

ORIGINAL ARTICLE

Biogenic Synthesis of Silver Nanoparticles from Aqueous Leaf Extract of *Psidium guajava* and their Antibacterial Activity

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ABSTRACT

The green synthesis of silver nanoparticles (AgNPs) utilizing *Psidium guajava* extracted is a sustainable and eco-friendly method with important biomedical and environmental implications. The mechanisms of the synthesized nanoparticles were investigated for its antibacterial activity. UV-visible spectroscopy, X-ray diffraction (XRD), and high-resolution transmission electron microscopy (HR-TEM) were used to characterize the synthesized silver NPs, confirming their homogeneity and physical properties. The plant metabolites played a key role in decreasing Ag⁺ to Ag⁰ and as capping agent for AgNPs. In UV-visible spectroscopy, a typical Ag⁰ Surface Plasmon Resonance band was found at 428 nm, confirming the formation of AgNPs. The AgNPs were face-centered cubic (FCC) crystalline, with an average size of 6.66 nm, as reported through XRD analysis. HR-TEM confirmed that the particles were homogenous and spherical, ranging between 40 to 100 nm. The FTIR spectra revealed information about the extract's functional groups, which helped in stabilizing AgNPs. The strong and wide peak at 3326 cm⁻¹ was observed in the plant extract. The participation of the hydroxyl group (-OH) present in the extract's compounds in the reduction of silver ions. The presence of silver in the nanostructure was verified by the EDX spectrum peak at around 3 keV. In addition, the biological potential of AgNPs was evaluated for their antibacterial activities. AgNPs showed significant zone of inhibition against all selected bacterial strains such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. The zone of inhibition produced by AgNPs exhibited remarkably in conjugation with gentamycin and chloramphenicol even at substantially lower concentrations. With further research, the green synthesis of AgNPs using *P. guajava* leaf extracts could lead to the development of effective antimicrobial agents for various biomedical applications.

Keywords: antibacterial activity; *Psidium guajava*; silver nanoparticles; characterization.

Received 25.05.2025

Revised 23.06.2025

Accepted 27.07.2025

How to cite this article:

Mayank R, Shad K, Suraj P, Sushil K U, Manoj S, Mukesh Y, Sunil K, Vandana S, Jatinder K S, Yogendra P P and Deepak Y. Biogenic Synthesis of Silver Nanoparticles from Aqueous Leaf Extract of *Psidium guajava* and their Antibacterial Activity. Adv. Biores., Vol 16 (4) July 2025: 441-450

INTRODUCTION

Nanotechnology represents the most significant approach in modern scientific endeavors globally. Researchers have significantly advanced electronics, environmental science, and human health in medical research using nanotechnology [1]. The application of nanoparticles (NPs) in nanotechnology is increasing steadily [2]. To meet the demand, different synthesis methods are being created over time. Traditional chemical or physical methods employed for nanoparticle synthesis are detrimental to environmental and human health due to chemical risks and contamination from reactions [3,4].

