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Green Synthesis of Iron Nanoparticles Using *Punica granatum* Extract and Evaluation of Their Biological Potential

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Abstract

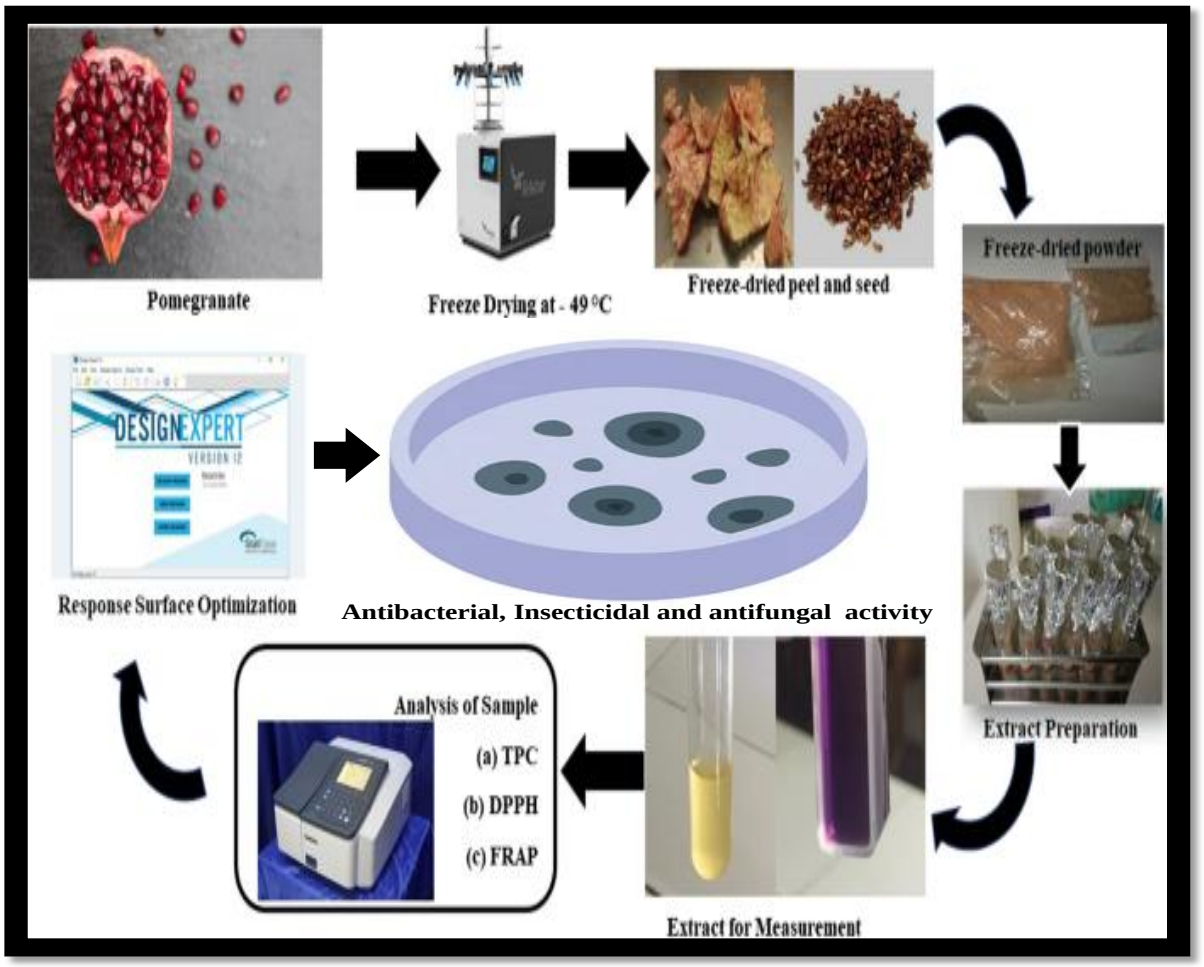
This study investigates the phytochemical composition and biological activities of *Punica granatum* peel extract and green-synthesized iron nanoparticles (FeNPs) were explored. The FeNPs were synthesized using an aqueous extract of *P. granatum* peel, with methanol and deionized water as solvents. Phytochemical analysis revealed the presence of phenols, tannins, alkaloids, flavonoids, and steroids. The FeNPs were characterized by UV-Vis spectroscopy, which showed a distinct absorption peak at 366 nm, indicating the presence of Fe-NPs. Fourier-transform infrared (FT-IR) spectroscopy revealed bands corresponding to phenolic and methyl C-H groups, confirming the role of the extract in reducing $FeCl_3$ to Fe-NPs. Energy dispersive X-ray (EDX) analysis identified iron, carbon, and oxygen, suggesting the formation of iron oxide (Fe_2O_3) nanoparticles. X-ray diffraction (XRD) patterns displayed sharp peaks, confirming the crystalline nature of the Fe-NPs, with additional peaks indicating the presence of carbon. Scanning electron microscopy (SEM) images revealed uniformly distributed, nearly spherical nanoparticles ranging from 19.8 nm to 48 nm in size.

