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Research Article

Keywords: Nanoparticles, Iron-oxide, *Gomphostemma mastersii*, Characterization, Antimicrobial, Antioxidant

Posted Date: September 23rd, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10535727/v1>

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Additional Declarations: No competing interests reported.

Green synthesis of iron oxide nanoparticles using *Gomphostemma mastersii* leaf extract and evaluation of structural antioxidant and antimicrobial properties

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Abstract

The purpose of this work is to demonstrate the eco-friendly production of iron oxide nanoparticles (FeONPs) by using *Gomphostemma mastersii* leaf extract as a capping and stabilizing agent for the first time. This study uses a microwave-assisted method and the synthesized FeONPs were characterized using different techniques, and were subsequently assessed for their antimicrobial, antifungal, and antioxidant properties. The synthesized FeONPs exhibited substantial free radical scavenging activity, with an IC₅₀ value of 174.52±2.38 µg/ml and a FRAP value of 346.86±6.57 µM AAE/g. Additionally, the FeONPs displayed antimicrobial activity against bacterial (*E. coli* and *B. subtilis*) and fungal strain (*A. niger* and *S. commune*). The highest and lowest antibacterial activity was observed against *B. cereus* (14.84 mm) and *Salmonella* spp. (12.73 mm), respectively. The current work therefore verified the antibacterial, antimicrobial, and antioxidant qualities of the green nanoparticles that were synthesized from *G. mastersii*. Green synthesis of nanoparticles using plant extracts has emerged as an eco-friendly and cost-effective alternative to conventional methods. Plant phytochemicals act as natural reducing and stabilizing agents, enhancing the biological functionality of nanoparticles. Iron oxide nanoparticles are widely studied for their biomedical and antimicrobial applications; however, the use of novel plant sources and rapid synthesis techniques remains limited. The aim of this study is to synthesize iron oxide nanoparticles using *Gomphostemma mastersii* leaf extract through a microwave-assisted green method, characterize their physicochemical properties, and evaluate their antioxidant and antimicrobial activities for potential biomedical applications.

Keywords: Nanoparticles; Iron-oxide; *Gomphostemma mastersii*; Characterization; Antimicrobial; Antioxidant.

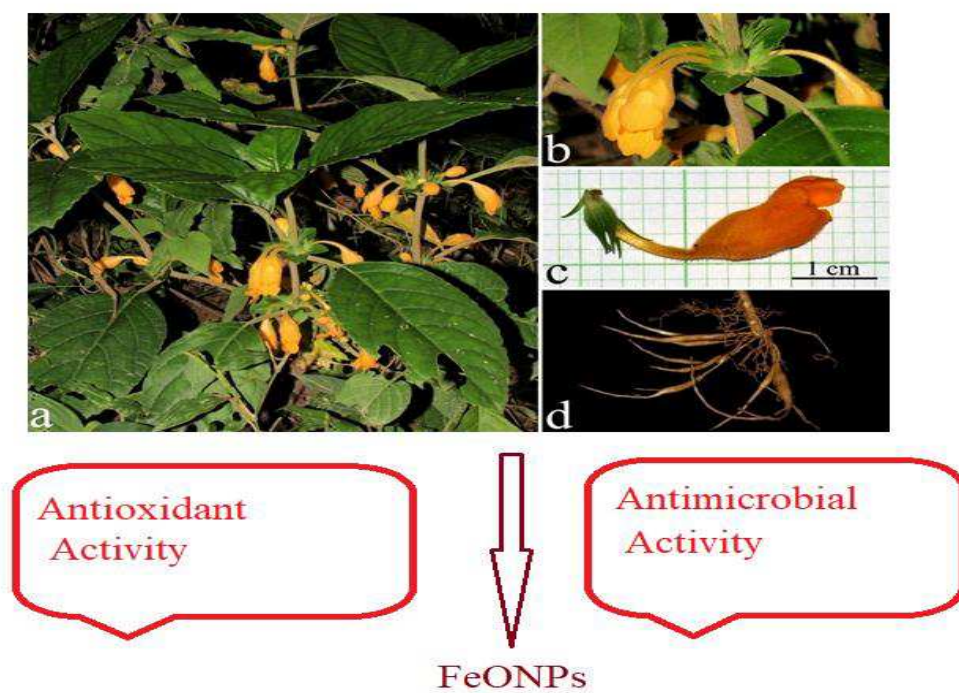


Figure 1: *Gomphostemma mastersii*. a. Habitat b. Inflorescence c. Flower d. Tuber like root.

1.Introduction:

The green synthesis of nanoparticles represents a novel approach that employs natural and environmentally sustainable resources, including plant extracts, microorganisms, or organic compounds, to reduce and transform metal ions into nanoscale particles [1,2]. The reduction in material size leads to diverse physicochemical properties and a wide array of potential applications, including nanoscience and various medical fields [3-4]. Meanwhile, Phyto-nanotechnology has significantly contributed to enhance the prospects for the eco-friendly production (green synthesis) of metallic nanoparticles (MNPs).

