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ANTI MICROBIAL ACTIVITY OF IRON OXIDE NANO PARTICLES USING SELECTED MEDICINAL PLANT EXTRACT

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Abstract: The investigation focused on synthesizing iron oxide (III) oxide nanoparticles (Fe3O4-NPs) using neem leaf extract, aiming for a straightforward and environmentally sustainable method. The synthesized nanoparticles showed distinct characteristics suitable for various applications, including antimicrobial and antifungal coatings. Transmission Electron Microscopy (TEM) confirmed the properties of the nanoparticles. Phytochemical screening revealed the presence of bioactive compounds in the synthesis process. The examination of antibacterial activity showed the effectiveness of Fe3O4 against microbial strains. This study lays the groundwork for environmentally sustainable antibacterial solutions with diverse applications.

Keywords: Iron oxide nanoparticles (Fe3O4 NPs), Medicinal plant extracts, Antimicrobial activity, Transmission electron microscopy (TEM).

Introduction

In recent years, the rise of antimicrobial resistance (AMR) has presented a major global health issue, prompting the investigation of alternative antimicrobial agents. Utilising the distinctive characteristics of nanoparticles, nanotechnology has become a promising solution in the battle against AMR [1]. These tiny particles possess a remarkable surface area-to-volume ratio and heightened reactivity, making them an ideal candidate for this purpose. Iron oxide nanoparticles (IONPs) have gained significant interest for their biocompatibility, affordability, and straightforward synthesis [2]. In recent years, there has been growing interest in utilising plant extracts as reducing and stabilising agents in the synthesis of IONPs. This approach is particularly appealing due to its sustainability and eco-friendly nature. Iron oxides that are not functionalized often degrade and leach, causing them to be biocompatible and prone to clumping together due to magnetic dipole-dipole attraction. Consequently, their stability and dispersity are compromised [3]. As a result, their usefulness is limited. It's important to be aware that the colloidal suspension of iron oxide, particularly magnetite, can undergo oxidation when exposed to air, resulting in a decrease in magnetism [4]. To enhance the dispersibility of iron oxides in a biological system, one can improve the materials by incorporating both organic and inorganic surfactants. Various surfactants are used in this context, such as biopolymers like nucleic and citric acids, albumin, collagen, dextran, chitosan, alginate, starch, chitin, lignin, gelatin, ethylcellulose, and liposomes [5]. Many medicinal plants have been studied for their antimicrobial properties, including neem,

turmeric, aloe vera, and garlic, among others. These plants have a wide range of bioactive compounds that have been discovered to possess antimicrobial properties. These compounds have the ability to disrupt microbial cell membranes, inhibit cell wall synthesis, or interfere with cellular metabolism [6]. Plant extracts have long been recognised for their antimicrobial properties, which have been extensively studied in traditional medicine. Many research studies have confirmed their effectiveness in combating various pathogens. The combination of IONPs with medicinal plant extracts is intriguing, as it has the potential to enhance the antimicrobial activity of both components through synergistic effects. In addition, the incorporation of plant extracts can help decrease the toxicity of nanoparticles, thus enhancing their safety for various biomedical uses [7]. Plant extracts are frequently employed in the creation of IONPs. Through a careful scientific process, iron salts are reduced in the presence of the plant extract, resulting in the formation of nanoparticles that are highly stable. Through careful manipulation of the reaction parameters, including pH, temperature, and reaction time, it is possible to precisely regulate the size, shape, and surface properties of the nanoparticles. Once the synthesis process is complete, it becomes feasible to evaluate the antimicrobial activity of the IONPs using well-known microbiological techniques like agar diffusion assays and determining the minimum inhibitory concentration (MIC) [8]. The antibacterial properties of nanoparticles, such as iron oxide nanoparticles (IONPs), can be linked to several factors, including their small size, large surface area-to-volume ratio, and unique physicochemical characteristics [9]. Nanoparticles exhibit distinctive characteristics that allow them to interact with microbial cells in different ways compared to conventional antibacterial drugs. Consequently, they demonstrate effectiveness against a broad spectrum of microorganisms, including bacteria, fungus, and viruses [10]. It has been observed that nanoparticles possess the ability to induce damage to microbial cell membranes, thereby exhibiting a notable antibacterial effect. It has been observed that nanoparticles possess the remarkable capability to penetrate the cell membrane, thereby compromising its structural integrity [11]. This phenomenon leads to the subsequent release of intracellular substances, ultimately culminating in the demise of the cell. The method under investigation demonstrates remarkable efficacy against bacteria, primarily attributed to the relatively simple structure of their cell membranes when compared to eukaryotic cells. It has been observed that nanoparticles possess the remarkable capability to disrupt enzyme activity and impede crucial metabolic pathways, thereby causing interference with the metabolic processes of microbial cells. Ultimately, the combination of IONPs and medicinal plant extracts holds immense promise for developing groundbreaking antimicrobial agents [12]. By harnessing the antimicrobial properties of both components, an impressive synergy can be achieved, leading to increased effectiveness against a wide range of pathogens. Furthermore, the use of plant extracts as reducing and stabilising agents offers a sustainable and eco-friendly alternative to traditional synthesis methods [9]. Further research is required to thoroughly examine the possibilities of this approach and its potential in the fields of biomedicine and the environment.