Biological activity tests showed that the peel extract exhibited significant antibacterial effects, inhibiting Staphylococcus aureus (61.53%), Pseudomonas aeruginosa (51.58%), *Klebsiella pneumoniae* (57.54%), *Bacillus pumilus* (48%), *Salmonella Typhi* (33.33%), and *Escherichia coli* (30.43%). The FeNPs demonstrated enhanced antibacterial activity, with inhibition rates of 73.07%, 66.66%, 76.19%, 56%, 40.74%, and 34.78%. Additionally, both the FeNPs and methanolic extract showed notable insecticidal activity against *Tribolium castaneum* (60%), *Carpophilus analis* (70%), and *Rhizopertha dominica* (50%). Phytotoxic effects were observed at higher concentrations, highlighting their potential for antibacterial, insecticidal, and phytotoxic applications

KEYWORDS

Nanoparticles, Antibacterial, Phytotoxic Activity, Insecticidal Activity, Green Synthesis

Graphical Abstract



1.0 INTRODUCTION

Nanotechnology is a rapidly evolving field that involves the manipulation and application of materials at the atomic, molecular, and supramolecular levels. It is defined as the science and engineering of structures, devices, and systems with critical dimensions in the nanometer range, typically under 100 nm [1] . With applications spanning various scientific disciplines such as biology, physics, chemistry, and engineering, nanotechnology has the potential to revolutionize multiple industries, including energy, medicine, electronics, and catalysis [2] . The ability to create materials at the nanoscale enables the development of products with enhanced properties due to the increased surface area, reactivity, and quantum effects that are exhibited by these tiny entities. One of the key aspects of nanotechnology is the ability to design and fabricate nanoparticles that exhibit unique properties compared to bulk materials, which is why they have garnered significant attention in recent years for their potential applications in fields

such as drug delivery, cancer treatment, gene therapy, and environmental remediation [3] .

Among the wide variety of nanoparticles, metal nanoparticles, and in particular iron nanoparticles (FeNPs), stand out due to their excellent chemical, mechanical, and optical properties, which make them suitable for a range of applications, including environmental cleanup and therapeutic purposes. FeNPs are especially useful because of their high surface-to-volume ratio, magnetic properties, and biocompatibility, which make them ideal candidates for use in catalysis, wastewater treatment, and as agents in biomedical applications [4]. The synthesis of FeNPs can be achieved through various methods, including physical, chemical, and biological processes. While chemical and physical methods offer precise control over particle size and morphology, they often involve the use of toxic chemicals and harsh reaction conditions that are harmful to the environment and living organisms. As a result, there has been growing interest in using biological sources, such as plant extracts, to synthesize nanoparticles in a more environmentally friendly manner [5].

The green synthesis of nanoparticles has emerged as a promising alternative to conventional methods. This approach utilizes plant extracts, which contain a variety of bioactive compounds such as polyphenols, flavonoids, and alkaloids, to reduce metal ions and stabilize nanoparticles. This method is cost-effective, sustainable, and does not require toxic chemicals or high-energy input [6]. Plants offer numerous advantages over other biological sources for nanoparticle synthesis, including ease of availability, low cost, and minimal environmental impact. Furthermore, the presence of various phytochemicals in plant extracts can also enhance the biological activity of the resulting nanoparticles, making them suitable for a range of applications in medicine, agriculture, and environmental protection[4].

Among the many plants that have been explored for nanoparticle synthesis, *P. granatum* (pomegranate) has gained considerable attention due to its rich chemical composition and potential therapeutic properties. Native to the Mediterranean region, *P. granatum* has been widely studied for its bioactive compounds, including polyphenols, flavonoids, tannins, and alkaloids, which have been shown to possess antioxidant, anti-inflammatory, antibacterial, and anticancer activities [19][20]. Pomegranate peel, which is often discarded as waste, is particularly rich in these bioactive compounds and has been identified as a valuable resource for nanoparticle synthesis. Previous studies have demonstrated the ability of *P. granatum* peel extract to successfully reduce metal ions and form nanoparticles with desirable properties [7].

The green synthesis of FeNPs using *P. granatum* peel extract involves the use of its phytochemicals as both reducing agents and stabilizing agents. The process is environmentally benign, as it occurs under mild conditions of pH, temperature, and pressure, avoiding the use of hazardous chemicals typically required in traditional methods. Characterization of the synthesized nanoparticles through techniques such as UV-Vis spectroscopy, X-ray diffraction (XRD), and scanning electron microscopy (SEM) reveals the size, morphology, and crystalline structure of the particles, providing insights into their potential applications [8]. The resulting FeNPs have been shown to possess significant antibacterial and antifungal properties, making them suitable for use in medical and agricultural applications.

In addition to their antimicrobial properties, *P. granatum* peel extract and its synthesized FeNPs have been evaluated for other biological activities, including phytotoxicity and insecticidal activity. Pomegranate extract has long been used in traditional medicine for its ability to treat a variety of ailments, including gastrointestinal disorders, diabetes, and infections. Recent studies have confirmed that the plant's bioactive compounds, particularly those found in the peel, contribute to its medicinal efficacy. The insecticidal activity of *P. granatum* extract has been demonstrated against several insect species, making it a promising candidate for use in natural pest control [5]. Furthermore, the FeNPs synthesized from *P. granatum* extract have been shown to exhibit enhanced

insecticidal and phytotoxic effects, which may be attributed to their small size and high surface area, which increase their interaction with biological systems.