Nanoscale iron oxide materials are susceptible to agglomeration and oxidation due to their elevated surface energy and significant surface metal chemical activity; consequently, the magnetic characteristics and dispersibility of the superparamagnetic nanoparticles are lessened [5]. Iron oxide nanoparticles (FeONPs) exhibit remarkable characteristics, including substantial catalytic properties, higher adsorption capacity, significant reactivity, enhanced mechanical properties, biocompatibility, and both chemical and thermal stability [6]. The applications of these particles are varied and extensive, encompassing areas such as drug delivery, magnetic targeting, stem cell sorting and manipulation, gene therapy, food preservation, bio-separation, etc., [7]. Plant-mediated synthesis has garnered major academic interest within the realm of established biological systems due to its expeditious, simple, and easily scalable approaches for commercial production [8].

In this work, an aqueous leaf extract from *Gomphostemma mastersii* Benth. ex Hook.f. (Lamiaceae) was used to assess the formation of iron oxide nanoparticles (FeONPs). The plant material was collected from Dima Hasao (North Cachar Hills), Assam, India. The species was taxonomically identified by a qualified taxonomist Dr Selim Mehmud on chargeability. We regret that a voucher specimen number was not obtained at the time of plant collection. The plant material used in this study was authenticated as *Gomphostemma mastersii* based on its morphological characteristics and taxonomic identification. Since no voucher specimen was deposited during the study, a voucher specimen number is not available yet. It will take some time to prepare the voucher specimen and then will be deposited at the Botanical Herbarium, Gauhati University, Assam, India, for future reference.

The genus *Gomphostemma* comprises approximately 31 species, predominantly distributed in evergreen forests across regions such as Brunei, Cambodia, China, India, Indonesia, Laos, Malaysia, Myanmar, Philippines, Thailand, and Vietnam. It is well known for its traditional medicinal applications in treating various ailments, including tuberculosis, diarrhea, arthritis, inflammation, and malaria [9]. The genus is rich in diverse bioactive compounds such as tannins, alkaloids, terpenoids, phenols, flavonoids, and saponins [10]. However, there is a paucity of scientific reports on the synthesis of FeONPs using *G. mastersii* and their pharmacological potential. Therefore, the present study aims to address this research gap by synthesizing FeONPs from *G. mastersii* and evaluating their potential applications. The research also evaluates the possibility of using the nanoparticles made in this manner

in the medical and health fields because they don't contain any dangerous chemicals. However, further thorough research using in vivo techniques is strongly advised to confirm the potential of *G. mastersii*-mediated FeONPs for the creation of new antioxidants, antibiotics, and anticancer drugs.

2. Experimental:

2.1 Materials

The plant sample (leaf of *G. mastersii*) was collected from Rani, Kamrup district, Assam, India (25° 47' 25.8" N, 91° 15' 45.36" E). The species was identified following the recent revision of the Genus *Gomphostemma* carried out by Bongcheewin et al. 2022 [11]. Self-authentication of the plant species was done by comparing the prepared herbarium of the species (*G. mastersii*) with the available herbarium specimen of the identical species housed at the Gauhati University Botanical Herbarium. All the chemicals and solvents were procured from Merck, India.

2.2 Extract preparation

The fresh leaves of *G. mastersii* were collected and thoroughly rinsed with deionised water to eliminate dust particles, then shade-dried. The dried leaves were finely grounded. 20 gm of the finely ground leaf powder was boiled in 1000mL of Milli-Q water at 70°C for 30 min. The extract was filtered and stored at 4 °C for subsequent analysis.

2.3 Synthesis of FeONPs

Briefly, 30 mL of *G. mastersii* leaf extract was gradually added to 30 mL of 0.1M FeCl₃.6H₂O solution in a 1:1 ratio at ambient temperature. 1M NH₄OH was subsequently added to the solution. [12]. The solution was stirred for 45 min. Appearance of a dense black solution implied the formation of iron oxide nanoparticles. The reaction mixture was then kept in a microwave reactor at 60 °C for 10 min. The precipitate was collected, rinsed with CH₃CH₂OH and water, and subsequently dried in an oven at 85 °C for 3 h. The powdered sample was stored in air tight containers for further analysis.

