

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: 09.04.2026

Accepted: 30.05.2026

Published: 03.06.2026

Abstract: **Background:** In recent years, green nanotechnology has proven to be as a viable way to create metallic nanoparticles with the use of phytochemicals. **Objective:** This paper aims to explore bacterial synthesis of silver nanoparticles (AgNPs) with plant extracts of *Angelica glauca* and *Paeonia suffruticosa* and characterize their properties, structure, antimicrobial activity, and anticancer. **Methodology:** The silver nanoparticles were synthesized through plant-based reduction of silver nitrate and characterized by several technologies. Agar-well diffusion assay was used in order to determine the antimicrobial activity whereas the anticancer activity on human breast cancer cells (MCF-7) was evaluated. **Results:** GC-MS study of *Paeonia suffruticosa* extract identified several of bioactive phytochemicals. The findings revealed the change of color of light yellow to dark brown, which indicated the creation of silver nanoparticles. Spectrophotometric analysis indicated the formation of these nanoparticles as it yielded the absorption peak at around 420-440 nm. The existence of intense, broad (FTIR) peaks was a good testament to the existence of hydroxyl (OH) groups as well. The SEM and TEM obtained results indicated that a majority of the synthesized nanoparticles had an angular spherical shape and was between the 30 and 98 nm sizes. Plant-based silver nanoparticles have demonstrated antibacterial effects superior to crude extracts on Gram-positive and Gram-negative bacteria, in bioactivity assays. **Conclusion:** Silver nanoparticles prepared with extracts of *Angelica glauca* and *Paeonia suffruticosa* roots are attractive multifunctional nanomaterials with strong antimicrobial and anticancer activities. Their characteristics indicate that they are promising to biomedical and pharmaceutical applications in the future.

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

INTRODUCTION

Nanotechnology has made a great impact on the field of biomedical research specifically in creation of antimicrobial and anticancer materials. Silver nanoparticles (AgNPs) have been of specific interest among metallic nanoparticles because they possess a broad-spectrum antimicrobial property and might have anticancer effects. Nanotechnology is an new area with promising outcomes to manage diseases in plants which are caused by phytopathogens [1]. Moreover, metal nanoparticles play an important role in the control of plant diseases, and are desirable to be utilized due to their facile perpetration and organic changes at the nanoscale level. Chemical, physical, and biological methods can be used to prepare metal nanoparticles [2, 3]. The field of nanotechnology has transformed biomedical research in that it has allowed the creation of materials with distinct physicochemical characteristics on a nanoscale. Nanoparticles of silver (AgNPs) are one nanomaterial which has been of significant interest, owing to its tremendous antimicrobial, anti-inflammatory and anti-cancer properties [2, 3].

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Citation: Suha M.A. Al-Mudhafar (2026). Green Synthesis, Structural Characterization, and Biomedical Evaluation of Silver Nanoparticles Using *Angelica glauca* and *Paeonia suffruticosa* Plant Extracts. *South Asian Res J Bio Appl Biosci*, 8(3), N/A. 268

The rising prevalence of multidrug-resistant (MDR) bacterial infections poses an enormous health challenge across the world. The high use of antibiotics that has been excessive and misplaced has led to increased evolution of resistant groups of microbes which have required the formulation of alternative antimicrobial measures [4].

AgNPs have a broad-spectrum antimicrobial activity against Gram-positives and Gram-negatives by using a variety of mechanisms services such as the cell membrane integrity breaking, the creation of reactive oxygen species (ROS), interruption of the protein synthesis cycle, and damage on DNA. Such a combination of mechanisms diminishes the chances of developing resistance as opposed to traditional antibiotics. Besides the antimicrobial action, anticancer action of silver nanoparticles has also shown promising results. Cancer is actually one of the most significant causes of death in the world, and the aspects of conventional chemotherapy related to systemic toxicity, drug resistance, and the lack of specificity of action, demand the design of new 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].

Among all 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, antiangiogenic, 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 synthesize 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 1mM 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 ($400\text{--}4000\text{ cm}^{-1}$) 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 K α radiation (2θ range $20\text{--}80^\circ$).

4. Scanning Electron Microscopy (SEM)

SEM and TEM were used to determine morphology and size distribution. Mean nanoparticle diameters were obtained from TEM images ($n \geq 100$ 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 ($0.1\text{ }\mu\text{g/mL}$) 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 ($50\text{ }\mu\text{L}$) of Ag-NPs and extract were put into 6 mm-diameter by using micropipette on Petri plates with the bacterial culture in Mueller Hinton agar. Plates were incubated at $37\text{ }^\circ\text{C}$ 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 $100\text{ }\mu\text{g/mL}$. 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 \pm 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).

