

Green synthesis of Silver nanoparticles using *Pyrenacantha volubilis* fruit extract with potent Antibacterial, Antifungal, and Anticancer activities.

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ABSTRACT

This study focused on the green synthesis and physicochemical characterization of silver nanoparticles synthesized using the fruit extract of the rare anticancer plant *Pyrenacantha volubilis*, followed by an assessment of their potential biological activities. The PV-Ag NPs were characterized using UV-Visible spectroscopy, FTIR, XRD, FESEM-EDS, TEM, and a particle size analyzer to determine their formation, size, crystallinity, morphology, elemental composition, and the involvement of phytochemicals in reduction, capping, and stabilization. The antibacterial potential of biosynthesized PV-Ag NPs was evaluated against five bacterial strains, with *Pseudomonas aeruginosa* showing the lowest MIC and MBC of 12.5 µg/mL and 25 µg/mL, respectively, followed by *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Bacillus subtilis*. Antifungal activity was determined against four pathogenic fungal species, with *Fusarium oxysporum* showing the highest mycelial inhibition (37.78%), followed by *Aspergillus flavus* (37.50%), *A. niger* (27.78%), and *F. solani* (10.53%). Further, antiproliferative effects were evaluated against four cancer cell lines, including A549, Caco2, MCF7, and Pa-1, with IC₅₀ values of 662.46 µg/ml, 364.58 µg/ml, 399.26 µg/ml, and 288.75 µg/ml, respectively. Overall, our findings demonstrate the successful use of the fruit extract as a reducing agent to synthesize silver nanoparticles with promising biological activity, highlighting their potential for biomedical applications.

KEYWORDS: Silver nanoparticles, *Pyrenacantha volubilis*, Icacinaceae, Green synthesis, Anticancer activity, Antibacterial activity, Antifungal Activity.

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INTRODUCTION

Nanotechnology is a rapidly developing field with applications across diverse areas of science, encompassing the design, synthesis, and characterization of nanomaterials typically ranging from 1 to 100 nanometers. Metal nanoparticles, owing to their small size and high surface area-to-volume ratio, can pass through cell membranes and reach various organs, making them highly mobile in biological systems (Shahlaei et al., 2024). Among different metallic nanoparticles, silver nanoparticles (AgNPs) have gained considerable scientific attention because of their distinct optical, catalytic, and biological properties.

Historical records indicate that silver has been utilized as an antimicrobial substance in various medical practices since ancient civilizations. Silver was used for suturing surgical wounds and promoting wound healing due to its germicidal properties. In addition, silver salts were used to treat infections such as conjunctivitis, syphilis, gonorrhea, and gastroenteritis (Rodrigues et al., 2024). Silver nanoparticles (AgNPs), a prominent category of metallic nanoparticles, have garnered significant attention in biomedicine due to their broad biocidal spectrum against bacteria, fungi, viruses, and mycobacteria. AgNPs combine antimicrobial activity with the ability to boost healing,

enable magnetic targeting, deliver genes and drugs, support bone health, enhance vaccine responses, and exhibit anticancer and antidiabetic properties. (Xu et al., 2020; Meher et al., 2024). Multiple factors influence the chemical properties of silver nanoparticles, including size, shape, aggregation tendency, surface charge, and distribution. These characteristics critically determine the nanoparticle interactions with other substances and their behavior in various environments. (Mamataj et al., 2025).

Nanoparticles can be synthesized using physical and chemical methods; however, these approaches are expensive and use toxic chemicals that pose environmental and health risks. In recent years, biological or green synthesis techniques have emerged as safer, more sustainable alternatives in nanotechnology research (Makwana et al., 2025). This method uses plant extracts, microorganisms, and other biological agents as reducing agents to produce nanoparticles. Compared to conventional chemical synthesis, plant-mediated nanoparticle production is simpler, cost-effective, rapid, and non-toxic, offering a biocompatible route to generate nanoparticles with desirable stability and uniformity for diverse applications in medicine, agriculture, and environmental science (Babu et al., 2025). Several

medicinal plant parts have been used in green synthesis of AgNPs (Vanlalveni et al., 2021). Medicinal plants possess healing properties due to a diverse array of complex chemical compounds, including metallothioneins, phytochelators, and various secondary metabolites such as polyphenols, tannins, alkaloids, flavonoids, glycosides, saponins, sterols, and terpenoids, facilitating the non-toxic and sustainable synthesis of nanoparticles by acting as reducing and capping agents, ensuring the particles are stable and uniformly shaped. (Iravani, 2011; Datkhile et al., 2017).

Pyrenacantha volubilis (Family: Icacinaceae) is a highly branched climbing shrub native to southeastern India and restricted to the drier forests of the Coromandel Coast. It has attracted significant research and commercial interest as a rich, sustainable source of the major plant-derived anticancer alkaloid camptothecin. Camptothecin is one of the three principal natural anticancer compounds utilized worldwide for chemotherapy. Remarkably, cotyledons and ripened fruits of *P. volubilis* contain approximately 1.35% and 0.60% camptothecin by dry weight, respectively, which are among the highest concentrations reported in plant sources. Seed extracts exhibit antiproliferative activity against several cancer cell lines (Suma et al., 2014) and leaf extract exert anti-inflammatory and anti-arthritis activities (Anjali et al., 2020).

This is the first study to utilize *P. volubilis* fruit pulp extract as a natural catalyst to mediate the bioreduction of silver ions from silver nitrate to elemental silver nanoparticles, providing a sustainable and efficient method for synthesis of AgNPs. The synthesized nanoparticles were characterized by UV-Vis, XRD, TEM, Zeta-PALS, FESEM-EDS, FTIR, and evaluated for antibacterial, antifungal, and anticancer activities, showing promising multifunctional bioactivity.

MATERIALS AND METHODS

Plant material and synthesis of PV-Ag NPs: The Fruits were collected from the Palur region of Chengalpattu District, Tamil Nadu, near National Highway 132B (12°45'09.0" N, 79°55'50.1" E). Fruits were washed and surface-sterilized with 70% alcohol, then washed thoroughly in sterile water. Approximately 20 g of *P. volubilis* fruit pulp along with peel was homogenised in a mixer blender in sterile distilled water, incubated at 80° C for 3 h in water bath with constant stirring and filtered with Whatman No.1 filter paper and made upto 100 ml, and kept at 4° C until further use. The aqueous extract (60 ml) was mixed with 10 mM silver nitrate at 80° C, stirred magnetically at 200 rpm and incubated at 80° C for 3 h (**Figure 1**). Then it was centrifuged at 10000 rpm for 10 minutes and washed with sterile deionised water 2 to 3 times, centrifuged at 4000 rpm for 5 min,

and dried at room temperature for two days. Then the sample was kept in refrigerator at 4° C for further use.

