

Lantana camara-Mediated Green Synthesis of Fe₂O₃-NPs: Structural Characterization and Biological Activity Assessment

Kadir Rafik Khan¹, Neha Samreen Gulam Dastageer², Samadhan Sakharam More³ and Momin Nishat Parveen^{1*}

^{1, 2, 1*}Department of Chemistry, Sir Sayyed College of Arts, Commerce and Science, Chh.Sambhajinagar, (Aurangabad), 431001 M.H., India.

³Department of Chemistry, Milind College of Science, Chh.Sambhajinagar (Aurangabad), 431001 M.H., India.

Corresponding Author: kadir75khan@gmail.com

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ABSTRACT

Green nanotechnology offers a sustainable route to functional nanomaterials using plant-derived phytochemicals as reducing and capping agents. In this study, α -Fe₂O₃ nanoparticles (Fe₂O₃-NPs) were synthesized using an aqueous leaf extract of *Lantana camara* followed by calcination at 700°C. The nanoparticles were characterized by XRD, UV–Vis spectroscopy, FTIR, EDS, SEM, and TEM, and evaluated for antibacterial and antifungal activity (disc diffusion) and DPPH radical scavenging activity (3.9–500 μ g/mL). XRD confirmed a crystalline rhombohedral hematite phase (JCPDS No. 33-0664) with an average crystallite size of $\sim$ 28 nm. The UV–Vis spectrum showed an absorption peak at 268 nm, and FTIR showed an Fe–O band at 576 cm⁻¹. EDS showed the presence of Fe, O, and C. TEM revealed irregular, quasi-spherical, agglomerated particles with a broad size distribution and a mean particle size of 79.87 ± 43.06 nm. The Fe₂O₃-NPs showed species-dependent antibacterial activity, with inhibition zones of 17 mm (*Staphylococcus aureus*), 14 mm (*Bacillus subtilis*), 20 mm (*Klebsiella pneumoniae*), and 13 mm (*Escherichia coli*), but no activity against *Aspergillus niger*. In the DPPH assay, scavenging activity generally increased with concentration, reaching $99.21 \pm 0.02\%$ at 500 μ g/mL, comparable to ascorbic acid (98.65%) at the same concentration. These results suggest that *L. camara*-mediated Fe₂O₃-NPs have potential as antibacterial and antioxidant agents, warranting further investigation.

Keywords: Green synthesis, *Lantana camara*, Hematite (α -Fe₂O₃), Nanoparticles, Antibacterial activity, Antioxidant efficiency, DPPH assay.

1. INTRODUCTION

Nanotechnology has become one of the most active areas of modern science, with applications in medicine, electronics, environmental science, and materials engineering.¹ Advances in synthesis now allow the size, shape, and surface properties of nanomaterials to be tailored for specific uses.² Metal oxide nanoparticles are of particular interest because their optical, magnetic, electrical, and chemical properties differ markedly from those of their bulk counterparts, which makes them useful in a wide range of technological and biomedical applications.^{3,4}

Among them, iron oxide nanoparticles, typically in the size range of 1–100 nm, are especially important because of their magnetic behavior, biocompatibility, and chemical stability.⁵ They have been explored for targeted drug delivery, magnetic resonance imaging, cancer therapy, and environmental remediation. Iron oxides are abundant in nature, inexpensive, and play important roles in geological and biological processes, and they have long been used in pigments, coatings, and catalysts.^{6,7} These attributes make iron oxide nanoparticles attractive for both research and practical use.⁸

However, conventional physical and chemical routes for synthesizing iron oxide nanoparticles often require toxic reagents and high energy input and generate hazardous waste, which raises environmental and safety concerns.^{9,10} This has led to growing interest in green synthesis approaches that are environmentally benign and safer for humans.^{11,12} In particular, biological materials such as plant extracts can serve as natural sources of reducing and capping agents, allowing nanoparticle formation without harmful chemicals.¹³ Plants are rich in phytochemicals such as flavonoids, polyphenols, terpenoids, and alkaloids, which act as efficient reducing agents.^{14,15} A variety of plants have been used for this purpose, including *Adhatoda vasica*,¹⁶ *Ananas comosus*,¹⁷ *Gardenia resinifera*,¹⁸ *Yucca gloriosa*,¹⁹ *Viola betonicifolia*.²⁰

