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Green Synthesis of Iron Oxide Nanoparticles using *Jatropha tanjorensis* Aqueous Leaf Extract and Evaluation of Their Antimicrobial Activity

Rabiu Sabo[1], Salihu Suleiman[2], Yahaya A. Adamu[3]

[1,2,& 3]Department of Chemistry,
Federal University Dutse

Email: salihu.s@fud.edu.com

Abstract
The need for environmentally benign nanoparticle production techniques is increasing as a result of existing technologies' negative effects on the environment. An environmentally responsible and sustainable method of producing iron oxide nanoparticles (IONPs) is the green synthesis, which uses an aqueous leaf extract from Jatropha tanjorensis. Because of its rich phytochemical content, an aqueous leaf extract of Jatropha tanjorensis (J.T.) was used as a reducing and stabilizing agent to enable the successful environmentally friendly synthesis of stable and evenly dispersed iron oxide nanoparticles (IONPs). A 0.6 M ferric chloride solution was combined with prepared Jatropha aqueous leaf extract in an optimal 1:4 ratio, causing the solution to become brown instead of green. X-ray diffraction (XRD), scanning electron microscopy (SEM), Fourier transform infrared (FTIR) spectroscopy, and ultraviolet-visible (UV-Vis) spectrophotometry were used to characterize the green-synthesized iron oxide nanoparticles (IONPs). According to these investigations, IONPs were spherically formed, crystalline, naturally stabilized, and within nanoscale dimensions. Gram-positive and gram-negative bacteria and fungi were used to test the synthetic IONPs' antimicrobial potential. Escherichia coli and Staphylococcus aureus were found to have an obvious zone of inhibition, however Candida albicans did not exhibit a defined zone. Iron oxide nanoparticles can be thought of as broad-band antibiotics because they were effective against both gram-positive and gram-negative bacteria. As a result, it can be applied as an antibacterial agent to prevent detrimental bacteria from growing. Such findings provide a promising substitute for traditional chemical synthesis techniques and add to the expanding corpus of research on green nanotechnology and its uses in environmental science and medicine.

Keyword: Antimicrobial activity, green synthesis, iron oxide nanoparticles, *Jatropha tanjorensis* leaf extract, nanotechnology.

INTRODUCTION
The rapid advancement of nanotechnology has significantly increased the demand for nanoparticles due to their unique properties and diverse applications. This growing demand presents challenges, such as the need for scalable and cost-effective production methods, ensuring the uniformity and stability of nanoparticles, and addressing potential environmental and health risks. Nanotechnology is transforming a wide range of industries, including healthcare, energy, and electronics, due to its ability to modify materials at the atomic or molecular level (Logothetidis, 2012). Richard Feynman first proposed the idea of nanotechnology in his well-known 1959 lecture, "There's Plenty of Room at the Bottom," where he discussed the possibility of manipulating individual atoms and molecules. This field

Author for Correspondence

has grown immensely since the pioneering work on carbon nanotubes and fullerene structures in the early 1990s (Iijima, 1991). For nanoparticles to have such a broad range of uses, their production is essential. Methods like chemical vapor deposition (CVD) are employed to create various nanostructures and carbon nanotubes (Bansal *et al.*, 2016). Since they have special optical, electrical, and mechanical capabilities, nanomaterials like metal nanoparticles and quantum dots are being explored in great detail (Ayanda *et al.*, 2024). The majority of iron oxide nanoparticles (IONPs) were synthesized using co-precipitation prior to the usage of green techniques, where iron salts (usually Fe^{2+} and Fe^{3+}) precipitate in an alkaline solution to generate iron oxide nanoparticles (Laurent *et al.*, 2008).

The green synthesis of nanomaterials has drawn interest recently as a viable substitute for traditional techniques, tackling concerns about stability, cost, and environmental impact. Unlike conventional methods, green synthesis does away with complicated synthesis pathways and uses biological resources (especially plants) to synthesise nanomaterials with therapeutic qualities (biocompatibility and low toxicity) in large part, often used in biomedicine, agriculture, and environmental remediation (Huston *et al.*, 2021; Mughal *et al.*, 2021; Ahmed *et al.,* 2022; Malik *et al.,* 2023).