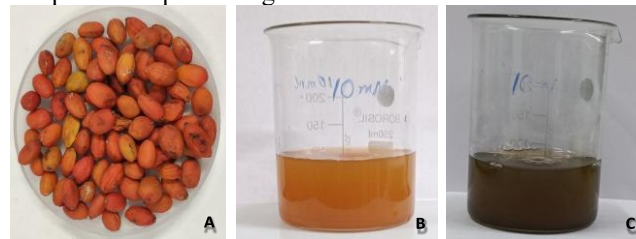


Figure 1. Formation of PV-Ag NPs. A- *Pyrenacantha volubilis* fruits, B- Aqueous Fruit extract, and C- PV-Ag NPs

Characterization of PV-Ag NPs:

a. UV-Vis: PV-Ag NPs were suspended in water and sonicated for 20 mins to obtain uniform suspension. The resulting suspension was analysed using Shimadzu UV-1800 double-beam spectrophotometer at 1 nm resolution with the wavelength range from 200-800 nm to confirm the formation of silver nanoparticles.

b. XRD: The phase and crystalline structure of PV-Ag NPs were investigated using an X-ray diffractometer (PANalytical XPERT-3, Malvern Panalytical, United Kingdom). The wavelength of Cu K α was 1.54059 Å, and the instrument was operated at 40 kV and 30 mA with a scanning range of 10-90° at room temperature.

c. FTIR: We analyzed the functional groups in the aqueous extract responsible for capping in PV-Ag NPs using an FTIR spectrophotometer (Spectrum Two with LiTaO₃ Detector, PerkinElmer, USA) with the KBr pellet method, in which we ground the nanoparticles and KBr in a 1:10 ratio using a mortar and pestle. They were pressed at 500 psi to obtain a pellet. The pellet was then analyzed on the instrument to obtain absorption peaks in the 4000- 400 cm⁻¹ range.

d. FESEM and Energy Dispersive X ray spectroscopy (EDS): FESEM integrated with EDS was used to determine the morphological features, size, and elemental composition of PV-Ag NPs. We imaged the surface morphology of the gold-coated nanoparticles using a field-emission scanning electron microscope (Ultra55, Zeiss, Jena, Germany) at different magnifications.

e. TEM: The morphology, size and size distribution of PV-Ag NPs were examined using TEM (ThermoFisher) operated at accelerating voltage of 60-300 kV. PV-Ag NPs were sonicated for 20 min and was placed on a copper grid and allowed to dry at room temperature. After drying, TEM micrographs were recorded at multiple magnifications to study their morphological features. For the analysis of particle size, 50 individual PV-Ag NPs were measured randomly using ImageJ. The mean size and standard deviation were calculated and a histogram showing size distribution was constructed.

f. Dynamic light scattering (DLS) and Zeta potential: Zeta PALS (Phase Analysis Light Scattering), Brookhaven Instruments Corporation,

USA was used to determine the hydrodynamic particle size distribution, Polydispersity index and zeta potential using DLS technique. PV-Ag NPs were suspended in MilliQ water and sonicated for 20 mins to measure the size with a 658 nm laser at 25° C. Electrophoretic cell under electric field was used to determine the surface charge and stability of nanoparticles.

Biological activities:

a. Antibacterial activity: we tested the antibacterial activity of PV-Ag NPs and determined the Minimum Inhibitory Concentration (MIC) using the resazurin broth microdilution method described by Elshikh et al. (2016) and Sarker et al. (2007) with slight modifications. PV-Ag NPs (1 mg/mL) were prepared in 10% DMSO and sonicated for 15 min. The samples were serially diluted in Mueller–Hinton broth, and kanamycin was used as the positive control. Bacterial inoculum (10 µL) was added to the test wells, and the plate was incubated at 37 °C for 18 h. Resazurin (40 µL of 0.015%) was added to each well and incubated for another 3–4 h at 37 °C. Antibacterial activity was determined by the resazurin response in each well, with pink indicating growth and blue indicating inhibition. MIC concentration was identified as the lowest concentration with no color change. Minimum bactericidal concentration (MBC) was determined by following the method described by Loo et al. (2018) with slight modification. The inocula from a 96-well microtiter plate (without Resazurin dye) were inoculated onto uncontaminated, sterile Mueller-Hinton (MH) agar plates by the streak plate method. The plates were incubated at 37 °C for 24 hours, and the lowest concentration that completely inhibited growth on the agar surface was determined as the MBC.

b. Antifungal activity: We evaluated the antifungal activity of PV-Ag NPs using the poisoned food technique (Satish et al., 2007). Briefly, 0.5mL of PV-Ag NPs were sonicated for 15 min, added it to sterile

Petri plates, and then poured 5 mL of molten potato dextrose agar (PDA). The plates were gently mixed and allowed to solidify. Fluconazole (100 µg/mL) served as the positive control, while PDA without PV-Ag NPs served as the control. A 5 mm mycelial disc obtained from a 7-day-old fungal culture was placed at the center of each plate. The plates were sealed and incubated at room temperature in the dark for 7 days. Mycelial growth was measured after incubation, and the percentage inhibition was calculated using the following formula:

$$\text{Mycelial growth inhibition (\%)} = \frac{(C-T)}{C} \times 100$$

where,

C = Mycelial growth in the control plate and T = Mycelial growth in the treatment plate

c. Anticancer activity:

Maintenance of cell lines: Different cancer cell lines such as MCF7 (human breast cancer), Pa-1 (human ovarian cancer), A549 (Human lung cancer) and Caco2 (Human colon cancer) were procured from the National Center for Cell Science (NCCS), Pune, India, and used to study the antiproliferative effect of PV-Ag NPs. MCF7 and Pa-1 cells were cultured in DMEM (Dulbecco's modified Eagle's medium) with high-glucose medium, whereas A549 and Caco2 Cells were cultured in MEM (Minimum Essential Medium) supplemented with an NEAA (Non-essential amino acids) mixture. All the cell lines were supplemented with 10% FBS (fetal bovine serum) and 1% antibiotic-antimycotic solution and were maintained in a humidified atmosphere containing 5% CO₂ and 18–20% O₂ at 37 °C in a CO₂ incubator. Sub-culturing was performed every three days. For the present study, MCF7, Pa1, A549, and Caco2 cells at passage numbers P69, P46, P74, and P37 were used.

Antiproliferative activity against cancer cell lines: 200 µL (15,000 cells per well) of MCF7, Pa1, A549, and Caco2 cell lines were seeded in a 96-well plate and allowed to grow for 24 h. They were treated with different concentrations of PV-Ag NPs (31.25, 62.5, 125, 250, 500 µg/ml) and incubated for 24 h at 37°C in a 5% CO₂ atmosphere. After incubation, morphology was observed under a microscope, and MTT reagent was added to a final concentration of 0.5 mg/ml. The plates were wrapped in aluminum foil to avoid light exposure and incubated for 3 hours at 37 °C. The formazan produced after incubation was dissolved in 100 µl of DMSO. The absorbance was read on a 96-well plate spectrophotometer at 570 nm wavelength and percent cell viability was calculated by dividing mean absorbance of treated cells by mean absorbance of untreated cells and multiplied by 100.

The half-maximal inhibitory concentration (IC₅₀) was determined via linear regression analysis ($Y=MX+C$), where $Y=50$, and the values for M and C were derived from the resulting viability dose-response curves. Doxorubicin at 1 µg/ml was used as the standard for Caco2, MCF7, and Pa1 Cell lines, whereas Cisplatin (10 µM/ml) was used for A549.

