no significant results in all three concentrations of the paraquat (10mM, 20mM, 40mM), the lower dose concentration,10mM is taken as the final working concentration for further analysis.

3.5 Protein estimation of the different treatment groups

From the standard graph, the amount of protein present in the control group, pre-treatment group, co-treatment group and paraquat group are 50µg, 14.28µg, 21.42µg and 69.82µg respectively.

Table 2: Concentration of protein in each treatment group

Groups	Protein Concentration
Control	2.5mg/500µl
Pre-treatment	1.4mg/1ml
Co-treatment	2.2mg/ml
Paraquat	6.6mg/ml

3.6 SOD activity:

From the graph, the paraquat group shows a higher SOD activity as compared to the control group which indicates that the paraquat has induced oxidative stress. The pre-treatment group shows a reduction in the SOD activity when compared to the paraquat group which suggests that the plant extract has induced neuroprotective effects on the pre-treated flies. This is not observed in the co-treated flies as the SOD activity is more or less similar to that of the paraquat group. Hence the pre-treated flies show positive results than the co-treated flies in the neuroprotective activity.

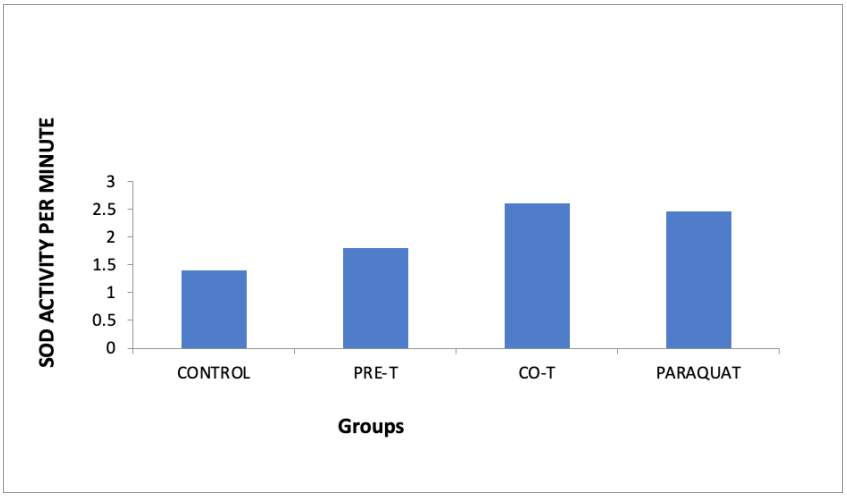

Fig. 3: Effect of *A.Calamus* on SOD activity in flies exposed to PQ with or without *A.Calamus* pretreatment and cotreatment. PQ increased the activity of SOD. *A.Calamus* treated flies showed a decrease in the activity of SOD after PQ exposure.

DISCUSSION

Oxidative stress is caused due to the imbalance in the formation of reactive oxygen species (free radicals) or dysfunctional antioxidant defences. Hydroxyl (OH·), superoxide (O2–·) and nitric monoxide (NO·) are the most described cellular free radicals are. Molecules like hydrogen peroxide (H2O2) and peroxy-nitrite (ONOO–) are testified to generate free radicals through various chemical reactions even though they are not free [18].

The brain is one such organ that is at risk of the effects of oxygen demand and its abundance of peroxide-susceptible lipid cells [19]. Increased oxidative stress induces neuronal deterioration leading to various neurodegenerative disorders like Alzheimer's disease, Parkinson's disease (PD) etc.

Ample evidence suggests that humans are repetitively exposed to free radicals created from the man-made environment such as pollutants and cigarette smoke. Agricultural chemicals such as paraquat (PQ) herbicides and heavy metals can react with DNA causing DNA damage which is implicated as a causative factor in PD [20]. Results show that PQ of 10mM is the safe dose to induce toxicity in the Drosophila melanogaster. However, the precise mechanism of paraquat action that leads to neurodegeneration is not well understood.

A. calamus toxicity study indicated the dose-dependent decrease in the mortality rate of the Drosophila melanogaster observed that feeding at 0.01% or lesser concentration does not affect the viability of Drosophila melanogaster. Hence, in co- and pre-treatment regimen experiments of the present study, concentrations lower than 0.01% were administered to avoid drug-related toxicity.

Superoxide dismutase is an enzyme that scavenges the superoxide anion that is expressed in any of the tissues. An enzyme that first comes to rescue during oxidative damage is the SOD, the first line of defence against oxygen radicals. It prevents endothelial

and mitochondrial dysfunction by inactivating nitric oxide and inhibiting peroxynitrite formation. It also clears oxygen radicals produced in the respiratory chain [21]. Whenever there is oxidative stress, this will be the first line of defence. In our current study, the activity of the SOD enzyme induced by PQ exposure was significantly lower in fly groups pre-treated with the extracts of A. calamus compared to the paraquat group, implicating that the pre-treatment with the A. calamus can prevent the neurodegeneration caused by the paraquat. The co-treated group fed with the A calamus and also exposed to paraquat showed increased SOD activity compared to the paraquat-treated flies, indicating co-treatment is not protective against paraquat-induced neurodegeneration.

Based on our biochemical outcome we propose that the *A calamus* extract could act as a prophylactic agent in the risk-prone population. A further detailed investigation is required to reconfirm the results obtained. Further, it confirms the *Drosophila melanogaster* system as a primary model to rapidly screen neuro-pharmacological properties before testing *in vivo* model systems.

CONCLUSION

Phytochemical analysis of the aqueous extract from Acorus calamus rhizome showed positive results for carbohydrates, flavonoids, phenol, flavanones, and anthocyanin. Phenols and flavonoids are known to contain strong antioxidant properties and their quantitative estimation showed that the total phenol content in aqueous A. calamus is 236μg/ml, and the total flavonoid is 139.4μg/ml. This is indicative of their natural potential to protect against oxidative stress. The plant extract toxicity test confirmed the LC50 of the aqueous *A. calamus* which showed protective effects at 0.01% concentration and the higher concentrations showed lethal effects on the flies. The paraquat resistance assay indicated there was no significant mortality in higher doses and considered 10 mM as the safe lower dose concentration to be used for further analysis. The SOD activity in the different treatment groups suggested that the paraquat treatment increased the SOD activity which was restored to the normal levels in the *A. Calamus* pre-treated flies as

compared to the co-treatment. Hence pre-treatment alone is considered to be an effective treatment method than co-treatment in controlling the oxidative stress induced by paraquat.