Iron oxide nanoparticles have exceptional properties that make them adaptable for a variety of pharmaceutical applications, particularly in the areas of drug delivery, where they can release pharmaceutical substances in a controlled and targeted manner to increase therapeutic effectiveness while lowering side effects; their magnetic properties allow for clearer and more detailed pictures for diagnostic purposes that are essential in improving the accuracy of disease detection and monitoring; hyperthermia therapy, which uses heat to treat certain conditions; and cancer treatment, among other biomedical fields (Attia *et al.*, 2022; Montiel-Schneider *et al*., 2022).

New antimicrobial drugs must be developed since antimicrobial resistance (AMR) poses a serious danger to public health worldwide. According to the World Health Organization (WHO, 2020), it is the capacity of microorganisms to endure and withstand exposure to antimicrobial medications, endangering the efficacy of infection therapy. Antimicrobial resistance is caused by a variety of factors, such as inadequate infection prevention and control practices and the overuse and abuse of antibiotics in agriculture and healthcare. However, by bringing sustainable techniques like green synthesis, nanotechnology has had a big impact on medical inorganic chemistry. By using eco-friendly practices, nanotechnology is making a big impact on how we treat illnesses, making it safer for both humans and the environment (Kazemi *et al.*, 2023). This study aims to develop an environmentally friendly protocol for synthesizing IONPs using fresh aqueous leaf extract from *Jatropha tanjorensis* and to evaluate their antimicrobial activity against pathogenic bacterial strains.

MATERIALS AND METHODS

Collection of Plant Material

Jatropha tanjorensis (Figure 1) leaves were collected from Minna, Nigeria (longitude 6°33 E and latitude 9°27 N) and subsequently identified in the Botany Unit, Federal University Dutse. Its identification key is GDT I JSTOR. [11] 03. The fresh leaves were used for all experimental procedures.

Figure 1: ***Jatropha tanjorensis*** **Leaf Extracts**

Materials

All reagents used were of analytical-grade purity. Iron(III) chloride hexahydrate ($FeCl_3.6H_2O$) was purchased from Toteil Limited Nigeria.

Preparation of Leaf Extract

Jatropha tanjorensis leaf extract was prepared by adopting procedures by Korbekandi *et al*. (2016) and Habila *et al*. (2021) after slight modification.

Synthesis of Iron Oxide Nanoparticles

Synthesis of iron oxide nanoparticles was carried out by modifying and adopting the methods described by Aisida *et al*. (2020) and Kiwumulo *et al*. (2022). Approximately, 60 mL of 0.6 M $FeCl_3.6H_2O$ solution was mixed with 15 mL of the leaf extract, followed by the addition of 2 M NaOH solution to adjust the pH. The resulting solution was placed on a magnetic stirrer hot plate at 70 °C for 50 minutes. IONPs were gradually obtained as the solution changed from green to brown. Finally, it was centrifuged at 4000 rpm for 10 minutes. The obtained nanoparticles were washed with distilled water and dried at room temperature.

Characterization of Nanoparticles

The optical properties were examined via UV-vis spectrophotometry; Fourier-transform infrared (FTIR) spectroscopy was employed in identifying the functional groups present on the nanoparticle surface. To verify the crystalline structure and estimate the crystallite size of the nanoparticles, X-ray diffraction (XRD) was performed using an XRD machine (model ARL'XTRA X-ray) at Umaru Musa Yar Adua University, Katsina, Nigeria. Morphology was investigated through scanning electron microscopy (SEM) conducted at the university using a Phenom World PRO: X 800-07334 scanning electron microscope.

Antimicrobial Activity

The antimicrobial activity of the synthesized iron oxide nanoparticle was carried out with the help of a certified laboratory personnel at Rasheed Shekoni Federal University Teaching Hospital's Laboratory, Dutse, Jigawa State, Nigeria. JT-IONPs were screened for their antimicrobial activities against some clinically isolated G^{+ve} bacteria, G^{-ve} bacteria, and fungus by the disc diffusion method.

RESULTS AND DISCUSSION

Results

The findings of the research are presented below.

