Research Article

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Antimicrobial Activity of Iron Nanoparticles Synthesized using *Bambusa vulgaris* Leaves

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ABSTRACT

The current study synthesized iron nanoparticles (FeNPs) through a sustainable green synthesis method which utilized aqueous extract of *Bambusa vulgaris* leaves. This method offers environmentally friendly as well as economically viable benefits. UV-Visible spectroscopy was used to confirm FeNPs formation, stating a distinct peak of absorption at 278 nm. Fourier Transform Infrared Spectroscopy was performed to identify the functional groups present in the synthesised FeNPs. Scanning Electron Microscopy examined the morphology, revealing mostly spherical FeNPs with slight agglomeration due to high surface energy. Energy-Dispersive X-ray spectroscopy was used to determine the elemental composition confirming the presence of iron along with oxygen and chlorine, suggesting the phytochemical involvement in synthesis. X-ray Diffraction established crystalline nature of FeNPs at nanometric dimensions. In addition, the FeNPs revealed significant antibacterial activity against *Escherichia coli* (*E. coli*), *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The FeNPs showed inhibition zones of 14 mm against *E. coli* and 20 mm against *S. aureus* and *P. aeruginosa* which demonstrated equivalent effectiveness to the standard antibiotic amikacin. The study results demonstrate that *Bambusa vulgaris* functions as a potential biological source which can produce active antimicrobial FeNPs with superior properties.

Keywords: Iron nanoparticles; *Bambusa vulgaris*; Antimicrobial activity; Green synthesis.

1. INTRODUCTION

Nanotechnology is a promising versatile technology with numerous applications to address global challenges. Its potential in the medical and environmental sectors makes it particularly adept at identifying efficient methods for pilot-scale nanoparticle synthesis (Manikandan *et al.* 2022). Biological processes are emerging as a means to carry out green nanoparticle production more effectively. Different plant derived components can act as stabilising and reducing agents in the synthesis of these green nanoparticles. Plant-based methods for synthesizing green nanoparticles offer easier, affordable, and environmentally friendly solutions than microbial methods that utilize bacteria, fungi, actinomycetes, and algae (Brar *et al.* 2022). Green synthesis method tends to be cost-efficient, non-toxic, and more stable in comparison to other biological, physical, and chemical methods (Mustapha *et al.* 2022). Green synthesised nanoparticles display potential antibacterial, antifungal, and antiviral activities (Pandit *et al.* 2022; Madani *et al.* 2022). Researchers study green-synthesized metal nanoparticles for environmental cleanup because these particles can remove dye pollutants and break down hazardous substances that exist in water bodies (Sasidharan *et al.* 2018; Vishalatchi *et al.* 2022). Components. (Vitta *et al.* 2020) synthesized iron nanoparticles (FeNPs) using aqueous extract of *Eucalyptus robusta* leaves and investigated their antibacterial activity against certain pathogens. Varying quantities of the precursor and leaf extract ratio were maintained at 1:1. Results indicated a clear relationship between the size of FeNPs and the specific conditions of their synthesis. The study also measured flavonoid and total polyphenol content while testing antioxidant activity from *E. robusta* extract. Green synthesis methods were used to synthesise iron nanoparticles from *Chlorophytum comosum* leaves. The TEM analysis confirmed the spherical shape of FeNPs and their amorphous nature was confirmed by X-ray powder diffraction, with particle sizes under 100 nm (Shaker *et al.* 2021; Kiwumulo *et al.* 2022). (Batool *et al.* 2021) investigated the synthesis of FeNPs using *Phoenix dactylifera,* where extract was the reducing agent while iron sulfate heptahydrate functioned as the substrate. Iron nanoparticles were synthesized using *Camellia sinensis* leaf extract, in which iron ions were reduced at room temperature to form nanoparticles *via* a sustainable, green synthesis approach (Gottimukkala, 2017). The objective of current research is to synthesize iron nanoparticles from *Bambusa vulgaris* leaf extract. Characterisation techniques were employed to confirm the formation of nanoparticles. The antimicrobial

properties of the synthesized FeNPs were also evaluated.