Lantana camara L. (Verbenaceae) is a medicinal plant native to tropical and subtropical regions that has attracted attention because of its abundance of phytochemicals.^{21,22} In traditional medicine, various parts of the plant have been used to treat toothache, skin infections, respiratory complaints, and wounds.^{23–25} *L. camara* extracts have been reported to show antioxidant, antibacterial, antifungal, insecticidal, and nematicidal properties, and shoot extracts in particular have shown strong radical scavenging capacity. Given the phytochemical richness of *L. camara* and the demand for sustainable nanoparticle synthesis, the present study aimed to synthesize α -Fe₂O₃ nanoparticles (Fe₂O₃-NPs) using an aqueous leaf extract of *L. camara* followed by calcination. The nanoparticles were characterized by X-ray diffraction (XRD), UV–Vis spectroscopy, Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), transmission electron microscopy (TEM), and energy-dispersive X-ray spectroscopy (EDS), and their antibacterial, antifungal, and antioxidant (DPPH) activities were evaluated.

2. EXPERIMENTAL

2.1 Material and Methods

Lantana camara L. plant leaves (Figure 1a), in fresh form were collected from Milind College of Science, Chh. Sambhajinagar (Aurangabad), Maharashtra. First, they were washed properly; that is, they were washed several times with tap water followed by washing with distilled water to remove dust particles from their surface. Then, after proper washing, they were dried under the shade at room temperature for some days till they became dry. After drying, they were powdered mechanically using a grinder.

All reagents utilized in the experiment were analytical grades and purity level reached up to 98.99%. They were procured from Aldrich and Rankem. Iron sulfate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) was used as the salt precursor of iron oxides nano particles. The NaOH solution was used to alter the pH value of the solution. Distilled water was used in all the experiments.

2.2 Aqueous Leaf Extract Preparation Procedure

In order to obtain an aqueous leaf extract, 10 g of the powdered *L. camara leaf* was loaded into the Soxhlet extractor that was attached to a 250 mL round bottom flask. Water was the extracting agent used (100 mL), and extraction was performed at a constant temperature of 60°C until completion. The aqueous extract obtained was kept in a 250 mL beaker and refrigerated at 4°C for use in the synthesis of Fe_2O_3 -NPs (see Figure 1b).



Figure 1(a) *L. camara* Leaves and (b) *L. camara* Leaves extract

2.3 Synthesis of iron oxide nanoparticles through green route

The nanoparticles were synthesized through bio-synthesis method by dissolving iron sulfate $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ in distilled water to obtain 0.1 M in 100 mL concentration, using 250 mL round bottomed flask with magnetic stirrer. Leaf extract of *Lantana camara* (50 mL) was slowly added to the iron salt solution under constant stirring at $70\text{--}80^\circ\text{C}$. The pH of the reaction mixture was adjusted to basic conditions, and the mixture was continuously stirred for 90 min to complete the process. Centrifugation of the solution was done at 5000 rpm for 10 minutes and the particles were separated from the liquid solution. Supernatant was removed and the particles were cleaned thoroughly with ethanol and distilled water in order to eliminate any unreacted precursors and other impurities. Drying was done in a hot air oven

followed by calcination in muffle furnace at 700°C for two hours. The product obtained was allowed to cool down to room temperature and was then grounded into fine powder and kept in air tight vials for characterization and biological evaluation (Figure 2).

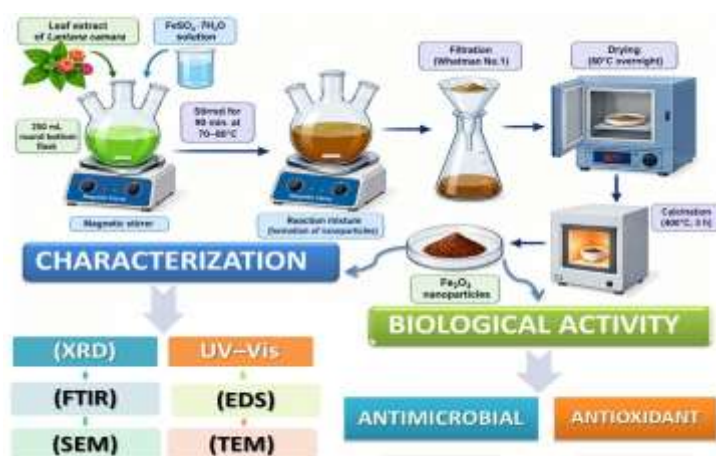


Figure 2. Preparation, characterisation and Biological analysis of Fe₂O₃ nanoparticles

