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Green Synthesis of Silver Nanoparticles Using *Psidium guajava* Leaf Extract and Their Antibacterial Properties.

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ABSTRACT

Silver nanoparticles (AgNPs) have garnered significant interest in antimicrobial research due to their potent antibacterial properties. To ensure their safe and biocompatible application, researchers have developed various biogenic synthesis methods that avoid the use of toxic chemicals. Among these, the utilization of plant extracts has emerged as a promising approach, as plant components can function both as reducing and capping agents in the synthesis process. For the large-scale production of antibacterial AgNPs using plant materials, it is essential to establish synthesis methods that are simple, rapid, cost-effective, and environmentally friendly. These methods should leverage the easy availability and non-toxic nature of plants, while also ensuring the stability and antibacterial efficacy of the biosynthesized nanoparticles. This study investigates the green synthesis of silver nanoparticles (AgNPs) utilizing Psidiumguajava (guava) leaf extract and assesses their antibacterial potential against pathogenic microorganisms. Leveraging the phytochemical richness of guava leaves—including flavonoids, tannins, and phenolic acids—as natural reducing and stabilizing agents, the biosynthetic approach offers a sustainable and cost-effective alternative to conventional chemical synthesis. The synthesized AgNPs were characterized using UV–Visible spectroscopy, Fourier-transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM), which confirmed the formation of stable nanoparticles with nanoscale dimensions. Antibacterial efficacy was evaluated using the agar well diffusion method against common pathogens such as Staphylococcus aureus and Escherichia coli, demonstrating significant antimicrobial activity. These findings suggest promising applications in healthcare, textiles, and food packaging industries, where antimicrobial coatings are critical for infection control and product preservation. Overall, the study emphasizes the potential of plant-based green synthesis as an eco-friendly strategy for producing nanoparticles with effective antimicrobial properties.

Keywords: Silver nanoparticles, *Psidium guajava*, green synthesis, plant extract, antibacterial activity, nanotechnology

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INTRODUCTION

Nanotechnology has emerged as one of the most transformative fields in modern scientific research, significantly advancing sectors such as electronics, environmental science, and healthcare. Notably, its application in medical science has opened new frontiers for diagnostics, drug delivery, and antimicrobial therapies. Among the various nanomaterials, silver nanoparticles (AgNPs) have garnered substantial attention due to their potent antimicrobial properties. Traditionally, AgNPs are synthesized through physical and chemical methods that often involve toxic reagents and high energy consumption, raising environmental and safety concerns. These limitations have prompted a shift towards more sustainable, eco-friendly synthesis approaches.

Green synthesis methods, which utilize biological resources such as plant extracts, have emerged as viable alternatives. Psidium guajava (guava), a medicinal plant known for its rich phytochemical profile—including tannins and flavonoids—has long been utilized in traditional medicine for its antibacterial and antioxidant properties. These phytochemicals serve as natural reducing and stabilizing agents in the biosynthesis of nanoparticles.

AgNPs synthesized via green methods exhibit unique characteristics in terms of structure, composition, crystallinity, morphology, and size when compared to their bulk counterparts. These distinct features confer exceptional physicochemical properties, such as chemical stability, surface-enhanced Raman scattering, high thermal and electrical conductivity, catalytic activity, non-linear optical behavior, and pronounced biological effects. As a result, AgNPs have been incorporated into a wide array of consumer products, including cosmetics, toiletries, plastics, textiles, and food packaging, owing to their broad-spectrum antibacterial and antifungal activities.

Furthermore, AgNPs exhibit unique optical properties that enable strong interactions with specific wavelengths of light, enhancing their utility in imaging and diagnostic applications.

Despite these advances, further research is required to comprehensively understand the synthesis mechanisms, structural properties, and comparative antibacterial efficacy of guava leaf-mediated AgNPs.

Challenges such as the standardization of synthesis protocols, scalability, long-term stability, and safety evaluation remain critical barriers to broader application.The primary objective of this study is to synthesize environmentally friendly silver nanoparticles using P. guajava (from the myrtle family) leaf extract and to evaluate their antibacterial activity. This research is of considerable importance, as it contributes to the development of safer and more sustainable antimicrobial agents, paving the way for their practical application across various industries.