2. MATERIALS AND METHODS

2.1 Preparation of *Bambusa vulgaris* Leaves Extract

Bambusa vulgaris leaves were collected from Sevalpatti and cleansed completely with distilled water to ensure all impurities were removed from the leaves. About 30 g of washed leaves were taken and cut into small pieces and placed in a 250-mL beaker that contained 100 mL of deionized water. The mixture was heated at 80°C for 15 minutes on a heating mantle (Raja *et al.* 2018). Whatman filter paper was used to filter the extract, and the resulting filtrate was stored for subsequent nanoparticle synthesis.

2.2 Synthesis of Iron Nanoparticles

The process of iron nanoparticle synthesis utilized ferric chloride ($FeCl_3$) solution together with aqueous extract of *Bambusa vulgaris* leaves. About 50 mL of leaf extract was combined with 50 mL of 0.1M $FeCl_3$ solution, in a 1:1 ratio.

The mixture was continuously agitated at 37°C using a magnetic stirrer. Distinct color change from brown to black indicated the presence of iron nanoparticles. Then the precipitate was centrifuged for 15 minutes at 15,000 rpm; the resulting pellets were collected and dried through a two-hour drying process at 65°C in a hot air oven.

2.3 Characterization of Biosynthesized Iron Nanoparticles

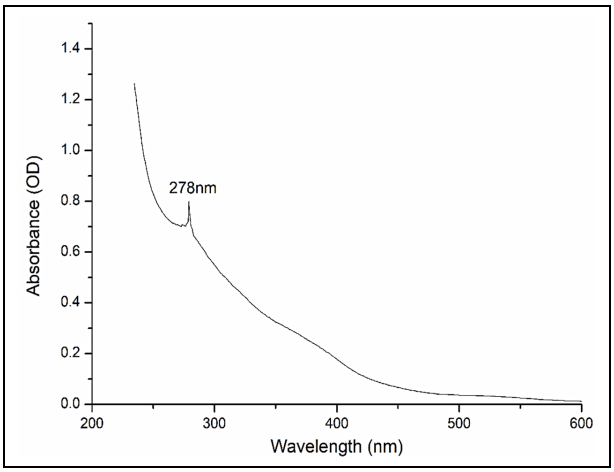


Fig. 1: UV spectra of synthesised FeNPs

Standard analytical techniques were used to characterize the synthesized FeNPs (Mareedu *et al.* 2021). UV-vis spectroscopy (LABMAN, UV-1000B) was employed to monitor the formation of iron nanoparticles over a wavelength range of 200–800 nm, using deionized water as the blank reference. The functional groups present in nanoparticles were determined by Fourier Transform Infrared (FTIR) spectroscopic analysis (SHIMADZU, 8400S). Scanning Electron Microscopy was used to examine the morphology of the FeNPs (Carl Zeiss NTS GMBH, Germany, SUPRA 55), while the elemental compositions were determined by Energy Dispersive X-ray spectroscopy (EDX). X-Ray Diffraction (XRD – RIKAGU Japan, Smartlab 9KW) was employed to study the crystalline structure of the FeNPs according to the procedure established by (Zakariya *et al.* 2022).

2.4 Antimicrobial Activity Assessment

Antibacterial activity of FeNPs was assessed using agar well diffusion method. The antibacterial activity against *P. aeruginosa*, *E. coli*, and *S. aureus* was evaluated. A nutrient agar Petri plate was inoculated with 100 µL of the microbial culture, ensuring uniform distribution across the medium. Next, 50 µL of iron nanoparticle solution was added to each well in the agar, prepared using the well puncture technique. The inoculated plates were incubated for 24 hours at 30°C in an incubator. Following incubation, the zone of inhibition was measured to ascertain the antibacterial efficacy (Vitta *et al.* 2020).

3. RESULTS AND DISCUSSION

3.1 Synthesis of Iron Nanoparticles

Biological route was adapted for synthesizing FeNPs employing aqueous leaf extract of *Bambusa vulgaris*. The original brown color of the leaf extract changed to deep black during the process due to successful production of FeNPs.

