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Synthesis, Characterization and Applications of Biogenic Iron Oxide Nanoparticles

R. Kanchana[1], Pranjita Zantye[2]
[1, 2] *Department of Biotechnology, Goa University, Goa 403 206, India*
[1]*Present address: Dept of Biotechnology, Parvatibai Chowgule College of Arts & Science, Autonomous, Margao, Goa – 403602, India*

Abstract: ***Biosynthesis of iron oxide nanoparticles (FeONPs) using the leaf extracts of Simarauba glauca (Sg) and Artocarpus altilis (Aa) was investigated in this work. The FeONPs were characterized by UV– Vis spectroscopy, Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM), and evaluated for antibacterial, biofilm inhibition and dye degradation activities. The crystalline polydispersed FeONPs with size range of 43-48nm prepared from Sg and 36-44nm prepared from Aa leaf extracts absorbed maximally at 250 and 220nm for Sg FeONPs and Aa FeONPs respectively. The FT-IR study indicated that the carboxyl (−C=O), hydroxyl (−OH), and amine (N-H) groups in Sg and Aa leaf extracts are mainly involved in reduction of iron to Iron oxide nanoparticles. The FeONPs showed potent antibacterial activities against Bacillus subtilis, Proteus vulgaris. E. coli and S. aureus. Bacillus biofilms treated with nanoparticles synthesized from the leaf extracts of Simarauba glauca (Sg) and Artocarpus altilis (Aa), showed biofilm dispersion with more than 75 % reduction within 24 h. Furthermore, the FeONPs degraded the dyes malachite green and methyl orange to the tune of >80 %. This study has shown that the biosynthesized FeONPs could be deployed for biomedical and environmental applications.***

Keywords: Antimicrobial activity, Biofilm, green nanotechnology, Iron oxide nanoparticles, Malachite green

I. INTRODUCTION

Nanoparticles can be synthesized using various approaches including chemical, physical, and biological. Although chemical method of synthesis requires short period of time for synthesis of large quantity of nanoparticles, this method requires capping agents for size stabilization of the nanoparticles which are expensive, toxic and lead to non-ecofriendly byproducts. The need for environmentally non-toxic synthetic protocols for nanoparticles synthesis leads to the developing interest in biological approaches which are free from the use of toxic chemicals. Thus, there is an increasing demand for 'green nanotechnology". Many biological approaches for both extracellular and intracellular nanoparticles synthesis have been reported till date using microorganisms including bacteria, fungi and plants.

The synthesis of nanoparticles using plant extract could be advantageous over other biological processes since it eliminates the elaborate process of maintaining cell cultures. Plants provide a better platform for nanoparticles synthesis as they are easily available, free from toxic chemicals and contain a broad range of biomolecules such as alkaloids, terpenoids, phenols, flavanoids, tannins, quinones which serve as natural reducing and capping agents. The experimental setup can be easily scaled for large scale synthesis and there is no need to use extreme conditions of pressure, temperature, energy [1].

Biological synthesis of nanoparticles has upsurge in the field of nano-biotechnology to create novel materials that are eco-friendly, cost effective, stable nanoparticles with a great importance for wider applications in the areas of electronics, medicine and agriculture. Though various biological entities have been exploited for the production of nanoparticles, the use of plants for the facile robust synthesis of nanoparticles is tremendous.

Nanoscale iron particles represent a new generation of environmental remediation technologies that could provide cost-effective solutions to some of the most challenging environmental cleanup problems. Iron oxide nanoparticles play an important role in environmental remediation circles [2], [3]. Iron oxide nanoparticles have been synthesized from *Eucalyptus globules,* [4], leaf litter [5], Oolong tea extract [6] and Pomegranate leaf extract [7]. However, plant-mediated synthesis of magnetic nanoparticles has remained a relatively unexplored research area with the majority of papers being published only in the last two years [8]. Thus the present study envisions the importance of plant mediated nanoparticles productions.

With this back ground, the present study reports synthesis of Iron oxide nanoparticles(FeO NPs) from leaf extracts of Simarauba glauca (Sg) and Artocarpus altilis (Aa) carried out with the main objective of evolving a simple biological method for the synthesis of nanoparticles with multi applications.

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II. MATERIALS AND METHODS

All chemicals used were of analytical grade and obtained from Sigma Chemical Co. (USA) or Hi-Media/ Merck/ Qualigens (India). In all experiments, the measurements were carried out with duplicated parallel sets.