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Mitochondria: The Cause of Pancreatic Cancer

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Abstract : *Mitochondria are called the "powerhouse of the cell". It has attracted many fields of science, including Life Sciences and Medicine, because it plays an integral role in all the major processes of the body, like energy generation, apoptosis, TCA cycle, etc. Any abnormality inthe mitochondria can be closely associated with pancreatic cancer. Pancreatic cancer has the highest mortality rate. It is the 7th most common cause of death worldwide. The present workis an attempt to study the various reasons and pathways by which mitochondria cause pancreatic cancer and if mitochondria can be used as targets for pancreatic cancer therapy.*

Keywords: *Pancreatic cancer, mtDNA, mitophagy, apoptosis*

INTRODUCTION

Pancreatic cancer is the 7th most common cancer-related cause of death worldwide. The reason for the higher mortality rates by pancreatic cancer can be associated with inappropriate diagnosis, treatment and because patients do not show symptoms until an advanced stage. Thus, it is the most lethal form of malignant neoplasm and has caused 432,242 deaths in the year 2018 (GLOBOCAN 2018 estimates). It has been studied that pancreatic cancer is closely associated with abnormalities in mitochondria such as disruptedCa^{2+} signaling, mitophagy, mutations in mitochondrial DNA (mtDNA), abnormal apoptosis,and a shift in mitochondrial metabolism. Thus, to treat pancreatic cancer, it is very importantto know the pathology and mechanisms related to it.

Ca^{2+} Levels and Acute Pancreatitis

Mitochondria play a crucial role in the physiology of Ca^{2+} signals. They also synchronize high energy demand with ATP supply [1]. The secretagogues secrete Ca^{2+}in oscillatory patterns which are coupled to mitochondrial metabolism. This is necessary for fuel secretion.Alcohol and its metabolites cause disrupted signaling of physiological Ca^{2+}. This induces sustained Ca^{2+}responses in the cytosol leading to pathology involving mitochondria. In situations of oxidative stress, mitochondria become depolarized in response to the pathologically increased energy demand. This results in subsequent PTP opening due to which Ca^{2+}gets released from the mitochondria. [2, 3, 4, 5]. This increases the cellular ATP degradation and an overload of Ca^{2+}. The depletion of ATP causes the death of the cell by necrosis which is an indication of acute pancreatitis. The digestive enzymes get prematurely activated by Ca^{2+} dependent pathways [6]. But, when the stress is less, the cells undergo apoptosis by the action of mitochondria. Targeting the components of cells like the intracellular Ca^{2+} channels can potentially reduce the overload of Ca^{2+} which is the main reason behind mitochondrial pathology in case of acute pancreatitis.

Mitophagy

The process by which degradation of cellular structures occurs by the action of lysosomes is called macro autophagy or autophagy. This process involves the formation of membrane structures that will be degraded, engulfed, and recycled. A Similar process is mitophagy that involves the autophagy of defective mitochondria. Mitophagy involves 2 mechanisms the PINK1-PRKN pathway which is ubiquitin-dependent and the BNIP3L/NIX, FUNDC1, and BNIP3 pathway which is independent of ubiquitin. Both these pathways will regulate mitochondrial turnover and play an integral role in metabolism as well as cell death. The Ub dependent pathway of mitophagy can be further divided into 2 types, i.e, the classical pathway, and the non-classical pathway [7]. The Classical pathway is controlled by PINK1 or PTEN induced kinase 1 which is a serine-threonine protein kinase and PRKN/PARK2 which is Parkin RBR E3 ubiquitin-protein ligase. [8]. Any sort of impairmentin this mitophagy pathway can lead to the formation of various forms of tumor including PDAC (Pancreatic ductal adenocarcinoma). It is seen that pancreatic

cancer cells have a high number of degraded mitochondria which can be attributed to the increased mitochondrial fission and increase in glycolysis or mitochondrial oxidative phosphorylation [9–11]. Also, many studies revealed that pancreatic cells undergo increased mitophagy and autophagy [12–14]. Autophagy plays a dual role here. In the early stages of PDAC,autophagy prevents DNA damage and inflammation thus inhibiting the growth of PDAC. Inthe later stages, upregulation of autophagy can lead to anti-tumor immunity or limitation in cell death which in turn increases the survival time of PDAC [15–19].

Apoptosis and Cancer

Apoptosis or programmed cell death is the process by which our body gets rid of the damaged and abnormal cells by undergoing a series of sequential steps which characteristically involves loss of adhesion to nearby cells, shrinkage of the cell, condensation of the chromatin, blebbing of the cell membrane, the disintegration of the organelles, degradation of the chromatin and organelles and finally the formation of apoptoticbodies which will be rapidly engulfed by the macrophages. It has two pathways-extrinsic (when the stimulus for apoptosis comes from the exterior of the cell) and intrinsic (when the stimulus of cell death comes from within the cells). Mitochondria play the most important role in the prevention of cancer. It is involved in the intrinsic pathway of apoptosis.