3.2 Characterization of Synthesised Iron Nanoparticles

3.2.1 UV-visible Spectroscopy

The formation of FeNPs using *Bambusa vulgaris* aqueous leaf extract was confirmed with UV-Visible spectrophotometric analysis between 200–800 nm range, with a unique absorption peak at 278 nm (Fig. 1). The absorption peak is the indicative of the presence of FeNPs. (Raja *et al.* 2018) also reported similar spectral properties during the synthesis of plant-mediated iron nanoparticles. The FeNPs prepared from *Mangifera indica* leaf extract showed a band at 223.62 nm, whereas other studies showed bands within the range 216 to 265 nm. The disparity in absorbance wavelengths can be attributed to the variations in phytochemical content of the plant extracts that can influence the nanoparticle size and stability and

reduction efficiency. The higher wavelength reported in the present study, in comparison to others, symbolizes the involvement of novel bioactive molecules within *Bambusa vulgaris* that efficiently mediate the reduction of iron ions and facilitate the formation of nanoparticles.

3.2.2 Fourier Transform Infrared Spectroscopy

Fourier Transform Infrared spectroscopy analysis determined the functional groups of specific biomolecules in aqueous extract of *Bambusa vulgaris* which were responsible for reduction and stabilization of FeNPs. The FTIR spectrum (Fig. 2) indicates several absorption peaks with distinct nature, each corresponding to different phytochemical constituents actively participating in the nanoparticle biosynthesis of FeNPs. An intense absorption band detected at 3350 cm^{-1} is ascribed to O–H stretching vibrations representing the existence of alcohols or phenolic substances in the leaf extract. The notable peak of absorbance at 1059 cm^{-1} is due to C–C stretching vibrations, possibly caused by aliphatic chains or saccharides in the plant extract. A sharp peak at 1613 cm^{-1} is attributed to C=O stretching, which points towards the carbonyl groups of flavonoids or other polyphenolic compounds. Another peak at 1428 cm^{-1} is for O–H bending, further validating the use of phenolic compounds as reducing and capping agents in the synthesis of FeNPs. The presence of aromatic compounds can be confirmed through the detection of a common spectral peak which occurs at 1513 cm^{-1} because of C=C stretching vibrations found in aromatic rings. The presence of carboxylic acids or aldehydes is shown through the C–H stretching and C=O asymmetrical stretching vibrations which reach their maximum at 2917 cm^{-1} and 1370 cm^{-1}. These implications verify that different bioactive molecules, especially polyphenols, flavonoids, and aromatic compounds in *Bambusa vulgaris* play a significant role in green synthesis and stabilization of FeNPs (Mareedu *et al.* 2021).