A. Synthesis of Iron Oxide Nanoparticles

Fresh and healthy leaves of Artocarpus altilis (Aa) and Simarauba glauca (Sg) were collected and thoroughly washed with distilled water. 5 grams of leaf sample was ground with mortar and pestle and boiled with 100ml of distilled water for 15 min. The suspension was cooled and filtered. Iron oxide nanoparticle synthesis was carried out by mixing 90 ml of the filtrate with 10 ml of aqueous solution of 1 mM ferric chloride. The mixture was incubated till a colour change was obtained. Then reaction mixture was centrifuged at 10000 rpm for 20min. The supernatant was discarded and the pellet obtained was washed repeatedly with sterile distilled water, dried and finely powdered for characterization [9]. The effect of various concentrations of ferric chloride (0.5 – 25 mM) on the synthesis of Iron oxide nanoparticles was also studied.

B. Characterisation of Iron Oxide Nanoparticles

UV-vis spectral analysis was performed to confirm the biosynthesis of FeONPs using UV-Vis spectrophotometer at wavelength of 350–600 nm (Chemito UV2300). SEM (Scanning Electron Microscope) analysis was carried out to characterize particle shape and approximate size. Dry powdered sample was coated with 80% gold and 20% palladium with quorum SC7620 sputter coater to make them conductive, and analysed with Zeiss Evo 18, Scanning Electron Microscope. FTIR (Fourier Transform- Infrared spectroscopy) analysis of the dried FeONP was done using Shimadzu Fourier transform infrared spectrometer and spectrum was recorded in the range of 500-4000cm-1 at a resolution of 4 cm^{-1}. Spectral absorption bands were identified in relation to published information.

C. Applications of Iron Oxide Nanoparticles

1) Evaluation of Antibacterial Activity: The antibacterial activity of Iron oxide nanoparticles was carried out using the well diffusion assay on Mueller-Hinton agar plates against *Bacillus subtilis*, *Staphylococcus aureus* , *Escherichia coli* and *Proteus vulgaris* [10]. Dose dependent anti bacterial activity of the nanoparticle was also analysed by taking different concentrations of Iron oxide nanoparticle solutions (2, 4, 6, 8 10, and 12 µg/mL) by well diffusion method. The plates were incubated at 37ºC for 24 h and the zone of clearance around the well was measured.

2) Biofilm Inhibition: Biofilm inhibition study was performed as described by Ref [11] using *the* biofilm formed by *Bacillus subtilis*. Experiments were performed in triplicate.

3) Reduction of Dyes Using Iron Oxide Nanoparticles: Azo dyes are a major class of synthetic, colored organic compounds that account for about half of the textile dyestuffs used today which are difficult to degrade and result in the threat of environmental pollution. Nanoscale particles are recently gaining great interest in environmental remediation circles [12]. In the current study, applicability of iron oxide nanoparticles in the removal of Methyl orange and Malachite green dyes were investigated.

The ability of iron oxide nanoparticles to act as fenton like catalysts in the degradation of dyes was evaluated by a method described by Ref [13]. 1 g/L stock solutions of methyl orange and malachite green was prepared in distilled water. 30 % stock solution of H_2O_2 was also prepared as an oxidizing agent. In polypropylene tubes, 45 ml of 50 mg/L of dye, 5 ml of 10% H_2O_2 and 25 mg of respective iron oxide nanoparticles was added. The absorbance readings were taken using Chemito UV2300 spectrometer at 465 nm for methyl orange and 617 nm for malachite green. The absorbance readings were obtained every 30 minutes. Control set without the addition of nanoparticles was carried out in parallel. The degradation efficiency was calculated using the formula:

$$R\ (\%) = [(C_0 - C_t)/C_0] \times 100$$

where R (%) represented the dye degradation efficiency, and C_0 and C_t (mg/L) represented the concentration of dye at initial and t time respectively.

III. RESULTS AND DISCUSSION

A. Synthesis of Iron Oxide Nanoparticles

The present work focused on the development of a biosynthetic method for the production of FeONPs. It was observed that the colour of the mixture containing leaf extract and ferric chloride turned from pale yellow to dark brown/ black immediately indicating the spontaneous formation of FeONPS (Fig 1 & 2). The color change is the most easy and commonly used indication of the metal. nanoparticles formation [8]. The alkaloids and secondary metabolites present in the leaf extract readily served as bioreductant molecules for the green synthesis of nanoparticles.

