

Original Research Article

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Biogenic Synthesis of Silver and Gold Nanoparticles and Evaluation of their Antibacterial and Cytotoxic Properties

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ABSTRACT

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The ecofriendly synthesis of silver and gold nanoparticles were successfully achieved using plant derived extracts. Mango (*Mangifera indica*) and guava (*Psidium guajava*) leaves facilitated silver nanoparticle formation, confirmed by distinct color changes and UV–Vis peaks at 353–448 nm. Pistachio shells (*Pistacia vera*), rich in polyphenols, demonstrated superior reducing capacity for gold nanoparticle synthesis, yielding stable particles with absorption maxima at 536–545 nm. Antioxidant activity was evaluated using the DPPH radical scavenging assay, where pistachio extracts showed strong inhibition from 69.97% at 20 µg/mL to 91.45% at 100 µg/mL. Antibacterial assays revealed that mango and guava extract inhibited *Staphylococcus aureus*, while antifungal ranging evaluation confirmed clotrimazole efficacy against *Candida albicans*. Preliminary anticancer studies using MG63 bone cancer cell lines demonstrated dose dependent cytotoxic effects, with reduced cell viability observed at concentrations up to 100 µg/mL. Collectively, these findings highlight the role of phytochemicals such as flavonoids, tannins, and phenolics in nanoparticle formation and bioactivity. The study concludes that plant mediated green synthesis offers a sustainable, cost-effective approach to producing nanoparticles with significant biomedical potential, reinforcing the importance of agricultural byproducts in advancing ecofriendly nanotechnology.

Introduction

Nanotechnology has emerged as a transformative field in biomedical and environmental sciences, with metallic nanoparticles such as silver (AgNPs) and gold (AuNPs) receiving particular attention due to their unique physicochemical and biological properties. Among the various approaches to nanoparticle synthesis, plant-mediated “green synthesis” has gained prominence as an eco-friendly, cost-effective, and sustainable

alternative to conventional chemical and physical methods (Ahmad *et al.*, 2022; Thumwong *et al.*, 2026). Plant extracts are rich in phytochemicals such as flavonoids, tannins, terpenoids, and polyphenols, which act as reducing and stabilizing agents, enabling nanoparticle formation under mild conditions (Gwada *et al.*, 2025; Mwatope *et al.*, 2026). Silver nanoparticles synthesized using diverse plant sources have demonstrated potent antibacterial, antifungal, antioxidant, and anti-inflammatory activities,

underscoring their biomedical relevance (Elias *et al.*, 2025; Shinde *et al.*, 2025; Pradhan *et al.*, 2025). Similarly, gold nanoparticles derived from plant extracts exhibit promising cytotoxic, anticancer, and antioxidant properties, making them valuable candidates for therapeutic applications (de Resende Marques *et al.*, 2026; Demir Becerekli *et al.*, 2026; Jiao *et al.*, 2025). Recent studies highlight the versatility of green synthesis across a wide range of plant species, including *Centella asiatica*, *Annona muricata*, *Phyllanthus emblica*, and *Swertia chirata*, each contributing distinct bioactivities (Sharma *et al.*, 2025; Al-Radadi, 2024; Hossain *et al.*, 2024; Shereen *et al.*, 2024). The integration of green chemistry principles with nanotechnology not only minimizes environmental hazards but also valorizes agricultural by-products, aligning with sustainable development goals (Marković *et al.*, 2024; Azadmanesh *et al.*, 2025). In this study, mango (*Mangifera indica*) and guava (*Psidium guajava*) leaves were selected because they are widely available, traditionally used in medicine, and rich in phytochemicals such as flavonoids, tannins, and phenolics, which have been shown to act as reducing and stabilizing agents in silver nanoparticle synthesis (Elias *et al.*, 2025; Shinde *et al.*, 2025; Pradhan *et al.*, 2025).

Their phytochemical profile not only facilitates nanoparticle formation but also contributes to antimicrobial activity, making them suitable candidates for ecofriendly synthesis. Pistachio (*Pistacia vera*) shells were chosen as a sustainable agricultural byproduct, notable for their high polyphenolic content and strong reducing potential. Prior studies have demonstrated that phenolic-rich plant materials, including *Virola oleifera* resin and jujube fruit extracts, enable efficient gold nanoparticle synthesis with significant antioxidant and cytotoxic properties (de Resende Marques *et al.*, 2026; Demir Becerekli *et al.*, 2026; Jiao *et al.*, 2025).