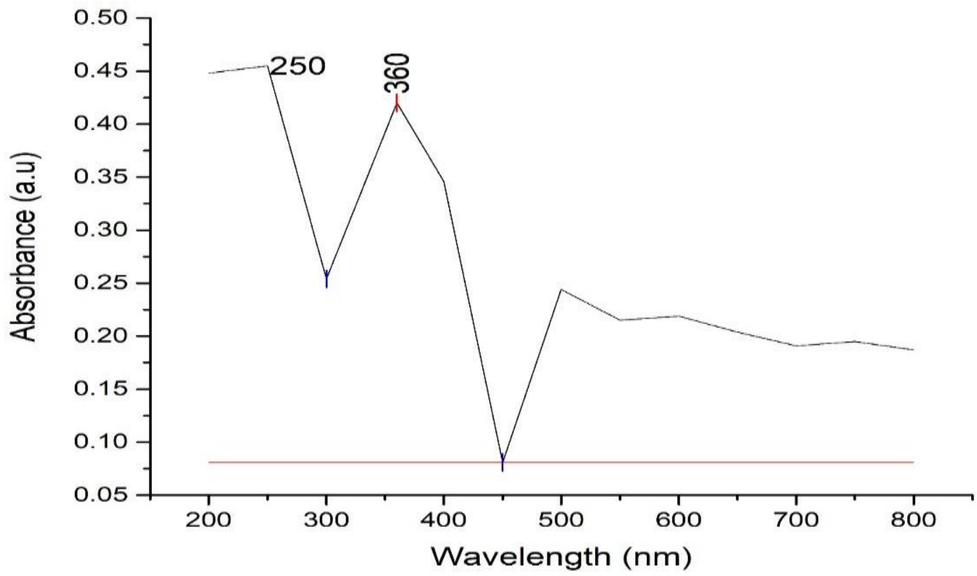

Figure 2: UV-Visible Graph of Iron Oxide Nanoparticles

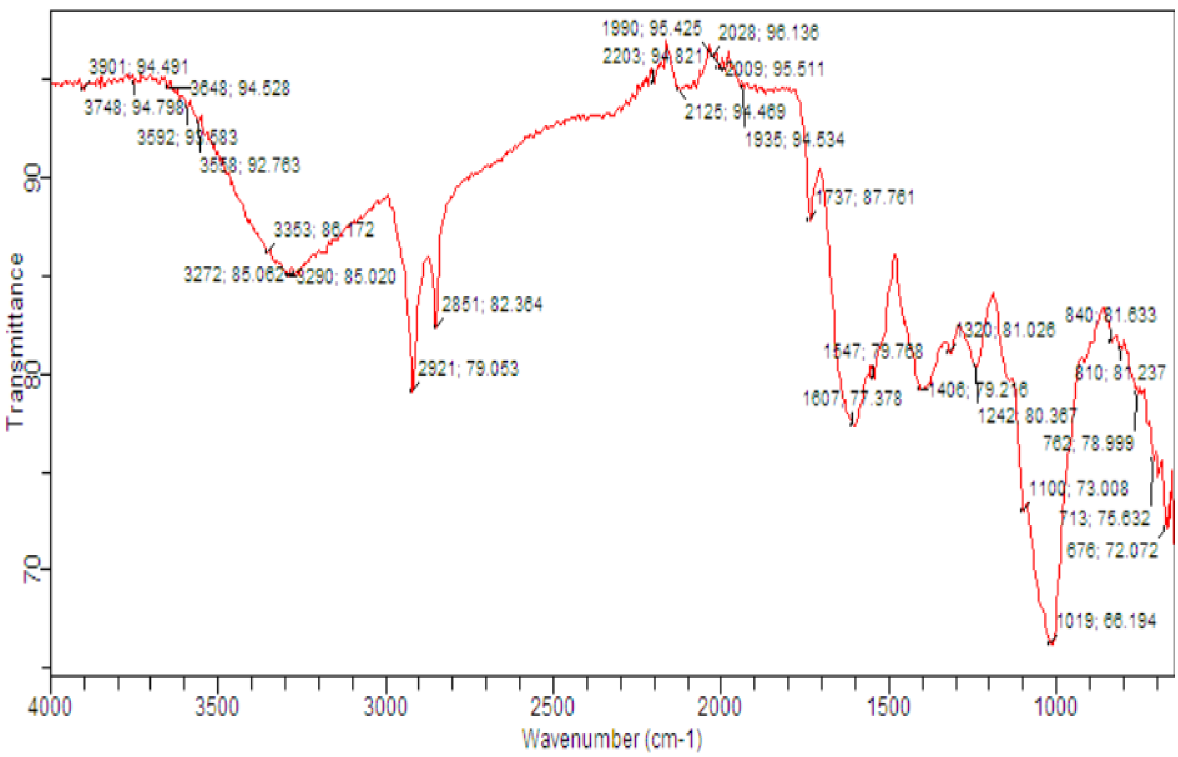

