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Characterization, antibacterial potential and photocatalytic activity of iron oxide nanoparticles biosynthesized from the leaves of *Ficus auriculata*

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ABSTRACT

The green synthesis of iron oxide nanoparticles (FeO NPs) from *Ficus auriculata* leaves have been carried out using aqueous extract. UV visible spectrometer was used to study the optical properties, it revealed spectral absorption band occurred at 416 nm, which is a characteristic feature of (FeO NPs). X ray diffraction pattern reveals crystalline structure with average crystallite size D = 39.2613 × 10^{-9} nm using Scherer's equation. In Fourier-transform infrared spectrometer analysis of functional groups, the appearance of peaks at (1086.76, 810.98, 712.10, 546.35) cm^{-1} which confirms the formation of (FeO NPs). The Raman Spectroscopy shows major peak at 325 nm. The synthesised (FeO NPs) shows antibacterial activity with minimum inhibitory constant at 100 µl/ml with *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia Coli*, *Salmonella typhi*, *Streptococcus pneumoniae.* It also shows maximum photocatalytic activity with Congo red dye concentration of 10 mg/L at 40 mins with 10 mg of synthesised FeO NPs.

Keywords: *Ficus auriculata,* green synthesis, iron oxide nanoparticle (FeONPs).

INTRODUCTION

Nanoparticles are defined as particles with a size between 1-100 nm undetectable by the human eye. Due to their small size, they behave differently from larger particles. Owing to their large surface area, it has properties like increased electrical, thermal conductivity, lowered melting points, stronger magnetism or unique optical properties even they are small enough to confine their electrons and produce quantum effects. It has its application in various field like environment, medicine, agriculture, chemistry, information, communication, heavy industry and consumer goods etc. Intentionally produced nanomaterials are of four types mainly: carbon-based, metal-based, dendrimers, and nanocomposites. Synthesis of nanoparticle can be done with various mechanism which include topdown approach (physical approach) like laser ablation, milling, sputtering, engraving and electro-explosion. The second is bottom-up approach which divide into two parts i.e. chemical and biological process. Coprecipitation, homogeneous phase, chemical reduction, solgel, pyrolysis, solvo/ hydrothermal, photoelectrochemical, molecular condensation and etching are chemical process. Among biological process the green synthesis of nanoparticle from various sources like plant, bacteria, fungi, and algae **(1).**The synthesis of oxide nanoparticles had drawn attention due to displayed electrical, optical, and magnetic properties that differed from their bulk counterparts. Photocatalytic processes involving semiconductors received remarkable attention for their potential applications in converting solar energy into chemical energy and controlling pollution. The degradation of dyes via photo assisted catalysis took place through active species formed on the surfaces of metal-oxide semiconductor nanostructures in aqueous solutions **(9).**The use of metal oxide nanoparticles is one of the promising ways for overcoming antibiotic resistance in bacteria. FeO NPs have found wide applications in different fields of biomedicine due to their antimicrobial potential. Iron is one of the key microelements and plays an important role in the function of living systems of different hierarchies. Iron abundance and its physiological functions bring into question the ability of iron compounds at the same concentrations, to inhibit the microbial growth,