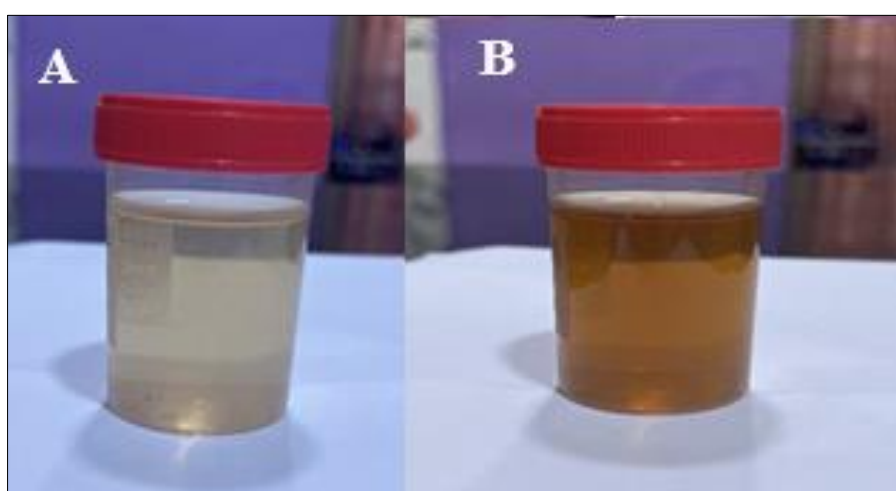


Fig. 1. A) *A. glauca*-Ag-NPs after 1 hrs. B) *A. glauca*-Ag-NPs after 4 hrs



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

The efficient green synthesis of AgNPs with the *A. glauca* and *P. suffruticosa* extracts is consistent with the recent findings that have shown the formation of nanoparticles via phytochemicals and their increased biological activities [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 shown 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 shown 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	$\text{C}_{10}\text{H}_{16}$	12.4
2	6.03	β -Pinene	$\text{C}_{10}\text{H}_{16}$	4.7
3	7.21	Myrcene	$\text{C}_{10}\text{H}_{16}$	5.2
4	8.05	Limonene	$\text{C}_{10}\text{H}_{16}$	3.8
5	10.45	p-Cymene	$\text{C}_{10}\text{H}_{14}$	2.6
6	12.12	n-Butylphthalide	$\text{C}_{12}\text{H}_{14}\text{O}_2$	2.0
7	13.67	Z-Ligustilide	$\text{C}_{12}\text{H}_{14}\text{O}_2$	-6045
8	14.20	Butylidene-phthalide	$\text{C}_{12}\text{H}_{12}\text{O}_2$	5-9
9	15.75	Spathulenol	$\text{C}_{15}\text{H}_{24}\text{O}$	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	$\text{C}_7\text{H}_6\text{O}$	4.3
2	8.35	Linalool	$\text{C}_{10}\text{H}_{18}\text{O}$	2.1
3	10.42	Paeonol	$\text{C}_9\text{H}_{10}\text{O}_3$	18.7
4	12.56	Eugenol	$\text{C}_{10}\text{H}_{12}\text{O}_2$	5.4
5	15.73	Hexadecanoic acid	$\text{C}_{16}\text{H}_{32}\text{O}_2$	9.6
6	17.20	Linoleic acid methyl	$\text{C}_{19}\text{H}_{34}\text{O}_2$	7.8
7	18.64	Oleic acid	$\text{C}_{18}\text{H}_{34}\text{O}_2$	6.2
8	21.30	Phytol	$\text{C}_{20}\text{H}_{40}\text{O}$	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. These phytochemicals might have a synergistic effect itself which would support the stability, bioactivity, and the therapeutic potential of the synthesized nanoparticles. In general, the findings indicate that extracts of *A. glauca* and *P. suffruticosa* are promising natural sources of bioactive compounds that have great potential in biomedical and nanotechnological uses. 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 ($\approx 45\text{--}69\%$), 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 $\sim 420\text{--}440$ nm (UV–Vis), confirming nanoparticle formation due to surface plasmon resonance, (**Fig. 3**).