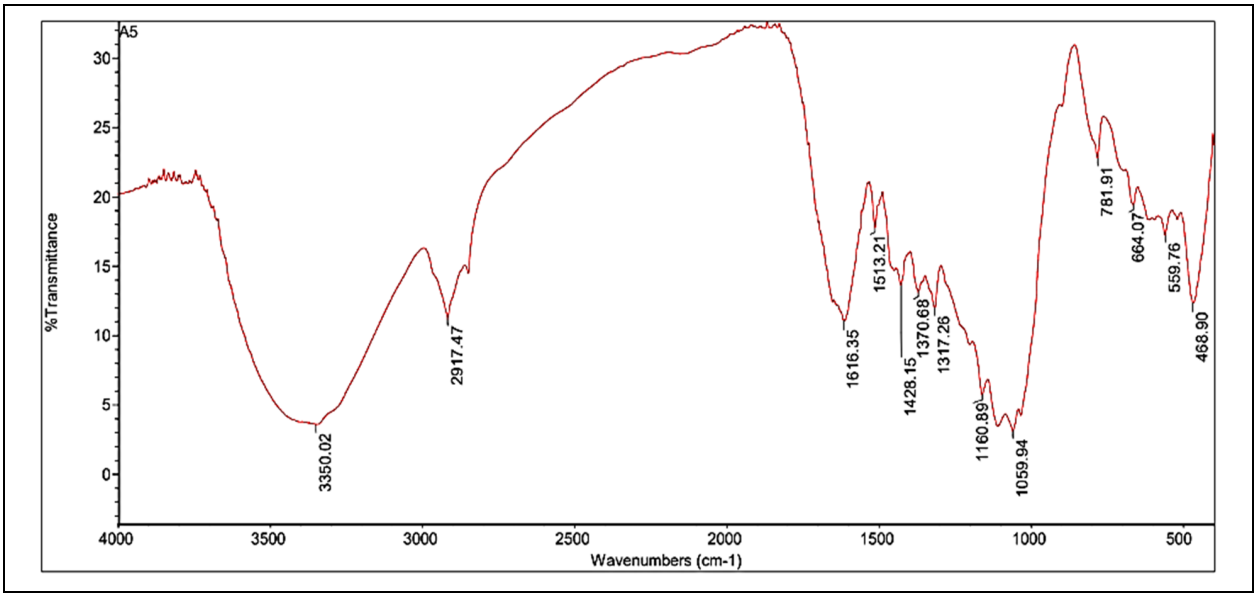


Fig. 2: FTIR spectrum of synthesised FeNPs

3.2.3 X-ray Diffraction Analysis

X-ray diffraction was employed to study the crystal structure and properties of FeNPs produced from the hydro extract of *Bambusa vulgaris*. The XRD pattern in Fig. 3 shows distinct and sharp diffraction peaks, which clearly denoted that the nanoparticles possess a crystalline nature. The crystal size was calculated using Scherrer equation 1 (Kiiamov *et al.* 2023). The most intense peak at 2θ = 31.67º was employed to determine the crystal size of the FeNPs as 27.9 nm. The diffraction peaks observed at 2θ values of 45.39º and 75.49º and had crystal sizes of 25.8 nm and 11.9 nm, respectively. The peaks indicate that there are highly ordered crystal structures in the FeNPs. The average crystal size was around 21.9 nm, indicating that the nanoparticles were in the anticipated nanoscale range. The biosynthesized iron nanoparticles from *Bambusa vulgaris* extract produced stable, crystalline FeNPs as reported in previous studies as well (Zangeneh and Zangeneh, 2020) and (Muthumari *et al.* 2024). The nanoparticles maintain their stability because of their crystallinity, which makes them appropriate for various biomedical and environmental uses.

$$D = \frac{K\lambda}{\beta \cos\theta} \quad (1)$$

where K is Scherrer constant, λ is wavelength, β is FWHM and θ is Bragg angle.

3.2.4 Scanning Electron Microscopy and Energy Dispersive X-ray Spectroscopy

The morphology of green produced FeNPs was characterized through Scanning Electron Microscopy (SEM), as indicated in Fig. 4. The FeNPs are predominantly spherical in morphology and tend to cluster in groups due to a common phenomenon called agglomeration, which is caused by strong electric forces between particles and surfaces coupled with the large surface area to volume. To determine the elemental composition of FeNPs, EDX was applied. The EDX spectrum is shown in Fig. 5. The elemental composition of FeNPs is shown in Table 1. The spectrum shows the presence of elemental iron, with peaks at energy levels of 0.5 and 6.5 keV. The results confirmed that iron constitutes a high percentage at 47.14%, affirming that the nanoparticles were successfully synthesized. In addition, the spectrum indicated oxygen (4.76%), chlorine (16.87%) sodium (27.46), likely to have originated from the plant extract and residual salts/ionic species used in the process (Devi and Ahmaruzzama, 2018). The insights affirm that bioactive compounds and alkali elements in the *Bambusa vulgaris* extract are responsible for reducing and stabilizing the FeNPs during unification.

3.3 Antimicrobial Activity Against Bacterial Strains

Table 1. Elemental composition of synthesised FeNPs

Element	Weight %	Atomic %
O	4.76	10.23
Na	27.46	41.13
Al	0.22	0.28
Si	0.45	0.55
Cl	16.87	16.38
K	0.51	0.45
Ca	1.44	1.24
Mn	0.45	0.28
Fe	47.14	29.06
Co	0.23	0.13
Cu	0.46	0.25
Total	100	100