2.4 Characterization of Fe₂O₃-NPs

The crystal structure of the green-synthesized Fe₂O₃ nanoparticles (Fe₂O₃-NPs) was analyzed by powder X-ray diffraction using a Rigaku MiniFlex 600 diffractometer operated at 40 kV and 50 mA with Cu-K α radiation ($\lambda = 1.5406 \text{ \AA}$) over a 2θ range of 10° – 80° . UV-visible spectra were recorded on a JASCO V-750 spectrophotometer fitted with a USE-753 accessory over 200–900 nm. Functional groups were identified using a Shimadzu FT-IR spectrometer in the range 400–4000 cm^{-1} . Surface morphology was examined by scanning electron microscopy (SEM, Zeiss Sigma/Gemini), elemental composition was determined by energy-dispersive X-ray spectroscopy (EDS), and particle size and lattice structure were studied by high-resolution transmission electron microscopy (TEM, JEOL JEM-2100 Plus, JEOL Asia Pte. Ltd.).

2.5 Antibacterial and Antifungal Activity

The antibacterial and antifungal activities of the prepared Fe₂O₃-NPs were evaluated by the agar disc diffusion method (6 mm discs) against four bacterial strains, namely *Staphylococcus aureus* (NCIM 2079), *Bacillus subtilis* (NCIM 2250), *Klebsiella pneumoniae* (NCIM 2719), and *Escherichia coli* (ATCC 25922), and one fungal strain, *Aspergillus niger* (ATCC 16404). Bacteria were cultured on Nutrient Agar (pH 7.2) and the fungus on Potato Dextrose Agar (pH 5.6 ± 0.2). A stock suspension of Fe₂O₃-NPs (1000 $\mu\text{g/mL}$) was prepared in DMSO, and each disc was loaded with 100 μg of the sample. The diameter of the zone of inhibition (ZOI) was measured in millimeters using a Vernier caliper. Streptomycin (10 $\mu\text{g/disc}$) was used as the positive control for bacteria, amphotericin B as the positive control for the fungus, and DMSO as the negative control.

2.6 Antioxidant Activity — DPPH Radical Scavenging Assay

Antioxidant potential of Fe₂O₃-NPs was assessed by using DPPH (2,2-diphenyl-1-picrylhydrazyl), which is a known method of measuring free-radical scavenging. This reagent

can accept electrons or hydrogen atoms from any antioxidant causing reduction in absorbance values at 517 nm. Such effect resembles the ability to scavenge radicals. In our study, the DPPH scavenging ability of Fe₂O₃-NPs was determined over a concentration range from 3.9 to 500 µg/mL. Ascorbic acid was used as a positive control for all tests performed. The calculation of percent inhibition in this test is represented by the formula below: % Scavenging activity = [(A₀ – A₁) / A₀] × 100, where A₀ is the absorbance value of DPPH and A₁ is the absorbance value of the tested substance.

3. RESULTS AND DISCUSSION

3.1 Visual Observation

The aqueous leaf extract of *Lantana camara* initially appeared yellow, which is attributed to the presence of phytochemicals such as flavonoids and polyphenols. Upon addition of the extract to the iron(II) sulfate solution, the colour gradually changed from yellow to brown. Such a colour change has been reported during the green synthesis of iron oxide nanoparticles and is considered an initial visual indication of nanoparticle formation, arising from the interaction of iron ions with phytochemicals and the subsequent formation of iron hydroxide/oxide species²⁶. The formation of the α-Fe₂O₃ phase was subsequently established by XRD and the other characterization techniques.

3.2 XRD Analysis of Fe₂O₃-NPs

Powder XRD was used to examine the crystal structure and phase of the synthesized Fe₂O₃-NPs. As shown in Figure 3, the XRD pattern displays sharp, well-resolved diffraction peaks at 2θ = 33.2° (104), 35.7° (110), 40.9° (113), 49.5° (024), 54.1° (116), 62.4° (214), 64.0° (300), and 75.4° (220). These reflections match the standard JCPDS card No. 33-0664 and confirm the formation of rhombohedral α-Fe₂O₃ (hematite).^{27, 28} No additional peaks corresponding to crystalline impurities or unreacted precursors were detected, and the most intense reflection was observed at (104). The crystallite size was estimated from the (104) peak using the Debye-Scherrer equation, $D = K\lambda/(\beta \cos \theta)$, where K = 0.89 is the shape factor, λ = 1.5406 Å is the wavelength of the Cu-Kα radiation, β is the full width at half maximum (FWHM) in radians, and θ is the Bragg angle. The calculated crystallite size was approximately 28 nm, indicating that the Fe₂O₃-NPs synthesized by this environmentally friendly approach are nanocrystalline with good crystallinity.^{29, 30}