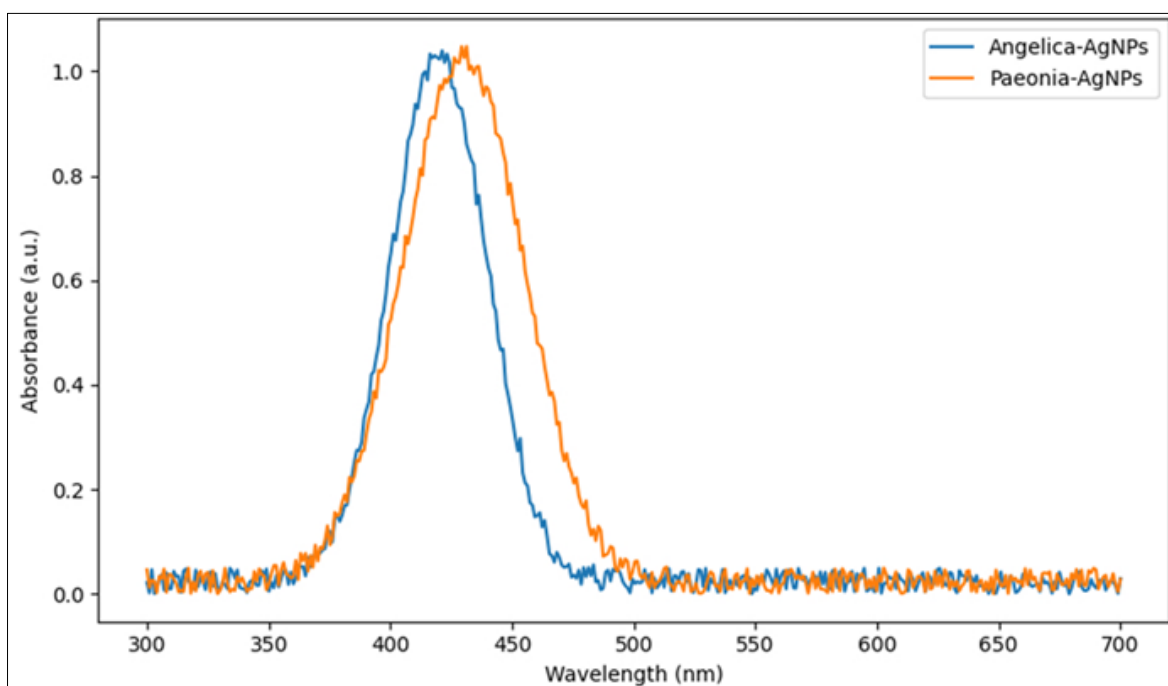


Fig. 3: UV-Vis of AG-NPs

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 $400\text{--}450$ 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**).