Intrinsic pathway activation is controlled by Bcl-2 proteins. They can be further subdivided into Pro-apoptotic members (Bax, Bak) that promote apoptosis, Anti-apoptotic members (Bcl-xl, Bcl-w) that protect cells from apoptosis, and BH3 only proteins that promote apoptosis by 2 ways. It can either activate the pro-apoptotic members. It can also inhibit anti-apoptotic members. The cellular stimulus causes proapoptotic members like Bax to get inserted into the mitochondria. This leads to the formation of pores which release cytochrome-c present in the intermembrane space of mitochondria through pores and come tothe cytosol where it binds with Procaspase-9 and Apaf-1 to form apoptosome. This causes a conformational change in the procaspase-9 to form Caspase 9. Caspase 9

activates Caspase 3 which degrades the inhibitor of nuclease and thus leading to nuclear degradation and apoptosis. It is seen that in pancreatic cancer cells, the expression of Bcl-2 is either normal or is decreased when compared to normal pancreatic cells [20,21,22]. Also, there is an overexpression of Bcl-xl in Pancreatic cancer patients. This is associated with shortened survival time of patient [23], accelerated carcinogenesis, decreased apoptosis in Pancreatic Intraepithelial Neoplasia causing shortened survival time of K-Ras mutant mice. However,reduced Bcl-xl causes senescence in PanIN cells. [24].

mtDNA and Pancreatic Cancer

The genome of mitochondrial is called mtDNA. It is 16.6Kb circular and closed DNA. It has many subunits of the respiratory chain complexes. Also, it comprises a displacement loop or D loop in the non-coding region which plays an integral role in mtDNA replication and transcription. [25]. Many direct, as well as indirect relations, have been encountered concerning mtDNA mutations and Pancreatic cancer. An analysis of mtDNA mutation showsthat out of all 159 patients, 99 of them had Pancreatic cancer, 42 of them had chronic pancreatitis and 18 of them had pancreato-biliary tract tumors. This survey indicated that almost 16% of the PC patients had a mutation in the D-Loop of mtDNA. It was also seen that there is an onset of diabetes Mellitus in PC patients which can be attributed to a single nucleotide polymorphism at position 16,519 in the D-loop [26]. In some pancreatic cells, thisSNP is also responsible for altering the levels of mitochondrial OXPHOS mRNA [27,28].

There is another indirect relation between the mtDNA mutations and pancreatic cancer whichwas studied in a cohort of smokers who were males. [29,30]. These men had higher mtDNA copies than non-smokers caused by the oxidative stresses due to smoking. [31].

Another study conducted on 532 Pancreatic cancer cases and 1701 controls, reveals a more direct form of a relation between mtDNA mutations and pancreatic cancer. Some

low- frequency variants occurred mostly in genes coding for proteins in OXPHOS like Cyt B, ND4, and ND5 (NADH- ubiquitone oxidoreductase core subunit 4 and 5) and in a few non- coding regions too. These caused severe alterations in OXPHOS which directly increases therisk of developing Pancreatic cancer. [32].

Mitochondrial Dysfunction

Cancer is characterized by the shift of metabolism- Oxidative phosphorylation which takes place in the mitochondria shifts to glycolysis which takes place in the cytosol. This is called mitochondrial dysfunction. This can occur under the influence of chemical or genetic factors which leads to an increase in the production of ROS. Genetically, mitochondrial dysfunction occurs due to oncogenic Ras expression and loss of p53 in the pancreatic cancer cells of primary tissue [33]. The KRas – driven ROS homeostasis has an integral role in the development of pancreatic cancer. A mutation in the oncogenic KRas in the acinar cells of the pancreas leads to the transition of acinar cells to duct-like cells. This trans-differentiation process called the acinar-to-ductal metaplasia (ADM) will later contribute to the formation of Pancreatic Intraepithelial Neoplasia lesions (a morphological marker of pancreatobiliary typeof Ampullary Carcinoma). The changes in the cell's metabolism led to KRas- induced formation of ROS which not only helps in the process of ADM but also helps in the growth of PanIN cells. The cancer cells maintain the ROS levels in a way that is beneficial for the growth of a tumor. But when the ROS level is below the threshold, it is accompanied by the death of the cells [34].

CONCLUSION

There is a very high mortality rate in Pancreatic cancer. This is since patients donot show any symptoms till the later advanced stages of it when it is very difficult to treat. Mitochondria is the cell organelle that has essential functions like producing ATP, cell death by autophagy and apoptosis, and maintain Ca^{2+} signaling. If these factors of mitochondria are targeted, then there is a probability of increased sensitivity of Pancreatic cancer cells to therapeutic agents. Thus, targeting the mitochondria can be a very promising way to overcome Pancreatic cancer.

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Female Genital Mutilation: How are women at the receiving end of Mental and Gynaecologic Ill-health?

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Abstract : *This paper analyses how female genital mutilation is associated with several health complications which continue to cause an overriding burden on women's healthcare in many countries of the African continent. In women who have undergone this practise, gynaecologic, obstetric as well as mental health issues have been observed. Apart from the physiological damage, it is also known to cause physical symptoms such as bleeding, infection, infertility, damage to the external genitalia and even death. However, due to lack of knowledge, women who have undergone this practise do not have access to quality healthcare.*

A scoping review of FGM/C in different parts of the world was conducted. The keyword 'gynaecologic ill-health' and 'mental health' were used in combination with the terms 'female genital mutilation,' 'female genital cutting' and 'female circumcision,' to search various databases. The content of various studies was analysed and categorised according to the themes.

The results showed that despite several efforts, the practise of female genital mutilation causes high morbidity and mortality rates among women in Africa. Since this practise is associated with several draconian and religious beliefs, it is important for us to understand it's adverse impact on women's health and spread awareness about the same.

In this study, we systematically review the gynaecologic, obstetric, and mental health impacts of female genital mutilation on women and how it is still a significant cause of high morbidity and mortality in women.

Keywords*: Female Genital Mutilation, Female Circumcision, gynaecologic ill-health, mental health, sexual dysfunction*

INTRODUCTION:

According to the World Health Organisation, female genital mutilation has been categorised into four degrees based on its severity. Type I consists of partial or total removal of the clitoris and its prepuce. It is also known as clitoridectomy. FGM/C Type II is also known as excision and it consists of the removal of the clitoris in the labia minora, Type III (the most severe form) incorporates the infibulation and narrowing of the vaginal orifice and Type IV, which includes "all other harmful procedures to the female genitalia for non-medical purposes," such as "pricking, pulling, piercing, incising, scraping and cauterization" [1].

Due to the transnational migration [2] of women living in conflict prone regions, more and more healthcare professionals are encountering women patients who have undergone the practice of female genital mutilation. However, the knowledge regarding the several impacts of FGM/C is scarce and healthcare providers aren't familiar with the many facets of issues that this practice brings with itself [3]. As such, clinicians residing in high income countries ought to familiarise themselves with the painful outcomes in women who have experienced FGM/C.