The growing interest in green synthesis methods for FeNPs reflects a broader trend towards environmentally sustainable and cost-effective approaches to nanomaterial production. In addition to their antimicrobial, phytotoxic, and insecticidal properties, the synthesized FeNPs have also been explored for their potential in environmental applications, particularly in the removal of contaminants from water. Due to their high adsorption capacity and ability to interact with pollutants at the molecular level, FeNPs have been proposed as effective agents for the removal of heavy metals and organic contaminants from aqueous solutions [8]. The combination of *P. granatum* extract's natural bioactive compounds and the unique properties of FeNPs positions this green synthesis method as a promising solution for a range of environmental and therapeutic applications.

The present study aims to explore the green synthesis of FeNPs using *P. granatum* peel extract and evaluate their biological activities, including antimicrobial, insecticidal, and phytotoxic effects. By utilizing a plant-based approach, this research contributes to the growing body of knowledge on the environmental and health benefits of green synthesis techniques. The findings may have significant implications for the development of sustainable and effective nanomaterials for use in medicine, agriculture, and environmental remediation.

2.0 MATERIALS AND METHODS

2.1 Sample Collection

Pomegranate peels (*P. granatum*) were collected from the Charsadda district. After being dried in a shaded environment, the peels were ground into a fine powder. The reagents used in this study, including methanol, deionized water, and iron chloride ($FeCl_3$), were procured from Merck. To extract bioactive compounds from the peels, 50g of the dried powder was soaked in methanol and distilled water for seven days. The mixture was then filtered through Whatman No. 1 filter paper, and the extract was concentrated using a rotary evaporator at 45–50°C[5].

2.2 Preparation of Stock Solutions

Nanoparticle synthesis involved the preparation of two distinct solutions: an aqueous extract of *P. granatum* peels and an iron chloride solution. One gram of the dried peel extract was dissolved in 100 mL of distilled water and stirred for 30–50 minutes to form the extract stock solution. A salt solution was prepared by dissolving 0.0162g of $FeCl_3$ in 100 mL of distilled water, followed by 30 minutes of stirring. These two solutions were then used for the synthesis of iron nanoparticles (FeNPs) [9, 10].

2.3 Chemicals Used

The chemicals used in the experiment were of analytical grade, including methanol (Sigma-Aldrich), $FeCl_3$ (Merck), deionized water, and distilled water. Detergents were used for cleaning purposes [11].

2.4 Apparatus and Instruments

The following apparatus and instruments were employed in the study: fume hood, water bath (HH-S6-220v/50Hz), spatula, hot plate, digital balance (OHAUS), UV-Vis spectrophotometer, heat gun, and scanning electron microscope (SEM) [12, 13].

2.5 Phytochemical Screening of *P. granatum* Extract

Phytochemical screening was performed to identify key compounds in *P. granatum* peel extract, including steroids, alkaloids, flavonoids, tannins, and saponins. The test for steroids involved the addition of acetic anhydride and sulfuric acid to the extract, with a color change from violet to blue or green indicating the presence of steroids. Alkaloids were detected by the formation of an orange-red precipitate when the extract was treated with Dragendroff's reagent [14]. Flavonoids showed a red to purple or pink to orange color when treated with magnesium crystals and hydrochloric acid [[15]]. Tannins were confirmed by a dark green color after reacting with ferric chloride, and saponins were detected by the formation of a froth layer when the extract was shaken with water [16].

2.6 $FeCl_3$ Nanoparticles Synthesis

For the synthesis of iron nanoparticles, 50g of pomegranate peel was boiled in 250 mL of distilled water for 30 minutes. The resulting extract was preserved at 4°C. To synthesize the nanoparticles, 0.0162g of $FeCl_3$ was dissolved in 100 mL of distilled water, and this solution was mixed with the pomegranate peel extract. The formation of FeNPs was indicated by a color change in the reaction mixture, which was then subjected to characterization using various techniques [[17]]. Characterization of the synthesized FeNPs was carried out using UV-Vis spectroscopy, XRD, SEM, and FTIR [[18]]. UV-Vis spectra were recorded to observe the characteristic peak of the nanoparticles, typically in the range of 300-450 nm [[19]].

2.7 Antibacterial Activity

The antibacterial activity of *P. granatum* methanol extract and FeNPs was evaluated against various bacterial strains, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Bacillus pumilus*, *Salmonella typhi*, and *Escherichia coli*. Nutrient agar plates were inoculated with the bacterial strains, and wells were created for the test samples. The plates were incubated at 37°C overnight, and the zones of inhibition were measured. The antibacterial properties of the samples were compared to the effects of amoxicillin, which served as a positive control [5].

2.8 Antifungal Activity

Antifungal activity was tested using Sabouraud Dextrose Agar (SDA) against fungal strains such as *Fusarium fumigatus*, *Fusarium solani*, *Aspergillus flavus*, *Aspergillus niger*, *Penicillium notatum*, and *Aspergillus oryzae*. The stock solutions of *P. granatum* methanol extract and FeNPs were prepared in DMSO, and 60 µL of each solution was added to SDA. The test tubes were incubated at 27±1°C for 3–7 days, and the percentage of fungal growth inhibition was calculated by comparing the test samples with the control groups [9].

