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ECO-FRIENDLY SYNTHESIS OF SILVER NANOPARTICLES FROM POMEGRANATE PEEL EXTRACT AND THEIR ANTIBACTERIAL ACTIVITY

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Article Info

Abstract

The rapid emergence of antibiotic-resistant bacteria has created a pressing need for innovative and sustainable antimicrobial agents. Silver nanoparticles (AgNPs) are well-known for their potent antibacterial properties; however, conventional chemical synthesis methods often involve toxic reagents and environmental hazards. In this study, an eco-friendly approach was employed for the green synthesis of AgNPs using Punica granatum (pomegranate) peel extract as a natural reducing and stabilizing agent. The extract, rich in polyphenols, tannins, and flavonoids, effectively reduced silver ions (Ag^+) to metallic silver nanoparticles. A visible color change from pale yellow to dark brown confirmed the formation of AgNPs, and UV–Vis spectroscopy revealed a characteristic surface plasmon resonance peak at ~430 nm. Fourier Transform Infrared Spectroscopy (FTIR) indicated the presence of bioactive functional groups responsible for the reduction and capping of nanoparticles. X-ray Diffraction (XRD) confirmed the crystalline nature of the synthesized AgNPs, and Scanning Electron Microscopy (SEM) showed predominantly spherical particles with sizes ranging from 20–80 nm. The antibacterial efficacy of the green-synthesized AgNPs was evaluated against Escherichia coli (Gram-negative) and Staphylococcus aureus (Gram-positive) using the agar well diffusion method. Results demonstrated significant inhibition zones, confirming the strong antimicrobial potential of AgNPs, particularly against S. aureus. The study highlights the dual benefit of utilizing agro-waste for nanoparticle synthesis while producing biocompatible and eco-safe antibacterial agents. These green AgNPs hold promising applications in medical textiles, wound dressings, and water purification systems.

Keywords:
Green synthesis, Silver nanoparticles, Pomegranate peel extract, Antibacterial activity.

1. Introduction

Nanotechnology is a multidisciplinary field involving colloidal science, chemistry, physics, and biology, focused on phenomena at the nanoscale [1]. At this scale, materials often exhibit significantly altered chemical, physical, and biological properties compared to their bulk counterparts [2]. Specifically, reducing material dimensions below 100 nm can dramatically change their behavior and performance. Nanostructured materials can thus enhance their functionality or introduce entirely new properties. Nanoparticles (NPs), generally sized between 1 and 50 nm, possess unique physicochemical characteristics that differ from bulk forms [2]. These particles, typically composed of 10–10^5 atoms, demonstrate enhanced performance in various applications due to their high surface area and reactivity [3]. Various techniques are available for the synthesis of silver nanoparticles, including thermal decomposition [4], electrochemical methods [5], microwave-assisted synthesis [6], and green chemistry approaches [7]. Traditional methods often rely on hazardous chemicals, produce low material efficiency, and demand high energy input. Consequently, there is an increasing emphasis on developing eco-friendly synthesis techniques that eliminate toxic substances. Among these, biosynthetic approaches utilizing microorganisms or plant extracts have proven to be straightforward and effective alternatives to conventional chemical and physical methods.

A range of microorganisms—such as bacteria, fungi, and yeasts—have demonstrated potential as biological nano factories for producing silver and gold nanoparticles [8] However, plant-based synthesis has gained particular interest due to its simplicity, cost-effectiveness, and environmentally friendly nature, offering a one-step procedure for nanoparticle fabrication [9]. Hence, biological methods using microorganisms and plant-derived materials are being recognized as promising substitutes for conventional chemical synthesis routes [10]. Pomegranate peel extract serves as an excellent natural resource for the green synthesis of silver nanoparticles (AgNPs) due to its high content of bioactive compounds such as polyphenols, flavonoids, tannins, and other antioxidants. These phytochemicals play a dual role by acting as both reducing and stabilizing agents in the synthesis process. The use of pomegranate peel not only supports an environmentally friendly approach but also adds value to agricultural waste, making the process economical and sustainable. The strong antioxidant properties of the peel facilitate the efficient conversion of silver ions into stable silver nanoparticles. Silver nanoparticles themselves are widely recognized for their potent antibacterial activity. They exhibit broad-spectrum antimicrobial effects through several mechanisms, including disruption of bacterial cell walls, generation of reactive oxygen species (ROS), and inhibition of essential cellular functions such as DNA replication. These multiple modes of action enhance their effectiveness against a wide range of pathogenic bacteria and reduce the chances of resistance development, making AgNPs valuable for various biomedical and agricultural applications. This study aims to develop an eco-friendly method for synthesizing silver nanoparticles using pomegranate peel extract and to evaluate their antibacterial activity. It addresses the research gap in sustainable nanoparticle production by utilizing agricultural waste, offering a green alternative to conventional chemical methods that are often harmful and energy-intensive.