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

positively affect mammalian cells. Several studies have established that FeO NPs have a low toxicity to eukaryotic cells which can be considered as potential antimicrobial agents of the new generation **(8).**During dye production and textile manufacturing, considerable amounts of wastewater containing highly coloured and toxic dyestuffs were introduced into aquatic systems. The synthesis of Fe_2O_3 as a photocatalyst for the degradation of the azo dye congo red. The ferric oxide prepared by the combustion method was selected due to its semiconductor properties, which included high photochemical reactivity, affordability and environmental safety. FeO NPs displayed advantageous optical properties, such as a good alignment between its band gap and the solar spectrum and a high absorption coefficient **(5).**Nanomaterials are utilized in various fields like to deliver drugs to areas of arteries that are damaged in order to fight cardiovascular disease, nanoparticles are being developed to assist the transportation of chemotherapy drugs directly to cancerous growths. Carbon nanotubes are also being developed in order to be used in processes such as the addition of antibodies to the nanotubes to create template-assisted synthesis, reverse micelle, hydrothermal, coprecipitation etc methods. Various shapes of iron oxides (i.e, nanorod, porous spheres, nanohusk, nanocubes and distorted cubes) can be synthesized using nearly matching synthetic protocols, by simply changing the precursor iron salts. The use of nano particle includes in agriculture industry, food industry etc **(12).**The biosynthesis of FeO NPs has garnered significant interest in the realm of food nanotechnology due to their non-toxic nature, high efficiency, strong antibacterial properties, and ability to aid in decontamination nanoparticles (NPs) have a large surface area and a small size, which typically results in greater chemical and biological activity compared to large ones. These properties make them applicable in different fields such as environment, medicine, food, catalysis, electronics, biosensors, and agriculture **(7).**The use of metal oxide nanoparticles is one of the promising ways for overcoming antibiotic resistance in bacteria. FeO NPs have found wide applications in different fields of biomedicine due to their antimicrobial potential. Iron is one of the key microelements and plays an important role in the function of living systems of different hierarchies. Iron abundance and its physiological functions bring into question the ability of iron compounds at the same concentrations, to inhibit the microbial growth, positively affect mammalian cells. Several studies have established that iron oxide nanoparticles have a low toxicity to eukaryotic cells which can be considered as potential antimicrobial agents of the new generation **(8).**In our present study we have chosen leaves of *F. auriculata* for the green synthesis of FeO NPs. The synthesised nanoparticles were used for assay of antimicrobial activity with *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia Coli*, *Salmonella typhi*, *Streptococcus pneumoniae* by MIC (mimimum inhibitory concentration) method. With Congo red dye, different dye concentrations, time interval and nanoparticle dosage show photocatalytic activity under sunlight.

MATERIAL AND METHODS:

Materials: Fresh, matured, and healthy leaves of *Ficus auriculata* was collected from Jnana Bharathi Campus Bangalore University, Bangalore 560056 and taxonomically authenticated by Botany Department of the same University. Leaves were thoroughly washed with tap water followed by distilled water for removing unwanted impurities such as dust and scum. The leaves were dried at room temperature for a week, ground into fine powder using mixer grinder and kept in air tight plastic bag till the end of experiment.

METHODS:

Green synthesis of nanoparticle: Synthesis of nanoparticle were carried out according to **(8)** with little modification. 10 gm of the dried powdered leaves (*Fi auriculata*) was boiled at 80 °C for about 60 mins with 200 ml of deionized water in Erlenmeyer flask. The extract was cooled to room temperature and filtered using Whatman No.1 filter paper and stored at 4 °C for future use. 0.1M solution of Fe $(NO_3)_3 \cdot 9H_2O$ was prepared in deionized water while stirring for about 15 minutes. Aqueous leaf extract was added to the 0.1M Fe $(NO_3)_3 \cdot 9H_2O$ solution in 1:2 ratios by volume until the mixture reaches light black in colour. Then the following mixture was continuously stirred for about 30 mins at 50 °C until the solution changes from light black to brownish-black colour. The solution was cooled to room temperature and centrifuged at 6,000 rpm for 10 mins and the precipitate obtained were collected on a

petri dish and oven-dried at 80 °C. The powder was collected and ground into fine powder using mortar and pestle and then subjected to calcination in muffle furnace at 400 °C for 4 hrs to get the black iron oxide nanostructures. Nanoparticles were stored at room temperature for further assay.

Characterization: The synthesized nanostructures were characterized using a UV−Visible spectrophotometer at a wavelength range of 200−900 nm to study the optical properties. To analyse physical properties such as phase composition, crystal structure and orientation of biosynthesized iron oxide nanostructures were confirmed by an X-ray diffraction (XRD). Fourier Transform Infrared Spectroscopy (FTIR) was used to characterize the chemical composition of NPs, their vibration and rotation of molecules. The presence of functional groups responsible for the reduction and stabilization of the FeO NPs nanostructures was confirmed. Energy Dispersive X-ray Analysis (EDAX) is a technique used for the measurement of nanoparticles by SEM. In this technique, the nanoparticles are analysed by activation using an EDAX-ray spectrophotometer, which is generally present in modern SEM (Scanning Electron Microscopy) and Raman Spectroscopy.