2.9 Insecticidal Activity

The insecticidal activity of *P. granatum* extract and FeNPs was assessed using *Tribolium castaneum*, *Rhizopertha dominica*, and *Carpophilus analis*. Filter paper discs were impregnated with 200 mg of crude methanol extract or its fractions. After allowing the organic solvents to evaporate, ten healthy insects from each species were placed on the filter papers. The plates were incubated at 27°C for 24 hours, and the number of surviving insects was counted. The mortality rates were calculated, and the effectiveness of the samples was compared to permethrin, which was used as a positive control [10].

2.10 Phytotoxic Activity

Phytotoxic activity was evaluated using *Lemna minor* (duckweed). Stock solutions of *P. granatum* extract and FeNPs were prepared in methanol, and various concentrations (10, 100, and 1000 µg/mL) were tested. After the organic solvent evaporated, 20 mL of E-media was added to each flask containing the plant. The flasks were incubated at 27±1°C for 7 days, and the growth of the plants was recorded to assess the phytotoxicity of the samples [11].

2.11 Statistical analysis

A one-way analysis of variance (ANOVA) was conducted using SPSS 18.0 to assess significant differences among the experimental groups. Post hoc comparisons were performed using Duncan's multiple range test to identify specific group differences at a 5% significance level ($P < 0.05$). This test is suitable for comparing multiple group means while controlling for Type I error, ensuring reliable pairwise comparisons when ANOVA indicates significant overall variation.

3.0 RESULTS AND DISCUSSION

3.1 Synthesis of Iron Nanoparticles (FeNPs)

The synthesis of iron nanoparticles followed a systematic approach, utilizing the pomegranate peel extract as both the stabilizing and reducing agent. $FeCl_3 \cdot 6H_2O$ was used as the primary precursor. Initially, $FeCl_3$ salt solutions were prepared by dissolving 0.0162g of iron salt in 100 mL of distilled water and stirring for 30 minutes. Following this, 30 mL of a 1 mM $FeCl_3$ salt solution containing 1% plant extract was mixed in various ratios (1:1, 1:2, 1:4, 1:6, 1:8, 1:10, 1:12, 1:14, and 1:16) in separate containers, with

continuous stirring for one hour. The optimal ratio for synthesizing iron nanoparticles was found to be 1:16, which was confirmed by UV-Vis spectroscopy. At a wavelength of 435 nm, the UV-visible spectrum exhibited a characteristic peak for the formation of iron nanoparticles. Additionally, a distinct color change (Figure 1) in the reaction mixture was observed, further confirming the successful synthesis of iron nanoparticles.

Figure 1. Synthesis of iron oxide nanoparticle

3.2 Phytochemical Screening of *P. granatum*

Phytochemical screening of aqueous, chloroform, methanolic, and ethyl acetate extracts of *P. granatum* was conducted, with the results shown in Table 1. The methanolic and aqueous extracts revealed the presence of flavonoids, steroids, alkaloids, tannins, and saponins. These phytochemicals play a crucial role in the synthesis and stabilization of iron nanoparticles.

Table 1. Phytochemical analysis of *P. granatum.*

Phytochemicals	Aqueous Extract
Steroids	+
Alkaloids	+
Tannins	+
Saponins	+
Flavonoids	+

3.3.1 UV-Vis Spectrum Analysis

The bio-reduction of Fe-NPs in aqueous solutions was investigated using UV-Vis spectroscopy. The spectrum displayed absorption peaks in the 200–400 nm range, attributed to the iron oxide nanoparticles. A distinct peak at 366 nm was observed for the synthesized pomegranate nanoparticles, as shown in Figure 2, indicating the presence of Fe-NPs.

