

## Biogenic mediated synthesis, characterization, antimicrobial and radical scavenging studies of iron oxide nanoparticles ( $\text{Fe}_3\text{O}_4$ -NPs) using *Eichhornia crassipes* leaves extract

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### Abstract

The plant *Eichhornia crassipes* (commonly known as water hyacinth) was obtained from Lake Geriyo, Yola, Adamawa State, Nigeria. The sampled leaves were gently washed with deionized (DI) water and air-dried at room temperature (25–30 °C). Iron nanoparticles were synthesized using *Eichhornia crassipes* (water hyacinth extract) and characterized using XRD, SEM, TEM, SEM-EDX, and FTIR. The antioxidant activity of the iron nanoparticles was analyzed using DPPH scavenging activity.

The plant extracts and iron nanoparticles were tested for antibacterial efficiency against *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Salmonella typhi*, and *Klebsiella pneumoniae*. The results

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revealed the proximate composition of the water hyacinth plant, including moisture content, ash content, fiber, fat content, protein, and carbohydrates. The proximate composition followed the order: carbohydrates > lipids > fiber > moisture > protein > ash content.

Qualitative phytochemical screening of the leaf revealed the presence of carbohydrates, terpenoids, phenolics, and saponins, while amino acids, terpenes, alkaloids, steroids, and flavonoids were absent. SEM-EDX, TEM, XRD, and FTIR confirmed the formation of iron nanoparticles. The iron nanoparticles exhibited higher percentage inhibition with varied concentrations of 25% FeNPs, 50/50 FeNPs, and 25/75 FeNPs, with 25/75 FeNPs showing significant scavenging activity of 24%, 39%, 47%, 55%, and 73% at 10, 20, 30, 40, and 50 µl/ml, respectively.

The minimum inhibitory concentration (MIC) of 25% FeNPs for *Klebsiella pneumoniae* and *Staphylococcus aureus* showed that the extract had a higher inhibitory effect on *Klebsiella pneumoniae* compared to *Staphylococcus aureus*. The inhibition sequence showed similar inhibition for all pathogens except *Salmonella typhi* and *Klebsiella pneumoniae*, which exhibited the least inhibition among all FeNP concentrations. An increase in material concentration resulted in higher inhibition for four organisms, while *Klebsiella pneumoniae* showed a different trend, with the highest inhibition observed at a 200 µg/L concentration.

## 1. Introduction

In recent years, there has been a gradual emergence and increasing application of nanomaterials across various fields. This is primarily due to the unique properties of nanoparticles, such as their small size, large surface area-to-volume ratio, quantum effects, optical properties, and enhanced mechanical, magnetic, and thermal properties compared to bulk materials. Additionally, nanoparticles possess flexible surface chemistry, allowing for surface functionalization tailored to specific applications [1-5].

These distinctive properties make nanoparticles highly versatile and valuable for a wide range of applications in fields such as medicine, electronics, catalysis, environmental remediation, and materials science [6-9]. Among the many types of nanoparticles, iron oxide nanoparticles ( $\text{Fe}_3\text{O}_4$ -NPs) have garnered significant attention due to their unique superparamagnetic properties, large surface area-to-volume ratio, ease of synthesis, biocompatibility, low toxicity, and cost-effectiveness [9-14].

Several methods have been employed to synthesize  $\text{Fe}_3\text{O}_4$ -NPs, including hydrothermal [15], solvothermal [16], precipitation [17], and biogenic (also referred to as green synthesis) approaches. However, recent research has increasingly focused on green synthesis methods due to their cost-effectiveness, sustainability, and environmental friendliness. Various plant extracts, such as *Aloe vera* [18], *Moringa oleifera* [19], *Graptophyllum pictum* [20], and *Zanthoxylum armatum* [21], have been utilized for the green synthesis of nanoparticles.

$\text{Fe}_3\text{O}_4$ -NPs have demonstrated varying degrees of biological activity against a range of pathogenic microbes, including bacteria [22], fungi [23], viruses [24], and protozoa [25]. Like most nanoparticles,  $\text{Fe}_3\text{O}_4$ -NPs exhibit unique mechanisms of action against microbial pathogens, including disruption of the cell membrane, interference with metabolic pathways, and generation of reactive oxygen species (ROS) [26-28].

*Eichhornia crassipes*, commonly known as water hyacinth, is an aquatic plant native to South America that has naturalized worldwide [29]. Since the 1800s, it has spread primarily to tropical and subtropical regions and is considered invasive due to its rapid growth beyond its native range. While water hyacinth has beneficial applications, such as phytoremediation and biomass production, its invasive nature poses significant environmental and socio-economic challenges. These include waterway obstruction, ecological disruption, habitat alteration, and water quality degradation [30, 31].