Figure 3a: FTIR Spectrum of *Jatropha tanjorensis* Leaves

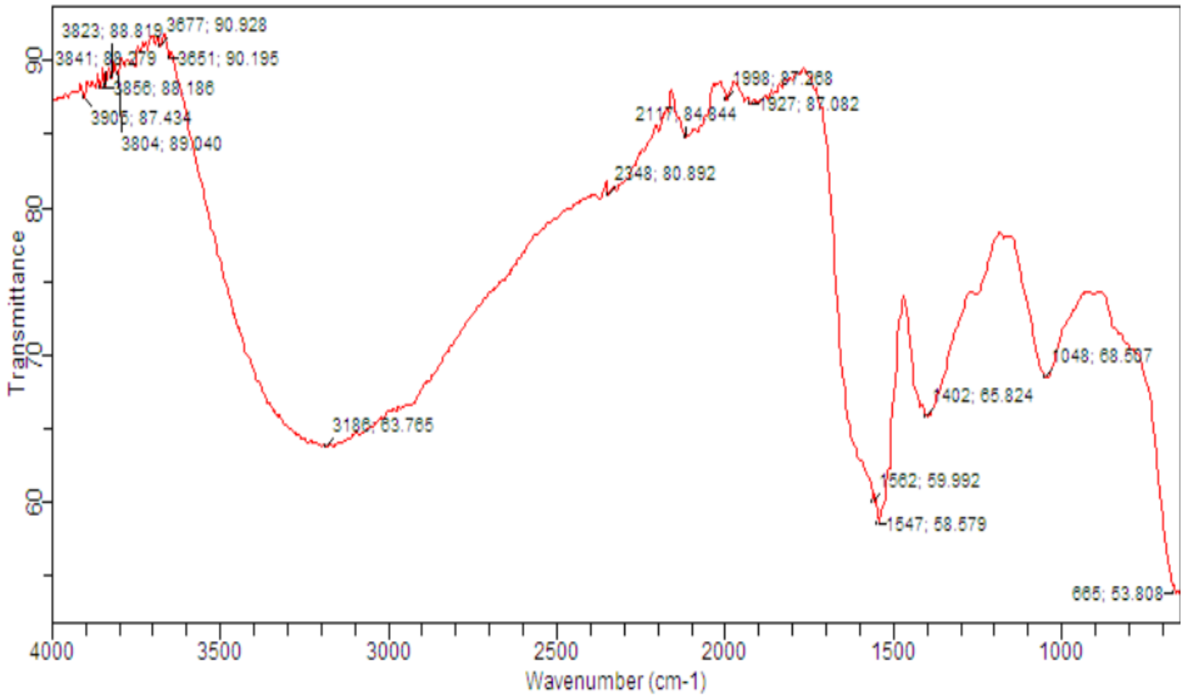

Figure 3b: FTIR Spectrum of Iron oxide nanoparticles

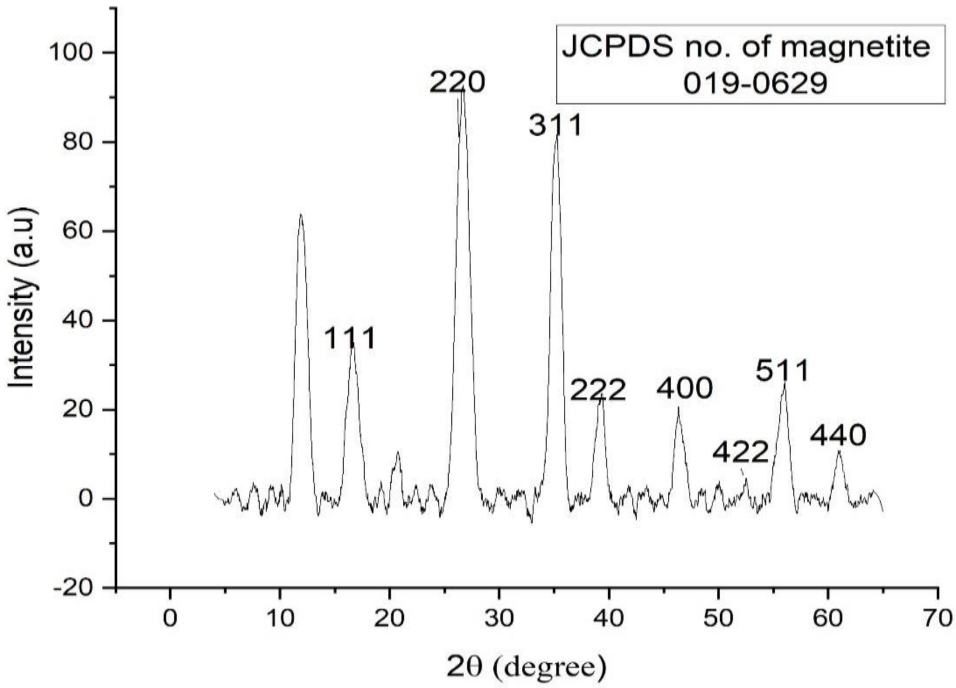