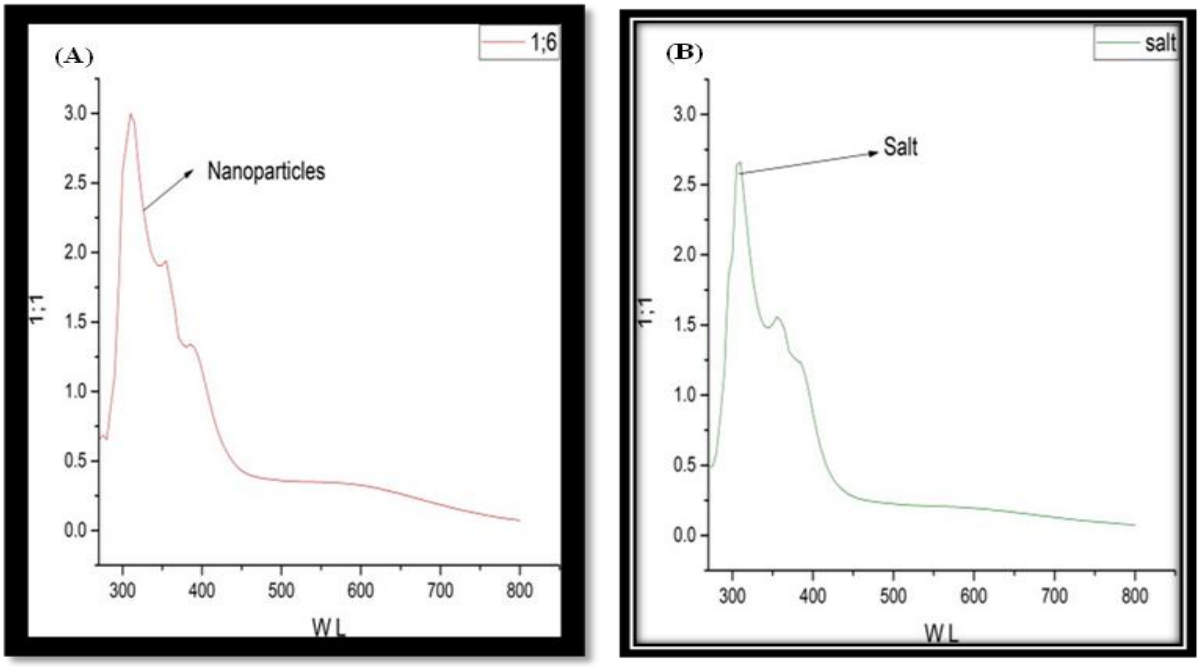


Figure 2. UV spectra of the iron nanoparticles (A) and iron salt (B).

3.3.2 FT-IR Examination

Fourier-transform infrared (FT-IR) analysis was employed to identify the biomolecules in the plant extract that may act as stabilizing agents for the metal precursor ions and nanoparticles. The FT-IR spectrum in Figure 3 shows a broad band at 3400 cm−1, corresponding to the O–H stretch of phenolic groups, and a band at 2900 cm−1, indicating methyl C-H stretching. The carbonyl groups were observed at 1600 cm−1, which further suggests that the phenolic compounds in the extract were involved in the reduction of $FeCl_3$ to Fe-NPs.

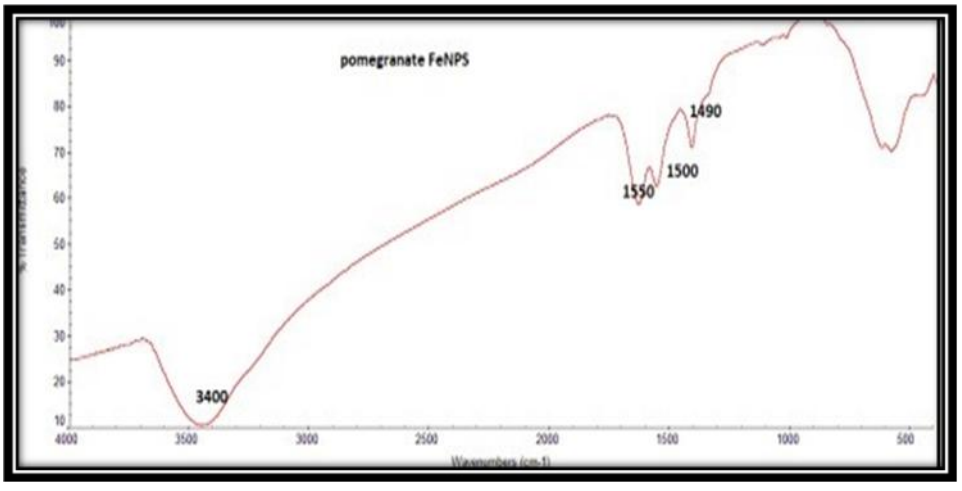


Figure 3. FT-IR spectra of the synthesized iron nanoparticles using *P. granatum* peel extract

3.3.3 Energy Dispersive X-ray (EDX) Analysis

Energy dispersive X-ray (EDX) analysis confirmed the elemental composition of the nanoparticles. The EDX profile as in Figure 4 a significant peak at 6.400 keV, indicating the presence of iron (Fe), along with carbon (C) and oxygen (O), at atomic percentages of 15.33%, 34.64%, and 39%, respectively. These results suggest that the iron

nanoparticles were in the oxide state, with the presence of oxygen supporting the formation of iron oxide (Fe_2O_3) nanoparticles.

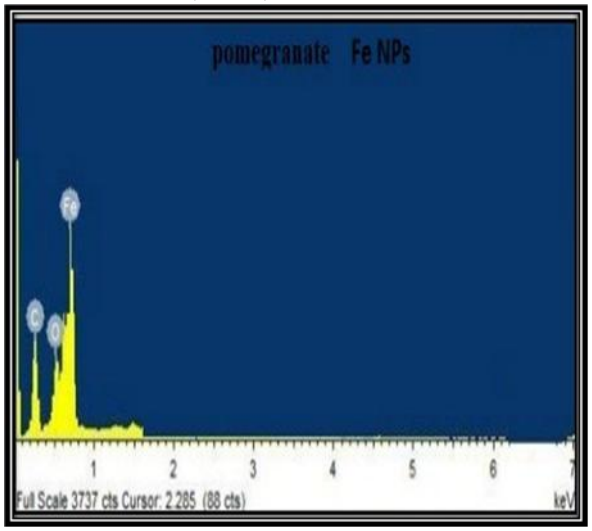


Figure 4. EDX profile of iron nanoparticles synthesized using *P. granatum* peel extract.

3.3.4 X-ray Diffraction (XRD) of Iron Nanoparticles

X-ray diffraction (XRD) was used to assess the crystalline nature of the synthesized nanoparticles. The XRD patterns of pomegranate-Fe_2O_3 nanoparticles, shown in Figure 5, revealed sharp peaks at 2θ values around 30°, 34°, and 45°, confirming the crystalline structure of the nanoparticles. The presence of peaks corresponding to carbon (C) at 24.7° further supports the idea that the pomegranate extract stabilizes the nanoparticles.