2.4. Characterization of FeONPs

The synthesis of FeONPs by the modified microwave technique was performed using the MONOWAVE 400 microwave synthesis reactor (ANTON PAAR, Austria). The absorbance spectrum was obtained using a UV-visible spectrophotometer (UV 1900 SHIMADZU, Kyoto, Japan). The measurement and comparison of the structure-property relationship was done using X-ray diffraction (XRD) spectroscopy (Rigaku Technologies, Japan). The transmission electron microscope (TEM, JEM-2100 JEOL, Japan) was used to ascertain the particle size, morphology, and distribution. A two-dimensional (2D) electron

diffraction patterns was obtained using single area electron diffraction (SAED) using JEM-2100 (JEOL, Japan). The microstructure image of the FeONPs was captured using FESEM (GEMINI 300, USA). The functional groups present in the synthesized FeONPs was identified by FTIR (IMPACT 410, NICOLET, USA) analysis. Thermal properties were determined using thermogravimetric analyzer (TGA-50 & DSC-60, SHIMADZU, Japan). Photoluminescence (PL) spectroscopy was performed employing the LS55 (PERKIN ELMER, USA) to investigate the dissociation of photogenerated charge carriers. The Zeta potential of the synthesized FeONPs was characterized using a Dynamic Light Scattering (DS) Litesizer™ 500 (ANTON PAAR, Austria).

2.5 Determination of Antioxidant capacity

2.5.1 DPPH Assay

1, 1-diphenyl-2-picryl-hydrazil (DPPH) free radical scavenging assay of the synthesized FeONPs was performed following the method mentioned in a previous study [13] with slight modifications. Briefly, 1 ml of the sample at varying concentrations (20, 50, 100, 150, and 200 µg/ml) was mixed with 3 ml of a 0.2 mM DPPH solution, vortexed, and kept for 30 min at 37 °C in a dark chamber. The control was formulated by mixing 1 ml of 95% CH₃OH and 3 ml of DPPH solution. The solution's absorbance was measured at 517 nm with a UV-VIS spectrophotometer. The percentage of radical scavenging activity was determined using the formula provided in equation (1), and the IC₅₀ value was calculated. The presented results are the mean values of triplicates

$$\%RSA = \left(\frac{C\alpha - S\alpha}{C\alpha} \right) 100 \quad (1)$$

Whereas % RSA is the percentage of the free radical scavenging activity, Cα is the control's absorbance and Sα is the sample's absorbance.

2.5.2. FRAP assay

Briefly, 0.1 ml of sample (FeONPs dissolved in 95% CH₃OH) was added to 3 ml of FRAP reagent and incubated for 20 min in dark chamber at ambient temperature [14]. The absorbances were taken using a UV-VIS spectrophotometer at 593 nm. Ascorbic acid at varying concentrations (0.2-1.0 mM) was employed for constructing a standard calibration curve. The FRAP value of the FeONPs was determined using the formula provided in equation (2), with results expressed in µM ascorbic acid equivalent (AAE)/g of powdered nanoparticles.

$$\text{FRAP value } (\mu\text{M AAE/g}) = c \times V \times \frac{t}{m} \quad (2)$$

where, c is the ascorbic acid equivalent in µM/ml, V is the sample volume, t is the dilution factor, m is the weight of powdered nanoparticles in gram (g). The reported results are the mean values of triplicates.

2.6 Determination of antimicrobial activity

2.6.1 Agar well diffusion method

The antimicrobial activity of the synthesized FeONPs was examined by the agar well diffusion method [15] against gram positive (*Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*), gram negative (*Escherichia coli*, *Salmonella enterica*, *Salmonella spp.* and *Yersinia pestis*) and a fungus (*Candida albicans*). The synthesized FeONPs were dissolved in Dimethyl sulfoxide (DMSO) at a concentration of 50 mg/ml. A bacterial inoculum with a concentration of 1×10^8 cells/ml was obtained from a 24 h culture. 100 μ l of the prepared inoculum was evenly spread on solidified Mueller Hinton Agar, and wells of 8 mm were bored using a sterile cork borer. 100 μ l of the sample (FeONPs in DMSO) and DMSO as negative control were added to the wells. A ciprofloxacin disc and a fluconazole disc served as positive controls for bacteria and fungi, respectively, and were placed on the test plate using sterile forceps. The test plates were incubated for 24 h (bacteria) and 48 h (fungi) at 37 °C. The diameter of the inhibition zone (mm) was determined at the end of the incubation period. The tests were performed in triplicates.

2.6.2 Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC)

The minimum inhibitory concentration (MIC) was determined by the microbroth dilution method as described in a previous study [16] with slight modifications. An inoculum was prepared by transferring bacterial colonies to sterile distilled water from a 24 h culture and subsequently diluted to achieve a turbidity equivalent to the 0.5 McFarland standard, resulting in a working microbial suspension of $1-5 \times 10^5$ CFU/ml. The working FeONPs solution was prepared in 100% DMSO at a concentration of 5000 μ g/ml. 100 μ l of Mueller Hinton broth supplemented with 2% glucose and 0.5% phenol red was placed in all the 96 wells of a sterile microplate. 100 μ l of the working sample was placed on the wells of the first row of the microplate. By using multichannel micropipette identical serial dilution (1:2) was performed till the wells of 10th column. 50 μ l of the microbial suspension was placed in all the wells till the 11th column. The microplate was sealed and incubated for 18 h at 37 °C. The absence of microbial growth was inferred from the presence of a red colour, while the presence of microbial growth was inferred from the presence of a distinct yellow colour. The MIC was defined as the lowest concentration of the sample that successfully inhibits visible growth or causes a color change from red to yellow.