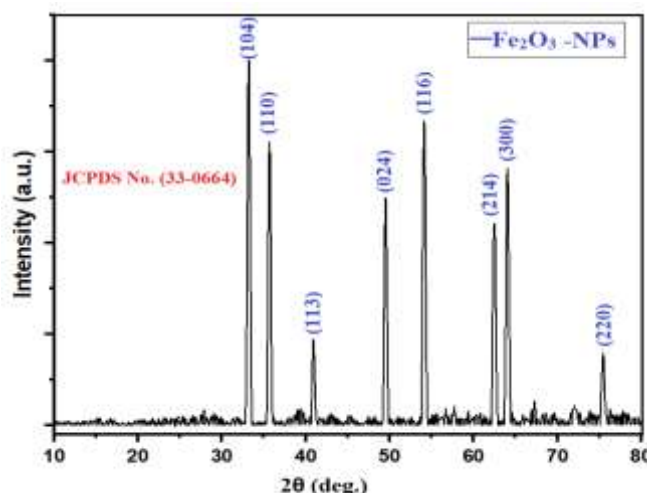


Figure 3 XRD spectrum of Fe₂O₃-NPs

3.3 UV-Visible Spectroscopic Analysis of Fe₂O₃-NPs

The optical properties of the biosynthesized iron oxide nanoparticles (Fe₂O₃ NPs) were investigated using UV-Visible spectrophotometry in Fig. 4. The wavelength range of 200-900 nm. The obtained absorption spectrum shows a sharp and intense absorption peak at 268 nm, along with a broad absorption band centered at around ~380 nm that extends into the visible region. The peak at 268 nm is attributed to the ligand-to-metal charge transfer (LMCT) transition from O²⁻ to Fe³⁺, while the broad band arises from the combined contribution of charge transfer and Fe³⁺ d-d transitions. These features are characteristic of iron oxide nanoparticles and confirm their successful formation, in good agreement with previously reported results^{31,32}. The exact position of the absorption peak depends on parameters such as particle size, shape, and the surrounding chemical environment, which may vary with the synthesis method and the plant extract used.

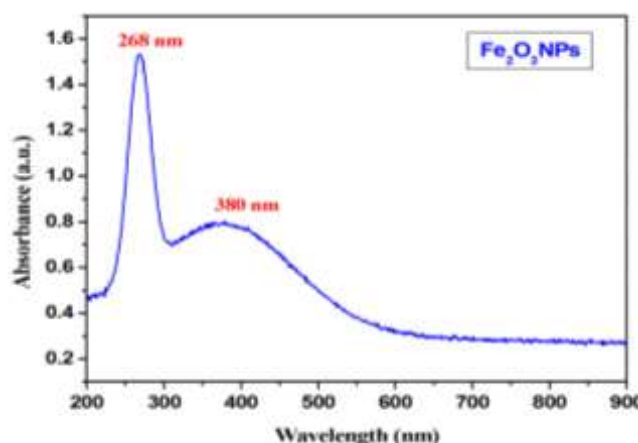


Figure 4 UV-vis spectrum of Fe₂O₃-NPs

3.4 FTIR Analysis of Fe₂O₃-NPs

The FTIR spectrum of the Fe₂O₃ nanoparticles synthesized using *Lantana camara* leaf extract, recorded in the range of 4000-400 cm⁻¹, is shown in Fig. 5. The broad band centered

at 3408 cm^{-1} is attributed to the O-H stretching vibration of surface hydroxyl groups and water molecules adsorbed on the nanoparticle surface. The band at 1632 cm^{-1} is assigned to the H-O-H bending vibration of adsorbed water, with a possible minor contribution from C=O/C=C vibrations of trace residual organic species originating from the leaf extract. The strong and sharp band at 576 cm^{-1} , together with a shoulder at lower wavenumber, is characteristic of the Fe-O stretching vibrations of the $\alpha\text{-Fe}_2\text{O}_3$ lattice^{33, 34}, indicating the formation of iron oxide. The absence of other intense bands suggests that most of the organic matter was removed during calcination at 700°C . These observations are consistent with the XRD results, which indicated the formation of the rhombohedral $\alpha\text{-Fe}_2\text{O}_3$ phase (JCPDS No. 33-0664).