The use of pistachio shells also highlights the valorization of waste materials, aligning with green chemistry principles and promoting cost-effective nanotechnology development (Azadmanesh *et al.*, 2025; Marković *et al.*, 2024). Furthermore, the biomedical potential of these nanoparticles has been demonstrated in antibacterial assays against *Staphylococcus aureus*, antifungal studies on *Candida albicans*, and anticancer evaluations using human cell lines (Dagher *et al.*, 2025; Wehbe *et al.*, 2025; Jeon *et al.*, 2024). Collectively, these findings establish plant-mediated nanoparticle synthesis as a promising frontier in nanomedicine.

Materials and Methods

Collection and Preparation of Plant Materials

Fresh mango (*Mangifera indica*) and guava (*Psidium guajava*) leaves were collected from healthy, disease-free trees in non-polluted areas, while pistachio (family *Anacardiaceae*) shells were obtained from freshly consumed nuts. The selection was based on their phytochemical richness, particularly flavonoids, tannins, terpenoids, and polyphenols, which are widely reported to act as reducing and stabilizing agents in nanoparticle synthesis (Ahmad *et al.*, 2022; Gwada *et al.*, 2025; Mwamatope *et al.*, 2026). Samples were washed under running tap water for 5–10 minutes to remove dust and debris, followed by rinsing with distilled water to eliminate residual ions. Shade drying was preferred over sun drying to preserve heat-sensitive compounds such as ascorbic acid and phenolic acids. Dried materials were ground into fine powders using sterile grinders, sieved for uniform particle size, and stored in amber-colored containers to prevent photodegradation and oxidation (Pradhan *et al.*, 2025; Shinde *et al.*, 2025).

Preparation of Aqueous Extracts

Preparation of aqueous extract was carried out following the method described by Sharma *et al.*, (2025), Elias *et al.*, (2025), Pradhan *et al.*, (2025), and Shinde *et al.*, (2025), who reported similar fixed weight-to-volume approaches for plant extract preparation in green synthesis studies. Two grams of each powdered sample were mixed with 50 mL of distilled water in sterile conical flasks. The mixtures were incubated in a water bath at 60 °C for 30 minutes, cooled, and then placed on an orbital shaker overnight (12–16 hours) to maximize extraction efficiency. Filtration was performed using Whatman No.1 filter paper, yielding phytochemically rich aqueous extracts. These extracts were stored at 4 °C in sterile amber bottles until further use.

Synthesis of Silver Nanoparticles (AgNPs)

Biosynthesis of silver nanoparticles was carried out following the method described by Sharma *et al.*, (2025) and Elias *et al.*, (2025), with modifications to suit mango and guava extracts. For AgNP synthesis, 1 mL of mango or guava extract was added to 8–9 mL of distilled water, followed by 30 µL of freshly prepared 1 mM silver nitrate (AgNO₃). The mixtures were incubated at room temperature (~25 °C) under dark conditions to prevent

photodegradation. Formation of AgNPs was indicated by distinct color changes (dark brown for mango, orange-red for guava) due to surface plasmon resonance (SPR). UV–Vis spectrophotometry confirmed nanoparticle formation, with peaks observed between 353–448 nm, consistent with earlier reports of plant-mediated AgNP synthesis (Pradhan *et al.*, 2025; Shinde *et al.*, 2025).

Synthesis of Gold Nanoparticles (AuNPs)

For biosynthesis of gold nanoparticles, the method described by Sharma *et al.*, (2025) and Elias *et al.*, (2025) was followed with modifications. Gold nanoparticles were synthesized using pistachio shell extract, as mango and guava extracts lacked sufficient reducing capacity. In sterile test tubes, 1 mL of pistachio extract was mixed with 4 mL distilled water and 30 μ L of 1 mM chloroauric acid (HAuCl₄). Successful synthesis was indicated by wine-red coloration, with UV–Vis peaks at 536–545 nm confirming SPR. The strong reducing potential of pistachio polyphenols facilitated stable AuNP formation, in agreement with previous reports highlighting phenolic-rich plant materials as effective reducing agents (Pradhan *et al.*, 2025; Shinde *et al.*, 2025).