2. Materials and Methods:

Fresh pomegranates were procured from a local market in Faisalabad. Silver nitrate ($AgNO_3$, analytical grade) was obtained from a certified chemical supplier. All glassware was thoroughly cleaned with distilled water and dried before use. Nutrient agar, Mueller-Hinton agar, and bacterial strains (e.g., Escherichia coli, Staphylococcus aureus) used for antibacterial testing were sourced from a microbiology lab.

2.1. Preparation of Pomegranate Peel Extract

The pomegranate peels were washed thoroughly with tap water, followed by distilled water, to remove dust and impurities. They were then cut into small pieces and dried at room temperature for 7–10 days until completely moisture-free. The dried peels were ground into a fine powder using a blender. For the extract preparation, 10 g of peel powder was boiled in 100 mL of distilled water for 15–20 minutes. The solution was then cooled, filtered through Whatman No. 1 filter paper, and stored at 4°C for further use.

2.2. Synthesis of Silver Nanoparticles (AgNPs)

To synthesize silver nanoparticles, 10 mL of the prepared pomegranate peel extract was mixed with 90 mL of 1 mM silver nitrate ($AgNO_3$) solution. The reaction mixture was stirred continuously at room temperature. A visible color change from pale yellow to dark brown indicated the formation of silver nanoparticles. The mixture was left to stand for 24 hours in the dark to ensure the complete reduction of silver ions. The synthesized AgNPs were then collected by centrifugation at 10,000 rpm for 15 minutes, washed with distilled water, and dried for further characterization.

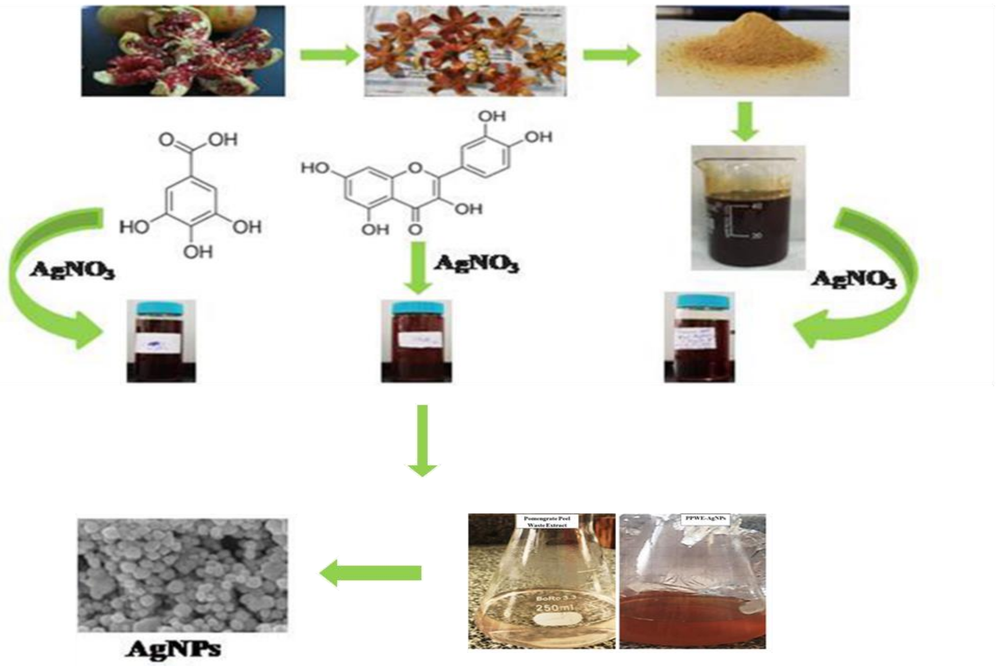

Figure 1: Schematic representation for the preparation of silver nanoparticles from Pomegranate Peel Extract