MATERIALS AND METHODS

Materials

Fresh leaves of *Psidiumguajava* (guava) were collected and thoroughly washed to eliminate dust and other surface contaminants. Silver nitrate ($AgNO_3$) served as the precursor for nanoparticle synthesis, while distilled water was used throughout the procedure as the solvent. Standard laboratory glassware, including beakers, conical flasks, and Whatman filter paper, were utilized for extraction and filtration processes. For characterization and antibacterial analysis, nutrient agar plates and bacterial strains of *Escherichia coli* and *Staphylococcus aureus* were employed. Additional equipment included a magnetic stirrer, centrifuge, oven, and spectrophotometer, which were essential for nanoparticle synthesis, purification, and analysis.

Preparation of *Psidiumguajava* Leaf Extract

Mature, healthy, and fully expanded guava leaves were selected, rinsed under running tap water to remove surface debris, and subsequently washed three times with distilled water to eliminate any remaining impurities. The cleaned leaves were air-dried in the shade at room temperature to preserve the integrity of bioactive compounds. Once fully dried, the leaves were ground into a fine powder using a clean, sterile mortar and pestle. The powdered leaf material was stored in an airtight container, protected from moisture and light.

For extract preparation, 20 grams of the powdered leaf material were boiled in 100 mL of distilled water for 15–20 minutes with intermittent stirring. After cooling to room temperature, the mixture was filtered using Whatman filter paper to obtain a clear aqueous extract. The extract was stored at 4 °C in a container wrapped with aluminum foil for future use.

(Adapted from: Reference 6)

Green Synthesis of Silver Nanoparticles (AgNPs)

A 1 mM$AgNO_3$ solution was prepared by dissolving 0.017 g of $AgNO_3$ in 100 mL of distilled water and stored in an amber-colored bottle to prevent light-induced degradation. Equal volumes (50 mL each) of the guava leaf extract and the $AgNO_3$ solution were mixed and stirred at room temperature for 20–30 minutes. The mixture was then incubated for 24–48 hours. A visible color change from greenish to brownish indicated the reduction of silver ions and the formation of silver nanoparticles, mediated by the phytochemicals present in the guava leaf extract.

Figure 1: Schematic representation of the synthesis methodology

Characterization of Silver Nanoparticles

The formation of silver nanoparticles was initially confirmed using UV–Visible spectroscopy, with absorbance measured in the range of 300–500 nm to detect the characteristic surface plasmon resonance peak (typically observed around 400–450 nm). Scanning electron microscopy (SEM) was employed to examine the shape, size, and surface morphology of the synthesized nanoparticles. Fourier-transform infrared spectroscopy (FTIR) was used to identify the functional groups present in the guava extract that contributed to the reduction and stabilization (capping) of the AgNPs.

Evaluation of Antibacterial Activity

The antibacterial activity of the synthesized AgNPs was assessed against *Escherichia coli* and *Staphylococcus aureus* using the agar well diffusion method. Bacterial cultures were inoculated onto nutrient agar plates, and wells with a diameter of 6 mm were made using a sterile cork borer. Different volumes of AgNP solution (25 µL, 50 µL, 75 µL, and 100 µL) were added to the wells. The plates were incubated at 37 °C for 24 hours. The antibacterial efficacy was evaluated by measuring the diameter of the zones of inhibition surrounding each well.

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RESULTS AND DISCUSSION

Characterization of Silver Nanoparticles (AgNPs)

Visual Observation

Figure 2: Color change observed during AgNP synthesis at different time intervals: (a) green before incubation, (b) brown after 24 hours of incubation.

The formation of AgNPs was initially indicated by a distinct color change. Upon mixing *Psidiumguajava* leaf extract with a 1 mM$AgNO_3$ solution, a gradual color transition from green to brown was observed within two hours of incubation, signifying the reduction of Ag^+ ions to elemental silver by phytochemicals present in the extract.