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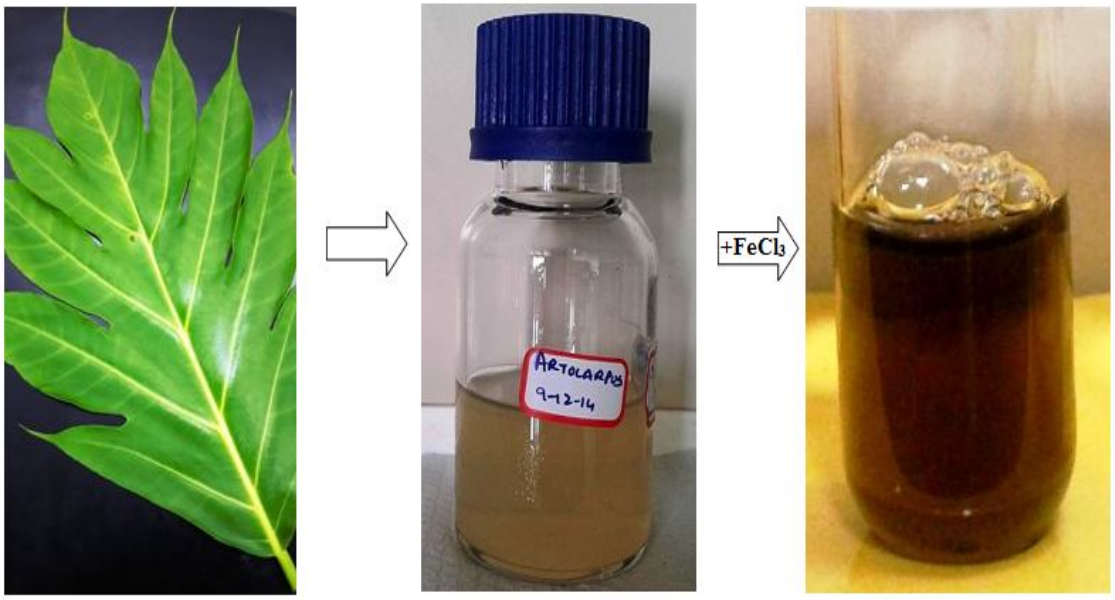
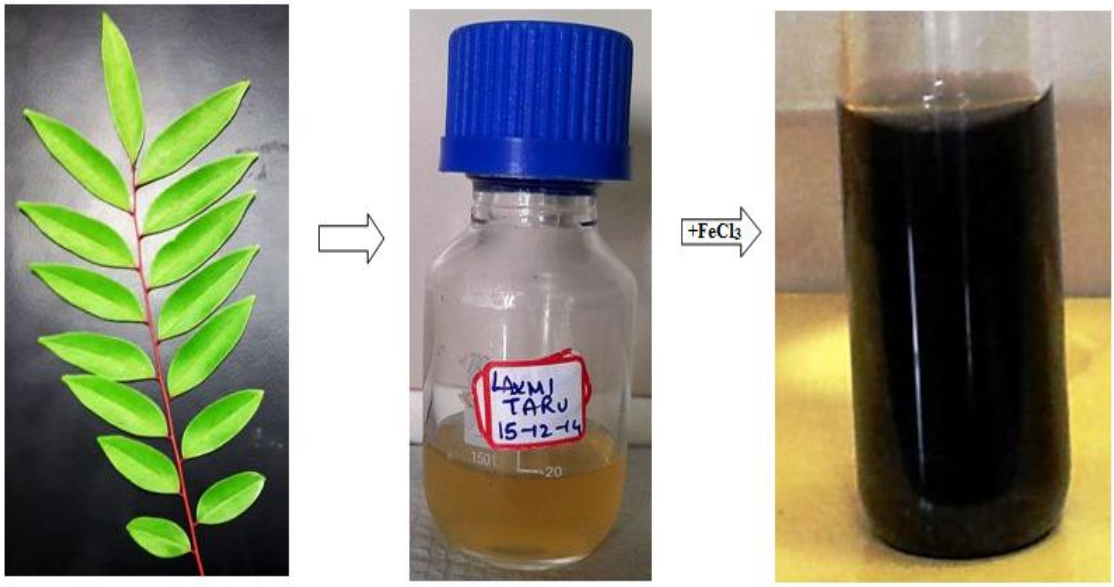


Artocarpus altilis leaf Leaf extract Biosynthesized FeONPs

Fig. 1 Biosynthesized FeONPs using Artocarpus altilis leaf extract. Spontaneous colour change from pale yellow to dark brown indicating the spontaneous formation of FeONPS