Simultaneously, traditional methods for nanoparticle synthesis are laborious, costly, and hazardous in terms of toxicity [5]. Conversely, green synthesis is an environmentally benign and sustainable novel process due to the use of numerous ingredients derived from organic materials [6]. The green synthesis of nanoparticles is a time-efficient and cost-effective biogenic process that minimizes chemical hazards. Both metallic and oxide nanoparticles exhibit significant potential. This synthesis method employs diverse components derived from organic sources [7–9]. Typically, wood and bones are utilised to make nanoparticles; nevertheless, in the green methodology, plants are favored above other materials. Plant elements such as barks, roots, fruits, and leaves are commonly utilised to synthesize nanoparticles [10]. Furthermore, extracts from green leaves of several medicinal plants are utilised due to their notable lowering and capping properties for the conversion of metallic ions into nanoparticles. *Azadirachta indica* (neem), *Moringa oleifera*, *Justicia adhatoda*, *Psidium guajava* (*P. guajava*) and similar species are prevalent medicinal plants utilised for the green production of nanoparticles (NPs). The extract of *P. guajava* (guava) is predominantly utilised due to its content of glycosides and polyphenolics (flavonoids and tannins), which function as reducing and capping agents to transform metallic ions into nanoparticles [12–14]. *P. guajava*, widely referred to as guava, is a favored fruit in Bangladesh. This perennial tree is distributed across Asia and is extensively farmed in India, Bangladesh, and Malaysia. The synthesis of different nanoparticles, including Au, Cu, Ni, Al, and Ag, can be achieved using guava leaf extract [15,16]. Numerous researchers have endeavored to synthesize metallic nanoparticles using aqueous extracts of *P. guajava* leaves, particularly silver nanoparticles, due to the extensive benefits of green synthesis. Silver nitrate (AgNO_3) served as a precursor, with the majority of studies employing a silver ion solution concentration ranging from 1 to 30 mM, decreased by varying amounts of aqueous extract from *P. guajava* leaves. During the synthesis process, silver ions (Ag^+) are reduced to metallic silver (Ag^0) by biological molecules present in the leaf extract, which may include reducing agents and metal salt reducers. The extract of *P. guajava* leaves comprises biochemicals, including polyphenols, carotenoids, terpenoids, flavonoids, tannins, and triterpenes, which may facilitate the reduction of metal ions to nanoparticles. Bose et al. synthesised AgNPs utilising *P. guajava* and examined their antibacterial efficacy against a Gram-negative bacteria strain [9]. Sharmila et al. report results for both Gram-negative and Gram-positive bacteria with a zone of inhibition measuring less than 10 mm [17]. Geetha et al. observed an inhibitory zone of less than 10 mm when analysing positive microorganisms in AgNPs solutions [18]. Dama et al. synthesized spherical AgNPs utilizing methanol as a solvent and evaluated their antimicrobial activity against *E. coli*, *S. aureus*, and *P. aeruginosa* [10,19]. AgNPs have been utilised in several fields including engineering, communication, photochemistry, biomedical applications, and antibacterial packaging protection. Moreover, it is utilised in biomedical devices, environmental cleanup, pharmaceuticals, food packaging, cosmetics, and several industrial applications. AgNPs exhibit unique composition and structure, crystallinity, morphology, and dimensions in contrast to their bulk counterparts. As a result, it demonstrated superior physicochemical features, including chemical stability, surface-enhanced Raman scattering, high thermal and electrical conductivity, catalytic activity, nonlinear optical behaviour, and biological properties in comparison to other nanoparticles. AgNPs have a broad range of antibacterial and antifungal properties, commonly utilised in diverse consumer products such as cosmetics, toiletries, plastics, food, and textiles [20]. This research aims to synthesize environmentally friendly silver nanoparticles (AgNPs) from the leaf extract of *P. guajava* and to investigate their antibacterial properties. Synthesized AgNP solutions with varying concentrations of AgNO_3 were evaluated for antibacterial efficacy against Gram-negative bacteria and also in conjugation with Chloramphenicol and Gentamicin.

MATERIAL AND METHODS

This research work, healthy and fresh leaves of *P. guajava* plant was collected in sterile plastic bags from three distinct locations in the Maharishi Markandeshwar (Deemed to be University) campus in Mullana. At each location, a sample was taken randomly from a chosen location. The tree was medium-sized; has leaves of 7–10 cm in length and 4–5 cm in width and remains green throughout the year. Analytical grade silver nitrate (AgNO_3) and sodium hydroxide (NaOH) were purchased from Merck, Germany (98% purity). For the preparation of leaf extract deionized water and Whatman no. 1 filter papers were used for erosion and filtration purpose. Moreover, for cleaning glassware (pipette, beaker, etc.) ethanol and distilled water were applied.

Preparation of *P. guajava* Leaf Extract: 20gm of fresh *P. guajava* leaves were used to prepare the aqueous extracellular extract of leaves. The leaves were thoroughly rinsed with double-distilled water and then cut into small pieces. Then, 100 mL of double-distilled water was added to the finely chopped pieces, and the mixture was allowed to boil for 5 minutes. It was passed through Whatman Filter Paper

No. 1 and stored at freezing temperature. The obtained aqueous leaf extract was subsequently added to AgNO_3 solution at several concentrations. The remaining extract was stored in the refrigerator at 4°C , sealed with aluminium foil for future use.

Biosynthesis of Silver Nanoparticles: To synthesize silver nanoparticles, 10 mL of aqueous leaf extract from *P. guajava* and 10 mL of AgNO_3 solution at several concentrations were combined in a beaker. The solution was subjected to a hot plate, elevating the temperature to 60°C while maintaining continuous magnetic stirring for 10 minutes. Subsequently, it was maintained at room temperature for 2 hours, during which the reduction of Ag^+ ions to metallic Ag^0 occurred. The solution was meticulously examined, and without any pulsation, its colour transitioned from pale yellow to reddish brown, indicating the initial synthesis of AgNPs. A little aliquot of colored suspension was centrifuged at 10,000 rpm for 15 minutes to get pellets. The collected items were repeatedly rinsed with deionized water to eliminate any contaminants. Figure 1 illustrates the complete process of synthesizing silver nanoparticles (AgNPs) mediated by *P. guajava* leaf extract.

