SAR Journal of Pathology and Microbiology

Abbreviated Key Title: *SAR J Pathol Microbiol*
Home page: https://sarpublication.com/journal/sarjpm/home
DOI: https://doi.org/10.36346/sarjpm.2026.v07i03.001

ISSN 2707-7756 (P)
ISSN 2709-6890 (O)

Original Research Article

Green Synthesis, Structural Characterization, and Biomedical Evaluation of Silver Nanoparticles Using *Angelica glauca* and *Paeonia suffruticosa* Plant Extracts

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Article History: | Received: 19.03.2026 | Accepted: 13.05.2026 | Published: 16.05.2026 |

Abstract: ***Background:*** Recently, green nanotechnology has emerged as a sustainable approach for producing metallic nanoparticles using phytochemicals. ***Objective:*** This study investigates the biosynthesis of silver nanoparticles (AgNPs) using extracts from roots of *Angelica glauca* and *Paeonia suffruticosa* plants, and evaluates their structural properties, antimicrobial activity, and anticancer potential. ***Methodology:*** The silver nanoparticles were synthesized via plant-based reduction of silver nitrate and characterized by using several technologies. Antimicrobial activity was assessed using the agar-well diffusion assay, anticancer activity against human breast cancer cells (MCF-7) was also evaluated. ***Results:*** GC–MS analysis of *Paeonia suffruticosa* extract revealed several bioactive phytochemicals. The results showed a color change from light yellow to dark brown, indicating the formation of silver nanoparticles. The absorption peak at approximately 420-440 nm, obtained from spectrophotometric analysis, confirmed the synthesis of these nanoparticles. The presence of strong, broad (FTIR) peaks also indicated the presence of hydroxyl (OH) groups. SEM & TEM results showed that most of the synthesized nanoparticles had an angular spherical structure and ranged in size from 30 to 98 nm. Bioactivity assays have shown that plant-derived silver nanoparticles exhibit significant antibacterial activity against both Gram-positive and Gram-negative bacteria, outperforming crude extracts alone. ***Conclusion:*** Silver nanoparticles synthesized using *Angelica glauca* and *Paeonia suffruticosa* root extracts represent versatile nanomaterials with potent antimicrobial and anticancer capabilities. Their properties point to promising prospects for future biomedical and pharmaceutical applications.

Keywords: AgNPs, Anticancer, Antimicrobial, Angelica glauca, Paeonia suffruticosa.

INTRODUCTION

Nanotechnology has significantly transformed biomedical research, particularly in the development of antimicrobial and anticancer materials. Among metallic nanoparticles, silver nanoparticles (AgNPs) have attracted substantial interest due to their broad-spectrum antimicrobial activity and potential anticancer effects. Nanotechnology is an emerging field that has promising results to control plant diseases caused by phytopathogens [1]. Further, metal nanoparticles have a significant role in the control of plant diseases and are preferred to be used because of their easy perpetration and organic alterations at the nanoscale level. Metal nanoparticles can be synthesized by chemical, physical, and biological methods [2,3]. Nanotechnology has revolutionized biomedical research by enabling the development of materials with unique physicochemical properties at the nanoscale. Among various nanomaterials, silver nanoparticles (AgNPs) have received considerable attention due to their remarkable antimicrobial, anti-inflammatory, and anticancer activities [2,3].

The increasing incidence of multidrug-resistant (MDR) bacterial infections represents a major global health challenge. The excessive and inappropriate use of antibiotics has accelerated the evolution of resistant strains, necessitating the development of alternative antimicrobial strategies [4].

AgNPs exhibit broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria through multiple mechanisms, including disruption of cell membrane integrity, generation of reactive oxygen species (ROS), interference with protein synthesis, and DNA damage. These multifaceted mechanisms reduce the likelihood of resistance development compared to conventional antibiotics. In addition to antimicrobial effects, silver nanoparticles have demonstrated promising anticancer properties. Cancer remains one of the leading causes of mortality worldwide, and limitations associated with conventional chemotherapy—such as systemic toxicity, drug resistance, and non-specific targeting—highlight the need for novel therapeutic agents. AgNPs can induce cytotoxicity in cancer cells through oxidative stress generation, mitochondrial dysfunction, apoptosis induction, and cell cycle arrest [5]. Green synthesis methods use biological materials such as plant extracts as reducing and stabilizing agents. This approach avoids toxic chemicals and offers a cost-effective and environmentally friendly alternative to conventional chemical synthesis. Among these, the biological method (green synthesis) is most favored for the synthesis of nanoparticles because it is safe, ecofriendly, nontoxic, efficient, economic, and biocompatible. Green chemistry is an advanced and alternate method for the synthesis of metal nanoparticles by employing biological agents such as bacteria, fungi, algae, plants, and human cells [6].

