Solvothermal-Assisted Green Synthesis of *Ziziphus nummularia* Leaf Extract Capped Fe_3O_4 Nanocomposites Promising Antibacterial Applications

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Abstract:

This study investigates the synthesis of biogenic iron oxide nanoparticles (Fe_3O_4NPs) using a solvothermal-assisted green chemistry approach and assesses their antibacterial properties. The biosynthesized Fe_3O_4NPs were characterized through multiple analytical techniques, including UV-visible spectroscopy, Fourier-transform infrared spectroscopy (FT-IR), X-ray diffractometry (XRD), energy-dispersive X-ray spectroscopy (EDX), and field emission scanning electron microscopy (FESEM). The resulting nanoparticles were spherical in shape. The antibacterial effectiveness of these Fe_3O_4NPs, derived from *Ziziphus nummularia*, was evaluated, with the highest inhibition zone observed at a concentration of 100 µg/mL against *Escherichia coli* and *Staphylococcus aureus*. These promising antibacterial characteristics highlight the potential application of Fe_3O_4NPs in medicinal fields..

Keywords: Fe_3O_4 NPs. ziziphus nummalariya .Vsm. Antibacterial activity .

1.Introduction

Nanotechnology is a modern and rapidly advancing field of science, recognized as one of the most swiftly expanding areas of research. It involves constructing innovative nanostructures, exploring their unique properties, and developing ways to arrange these nanostructures into more complex and functional systems. Nanoparticles can be produced from various metals, including silver, gold, zinc, copper, iron, and titanium, through different physical and chemical methods. However, the synthesis of nanoparticles from noble metals is particularly popular due to their extensive applications in areas such as biomedical sciences, electronics, optics, chemical industries, drug and gene delivery, environmental protection, cosmetics, energy conservation, antibacterial, antifungal, antioxidant and anticancer agents and catalysis(1).

There are several ways to synthesize metal nanoparticles, including physical, chemical, and biological methods. However, physical and chemical methods are often difficult, time-consuming, and harmful to the environment because they produce toxic chemicals. They are also not suitable for large-scale production (2).

This method offers several advantages, including being cost-effective, simple, environmentally friendly, and easily scalable for large-scale production. Plants contain various phytochemicals, such as polyphenols, alkaloids, and flavonoids, which help reduce metal ions into metal nanoparticles. These phytochemicals also act as reducing, capping and stabilizing agents for the nanoparticles, eliminating the need for additional chemicals or compounds(3).

Ziziphus nummularia is a plant from the Rhamnaceae family, commonly found in dry areas. It is a multipurpose plant, traditionally used to make different herbal remedies, and has great potential for medicinal use (4)*Ziziphus nummularia* exhibits several pharmacological properties, including antioxidant, analgesic, anti-inflammatory, antinociceptive, and antipyretic activities. The leaves are traditionally used to treat coughs, colds, and typhoid, as well as to heal cuts and skin diseases. Notably, the DPPH radical scavenging activity in *Ziziphus nummularia* extracts has also been demonstrated(5).

In recent years, there has been a growing focus on iron oxide nanoparticles, such as magnetite nanoparticles (Fe_3O_4 NPs), hematite (α-Fe_2O_3 NPs) and maghemite (γ-Fe_2O_3 NPs), for removing organic and inorganic contaminants. Among the various magnetic nanoparticles, Fe_3O_4

NPs have gained attention as promising candidates for a wide range of technological applications, including magnetic fluids, high-density magnetic storage, environmental remediation, cancer therapy, MRI contrast enhancement, drug delivery, tissue repair engineering, antibacterial treatments, catalysis, and lithium-ion batteries. This is due to their small size, biocompatibility, low toxicity, and high saturation magnetism (6-12).