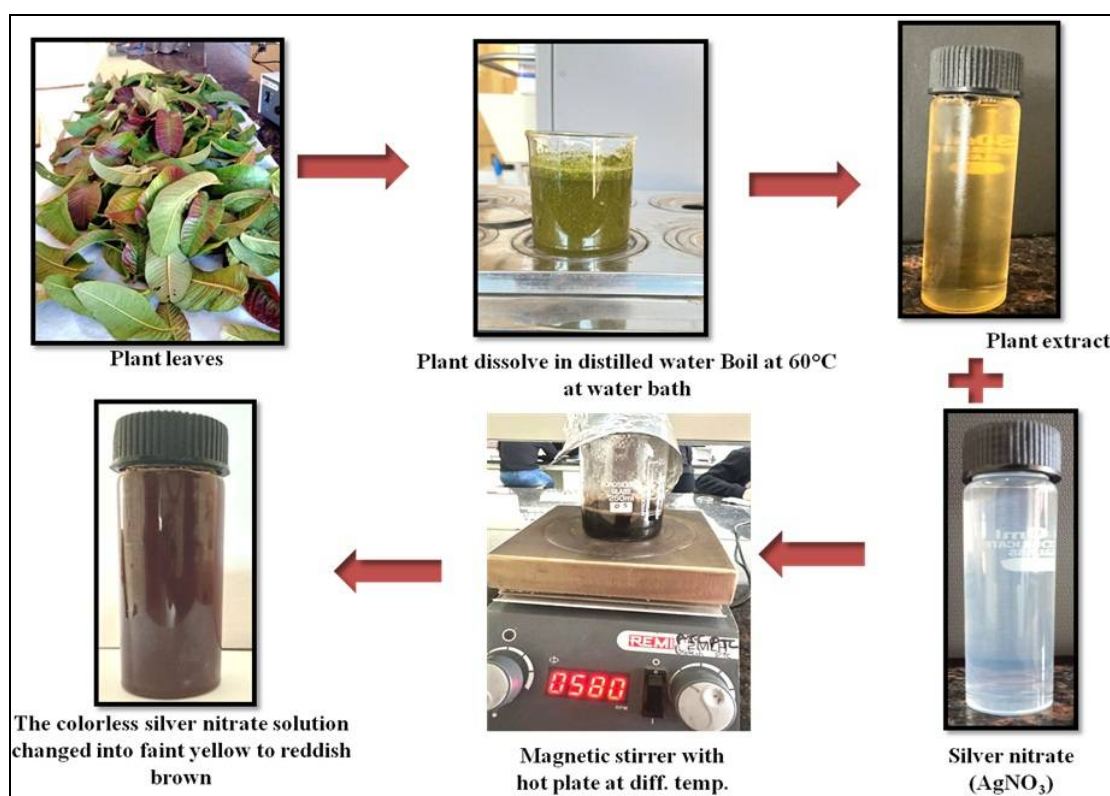


Figure 1: Schematic representation for the synthesis of *P. guajava* leaf extract mediated AgNPs.

Instrumentation: The characteristic of synthesized AgNPs was investigated by different characterization techniques. The bioreduction of AgNO_3 in aqueous solution was observed with mixed plant extracts studies by using ultraviolet-visible near-infrared (UV-Vis-NIR) spectrophotometer (UV-2600, Shimadzu, Japan) ranging 200–800 nm. X-ray diffraction (XRD) (Rigaku Smart Lab, Japan) with Cu-K α radiation ($\lambda = 1.5406 \text{ \AA}$) was used to confirm the crystalline of AgNPs at room temperature. The scanning rate was $10^\circ \text{ min}^{-1}$; power 40 kV at 40 mA and 0.02° step. A high resolution transmission electron microscope (HR-TEM) (JSM-7610F, Japan) and energy dispersive X-ray (EDX) were used to study the surface morphology of AgNPs to identify the elemental composition of materials. Highly pure KBr powder was mixed with 1% (w/w) samples and then pressed into pellets to identify the bioactive functional group of AgNPs by Fourier transform infrared spectroscopy (FTIR, PerkinElmer, USA) recorded within $4000\text{--}350 \text{ cm}^{-1}$.

RESULTS AND DISCUSSION

Characterization of AGNPs: AgNPs possess distinctive optical characteristics that enable them to interact effectively with particular wavelengths of light [21]. The creation of silver nanoparticles was primarily assessed by observing the colour of the solution. Following a two-hour mixture of the leaf extracts with AgNO_3 , the solution transitioned from a light yellowish colour to a brownish colour, indicative of the existence of AgNPs due to surface plasmon resonance (SPR). Figure 2 illustrates the

colour change observed in AgNPs solutions, which yielded results consistent with prior research. To further validate the creation of nanoparticles, UV-Vis spectroscopic investigation was conducted.