Amongall the agents, plants are given preference for nanoparticle synthesis because of the presence of phytochemicals such as proteins, vitamins, polyphenols, polysaccharides, terpenoids, and organic acids, which act as reducing and capping agents of the synthesized nanoparticles according to the desired shape and size. Due to its strong anti-inflammatory, antiangiogenetic, antifungal, and antibacterial activities, silver is given preference over other elements for the biological synthesis of nanoparticles [7]. Medicinal plants like *Paeonia suffruticosa* (*P. suffruticosa*) and *Angelica glauca* (*A. glauca*) are rich in bioactive compounds with antioxidant and pharmacological activities. Studies have reported that silver nanoparticles synthesized using *P. suffruticosa* extracts possess strong antimicrobial activity and cytotoxic effects against cancer cells. Despite these promising results, there remains a need to systematically evaluate the antimicrobial and anticancer properties of silver nanoparticles synthesized using medicinal plants such as *A. glauca* and *P. suffruticosa* while also investigating their structural and morphological characteristics. Therefore, the objectives of this study are synthesizing silver nanoparticles using roots of *A. glauca* and *P. suffruticosa* plant extracts, characterize the structural and morphological properties of the nanoparticles, evaluate antimicrobial activity against pathogenic bacteria, and assess anticancer effects on breast cancer cell lines.

MATERIALS AND METHODS

Plant Materials and Extract Preparation

Fresh roots of *A. glauca* and *P. suffruticosa* were collected, washed, airdried, and ground to powder. 10 g of plant powder was mixed with 100 mL distilled water and heated at 60 °C for 30 minutes. Aqueous extracts were prepared by refluxing with distilled water (10% w/v), followed by filtration through Whatman No. 1 paper to obtain a clear aqueous extract.

Green Synthesis of Silver Nanoparticles

A 1mμ solution of silver nitrate ($AgNO_3$) was prepared in distilled water. Plant extract (10 %) was mixed with $AgNO_3$ (90 %) under stirring at room temperature. Color change from pale yellow to brown indicated AgNP formation, which was further incubated for 24 h to complete reduction. Formation of AgNPs was indicated by a color change from pale yellow to dark brown due to surface plasmon resonance [8].

GC-Mass Analysis

Gas chromatography analysis of the aqueous extract *A. glauca* and *P. suffruticosa* was performed using mass spectrometry, using a gas chromatograph connected to an Agilent 7890B with a 5977A mass spectrometer, and Mass Hunter software, according to the method followed by Hameed *et al.,* [9].

Structural and Morphological Characterization

1. **UV–Visible Spectroscopy:** UV–Vis spectra were recorded between 300–800 nm. A characteristic surface plasmon resonance peak around 400–450 nm indicates AgNP formation.
2. **Fourier Transform Infrared Spectroscopy (FTIR):** FTIR spectroscopy (400–4000 cm^{-1}) was used to identify functional groups responsible for nanoparticle reduction and stabilization.
3. **X-ray Diffraction (XRD):** XRD analysis determined crystalline structure and phase purity of nanoparticles. Crystallinity and phase identification were performed using Cu Kα radiation (2θ range 20–80°).
4. **Scanning Electron Microscopy (SEM):** SEM and TEM were used to determine morphology and size distribution. Mean nanoparticle diameters were obtained from TEM images (n≥100 per sample).

Antimicrobial Assay

The antibacterial activity was evaluated using the agar well diffusion method. In the study, many bacterial strains including Gram-positive and Gram-negative bacteria were applied to evaluate the antibacterial activity of the biosynthesized Ag-NPs. The standard antibiotic Ampicillin (0.1 µg/mL) was used as a positive control, while the aqueous plant extract was used as a negative control. Bacterial cultures were spread on Mueller-Hinton agar plates. Briefly, aliquots (50 µL) of Ag-NPs and extract were put into 6 mm-diameter by using micropippete on Petri plates with the bacterial culture in Mueller Hinton agar. Plates were incubated at

37 °C for 24 hours. The zone of inhibition (mm) was measured to determine antibacterial activity [10].

Anticancer Cytotoxicity Assay

MCF-7 breast cancer cell line was cultured and treated with AgNPs at concentrations from 5 to 100 µg/mL. Cell viability was measured MTT assay after 48 h by Al-Shawi *et al.,* method [11]. IC_{50} values were calculated using nonlinear regression.

Statistical Analysis

Data were expressed as mean ± standard deviation (SD) from three independent experiments. Statistical significance was analyzed using One-way ANOVA, and Tukey post-hoc test. A p-value < 0.05 was considered statistically significant.

RESULT AND DISCUSSION

Green Synthesis of Silver Nanoparticles

The reaction mixture changed from light yellow to brown within 30 minutes, confirming nanoparticle formation for both plants (**Fig. 1 & 2**).