UV–Visible Spectroscopy Analysis

To confirm nanoparticle synthesis, UV–Visible spectrophotometry was performed on diluted AgNP samples over a wavelength range of 300–700 nm. A strong absorbance peak was observed between 400–450 nm, corresponding to the surface plasmon resonance (SPR) of silver nanoparticles, thus confirming their successful formation.

Figure 3: UV–Visible absorption spectrum of biosynthesized AgNPs.

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Field Emission Scanning Electron Microscopy (FE-SEM)

FE-SEM analysis was conducted to evaluate the morphology and size distribution of the synthesized AgNPs.

Morphology: The nanoparticles exhibited predominantly spherical shapes across all $AgNO_3$ concentrations.
Size: The average particle size ranged between 37–77 nm.
Uniformity: While slight size variations were noted, the majority of particles were within a comparable nanoscale range.

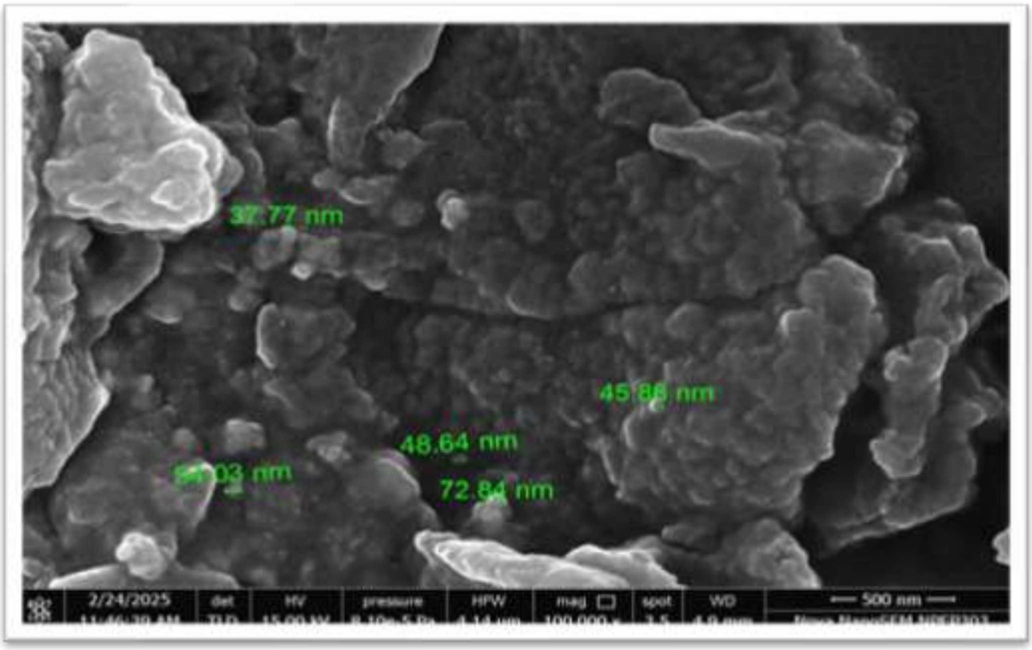
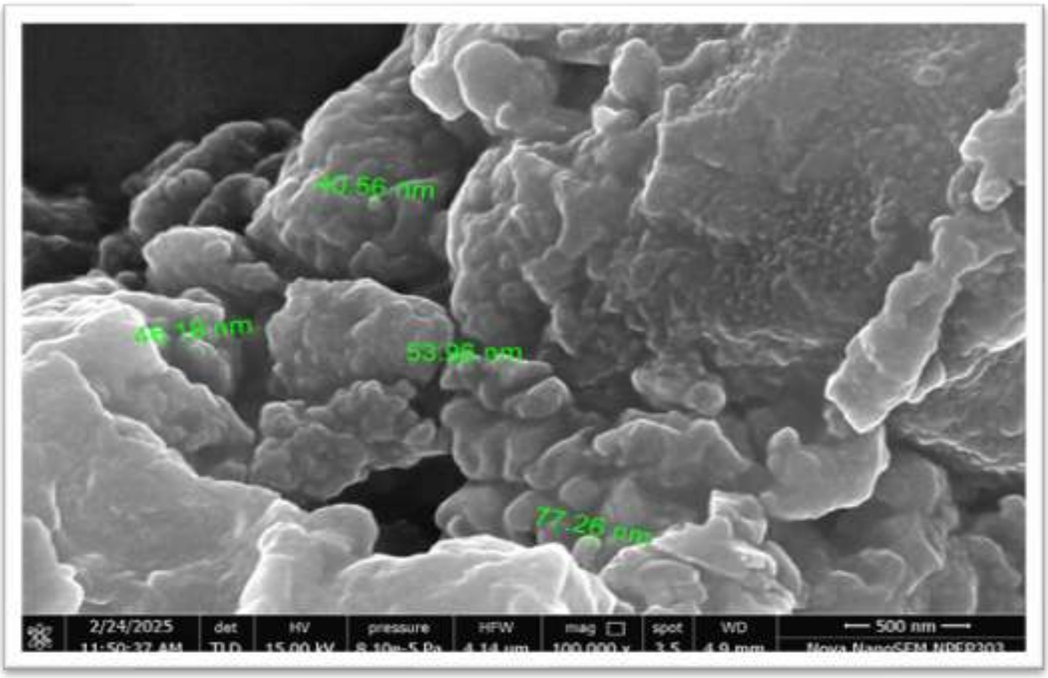