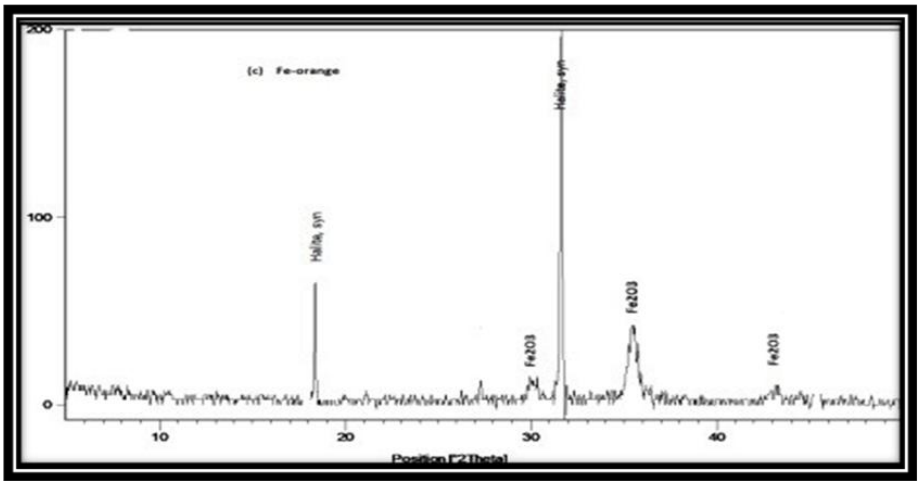


Figure 5. XRD pattern of iron nanoparticles synthesized using *P. granatum* peel extract.

3.3.5 Scanning Electron Microscopy (SEM) of Iron Nanoparticles

Scanning Electron Microscopy (SEM) images of the synthesized nanoparticles revealed that the particles were evenly distributed and nearly spherical in shape. The size of the particles ranged from approximately 19.8 nm to 48 nm, as shown in Figure 6 The uniformity of particle size indicates the effectiveness of the pomegranate peel extract in stabilizing the nanoparticles.

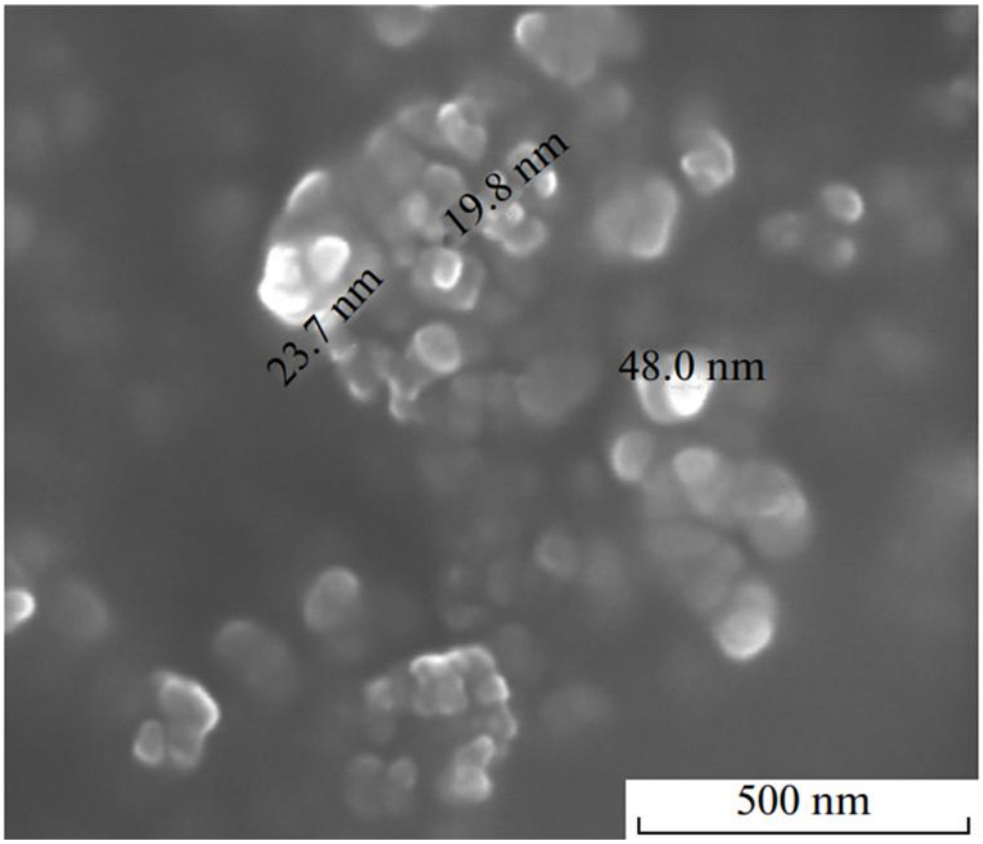


Figure 6. SEM images of the iron nanoparticles synthesized using *P. granatum* peel extract.