Therefore, there is a global need [4] for exploring the much-neglected areas of a sensitive topic such as that of FGM/C such that women who have undergone this practise can also have access to quality healthcare in case of chronic complications.

STUDY RESULTS STRATIFIED BY PHYSIOLOGICAL OUTCOMES:

Issues in obstetric and gynaecologic health:

The most common obstetric issue that was reported in participants who had experienced FGM/C was caesarean section. Other prominent obstetric health manifestations included episiotomy, instrumental delivery, prolonged labour, perineal tears, dyspareunia, dysmenorrhea, urinary tract infection and dysuria [5]. In a WHO collaborative prospective study, it was found that women who had undergone this practise

were also prone to postpartum haemorrhage, still-birth or early neonatal death, low birth weight and extended maternal hospital stay. In the same study it was also interpreted that these risks seemed to be greater with more extensive FGM/C [6].

According to bivariate logistic regression analysis conducted in data collected from Northern Tanzania, women who were subjected to FGM/C were also at an increased odd of delivering a baby with a low Apgar score (an assessment method to quickly check the health and vital signs of a neonate). It was also found that women who had experienced Type III and Type IV FGM/C were at three times more risk of delivering a baby with a low Apgar score [7].

In a rare worst-case scenario, a large inclusion cyst was also reported in one of the patients who had undergone FGM/C. The size of the cyst was so large that it restricted the movement of the patient [8]. Findings also show that women who've been subjected to this practise are at a greater risk of urinary tract infections and bacterial vaginosis. Moreover, with mutilation they're also exposed to the risks of pain during intercourse, burning/painful urination, problems with menstruation, and vaginal discharge and itching.

Another fact that can be drawn from the study is that there is a high chance of under-reporting of many of these issues because of a variety of healthcare and societal factors. [9] Many women have also reported being regarded with disgust and shock apart from all the offensive comments they received from their caregivers [10].

Issues in sexual functioning:

Female genital mutilation or circumcision has also been associated with sexual dysfunction in many patients. [11] This can be attributed to removal of women's genital parts which lead to damaged nerve endings around the excised areas. Studies have found that women who had been subjected to FGM/C were more prone to no sexual desire and reduced sexual satisfaction [12]. In another study conducted in Egypt, [13] sexual dysfunction was 83.8% of FGM/C cases in contrast to 64.5% of the control. This study also concluded that both Type I and Type II FGM/C were associated with sexual dysfunction.

The studies have reported that female genital mutilation has a negative impact on a woman's psychosexual life as well. Women who have undergone this practise reported many issues such as that of dysmenorrhea, vaginal dryness during intercourse, lack of sexual desire, less frequency of sexual desire per week, less initiative during sex, being less pleased by sex, being less orgasmic, and less frequency of orgasm, and having difficulty reaching orgasm than the uncircumcised women [14].

Issues in mental health:

Female Genital Mutilation is also responsible for causing mental stress and chronic mental health issues in women who've undergone this practise. In a study it was concluded that women who have undergone FGM/C showed a higher prevalence of Post-Traumatic Stress Disorder and other psychiatric syndromes than their non-circumcised counterparts. [15] Memory and cognitive issues were also observed in these individuals[16].

In another study conducted on adolescent Egyptian girls [17], it was concluded that circumcised girls suffered a significantly higher level of depression, anxiety, phobic anxiety, and somatisation than their non-circumcised counterparts. Additional mental health consequences in women who have undergone FGM/C include suppression, inferiority, incompleteness, fear, chronic irritability, and nightmares[18].

In cases where patients chose surgical intervention for clitoral reconstruction, they were reminded of the pain experienced during their traumatic mutilation. This resulted in a relapse of their PTSD symptoms along with symptoms of anxiety, depression, and hypervigilance[19].

CHALLENGES:

Female genital mutilation has been regarded as an extreme form of discrimination against women by several experts. This is because, it abrogates the recipients' right to attain the highest standard of health. It also violates other rights such as the right to security and physical integrity of women and the right to be free from torture and degrading treatment.

Since this practise may also correspond to fatality, it violates the right to life as well.[20] However, despite the discussion regarding the ill effects of this practise for more than 40 years, the topic remains emotive for the practising communities.

Low frequency of prosecution in case of practising FGM/C in countries where it is prohibited is also a challenge in eradicating this practise. This is because, unless the recommendations of the international organisations as well as legal frameworks are not enforced, FGM will continue to be practised through the populations.

Another facet to this practise is that the different forms of FGM/C classified by WHO exhibit a substantial overlap in the anatomical sense. According to studies, women who had undergone the "*sunna* circumcision" (excision of prepuce and part or all of clitoris, equivalent to WHO type I) had a form of FGM which extended beyond the clitoris. A significant chunk of this percentage had undergone WHO type III (infibulation and excision of part or all of external genitalia). Moreover, in many women and girls who were classified as having WHO Type III circumcision, the labia majora was not involved. [21]

DISCUSSION:

Female genital mutilation has no value but still the value of a girl in some societies is tied to this tradition. This practise aims to define a girl in terms of her society since it is often thought that a family or clan's honour depends upon a girl's sexual restraint. The is precisely the reason given by societies in Somalia, Sudan and Egypt where premarital sex is considered a societal crime. It is often said that the true statistics pertaining to FGM/C are unknown since it is illegal in most countries but is still practised cryptically.

One important fact to note is that FGM/C is not a religious practise. It is a cultural practise that is practised by Jews, Christians, Muslims and a variety of other religious groups in Africa and globally. In 2012, the UN General Assembly designated February 6th as the International Day of Zero Tolerance for Female Genital Mutilation/Cutting

(FGM/C), with the aim to amplify and direct the efforts on the elimination of this practice. This is also because FGM/C is considered an act of gender-based violence by major humanitarian organisations. And rightly so, since this practise promulgates the ideas of discrimination, infringes the right to bodily autonomy and reduces the personality of a woman to suit the ideals of the society [22].

CONCLUSION:

From the data mentioned above, it is quite clear that FGM/C has no health benefits whatsoever. On the other hand, it causes several obstetric, gynaecologic, and mental health issues in women who have undergone the procedure. Also, the reliability of the WHO data regarding the reported forms of FGM is also low. When it comes to a practise like this, underreporting is also an issue. Therefore, to address the issue properly it is imperative to improve the quality of the statistical data by revising the pre-existing WHO norms.