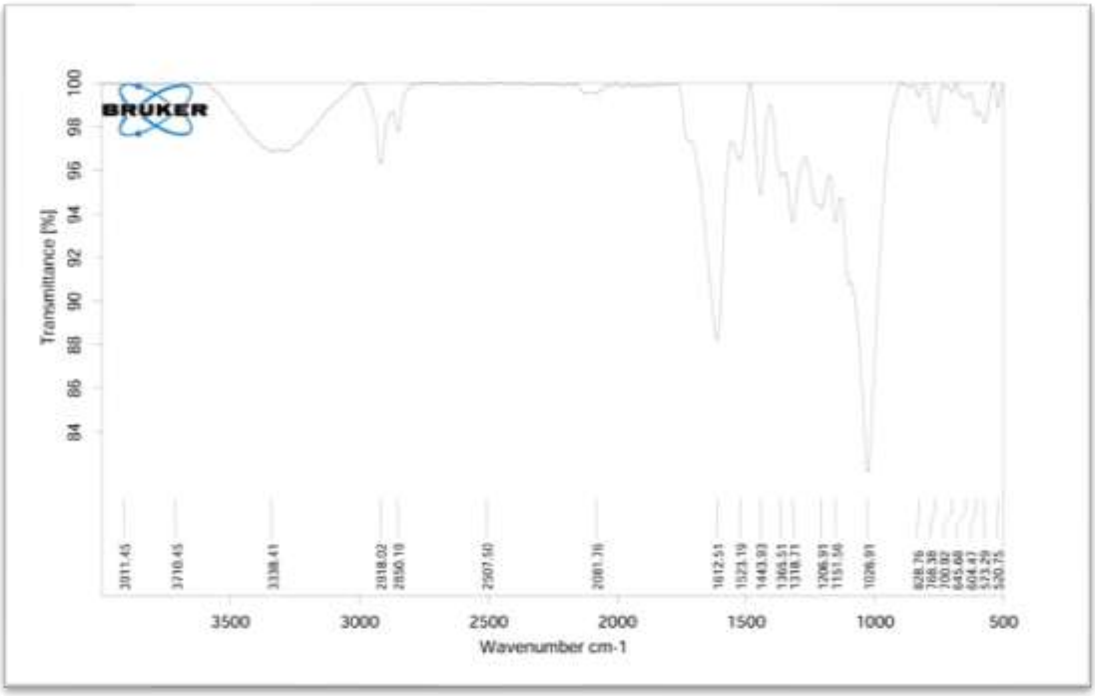
Figure 4: FE-SEM micrographs of AgNPs synthesized using guava leaf extract.



Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis was performed to identify the functional groups responsible for the reduction and stabilization of AgNPs. Key absorption peaks were detected at:

3338 cm^{-1} (O–H stretching),
2918 & 2850 cm^{-1} (C–H stretching),
1612 cm^{-1} (C=C stretching),
1026 cm^{-1} (C–O stretching),
1365 & 1289 cm^{-1} (C–N stretching),



These peaks suggest the involvement of hydroxyl, carbonyl, and amine groups in the biosynthesis process.

Sample	O–H (cm^{-1})	C–H (cm^{-1})	C=C (cm^{-1})	C–O (cm^{-1})	C–N (cm^{-1})
AgNPs	3338	2918, 2850	1612	1026	1365, 1289

Antibacterial Activity of Synthesized AgNPs

The antibacterial potential of the synthesized AgNPs was evaluated using the agar well diffusion method against two clinical pathogens: *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive). Wells of 6 mm diameter were loaded with AgNP solutions at varying concentrations (25 µL, 50 µL, 75 µL, and 100 µL). After 24 hours of incubation at 37 °C, the zones of inhibition (ZOI) were measured.

Results demonstrated a dose-dependent antibacterial effect. No inhibition was observed at 25 µL, while higher volumes resulted in progressively larger inhibition zones, confirming the antimicrobial efficacy of the synthesized AgNPs.

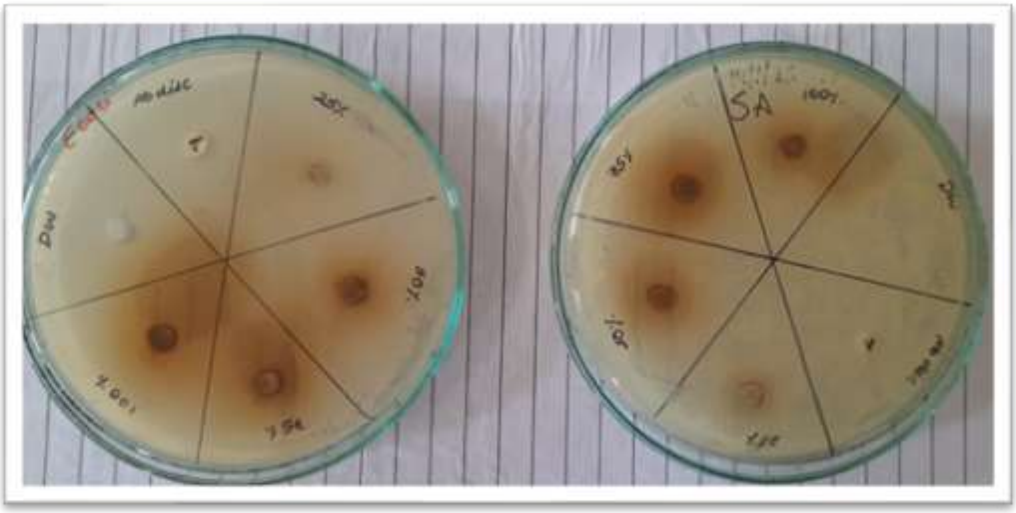


Figure 6: Zones of inhibition against E. coli and S. aureus after treatment with AgNP solutions.

$AgNO_3$ Concentration (μL)	ZOI - *E. coli* (mm)	ZOI - *S. aureus* (mm)
25	0	0
50	10	10
75	17	15
100	25	21

CONCLUSION

This study successfully demonstrates the green synthesis of silver nanoparticles using *Psidiumguajava* leaf extract, offering a sustainable and eco-friendly alternative to traditional chemical synthesis methods. The synthesized AgNPs exhibited notable antimicrobial activity against both Gram-positive and Gram-negative bacteria. The approach is not only simple and cost-effective but also aligns with the principles of green chemistry. These findings suggest that plant-based AgNPs hold promise for applications in antimicrobial coatings across healthcare, food safety, and environmental protection sectors.

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