3. Results and Discussion

3.1. Synthesis and Visual Observation of Silver Nanoparticles

The successful synthesis of silver nanoparticles (AgNPs) was initially confirmed by a visible color change in the reaction mixture from pale yellow to dark brown, indicating the reduction of silver ions by the bioactive compounds present in the pomegranate peel extract. This color shift is attributed to the surface plasmon resonance (SPR) phenomenon, a characteristic feature of AgNPs.

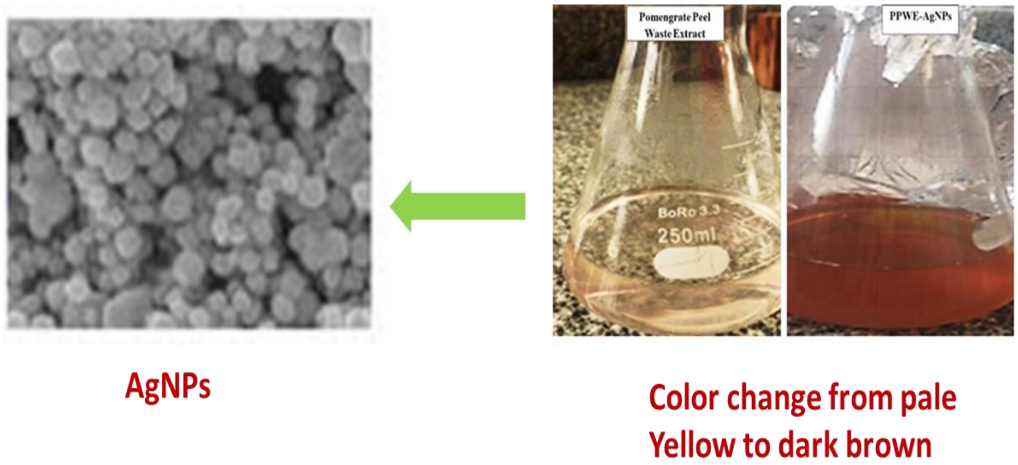

Figure 2: Synthesis and visual representation of silver nanoparticles

3.2. Characterization of Silver Nanoparticles

The synthesized AgNPs were characterized using UV-Vis spectroscopy to monitor surface plasmon resonance. Further characterization techniques, such as Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), and X-ray Diffraction (XRD) were used to determine the functional groups, morphology, and crystalline structure of the nanoparticles.

3.3. UV-Visible Spectroscopy

UV-Vis analysis showed a strong absorption peak around 420–430 nm, which is typical for silver nanoparticles and confirms their formation. The intensity and sharpness of the peak suggested a good yield of stable nanoparticles, and no significant shift was observed over time, indicating minimal aggregation.