Antioxidant Assay (DPPH Method)

The antioxidant potential of pistachio extracts was evaluated using the DPPH radical scavenging assay. A 0.4 mM DPPH solution in methanol was prepared, and varying concentrations of pistachio extract (20–100 μ g/mL) were added. After 15 minutes of incubation in the dark, absorbance was measured at 517 nm. Percentage inhibition was calculated relative to the control, demonstrating strong radical scavenging activity (Azadmanesh *et al.*, 2025; Jiao *et al.*, 2025).

Antibacterial Activity

The antibacterial efficacy of mango and guava extracts was tested against *Staphylococcus aureus* using the agar well diffusion method on nutrient agar plates. Wells were loaded with 50 μ L and 100 μ L of plant extracts, while gentamycin served as the positive control.

Plates were incubated at 37 °C for 24 hours, and zones of inhibition were measured in millimetres to assess antibacterial potency as followed by Shereen *et al.*, 2024; Elias *et al.*, 2025.

Antifungal Activity

Antifungal activity was assessed against *Candida albicans* using potato dextrose agar (PDA) plates. Wells were loaded with clotrimazole solution as the test agent, and plates were incubated at 28–30 °C for 48 hours as followed by Kalantari *et al.*, 2024; Marković *et al.*, 2024. Clear zones of inhibition confirmed antifungal efficacy.

Anticancer Assay

Cytotoxicity was evaluated using MG63 bone cancer cell lines maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with fetal bovine serum. The MTT assay was performed by treating cells with varying concentrations of extracts for 24 hours. Formazan crystals formed were dissolved in DMSO, and absorbance was measured at 570 nm to determine cell viability. Morphological changes in treated cells were observed under an inverted microscope, confirming cytotoxic effects.

Results and Discussion

Synthesis of Silver Nanoparticles (AgNPs)

Mango (*Mangifera indica*) and guava (*Psidium guajava*) extracts successfully reduced silver nitrate (AgNO₃) to silver nanoparticles (AgNPs), as evidenced by distinct color changes and characteristic UV–Vis absorption peaks. The mango extract produced a dark-brown coloration, with absorption maxima at 353, 384, and 448 nm, whereas the guava extract exhibited an orange-red coloration with a prominent absorption peak at 416 nm. These spectral changes are attributable to surface plasmon resonance (SPR), confirming the formation of AgNPs. Similar SPR absorption ranges have been reported for plant-mediated AgNP synthesis, in which phytochemicals such as flavonoids and phenolic compounds contribute to the reduction of silver ions and stabilization of the resulting nanoparticles. UV–Vis spectroscopy alone cannot conclusively establish these structural characteristics, and further analyses such as transmission electron microscopy (TEM), scanning electron microscopy (SEM), dynamic light scattering (DLS), and X-ray diffraction (XRD) would be required for detailed characterization. Overall, the observed color changes and SPR-associated UV–Vis absorption bands confirm the successful plant-mediated synthesis of AgNPs using both *M. indica* and *P. guajava* extracts. The

results are consistent with previous reports of green synthesis of AgNPs using plant extracts, in which phytochemicals such as flavonoids and phenolic compounds have been proposed to function as reducing and stabilizing agents (Ahmad *et al.*, 2022; Gwada *et al.*, 2025; Shinde *et al.*, 2025).

Synthesis of Gold Nanoparticles (AuNPs)

Attempts to synthesize gold nanoparticles (AuNPs) using mango (*Mangifera indica*) and guava (*Psidium guajava*) extracts did not result in an observable AuNP formation under the experimental conditions employed. This outcome may be associated with differences in the concentration, composition, or reducing capacity of the phytochemicals present in the extracts. In contrast, pistachio (*Pistacia vera*) shell extract, rich in polyphenols, successfully reduced chloroauric acid to gold nanoparticles. The diluted extract produced a wine-red color with a peak at 536 nm, while undiluted extract yielded a deeper red with a peak at 545 nm, indicating larger particle size or aggregation. The findings are consistent with previous reports demonstrating the potential of phenolic-rich plant materials as reducing and stabilizing systems for green AuNP (de Resende Marques *et al.*, 2026; Jiao *et al.*, 2025; Demir Becerekli *et al.*, 2026). UV-Vis spectroscopy alone cannot conclusively distinguish between these possibilities. Morphological and size-related techniques such as transmission electron microscopy (TEM) or scanning electron microscopy (SEM) would be required to verify this interpretation.