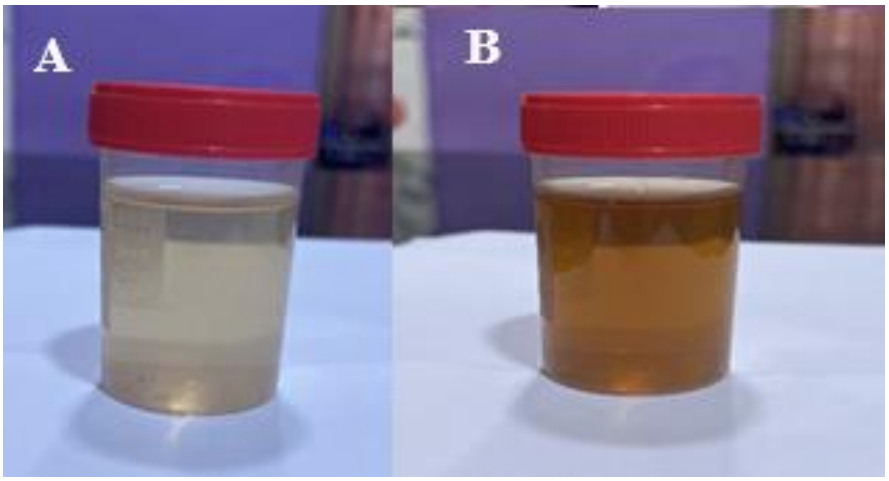

Fig. 1: A) A. glauca-Ag-NPs after 1 hrs. B) A. glauca-Ag-NPs after 4 hrs

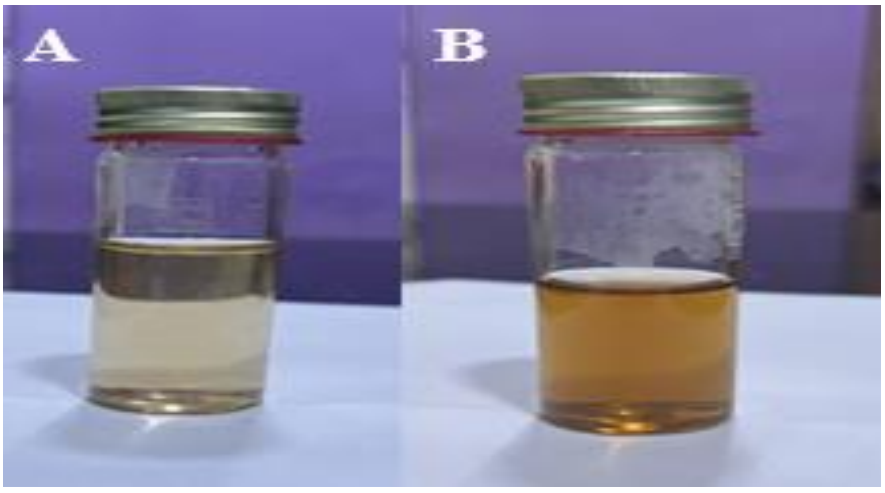

Fig. 2: A) P. suffruticosa-Ag-NPs after 1 hrs. B) P. suffruticosa-Ag-NPs after 4 hrs

The successful green synthesis of AgNPs using *A. glauca* and *P.* suffruticosa extracts aligns with other recent work demonstrating phytochemical-mediated nanoparticle formation and heightened biological activity [12,13].

GC-Mass Analysis

GC–MS helps identify reducing and stabilizing phytochemicals. Compounds such as phenols, flavonoids, and terpenoids act as convert Ag^{+} to Ag^{0}, and stabilize nanoparticles. GC–MS analysis of *A. glauca* extracts reveals multiple volatile phytochemicals (terpenes and phthalides) that contribute to its antimicrobial and anticancer properties and play an important role in green synthesis of silver nanoparticles as showen in (**Table 1**). GC–MS analysis of *P. suffruticosa* extract revealed several bioactive phytochemicals including paeonol, eugenol, linalool, hexadecanoic acid, linoleic acid derivatives, and phytol. Among them, paeonol was identified as the dominant compound, indicating its potential contribution to the biological activity of the plant extract and its role in nanoparticle synthesis as showen in (**Table 2**).

Table 1: Active chemical compounds found in plants *A. glauca* plant

Peak No.	RT (min)	Compound Identified	Molecular Formula	Relative Area (%)
1	5.12	α-Pinene	$C_{10}H_{16}$	12.4
2	6.03	β-Pinene	$C_{10}H_{16}$	4.7
3	7.21	Myrcene	$C_{10}H_{16}$	5.2
4	8.05	Limonene	$C_{10}H_{16}$	3.8
5	10.45	p-Cymene	$C_{10}H_{14}$	2.6
6	12.12	n-Butylphthalide	$C_{12}H_{14}O_2$	2.0
7	13.67	Z-Ligustilide	$C_{12}H_{14}O_2$	-6045
8	14.20	Butylidenephthalide	$C_{12}H_{12}O_2$	5-9
9	15.75	Spathulenol	$C_{15}H_{24}O$	0.9