Despite its negative impacts, the plant has been reported to exhibit antibacterial, anti-inflammatory, and antifungal properties [32], as well as anti-aging [33] and anticancer activities [34].

In the pursuit of addressing the growing global concern of antimicrobial resistance, we report here the eco-friendly, biogenic-mediated synthesis of Fe<sub>3</sub>O<sub>4</sub>-NPs using varying concentrations of an iron solution and water hyacinth leaf extract. The nanoparticles were characterized using UV-Visible spectroscopy, X-ray diffraction (XRD), Fourier Transform Infrared (FTIR) spectroscopy, Scanning Electron Microscopy (SEM), and Transmission Electron Microscopy (TEM). Additionally, there in vitro antimicrobial activity was evaluated against *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, *Salmonella typhi*, and *Klebsiella pneumoniae*, along with an assessment of their radical scavenging potential.

## 2. Materials and Methods

### 2.1. Extraction of the plant metabolite

The plant (*Eichhornia crassipes*, commonly known as water hyacinth) was obtained from Lake Geriyo, Yola, Adamawa State, Nigeria. The sampled leaves were gently washed with deionized (DI) water and air-dried at room temperature (25–30 °C). The dried leaves were then blended and sieved using a 200-mesh sieve (pore diameter: 0.074 mm). An aqueous extract of the plant leaves was prepared by boiling 10 g of the powdered leaves in 100 mL of DI water with continuous stirring for 10 minutes. The extract was then filtered and stored in a refrigerator for further use.

### 2.2. Synthesis of nanoparticles

Three different ratios—25 mL/75 mL, 50 mL/50 mL, and 75 mL/25 mL—of the plant extract and a 1 mM solution of iron (II, III) oxide (Fe<sub>3</sub>O<sub>4</sub>) were mixed and stirred at 80 °C for 1 hour. The resulting precipitate was then centrifuged, washed several times with water, and dried in a desiccator over silica

### 2.3. Characterization of the iron oxide (Fe<sub>3</sub>O<sub>4</sub>) nanoparticles

The spectroscopic analysis, namely UV-Visible spectroscopy, was performed using a Cary 60 spectrophotometer by Agilent Technologies. Fourier Transform Infrared (FTIR) spectroscopy was conducted using a Cary 630 spectrophotometer by Agilent Technologies. Morphological analysis, including scanning electron microscopy (SEM) and transmission electron microscopy (TEM), was carried out using the Phenom-ProX system. X-ray diffraction (XRD) analysis was performed using a MiniFlex 600 X-ray diffractometer.

### 2.4. Radical scavenging activity of the Fe<sub>3</sub>O<sub>4</sub>-NPs

The radical scavenging activity of Fe<sub>3</sub>O<sub>4</sub>-NPs was studied using the 1,1-Diphenyl-2-picrylhydrazyl (DPPH) method. In this method, 200, 400, 600, 800, and 1000 µL of the sample were taken in a series of test tubes, along with 1 mL of 100 µM DPPH solution dissolved in methanol. The mixture was adjusted to a final volume of 2 mL using methanol and incubated in the dark for 30 minutes to allow the reaction to occur. After incubation, the absorbance was measured using a UV-Vis spectrophotometer (UV-1800, Shimadzu) at a specific wavelength (nm).

In this experiment, methanol served as the blank, the DPPH solution without any test sample acted as the positive control, and ascorbic acid was used as the standard. The experiment was repeated three times, and the mean absorbance values were recorded. The inhibition percentage was determined using the following equation.

## 2.5. Antimicrobial studies

The antibacterial activities of all extracts of *Ziziphus spina-christi* and *Hymenocardia acida* were evaluated using the paper disc diffusion method [35]. Extended-spectrum beta-lactamase (ESBL)-producing *Pseudomonas aeruginosa* was first reactivated by inoculating it into freshly prepared peptone water and incubating it for 48 hours at 37 °C. The broth cultures of ESBL-producing *P. aeruginosa* were then adjusted to a 0.5 McFarland standard using a sterile 0.9% normal saline solution and inoculated onto ceftrimide agar.

Sterilized filter paper discs (Whatman No. 1; 6 mm in diameter) were soaked in test tubes containing the dissolved extracts at different concentrations, picked up with sterilized forceps, air-dried, and placed on agar plates inoculated with the bacteria. The plates were then incubated for 24 hours at 37 °C.