Figure 4: X-ray Diffraction Pattern of Iron Oxide Nanoparticles

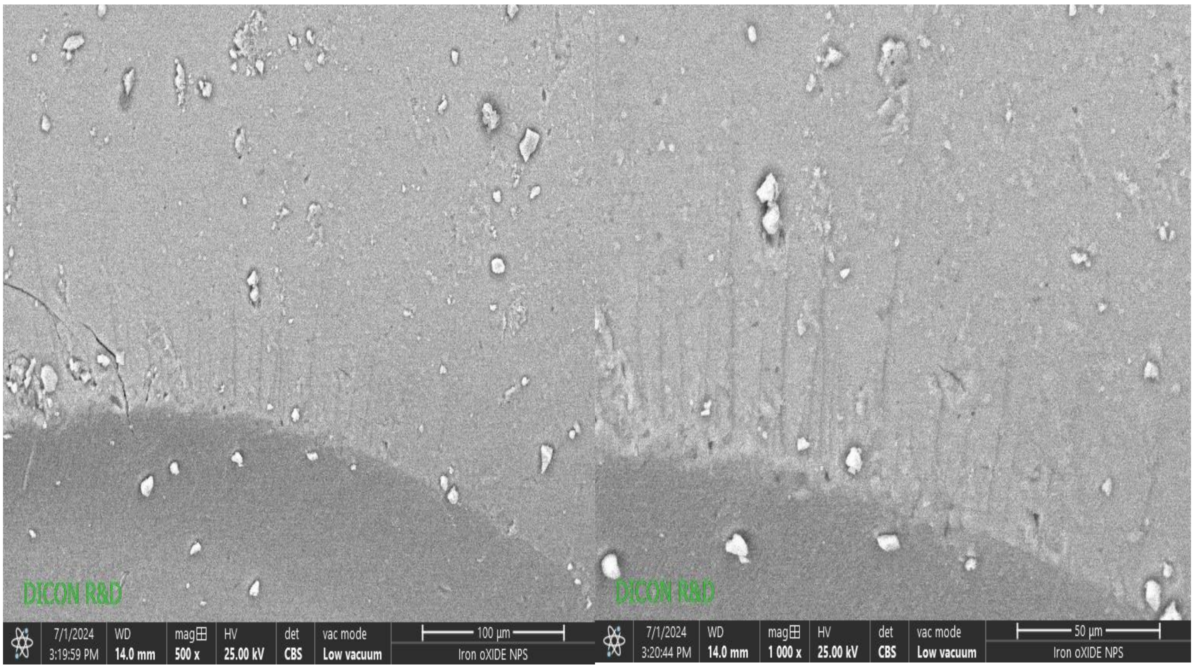

Figure 5: Scanning Electron Microscope