Materials & Methods

Characteristics of synthesized Fe3O4 nanoparticles: Iron oxide nanoparticles (IONPs) produced from neem leaf extract exhibited unique properties suitable for various applications. Their consistent size and spherical shape made them ideal for drug delivery and imaging. Primarily composed of magnetite (Fe3O4), these nanoparticles possessed magnetic properties and stability. They were superparamagnetic, meaning they could be magnetized in a magnetic field and lose magnetism when the field was removed, crucial for applications like magnetic targeting and hyperthermia. Surface functionalization with molecules or coatings could enhance their stability, biocompatibility, and targeting ability for drug delivery or imaging. Although further research on biocompatibility is necessary before clinical use, neem leaf extract-synthesized IONPs generally showed biocompatibility. The combined antimicrobial action of neem extract and IONPs could benefit applications such as antimicrobial coatings and wound healing. Proper surface functionalization and storage conditions are essential for maintaining the stability of synthesized IONPs for long-term use. While neem leaf extract-synthesized IONPs hold promise for biomedical, environmental, and other applications, additional studies are needed to optimize their properties.

Bacterial growth conditions:
The growth conditions for Escherichia coli and Bacillus subtilis were fine-tuned to achieve reliable and consistent results. E. coli cultures were cultivated in Luria-Bertani (LB) broth at 37°C with constant shaking at 180 rpm to maintain aeration. For B. subtilis, cultivation was performed in nutrient broth at 30°C under similar shaking conditions to facilitate aerobic growth. Both bacterial strains were grown until they reached the mid-logarithmic phase, ensuring active cellular division. Growth was monitored by measuring the optical density at 600 nm (OD600). These optimized conditions created a favorable environment for the respective bacteria, ensuring reproducibility in experimental results.

In order to conduct this experimental procedure, nutrient agar plates are meticulously prepared. The primary objective of this investigation is to assess the impact of these nanoparticles on the growth of Escherichia coli (E. coli) and Bacillus subtilis bacterial strains. The dissolution of nutrient agar powder in distilled water is a common method employed to generate an agar solution that is abundant in nutrients. The aforementioned solution is subsequently transferred into sterile Petri dishes and allowed to undergo the process of solidification. The incorporation of Fe3O4 nanoparticles (NPs) into the agar prior to solidification leads to the formation of plates with varying concentrations of Fe3O4 NPs. As part of the experimental procedure, a suspension of E. coli is prepared. This involves inoculating the agar plates with the bacterial culture. To ensure sterility, the agar plates are then subjected to autoclaving. Following the cooling of the plates, the introduction of E. coli is conducted with utmost caution utilising a sterile loop. The growth of Escherichia coli (E. coli) is subsequently monitored in a meticulous manner in order to investigate the potential effects of iron (III) oxide nanoparticles (Fe3O4 NPs) on bacterial proliferation.

Antimicrobial activity: The antimicrobial properties of iron (III) oxide nanoparticles (Fe3O4 NPs) were evaluated against Escherichia coli (a Gram-negative bacterium) and Bacillus subtilis (a Gram-positive, nonpathogenic bacterium). Fe3O4 NPs were synthesized and characterized to determine their size, morphology, structure, and optical properties.

To investigate their antimicrobial activity, nutrient agar plates were prepared by dissolving nutrient agar powder in distilled water to create a nutrient-rich medium. The agar solution was sterilized, poured into Petri dishes, and allowed to solidify. Broth cultures of E. coli and B. subtilis were spread uniformly across the surface of the solidified agar plates to serve as the test organisms.

Sterile discs (6 mm diameter) prepared from Whatman filter paper were impregnated with varying concentrations of Fe3O4 NPs and placed onto the agar plates. The plates were then incubated under optimal growth conditions, and bacterial growth was monitored. The impact of Fe3O4 NPs on bacterial proliferation was assessed by measuring the zones of inhibition around the discs.