Antioxidant Activity

The DPPH radical scavenging assay demonstrated strong antioxidant potential of pistachio shell extract. Inhibition increased with concentration, ranging from 69.97% at 20 µg/mL to 91.45% at 100 µg/mL, confirming dose-dependent activity. This correlation between phytochemical concentration and radical scavenging efficacy agrees with earlier studies on plant-derived nanoparticles (Azadmanesh *et al.*, 2025; Demir Becerekli *et al.*, 2026). The high polyphenolic content of pistachio shells likely contributed to their superior antioxidant performance.

Antibacterial Activity

Agar well diffusion assays demonstrated that both mango (*Mangifera indica*) and guava (*Psidium guajava*) extracts

exhibited antibacterial activity against *Staphylococcus aureus*. Clear zones of inhibition were observed at extract volumes of 50 and 100 µL, indicating that both plant extracts were capable of inhibiting the growth of the tested bacterial strain under the experimental conditions. The observed antibacterial activity may be attributed to bioactive phytochemicals present in the extracts, particularly phenolic compounds, flavonoids, and other secondary metabolites known to possess antimicrobial properties.

Gentamicin was used as the positive control and produced the largest zone of inhibition among the tested samples. This confirmed the susceptibility of *S. aureus* to the standard antibiotic and provided a reference for evaluating the antibacterial activity of the plant extracts. Although the mango and guava extracts produced measurable inhibition zones, their activity cannot be considered equivalent to that of gentamicin based solely on agar diffusion results.

The increase in extract volume from 50 to 100 µL resulted in observable inhibition, suggesting that the antibacterial effect may be influenced by the number of bioactive compounds introduced into the agar medium. However, inhibition-zone diameter is affected not only by antimicrobial potency but also by factors such as compound diffusion, molecular size, extract composition, and agar properties. Therefore, the results provide evidence of antibacterial activity but do not by themselves establish the minimum inhibitory concentration or bactericidal concentration of the extracts.

The antibacterial effects observed against *S. aureus* are consistent with previous reports describing the antimicrobial properties of plant-derived extracts and their phytochemical constituents (Thumwong *et al.*, 2026; Dagher *et al.*, 2025; Sharma *et al.*, 2025). Phenolic and flavonoid compounds may contribute to bacterial inhibition.

Overall, the agar well diffusion assay confirms that mango and guava extracts possess measurable antibacterial activity against *S. aureus*. Further investigations, including minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), phytochemical profiling, and testing against a broader range of bacterial pathogens, would be useful to further characterize their antimicrobial properties.

Table.1 (Synthesis of Silver Nanoparticles) (AgNPs)

Extract type	Colour observed	UV- Spectrophotometer
Mango extract	Dark brown	353,384,448 nm
Guava extract	Orange brick red	416nm

Table.2 (Synthesis of Gold Nanoparticles (AuNPs))

Extract type	Colour observed	UV – spectrophotometer
Pistachio (diluted)	Wine red	536
Pistachio (undiluted)	Deep red	545

Table.3 (Readings under UV (Single Beam))

Test Tube	DPPH	Concentration of Sample	Absorbance	% of Inhibition
Control	2ml	1ml of methanol	0.936	-
T1	2ml	20 µL	0.281	69.97 %
T2	2ml	40 µL	0.176	81.19 %
T3	2ml	60 µL	0.137	85.36 %
T4	2ml	80 µL	0.098	89.529 %
T5	2ml	100µL	0.080	91.452 %

Fig.1 Antibacterial activity of mango and guava extracts against *Staphylococcus aureus* using agar well diffusion assay.



Fig.2 Antifungal activity of mango and guava extracts against *Candida albicans* on PDA medium.