Various methods have been reported for synthesizing Fe_3O_4 nanoparticles, including co-precipitation, thermal decomposition of organic iron precursors, hydrothermal ,solvothermal method, the sol–gel method, the polyol method, and surfactant- or polymer-assisted precipitation techniques such as reverse micelles. Other approaches include co-polymer template-assisted synthesis, solvothermal synthesis, and hydrothermal synthesis(13-19).While some physical and chemical methods have successfully produced uniformly sized nanoparticles, a major drawback is their lack of stability and the high tendency of the particles to agglomerate, leading to altered physical properties. Additionally, these methods often involve toxic chemicals. In contrast, biological methods using leaf extracts are more environmentally friendly and result in nanoparticles that are highly stable with minimal agglomeration.(20-23). The concentration of the biological extract significantly influences the nanoparticle size. Lower extract concentrations tend to produce particles with an average size of less than 50 nm, while higher concentrations lead to larger particles exceeding 70 nm(24). Interestingly, in some cases, higher extract concentrations have also produced smaller nanoparticles.(25). This suggests that the amount of phytochemicals, functional groups, and metabolites in the extract has a greater influence on nanoparticle size than the extract concentration itself(26).

This study presents the development of a magnetite-ziziphus nummulariya leaf extract nanocomposite using a solvothermal-assisted green synthesis method, followed by characterization with various modern techniques. To the best of our knowledge, this is the second report on the solvothermal-assisted green synthesis of a -magnetite nanocomposite using zizinummalariya leaf extract. The primary goal of this research is to create a stable, surface charge-modified magnetite nanocomposite and evaluate its enhanced properties, surface interactions, and potential biological applications (27).

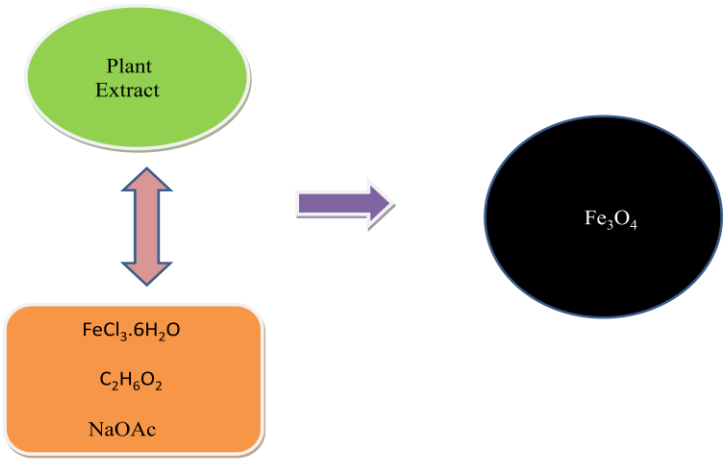


Figure.1. Schematic representation of biosynthesis of Fe_3O_4 NPs using ziziphus nummularia leaf extract

2.Materials methods

2.1 Materials

Iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$, 99.9%), sodium acetate (NaOAc, 99.9%), and ethylene glycol ($C_2H_6O_2$) were procured from Sigma-Aldrich and used as precursors in this study. Ziziphus nummularia leaves, (jujube) commonly found along roadsides, were collected from areas around Chidambaram town in Cuddalore district, Tamil Nadu, India, and used as a bio-agent.

2.2 Synthesis of Fe_3O_4 capped ziziphus nummalariya leaf extract

The plant extract was prepared by boiling 20 g of fresh leaves in 100 mL of distilled water at 80°C for 30 minutes. And fnally taken 10 ml plant extract solution. Vigorous stirring was used

to dissolve 2.70 g of $FeCl_3.6H_2O$ and 7.20 g of sodium acetate in 100 mL of ethylene glycol. and mixed with 10 mL of the plant extract. during which a yellowish color developed. Next, 10 mL of freshly prepared leaf extract solution was added dropwise allowing the magnetite to precipitate under constant stirring. After 30 minutes, the solution changed color from yellow to black. The mixture was then transferred to a Teflon-lined stainless-steel autoclave and heated to 200°C for 12 hours. After cooling to room temperature gradually, the Fe_3O_4 nanoparticles (magnetite NPs) were collected through continuous centrifugation and washed with deionized water to remove any remaining plant extract. The concentrated nanoparticles were dried at 60°C for 12 hours and stored in an airtight container for further analysis.(28)