Simarauba glauca leaf leaf extract Biosynthesized FeONPs

Fig. 2 Biosynthesized FeONPs using Simarauba glauca (Sg) leaf extract. Spontaneous color change from pale yellow to black indicating the spontaneous formation of FeONPS

B. Characterisation of Feo NPS

Absorption maxima observed as sharp peak at 250 and 220nm for Sg FeONPs and Aa FeONPs respectively confirming the synthesis of FeONPs due to the excitation of surface plasmon vibrations (Fig.3a & b). Ref [14] had reported identical UV-Vis spectra for FeONPs synthesized using A. annua and P. frutescens extracts. The optimized concentrations of ferric chloride for the synthesis of Sg FeONPs and Aa FeONPs were found to be 1mM and 25mM respectively (Fig. 4a &b) since higher concentration led to the aggregation of nanoparticles.

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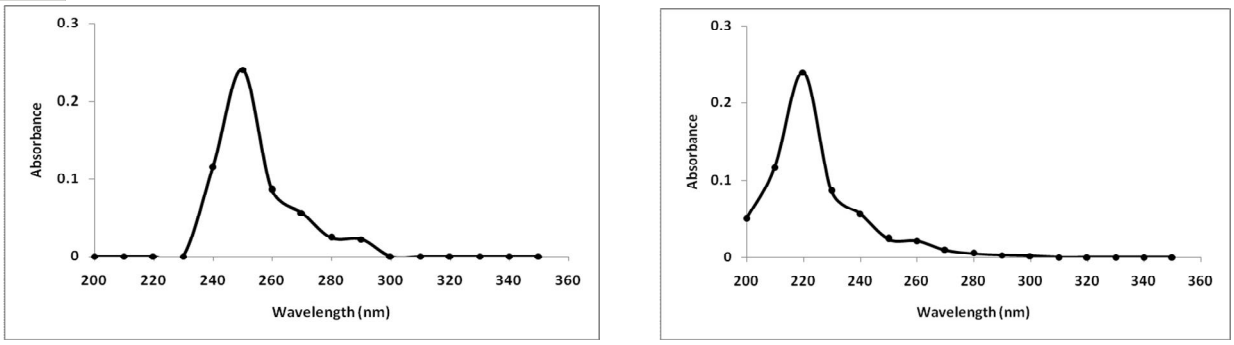

Fig. 3 a&b UV-vis spectra of FeONPs synthesized from (a) Simarauba glauca leaf extract and (b) Artocarpus altilis leaf extract

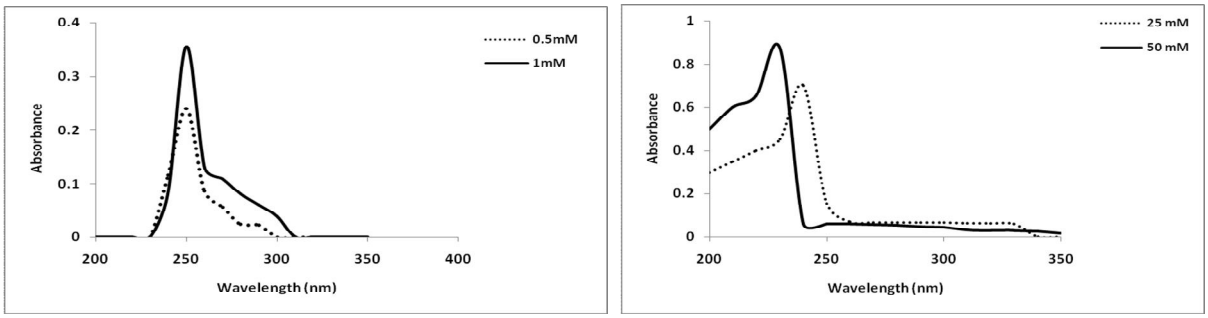

Fig. 4 a&b UV-vis spectra of FeONPs synthesized from (a) Simarauba glauca leaf extract and (b) Artocarpus altilis leaf extract as a function of the different concentration of ferric chloride

SEM provided further insight into the morphology and size details of the nanoparticles. Experimental results showed that the size of the prepared nanoparticles was in the range of 43-48nm for Sg FeONP's and 36-44nm for Aa FeONP's (Fig. 5a and b). The morphology of iron oxide nanoparticles was nearly spherical and is in agreement with the shape of surface plasma resonance band in the UV – Visible spectra [15].