RESULTS AND DISCUSSION

Characterization of PV-Ag NPs:

a. UV-Vis: The formation of PV-Ag NPs was initially confirmed by UV-Visible spectroscopy. A distinct absorption peak was formed at 437 nm, which can be

attributed to surface plasmon resonance (SPR) of electrons on the nanoparticle surface. SPR is the characteristic optical feature of AgNPs, confirming the formation of silver nanoparticles. Typically, for a green-synthesized nanoparticle, the SPR maxima is usually seen between 400-500 nm, but this may vary because of nanoparticle morphology, size, concentration, aggregation, and surface-associated biomolecules (Poudel et al., 2022). A comprehensive review by Mikhailov & Mikhailova (2019) also reported that AgNPs display an absorption maximum between 400-460 nm, supporting our results. The direct band gap energy was

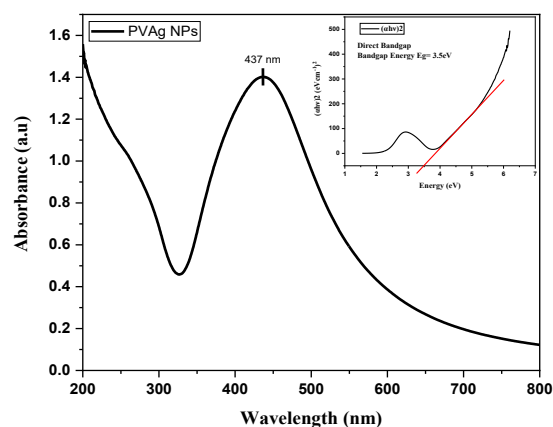


Figure 2. UV-Visible spectra of PV-Ag NPs along with tauc plot showing direct band gap energy.

determined from the Tauc plot and was found to be 3.5 eV, which is similar to AgNPs synthesized from *Psidium guajava* and *Azadirachta indica* (Kularia et al 2026). UV-visible spectra, along with the Tauc plot is shown in **Figure 2**.

b. XRD: The crystalline structure of PV-Ag NPs was determined using X-ray diffraction. It showed characteristic peaks at $2\theta = 38^\circ, 44^\circ, 64^\circ$ and 77° (**Figure 3**) corresponding to (111), (200), (220) and (311) planes respectively. These phases represent the face-centered cubic (fcc) structure of Ag NPs. The diffraction pattern observed in our study agrees well with JCPDS No. 04-0783, confirming the formation of PV-Ag NPs. Bharadwaj et al. (2021) and Poudel et al. (2022) also reported similar observations in plant-mediated synthesis of silver nanoparticles. Overall, the XRD pattern confirms the successful formation of FCC-structured PV-Ag NPs.

c. FTIR: FTIR analysis was used to identify the functional groups of phytochemicals involved in stabilizing and capping PV-Ag NPs. The FTIR

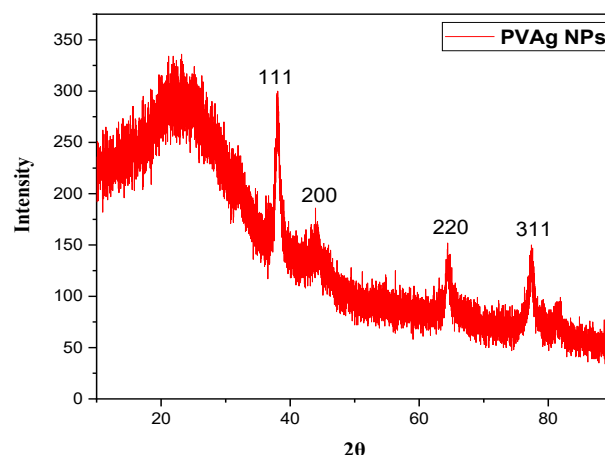


Figure 3. XRD pattern of PV-Ag NPs synthesized using *P. volubilis* fruit extract.

spectrum of the fruit extract used to obtain nanoparticles showed prominent peaks at 3415, 2927, 1641, 1382, 1072, and 616 cm^{-1} (**Figure 4**), whereas the major absorption peaks of PV-Ag NPs were at 3420, 2927, 1634, 1382, 1059, and 616 cm^{-1} . The close similarity of absorption peaks between the extract and the nanoparticles, along with slight shifts, suggests that phytochemicals in the fruit extract were involved in the reduction and stabilization of PV-Ag NPs. The broad band at 3415 cm^{-1} in the fruit extract shifted slightly to 3420 cm^{-1} , which may be assigned to O-H stretching in hydroxyl groups found in phenolics, flavonoids, or other phytochemicals. This may also arise due to N-H vibrations from proteins. A similar interpretation was reported by Hemlata et al. (2020) in *Cucumis prophetarum*-mediated silver nanoparticle synthesis, with small shifts from 3327 cm^{-1} to 3309 cm^{-1} for O-H and N-H stretching.

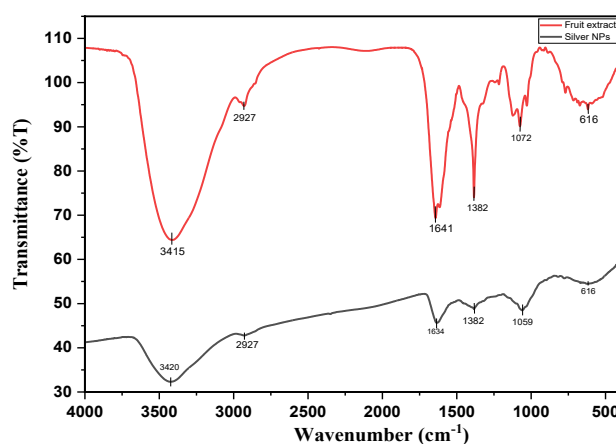


Figure 4. FTIR spectra of *P. volubilis* fruit extract (red) and PV-Ag NPs (black).

C–H stretching vibrations of aliphatic hydrocarbons correspond to 2927 cm^{-1} and remained in the same position in both the extract and PV-Ag NPs. Sakthi Sri et al. (2024) also reported the same band at 2927 cm^{-1} . The peak at 1641 cm^{-1} in the fruit extract shifted to 1634 cm^{-1} and is associated with C=O or C=C stretching of amides or aromatic compounds. The band at 1382 cm^{-1} was unchanged and involves C–N stretching, C–H bending, and O–H vibrations. The band at 1072 cm^{-1} in the fruit extract shifted to 1059 cm^{-1} in the AgNPs and belongs to C–O and C–O–C stretching vibrations of ethers, alcohols, and other oxygen-containing phytochemicals. This shift in our study may suggest that oxygen-containing phytochemicals changed during nanoparticle formation. We observed the same band at 1382 cm^{-1} with similar observations and minor shifts, which agreeing closely with Baradwaj et al., (2021) in *Diospyros malabarica*-mediated AgNPs synthesis.

d. FESEM and Energy Dispersive X ray spectroscopy (EDS): The morphology of the green-synthesized PV-Ag NPs was examined using FESEM. **Figure 5** shows polymorphic morphologies, including irregularly granulated, highly aggregated, and roughly spherical and ellipsoidal shapes. The nanoparticle size ranged from 28.01 nm to 49.91 nm. Hemlata et al. (2020) reported similar observations of Ag shapes and aggregates in *Cucumis prophetarum*-mediated Ag nanoparticles. Dhand et al. (2016) synthesized AgNPs using *Coffea arabica* seed extract and reported different polymorphic forms in SEM analysis but predominantly spherical and ellipsoidal shapes in TEM. Elemental analysis by energy-dispersive X-ray spectroscopy (**Figure 5**) revealed a stronger absorption peak at 3 keV, mainly due to silver (50.70% by weight). Carbon, Oxygen, along with traces of Nitrogen, Sulfur, and chlorine, were also detected, which may be associated with the phytochemicals of the fruit extract. Shrestha et al. (2024) observed a similar Ag peak at 3 keV in *Persicaria perfoliata*-mediated AgNPs, along with C, N, and O signals. They associated their presence with secondary metabolites on the nanoparticle surface. Further, the presence of other elements in our results can be compared with literature reports such as Bhusal et al. (2024), who reported Ag, C, O, N, and Cl signals in *Rhus chinensis*-mediated AgNPs. Thus, the EDX profile confirms that Silver was the principal element, along with C, O, N, S, and Cl, suggesting the presence of plant-derived chemicals that support the reduction, capping, and stabilization of PV-Ag NPs.