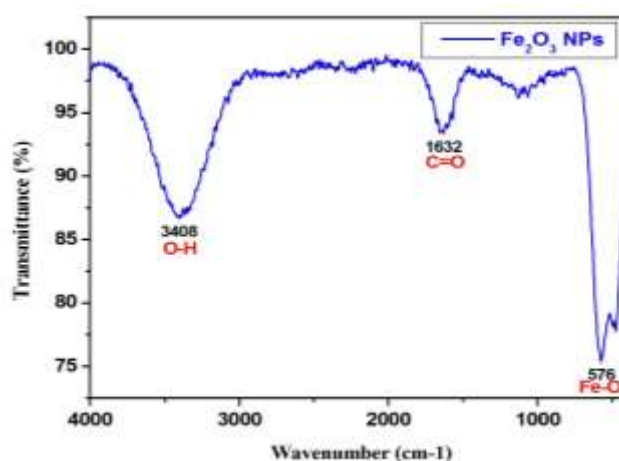
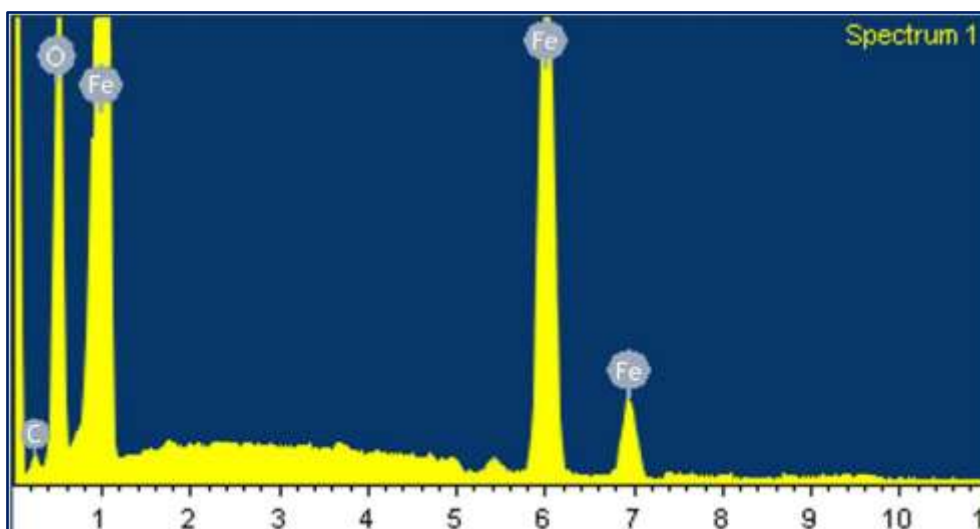


Figure 5 FTIR graph of Fe₂O₃ NP's

3.5 Elemental Analysis (EDS) of Fe₂O₃-NPs

EDS was employed to determine the elemental composition of the synthesized Fe₂O₃-NPs. The EDS spectrum in Fig. 6 shows signals of iron (Fe), oxygen (O), and carbon (C), with no other elemental peaks detected. The quantitative results in Table 1 show Fe as the dominant element at 70.67 wt% (38.76 at%), followed by O at 21.31 wt% (40.79 at%) and C at 8.02 wt% (20.45 at%). The strong Fe and O signals are consistent with the iron oxide phase identified by XRD. The carbon signal may be attributed to residual organic phytochemicals from the leaf extract acting as capping agents, with a possible contribution from the carbon tape or conductive coating used for sample mounting.³⁵ As EDS is semi-quantitative, particularly for light elements such as O and C, these values are indicative only and the measured Fe:O ratio need not match the ideal Fe₂O₃ stoichiometry exactly.

Figure 6 EDS of Fe₂O₃-NPsTable 1 Elemental composition of green-synthesized Fe₂O₃-NPs obtained from EDS analysis.

Element	Weight (%)	Atomic (%)
Fe K	70.67	38.76
O K	21.31	40.79
C K	8.02	20.45

3.6 SEM Analysis of Fe₂O₃-NPs

The surface morphology of the biosynthesized Fe₂O₃-NPs was examined by scanning electron microscopy (SEM). The micrograph (Fig. 7) reveals densely packed, irregular agglomerates composed of smooth, rounded, ovoid to short rod-like aggregates with a noticeably polydisperse size distribution. Adjacent aggregates appear partially fused through interparticle necking, giving rise to interconnected networks with voids and pores between them. The formation of such agglomerates can be attributed to the high surface energy of the nanoparticles and interparticle van der Waals forces, with possible magnetic interactions also contributing. The fused boundaries and rounded surfaces are consistent with particle coarsening and sintering during calcination at 700 °C.³⁶⁻³⁸ The features observed by SEM are therefore considered to be may consist of agglomerated and partially fused particles rather than individual particles. Owing to the extensive overlap, reliable size analysis could not be carried out from the SEM image; the primary particle size and shape were evaluated by TEM (Fig. 8), and the crystalline phase was confirmed by XRD.