Image of Iron Oxide Nanoparticles

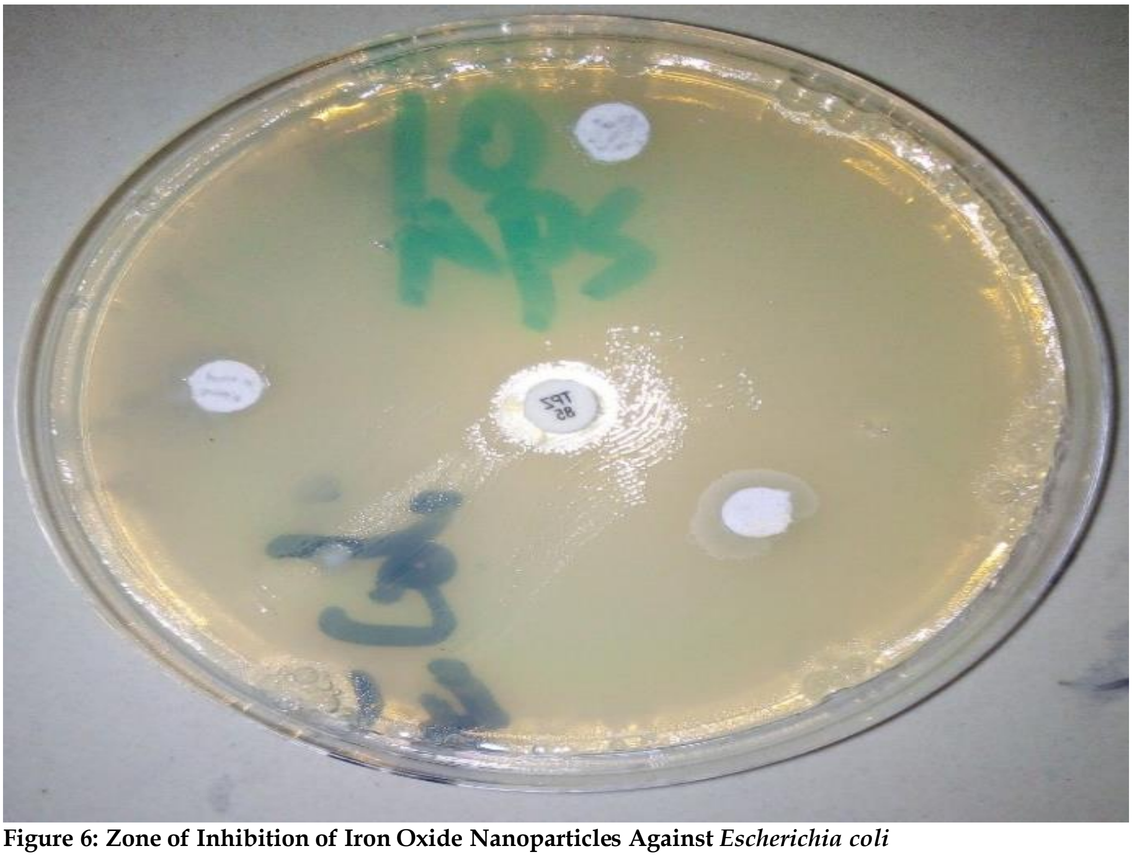

Figure 6: Zone of Inhibition of Iron Oxide Nanoparticles Against *Escherichia coli*