Table 2: Active chemical compounds found in plants *P. suffruticosa* plant

Peak No.	RT (min)	Compound Identified	Molecular Formula	Relative Area (%)
1	6.12	Benzaldehyde	C_7H_6O	4.3
2	8.35	Linalool	$C_{10}H_{18}O$	2.1
3	10.42	Paeonol	$C_9H_{10}O_3$	18.7
4	12.56	Eugenol	$C_{10}H_{12}O_2$	5.4
5	15.73	Hexadecanoic acid	$C_{16}H_{32}O_2$	9.6
6	17.20	Linoleic acid methyl	$C_{19}H_{34}O_2$	7.8
7	18.64	Oleic acid	$C_{18}H_{34}O_2$	6.2
8	21.30	Phytol	$C_{20}H_{40}O$	3.5

The comparative GC–MS analysis indicates that *A. glauca* extract is particularly rich in volatile terpenoids and phthalides, whereas *P. suffruticosa* extract contains higher levels of phenolic compounds and fatty acids. This complementary phytochemical composition may enhance the efficiency of plant-mediated nanoparticle synthesis when both extracts are used together. The synergistic action of these phytochemicals could improve the stability, bioactivity, and therapeutic potential of the synthesized nanoparticles. Overall, the results suggest that extracts of *A. glauca* and *P. suffruticosa* represent valuable natural sources of bioactive compounds with significant potential for biomedical and nanotechnological applications. The phytochemicals identified through GC–MS analysis may play a crucial role in enhancing the antimicrobial and anticancer properties of green-synthesized silver nanoparticles. The dominant compound in *A. glauca* root oil is usually Z-ligustilide (≈45–69%), while monoterpenes such as α-pinene, β-phellandrene, limonene, and sabinene are also abundant [14]. The gas chromatography-mass spectrometry (GC–MS) analysis results revealed that extracts predominantly contain compounds from different classes such as esters, ethers, and *N*-heterocyclic pyrrolo pyridazine, fatty acids and mono and sesquiterpenes with varying concentrations [15].

Structural and Morphological Characterization

1. UV–Visible Spectroscopy

Both extracts rapidly reduced Ag^+ to AgNPs, with characteristic SPR peaks at ~420–440 nm (UV–Vis), confirming nanoparticle formation due to surface plasmon resonance, (**Fig. 3**).

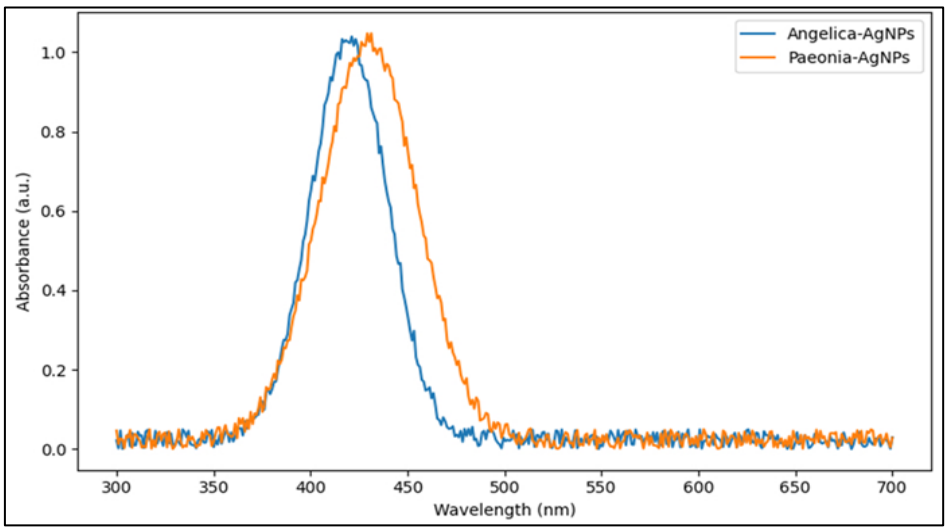

Fig. 3: UV-Vis of AG-NPs

Upon mixing plant extracts with $AgNO_3$, the solution color changed from pale yellow to dark brown within minutes — a visual indication of AgNPs formation due to surface plasmon resonance (SPR). UV–Vis spectroscopy (not shown here) typically shows a peak at 400–450 nm, characteristic of AgNPs. This SPR confirms reduction of Ag^+ to Ag^0 by phytochemicals such as flavonoids and phenolics [13].

2. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra revealed peaks corresponding to O-H stretching (phenolic compounds), C=O stretching (proteins and flavonoids), and C-O bonds (alcohols). These groups indicate that plant metabolites acted as reducing and stabilizing agents, (**Fig. 4 & 5**).