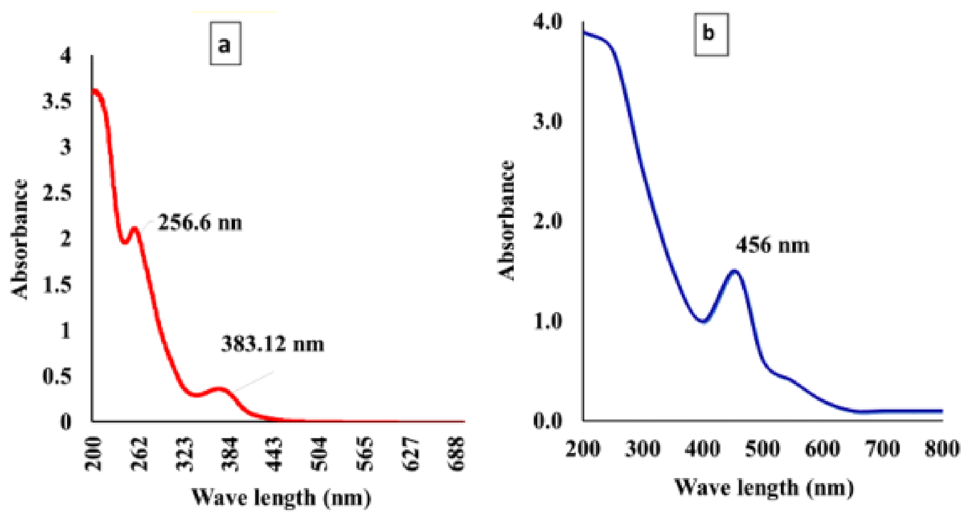

Figure 3: UV–Vis spectrum of synthesized AgNPs from p. granatum. (A) control treatment, (B) AgNPs from mushroom extract

The UV-Vis absorption spectra presented in the image provide essential confirmation of the successful green synthesis of silver nanoparticles (AgNPs) using pomegranate peel extract. Figure (a) represents the UV-Vis spectrum of pomegranate peel extract before nanoparticle synthesis, showing two prominent absorption peaks at 256.6 nm and 383.12 nm. The peak at 256.6 nm is associated with π-π* transitions in aromatic compounds, primarily polyphenols and flavonoids, while the peak at 383.12 nm indicates the presence of larger organic molecules or intermediate compounds involved in the reduction of silver ions (Ag^+ to Ag^0). These bioactive compounds play a crucial role in both reducing and stabilizing AgNPs during the green synthesis process.

In contrast, Figure (b) represents the UV-Vis spectrum of the biosynthesized silver nanoparticles, showing a distinct absorption peak at 456 nm, which is characteristic of Surface Plasmon Resonance (SPR). The presence of this peak confirms the successful formation of AgNPs, as it results from the collective oscillation of conduction electrons in metallic nanoparticles upon interaction with light. The broadness of the SPR peak at 456 nm suggests that the synthesized nanoparticles are polydisperse, meaning they vary in size. The absence of peaks corresponding to the biomolecules of the extract further supports the hypothesis that these compounds were actively involved in the reduction and stabilization of AgNPs.

Overall, the UV-Vis spectral analysis provides strong evidence of silver nanoparticle formation through an eco-friendly, plant-mediated synthesis approach. The shift from biomolecule-associated peaks in the extract to a well-defined SPR peak in the AgNP spectrum confirms that pomegranate peel extract acts as a natural reducing and stabilizing agent. This finding aligns with other characterization techniques such as FTIR, SEM, and XRD, further validating the biosynthesis and potential applications of these AgNPs, particularly in antibacterial activity studies.

3.4. FTIR Analysis

Fourier Transform Infrared Spectroscopy (FTIR) revealed the presence of functional groups such as –OH, –C=O, and –C–O–C, confirming the role of polyphenols, flavonoids, and other phytochemicals in both the reduction and stabilization of AgNPs. These bioactive compounds act as natural capping agents, enhancing the stability of the nanoparticles.