Antifungal Activity

The antifungal potential of the tested agent was evaluated against *Candida albicans* using the agar well diffusion method on Potato Dextrose Agar (PDA) medium. Clear zones of inhibition were observed around the wells containing clotrimazole, confirming its efficacy as a positive control. The presence of distinct inhibition zones validated the sensitivity of *C. albicans* to the antifungal compound and ensured that the experimental conditions were appropriate for assessing antifungal activity.

The observed inhibition can be attributed to the mechanism of clotrimazole, which disrupts fungal cell membranes by inhibiting ergosterol biosynthesis, ultimately leading to cell lysis and death. This aligns with previous reports that highlight clotrimazole's broad antifungal spectrum and its effectiveness against *Candida* species (Kalantari *et al.*, 2024; Marković *et al.*, 2024).

Although the present study primarily used clotrimazole as a reference drug, literature suggests that plant-derived silver nanoparticles (AgNPs) also exhibit antifungal properties. Several studies have reported that AgNPs synthesized from plant extracts can inhibit fungal growth by generating reactive oxygen species, altering membrane permeability, and interfering with fungal metabolic pathways (Shereen *et al.*, 2024; Marković *et al.*, 2024). This indicates that mango (*Mangifera indica*) and guava (*Psidium guajava*) extracts, which

successfully produced AgNPs in the current work, may possess unexplored antifungal potential. The agar well diffusion assay provides preliminary evidence of antifungal activity; however, it does not establish the minimum inhibitory concentration (MIC) or fungicidal concentration. Zone diameters are influenced not only by antifungal potency but also by diffusion rates, molecular size, and agar composition. Therefore, while the results confirm antifungal efficacy of clotrimazole and suggest potential for plant-mediated AgNPs, further investigations are required. Future studies should include MIC/MFC determination, time-kill assays, and direct testing of synthesized AgNPs against *Candida albicans* and other clinically relevant fungi to comprehensively evaluate their antifungal properties.

Overall, the antifungal assay demonstrates that clotrimazole remains a potent control against *C. albicans*, while literature evidence opens avenues for exploring mango- and guava-derived AgNPs as eco-friendly antifungal agents. This dual perspective strengthens the biomedical relevance of green-synthesized nanoparticles and highlights their potential role in combating fungal infections.

Anticancer Activity

The MTT assay on MG63 bone cancer cells demonstrated dose-dependent cytotoxic effects of the synthesized nanoparticles. Reduced cell viability was observed at concentrations up to 100 µg/mL, with

morphological changes confirming cytotoxicity. These findings indicate anticancer potential, though they represent cytotoxicity rather than a complete anticancer claim, as advanced assays (apoptosis markers, gene expression) were not performed. Similar nanoparticle-mediated cytotoxicity has been reported in other plant-based systems (Wehbe *et al.*, 2025; Al-Radadi, 2024).

In conclusion, this study demonstrated the green synthesis of silver nanoparticles (AgNPs) using mango (*Mangifera indica*) and guava (*Psidium guajava*) extracts, and gold nanoparticles (AuNPs) using pistachio (*Pistacia vera*) shell extract. Nanoparticle formation was confirmed by characteristic color changes and UV–Vis absorption peaks corresponding to surface plasmon resonance, indicating the involvement of plant-derived phytochemicals in nanoparticle formation.

Pistachio extract showed strong, dose-dependent antioxidant activity, with 69.97–91.45% radical-scavenging inhibition. Mango and guava extracts exhibited antibacterial activity against *Staphylococcus aureus*, while clotrimazole showed antifungal activity against *Candida albicans*.

Cytotoxicity assays using MG63 cells demonstrated reduced cell viability at concentrations up to 100 µg/mL, suggesting potential biological activity that requires further mechanistic investigation.

Overall, plant-mediated synthesis provides a simple, cost-effective, and eco-friendly approach to nanoparticle production using renewable plant materials and agricultural by-products. Further characterization using SEM, TEM, XRD, FTIR, DLS, and zeta-potential analysis, together with detailed biological and mechanistic studies, is required to establish nanoparticle properties and their potential biomedical applications.

Author Contributions

G. J Aswini: Original draft preparation, Data curation, methodology, writing-review & editing. C. Soundhari: Designing Protocols, Evaluation of Manuscript

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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