The bacterial sensitivity or susceptibility to the standard drug was tested by placing discs seeded with amoxicillin-clavulanic acid (amoxy-clav, 20/10 µg) on each plate at different concentrations, serving as the positive control. Sterilized paper discs with dimethyl sulfoxide (DMSO) were used as negative controls for all plates. The experiment was performed in triplicate.

The bactericidal activity was determined by measuring the zone of inhibition around each paper disc. The results for the anti-*Pseudomonas* agent were compared with those of the plant extracts [36].

## 3. Results and discussion

### 3.1. Characterization

The structural characterization, phase analysis, and crystallinity of the synthesized samples were determined using the XRD technique with Cu K $\alpha$  radiation ( $\lambda = 1.5406 \text{ \AA}$ ) in the  $2\theta$  range of 20–80°, along with scanning electron microscopy (SEM).

### 3.2. Phytochemical and proximate analysis of the extract used as capping and reducing agent

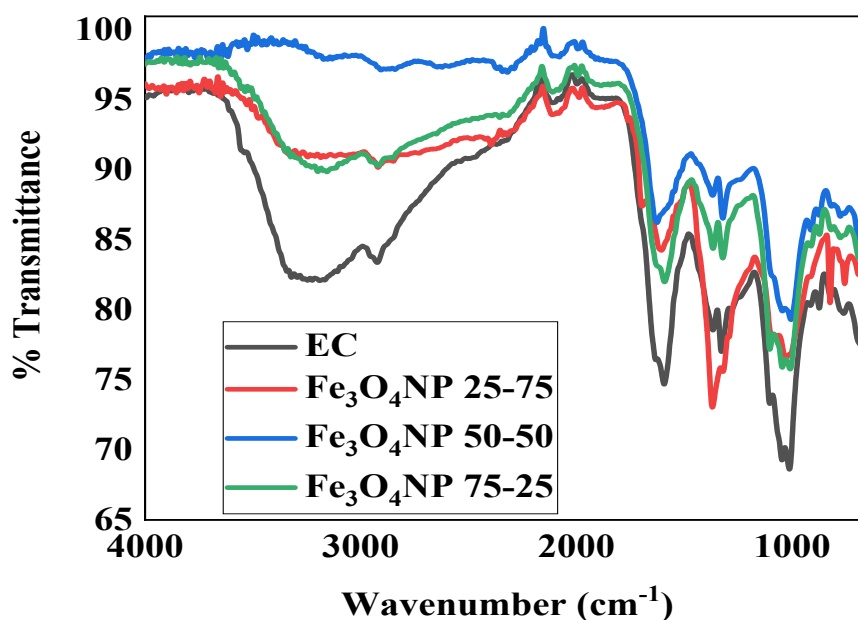
Table 1 presents the qualitative phytochemical analysis, which revealed that the leaf extract of *Eichhornia crassipes* contains carbohydrates, terpenoids, phenolics, and saponins, while amino acids, terpenes, alkaloids, tannins, steroids, and flavonoids were absent. The proximate composition analysis of *Eichhornia crassipes* showed that carbohydrates had the highest composition, followed by lipids and then fiber, while ash content had the lowest value. The composition values followed the order:

Carbohydrates > Crude Lipid > Crude Fiber > Crude Protein > Moisture Content > Ash Content

**Table 1.** Phytochemical and proximate analysis of the plant extract.

Phytochemical screening of the plant extract		Proximate analysis of the plant extract	
Phytochemical component	Aqueous extract	Parameter	% Composition
Saponins	+	Moisture	10.06
Tannins	-	Ash content	7.41
Alkaloids	-	Crude fiber	14.92
Flavonoids	-	Crude lipid	21.75
Terpenoids	+	Crude protein	8.60
Steroids	-	Carbohydrates	36.72
Phenols	+		
Amino acids	-		
Carbohydrates	+		
Terpenes	-		

### 3.3. Fourier transform infrared spectroscopy

**Figure 1.** FTIR spectra of the extract and the Fe<sub>3</sub>O<sub>4</sub>-NPs.

The FTIR analysis of *Eichhornia crassipes* (EC) before synthesis is shown in Figure 1. The spectrum exhibits broad absorption spanning from 3100 to 3600 cm<sup>-1</sup>, indicating the presence of O–H stretching vibrations. A peak at 3265 cm<sup>-1</sup> corresponds to the alcohol functional group, while weak absorption at 2920 cm<sup>-1</sup> is indicative of C–H stretching. The fingerprint region displays characteristic absorption peaks at 1600 cm<sup>-1</sup>, 1313 cm<sup>-1</sup>, and 1025 cm<sup>-1</sup>, corresponding to nitro, alcohol, and amine functional groups, respectively. The presence of N–O, O–H, and C–N bonds suggests nitrogen-containing, alcohol-containing, and amine-containing metabolites in EC.