Statistical analysis was conducted to determine the significance of the results, including the minimum inhibitory concentration (MIC) of Fe3O4 NPs. The findings provide valuable insights into the antimicrobial potential of Fe3O4 NPs and their applicability in controlling bacterial growth. This study underscores the relevance of Fe3O4 NPs as a potential antimicrobial agent.

Effect of Fe3O4 membrane activity: The investigation into the impact of Fe3O4 nanoparticles (NPs) on bacterial membrane activity is of considerable importance and is currently a subject of intense scientific inquiry. It has been reported in the literature that Fe3O4 nanoparticles (NPs) possess antimicrobial properties. These properties have the potential to impact the structural and functional integrity of bacterial membranes. Fe3O4 nanoparticles (NPs) have been found to exhibit antimicrobial properties, and one of the ways they achieve this is through the production of reactive oxygen species (ROS) upon exposure to biological environments. The reactive oxygen species (ROS) mentioned in the statement have the potential to induce oxidative stress within bacterial cells. This oxidative stress can result in detrimental effects on various cellular components, including the bacterial membrane. Moreover, it has been observed that Fe3O4 nanoparticles (NPs) have the capability to directly interact with the bacterial membrane, leading to the disruption of its structure and subsequent impairment of its normal function. The aforementioned disruption has the potential to result in the release of intracellular components, ultimately culminating in cellular demise. Moreover, it has been documented that Fe3O4 nanoparticles (NPs) possess magnetic hyperthermia characteristics, whereby they are capable of producing thermal energy upon exposure to an oscillating magnetic field. The hyperthermic effect observed in this context has the potential to significantly contribute to the occurrence of bacterial membrane damage and subsequent cell death. Upon careful examination, it becomes evident that the impact of Fe3O4 NPs on bacterial membrane activity is a multifaceted phenomenon that

encompasses various mechanisms. Additional investigation is required in order to gain a comprehensive understanding of the antimicrobial mechanisms exhibited by Fe3O4 NPs, as well as their potential utility in addressing bacterial infections.

Results and Discussion

The TEM pattern of the synthesized iron oxide nanoparticles (IONPs) confirmed the presence of the Fe3O4 Fe3O4 NPs) as shown in Figure 4. This crystalline structure is essential for their magnetic properties, suitable for applications such as drug delivery and imaging. Transmission electron microscopy (TEM) images showed that the photosynthesized iron oxide nanoparticles were predominantly spherical with a consistent size distribution, suitable for various applications.