Antimicrobial activity of FeNPs biosynthesized from aqueous extract of the *Bambusa vulgaris* was tested against three clinically important bacterial strains, *S. aureus*, *E. coli*, and *P. aeruginosa*. Antibiotic amikacin was used as the standard for comparison. Fig. 6 and Table 2 show the antibacterial activity and zone of inhibition, respectively. Clear and well-defined zones of inhibition were noticed around the bacterial colonies after proper incubation. The biosynthesized FeNPs exhibited strong antibacterial activity, producing inhibition zones of 20 mm against *S. aureus*, 14 mm against *E. coli*, and 20 mm against *P. aeruginosa*. For standard amikacin in comparison, the zones were recorded at 15 mm, 16 mm, and 18 mm against same bacterial strains. These results strongly validate the fact that the FeNPs biosynthesized from *Bambusa vulgaris* show good antibacterial activity, particularly with respect to the bacterial strain *S. aureus*, where the zones of inhibition were slightly greater than that of the standard antibiotic treatment. These results corroborate with the work of Raja *et al.* (2018) and provide compelling evidence for the potential use of biosynthesized FeNPs as potent antibacterial agents.

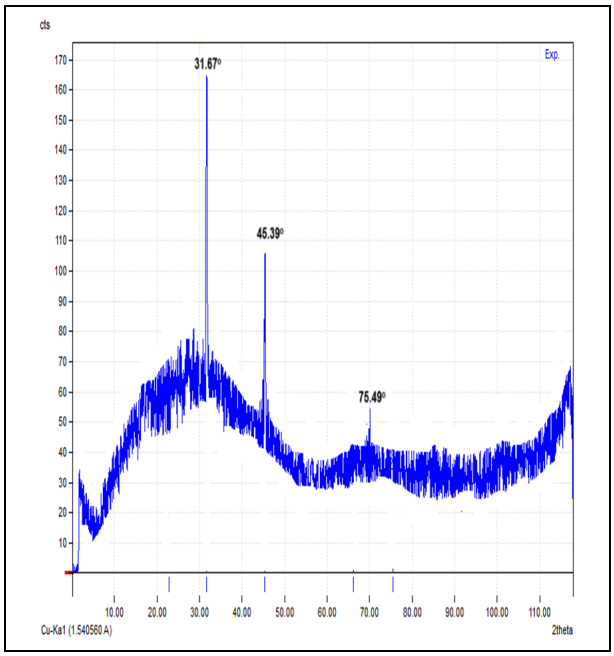


Fig. 3: XRD pattern of synthesised FeNPs

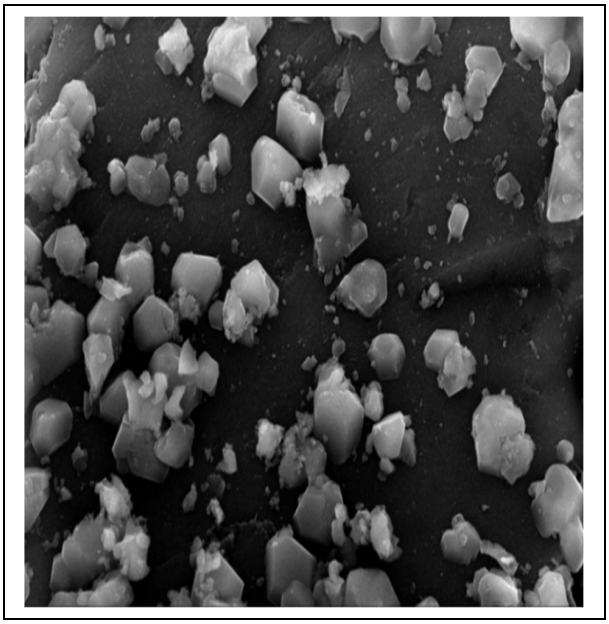


Fig. 4: SEM image of synthesised FeNPs

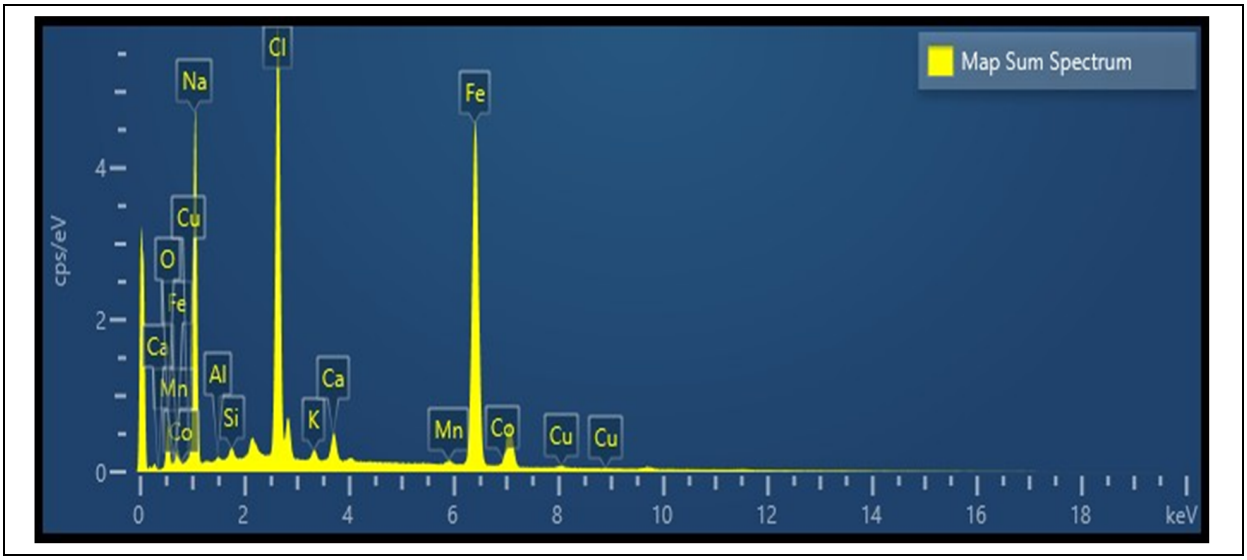


Fig. 5: EDX of synthesised FeNPs

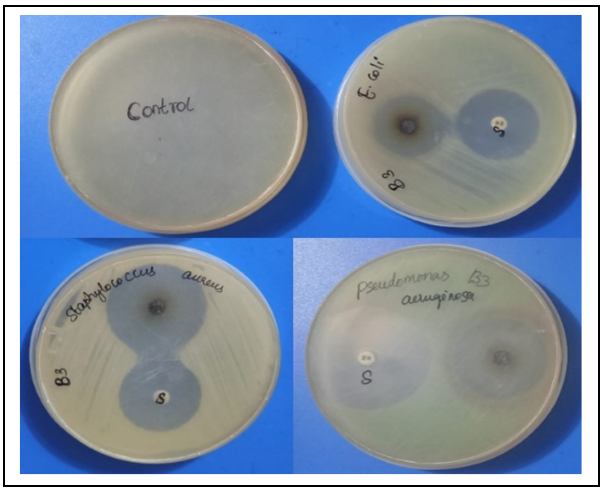


Fig. 6: Antimicrobial activity of FeNPs