For a 25:75 ratio of iron oxide to EC, the FTIR analysis of the synthesized nanoparticles reveals

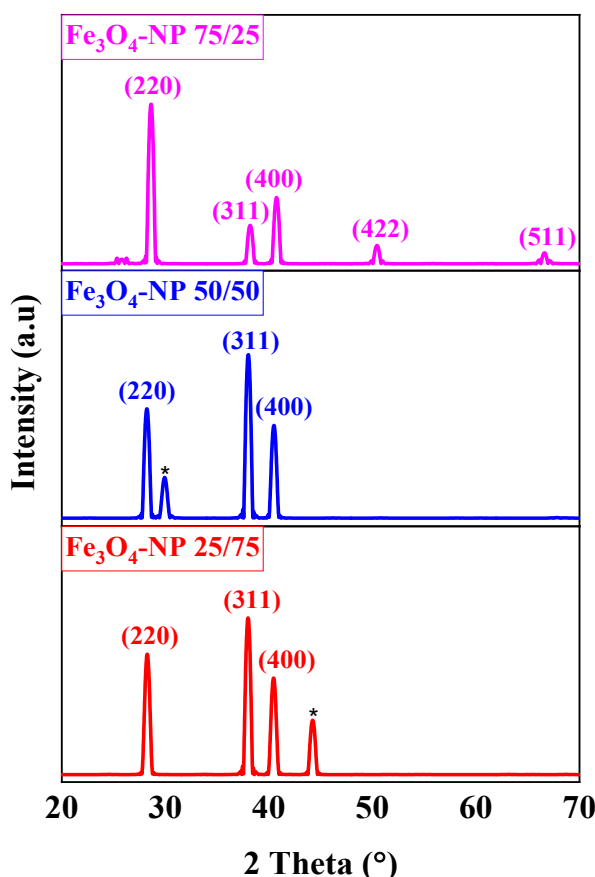
characteristic N–H stretching of aliphatic amines and C–C stretching of cyclic alkenes. N–O stretching at  $1541\text{ cm}^{-1}$  and S–O stretching at  $1030\text{ cm}^{-1}$  indicate functional groups typically found in iron oxide nanoparticles.

Vibrational frequencies observed at  $3268\text{ cm}^{-1}$  and  $1623\text{ cm}^{-1}$  suggest the presence of amine and alkene groups. A peak at  $2920\text{ cm}^{-1}$  corresponds to thiols, while peaks at  $1341\text{ cm}^{-1}$  and  $1030\text{ cm}^{-1}$  indicate the presence of sulfoxide and sulfonamide functional groups.

For a 50:50 iron oxide-based nanoparticle composition, the FTIR spectrum exhibits similar peaks to the 75:25 iron oxide-to-extract nanoparticle ratio. Iron oxide interactions contribute to weak peaks observed at  $1397\text{ cm}^{-1}$  and  $1235\text{ cm}^{-1}$ . The synthesized nanoparticles display irregular peak patterns, differing from those observed in the extract prior to synthesis.

### 3.4. XRD-diffraction spectroscopy

The formation of the EC- $\text{Fe}_3\text{O}_4$ NPs phase shift was confirmed by XRD analysis, as shown in Figure 2. The diffraction patterns reveal the presence of four distinct diffraction peaks. Strong peaks were observed at  $2\theta$  values of  $26.34^\circ$ ,  $29.92^\circ$ ,  $38.02^\circ$ , and  $40.50^\circ$ , corresponding to the (110), (220), (311), and (400) planes, respectively. These peaks are characteristic of Bragg's reflection based on the face-centered cubic (FCC) crystal structure of EC- $\text{Fe}_3\text{O}_4$ NPs, in accordance with the JCPDS standard data (11-0614). No additional diffraction peaks were detected other than those corresponding to  $\text{Fe}_3\text{O}_4$ NPs. However, the broadening of Bragg's peaks indicates the formation of EC- $\text{Fe}_3\text{O}_4$ NPs. The XRD pattern thus confirms that the EC- $\text{Fe}_3\text{O}_4$ NPs synthesized via the reduction of  $\text{Fe}^{3+}$  ions by EC extracts are crystalline in nature.