Since we live in an era where transnational migration is at an all-time high, it is necessary for healthcare professionals in high income countries to acquaint themselves with possible medical sequelae associated with female genital mutilation. This is important to ensure that these women also have access to quality healthcare in terms of pathologies related to all the four types of FGM/C.

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Reverse Vaccinology: A Promising Method to Develop Covid-19 Vaccine

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Abstract : *Reverse vaccinology (RV), the science that identifies vaccine antigens from the genome of pathogens, was first used in 2000 to identify novel antigens for vaccines against meningococcus B (MenB). Remarkable progress in genome-based vaccines was made in 2013 when an RNA vaccine against a potentially pandemic H7N9 influenza virus was produced in one week without culturing the virus but using the genome sequence available in databases. Now, during the COVID-19 outbreak, various web-based bioinformatics tools like Vaxign, ORFfinder, AllerTOP v2.0, VaxiJen v2.0, Chimera, IEDB-AR v.2.22, GRAMM-X, etc. have been used to find the structural epitopes of SARS-CoV-2, from which potential vaccine candidates were developed. This next-generation sequencing has helped save a lot of time, and money and prevents infection to the user when handled with BSL-4 organisms by using the bioinformatics tool. Not only for the COVID-19 vaccine but reverse vaccinology has also been used to develop vaccines for other potentially pathogenic organisms.*

Keywords: *Reverse Vaccinology (RV), SARS-CoV, Antigen, Next-Generation Sequencing (NGS), COVID-19 Vaccine.*

INTRODUCTION:

Vaccination has played a significant role in controlling and eliminating life-threatening infectious diseases throughout the world [1]. Reverse vaccinology is a part of vaccinology that studies the genomic sequence of pathogen by the utilization of bioinformatics for predicting the epitope of the antigen [2]. Till 20th Century vaccines

were prepared by using traditional methods. But now with the development of various techniques like recombinant DNA vaccine, conjugated proteins, and advances in novel adjuvant, it is possible to develop vaccines with the help of molecular biology and genetic engineering [3]. With the start of genomic era, various technological revolution has taken place in vaccine development [1]. Shotgun sequencing has been used in revealing genomic sequence of several pathogens. With this data sequence, vaccines were prepared against the organism. The complete genomic sequence of an organism is the reservoir of genes encoding for the proteins that can act as potential antigen and be used as a candidate for vaccine preparation. This technique identifies the antigen expressed on the surface by using the genomic sequence instead of the microorganism. This is known as Reverse Vaccinology (RV) [4]. This technique utilizes high-throughput in-silico screening of genomic sequence that encode for proteins with the attributes of a good vaccine candidate. With the increase in availability of genomic sequences has led to the development and application of additional technologies to vaccine discovery, including comparative genomics, transcriptomics, proteomics, immunomics and structural genomics. The first pathogen addressed by the reverse vaccinology approach was *Meningococcus B* (MenB), a pathogen that causes 50% of the meningococcal meningitis worldwide [5]. Reverse vaccinology was developed by Rino Rappuoli in 2000 and applied to develop vaccine against MenB [6]. This bacterium had been resistant to vaccine development due to its capsular polysaccharide which was identical to human self-antigen. Many attempts were made to develop vaccine against this bacterium using traditional methods but failed to do so. Gene sequence of MenB genome was sequenced and the data was used to develop vaccine that can kill the bacterium or at least stop its multiplication in the host. Gene sequences were analysed and over 600 potential antigens were tested for antigenicity. *E. coli* was used as a mode for expression of candidate sequences and sera was obtained from immunized mice against each of them. Analysis of the sera revealed quite 90 previously unknown surface proteins were located and out of which 29 were ready to induce antibodies to kill the bacteria in-vitro in the presence of complement [5]. The process of reverse technology has been applied to several pathogens like serogroup B *Neisseria meningitidis*, which causes meningitis in children, *Streptococcus agalactiae*,

Streptococcus pyogenes, *Streptococcus pneumoniae*, pathogenic *Escherichia coli* [7], *Bacillus anthracis, Staphylococcus aureus, Chlamydia pneumoniae* and *Mycobacterium tuberculosis* [6].

Genomes of infectious agents have been sequenced and reverse vaccinology utilizes this information to discover new antigens for the development of vaccines. The process involves sequencing the genome of the target protein or antigen and expressing them as library. The libraries are then scanning for genes in the genome that may be useful for the development of vaccine like those that encode for virulence factors or surface proteins. These proteins can be separately expressed and isolated. These proteins are screened for use in animal models, after which human clinical trials will be conducted. The proteins