Microbial analysis: Microorganisms used were *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia Coli*, *Salmonella typhi*, *Streptococcus pneumoniae.* Nutrient agar containing peptone, beef extract, and NaCl was used to maintain bacterial strains. Prior to the experiment, bacterial strains were inoculated in nutrient broth to for the growth of microorganisms in the exponential phase (optical density was <1 measured at 600nm. **Antibacterial assay:** According to **(13)** minimum inhibitory concentration (MIC) method was based on growing microorganisms at varying concentrations of FeO NPs in suspension. In laminar air flow, sterile test tubes with 5 ml of Nutrient broth were taken inoculated with freshly prepared bacterial 10 µl of broth culture suspension with optical density of <1 along with varying concentrations of FeO NPs (100, 200, 300 µ/ml). One test tube was taken as positive control (with microorganism and without FeO NPs) and one test tube as negative control (without microorganism and FeO NPs). The inoculated test tubes were incubated in a shaker incubator at 250 rpm and 37 °C for overnight. The next day, absorbance was taken using UV-visible spectrophotometer at 600 nm, and the graph was plotted against optical density and the concentration of FeO NPs. The concentration giving the least optical density corresponds to MIC of iron nanoparticles for that particular microorganism. The above method is repeated with all the microorganisms mentioned above. Assay were carried out in triplicate.

Photocatalytic Activity: Degradation of dye (congo red) were carried out according to **(15)** with little modification. The dye solution was prepared in aqueous solution (20mg/L). The photocatalytic degradation of Congo red (CR) dye was carried out by various dosages of the FeO NPs (6, 8, 10 and 12mg) in 10 ml at room temperature. The effect of initial dye concentration on the photocatalytic dye degradation was studied. Then 10 mg of FeO NPs was added to 10 ml of different dye concentrations (10, 20, 30 and 40mg/L). Then 10 mg of FeO NPs was allowed to react at different time (0, 20, 40, 60, 80, 100 and 120 mins) interval with 10 ml dye (concentration of 10 mg /L). The samples were withdrawn from the reaction medium at regular time intervals. The catalyst was separated from the solution using a centrifuge at 3000 rpm for 5 mins. The dye degradation was carried out under direct sunlight according to **(2).** The change of absorbance at a maximum wavelength of dye was monitored by UV Vis spectrophotometer. The effect of visible light irradiation on the removal of dyes was surveyed. The percentage of the photocatalytic degradation of dye checked out by the following equation, % of Degradation = A_0- A_t/ A_0 × 100 Where A_0 is the initial absorbance and A_t is the final absorbance.

RESULT AND DISCUSSION

Green synthesis of nanoparticle: The leaves of *F.auriculata* was ground into fine powder shown in Figure 1



Figure 1: Fine powder of *F. auriculata* leaves

CHRACTERIZATION

During the synthesis processes following colour change occurs i.e. light black to brownish-black colour is seen in Figure 2 which indicate the synthesis of iron oxide nanoparticles **(6).**

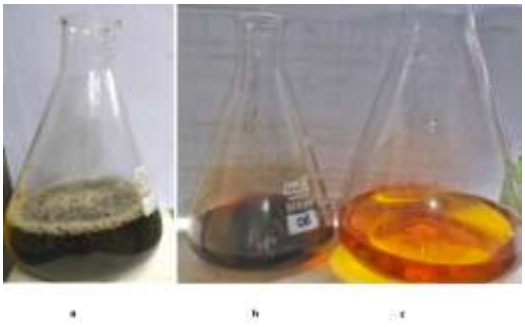


Figure 2: Synthesis procedure of iron oxide nanostructures. (a) leaf extract (b) leaf extract+ Fe $(NO_3)_3$•$9H_2O$ solution (c) Fe $(NO_3)_3$•$9H_2O$ solution

The synthesized nanostructures were characterized using a UV−Visible spectrophotometer working at a wavelength range of 200−900 nm to study the optical properties. The absorption in the visible range of the electromagnetic spectrum affects the perceived colour of the chemicals involved. The UV−Visible spectrum of FeO NPs synthesized by the extract of leaves of *F auriculata* shows its peak at 416nm (Figure 3).