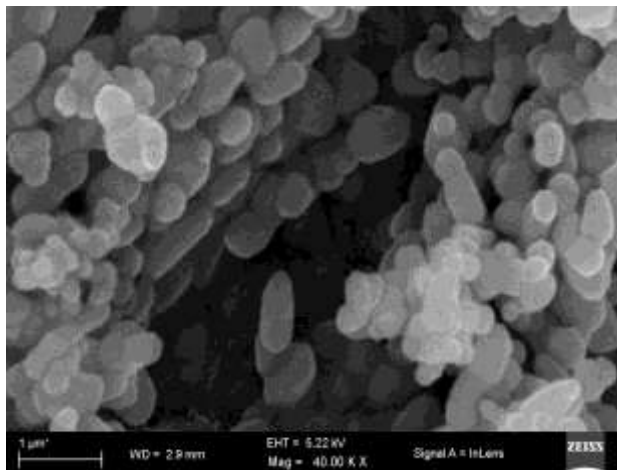


Figure 7 SEM image of Fe₂O₃-NPs

3.7 TEM Analysis of Fe₂O₃-NPs

TEM analysis was carried out to examine the morphology, size distribution, and microstructure of the biosynthesized Fe₂O₃-NPs. The TEM images (Figs. 8a and 8b) show irregular, rounded to quasi-spherical nanoparticles that are strongly agglomerated and partially overlapping, which can be attributed to their high surface energy and interparticle van der Waals interactions, with possible magnetic dipole interactions also contributing. Some neighbouring particles appear to be in contact or partially fused, in agreement with the necking observed by SEM. Variations in contrast within individual particles may indicate the presence of multiple crystalline domains, although thickness and diffraction contrast cannot be ruled out. A thin, low-contrast layer visible around some particles may correspond to organic residues from the plant extract. The particle size distribution obtained from ImageJ analysis (Fig. 8c) is broad, ranging from approximately 15 to 151 nm, with a mean particle size of 79.87 ± 43.06 nm ($n = 15$).³⁹⁻⁴² This is larger than the crystallite size of ~28 nm estimated from XRD, which suggests that the particles are polycrystalline, since XRD measures coherently diffracting domains rather than whole particles. The aggregates observed by SEM are considerably larger than the particles observed by TEM, which is attributed to agglomeration and sintering of particles during calcination at 700°C. Together, the SEM and TEM results provide complementary information on the morphology and size of the biosynthesized Fe₂O₃-NPs.