3.3 Antibacterial Activity

The antibacterial properties of FeNPs and methanol (MeOH) extract of *P. granatum* peel were tested against several bacterial strains, including *E. coli, B. pumilus, S. Typhi, K. pneumoniae, P. aeruginosa,* and *S. aureus*. The results showed that FeNPs exhibited significantly stronger antibacterial activity compared to the MeOH extract alone. The growth inhibition was 30.43% for *E. coli*, 48% for *B. pumilus*, 33.33% for *S. Typhi*, 57.54% for *K. pneumoniae*, 51.58% for *P. aeruginosa*, and 61.53% for *S. aureus* when using the MeOH extract. In contrast, the FeNPs of *P. granatum* peel extract inhibited the growth of *K. pneumoniae* (76.19%), *S. aureus* (73.07%), *P. aeruginosa* (66.66%), *B. pumilus* (56%), *S. Typhi* (40.74%), and *E. coli* (34.78%), demonstrating the enhanced antibacterial effect of the nanoparticles compared to the extract alone.

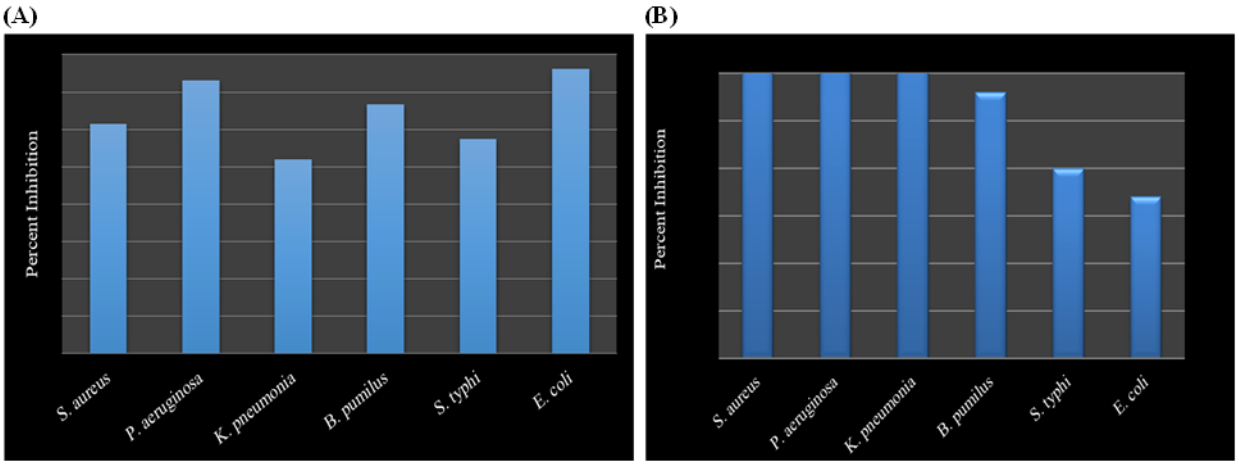


Figure 7. Comparison of antibacterial activity of (A) MeOH extract and (B) FeNPs of *P. granatum* peel extract.

3.5 Antifungal Activity

The antifungal activity of FeNPs and MeOH extract was evaluated against fungal strains such as *A. niger*, *P. notatum*, *A. fumigatus*, *A. oryzae*, *A. flavus*, and *F. solani*. The results showed no significant antifungal activity in either the FeNPs or the MeOH extract of *P. granatum* peel, indicating that these extracts do not possess antifungal properties under the experimental conditions tested.

3.6 Insecticidal Activity

The insecticidal activity of FeNPs and MeOH extract of *P. granatum* peel was assessed against *Callosbruchus analis*, *Rhizopertha dominica*, and *Tribolium castaneum*. The results, presented in Table 2, indicated that FeNPs exhibited significant insecticidal activity with mortality rates of 60% against *T. castaneum*, 70% against *C. analis*, and 50% against *R. dominica*. In comparison, the MeOH extract showed lower insecticidal activity with 40%, 40%, and 30% mortality, respectively. The organic solvent used as a negative control showed no mortality, while permethrin, used as a positive control, demonstrated 100% mortality in all cases.

Table 2. Insecticidal activity of FeNPs and MeOH extract of *P. granatum* peel extract

Name of Insect	+ive Control	MeOH Extract	FeNPs
T. castaneum	100%	40%	60%
R. dominica	100%	30%	50%
C. analis	100%	40%	70%

3.7 Phytotoxic Activity

The phytotoxic effects of FeNPs and MeOH extract of *P. granatum* peel were evaluated using *Lemna minor*. The results, shown in **Table 3**, revealed that FeNPs had a significant phytotoxic effect, with mortality rates of 87% at 1000 µg/mL and 62% at 100 µg/mL. In comparison, the MeOH extract exhibited moderate phytotoxicity with 50% mortality at 1000 µg/mL and 43% at 100 µg/mL.