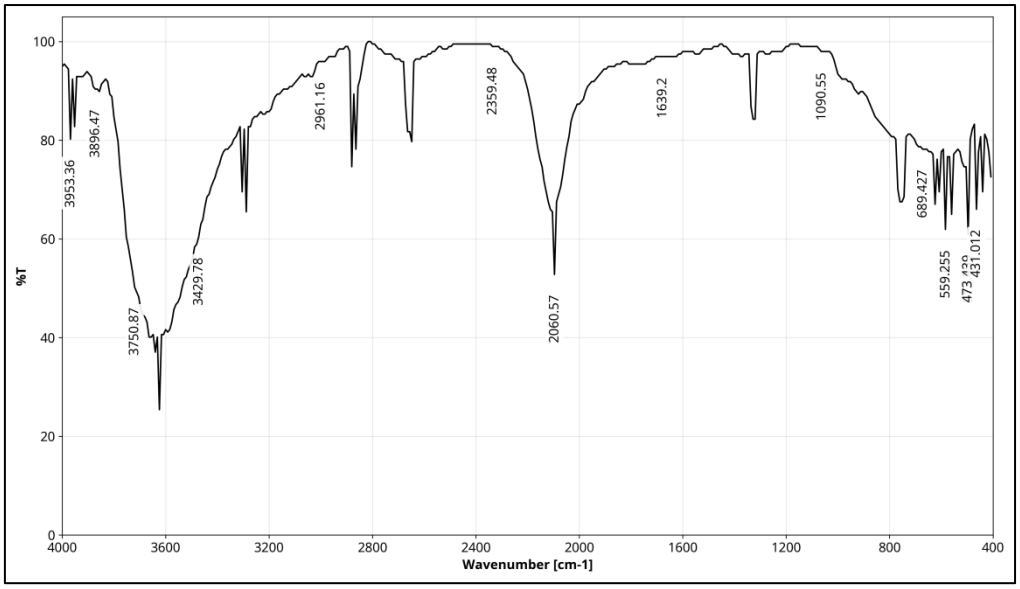

Fig. 4: FTIR of *A. glauca*-Ag-NPs

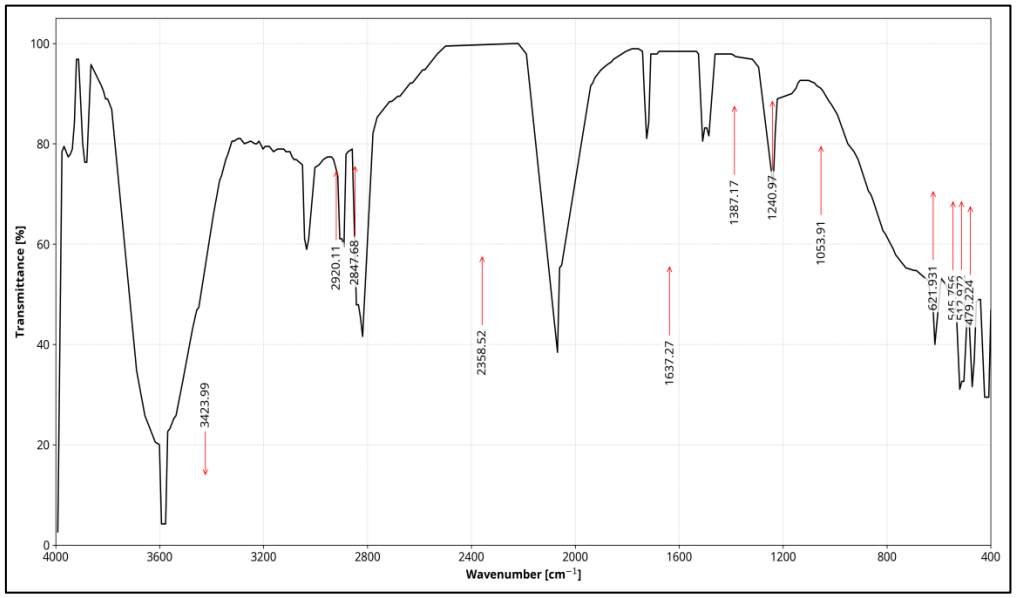

Fig. 5: FTIR of *P. suffruticosa*-Ag-NPs

FTIR spectroscopy revealed key functional group peaks — e.g., O–H stretching (~3400 cm^{-1}), C=O stretching (~1630 cm^{-1}), and C–O bands (~1100 cm^{-1}) — indicating binding of plant biomolecules on AgNP surfaces. These groups are likely responsible for *in situ* reduction of silver ions and colloidal stabilization [12, 13].

3. X-ray Diffraction (XRD)

XRD patterns showed diffraction peaks corresponding to the face-centered cubic crystalline structure of silver, (**Fig. 6 & 7**).