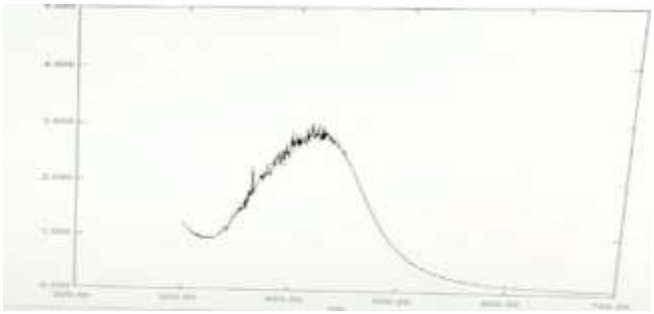


Figure: 3 UV visible spectra of synthesised FeO NPs synthesised from leaves of *F. auriculata*

The UV−Visible spectroscopic study shows the plasmonic resonance property resulting from electronic transitions of molecules and confirmed the reduction of metal ions and formation of iron oxide nanostructures with a sharp peak at 416nm. In *Borassus flabellifer* seed coat UV visible spectra show peak at 352nm with magnetic iron oxide **(14)**.The XRD peak for the sample was found at 14.1579, 26.4289, 35.5480 ˚2Ɵ were well matched with JCPDS no. 00-900-5841 and confirmed iron oxide nanostructures **(3).** The average crystallite size (D) of iron oxide was determined by Scherer's equation

$$D = \frac{K\lambda}{\beta \cos\theta}$$

and average crystalline structure was found to be 39.2613×10^{-9} nm from the leaf extract of *F auriculata* Figure 4**.**

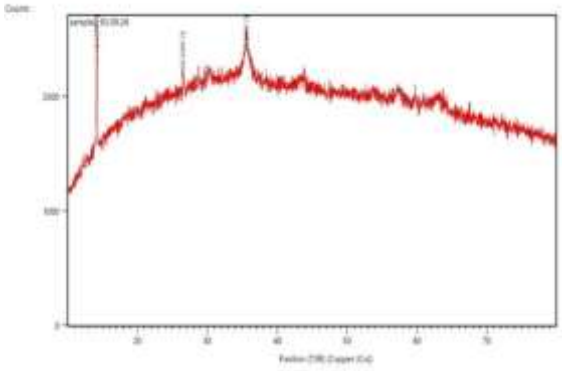


Figure 4: X ray diffraction of FeO NPs synthesised from the leaves of *F. auriculata*

The presence of functional groups responsible for the reduction and stabilization of the iron oxide nanostructures was confirmed by FTIR spectrometer. For FTIR spectral analysis, the as synthesized powder of was mixed with KBr to prepare pellets that suit to prepare pellets that suit the Bruker FTIR spectrometer instrument, and values were recorded in the wavenumber range of 400−4000 cm-1. In Figure 5 peak at 1086 cm^{-1} corresponds to the bending of C-H bonds 814 cm^{-1}, 712 cm^{-1} presence of calcium carbonate. An asymmetric banding vibration of the C-O-C in the calcite occurs in the 717–712 cm^{-1} range **(3).**

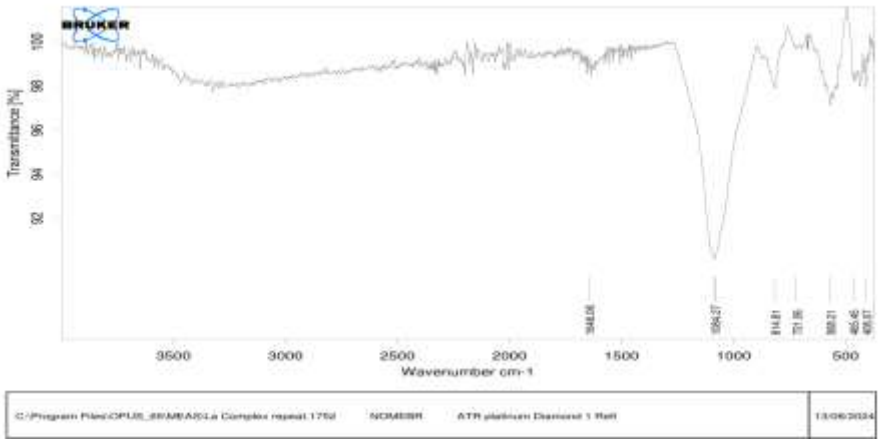


Figure 5: FTIR spectrum of FeO NPs synthesised from the leaves of *F. auriculata*