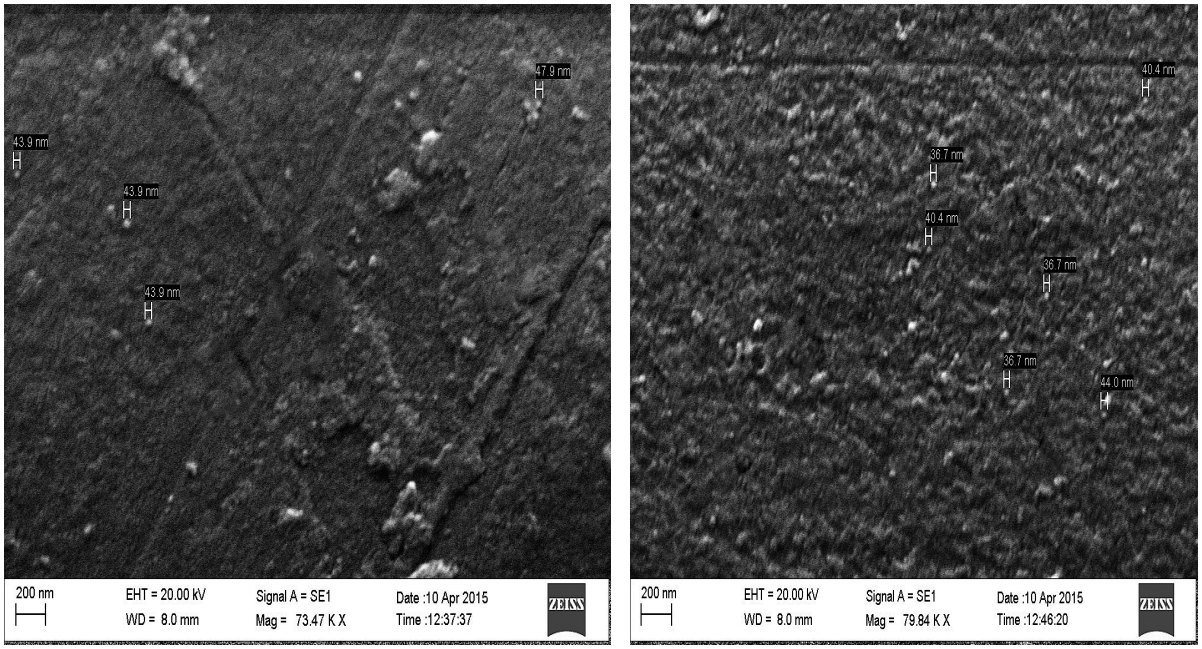

Fig. 5a & b Scanning electron microscope images of FeONPs synthesized from (a) Simarauba glauca leaf extract and (b) Artocarpus altilis leaf extract

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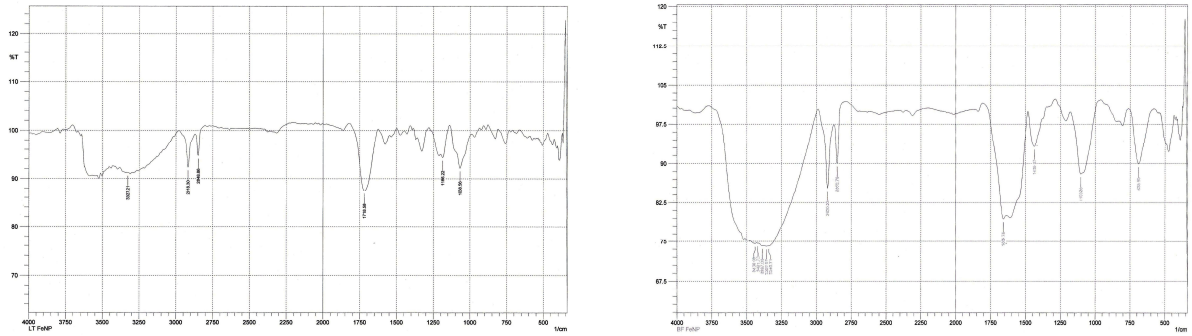

Fig. 6a & b Fourier transform-infrared spectroscopy (FTIR) spectra of FeONPs synthesized from (a) Simarauba glauca leaf extract and (b) Artocarpus altilis leaf extract