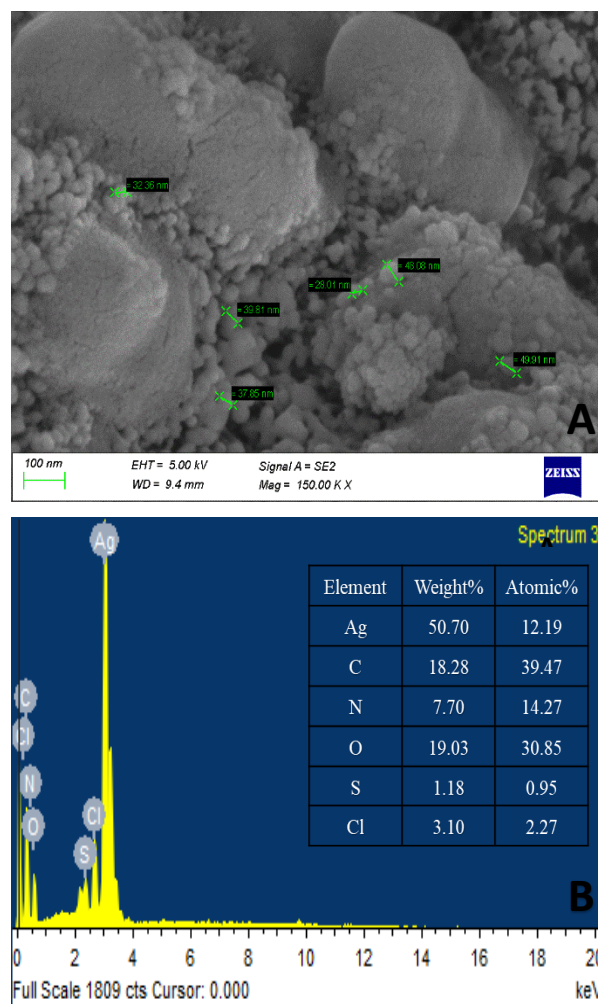


Figure 5. A- Scanning Electron Microscopic image of PV-Ag NPs at 150K magnification, B- EDX spectra with elemental composition of PV-Ag NPs.

e. TEM: TEM analysis comprehensively evaluated the morphology and size distribution of PV-Ag NPs (**Figure 6**). The PV-Ag NPs were roughly spherical, and histogram analysis showed the particle size between 3.416 nm to 19.352 nm with a mean diameter of $7.6672 \pm 3.3419\text{ nm}$ (N=50). This indicated a well-defined distribution, with most particles between 4–8 nm. Similar sized spherical AgNPs nanoparticles were synthesized from *Leonotis nepetifolia* flower-bud extract, with an average particle size of 24.50 nm (Nagaraja et al. 2022). Shankar et al., (2003) also reported spherical AgNPs using *Pelargonium graveolens* leaf extract, with a size of 16–40 nm.

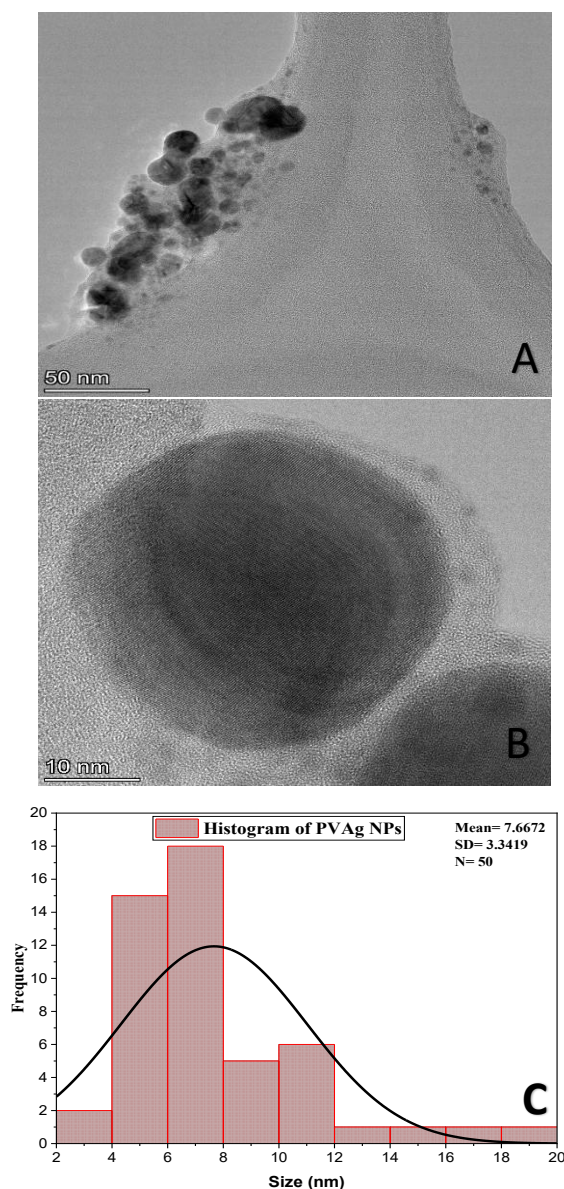


Figure 6. A & B-Transmission Electron Microscopic images of PV-Ag NPs at 50 nm and 10 nm scale. C-Histogram showing particle size distribution.

f. Dynamic light scattering (DLS) and Zeta potential: We determined the mean hydrodynamic diameter, polydispersity index, and zeta potential of PV-Ag NPs using dynamic light scattering. The nanoparticle suspension was suitably diluted to ensure proper scattering. The mean hydrodynamic diameter was 270.8 nm, with 96.6% intensity shown in the size distribution graph (Figure 7-A) and a moderately high polydispersity index of 0.4. A small peak with 3.4% intensity indicates particle aggregation. The zeta potential was -26.1 mV (Figure 7-B). The hydrodynamic diameter was much larger than the

TEM analysis because it measures the overall size of nanoparticles, along with capping agents and the hydration layer. Nayak et al, (2019) reported similar observations for *Azadirachta indica* and *Syzygium cumini*-mediated AgNPs, with hydrodynamic diameters of 123.4 and 128.8 nm, respectively, and a PDI of 0.4; these values were much larger than the FESEM results. *Teucrium polium*-mediated AgNPs exhibited a mean hydrodynamic diameter of 270.2 nm as reported by Ghrair et al. (2026), closely corresponds to our results. The zeta potential in our study was -26.1 mV, indicating a negative charge on the nanoparticle surface, which closely agrees with studies on *Ocimum tenuiflorum*-mediated AgNPs with a -26.20 mV zeta potential reported by Pandey et al. (2021) and attributed to the presence of negatively charged phytochemicals. The zeta potential was similar even in the studies by Nayak et al. (2019). Thus, the negative charge on PV-Ag NPs may be associated with negatively charged phytochemical constituents from the *P. volubilis* fruit extract.