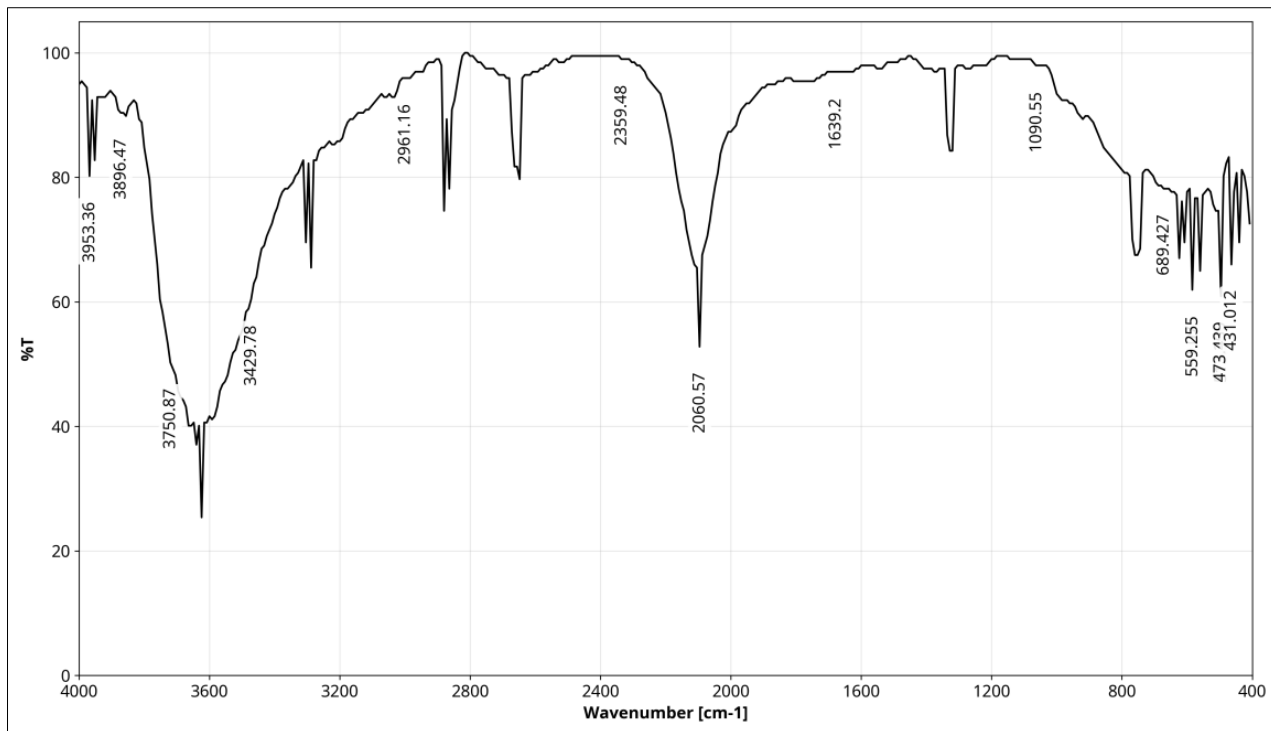


Fig. 4: FTIR of *A. glauca*-Ag-NPs

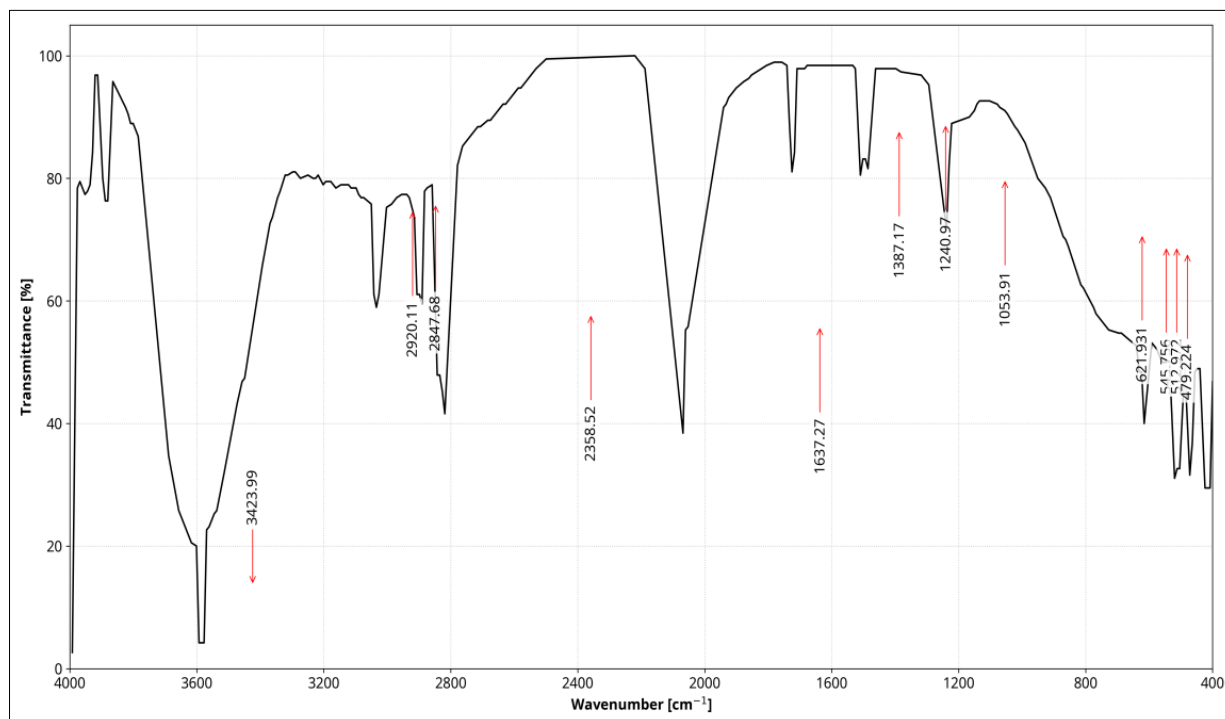


Fig. 5: FTIR of *P. suffruticosa*-Ag-NPs

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).