FTIR measurements were carried out to identify the biomolecules responsible for capping and efficient stabilization of the synthesised nanoparticles. The FTIR spectra of 3,327 cm^{-1} for Sg FeONP's and 3327.21 cm^{-1} for Aa FeONPS's which have resulted from stretching of the N-H band of amino groups or is indicative of bonded O-H hydroxyl group. Absorption peaks at 1,186cm^{-1} for LT FeONP's and 1103.26 cm^{-1} for BF FeONP's correspond to carboxylic acids and alcohols.
FT-IR study indicates that the carboxyl (−C=O), hydroxyl (−OH), and amine (N-H) groups in Sg and Aa leaf extracts are mainly involved in reduction of iron to Iron oxide nanoparticles. The FT-IR spectroscopic study also confirmed that the metabolites present in the leaf extracts act as reducing agents and stabilizers for nanoparticles and prevent agglomeration. This suggests that the biological molecules could possibly perform dual functions of formation and stabilization of silver and iron oxide nanoparticles in the aqueous medium [16]. The band 618 cm-1 indicated the Fe-O stretching of Fe2O3 nanoparticles, as reported earlier [17] confirming the formation of iron oxide nanoparticles. Based on these results, the presence of phenolic compounds and proteins were believed to be responsible for the formation and stabilization of synthesized iron oxide nanoparticles.

C. Applications of Feonps

1) Antibacterial Activity: The green synthesized iron oxide nanoparticles exhibited excellent antibacterial activity against all the tested pathogenic bacteria (Table 1). The mechanism of action may be due to the nanoparticles attachement to the surface of the cell membrane and disturbing permeability and respiration functions of the cell [18]. The results of the dose dependant antibacterial activity revealed the increase in antibacterial activity with increase in nanoparticle concentration (Fig. 7 a &b).

Table 1: Antibacterial Activity Of FeoNPS

Sample	Zone of Inhibition (mm)			
	Bacillus subtilis	Proteus vulgaris	E. coli	S. aureus
LT FeONP	13.67 ± 0.58	14 ± 0.70	13.67 ± 0.58	14.33 ± 0.58
Control (leaf extract)	9.0 ± 0.5	8.0 ± 0.5	6.0 ± 0.8	7.0 ± 0.6
BF FeONP	11 ± 0.5	13 ± 0.6	13 ± 0.9	11.67 ± 0.58
Control (leaf extract)	0	0	0	0

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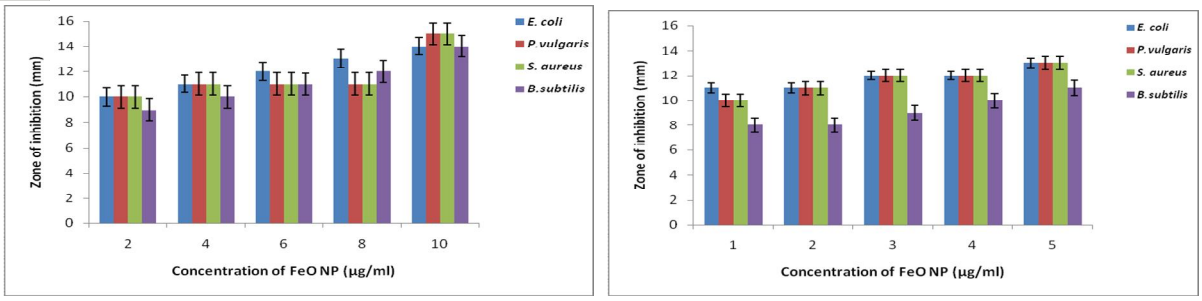

Fig. 7 a &b Dose-dependent antimicrobial activity of FeONPs synthesized from (a) Simarauba glauca leaf extract and (b) Artocarpus altilis leaf extract. Error bars are mean ± SE; (n=3)

2) Biofilm Degradation Activity: Biofilms are conglomerations of bacterial cells protected by self-synthesized extracellular polysaccharide matrices. Biofilm infections are extremely challenging to treat because antimicrobials are less effective than planktonic cells, thus making clearance more challenging. The presence of biofilms causes numerous problems in the field of medicine, interfering with clinical therapy of chronic and wound-related infections as well as persistent infections of various indwelling medical devices. Although numerous strategies have been established and are currently in use to control biofilms, the pursuit for novel, natural and effective anti-biofilm agents still continues.

In this study, the use of nanoparticles as alternatives to control the formation of biofilms has been explored. Silver nanoparticles have been shown to modify the surface properties of bacterial cells and reduce their adhesive properties [19]. Compared to the controls, preformed *Bacillus* biofilms treated with nanoparticles at 1 mg/mL concentration, for 24 hours showed increased biofilm dispersion with more than 75 % reduction by FeONPs (Fig. 8).