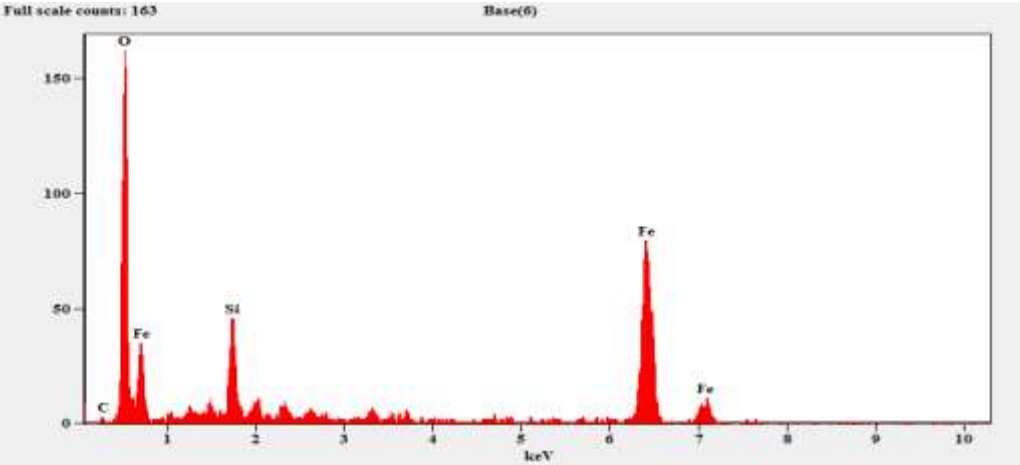


Figure 6 (a)

Element Line	Weight %	Weight % Error	Atom %
C K	0.99	±0.99	2.42
O K	32.42	±1.21	59.41
Si K	6.21	±0.63	6.48
Si L	—	—	—
Fe K	60.38	±2.87	31.69
Fe L	—	—	—
Total	100.00		100.00

Figure 6 (b)

Figure 6 EDAX of FeONPs from leaf extract of *F. auriculata*

From the EDAX data, it can be concluded that particles of iron oxide are crystalline in nature and have few impurities of Silicon. Figure 6a confirms the presence of Fe and O in the NPs system. Iron oxide NPs compose of Fe and O in weight ratio of 1:2 as given in Figure 6bThe studies on the morphology, surface details, and elemental composition of the fabricated FeO NPs are made using SEM images and EDAX. SEM images of FeO NPs are given in Figure 7 shows. iron oxide particles have rough surface and are composed of cavity like structures on the surface **(6).**

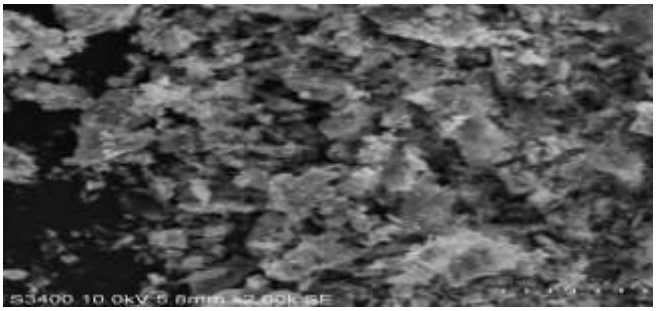


Figure 7: SEM images of FeO NPs synthesised from the leaves of *F. auriculata*

The green synthesis of FeO NPs utilized extracts from celery stalks and green tea leaves. The fresh suspension of the plant extracts from green-brown changed colour to dark brown with addition of Fe $(NO_3)_3$, within 20 mins. The formation of FeO NPs was confirmed through X-ray spectral analysis, which revealed characteristic Bragg peaks at the (111) plane for both green tea and celery extracts, indicating a face-Centered cubic (FCC) structure. SEM showed the FeO NPs as small particles and rod-like structures. Utilizing extracts from *Camellia sinensis* leaves and *Apium graveolens*, researchers synthesized FeONPs via a green method that prevented environmental contamination and provided a cost-effective solute **(4).**