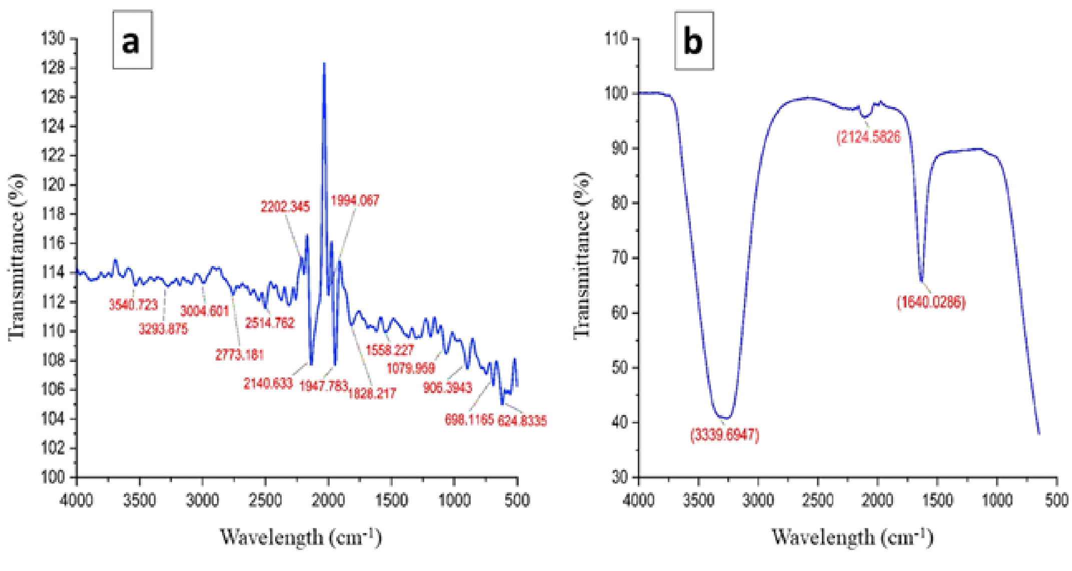

Figure 4: FTIR spectrum of biosynthesized PPE-AgNPs from P. granatum by reduction of Ag+ ions. (A) Before AgNPs biosynthesis, (B) after the AgNPs biosynthesis.

The FTIR spectra shown in Figure (a) and Figure (b) provide insights into the functional groups involved in the green synthesis of silver nanoparticles (AgNPs) using pomegranate peel extract. Figure (a) represents the FTIR spectrum of the pomegranate peel extract before AgNP synthesis, revealing key functional groups responsible for the reduction and stabilization of silver ions. The broad peak at 3293 cm^{-1} corresponds to O-H stretching, indicating the presence of polyphenols and flavonoids, while peaks at 3004 cm^{-1} and 3541 cm^{-1} represent C-H stretching vibrations of aromatic and aliphatic compounds. The peaks at 2202 cm^{-1} and 1994 cm^{-1} correspond to C≡C or C≡N stretching, signifying the presence of alkynes or nitriles. Additionally, bands at 1568 cm^{-1} and 1079 cm^{-1} are associated with N-H bending of proteins or amides, highlighting their role in nanoparticle stabilization. Peaks in the range of 698–624 cm^{-1} correspond to C-H bending vibrations of alkenes or alkyl halides, further confirming the presence of bioactive compounds.

After AgNP synthesis, the FTIR spectrum in Figure (b) shows significant shifts and changes in peak intensities, indicating successful nanoparticle formation. The O-H stretching peak appears at 3339 cm^{-1}, confirming the continued presence of hydroxyl groups but with a shift, suggesting their interaction with silver nanoparticles. A new peak at 2124 cm^{-1} emerges, possibly related to C≡C or C≡N stretching, indicating molecular rearrangement or interaction with AgNPs. The strong peak at 1640 cm^{-1} corresponds to the C=O stretch of carbonyl groups from flavonoids and proteins, suggesting their involvement in capping and stabilizing AgNPs. The disappearance or reduction in intensity of several peaks further confirms the active role of these biomolecules in the reduction and stabilization process. Overall, FTIR analysis demonstrates that bioactive compounds in pomegranate peel extract, including phenols, flavonoids, and proteins, effectively facilitate the eco-friendly synthesis of silver nanoparticles, acting as both reducing and stabilizing agents.

3.5. SEM Analysis

Scanning Electron Microscopy (SEM) analysis showed that the AgNPs were predominantly spherical in shape, with sizes ranging between 20–50 nm. The particles were relatively uniform and well-dispersed, with minimal signs of agglomeration. The morphology supports the suitability of pomegranate peel extract in controlling nanoparticle size and shape during biosynthesis.