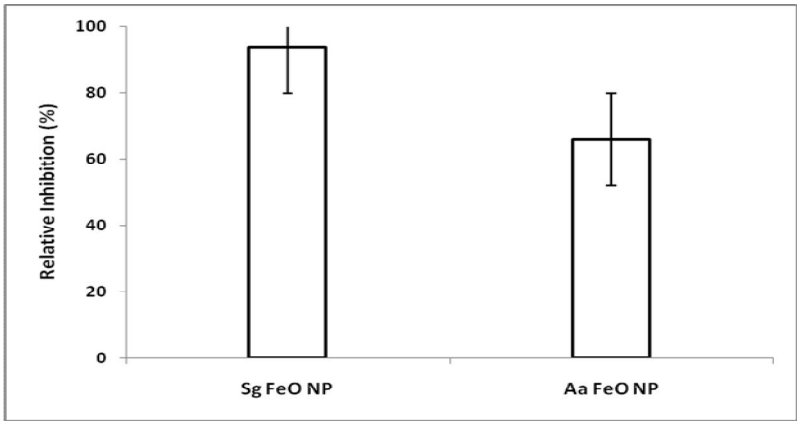

Fig. 8 Biofilm degradation using FeONPs

3) Reduction of Dyes Using Iron Oxide Nanoparticles: The reduction of Methyl orange and Malachite green by iron oxide nanoparticles is shown in Fig. 9 a & b. Sg FeONPs reduced Malachite green and Methyl orange in 480 minutes (91%) and 420 minutes (82%) respectively. Aa FeONP's reduced Malchite green in 480 minutes (92%) and Methyl orange in 420 minutes (86%). In the absence of catalyst, decolourization of the dyes was not noticed even after 24 h, indicating that there was no direct oxidation pathway by peroxide. However, decolourization occurred only when FeONPs was introduced into the solution mixtures indicating essentiality of the nanoparticles for promoting the decolourization which probably occur through free radicals pathway. The introduction of the nanoparticles may facilitate the formation of OH^* radical through which the degradation of the dye proceeds [20].

It has been reported that Iron nanoparticles synthesized from green tea extracts as a Fenton catalyst for the oxidation of bromothymol blue and cationic (methylene blue) and anionic (methyl orange) model dyes [13]. The oxidative decolorisation is carried out in the presence of hydrogen peroxide solution. The resulting free radicals then carry out secondary reactions on dyes [21], [22]. It was therefore demonstrated that the present Iron oxide nanopartilces synthesized through green route is potential agent in remediation of cationic and anionic dyes.

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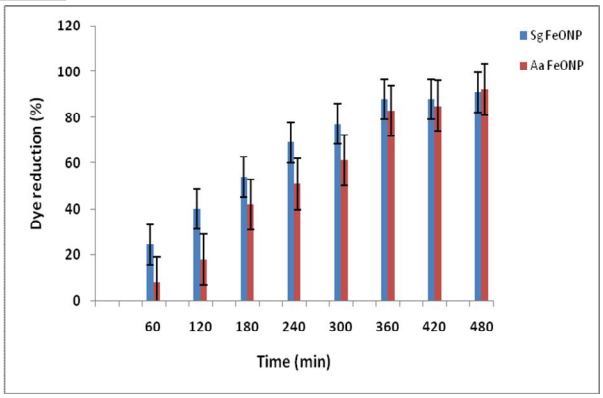

Fig. 9a. Reduction of Malachite green using FeONPs

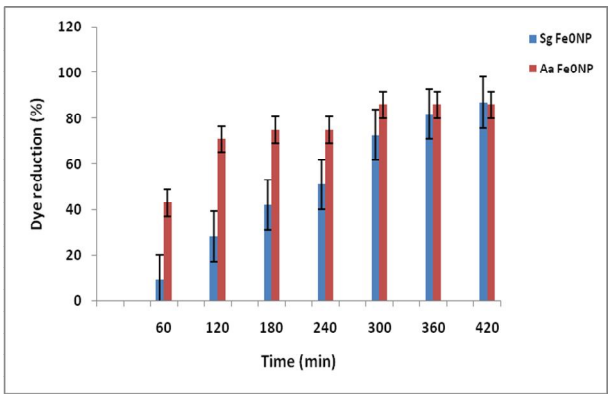

Fig. 9b. Reduction of Methyl orange using FeONPs

IV. CONCLUSION

This study has demonstrated the spontaneous and eco-friendly synthesis of iron oxide nanoparticles using the leaf extracts of Simarauba glauca and Artocarpus altilis. The synthesised nanoparticles displayed remarkable antibacterial activity, biofilm degradation and also degraded malachite green and methyl orange under ambient conditions to the tune of >90 %. These activities have shown that the plant mediated iron oxide nanoparticles can find useful biomedical and environmental applications. To the best of our knowledge, this is the first report of the biogenic synthesis of iron oxide nanoparticles using the metabolites of Simarauba glauca leaf extract and Artocarpus altilis leaf extract, which adds to the growing utilization of novel biomaterials of plant origin in nano-biotechnology.