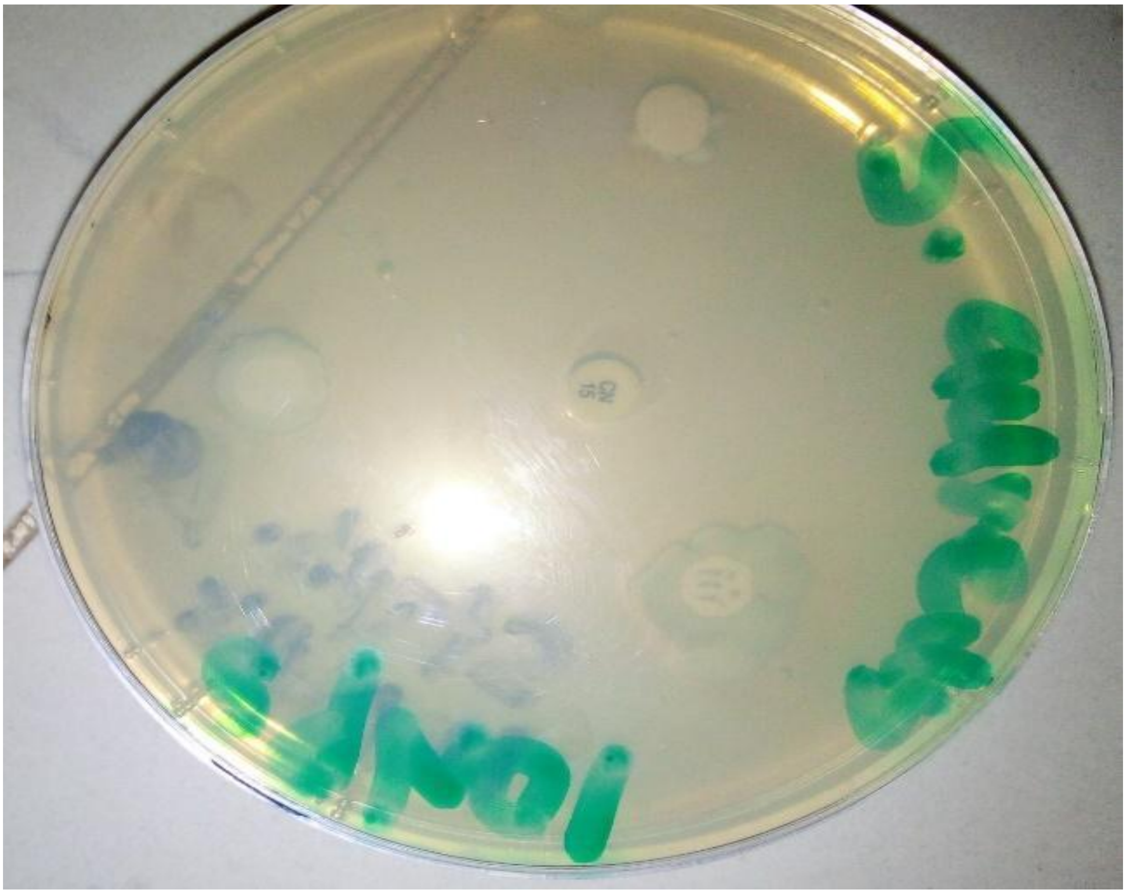

Figure 7: Zone of Inhibition of Iron Oxide Nanoparticles Against *Staphylococcus aureus*