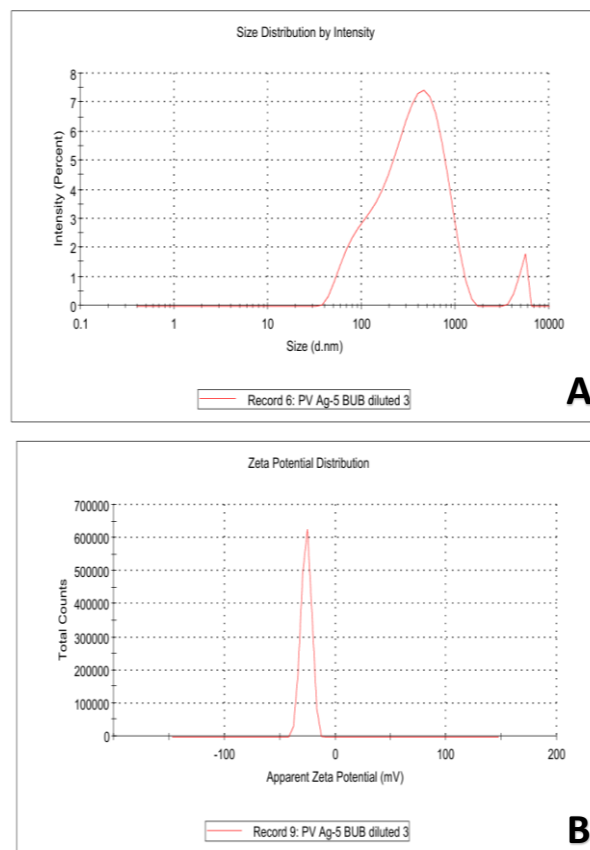


Figure 7. Dynamic Light Scattering. A- size distribution of PV-Ag NPs, B- Zeta potential of PV-Ag NPs.

Biological activities:

a. Antibacterial activity: Silver nanoparticles are considered next-generation antimicrobial agents (Khalifa et al., 2025), and green-synthesized AgNPs

offer an alternative approach to combat multidrug-resistant bacteria (Girma et al., 2024). We tested the antibacterial activity of PV-Ag NPs against five bacterial strains, as shown in **Figure 8**. The PV-Ag NPs showed antibacterial activity against all the tested bacteria, with MIC values ranging from 12.5 to 50 $\mu\text{g/mL}$. Among them, *Pseudomonas aeruginosa* was the most sensitive, showing the lowest MIC of 12.5 $\mu\text{g/mL}$ at both 24 and 48 h. *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Escherichia coli* showed an MIC of 25 $\mu\text{g/mL}$, while *Bacillus subtilis* showed an MIC of 50 $\mu\text{g/mL}$. The MBC values ranged from 25 to 50 $\mu\text{g/mL}$. *S. aureus*, *P. aeruginosa*, and *E. coli* had an MBC of 25 $\mu\text{g/mL}$, whereas *B. subtilis* had 50 $\mu\text{g/mL}$ and *K. pneumoniae* had 100 $\mu\text{g/mL}$. These results indicate that AgNPs are effective against both Gram-positive and Gram-negative bacteria, although their activity varied among the tested strains. The antibacterial effect of silver nanoparticles may be related to their interaction with the bacterial cell membrane and their ability to disturb normal cellular functions.

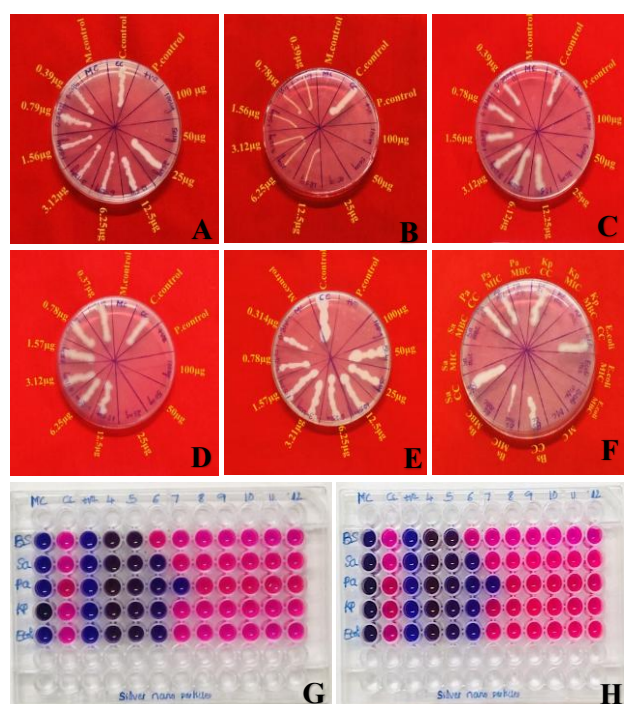


Figure 8. Antibacterial activity of PV-Ag NPs against the tested bacterial strains: (A) *Bacillus subtilis*, (B) *Staphylococcus aureus*, (C) *Pseudomonas aeruginosa*, (D) *Escherichia coli*, (E) *Klebsiella pneumoniae*, and (F) MIC and MBC assessment of all tested bacterial strains. G-H: Resazurin-based broth microdilution assay for determination of the minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of PV-Ag NPs against the tested bacterial strains after (G) 24 h and (H) 48 h of incubation.

PV-Ag NPs can also promote reactive oxygen species formation and interact with cellular proteins and DNA, which can eventually inhibit bacterial growth (Dakal et al., 2016). Differences in bacterial cell-wall structure may also contribute to variations in sensitivity among bacterial species (Bruna et al., 2021). Overall, the present findings suggest that PV-Ag NPs have promising antibacterial activity against the tested bacterial strains.

b. Antifungal activity: AgNPs have emerged as broad-spectrum antifungal agents, and green-synthesized AgNPs cause abnormal morphological changes in mycelia, induce cellular damage, leakage of intercellular substances, and alter pathogen gene expression (Li et al., 2022). The antifungal activity of silver nanoparticles (AgNPs) was evaluated against *Fusarium oxysporum*, *Fusarium solani*, *Aspergillus niger*, and *Aspergillus flavus* using the poisoned food technique (**Figure 9**). After seven days of incubation, AgNPs at 300 $\mu\text{g/mL}$ reduced mycelial growth in all tested fungi. Inhibition varied among the fungal strains, indicating differences in their sensitivity to the nanoparticles.