FeO NPs were biosynthesized effectively from the leaf extract of *Celosia argentea*. The phytochemicals within the leaf extract were analysed. The characterization of the synthesized nanoparticles was performed to assess their structural, elemental, and optical properties using a range of analytical techniques. Results validated the formation of iron oxide nanoparticles, which displayed a surface plasmon resonance (SPR) band at 299 nm and showcased a high degree of crystallinity. The bonding between Fe and O was confirmed, and the nanoparticles appeared spherical, with sizes between 5 and 10 nm. The mapping spectrum also confirmed the presence of Fe and O elements **(16).**

Raman spectroscopy is an important technique for identifying the functional groups in the sample and plays an important role in phase identification. In Figure 8 Raman spectra shows characteristic peak at 390 nm and 640 nm. Both the bands are attributed to the Fe-O band in the maghemite phases of the synthesized iron oxide **(17).**

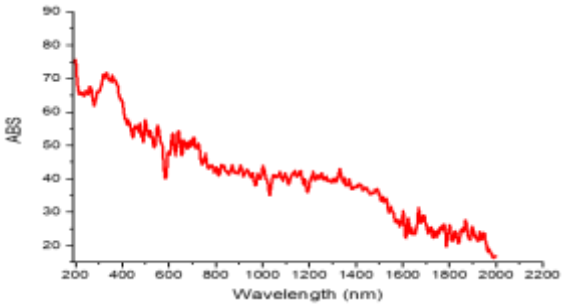


Figure 8: Raman spectra of of FeO NPs synthesised from the leaves of *F. auriculata*

MICROBIAL ANALYSIS:

MIC of FeO NPs against following microorganism like *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Streptococcus pneumoniae* was determined using Broth dilution method. After 24 hrs of incubation growth was observed in the test tubes supplemented with various concentration of nanoparticles (100, 200, 300 µl/ml) and optical density was measured at 600 nm. The results are shown in the following table1. With 100 µl/ml there is significant decrease in the growth of microorganism.

Table1: Absorbance of microbial culture in presence and absence of nanoparticles

Microorganism	***Pseudomonas aeruginosa***	***Staphylococcus aureus***	***Escherichia coli***	***Salmonella typhi***	***Streptococcus pneumoniae***
Without NP	0.846	1.507	0.942	0.425	0.845
Np 100 µl/ml	0.168	0.388	0.198	0.224	0.248
Np 200 µl/ml	0.127	0.301	0.162	0.192	0.194
Np 300 µl/ml	0.122	0.298	0.160	0.155	0.175

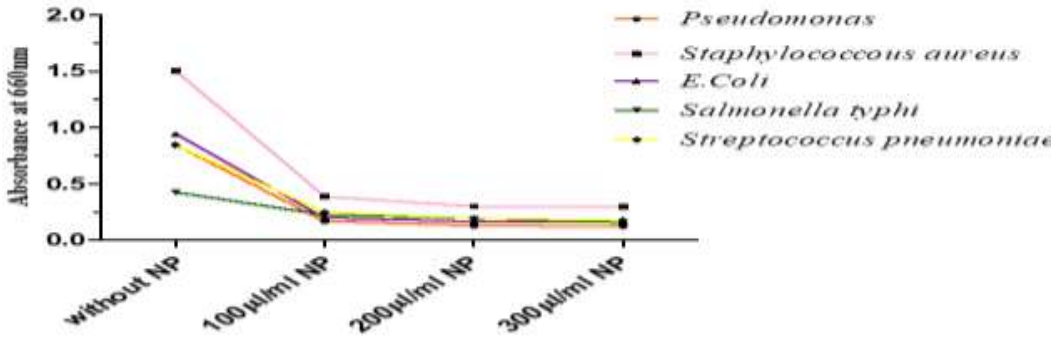


Figure 9: Absorbance of microbial culture in presence and absence of FeO NPs synthesised from the leaves of *F. auriculata*