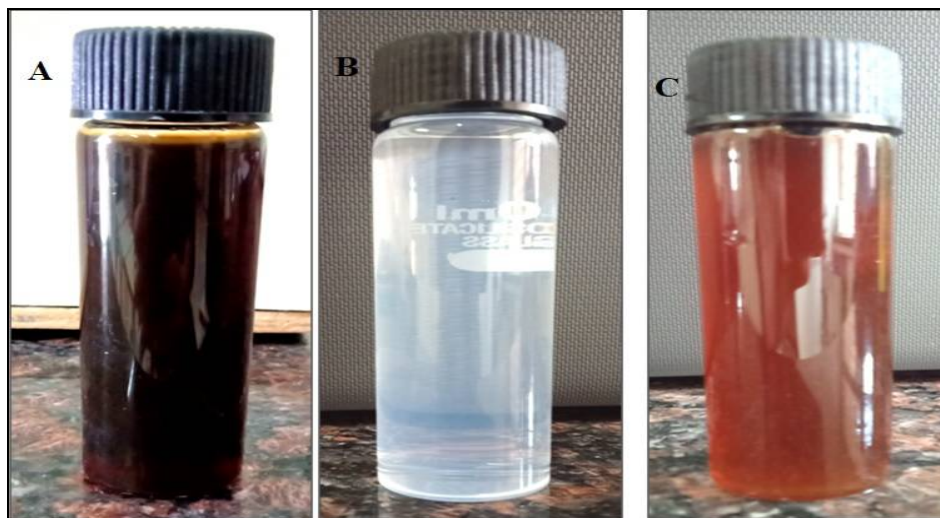


Figure 2: Preliminary Synthesis: (a) Brown color from aqueous leaf extract of *P. guajava*, (b) white color due to mixing of AgNO_3 (c) Dark colour change indicates the formation of silver nanoparticles.

UV-Vis Spectroscopic Analysis: UV-Vis spectroscopy is an effective, reliable, sensitive, and selective technique employed for the first identification of various types of nanoparticles. Furthermore, calibration is unnecessary for assessing the particle characteristics of the colloidal suspension [22,23]. Figure 3(a) illustrates the UV-Vis absorption spectra of silver nanoparticles (AgNPs) solution mediated by *P. guajava* leaf extract, within the wavelength range of 200–700 nm. The baseline data was obtained using deionized water, also referred to as the sample blank in this analysis. The colored samples were diluted with deionized water and placed in a quartz cell for UV spectroscopy, where absorption peaks were identified in the ultraviolet range of around 350–530 nm. The absorption peaks were identified at various time intervals of AgNO_3 salt, as illustrated in figure 3a. Figure 3b and figure 3c exhibit the effects of concentration and pH change on AgNO_3 solution. The absorption in the visible spectrum progressively increases with time and temperature. Figure 3(b) illustrates that the absorption intensity was red-shifted as a result of the increase in particle size over time, corroborated by the SPR intensity through the percentage reduction of Ag^+ to Ag^0 . The free electron between the conduction band and the valence band oscillates, producing a spectrum of absorption peaks due to the mass oscillation of the electrons in silver nanoparticles resonating with the optical wave at surface plasmon resonance (SPR) [24,25]. The primary factors influencing the absorption of AgNPs are particle sizes and the surrounding chemical and electrical insulation [18,26,27].

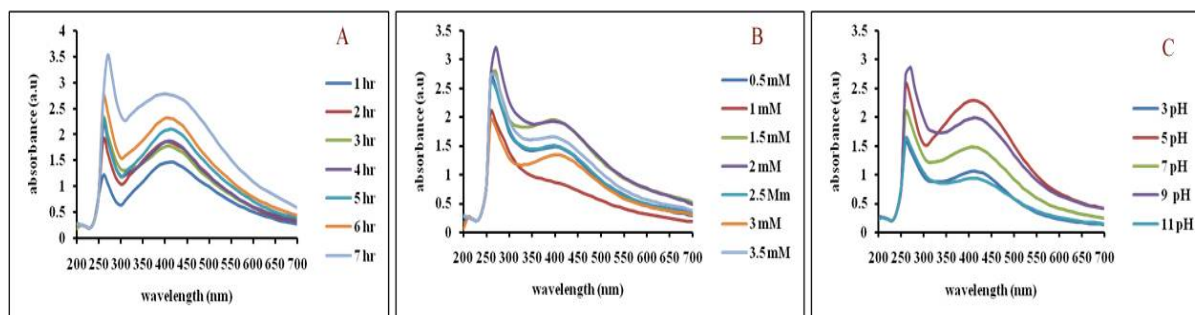


Figure 3: UV-Vis absorption spectra of AgNPs (a) at different time intervals for AgNO_3 solution (b) at different concentrations of AgNO_3 solution and (c) at different pH AgNO_3 solution.