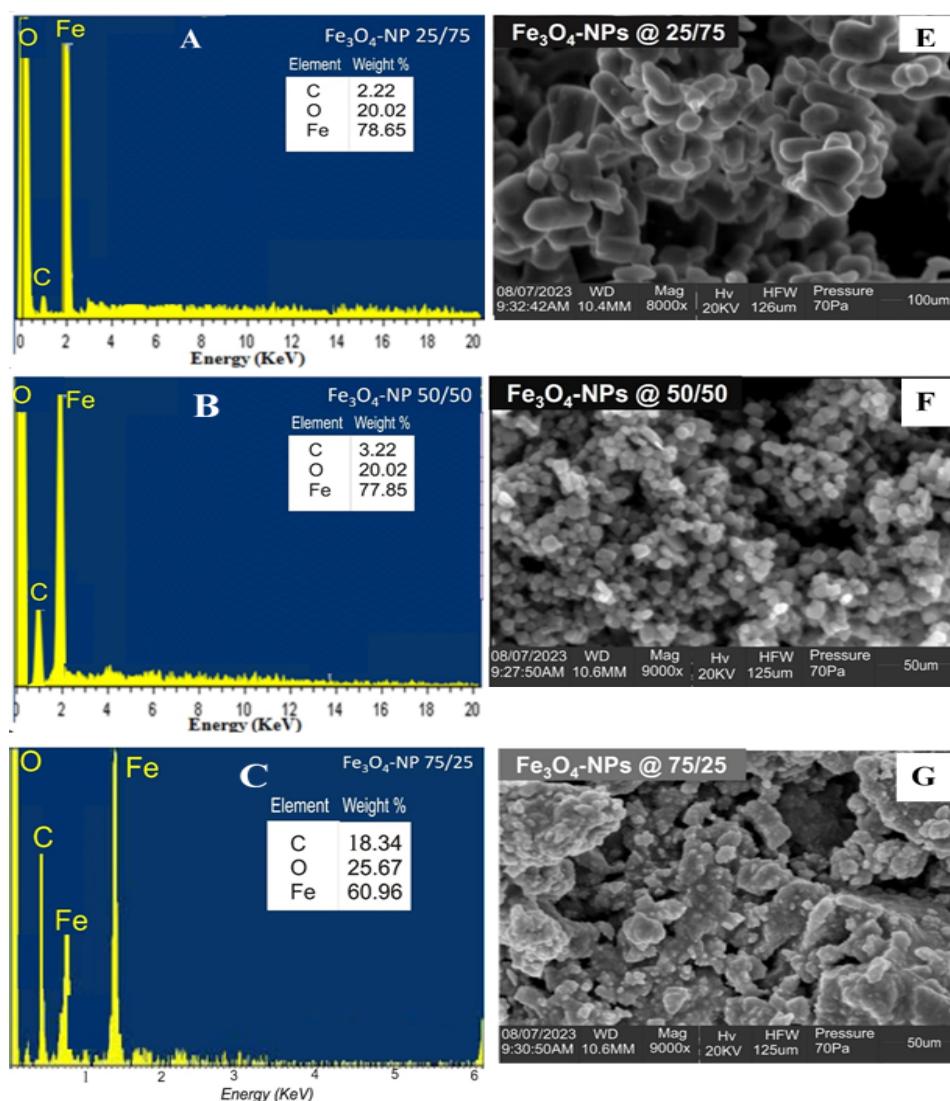
**Figure 2.** XRD of patterns of  $\text{Fe}_3\text{O}_4$ -NPs synthesized at different amount of the reducing agent and the metal salt solution.



### 3.5. Scanning electron spectroscopy (SEM) and energy dispersive X-ray (EDX) analysis

Surface morphological studies of EC-Fe<sub>3</sub>O<sub>4</sub> NPs were performed using scanning electron microscopy (SEM), and elemental analysis was obtained using EDX. As shown in Figure 3e, the Fe<sub>3</sub>O<sub>4</sub> nanoparticles are uniformly embedded in the EC matrix and remain stable with minimal aggregation, which is consistent with Figures 3f and 3g.

Therefore, the issue arising from magnetic dipole-dipole interactions between Fe<sub>3</sub>O<sub>4</sub> nanoparticles appears to be resolved due to their dispersion within the EC matrix. Figure 3a presents a typical EDX analysis of EC-Fe<sub>3</sub>O<sub>4</sub> NPs. The peaks around 0.5, 1, and 2.01 keV indicate that only oxygen (O), carbon (C), and iron (Fe) elements are present in the sample. The strong energy signal peak for Fe atoms around 2.01 keV is characteristic of metallic Fe nanocrystals, with EC acting as a reductant and capping agent. The strong single energy peak indicates the purity of Fe (77.85%) in the sample, while other studies have reported the presence of C (3.22%) and O (20.02%) in synthesized Fe<sub>3</sub>O<sub>4</sub> NPs.