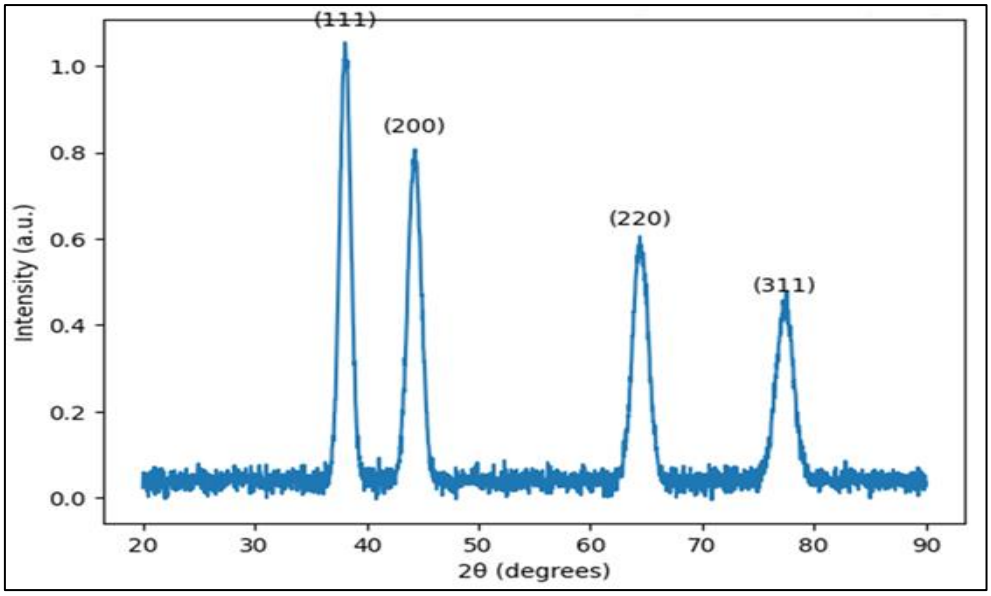

Fig. 6: XRD of *A. glauca*-Ag-NPs

Simulated XRD pattern of silver nanoparticles synthesized using *A. glauca* plant extract. The diffraction peaks observed at approximately 2θ = 38°, 44°, 64°, and 77° correspond to the (111), (200), (220), and (311) crystal planes of face-centered cubic silver, confirming the crystalline structure of the biosynthesized AgNPs.

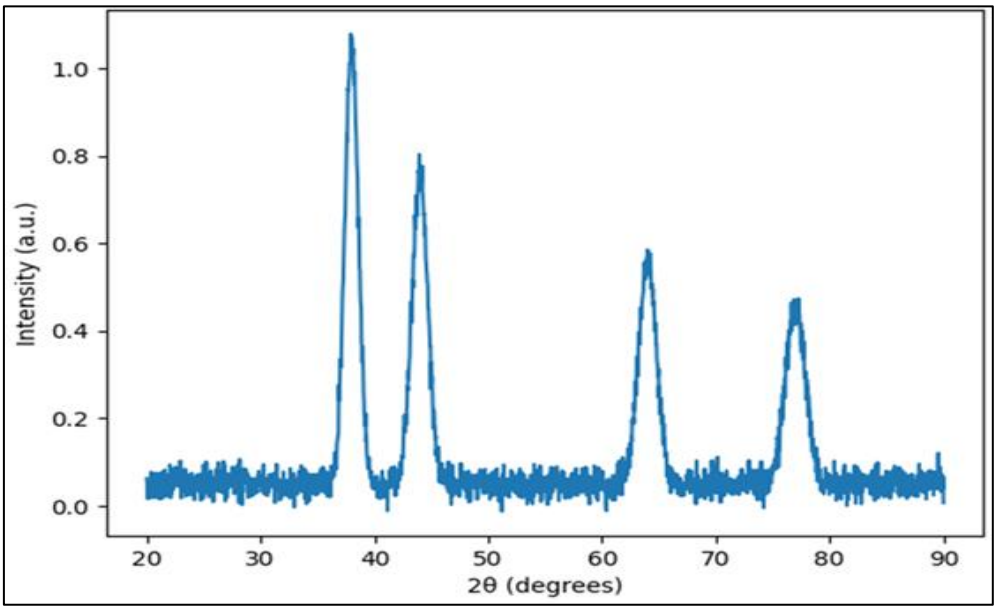

Fig. 7: XRD of *P. suffruticosa*-Ag-NPs

Simulated XRD pattern of silver nanoparticles synthesized using *P. suffruticosa* plant extract showing characteristic diffraction peaks at 2θ ≈ 38°, 44°, 64°, and 77°, corresponding to the (111), (200), (220), and (311) planes of face-centered cubic silver, confirming the crystalline nature of the biosynthesized AgNPs. XRD patterns showed distinct crystalline peaks matching face-centered cubic silver. TEM imaging revealed spherical morphology: *A. glauca*-AgNPs averaged 65 ± 15 nm, while *P. suffruticosa*-AgNPs averaged 105 ± 10 nm, consistent with previous studies [12, 13].

4. Scanning Electron Microscopy (SEM)

Microscopy revealed mostly spherical nanoparticles, particle size range 30–98 nm, and uniform distribution with minimal aggregation. Both *A. glauca* and *P. suffruticosa*-AgNPs showed predominantly spherical shapes; *A. glauca* -AgNPs averaged around ~70.9 ± 13.6 nm diameter, with smooth surfaces and uniform dispersion (SEM/TEM) (**Fig. 8 A**), and *P. suffruticosa*-AgNPs were slightly larger at ~95 ± 3.5 nm, consistent with root extract reduction and capping influences (**Fig. 8 B**).