Dynamic Light Scattering Analysis: The biofunctionalized silver nanoparticles formed from the aqueous solution of 0.5 mM with 5% aqueous leaves extract of *P. guajava* at 60°C temperature was characterized for particle size distribution. The peak number and peak amplitude obtained explained the size and size

distribution of silver nanoparticles. The calculated particle size distribution by intensity was observed in the 10-100 nm range (Fig. 4). It can be seen that the mean particle size was ca.49.63 nm, with some particles having diameters 100-1000 nm. The Poly dispersity index (pdl) was 0.418.

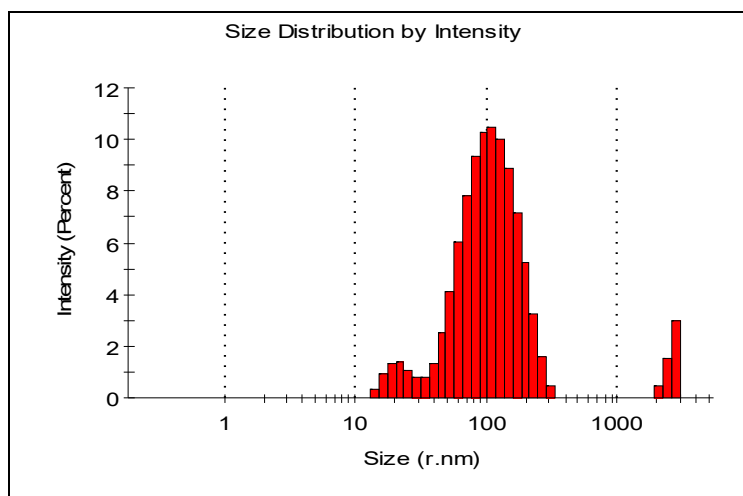


Figure 4: DLS size distribution of silver nanoparticles.

X-Ray Diffraction Analysis: The structural properties of particles were analyzed by a conversant technique, XRD to get information about crystallinity and phase formation. The XRD pattern with reflections at different angular positions of the synthesized AgNPs was shown in figure 5. The Bragg's peaks were observed at 2 positions 32.99°, 39.21°, 44.39°, 64.43° and 77.37° for (101), (110), (200), (201) and (112) crystal planes respectively. The formations of AgNPs were confirmed further by these characterization reflections. Also, the diffraction peaks of the XRD pattern correspond to the card (JCPDS No. 03-0921) which reflects those NPs are crystalline in nature [28]. The XRD pattern has confirmed that the synthesized samples were AgNPs with face-centered cubic crystal structure. Along with the characteristic peaks in the XRD pattern some marked reflections also exist at 2 (27.90°, 30.01°, 31.06, 46.34 and 54.97°) which were not known precisely. It may be due to the crystallization of bioorganic phases on the AgNPs surface that originates from the leaf extract or due to AgNO₃ that was not reduced and remained in the sample slightly. Many researchers also get similar patterns in XRD. The average crystallite size, D of the synthesized AgNPs were estimated from the diffractogram by using the Debye-Scherrer formula, $D = 0.9\lambda / b \cos \theta$, where λ and b are the wavelength of diffraction and full width at half maximum (FWHM) of a peak respectively. The interplanar spacing between the atoms, d , was calculated using Bragg's law, and lattice constant, a , was determined. It was found that the D values are nearly identical for all the peaks and the average crystallite size obtained was ~22 nm. Thus, the XRD analysis of silver nanoparticles showed well-defined dimensions has fair agreement with the standard value for silver. Moreover, the lattice parameters, d -spacing, and hkl value calculated from the characteristic peaks of the synthesized sample match with reports of other researchers [10,15].

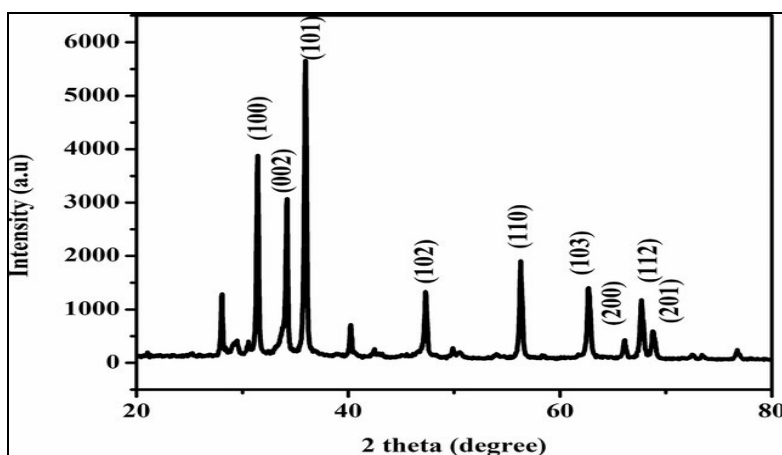


Figure 5: XRD patterns of synthesized leaf extract mediated AgNPs.