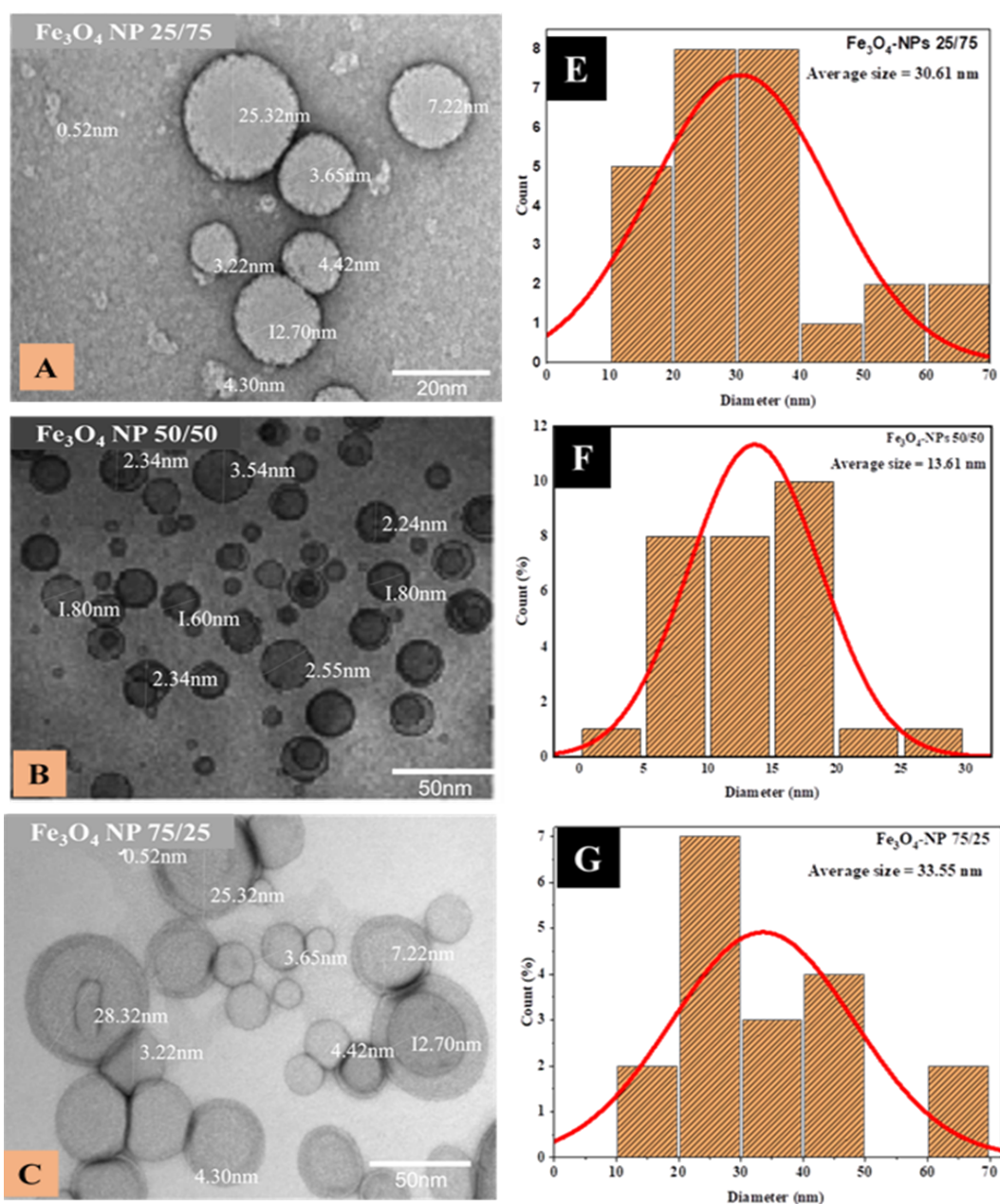


**Figure 3.** SEM Images and EDX plot of the Fe<sub>3</sub>O<sub>4</sub>-NPs.

### 3.6. Transmission electron microscopy and particle size distribution

In the assessment of the morphology and size of the synthesized EC-Fe<sub>3</sub>O<sub>4</sub> nanoparticles, a transmission electron microscope (TEM) and a particle size analyzer were used. Figures 4a, 4b, and 4c represent TEM images of EC-Fe<sub>3</sub>O<sub>4</sub> at different concentrations. As shown in these figures, the Fe<sub>3</sub>O<sub>4</sub> nanoparticles exhibit a nanocrystalline structure, and their shape is approximately spherical.