To validate MICs and determine the MBC, 50 μ l of each culture medium with no observable growth was taken from each well and subsequently inoculated into MHA or Sabouraud Dextrose agar plates. The number of surviving organisms was assessed after a period of 16-20 h of aerobic incubation at 37 °C. Each experiment was performed in triplicates.

3. RESULTS AND DISCUSSION

3.1 UV-Vis. analysis of FeONPs

The absorbance peak of iron oxide nanoparticles produced using *G. mastersii* leaf extract ranged from 230-250 nm Fig 2. The maximum absorbance ($\lambda_{\max}$) peak was recorded at 240 nm, signifying the synthesis of FeONPs [17]. This confirms the presence of iron oxide nanoparticles. Absorption was not detected beyond 500 nm, leading to a thorough reduction and the formation of FeONPs [18]. The Surface plasmon resonance (SPR) band of FeONPs was observed at 240 nm, confirming the formation of iron oxide NPs and is consistent with earlier reports.

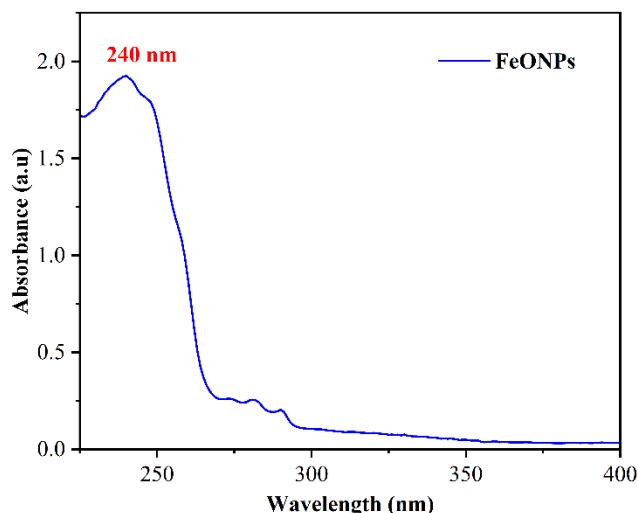


Figure 2: UV-Vis analysis of FeONPs

3.2 XRD

X-ray diffraction graph of synthesized FeONPs is shown in Fig. 3. FeONPs exhibited distinct diffraction peaks at 21.12° , 23.70° , 31.74° , 31.72° , 35.22° , 49.22° , and 62.64° 2θ angles, corresponding to inter-planar spacings associated with the (001), (012), (100), (101), (104), (211), and (300) crystallographic planes, respectively. Moreover, all these diffraction peaks are consistent with the previous findings, confirming the cubic crystallinity structures of the synthesized FeONPs [19]. The average crystallite size of the nanoparticles was calculated at 14.35 nm using Debye–Scherrer equation. Besides this, other characteristic peaks may be due to the crystalline nature of the bio-organic phase on the surface of FeONPs [20]. The strong and sharp peaks indicated that the FeONPs formed by the reduction of iron ions using *G. mastersii* leaf extract were highly crystalline.

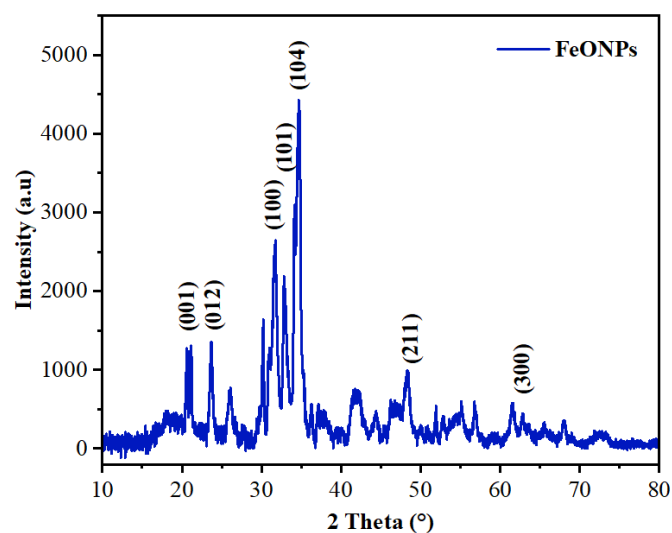


Figure 3: X-ray diffraction graph of FeONPs.

3.3 TEM

The internal morphology, shape, and size of the synthesised FeONPs were examined utilising TEM, as illustrated in Fig. 4. The micrographs validate the crystalline structure of the synthesized FeONPs. The synthesis approach successfully produced identical crystalline structures at the nanoscale. TEM micrograph of FeONPs revealed the existence of extremely small nanoclusters with uniform size distribution. The average particle size was 43 nm ascertained using high-resolution TEM analysis. The SAED pattern (Fig. 4) established the semi-crystalline and smoothed nature of the leaf extract synthesized FeONPs.