Table 3. Phytotoxic Activity and Percent Growth Regulation of *Lemna minor* by FeNPs and MeOH Extract of *P. granatum* Peel Extract

Sample (µg/mL)	Number of Fronds Killed (FeNPs)	Number of Fronds Killed (MeOH Ext)	Number of Fronds Killed (Acetone)	Number of Fronds Killed (Aqueous)	Control	Percent Growth Regulation (FeNPs)	Percent Growth Regulation (MeOH Ext)	Percent Growth Regulation (Control)
1000	14	8	9	6	7	87	50	100
100	10	7	5	16	16	62	43	100
10	8	2	4	3	2	50	12	

4.0 DISCUSSION

The green synthesis of iron oxide nanoparticles (FeNPs) has become an increasingly popular and well-discussed method in recent years due to its simplicity, non-toxicity, cost-effectiveness, and scalability [[20]]. This environmentally friendly approach has garnered attention because it involves fewer hazards compared to traditional chemical synthesis methods and can be executed on a large scale. Iron oxide nanoparticles synthesized through plant-based methods exhibit dual properties antibacterial and anti-inflammatory which make them highly desirable in fields like medicine and cosmetics [[21]]. In this study, FeNPs were synthesized using *Punica granatum* (pomegranate) extract, a plant known for its rich bioactive compounds, which served both as a reducing and stabilizing agent.

The synthesized nanoparticles were analyzed using TEM, FTIR, and EDX. The UV-Vis spectrum of the nanoparticles displayed a characteristic absorption peak around 298 nm, which is typical of iron oxide nanoparticles, indicating high purity and successful synthesis. The changes in the absorption peak between 290 nm and 455 nm suggest that the synthesis process involves complex physical, chemical, or biological mechanisms [22], [23]. Such fluctuations may be indicative of intermittent synthesis or variations in nanoparticle formation during the process.

Further morphological analysis using SEM revealed that the FeNPs synthesized from *P. granatum* were approximately 100 nm in diameter, exhibiting an irregular and monodisperse nature. These findings align with previous studies, where plant-based FeNPs have shown similar sizes and shapes [[24]]. The EDX analysis confirmed the presence of key elements, such as iron, carbon, and oxygen, with a prominent peak at 34 kV, signifying the successful formation of iron oxide nanoparticles. The elemental composition supports the presence of iron oxide in the synthesized particles, with oxygen indicating that the nanoparticles were in the oxide form, as corroborated by previous literature [25], [26].

FTIR spectroscopy was used to identify the functional groups involved in nanoparticle formation. The FTIR spectra showed several peaks, including a broad band around 3400 cm−1 associated with the O–H stretch of phenolic groups, which are known to participate in nanoparticle formation. The presence of other peaks, such as those at 2900 cm−1 (methyl C-H stretching) and 1600 cm−1 (carbonyl stretching), suggests the involvement of carboxylic acids, amines, and alkanes in stabilizing and encapsulating the nanoparticles during synthesis [[27], [28]].

To further validate the crystalline nature of the synthesized nanoparticles, XRD analysis was performed. The XRD patterns displayed characteristic diffraction peaks at 30°, 34°, and 45°, which are indicative of the crystalline structure of Fe_2O_3 nanoparticles, supporting the results from earlier studies [[29], [30]]. The diffraction data confirmed the formation of crystalline iron oxide nanoparticles, with the observed peaks aligning with the standard reflections of Fe_2O_3.

The antibacterial activity of the FeNPs was evaluated against six bacterial strains, including *Bacillus subtilis*, *Staphylococcus aureus*, *Enterobacter*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella Typhi*. The results demonstrated that FeNPs significantly inhibited

bacterial growth, with the highest inhibition observed for *S. aureus* (73.07%) and *K. pneumoniae* (76.19%), which is consistent with findings in other studies on iron oxide nanoparticles [[28]]. These nanoparticles exhibited effective bactericidal action, suggesting their potential application in combating multidrug-resistant (MDR) strains, which are a major global health concern.

Nanoparticles' antibacterial and antifungal effects depend not only on their size but also on their ability to penetrate bacterial cell membranes and disrupt cellular functions. Small-sized nanoparticles, such as those in this study, can penetrate the protective barriers of bacterial cells, leading to cellular damage and death [31]. Additionally, the size of the nanoparticles influences their surface area, reactivity, and interaction with biological systems, enhancing their antimicrobial efficacy.

The results of this study indicate that both FeNPs and their synthesis process from *P. granatum* extract enhance the nanoparticles' antioxidant capacity, further supporting their potential use in therapeutic applications. Moreover, the synthesis of FeNPs using *P. granatum* extract provides an eco-friendly alternative to conventional methods, offering a scalable, cost-effective, and sustainable approach for producing nanoparticles with significant biological activity.

5.0 CONCLUSION

This study demonstrates the successful synthesis of FeNPs using *P. granatum* peel extract, providing an eco-friendly, cost-effective, and scalable method for nanoparticle production. Characterization using UV-Vis spectroscopy, SEM, FTIR, EDX, and XRD confirmed the formation of high-purity nanoparticles with a size of approximately 100 nm. The presence of key phytochemicals in the extract played a crucial role in the reduction and stabilization of iron ions. The synthesized FeNPs exhibited significant antibacterial activity against pathogenic bacterial strains, including *S. aureus* and *K. pneumoniae*, highlighting their potential for medical applications. Furthermore, the nanoparticles demonstrated antioxidant properties, supporting their broader therapeutic potential. This green synthesis approach not only provides an effective method for producing nanoparticles but also contributes to the development of sustainable nanomaterials for biomedical and industrial use.

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CONFLICT OF INTEREST

Declare conflicts of interest or state “The authors declare no conflict of interest.” Authors must identify and declare any personal circumstances or interest that may be perceived as inappropriately influencing the representation or interpretation of reported research resultsThe authors declare that the research was carried out without any commercial or financial interests that could potentially lead to a conflict of interest.

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