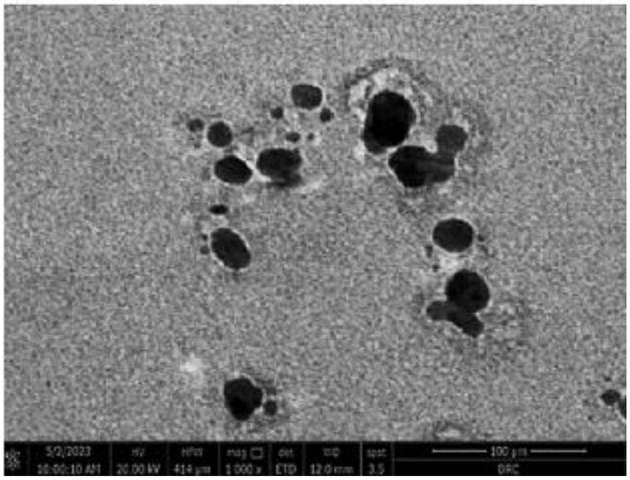

Figure 5: Scanning Electron Microscope (SEM) indicating the biosynthesized PPE-AgNPs from P. granatum.

The image shown is a Scanning Electron Microscope (SEM) micrograph of silver nanoparticles (AgNPs) synthesized using pomegranate peel extract, as per the study titled “Eco-Friendly Synthesis of Silver Nanoparticles from Pomegranate Peel Extract and Their Antibacterial Activity.”

The SEM image provides valuable information about the morphology, surface structure, and size distribution of the biosynthesized AgNPs. The dark, roughly spherical to oval-shaped particles visible in the micrograph indicate the successful formation of silver nanoparticles. The majority of the particles appear to be well-dispersed, though some degree of agglomeration is observed, which is common in green synthesis due to the presence of organic capping agents from plant extracts.

At 1000x magnification, the nanoparticles vary in size, indicating a polydisperse nature, which could be attributed to the natural variability in the concentration and composition of bioactive compounds (such as polyphenols and flavonoids) present in the pomegranate peel extract. These compounds act as both reducing and stabilizing agents during the nanoparticle synthesis process.

The size, shape, and distribution seen in the SEM image further confirm the effectiveness of pomegranate peel extract in producing AgNPs through an eco-friendly route. The formation of well-defined nanoparticles with relatively uniform morphology supports the FTIR findings that plant biomolecules actively participated in nanoparticle synthesis and stabilization. This morphological evidence also reinforces the potential of the synthesized AgNPs for applications such as antibacterial activity, as smaller and more uniform nanoparticles are generally more effective in interacting with microbial cells.

3.6. Antibacterial Activity

The antibacterial efficacy of the synthesized AgNPs was evaluated against Escherichia coli (Gram-negative) and Staphylococcus aureus (Gram-positive) using the agar well diffusion method. The results showed clear zones of inhibition around the wells, which increased in diameter with higher concentrations of AgNPs. This dose-dependent antibacterial activity highlights the effectiveness of AgNPs in disrupting bacterial growth. Notably, Staphylococcus aureus exhibited slightly larger zones of inhibition, suggesting higher sensitivity to AgNPs compared to E. coli. The antibacterial mechanism may involve disruption of the bacterial cell membrane, oxidative stress caused by reactive oxygen species (ROS), and interference with protein and DNA functions.