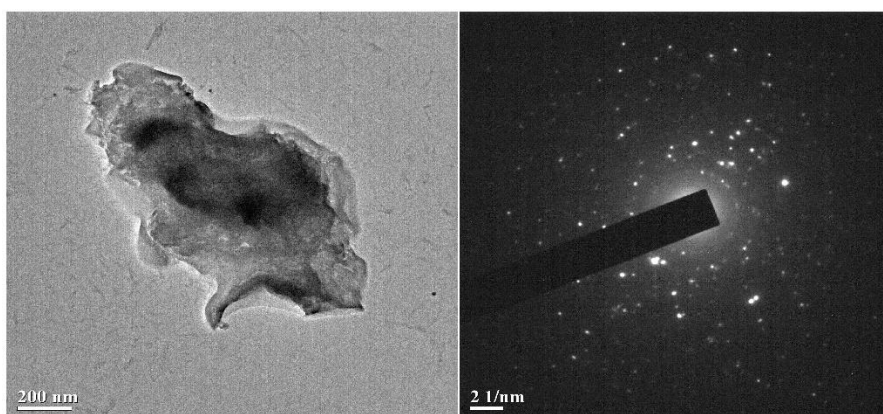


Figure 4: TEM micrograph and SAED pattern of FeONPs

3.4 FESEM

The FESEM image determined the external morphology of the synthesized FeONPs as shown in Fig. 5. The FeONPs were sphere-shaped, with a diameter ranging from 100-105 nm. The NPs nearby

interaction with each other is accredited to the magnetic properties of iron and is in agreement with SEM images.

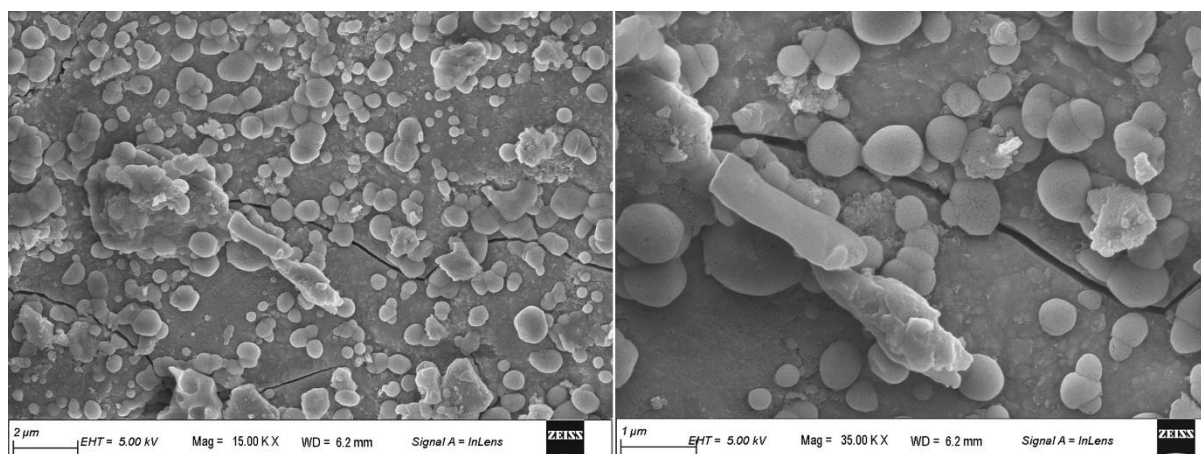


Figure 5. FESEM micrographs of synthesized FeONPs

3.5 EDX

EDX analysis was used to find the elemental composition of the synthesized FeONPs. The spectrum showed the presence of strong peaks of iron and oxygen, confirming the formation of Fe_2O_3 NPs (fig 6). The synthesized iron oxide nanoparticles have an elemental percentage of 77.4% iron and 22.6% oxygen.