2.3 Characterization techniques

The functional groups were characterized using FT-IR spectroscopy (SHIMADZU 8400) in transmittance mode, with a resolution of ±4 cm^{-1}, across the range of 4000–400 cm^{-1} through the KBr pellet method. The purity and crystalline structure were examined with an X-ray diffractometer (X'pert PRO), using CuKα radiation (λ = 1.5406 Å), with a 30 mA current and an operating voltage of 40 kV. The magnetic properties were assessed at room temperature using a vibrating sample magnetometer (VSM, EV X). The sample morphology was analyzed via Field Emission Scanning Electron Microscopy (FESEM, CARL ZEISS-SIGMA-300), operating with an accelerating voltage between 0.3 kV and 30 kV, and a resolution of 15 nm at 1 kV (HV). Elemental mapping and composition were determined by Energy-dispersive X-ray (EDX) analysis, conducted on a Brucker system with 129 eV energy resolution..

2.4 Antibacterial Activity

By using the Muller-Hinton diffusion method, the antibacterial activity of Fe_3O_4 nanoparticles was tested against both Gram-positive and Gram-negative bacteria, including Escherichia coli and Staphylococcus aureus. These cultures were created at Annamalai University's Department of Microbiology. First, sterilized Petri dishes were filled with autoclaved, sterile Muller-Hinton agar. To test the synthesis of the cultures, 15-20 ml of the inoculums were wiped on the surface of the solidified MHA medium and allowed to dry for 10 minutes. The Fe_3O_4 nanoparticles that were synthesized (concentrations of 25, 50, 75, and 100 µg/mL) were then impregnated onto 6 mm sterile disks.In order to allow for compound diffusion, the loaded disks were placed for 30

minutes at room temperature on the surface of the bacterial culture on MHA plates with the controls that were required. A positive control for microorganisms was chloramphenical(5 µg/disk). The sterile disk served as a negative control and was impregnated with DMSO. The disks were then incubated for a full day at 37 ºC. The strong inhibitory zone on the agar surface surrounding the disks was measured in millimeters to determine the bacterial susceptibility following the incubation. This finding was analyzed and contrasted with the results of the chloramphenical antibiotic.

3. Results and Discussion

3.1 XRD Analysis

The XRD pattern of the synthesized magnetite exhibited six distinct peaks at 2θ values of 30.222°, 35.603°, 43.448°, 53.318°, 56.953°, and 62.956°. These peaks correspond to the crystallographic planes [(220), (311), (400), (511), and (440)] and are consistent with the cubic spinel structure of Fe_3O_4 (Fig. 3). The results align well with the standard XRD pattern for Fe_3O_4 (PDF #890691). Using Scherrer's formula, the average crystallite size of the nanoparticles was calculated to be approximately 13 nm, similar to findings reported by Gerko..(29)

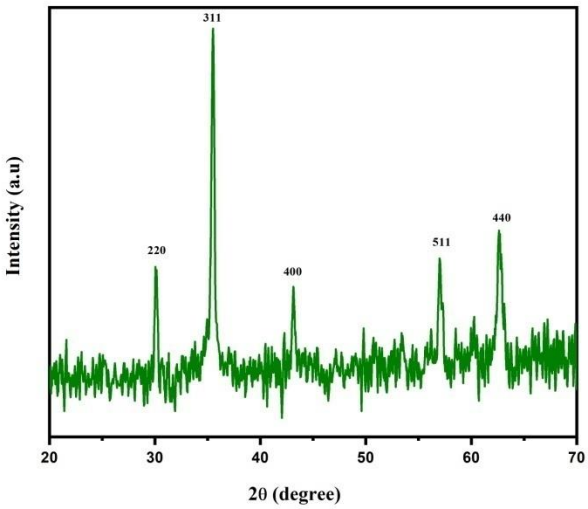


Figure.2. XRD pattern of ziziphus nummularia leaf extract mediated Fe_3O_4 NPs

3.2 FTIR Analysis

Figure 3 presents the Fourier transform infrared (FTIR) spectrum of Fe_3O_4 nanoparticles. To verify the purity and characteristics of the metal nanoparticles, an infrared analysis was conducted. The IR spectrum is divided into two regions: the functional group region and the fingerprint region. Organic compounds typically show absorption bands in the functional group region, while metals exhibit absorption spectra in the fingerprint region, which are attributed to the atomic vibrations of the molecules.