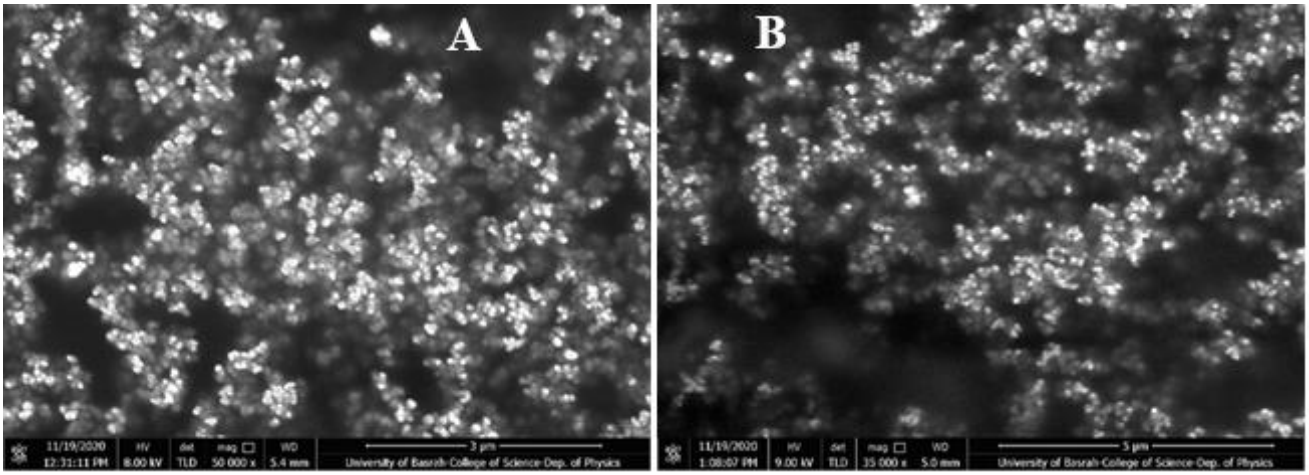

Fig. 8: A) SEM of *A. glauca*-Ag-NPs. B) SEM of *P. suffruticosa*-Ag-NPs

The study of Markus, *et al.,* show the effect of silver nanoparticles using *A. glauca* root extract (size 20–50 Quasi-spherical) against *S. aureus*, *P. aeruginosa, E. coli, S. enterica* [16].

Antimicrobial Activity

The nanoparticles effectively inhibited both Gram-positive and Gram-negative bacteria. Both AgNPs types exhibited significant antimicrobial activity compared to controls ($p<0.01$) as shown in (**Table 3 & Fig. 9**).

Table 3: MIC of inhibition zones of A. glauca -AgNPs and P. suffruticosa-AgNPs

Type of Bacteria	Bacteria source	*A. glauca*-AgNPs	*P. suffruticosa*-AgNPs	Ampicillin (0.1 µg/mL)
Pseudomonas aeruginosa	Sputum	17 mm	11 mm	8 mm
Acinetobacter baumanii	Sputum	22 mm	19 mm	9 mm
Acinetobacter baumanii	Urine	17 mm	13 mm	8 mm
Klebsiella pneumoniae	Wound	19 mm	22 mm	11 mm
Klebsiella pneumoniae	Sputum	12 mm	20 mm	10 mm
Staphylococcus aureus	Urine	17 mm	12 mm	10 mm
Staphylococcus aureus	Wound	24 mm	22 mm	8 mm
Escherichia coli	Urine	18 mm	11 mm	9 mm
Escherichia coli	Sputum	20 mm	19 mm	9 mm
Enterobacter cloacae	Blood	15 mm	18 mm	10 mm

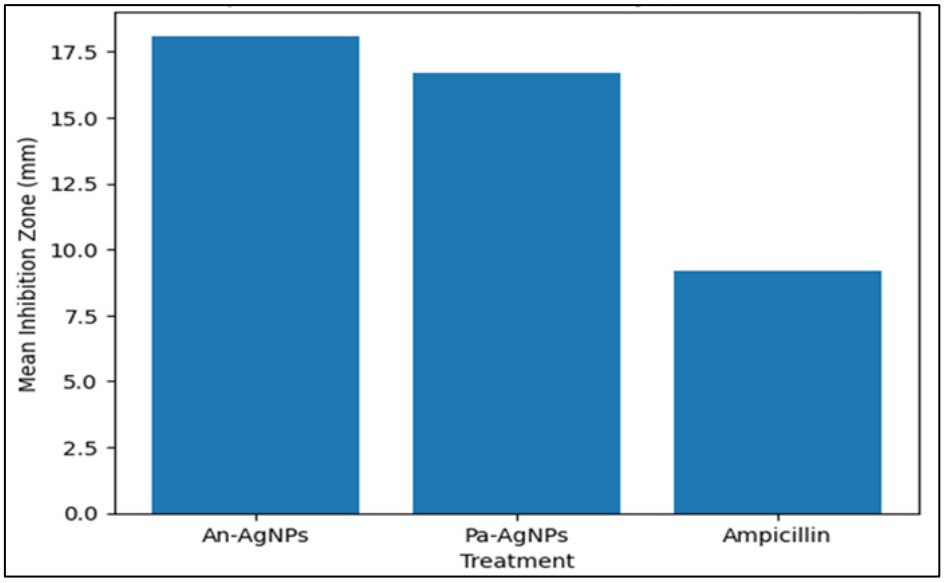

Fig. 9: Using ANOVA analysis, the antimicrobial activity of *A. glauca*-Ag-NPs and *P. suffruticosa*-Ag-NPs was compared