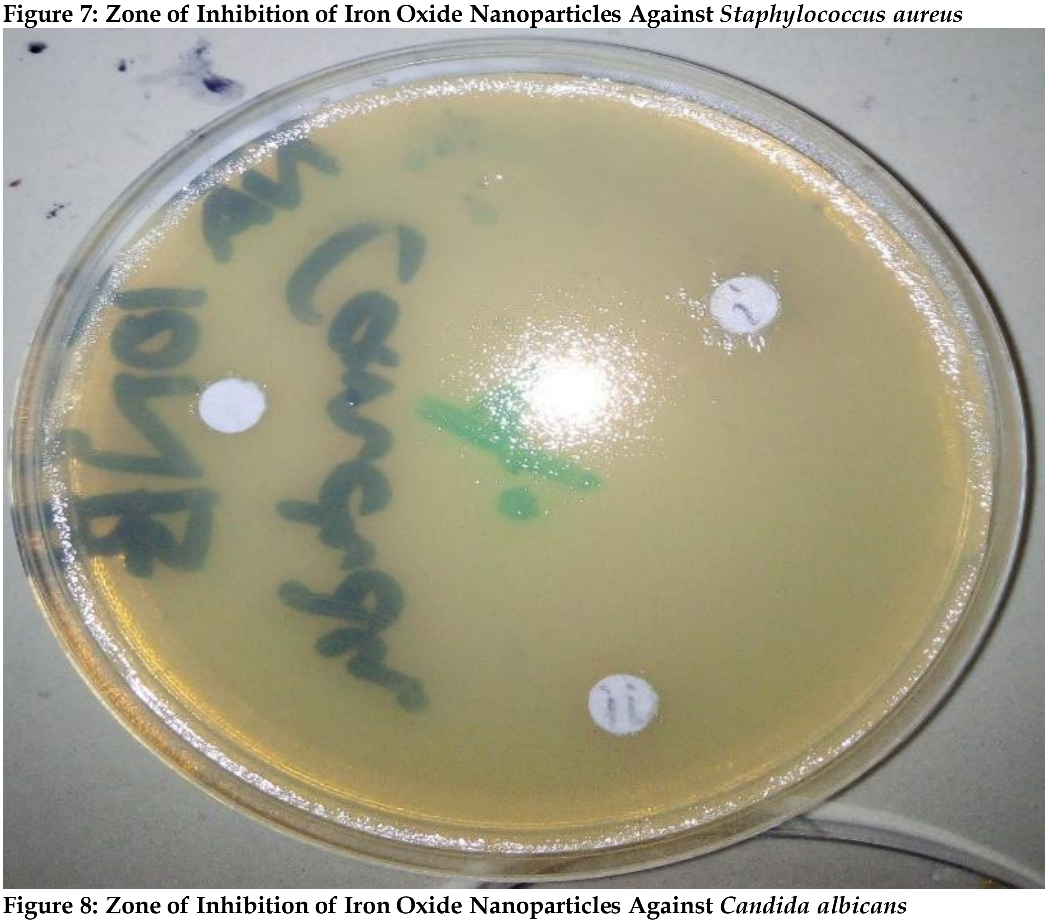

Figure 8: Zone of Inhibition of Iron Oxide Nanoparticles Against *Candida albicans*

Table 1: Summary of Antimicrobial Activity Result of IONPs

S/N	Isolates	0.4 mg/mL	0.8 mg/mL	1.2 mg/mL	ZOI and Control
1	*E. coli*	11 mm	11.5 mm	12 mm	10 mm (TPZ)
2	*S. aureus*	8 mm	10 mm	15 mm	-(CN)
3	*C. albicans*	-	-	-	- (None)

CN – Gentamicin TPZ – Piperacillin ZOI – Zone of Inhibition

DISCUSSION

The formation of IONPs was confirmed by a characteristic absorption peak at 250 nm and 360 nm, indicating the photosensitive nature of these nanoparticles (Figure 2). This is in line with the UV-Vis result by Adhikari *et al.* (2022). FTIR analysis revealed absorption bands around 600 cm^{-1}, corresponding to Fe-O bond stretching, while the presence of bands at 3343 cm^{-1} and 1607 cm^{-1} indicated O-H and C=O groups from the leaf extract, suggesting effective capping of the nanoparticles (Figure 3). Similar results were reported by Devi *et al.* (2019) and Mohamed *et al*. (2023). XRD patterns confirmed the crystalline nature of the nanoparticles and crystallite size of magnetite (Fe_3O_4) calculated using Scherrer's equation to be 2.05 nm (Figure 4). Hossen *et al.* (2020) synthesised iron oxide nanoparticles of similar crystallite size. SEM images showed that these IONPs are spherical nanoparticles with heterogeneous surfaces (Figure 5), consistent with previous reports on green-synthesised IONPs (Hossen *et al*., 2020; Abdulsada *et al*., 2023). With regards the antimicrobial activity of the green synthesised Iron oxide nanoparticles, the zone of inhibition was found to be 12 mm for *E. coli*, 15 mm for *S. aureus*, and 15 mm for *C. albicans*. From Table 1, it can be observed that the antimicrobial potency of iron oxide nanoparticles on *S. aureus* is greater than that of *E. coli*. Zhang and Miao (2024) explained this to be attributed to the strength of structure of the cell wall, generation of reactive oxygen species, and variation in surface charge and size.

CONCLUSION

This research successfully demonstrates the green synthesis of iron oxide nanoparticles using *Jatropha tanjorensis* aqueous leaf extract, offering a sustainable alternative to conventional methods. The synthesized Fe_3O_4 NPs exhibited promising antimicrobial activity, representing a promising avenue for addressing the escalating global challenge of antimicrobial resistance, thus suggesting potential applications in medical fields. The study emphasizes the importance of eco-friendly synthesis methods and the role of plant-based extracts in advancing nanotechnology.

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