High Resolution Transmission Electron Microscopy (HR-TEM) Analysis: The morphology of guava leaf extract-mediated AgNPs were investigated by HR-TEM. A mixture of the plate (spherical and hexagons) and spheres was illustrated in figure 6. Representative TEM images revealed that the size distribution of AgNPs was in the range range of 40 to 100 nm. The high-resolution TEM displayed clear lattice fringes on the particle surfaces. SAED patterns are interpreted by analyzing the diffraction spots or rings which corresponds to specific crystal planes.

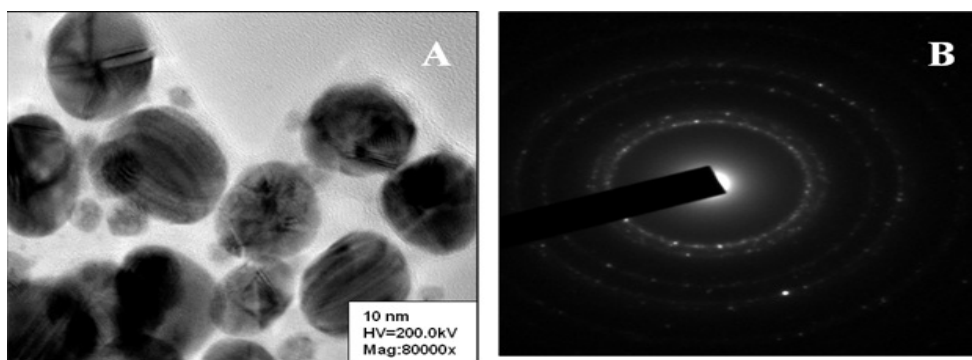


Figure 6: (a) HR-TEM image (b) SAED pattern of silver nanoparticles

Fourier Transforms Infrared Spectroscopy (FTIR) Analysis: To detect functional group properties for NPs, FTIR is the most significant spectroscopic technique. FTIR spectra were employed to identify the photochemical elements in leaf extract-mediated AgNPs which are responsible for reducing and capping agents on Ag^+ ions to AgNPs. Those bioactive different functional groups produce effective NPs. In our research, guavas leave extract contains bioactive high polyphenolics acid molecules whose presence was found in FTIR spectra. The FTIR spectra of AgNPs with different functional groups were shown in figure 7. It revealed that the characteristic peak at 3326 cm^{-1} can be attributed to the $-\text{OH}$ stretching indicating the capping and reducing agent. In addition, $-\text{C}-\text{H}$ stretching vibrations of alkane came from the carboxylic acid group at 2351 cm^{-1} . Highly conjugated carboxylic functional groups were found at 1995 cm^{-1} absorption band and the materialization oxygen group $-\text{C}-\text{O}$ vibrations were confirmed at 1638 cm^{-1} respectively. The peak 1633 cm^{-1} confirmed the formation of AgNPs. Thus, the fingerprint suggests that amid bonds belonging to aromatic, ethers and polyphenols are responsible bioactive elements that act as reducing and stabilizing agents for leaf extract-mediated AgNPs. The absorption peak was slightly shifted from guava leaf extract to AgNPs because of the formation of nanoparticles. Table 1 shows the different bioactive functional groups which confirm the formation of Ag^+ ions to AgNPs [33, 35].

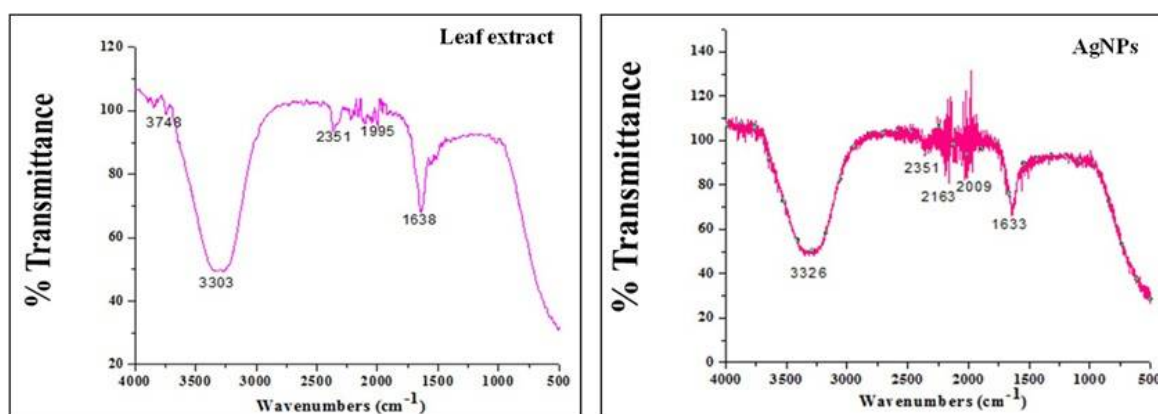


Figure 7: FT-IR spectra of *P. guajava*-mediated AgNPs and leaf extract.