Table 2. Zone of Inhibition of FeNPs

S NO.	Bacterial Species	Zone of Inhibition (Amikacin) mm	Zone of Inhibition (FeNPs) mm
1	*E. coli*	16	14
2	*S. aureus*	15	20
3	*P. aeruginosa*	18	20

4. CONCLUSION

This current work shows successful synthesis of iron nanoparticles from *Bambusa vulgaris* leaf extract through a green route. UV-vis spectrophotometry confirmed the formation of nanoparticles, with a peak at 278 nm. X-ray diffraction analysis showed that FeNPs have a crystalline framework and an average particle size of 21.9 nm. The SEM illustrated sphere-shaped morphology of FeNPs. Elemental profiling *via* EDX confirmed the existence of iron, oxygen, and chlorine (role of phytochemicals) in the synthesis of FeNPs. Fourier Transform Infrared Spectroscopy further found important functional groups like O–H, C=O, and C–H due to the presence of phenolic compounds and other bioactive molecules that help reduction and stabilization of FeNPs. Antimicrobial assays revealed that the FeNPs had higher antibacterial effectiveness than the conventional antibiotic amikacin (reference). Notably, FeNPs exhibited an inhibitory zone of 20 mm against *S. aureus* which surpasses the activity of amikacin. Thus, *Bambusa vulgaris* shows strong potential as a sustainable, long-term source for producing biologically active FeNPs. This environmentally benign approach is a promising option for biomedical applications, as it offers strong antibacterial activity.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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