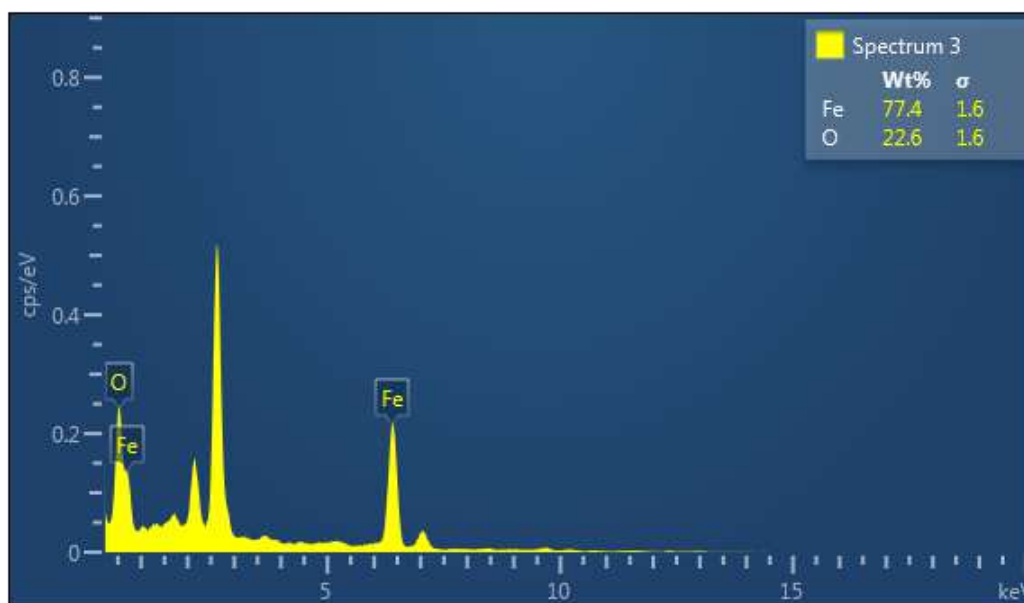


Figure 6: EDX analysis spectra for FeONPs.

3.6 FTIR

The functional groups of the synthesized plant extract-based FeONPs were studied using FTIR analyses as shown in Fig. 7. The broad absorption band in the region $3300\text{--}3100\text{ cm}^{-1}$ was attributed to O-H stretching. The absorption band observed at $620\text{--}600\text{ cm}^{-1}$ corresponds to the Fe-O stretching of iron oxide. This also confirms the formation of nanoparticles. In addition, the two peaks seen at 1584 and 1114 cm^{-1} are attributed to C = O and C-O stretching vibrations respectively. The band that corresponds to the OH bond stretching, signifies the aqueous phase, with a rise in the absorption band, representing reduction of ferric chloride [21]. The FTIR spectrum in the region of $3450\text{--}3200\text{ cm}^{-1}$ typically exhibits a broad absorption band associated with O-H stretching vibrations.

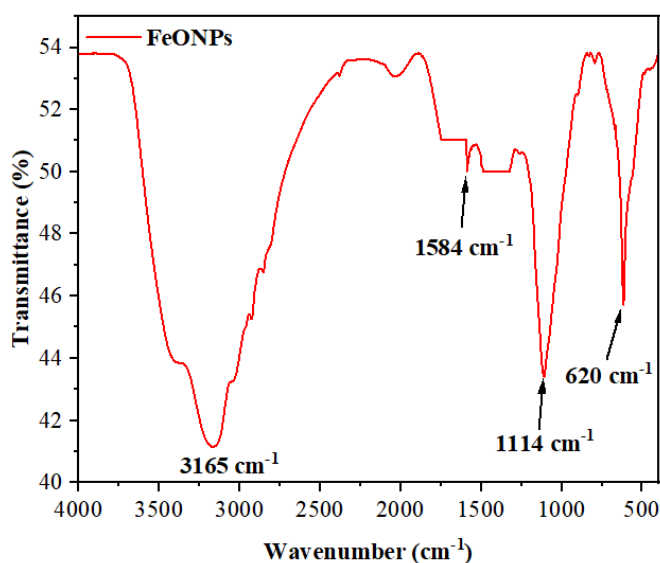


Fig. 7. FTIR spectra of FeONPs

3.6 TGA

The TGA curve of the synthesized iron oxide nanoparticle is shown in Fig. 8. TGA measurements were carried out at a heating rate of $10^{\circ}\text{C}/\text{min}$ within the temperature range of 20.71 to 600°C . An initial weight loss of 2.73% around 165°C occurred, followed by 1.72 and 1.05% at 410 and 540°C , respectively. This weight loss is due to the process of desorption and subsequent evaporation of the used plant extract. A slight weight loss of 1.05% was observed at $520\text{--}540^{\circ}\text{C}$ due to the conversion step of the iron oxide NPs [22]. Hence, no more changes were detected after 540°C , signifying that the synthesized FeONPs were thermally stable.