Furthermore, a careful review of these images reveals that the EC-Fe<sub>3</sub>O<sub>4</sub> nanoparticles tend to agglomerate due to their high surface area and the magnetic dipole-dipole interactions between them, as compared to Fe nanoparticles. To determine the mean size of the spherical EC-Fe<sub>3</sub>O<sub>4</sub> nanoparticles, we obtained the histogram of the particle size distribution, as shown in Figures 4e, 4f, and 4g. The graphical results in these figures indicate that the sizes of the EC-Fe<sub>3</sub>O<sub>4</sub> nanoparticles range from 1.6 to 30 nm, with an average particle size of  $15.03 \pm 7.39$  nm.



**Figure 4.** TEM micrographs and Average particle size histogram of the Fe<sub>3</sub>O<sub>4</sub>NPs.



### 3.7. Radical scavenging activity of the Fe<sub>3</sub>O<sub>4</sub>NPs

#### *Antioxidant Activity*

In this study, DPPH radical-scavenging activity was measured to identify the antioxidant activity of EC Fe Nano 25/75, EC Fe Nano 50/50, and EC Fe Nano 75/25 extracts. The DPPH radical-scavenging activity (%) of EC 25/75 Nano increased as the concentration increased from 10, 20, 30, 40, and 50 µL/mL, with values of 24, 39, 47, 55, and 73%, respectively. For EC 50/50 Nano, the values were 31, 32, 39, 47, and 63%. Summing up these data, this study concluded that water is the most suitable solvent to enhance radical-scavenging activity. The study results show that the EC Fe Nano 25/75 aqueous extract exhibited the highest DPPH radical-scavenging activity (73%), followed by EC Fe 50/50 (63%) and EC Fe 75/25 (68%). The antioxidant activity of the extracts also proportionally increased in the water extract. Turmeric leaf ethanol extract (1 mg/mL concentration) showed higher DPPH radical-scavenging activity (80.11%) than the water extract (74.65%). There was also a significant difference in the radical-scavenging activity between the ethanol extract and the water extract, similar to the pattern observed in DPPH radical-scavenging activity. However, this indicates only a slight difference, and we concluded that this distinction may be due to differences in solvent concentration.

### 3.8. Antimicrobial studies of the Fe<sub>3</sub>O<sub>4</sub>NPs

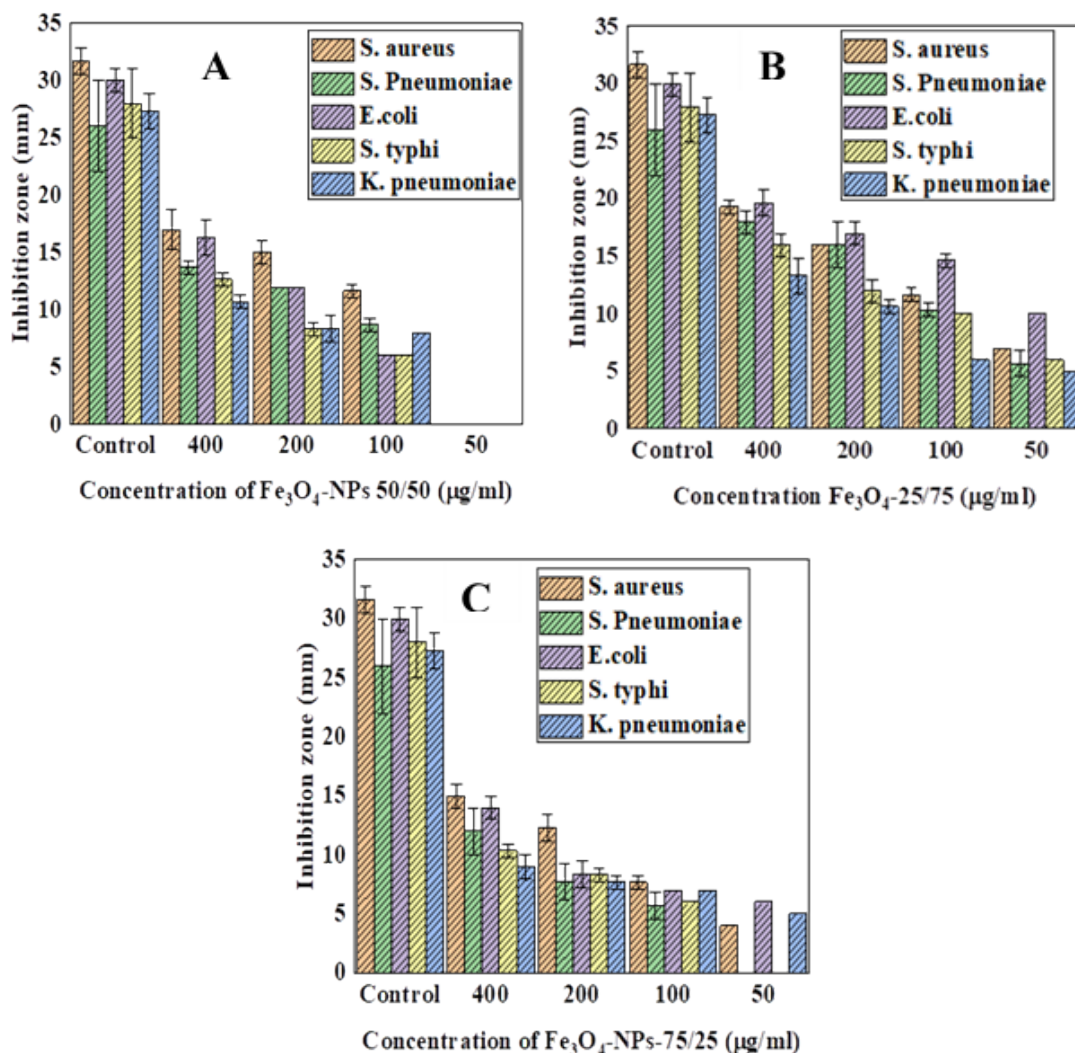
EC Fe 75/25 exhibits lower antimicrobial inhibition compared to the 25/75 nanoparticles. The inhibition trend is similar for all pathogens except *K. pneumoniae*, which shows the least inhibition among the 50/50 EC Fe samples. The inhibition pattern remains consistent for most pathogens, except for *S. typhi* and *K. pneumoniae*, which display the lowest inhibition across all EC Fe samples. The maximum inhibition observed is 20 mm, while the minimum is 5 mm. Increasing the material concentration generally results in higher inhibition for four organisms, whereas *K. pneumoniae* follows a different trend, showing the highest inhibition at a concentration of 200 µg/L.