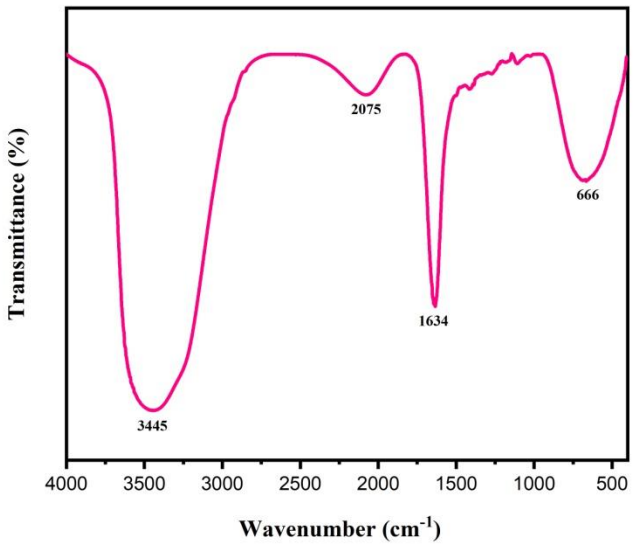


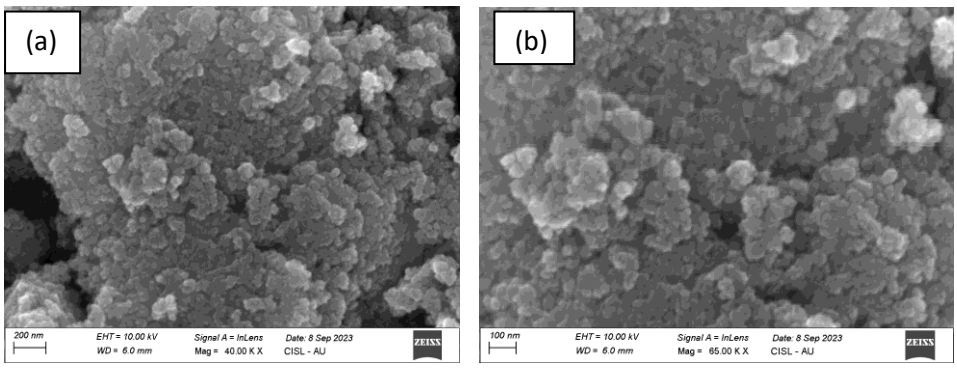
Figure.3.FTIR Spectra of ziziphus nummularia leaf extract mediated Fe_3O_4 NPs

FTIR spectra of Fe_3O_4 NPs with leaf extract of ziziphus nummularia plant. were presented in Figure . The FTIR spectrum of the aqueous leaf extract of ziziphus nummularia plant exhibited characteristic bands at 3445, 2075, 1634 and 666 cm^{-1} . The peak observed at 3445 cm^{-1} corresponds to the O-H group, which is attributed to stretching and deformation, indicating water adsorption on the metal surface(30)**.**The absorption peak at 1634 cm^{-1} indicates the presence of amide bonds in proteins due to C=O stretching, which aligns closely with native protein values, suggesting protein interaction with the synthesized nanoparticles. Additionally, the absorption peak at 3445 cm^{-1} is attributed to O-H stretching in alcoholic and phenolic compounds. IR analysis confirms that the carbonyl groups of amino acid residues have a strong

binding affinity with the metal nanoparticles, acting as a capping agent to stabilize the medium, thereby verifying that these proteins serve as stabilizing agents for the Fe_3O_4 nanoparticles.(31).The strong peak observed at 666 cm^{-1} corresponds to the inorganic stretching, indicating the presence of Fe-O vibration .(32)