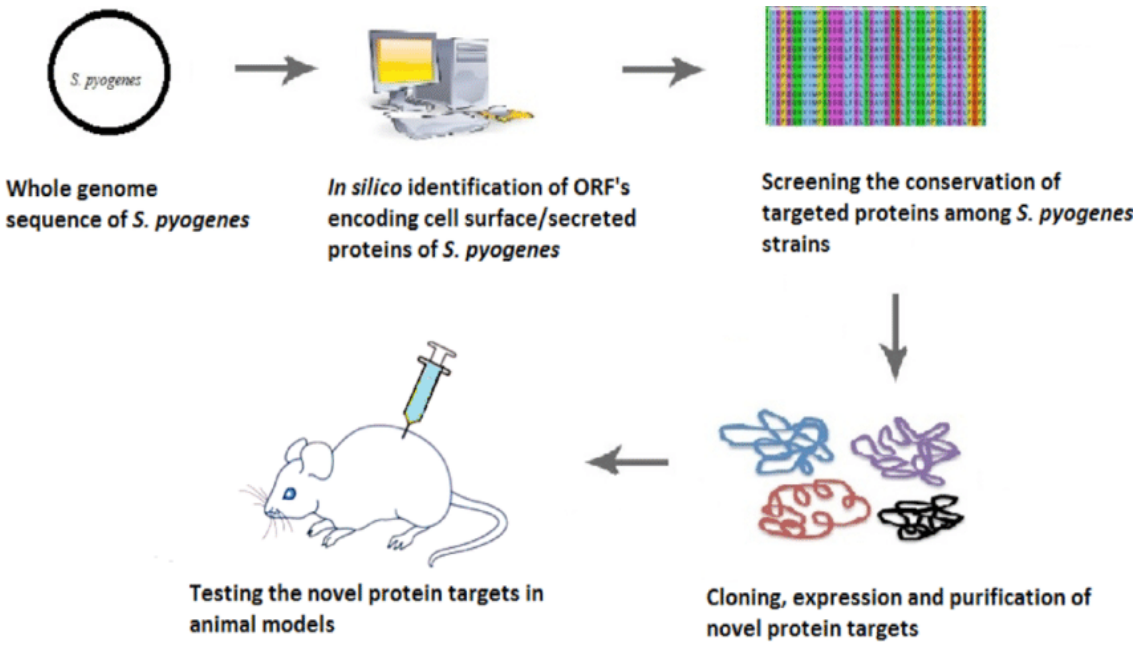

Fig 1: Process of Reverse vaccinology using the whole genome sequence of *Streptococcus pyogenes (Source: Gandhi, G. D. et al., (2017, May). Towards developing a vaccine for rheumatic heart disease).*

that induce the best immune response is selected and can be either used as subunit vaccine or used as separate vaccine. The main advantage of reverse vaccinology is that all the

proteins are available for testing rather than only one unsuitable protein expressed by the cell line [2,24].

Reverse vaccinology is modified to fit the use of vaccine making. The genome of different isolates of the same organism is examined by Pan-genomic reverse vaccinology and is compared with each other by using bioinformatics. It was first applied on *Streptococcus agalactica* [8]. Comparative reverse vaccinology uses the genomes of both pathogenic and non-pathogenic strains of the same species and are compared. It tells us about the differences in structure of proteins of different organisms. Reverse vaccinology is also used in T-cell and B-cell epitope mapping and prediction. It is well known that lymphocytes play a major role cell-mediated and humoral mediated immune response. The epitope prediction plays an important role in designing of epitope-based vaccine [9]. It utilizes structure-based prediction method to determine the antigen-antibody complexes using X-ray crystallography. DiscoTope is used for the determination of conformational B-cell epitope prediction. PEPITOPE uses combination of propensity value and half sphere exposure value of amino acid residues [10]. The whole genome sequence of the *Listeria monocytogenes*, which causes Listeriosis (food borne disease), is sequenced and available for the development of vaccine against the disease. The signal peptides, LPXTG (recognition) motifs, transmembrane helicases and many other surface proteins can be easily identified by these web-based tools like SignalP 3.0, TMHMN, LipoP, PSORTb etc. [11]. Vaccination against malaria is an efficient way to control it. Sequences of two chromosomes of *Plasmodium falciparum* out of 14 were sequenced and analysed using computer approach and the ORFs were selected that can be translated in proteins, studied for their properties, and be used in preparation of successful vaccine candidate [12]. Many other vaccines for diseases like Anthrax, endocarditis etc, were developed using reverse vaccinology.

Here, we will discuss how the various COVID-19 vaccines were predicted using Reverse Vaccinology in a short time as compared to traditional approach to develop a vaccine.

COVID-19: A pandemic of the 21st century

The novel coronavirus 2019 named as COVID-19 by World Health Organization (WHO) oversees the current outbreak of pneumonia that began at the beginning of December 2019 near Wuhan City, Hubei Province, China. COVID-19 is a pathogenic virus. Coronaviruses mostly cause gastrointestinal (GI) and respiratory tract infections and are inherently categorized into four major types: Gammacoronavirus, Deltacoronavirus, Alphacoronavirus, and Betacoronavirus. The first two mainly infect the birds, and the last two infect mammals. Six types of human CoVs have been formally recognized, these are HCoVHKU1, HCoV-OC43, Middle East Respiratory Syndrome coronavirus (MERS-CoV), severe acute respiratory syndrome coronavirus (SARS-CoV) which is a type of Betacoronavirus, HCoV229E and HCoV-NL63, which are the members of the Alphacoronavirus. Coronaviruses did not draw global concern until the 2003 SARS pandemic, preceded by the 2012 MERS and most recently by COVID-19 outbreak. SARS-CoV and MERS-CoV are known to be extremely pathogenic and spread from bats to humans. COVID-19 is spread by dust particles and fomites while close unsafe touch between the infector and therefore the infected individual. Faecal spreading has been seen in certain patients, and active virus has been reported in a small number of clinical studies. It was recorded that the maximum number of cases (77.8%) was between 30-69 years of age as of April 2020 [13]. SARS-CoV-2 is a single stranded (ss) RNA enveloped virus. An RNA-based metagenomic next-generation sequencing (MNGS) has been applied to decode its entire genome, that is of 29881 bp in length, coding for 9860 amino acids [14]. The S, E, M, and N genes encode structural proteins, such as 3-chymotrypsin-like protease, papain-like protease, and RNA-dependent RNA polymerase, are encoded by the Open Reading Frame (ORF) region [15]. Many glycosylated S proteins cover the surface of SARS-CoV-2 and bind to the host cell receptor Angiotensin-converting enzyme 2 (ACE2), mediating viral cell entry [16]. Efforts have been made to develop vaccines against COVID-19, many of which are targeted against the structural proteins.

Utilizing computational tools to detect vaccine candidates against COVID-19:

Computational immunological tools can be applied to examine viral antigens, prediction of its epitopes and evaluation of its immunogenicity [17]. Excessive respiratory infection can be resolved with T-cell reactions and antibodies [18]. Rapid identification, isolation, disease prevention, and control measures are required to hinder the spread of SARS-CoV-2 at homes, communities, and health care centres [19]. In a study that was conducted that the potential T-cell and B-cell epitopes from SARS-CoV-2 structural proteins can be joined through adjuvant and linkers to design a multi-epitope-based subunit vaccine (MESV). The genome of the SARS-CoV-2 was taken from National Centre for Biotechnology Information (NCBI) and the genes coding for the structural proteins were predicted. Many in-silico techniques, like ProParam, VaxiJen v2.0 etc. were used to evaluate the structural and physiochemical properties of the MESV. From here, the B- and T-cell epitopes were predicted using computational tools like IEDB-AR v2.22 consensus method. Molecular docking analysis has been utilized to examine the binding interaction and stability of MESV with human pathogenic receptors. In addition to this, in-silico immune simulation was also performed to evaluate the immunogenic potential of designed MESV. At the end, MESV codons were optimized for *Escherichia coli* system and in-silico cloning was performed to ensure its expression profiling [20].