The increase in material concentration results in higher inhibition for five organisms. EC Fe 50/50 shows a different trend, with the highest inhibition at a concentration of 400 µg/L and weak inhibition at 50 µg/L. EC Fe 75/25 exhibits very weak inhibition at concentrations below 100 µg/L. The maximum inhibition was observed at 12 mm for a nanoparticle concentration of 400 µg/L.

#### *Susceptibility test of the various*

The EC plant extract shows activity against *S. aureus*, *Streptococcus pneumoniae*, *E. coli*, *Salmonella typhi*, and *Klebsiella pneumoniae*. According to the World Health Organization (WHO) report on infectious diseases, overcoming antibiotic resistance is a major challenge for the WHO in the coming millennium. Hence, the last decade has witnessed an increase in the investigation of plants as a source for human disease management. This study shows that the investigation of plants as a source of antimicrobial agents was enhanced by increasing the concentration of the extract.

The activities of the plant extracts are ranked as follows: EC plant extract silver ranked first, followed by EC Fe Nano 25/75, EC Fe Nano 50/50, and EC Fe Nano 75/25 in second, third, and fourth places, respectively. Based on the organism, *Staphylococcus aureus* and *Escherichia coli* were found to be more sensitive to the extracts.



**Figure 5.** Graph of antimicrobial activity of Fe<sub>3</sub>O<sub>4</sub>-NP-25/75 (A), Fe<sub>3</sub>O<sub>4</sub>-NP-50/50 (B), Fe<sub>3</sub>O<sub>4</sub>-NP-75/25 (C).

## Conclusion

This study successfully reports the synthesis of FeNPs, as confirmed by FTIR, SEM-EDX, XRD, and UV-Spectrophotometry. FTIR analysis confirmed the presence of carbohydrate groups from the plant extract on the synthesized FeNPs. The characteristic peaks of FeNPs were observed in the XRD plots of the synthesized nanoparticles. The proximate composition revealed that water hyacinth has both nutritional and medicinal value.

The antioxidant study shows that FeNPs exhibit good scavenging activity, with the highest percentages recorded at 63% and 71%. DPPH free-radical scavenging activity tests were conducted to evaluate the antioxidant potential of the FeNP extracts, and the results indicated varying degrees of scavenging activity among different extracts, with FeNPs 25/75 demonstrating the highest activity.

Antimicrobial tests showed that the synthesized nanoparticles exhibited inhibitory effects against various pathogens, with inhibition zones increasing at higher nanoparticle concentrations. The efficacy of the nanoparticles varied depending on their composition and concentration, with EC Fe Nano and Fe Nano 25/75 demonstrating the highest inhibitory effects. The reusability study of these materials revealed that EC Fe Nano 25/75 was more stable compared to FeNPs 75/25.

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