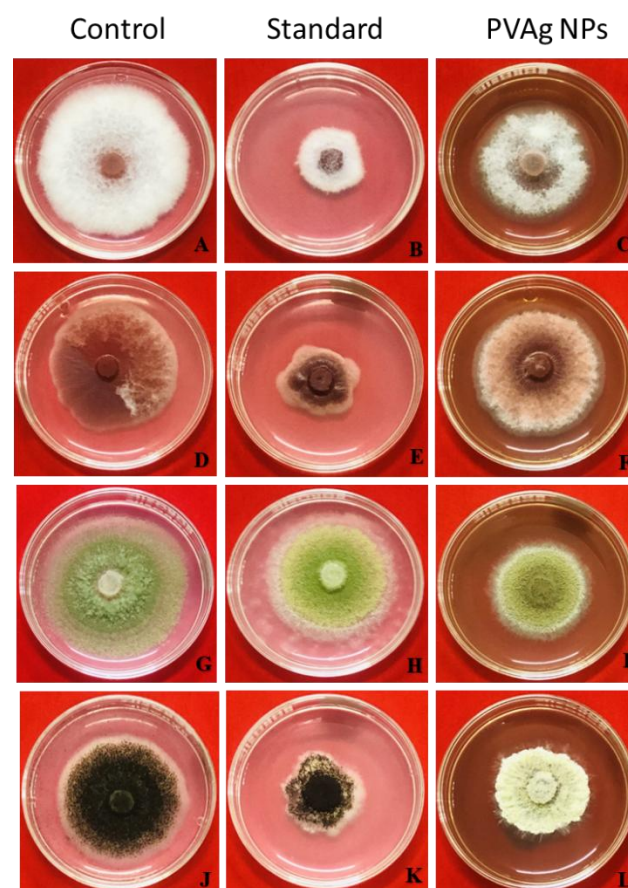


Figure 9. Antifungal activity of PV-Ag NPs against *Fusarium oxysporum* (A-C), *Fusarium solani* (D-F), *Aspergillus flavus* (G-I) and *Aspergillus niger* (J-L). Culture Control (A, D, G, J), Standard fluconazole at 100 $\mu\text{g/mL}$ (B, E, H, K) and PV-Ag NPs at 300 $\mu\text{g/mL}$ (C, F, I, L).

AgNPs showed the highest inhibition against *F. oxysporum* (37.78%) and *A. flavus* (37.50%), followed by *A. niger* (27.78%). A lower inhibition (10.53%) was observed for *F. solani*. Fluconazole (100 µg/mL) was used as the positive control. Previous studies have also reported the antifungal activity of AgNPs against species of *Aspergillus* and *Fusarium*. For Instance, silver nanoparticles have been shown to suppress the growth of *A. flavus* and *Fusarium* species, although their effectiveness varies with nanoparticle characteristics, concentration, and fungal species (Ahmad et al., 2024). Overall, the results suggest that AgNPs possess antifungal activity against the tested fungal strains.

c. Antiproliferative activity against cancer cell lines: Silver nanoparticles exert anticancer activity through multiple mechanisms across cell lines, and phytochemicals are the most effective and superior source for synthesizing AgNPs (Ratan et al., 2020). The cytotoxic potential of PV-Ag NPs was evaluated by treating four different human cancer cell lines, namely A549, Caco2, MCF7, and Pa-1, using the MTT assay after 24 hours. The assay demonstrated a concentration-dependent reduction in cell viability in all tested cell lines, indicating that PV-Ag NPs possess measurable anticancer activity. The IC₅₀ values for A549, Caco2, MCF7, and Pa-1 were 662.46 µg/ml, 364.58 µg/ml, 399.26 µg/ml, and 288.75 µg/ml, respectively (**Figure 10**).

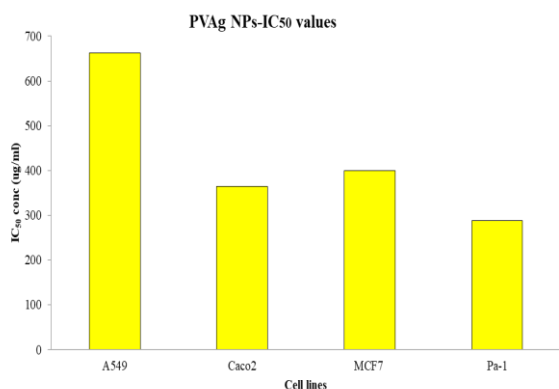


Figure 10. IC₅₀ values of PV-Ag NPs against the A549, Caco2, MCF-7 and Pa1 cell lines after 24 h of incubation as determined by MTT Assay.

We observed cell morphology at IC₅₀ under the microscope (**Figure 11**). Among all tested cell lines, PV-Ag NPs showed the highest toxicity towards Pa-1 cells, followed by Caco2, MCF7, and A549. We observed a marked reduction in Pa-1 cell viability, decreasing from 96.35% at 31.25 µg/ml to only 4.34% at 500 µg/ml. This sharp decline at higher concentrations indicates potent cytotoxic action of

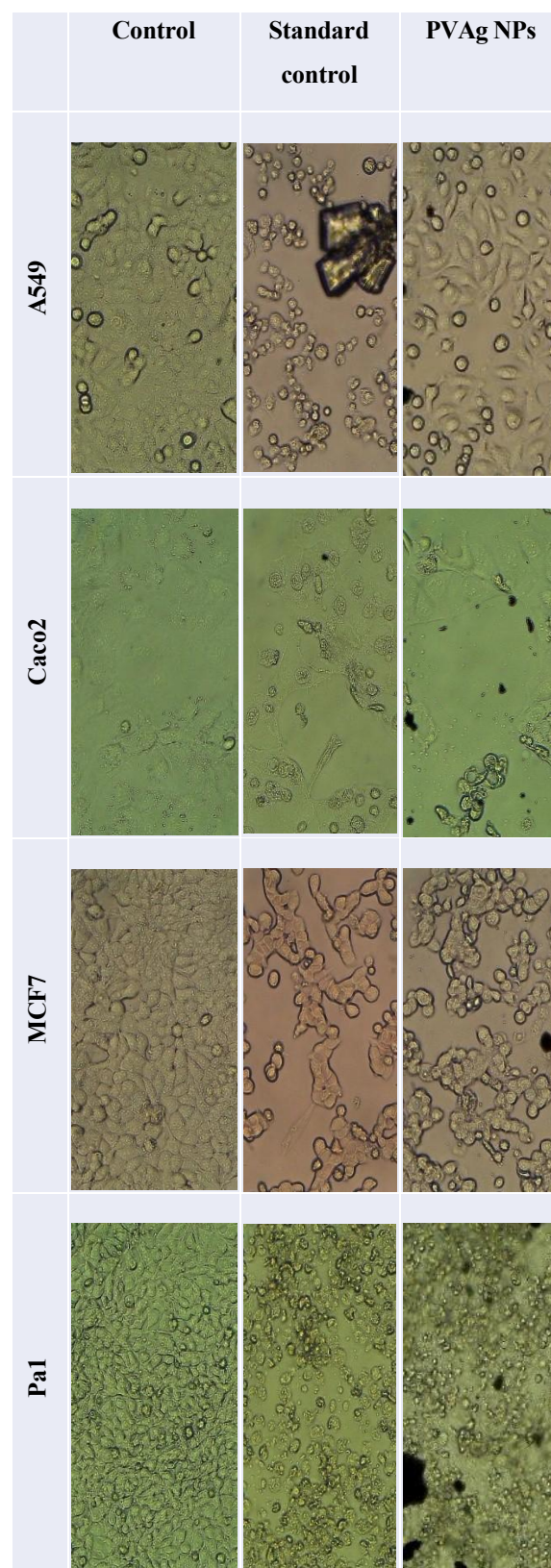


Figure 11. Antiproliferative activity against cancer cell lines. Microscopic images of cells under untreated, standard control and PV-Ag NPs treated conditions. A-C: A549, D-F: Caco2, G-I: MCF7, and J-L: Pa1.