Vaxign, a reverse vaccinology tool helped in the development of vaccine against SARS and MERS, and the Vaxign-ML machine learning tool was used to predict COVID-19 human coronaviruses causing mild symptoms. The S protein and five non-structural proteins (nsp3, 3CL-pro, nsp8, nsp9, and nsp10) of SARS-CoV-2, were found to be adhesins, which are important for the viral adhering and host invasion and were also found to induce high protective antigenicity by Vaxign-ML. Nsp3 was predicted to be more conserved among SARS-CoV- 1 & -2 and MERS-CoV coronaviruses than among other 15 coronaviruses infecting human and other animals. The protein was also predicted to contain promiscuous MHC-I and MHC-II T-cell epitopes were found to be localized on the surface of the protein. The predicted vaccine targets have the potential for effective

and safe COVID-19 vaccine development against SARS-CoV-2 by stimulating effective immune responses [21].

The SARS-CoV-2 N protein sequence was analysed by Vaxign and was found to be conserved with SARS-CoV and MERS-CoV but not from the four other computational programs like ORFfinder, Get Orf etc. are used to identify all ORFs in the sequences. The identified antigens then can be mapped and screened for the expression by the pathogen, in this case COVID-19, and for immunogenicity during infection. For a humoral response, bioinformatics databases like Immune Epitope Database (IEDB) are used to identify B-cell epitopes. For cellular responses, databases are used to search for antigens that can be identified by MHC molecules found in T-cells. Thereafter, in-vitro and in-vivo studies are carried out to confirm vaccine candidates [22]. In-silico vaccinology, which includes structural vaccinology and reverse vaccinology, takes less than a year to discover the same or even more relevant information [23].

Predicting Vaccine Candidates

Vaxign analysis framework was utilized to compare the genome of the seven human coronavirus strains. The structural proteins of SARS-CoV-2 was used as a source for the pan-genomic comparative analysis. It was reported that through the analysis only the N protein in SARS-CoV-2 has a high sequence similarity to the SARS-CoV and MERS-CoV, while having low sequence similarity towards members of the Alphacoronavirus. This suggested that the N protein could be used a potential vaccine candidate for the cross-protective vaccine against SARS and MERS. This protein is found to induce both humoral and cellular immune response [25]. However, a study suggested severe pneumonia and other serious liver failures caused by N-protein derived vaccines [26].

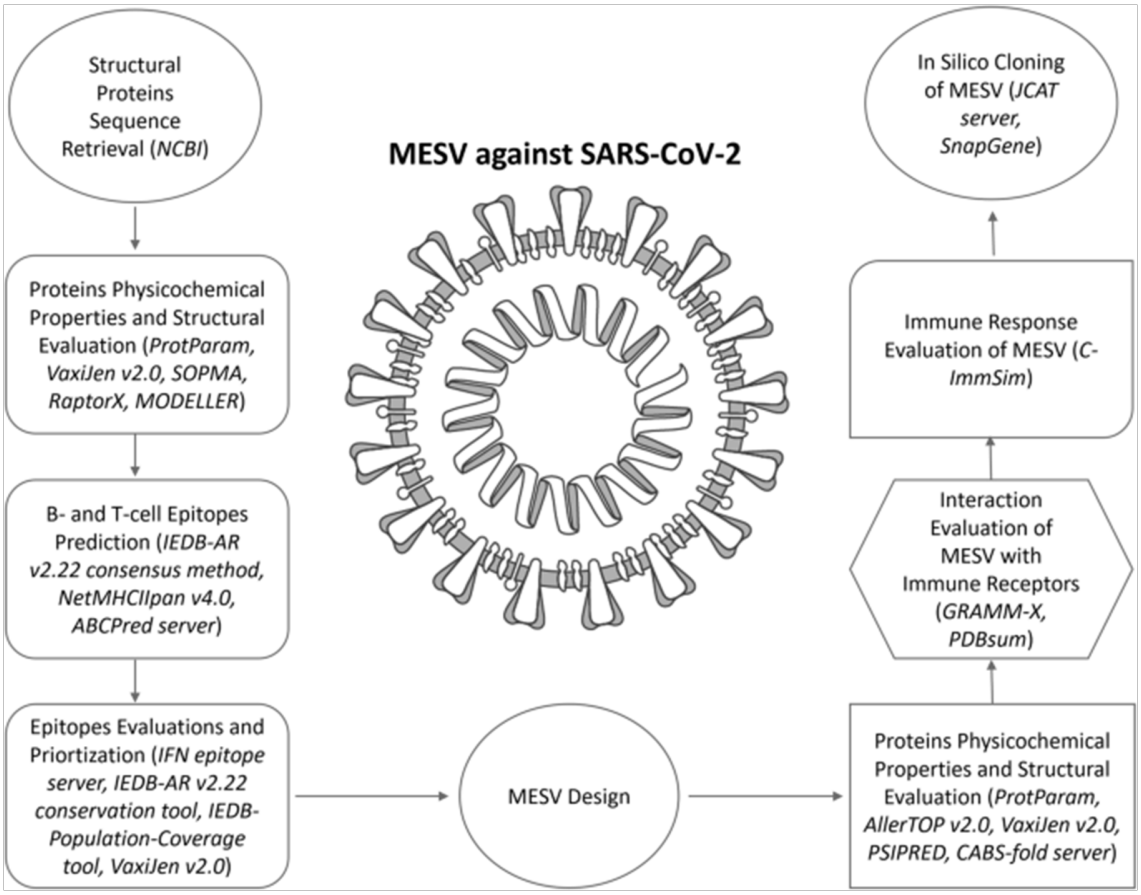

Fig 2: The schematic workflow used to develop MESV construct against SARS-CoV-2 structural proteins *(Source: Qamar, M. T. et al., (2020, September). Reverse Vaccinology assisted in designing of a multi epitope-based subunit vaccine against SARS-CoV-2. Infec Dis Poverty, 9: 132).*