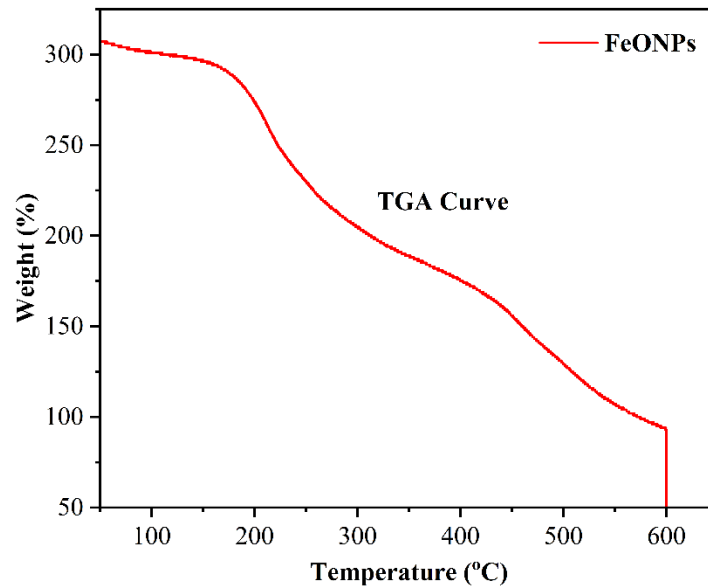


Figure. 8: TGA graph of FeONPs

3.7 PL

The photoluminescence absorption spectra, along with the measured band gap of the produced plant extract FeONPs, is presented in Fig. 9. PL spectra of the nanoparticles are self-reliant on the excitation density. A clear variation in the spectral shape was observed with the maximum emission for FeONPs recorded at 435 nm. Birusanti et al. reported a prominent band of luminescence at 450nm, which is alike to the result shown by our PL studies[23]. Similarly, Lassoued et al. reported that iron oxide nanoparticles showed a strong emission band at 450nm due to the electron transition within the nanoparticles [24]. In the current study, the PL analysis spectra of FeONPs synthesized using *G. mastersii* leaf extract showed a sharp emission at 435nm, due to electron/hole recombination of nanoparticles.

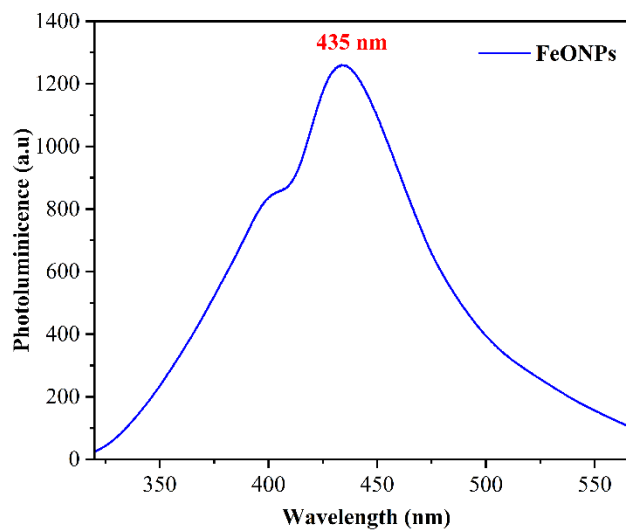


Figure. 9.PL spectra of the FeONPs.

3.8 Zeta Potential

Zeta potential analysis is used to study the stability of FeONP colloidal dispersions. The mean zeta potential of the synthesized FeONPs using *G. mastersii* leaf extract was -33.8mV and the distribution peak value was -32.5mV. Nanoparticles with zeta potential values greater or less than ± 25 mV typically have high degrees of stability [25]. Dispersions characterised by a low zeta potential value will progressively coalesce as a result of Van der Waals inter-particle interactions. The Zeta potential measurement of the synthesized FeONPs displays the surface charge magnitude of the colloidal particles. The magnitude of the surface charge step was determined to be higher, which specified good stability.

3.9 Antioxidant study

Free radicals produced within living organisms lead to membrane damage via lipid oxidation, resulting in a variety of illnesses [26]. Metal nanoparticles derived from plants possess the capability to eradicate these free radicals. Nevertheless, free radicals encompass a variety of chemical entities, necessitating different tests to demonstrate their scavenging capabilities through diverse mechanisms. In this context, the DPPH and FRAP assay methods are frequently used. The DPPH radical is a persistent free radical. The DPPH scavenging assay evaluates the capacity of antioxidants to counteract free radicals by using these radicals. Whereas, FRAP assay is based on the capability to reduce Fe^{3+} to Fe^{2+} by the antioxidant compound. The DPPH and FRAP assay revealed the antioxidant activity of FeONPs with an IC_{50} value of $174.52 \pm 2.38 \mu\text{g/ml}$ and $346.86 \pm 6.57 \mu\text{M}$ AAE/g of FRAP value respectively (Table 1). The synthesised FeONPs were shown to effectively scavenge 50% of the DPPH free radicals at a concentration of $423.14 \mu\text{g/mL}$. The synthesized FeONPs exhibit antioxidant activity that closely corresponds with the previously reported activities.