V. ACKNOWLEDGMENT

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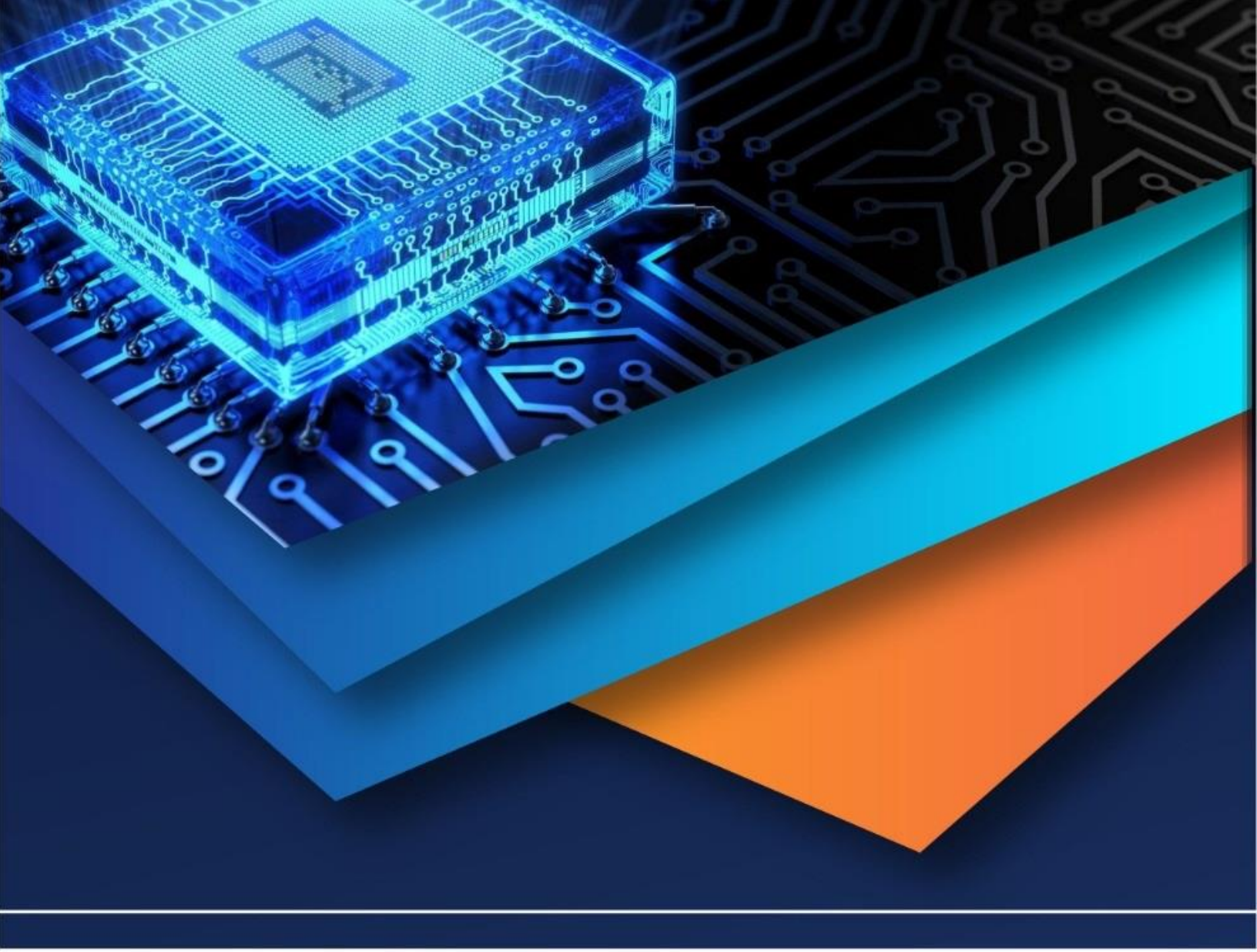

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