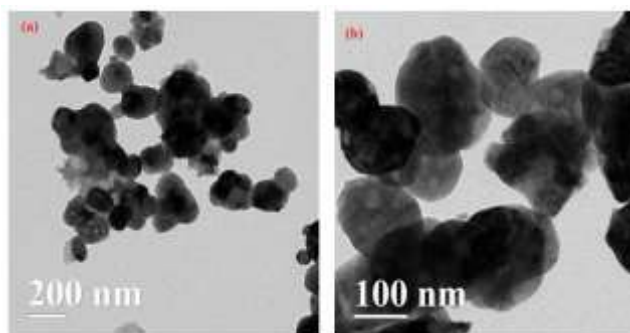


Figure 8 (a-b) TEM image of synthesized Fe₂O₃-NPs

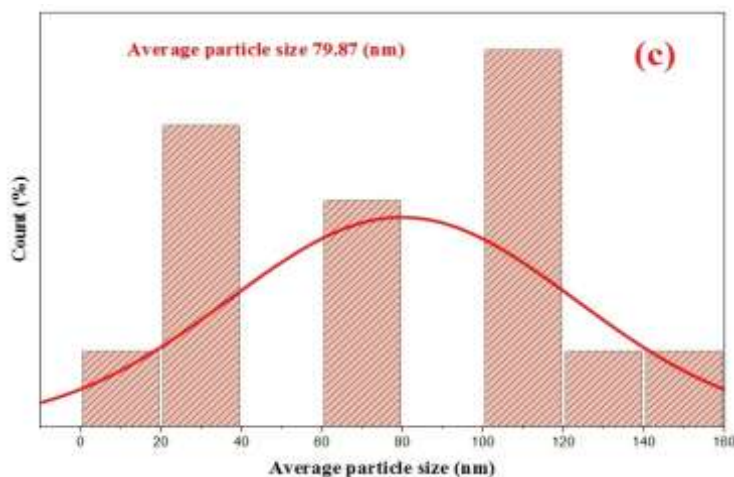


Figure 8 (c) Histogram Analysis Average Particle Size using OriginPro

3.8 Antibacterial and Antifungal Activity

The Fe_2O_3 -NPs (100 $\mu\text{g}/\text{disc}$) produced measurable inhibition zones against all four tested bacterial strains. The greatest zone of inhibition was observed for *K. pneumoniae* (NCIM 2719; 20 mm), followed by *S. aureus* (NCIM 2079; 17 mm), *B. subtilis* (NCIM 2250; 14 mm), and *E. coli* (ATCC 25922; 13 mm). These values were lower than those of the positive control streptomycin (21–29 mm; 10 $\mu\text{g}/\text{disc}$), although the *K. pneumoniae* zone (20 mm) was close to that of streptomycin (21 mm). As the NPs were tested at 100 $\mu\text{g}/\text{disc}$ and streptomycin at 10 $\mu\text{g}/\text{disc}$, the two are not directly comparable on a dose basis. The results indicate species-dependent inhibitory activity of the Fe_2O_3 -NPs. The antibacterial activity of Fe_2O_3 -NPs has been associated mainly with the generation of reactive oxygen species (ROS), which causes oxidative stress and disrupts the bacterial membrane, and nanoparticles have also been reported to penetrate directly into bacterial cells, enhancing their bactericidal effect.⁴³ In contrast, the Fe_2O_3 -NPs showed no inhibition against *A. niger* (ATCC 16404; ZOI = 0 mm), whereas amphotericin B produced a 14 mm zone. This may be related to the complex cell wall of *A. niger*, which contains polysaccharides such as chitin, glucans, and glycoproteins. The negative control (DMSO) showed no inhibition zone, indicating that the observed zones were due to the Fe_2O_3 -NPs (Table 2; Fig. 9).

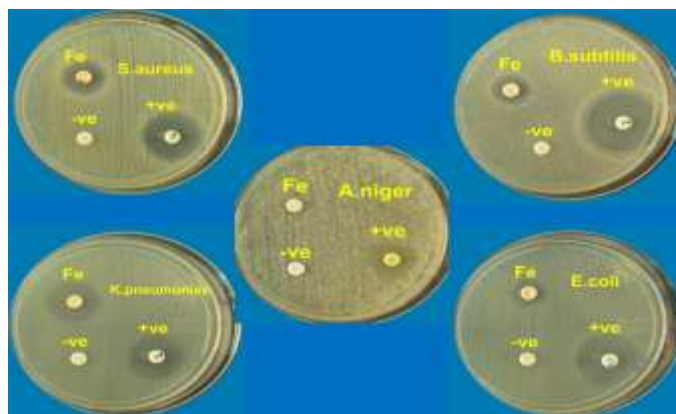


Figure 8 Antibacterial activity of green synthesized Fe_2O_3 -NPs against *S. aureus*, *B. subtilis*, *K. pneumoniae*, *E. coli* and *A. niger*

Table 1 Zone of inhibition (mm) of green-synthesized Fe₂O₃-NPs against bacterial and fungal strains.

Sr. No.	Sample ID	S. aureus NCIM 2079	B. subtilis NCIM 2250	K. pneumoniae NCIM 2719	E. coli ATCC 25922	A. niger ATCC 16404
1.	Fe ₂ O ₃ -NPs	17	14	20	13	0
2.	Streptomycin (+ve control)	25	29	21	25	-
3.	Amphotericin B (+ve control, fungus)	-	-	-	-	14
4.	DMSO (-ve control)	0	0	0	0	0

3.9 Antioxidant Activity — DPPH Radical Scavenging

The radical scavenging activity of the green-synthesized Fe₂O₃-NPs was evaluated by the DPPH assay over a concentration range of 3.9–500 µg/mL, using ascorbic acid as the standard (Table 3; Fig. 10). All measurements were performed in triplicate for the Fe₂O₃-NPs and are reported as mean ± SD. The scavenging activity generally increased with concentration, from 89.16 ± 0.54% at 3.9 µg/mL to 99.21 ± 0.02% at 500 µg/mL. At the lower concentrations (3.9–15.6 µg/mL), the Fe₂O₃-NPs showed higher scavenging than ascorbic acid (85.45–88.52%), while the two were similar at 31.3 and 62.5 µg/mL, slightly lower for the NPs at 125 µg/mL (94.00 ± 1.27% vs. 95.17%), and slightly higher for the NPs at 250 and 500 µg/mL (98.20 ± 0.71% and 99.21 ± 0.02% vs. 97.12% and 98.65%). Because all tested concentrations gave more than 85% inhibition, IC₅₀ values could not be determined. The antioxidant activity of the Fe₂O₃-NPs may be attributed to the redox-active iron oxide surface and to residual phytochemicals from the plant extract acting as capping agents, which can donate electrons or hydrogen atoms to the DPPH radical.^{44–47} These results suggest that the biosynthesized Fe₂O₃-NPs have free radical scavenging potential comparable to ascorbic acid under the in vitro conditions used.