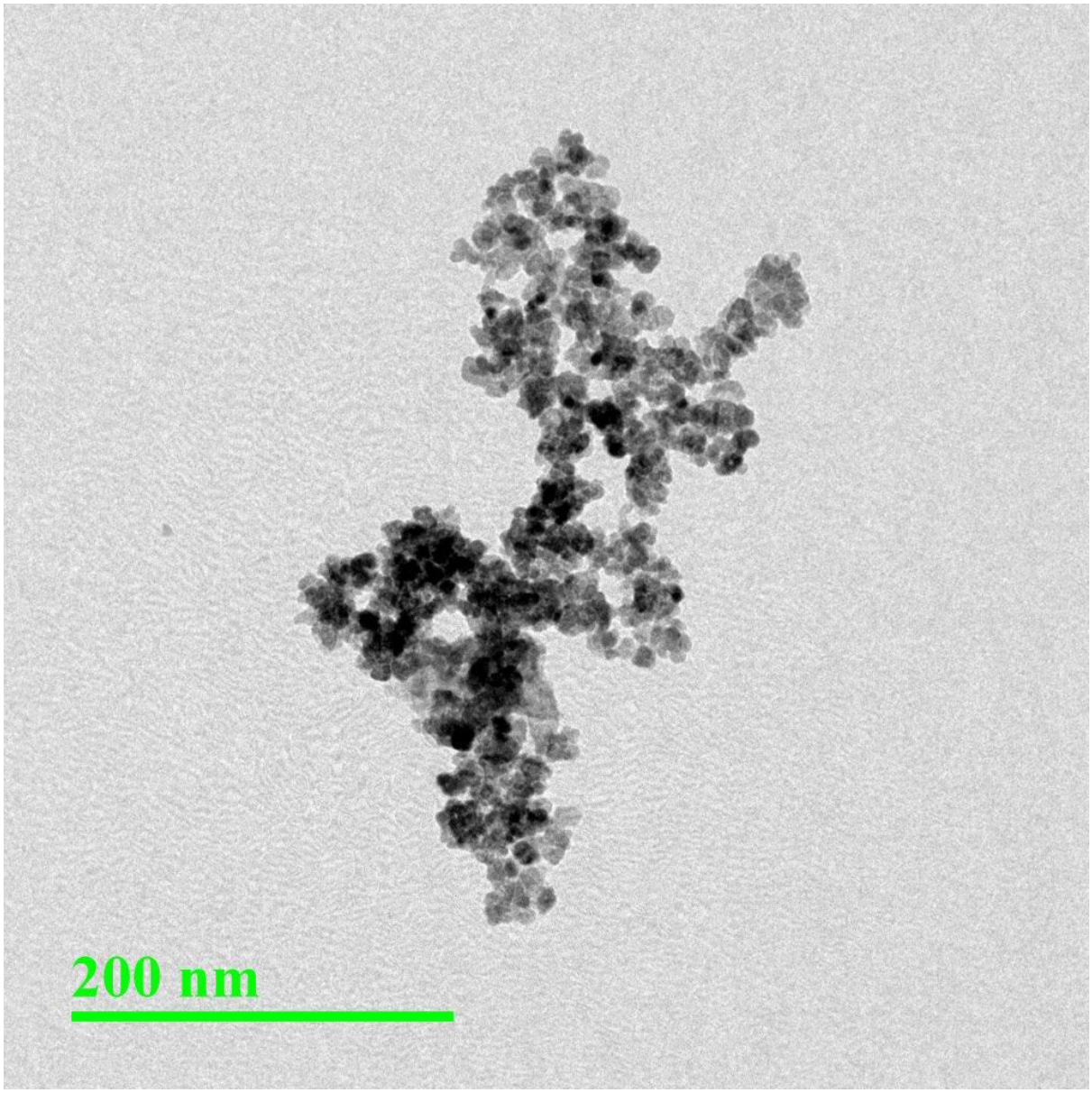
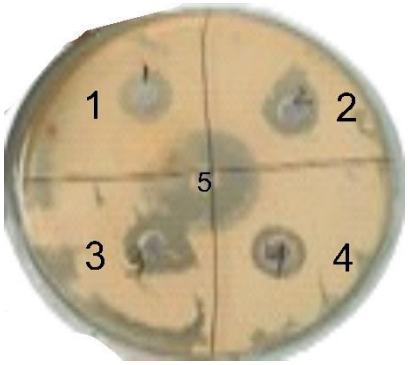


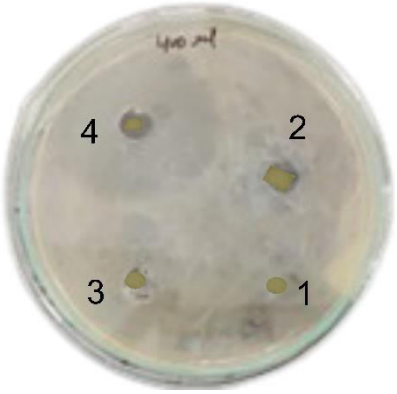
Figure 4 - TEM images of the Phyto synthesized iron oxide NPs

The antimicrobial activity of the synthesized Fe3O4 nanoparticles was assessed against both Gram-positive and Gram-negative bacteria. The disc and well diffusion method demonstrated in Figure 5 & 6 was used to test the nanoparticles' antimicrobial activity against Escherichia coli (E. coli) and Bacillus subtilis bacterial strains.



Bacillus subtilis Test (Figure 5a)

Bacillus subtilis Control (Figure 5b)



E. coli Test (Figure 6a)

E. coli Control (Figure 6b)

Figure 5 & 6 – Iron oxide (Fe3O4) NPs (disc diffusion method)

The disc diffusion assay for Escherichia coli (Figure 5) demonstrated the formation of distinct zones of inhibition surrounding the discs impregnated with Fe3O4 nanoparticles. The inhibitory effect was concentration-dependent, with larger zones of inhibition observed at higher concentrations of Fe3O4 nanoparticles, indicating enhanced antibacterial activity with increasing nanoparticle concentration.

Antimicrobial activity assays revealed significant antibacterial effects of Fe3O4 NPs against Bacillus subtilis and Escherichia coli. The disc diffusion assay demonstrated clear zones of

inhibition, with larger zones observed at higher nanoparticle concentrations, indicating a dose-dependent antibacterial response. This effect aligns with the production of reactive oxygen species (ROS) by Fe3O4 NPs, which disrupt bacterial cellular components and ultimately cause cell death. The efficacy against both Gram-positive and Gram-negative strains underscores the versatility of Fe3O4 NPs as effective antimicrobial agents.