3.3 FESEM

Field Emission Scanning Electron Microscopy (FESEM) analysis revealed that *Ziziphus nummalaria*-mediated Fe_3O_4 nanoparticles (Fe_3O_4NPs) exhibit a spherical morphology with uniform arrangement and noticeable agglomeration (Fig.4). These Fe_3O_4NPs were further characterized by FESEM-EDX, confirming their formation with an elemental composition by weight of 38.70% iron, 44.43% oxygen, 15.28% carbon, 0.64% silicon, 0.59% chloride, and 0.36% calcium. Similarly, Demirezen et al. (2019) observed Fe_3O_4NPs synthesized using carob pod (*Ceratonia siliqua*), which displayed an EDX profile in atomic percentages: 14.46% iron, 57.96% oxygen, 26.56% carbon, 0.47% silicon, 0.35% chloride, and 0.19% calcium(33). This profile suggests that the biological components associated with *Ziziphus nummalaria* may contribute to the elemental composition of the Fe_3O_4NPs .(34)



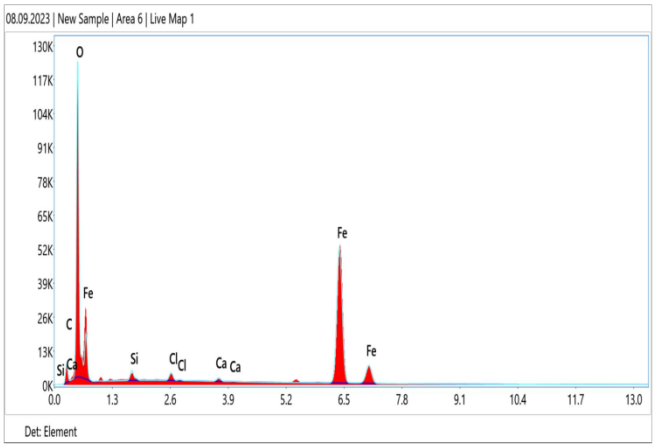


Figure.4.FESEM with EDX images of ziziphus nummularia leaf extract mediated Fe_3O_4 NPs

3.4 VSM

The magnetic properties of sample C (chosen for its representative characteristics) were analyzed using VSM, as shown in Fig. 5, by measuring the hysteresis loop [magnetization versus magnetic field (M–H)] at room temperature with an applied field of ±15 kOe. The M–H loops revealed a superparamagnetic behavior for the Fe_3O_4 nanoparticles (Fe_3O_4NPs) with a lower saturation magnetization (Ms) compared to the bulk value of 93.9 emu/g [35], making them suitable for biomedical applications such as hyperthermia. This indicates that Fe_3O_4NPs possess strong magnetic properties, ideal for biomedical use. The magnetization of magnetic nanoparticles (MNPs) is highly dependent on particle size. Therefore, in comparison to bulk materials, smaller particles exhibit lower Ms values [35]. The reduced Ms of the Fe_3O_4NPs can be attributed to their smaller particle size, which may be associated with a disordered surface spin layer [36].