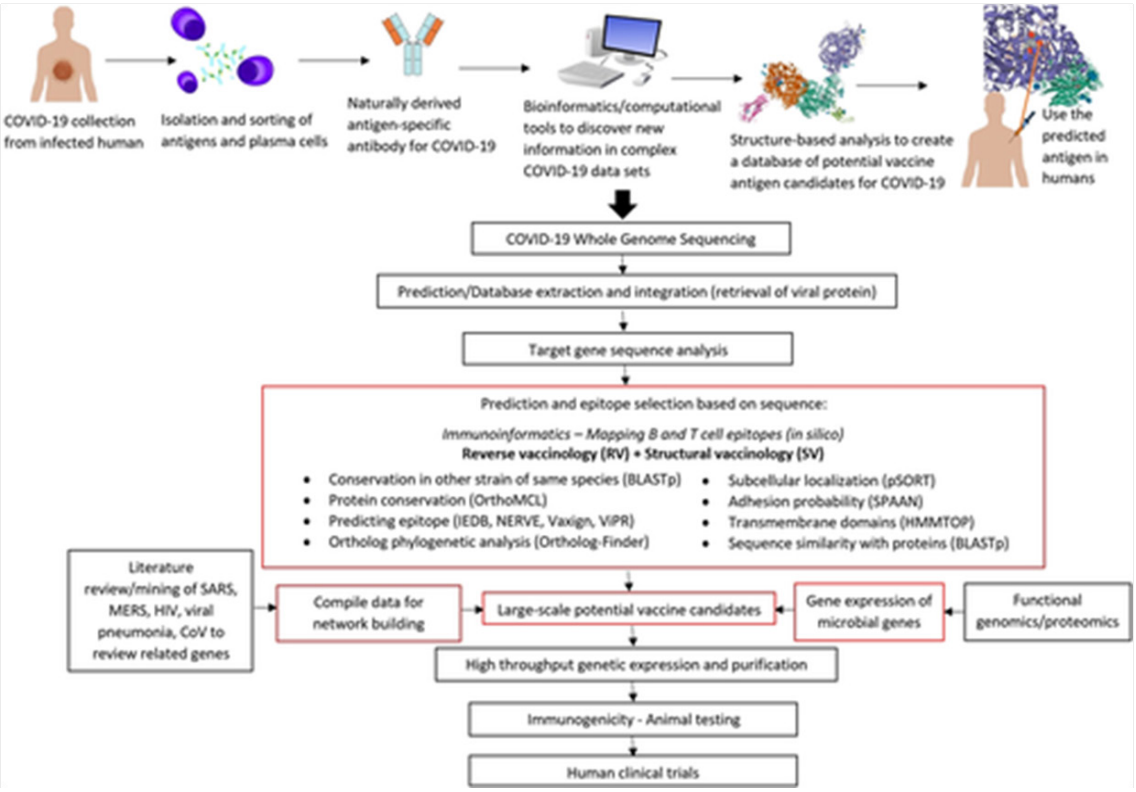

Fig 3: A complete sketch of COVID-19 epitope prediction using bioinformatics for the development of vaccines and testing them during clinical trials

(Source: Ishack, S., Lipner, S.R. (2021, April). Bioinformatics and immunoinformatics to support COVID-19 vaccine development. J Med Virol, 93(9): 5209-11).

The Vaxign RV analysis predicted the six SARS-CoV-2 proteins, namely nsp3, 3CL-PRO, S protein, and nsp8-10. These proteins are adhesive proteins that help the virus adhere to the host cell and help in virus entry into the host cell [27]. The S protein of SARS-CoV-2 was found to be interacting with Angiotensin-converting enzyme 2 (ACE2) for host cell entry [28]. The nsp3 protein was also found to be a promising vaccine candidate besides S protein. This protein was found active during the replication and pathogenesis of coronavirus infection [29]. The Multi-sequence Alignment (MSA) and the phylogeny analysis of nsp3 protein showed its close relation to SARS, MERS, and

bat coronaviruses. It was also found that there were 28 and 42 epitopes predicted to bind with reference to MHC- I and -II alleles [30].

Using Synthetic biology and computer modelling of the structure of the spike protein of similar coronaviruses, synthetic genes were utilized to develop more than 200 different vaccines against COVID-19, which included DNA- and RNA-based vaccines, viral vectors etc. [31]. Majority of the approved vaccines that were developed was found to be effective in stimulating production of neutralizing antibodies against Receptor-binding domains (RBDs) of SARS-CoV-2 [32]. However, there are limited data for inactivated and subunit vaccine candidates. This is due to the lack of clinical trials, limited sample size, geographical location etc.

Limitations of reverse vaccinology

Reverse and Structural vaccinology have several limitations. Several studies shows that the antigen residues may become epitopes under specific conditions which is not ideal during clinical trials [33]. Computational tools require advanced algorithms and prediction tools for effective development of vaccine by filtering non-epitope antigen surface residues. There also may be errors while inputting the data which can give incorrect results to the use and thereby affecting immunoinformatics data. The predicted epitope may be present deep within the protein, which effectively cuts off the immune system from detecting it and thus potential COVID-19 antibodies are not produced [33]. The major disadvantage of RV is that the strain under investigation does not represent the genetic diversity of its species. However, the use of genome of multiple strains of a species might help identify an efficient vaccine candidate [34].

CONCLUSION

Traditional method of vaccine development involves culturing of the pathogen in laboratory and is dangerous to culture if the pathogen is highly infectious for humans. Novel approaches like reverse vaccinology and structural vaccinology involves sequencing of the whole genome of the pathogen. It requires advanced prediction algorithms to filter

and analyse non-epitope antigen surface residues. Large number of isolates of the genome can be screened for prediction of epitopes and other surface proteins for the development of successful vaccine candidates. Although the complete removal of physical laboratory experiments is not possible, but reverse vaccinology saves enough time and effort as long the sequence of the gene desired is present in the databases.

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