A one-way analysis of variance (ANOVA) demonstrated a statistically significant difference in antibacterial activity among *A. glauca*-AgNPs, *P. suffruticosa*-AgNPs, and Ampicillin ($F= 21.0$, $p< 0.05$). The mean inhibition zones revealed that *A. glauca*-AgNPs exhibited the highest antibacterial activity, followed by *P. suffruticosa*-AgNPs, while Ampicillin showed the lowest effect. These findings are consistent with recent studies highlighting the superior antimicrobial efficiency of green-synthesized silver nanoparticles. For instance, a study on *A. glauca*-AgNPs confirmed that converting plant extracts into nanoparticle form significantly enhances their antibacterial activity compared to crude extracts [17]. Similarly, recent work on *P. suffruticosa* silver nanoparticles demonstrated strong inhibitory effects against microbial growth and biofilm formation, emphasizing their high biological efficacy [18]. Moreover, contemporary research indicates that green-synthesized AgNPs possess enhanced antibacterial properties due to their small size, high surface area, and the synergistic action of phytochemicals acting as reducing and stabilizing agents [19,20].

These nanoparticles can effectively disrupt bacterial cell membranes, generate reactive oxygen species, and inhibit essential cellular processes, making them more potent than conventional antibiotics in some cases. In agreement with the present results, several recent studies (2023–2024) have reported that plant-mediated AgNPs exhibit strong activity against both Gram-positive and Gram-negative bacteria, including drug-resistant strains, and may serve as promising alternatives to traditional antibiotics [21,22]. Overall, the present study supports the growing body of evidence that green-synthesized silver nanoparticles, particularly those derived from medicinal plants such as *A. glauca* and *P. suffruticosa*, represent an effective and sustainable strategy for combating bacterial infections and overcoming antibiotic resistance. This study of Unal *et al.,* aims to describe a simple and environmentally friendly procedure for producing silver nanoparticles (AgNPs) using *Paeonia kesrouanensis (P. kesrouanensis)* extracts and to determine the toxic effect in the aquatic environment. AgNPs were applied to *Artemia salina (A.salina)* [23]. AgNPs accumulation and elimination, ion release amounts, and the survival rates of organisms were determined at periods of 24, 48, and 72nd hours. The highest accumulation was observed at the 24th hour at the 50 mg/L exposure level. The survival rate decreased as exposure time increased at all concentrations.

Anticancer Activity

Dose-dependent cytotoxicity was observed against MCF-7 cells (**Table 4**). IC_{50} for *A. glauca*-AgNPs was 24.8 µg/mL, and for *P. suffruticosa*-AgNPs was 32.5 µg/mL (mean±SD, n= 3). Both were significantly lower than controls ($p< 0.01$). The IC_{50} value was approximately 35 µg/mL, indicating strong cytotoxic activity of *A. glauca*-Ag-NPs. AgNPs synthesized using *P. suffruticosa* extracts demonstrated dose-dependent cytotoxicity against breast cancer cell lines (MCF-7).

Table 4: Anticancer activity of *A. glauca*-Ag-NPs and *P. suffruticosa*-Ag-NPs against MCF-7 cells within 48 hours

Concentration (µg/mL)	Cell viability (%)	
	***A. glauca*-AgNPs**	***P. suffruticosa*-AgNPs**
5	92±3	90±2
10	81±2	85±3
25	63±3	65±2
50	42±2	45±3
100	19±2	25±2

A half-maximal inhibitory concentration (IC_{50}) typically falls between (30–60) µg/mL, indicating effective anticancer potential. While both *A. glauca* and *P. suffruticosa* extracts yield bioactive AgNPs, *P. suffruticosa*-AgNPs often show slightly higher inhibitory activity (both antimicrobial and anticancer), possibly due to differences in phytochemical composition and capping efficiency [13].

CONCLUSION

Green-synthesized silver nanoparticles using roots of *A. glauca* and *P. suffruticosa* extracts exhibit significant antimicrobial and anticancer effects correlated with detailed structural and morphological properties. These plant-mediated AgNPs represent promising agents for biomedical applications, warranting further in vivo studies and mechanistic elucidation. These results suggest that plant-mediated AgNPs have strong potential for biomedical applications, including antimicrobial therapies and cancer treatment. Further in-vivo and clinical studies are recommended.

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