Table. 1.Antioxidant activity of the FeONPs

	DPPH	FRAP value
	($\text{IC}_{50}\mu\text{g/ml}$)	(μM AAE/g)
FeONPs	174.52 ± 2.38	346.86 ± 6.57

3.10 Antimicrobial study

The antimicrobial activity of FeONPs was evaluated against pathogenic bacteria and fungi applying the agar well diffusion method. The antimicrobial efficacy of FeONPs was assessed by measuring the

diameter of inhibition zone (mm). The details of the antimicrobial activity of FeONPs against different bacteria and a fungus are displayed in Table 2. The growth inhibition zone of FeONPs against the tested bacteria was observed in the range of 12-15mm. The highest and lowest antibacterial activity was observed against *B. cereus* (14.84 mm) and *Salmonella spp.* (12.73mm) respectively. FeONPs also showed antifungal activity against *C. albicans* with an inhibition zone of 15.25mm (Table. 3). The reduced activity against *Salmonella spp.* and *S. enterica* could be related to the specificity of some phytochemicals or the strong tolerance of gram-negative bacteria to an array of antibiotics [27]. For example, coumarin, a polyphenolic compound, exhibits efficacy against gram-positive bacteria, also displays no activity against gram-negative bacteria [28-30]. The bacteria and fungus against which the FeONPs exhibited an inhibition zone of more than 11mm were further analyzed to determine minimum inhibition concentration and bactericidal potency. The MIC values of the FeONPs against tested bacteria and fungus were in the range of 625-2500 µg/ml. The lowest MIC value of 625µg/ml was observed against *S. aureus*, *E. coli*, *B. cereus* and *C. albicans*, whereas against *S. enterica* and *Salmonella spp.* the MIC values were high (> 1250µg/ml) (Table. 3). Further, the bactericidal potency of FeONPs was tested against bacteria showing an MIC value of 625µg/ml. The bactericidal activity was observed only against *S. aureus* and *B. cereus* with MBC values of 1250µg/ml. The effectiveness of the synthesised FeONPs as an antimicrobial agent can be ascribed to the activity of the loaded antimicrobial agents (bioactive compounds) derived from the *G. mastersii* leaf extract [31-32].



Figure 10 Antimicrobial activity of the FeONPs against gram-positive bacteria, gram-negative bacteria and fungus

Table. 2. Antimicrobial activity of the FeONPs

Diameter of inhibition zone (mm)	MIC (µg/ml)
----------------------------------	-------------

	FeONPs	Positive control	
<i>Staphylococcus aureus</i>	14.67±0.46	27.23±2.67	625
<i>Escherichia coli</i>	14.08±0.32	29.36±0.72	625
<i>Bacillus cereus</i>	14.84±0.85	28.76±0.51	625
<i>Bacillus subtilis</i>	13.72±0.29	28.76±0.51	1250
<i>Salmonella enterica</i>	12.11±0.54	31.23±3.09	> 1250
<i>Salmonella spp.</i>	12.03±0.44	32.34±4.37	> 1250
<i>Yersinia pestis</i>	14.09±0.76	34.18±1.02	1250
<i>Candida albicans</i>	15.25±0.89	37.85±2.30	625

4. Conclusion

In this study, the successful green synthesis of iron oxide nanoparticles (FeONPs) using the aqueous leaf extract of *Grewia mastersii* via a microwave-assisted approach underscores a sustainable and efficient alternative to conventional methods. The process not only proves to be rapid, cost-effective, and environmentally friendly, but also results in nanoparticles exhibiting desirable physicochemical properties, as evidenced by comprehensive characterization techniques including XRD, TEM, FESEM, FTIR, PL, and zeta potential analysis. The synthesized FeONPs demonstrated significant antioxidant and antimicrobial activities, likely attributed to the bioactive phytochemicals inherent in the *G. mastersii* extract, which enhance the nanoparticles' stability and functionality. These findings not only expand our understanding of plant-mediated nanoparticle synthesis but also highlight the therapeutic potential of FeONPs in biomedical applications, particularly in targeted drug delivery systems. Nonetheless, further in-depth studies, including cytotoxicity assessments, GC-MS profiling, and in vivo analyses, are imperative to fully elucidate their safety profile and broader pharmacological benefits. This work paves the way for future exploration into plant-based nanotechnology, bridging natural resources and advanced therapeutic strategies.

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Acknowledgments: The authors acknowledge department of chemistry, USTM for providing chemicals support to carry out this work. The Central Instrument Facility (CIF), at USTM, IIT, Guwahati and Sophisticated Analytical Instrumentation Centre (SAIC) at Tezpur University, Tezpur are gratefully acknowledged for assistance in characterization.

Funding: No Funding

Ethical Approval and accordance: Not Applicable

Conflict of interest: I, as corresponding author of this manuscript, declare that it is the following authors' original work. The authors declare that they have no conflicts of interest.

Consent to Publish: Not Applicable

Author contributions: RS: Conception, Design, Drafting and writing the manuscript; SS: Conception, Design, Drafting and writing the manuscript; SRS: Data analysis; SS: Data analysis; MMP: Data analysis and drafting.

Consent to participate: Not Applicable

Data availability statement:

All data supporting this study are included within the manuscript. Additional raw data can be made available by the authors upon reasonable request.