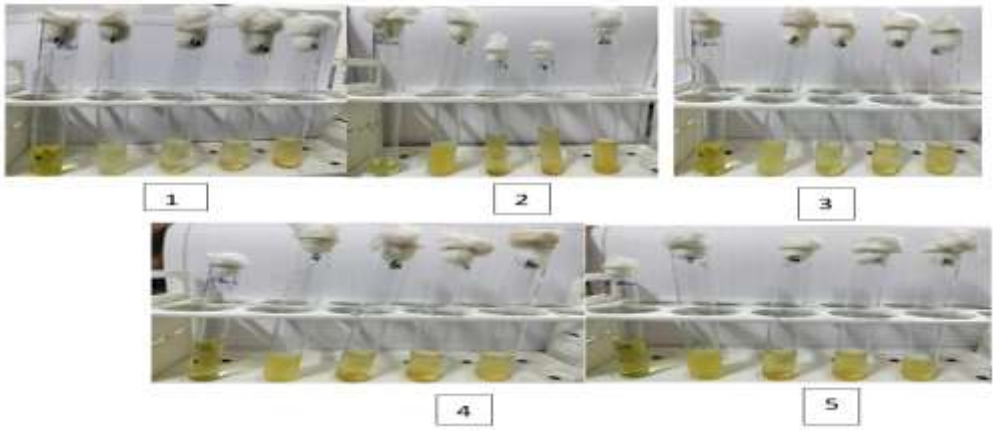


Figure 10: The minimum inhibitory growth shown by following organism (1) *Pseudomonas aeruginosa*, (2) *Staphylococcus aureus*, (3) *Escherichia coli*, (4) *Salmonella typhi* and (5) *Streptococcus pneumoniae.* Test tube no (1) Blank,(2) without FeO NPs, (3) 100 µl FeO NPs, (4) 200 µl FeO NPs (5) 300 µl FeO NPs.

FeO NPs exhibited significant potential against both Gram-negative and Gram-positive bacteria, including *Pseudomonas aeruginosa*, *Salmonella typhi*, *Xanthomonas oryzae* and *Lactobacillus*. Although *Lactobacillus* is non-pathogenic, the results demonstrated that FeO NPs had excellent inhibitory effects, with complete bacterial killing observed at concentrations between 950 µL and 150 µL **(10).**

Consequently, FeO NPs were synthesized using leaf extracts from various vegetables, including cabbage, turnips and moringa. The alcoholic extracts of these nanoparticles were subsequently tested for their antibacterial effects against *Staphylococcus aureus* and *Escherichia coli* **(7).**

The study established that the lowest concentration of FeO NPs that prevented the growth of *E. coli* (MIC) was 250 µg/mL. In contrast, the minimum bactericidal concentration (MBC) was measured at 500 µg/mL. FeONPs were generated through a simple, green, and affordable process. The results demonstrated that these nanoparticles effectively inhibited the growth of *E. coli* at 250 µg/mL, indicating their potential as antibacterial agents **(11).**

PHOTOCATALYTIC ACTIVITY

Photocatalytic activity by Congo Red dye was studied with different FeO NPs (5, 10, 15, 20 mg/10ml) with dye concentration of 20mg/ L and results were obtained were shown in Figure 11. Among this 10 mg of Fe NPs shows maximum decolourization.

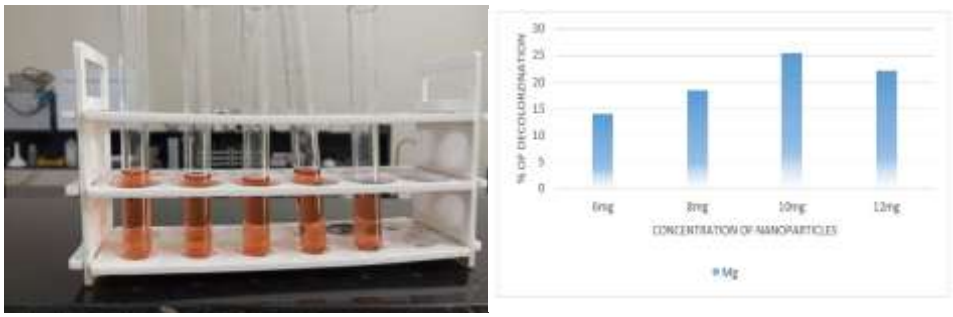


Figure 11 Effect of decolourization of Congo red dye with different concentration of Fe ONPs under sunlight