PV-Ag NPs against Pa-1 cells. The lowest IC₅₀ value of 288.75 µg/ml among all tested cell lines further supports the enhanced susceptibility of Pa-1 cells. The higher IC₅₀ value of 662.46 µg/ml for A549 cells indicates that they were less sensitive to PV-Ag NPs than the other tested cancer cell lines. Our results agree with the effectiveness of *P. volubilis* fruit extracts against breast, ovarian, and colon cancer cell lines, as reported by Suma et al. (2014). Ag NPs showed different sensitivities across cell lines, as shown by Wypij et al. (2022), who reported that A549 cells showed an IC₅₀ of more than 100 µg/ml, whereas MCF-7 cells showed an IC₅₀ of 63.07 µg/ml. Several fruit extract-mediated AgNPs (Alharbi and Alsubhi, 2022; Lakshmanan et al., 2018) exert varied cytotoxic effects across different cancer cell lines. This work is the first study to report the biosynthesis of AgNPs and evaluate their antiproliferative activity against different cell lines. We observed comparatively higher IC₅₀ values, which may be due to differences in NP synthesis and size, capping molecules, morphology, and cell-line properties.

CONCLUSIONS

This study demonstrated the successful green synthesis of silver nanoparticles (AgNPs) using *P. volubilis* fruit extract. UV-visible spectroscopy, FT-IR spectroscopy, XRD, FESEM, TEM, EDS, and zeta potential by DLS confirmed the formation and physicochemical characteristics of the synthesized AgNPs. The synthesized nanoparticles showed polymorphic morphologies, with an average particle size between 3.416 nm to 19.352 nm. Furthermore, the biosynthesized PV-Ag NPs exhibited significant biological activities, including antimicrobial and antiproliferative effects. Overall, the findings highlight the potential of *Pyrenacantha volubilis* fruit extracts as an effective biological resource for the sustainable synthesis of AgNPs with promising applications in biomedical sciences.

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REFERENCES:

1. Ahmad, N., Malik, M. A., Wani, A. H., & Bhat, M. Y. (2024). Biogenic silver nanoparticles from fungal sources: Synthesis, characterization, and antifungal potential. *Microbial Pathogenesis*, 193, 106742.
2. Alharbi, N. S., & Alsubhi, N. S. (2022). Green synthesis and anticancer activity of silver nanoparticles prepared using fruit extract of *Azadirachta indica*. *Journal of Radiation Research and Applied Sciences*, 15(3), 335-345.
3. Anjali, P., Vimalavathini, R., & Kavimani, S. (2020). In Vitro Anti-Inflammatory and Anti-Arthritic Activity of Ethanolic Extract Of Leaves Of *Pyrenacantha volubilis* (Eepv). *Asian Journal of Pharmaceutical and Clinical Research*, 156-158.
4. Babu, A. B., Rao, Y. S., Sravanthi, P., Miditana, S. R., Pulsingh, D., & Bhavani, A. D. (2025). Green synthesis of silver nanoparticles using *Azadirachta indica* (Neem) fruit pulp extract and their antioxidant, antibacterial, and anticancer activity. *Sustainable Chemistry for the Environment*, 100282.
5. Bharadwaj, K. K., Rabha, B., Pati, S., Choudhury, B. K., Sarkar, T., Gogoi, S. K., & Edinur, H. A. (2021). Green synthesis of silver nanoparticles using *Diospyros malabarica* fruit extract and assessments of their antimicrobial, anticancer and catalytic reduction of 4-nitrophenol (4-NP). *Nanomaterials*, 11(8), 1999.
6. Bhusal, M., Pathak, I., Bhadel, A., Shrestha, D. K., & Sharma, K. R. (2024). Synthesis of silver nanoparticles assisted by aqueous root and leaf extracts of *Rhus chinensis* Mill and its antibacterial activity. *Heliyon*, 10(13).
7. Bruna, T., Maldonado-Bravo, F., Jara, P., & Caro, N. (2021). Silver nanoparticles and their antibacterial applications. *International Journal of Molecular Sciences*, 22(13), 7202.
8. Dakal, T. C., Kumar, A., Majumdar, R. S., & Yadav, V. (2016). Mechanistic basis of antimicrobial actions of silver nanoparticles. *Frontiers in Microbiology*, 7, 1831.
9. Datkhile, K. D., Durgavale, P. P., & Patil, M. N. (2017). Biogenic silver nanoparticles from *Nothapodytes foetida* kills human cancer cells in vitro through inhibition of cell proliferation and induction of apoptosis. *J Bionanosci*, 11(5), 416-427.
10. Dhand, V., Soumya, L., Bharadwaj, S., Chakra, S., Bhatt, D., & Sreedhar, B. J. M. S. (2016). Green synthesis of silver nanoparticles using *Coffea arabica* seed extract and its antibacterial activity. *Materials Science and Engineering: C*, 58, 36-43.
11. Elshikh, M., Ahmed, S., Funston, S., Dunlop, P., McGaw, M., Marchant, R., & Banat, I. M. (2016). Resazurin-based 96-well plate microdilution method for the determination of minimum inhibitory concentration of biosurfactants. *Biotechnology letters*, 38, 1015-1019.
12. Ghair, A. M., Aldmour, S., Ateyyat, M., Qrunfleh, I. M., Jaradat, D. S. M., Natsheh, I. Y.,