Table 1- Antimicrobial Activity of Fe3O4

S.no	Compounds	Concentration of compounds		Zone of Inhibition (Bacillus subtilis) In mm	Antimicrobial activity of compound	Zone of Inhibition (Escherichia coli) In mm		Disc Diameter (in mm)	Antimicrobial activity of compound	Control (DMSO)
1	Fe3O4 Green Nanoparticles	5	2 mg	18	+++++	4	22	6	++++++	No activity found
		4	1 mg	14	++++	3	18	6	+++++	
		3	.5 mg	14	++++	2	12	6	+++	
		2	.25 mg	13	++++	1	9	6	++	
		1	.125 mg	12	+++					

The antimicrobial activity of Fe3O4 nanoparticles (Fe3O4 NPs) was evaluated against Bacillus subtilis and Escherichia coli using the disc diffusion method. The inhibitory effect of Fe3O4 NPs was quantified by measuring the diameter of the zones of inhibition in millimetres at concentrations of 0.125 mg, 0.25 mg, 0.5 mg, 1 mg, and 2 mg.

At a concentration of 2 mg, 1mg, 0.5 mg and 0.25 mg Fe3O4 NPs exhibited highest antimicrobial activity against Bacillus subtilis, with a zone of inhibition measuring 18 mm (+++++), 14 mm (++++), 14 mm (++++), 13 mm (++++) and 12 mm (+++) respectively. In contrast, E. coli showed a more pronounced response, with zones of inhibition measuring 22 mm at 2 mg, 18 mm at 1 mg, 12 mm at 0.5 mg, and 9 mm at 0.25 mg. Notably, Fe3O4 NPs displayed significant antimicrobial activity against Bacillus subtilis and E. coli at all tested concentrations, with the inhibition zones showing a dose-dependent trend.

This difference in activity suggests a selective antimicrobial effect of Fe3O4 NPs, with E. coli demonstrating higher sensitivity compared to B. subtilis.

A comparative analysis of the two bacterial strains revealed differences in susceptibility, with Escherichia coli exhibiting larger zones of inhibition. This variation may be attributed to differences in the structural composition and permeability of the bacterial cell walls, which influence the interaction and uptake of Fe3O4 NPs. analysis through TEM measurements confirmed the high purity and quality of the synthesized Fe3O4 NPs, ensuring reproducibility and consistent antimicrobial performance.

These findings establish Fe3O4 nanoparticles as a viable and effective antimicrobial agent, with potential applications in controlling bacterial infections and mitigating microbial contamination.

The antimicrobial activity evaluation revealed significant antibacterial properties of Fe3O4 nanoparticles. Using the disc diffusion assay, Fe3O4 NPs demonstrated notable inhibitory effects against Bacillus subtilis, with a maximum zone of inhibition measuring 18 mm observed at a concentration of 2 mg/ml. Inhibitory zones were detected at concentrations 1, 0.5 and 0.125 mg/mL for B. subtilis (Figure 5a).

Similarly, substantial antibacterial activity was recorded against Escherichia coli, with inhibition zones of 9 mm at 0.25mg/mL and increasing to 22 mm at 2 mg/mL (Figure 6a). These findings confirm the concentration-dependent antimicrobial efficacy of Fe3O4 NPs, highlighting their potential as effective antibacterial agents against both Gram-positive and Gram-negative bacterial strains.

No antimicrobial activity was observed in the DMSO-treated control group for either bacterium, confirming the specificity of Fe3O4 NPs in inhibiting bacterial growth (Figure 5b & 6b). These findings highlight the varying degrees of antimicrobial activity exhibited by Fe3O4 NPs, with substantial efficacy against E. coli and B. subtilis. The results underscore the potential of Fe3O4 NPs as effective antimicrobial agents, against Gram positive and Gram-negative bacteria.

Conclusion

This study successfully demonstrated the green synthesis of Fe3O4 nanoparticles using neem leaf extract, providing a sustainable and environmentally friendly approach to nanomaterial production. The bioactive compounds in neem played a crucial role in the synthesis, imparting unique properties to the nanoparticles. Characterization using TEM confirmed the nanoparticles' desirable morphology, crystallinity, and properties, while antibacterial activity tests showcased their effectiveness against microbial strains. These findings underscore the potential of Fe3O4-NPs as a versatile material for antimicrobial and antifungal applications. The research paves the way for developing eco-friendly solutions for healthcare, environmental protection, and other industrial applications.

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Author contribution:

SC: Drafting & Editing

MIN: Analysis & Writing
SZ: Conceptualization, data analysis, writing
FS: Editing
KKA: Supervision & Editing

Conflict of Interest:
Authors declare that there is no conflict of interest among the authors.

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