Table 3. DPPH radical scavenging activity (%) of green-synthesized Fe₂O₃-NPs (mean ± SD, n = 3) and ascorbic acid (mean of two replicates) at different concentrations.

Concentration (µg/mL)	Fe ₂ O ₃ -NPs	Ascorbic acid (mean, n = 2)
3.9	89.16 ± 0.54	85.45
7.8	91.03 ± 0.41	86.13
15.6	92.11 ± 0.55	88.52
31.3	93.11 ± 0.24	92.66
62.5	94.54 ± 0.60	94.55
125	94.00 ± 1.27	95.17
250	98.20 ± 0.71	97.12
500	99.21 ± 0.02	98.65

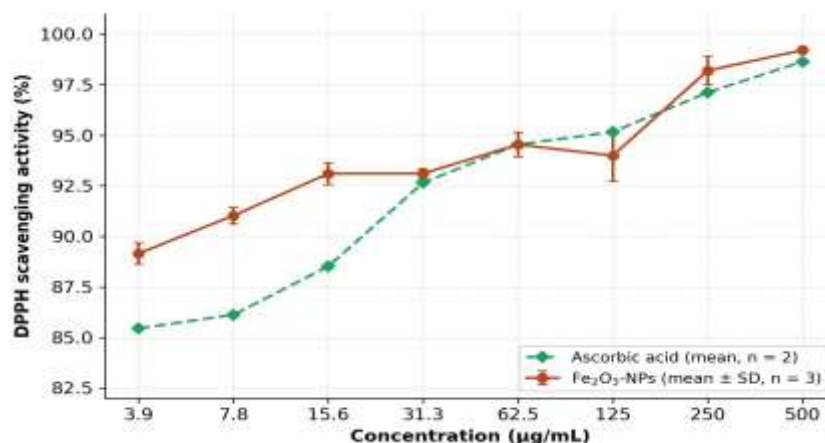


Figure 10. DPPH radical scavenging activity of green-synthesized Fe₂O₃-NPs (mean ± SD, n = 3) and ascorbic acid (mean of two replicates) at different concentrations.

4. CONCLUSION

Fe₂O₃ nanoparticles were successfully synthesized using *Lantana camara* leaf extract followed by calcination at 700 °C. XRD confirmed the crystalline rhombohedral α-Fe₂O₃ (hematite) phase with an average crystallite size of approximately 28 nm, while TEM revealed irregular, quasi-spherical, agglomerated particles with a broad size distribution and a mean particle size of 79.87 ± 43.06 nm. The larger TEM size relative to the XRD crystallite size indicates that the particles are polycrystalline and partly agglomerated. EDS confirmed the presence of Fe and O, along with carbon that may originate from residual phytochemicals of the plant extract.

The Fe₂O₃-NPs showed species-dependent antibacterial activity against Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram-negative (*Klebsiella pneumoniae*, *Escherichia coli*) bacteria, with inhibition zones of 13–20 mm and the largest zone against *K. pneumoniae*, although the zones were generally smaller than those of streptomycin. No inhibition was observed against *Aspergillus niger*. In the DPPH assay, scavenging activity increased with concentration and reached 99.21 ± 0.02% at 500 µg/mL, comparable to that of ascorbic acid (98.65%) at the same concentration under the in vitro conditions used.

Overall, these findings indicate that *L. camara*-mediated Fe₂O₃-NPs have potential as antibacterial and antioxidant agents. Further studies, including cytotoxicity, mechanism-based, and in vivo evaluations, are needed to assess their suitability for biomedical use.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

Khan Kadir Rafik  <http://orcid.org/0009-0001-9438-7810>

Momin Nishat Parveen  <http://orcid.org/0009-0005-0408-0815>

Neha Samreen Gulam Dastageer  <https://orcid.org/0009-0009-9647-9768>

Samadhan Sakharam More  <https://orcid.org/0009-0000-8783-526X>

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