Table 1: Functional group at different characteristics absorptions of FT-IR data (cm^{-1}).

Sample name	O-H (stretching)	C-H (alkane)	C=C (stretching)	-C-O (stretching)	O-H (stretching)
AgNPs	3326	2351	2163	2009	1633
Leaf extract	3748	3303	2351	1995	1638

EDX Analysis: The EDX profile showed a strong signal for silver along with some other signal peaks that might have originated from the biomolecules that are bound to the surface of AgNPs, representing the reduction of silver ions to elemental silver. The other peak of Cu in the EDX is an artifact of the Cu grid on which the sample was coated. No other signal peaks were noticed for silver [23]. This authorizes the thorough reduction of silver compounds to *P. guajava*, as revealed in the EDX spectrum in figure 8.

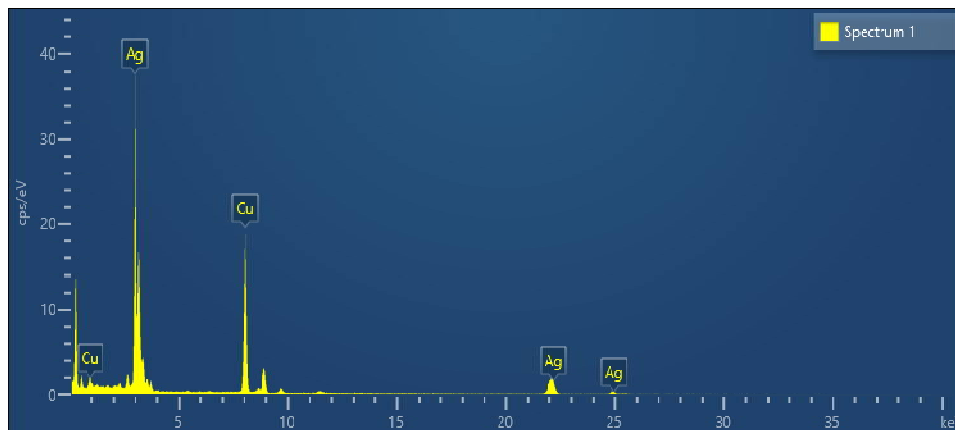


Figure 8: EDX graph of silver nanoparticles

Antibacterial Activity: The antibacterial screening of NPs is one of the important studies to find an antibacterial agent. NPs can be considered effective antibacterial agent when it exhibits a significant zone of inhibition (ZOI) against bacteria [36,37]. A well disk diffusion method was applied for the measurement of zone of inhibition of *P. guajava*-assisted AgNPs [38]. To attune the optical density (OD 0.1) of bacteria in culture UV-visible spectrophotometer (JASCO V-600, Japan) was used. Subsequently, we optimized the testing conditions and assessed the susceptibility of rapidly synthesized silver nanoparticles from *P. guajava* and commercial drugs against both Gram-negative bacteria (*E. coli*, *Pseudomonas aeruginosa*) and Gram-positive bacteria (*S. aureus*, *Bacillus subtilis*). The surface chemistry and size pattern of metallic ions of the nanometer range helped us investigate silver nanoparticles efficacy as drug carriers. To understand the mechanisms, a comparative analysis of drugs formulated with nanoparticles was performed. The in-vitro antibacterial activity of gentamicin and chloramphenicol, along with their nanoformulation, were tested against bacterial strains. The zone of inhibition for gentamycin formulated silver nanoparticles for *S. aureus* was more significant than that for chloramphenicol nanocolloids. Whereas, the zone of inhibition for both nanoformulated drugs against *E. coli* (gent~3.6 mm and chlo~3 mm), *S. aureus* (gent~3.9 mm and chlo~2.9 mm), *P. aeruginosa* (gent~2.8 mm and chlo~3.8 mm) and *B. subtilis* (gent~3.1 mm and chlo~3.2 mm) were highly significant than its pure form figure 9 and figure 10.

Furthermore, the mechanism underlying the action of silver nanoparticles on bacterial membranes has been elucidated based on the composition of the cell wall. Gram-negative organisms are more susceptible due to their easier permeability [25]. The attachment of metal nanoparticles and drug formulations to bacterial cells induces structural changes in the cell membrane and obstructs transport channels [18,26]. The inhibitory mechanism likely involves the impairment of genetic material replication and the immobilization of specific cellular proteins and enzymes crucial for ATP synthesis.