The dye decolourization experiment was carried out by various dosage of the dye (10,20,30,40 mg/L) and 10mg of iron oxide nanoparticle at room temperature shows maximum decolourization with 10 mg of Fe NPs (Figure 12)

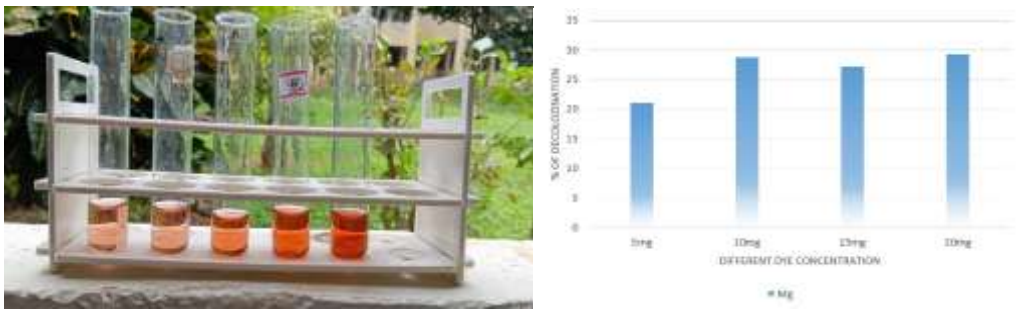


Figure 12 Effect of decolourization of Congo red dye with different concentration of dye with 10 mg of FeO NPs under sunlight

The 10 mg of FeO NPs were reacted with dye concentration (10mg/L) in 10ml of dye solution with various time interval (0, 20, 40, 60, 80, 100 mins) and following results were obtained shown in figure 13

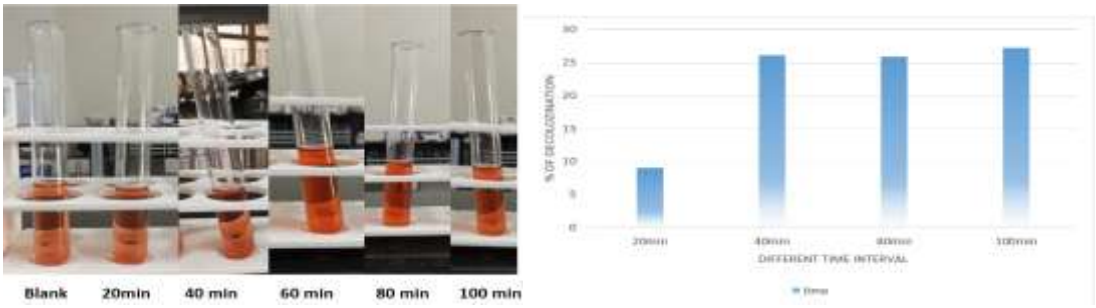


Figure 13 Effect of decolourization of Congo red dye with different time interval with 10 mg of Fe NPs and dye concentration of 10 mg/ml under sunlight

Congo red Dye shows approximately shows 25 % of decolourization with dye concentration of 10mg /ml and 10 mg of FeO NPs synthesised from the leaves of *F. auriculata* in 40 mins.

The photocatalytic activity of the α-Fe_2 O_3 nanoparticles was studied for degradation of Congo red dye under visible light irradiation. The results showed that 90% of Congo red dye was degraded within 90 min **(15)**

CONCLUSION

In our study, synthesis of FeO NPs were carried out from the *aqueous* leaves of *F. auriculata*. The nanoparticles composed of Fe and O in weight ratio of 1:2 confirmed by EDAX and average crystalline

structure was found to be 39.2613 × 10^{-9} nm confirmed by XRD. FeO NPs shows antibacterial activities and photocatalytic activity against Congo red. Further biochemical assays can be carried out in future.

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This research work has not been published anywhere.

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