- & Alsaleh, M. M. (2026). Dose-Reduction Potential of Spinosad via Combination with Green-Synthesized Silver Nanoparticles for Thrips Control. *Environments*, 13(4), 218.
13. Girma, A., Alamnie, G., Bekele, T., Mebratie, G., Mekuye, B., Abera, B., Workineh, D., Tabor, A., & Jufar, D. (2024). Green-synthesised silver nanoparticles: Antibacterial activity and alternative mechanisms of action to combat multidrug-resistant bacterial pathogens: A systematic literature review. *Green Chemistry Letters and Reviews*, 17(1), 2412601.
14. Hemlata, Meena, P. R., Singh, A. P., & Tejavath, K. K. (2020). Biosynthesis of Silver Nanoparticles Using *Cucumis prophetarum* Aqueous Leaf Extract and Their Antibacterial and Antiproliferative Activity Against Cancer Cell Lines. *ACS omega*, 5(10), 5520–5528.
15. Irvani, S. (2011). Green synthesis of metal nanoparticles using plants. *Green chemistry*, 13(10), 2638-2650.
16. Khalifa, H. O., Oreiby, A., Mohammed, T., Abdelhamid, M. A., Sholkamy, E. N., Hashem, H., & Fereig, R. M. (2025). Silver nanoparticles as next-generation antimicrobial agents: mechanisms, challenges, and innovations against multidrug-resistant bacteria. *Frontiers in cellular and infection microbiology*, 15, 1599113.
17. Kularia, G., Sharma, Y., Verma, A., Bhatia, R., Anand, V., & Heera, P. (2026). Comparative evaluation of green synthesized silver nanoparticles using *Psidium guajava* and *Azadirachta indica* leaf extracts for photocatalytic and antibacterial applications. *Next Materials*, 12, 102283.
18. Lakshmanan, G., Sathiyaseelan, A., Kalaichelvan, P. T., & Murugesan, K. (2018). Plant-mediated synthesis of silver nanoparticles using fruit extract of *Cleome viscosa* L.: assessment of their antibacterial and anticancer activity. *Karbala International Journal of Modern Science*, 4(1), 61-68.
19. Li, L., Pan, H., Deng, L., Qian, G., Wang, Z., Li, W., & Zhong, C. (2022). The antifungal activity and mechanism of silver nanoparticles against four pathogens causing kiwifruit post-harvest rot. *Frontiers in Microbiology*, 13, 988633.
20. Loo, Y. Y., Rukayadi, Y., Nor-Khaizura, M. A. R., Kuan, C. H., Chieng, B. W., Nishibuchi, M., & Radu, S. (2018). In vitro antimicrobial activity of green synthesized silver nanoparticles against selected gram-negative foodborne pathogens. *Frontiers in microbiology*, 9, 1555.
21. Magesh, M., Thadanki, M. L., Alam, M. W., Kishore, V., & Ragavendran, C. (2024). Sustainable synthesis of Ag-NPs from *Cassia auriculata* flower extract: synthesis, spectral characterization, its biomedical and environmental applications. *Nanotechnology for Environmental Engineering*, 9(2), 161-177.
22. Makwana, K., Binsuwaidan, R., Adnan, M., Alshammari, N., & Patel, M. (2025). Sustainable synthesis and functional profiling of *Ipomoea hederifolia* derived terpenoids-assisted silver nanoparticles: Mechanistic insights into anticancer, antioxidant, antibiofilm, and anti-quorum sensing activities. *Biophysical Chemistry*, 107524.
23. Mamataj, S., Roy, S., Ravindiran, G., Datta, D., & Das, B. (2025). Contemporary research on synthesis of silver nanoparticles and its applications. *Indian Chemical Engineer*, 67(1), 91-120.
24. Meher, A., Tandi, A., Moharana, S., Chakroborty, S., Mohapatra, S. S., Mondal, A., & Chandra, P. (2024). Silver nanoparticle for biomedical applications: A review. *Hybrid Advances*, 6, 100184.
25. Mikhailov, O. V., & Mikhailova, E. O. (2019). Elemental silver nanoparticles: biosynthesis and bio applications. *Materials*, 12(19), 3177.
26. Nagaraja, S. K., Niazi, S. K., Bepari, A., Assiri, R. A., & Nayaka, S. (2022). *Leonotis nepetifolia* flower bud extract mediated green synthesis of silver nanoparticles, their characterization, and in vitro evaluation of biological applications. *Materials*, 15(24), 8990.
27. Nayak, P. S., Pradhan, S., Arakha, M., Kumar, D., Saleem, M., Mallick, B., & Jha, S. (2019). Silver nanoparticles fabricated using medicinal plant extracts show enhanced antimicrobial and selective cytotoxic propensities. *IET nanobiotechnology*, 13(2), 193-201.
28. Pandey, V. K., Upadhyay, S. N., & Mishra, P. K. (2021). Light-induced synthesis of silver nanoparticles using *Ocimum tenuiflorum* extract: characterisation and application. *Journal of Chemical Research*, 45(1-2), 179-186.
29. Poudel, D. K., Niraula, P., Aryal, H., Budhathoki, B., Phuyal, S., Marahatha, R., & Subedi, K. (2022). Plant-mediated green synthesis of Ag NPs and their possible applications: A critical review. *Journal of Nanotechnology*, 2022(1), 2779237.

30. Ratan, Z. A., Haidere, M. F., Nurunnabi, M. D., Shahriar, S. M., Ahammad, A. S., Shim, Y. Y., & Cho, J. Y. (2020). Green chemistry synthesis of silver nanoparticles and their potential anticancer effects. *Cancers*, 12(4), 855.
31. Rodrigues, A. S., Batista, J. G., Rodrigues, M. Á., Thipe, V. C., Minarini, L. A., Lopes, P. S., & Lugão, A. B. (2024). Advances in silver nanoparticles: a comprehensive review on their potential as antimicrobial agents and their mechanisms of action elucidated by proteomics. *Frontiers in Microbiology*, 15, 1440065.
32. Sarker, S. D., Nahar, L., & Kumarasamy, Y. (2007). Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. *Methods*, 42(4), 321-324.
33. Satish, S., Mohana, D.C., Ranhavendra, M.P. and Raveesha, K.A. (2007) Antifungal Activity of Some Plant Extracts against Important Seed Borne Pathogens of *Aspergillus* sp. *Journal of Agricultural Technology*, 3, 109-119.
34. Shahalaei, M., Azad, A. K., Sulaiman, W. M. A. W., Derakhshani, A., Mofakham, E. B., Mallandrich, M., & Subramaniyan, V. (2024). A review of metallic nanoparticles: present issues and prospects focused on the preparation methods, characterization techniques, and their theranostic applications. *Frontiers in Chemistry*, 12, 1398979.
35. Shankar, S. S., Ahmad, A., & Sastry, M. (2003). Geranium leaf assisted biosynthesis of silver nanoparticles. *Biotechnology progress*, 19(6), 1627-1631.
36. Shrestha, D. K., Jaishi, D. R., Ojha, I., Ojha, D. R., Pathak, I., Magar, A. B., ... & Sharma, K. R. (2024). Plant assisted synthesis of silver nanoparticles using *Persicaria perfoliata* (L.) for antioxidant, antibacterial, and anticancer properties. *Heliyon*, 10(23).
37. Suma, H. K., Kumar, V., Senthilkumar, U., Kumara, P. M., Ravikanth, G., Santhoshkumar, T. R., & Shaanker, R. U. (2014). *Pyrenacantha volubilis* Wight, (Icacinaeae) a rich source of camptothecine and its derivatives, from the Coromandel Coast forests of India. *Fitoterapia*, 97, 105-110.
38. Swathi Pon Sakthi Sri, V., Aron Santhosh Kumar, Y., Savurirajan, M., Jha, D. K., Vinithkumar, N. V., & Dharani, G. (2024). Anticancer efficacy of magnetite nanoparticles synthesized using aqueous extract of brown seaweed *Rosenvingea intricata*, South Andaman, India. *Scientific Reports*, 14(1), 20255.
39. Vanlalveni, C., Lallianrawna, S., Biswas, A., Selvaraj, M., Changmai, B., & Rokhum, S. L. (2021). Green synthesis of silver nanoparticles using plant extracts and their antimicrobial activities: A review of recent literature. *RSC advances*, 11(5), 2804-2837.
40. Wypij, M., Ostrowski, M., Piska, K., Wójcik-Pszczola, K., Pękala, E., Rai, M., & Golińska, P. (2022). Novel antibacterial, cytotoxic and catalytic activities of silver nanoparticles synthesized from acidophilic actinobacterial SL19 with evidence for protein as coating biomolecule. *Journal of Microbiology and Biotechnology*, 32(9), 1195.
41. Xu, L., Wang, Y. Y., Huang, J., Chen, C. Y., Wang, Z. X., & Xie, H. (2020). Silver nanoparticles: Synthesis, medical applications and biosafety. *Theranostics*, 10(20), 8996.

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