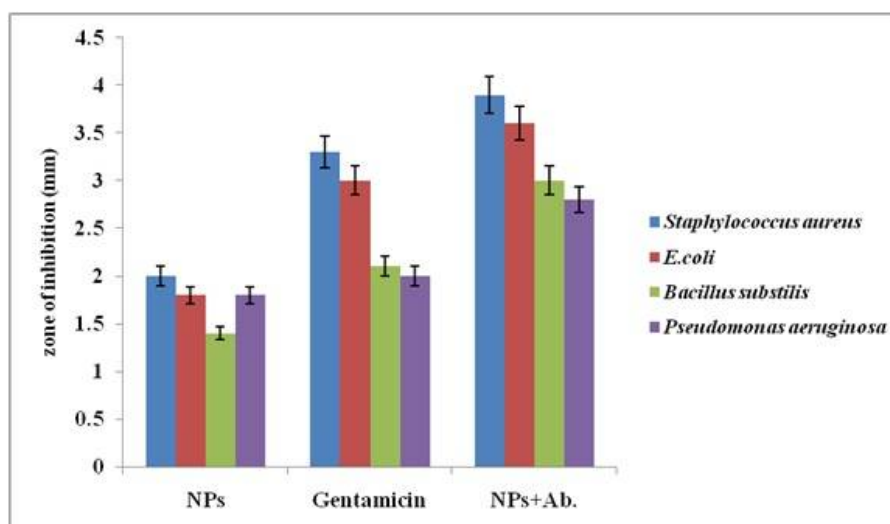


Figure 9: Graphical representation of zone of inhibition for gentamicin drug mixed with AgNPs against the test bacteria.

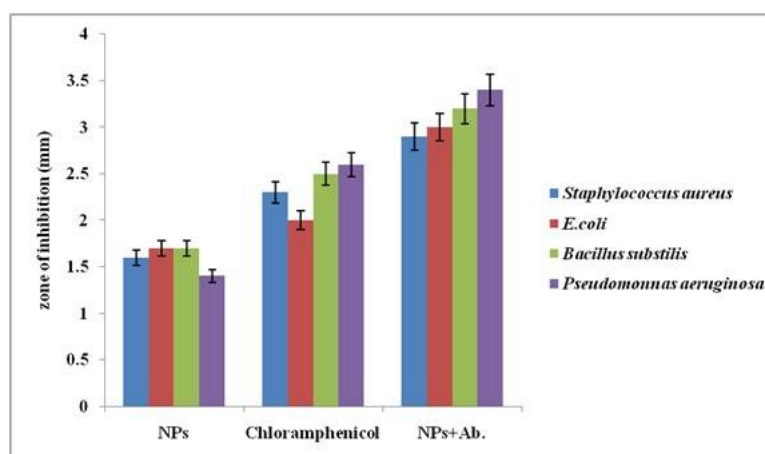


Figure 10: Graphical representation of zone of inhibition for chloramphenicol drug mixed with AgNPs against the test bacteria.

CONCLUSIONS

Silver nanoparticles were fabricated using aqueous leaf extract of *P. guajava* following green synthesis route. The reduction of silver ions from AgNO_3 to metallic Ag occurs due to leaf extract of *P. guajava*. The synthesized AgNPs were confirmed by observing the change of color initially and the absorbance peak at 430 nm in UV-Vis spectroscopic analysis. Moreover, the XRD data revealed that it corresponds to the face-centered cubic structure of crystalline silver. Thus, the present method leads to the formation of silver nanoparticles with well-defined dimensions. The average crystalline size was estimated 22 nm confirming the nanoparticle nature. However, HR-TEM analysis revealed that the material was formed in nano-dimension, with an average grain size 50 nm. The particles were almost spherical in shape though these have some agglomeration. In addition, the FTIR study showed the reduction of Ag^+ ions owing to the presence of biomolecules in the leaf extract, which acted as reducing as well as capping agents. Examination showed significant antibacterial activities of the synthesized AgNPs solution. The zone of inhibition in homogeneously showed a promising result against Gram-negative bacteria (*E. coli*, *P. aeruginosa*) and Gram-positive bacteria (*S. aureus*, *B. subtilis*) in combination with antibiotics. Moreover, Gentamicin-AgNPs and Chloramphenicol-AgNPs also significantly reduced the host cells cytotoxicity. The exact mechanism of action of these nanoparticles is not precisely understood and it is the subject of future studies along with testing their potential *in vivo*.

CONFLICT OF INTEREST

The authors claim no conflicts of interest because none financial support was received from any government, non-government agency or organization to conduct this research work.

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