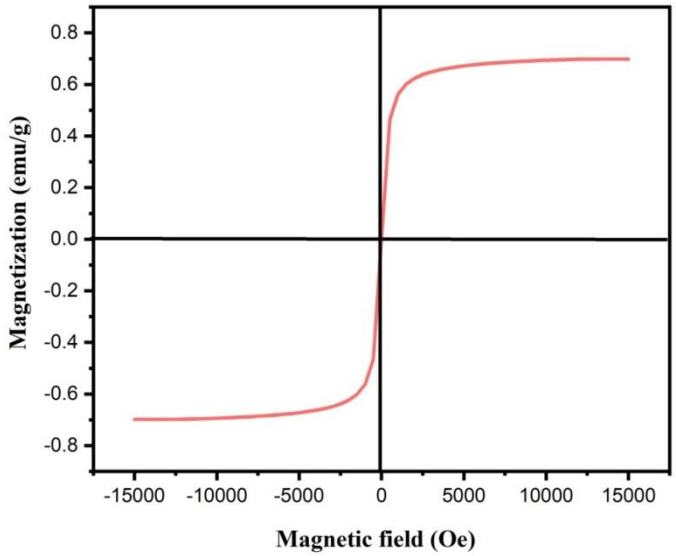


Figure.5. Hysteresis loop of Fe_3O_4 NPs using ziziphus nummularia leaf extract

3.5 Antibacterial activity

The antibacterial activity of iron oxide nanoparticles synthesized using *zizinummalaria* is demonstrated in Fig. 5. The results reveal the most significant zones of inhibition against *Staphylococcus aureus* (20.08±0.76), and *Escherichia coli* (22.04±0.17mm) at a concentration of 100 µg/ml Fe_3O_4 nanoparticles. The lowest inhibitory effects were observed in *Escherichia coli* (12.04±0.24) and *Staphylococcus aureus* no inhibition at 25 µg/ml. This indicates the strong biocidal properties of Fe_3O_4 nanoparticles, potentially due to membrane disruption caused by reactive oxygen species, leading to cell death, as suggested by Brayner et al. (2006). These findings confirm the efficacy of Fe_3O_4 nanoparticles against *S. aureus*, and *E. coli*. Naika et al. (2015) The zone of inhibition for the common antibiotic Chloramphenicol was measured to be between 27 and 28 mm (Table 1 and Figure 6).(37)

Table 1. Antibacterial activity ziziphus nummularia leaf extract mediated Fe_3O_4 NPs

Test bacterial pathogens	Zone of inhibition (mm)				
	25 µg	50 µg	75 µg	100 µg	Chloramphenicol (5 µg)
E. coli	12.04±0.24	13.10±0.08	17.06±0.26	20.08±0.76	27.42±0.05
S. aureus	-	16.34±0.12	19.15±0.19	22.04±0.17	28.08±0.62

The values are expressed in the mean ± standard deviation of three replicates.

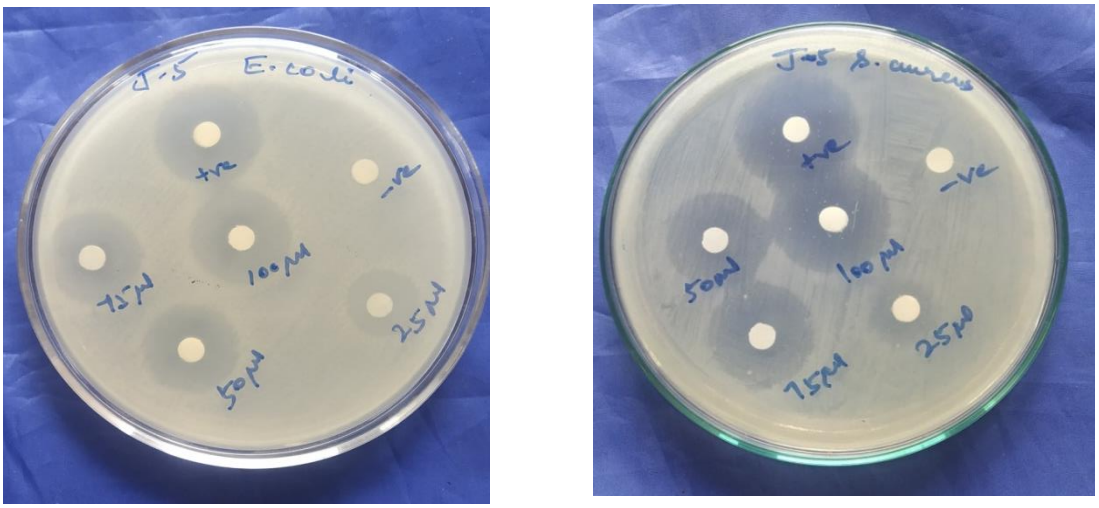


Figure.6. Antibacterial activity of Fe_3O_4 NPs using ziziphus nummularia leaf extract against Gram-negative and Gram –positive bacteria.

CONCLUSION

Fe_3O_4 nanoparticles were synthesized using a simple and cost-effective method involving *Ziziphus nummularia* leaf extract. XRD and FTIR analyses confirmed the successful formation of the Fe_3O_4 nanoparticles. Morphological studies using FESEM revealed semi-spherical structures with noticeable agglomeration. Additionally, VSM analysis confirmed the superparamagnetic nature of the synthesized nanoparticles. Due to the excellent antibacterial activity demonstrated by the nanopowder, it is concluded that Fe_3O_4 nanoparticles synthesized via *Ziziphus nummularia* leaf extract hold promising potential for medical applications.

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