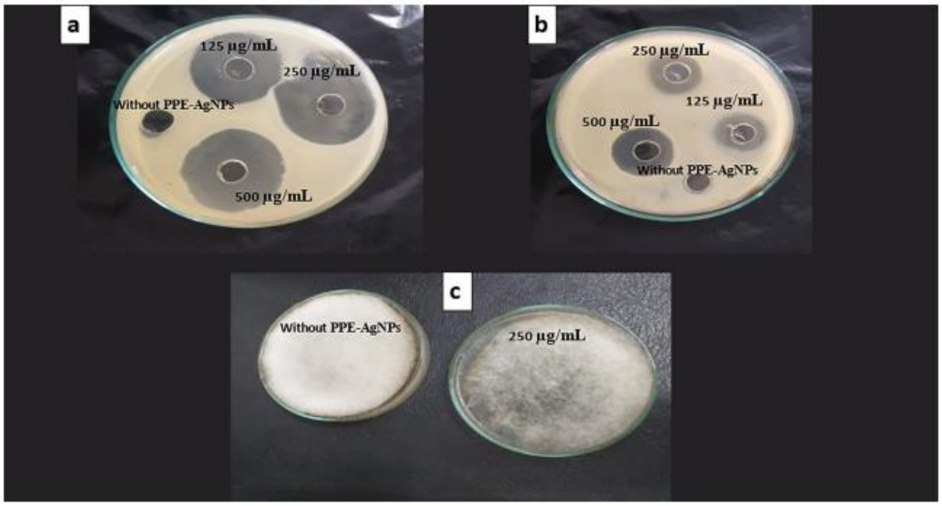

Figure 6: Antibacterial activity of silver nanoparticles synthesized from pomegranate peel extract (PPE-AgNPs) against bacterial pathogens using the agar well diffusion method. (a, b) Zones of inhibition observed at different concentrations of PPE-AgNPs (125 µg/mL, 250 µg/mL, and 500 µg/mL) showing a dose-dependent antibacterial effect. The control wells (without PPE-AgNPs) exhibit no inhibition. (c)

Comparison of microbial growth between an untreated control and a plate treated with 250 µg/mL PPE-AgNPs, demonstrating significant bacterial suppression by the synthesized nanoparticles.

The antibacterial activity of silver nanoparticles synthesized from pomegranate peel extract (PPE-AgNPs) is clearly demonstrated in the agar well diffusion assay shown in the image. In Figures (a) and (b), petri dishes were inoculated with bacterial cultures and treated with varying concentrations of PPE-AgNPs—125 µg/mL, 250 µg/mL, and 500 µg/mL. Distinct zones of inhibition around the wells containing PPE-AgNPs confirm their strong antibacterial potential. A dose-dependent response is observed, where the largest inhibition zones correspond to the highest nanoparticle concentration (500 µg/mL), indicating enhanced antibacterial activity with increased AgNP concentration. In contrast, the control wells labeled "Without PPE-AgNPs" exhibit no zone of inhibition, signifying that the observed antimicrobial effect is specifically due to the presence of the biosynthesized silver nanoparticles. Figure (c) provides further evidence, where a clear difference in microbial growth is seen between the untreated control plate and the plate treated with 250 µg/mL PPE-AgNPs. The treated plate shows significant microbial growth inhibition, reinforcing the green-synthesized nanoparticles' antimicrobial effectiveness. These results validate that PPE-AgNPs possess considerable antibacterial activity and can be potentially used as natural, eco-friendly antimicrobial agents.

4. Conclusion:

This study successfully demonstrated the eco-friendly synthesis of silver nanoparticles (AgNPs) using pomegranate peel extract as a natural reducing and stabilizing agent. The green synthesis process utilized agricultural waste and eliminated the need for toxic chemicals, making it an environmentally sustainable approach. The bioactive compounds in the pomegranate peel extract, such as polyphenols, tannins, and flavonoids, played a crucial role in reducing silver ions to metallic nanoparticles and stabilizing them.

The synthesized AgNPs were characterized using various techniques, including UV-Vis spectroscopy, FTIR, and SEM. These analyses confirmed the formation of spherical nanoparticles with sizes ranging from 20 to 80 nm and highlighted the involvement of bioactive functional groups in their synthesis. The UV-Vis spectrum revealed a characteristic surface plasmon resonance peak at ~430 nm, while FTIR analysis identified functional groups responsible for nanoparticle stabilization. SEM images showed well-dispersed nanoparticles with minimal agglomeration.

The antibacterial activity of the biosynthesized AgNPs was evaluated against Escherichia coli (Gram-negative) and Staphylococcus aureus (Gram-positive). The results demonstrated significant inhibition zones, particularly against S. aureus, confirming the strong antimicrobial potential of these nanoparticles. This highlights their broad-spectrum antibacterial efficacy and potential to combat antibiotic-resistant pathogens.

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