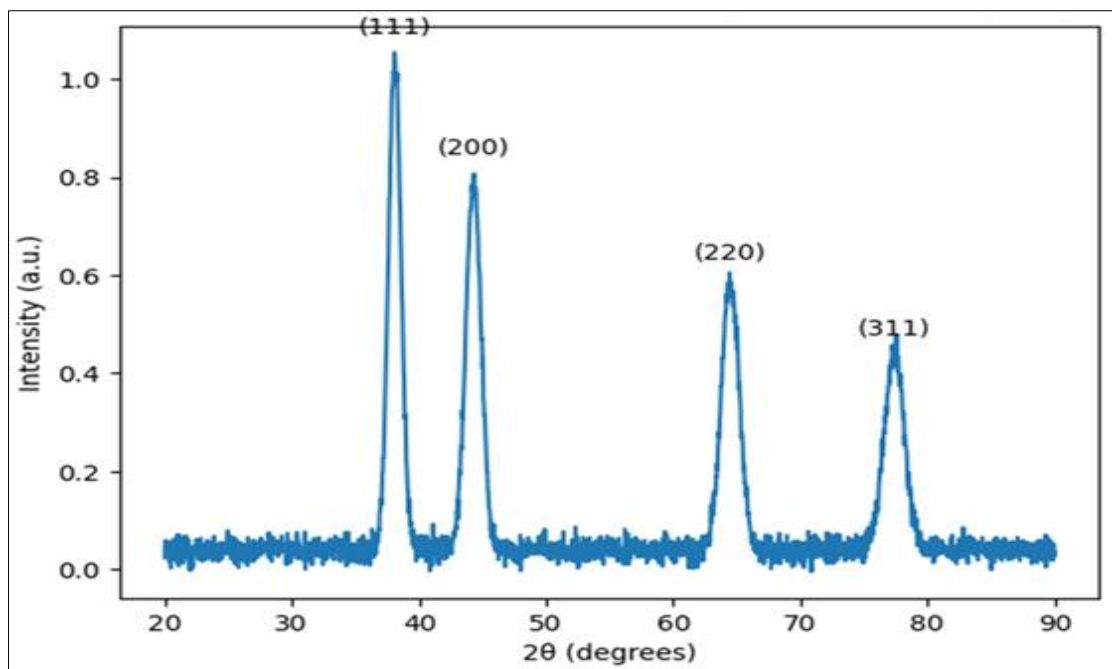


Fig. 6: XRD of *A. glauca*-Ag-NPs

Simulated XRD pattern of silver nanoparticles synthesized using *A. glauca* plant extract. The diffraction peaks observed at approximately $2\theta = 38^\circ$, 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.

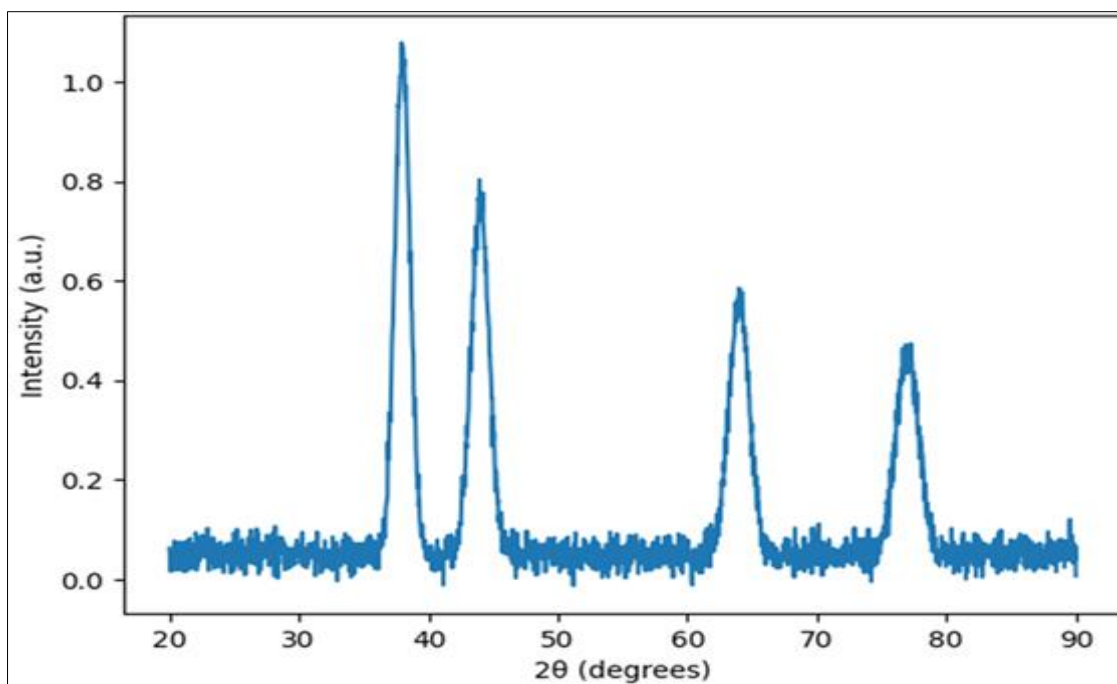


Fig. 7: XRD of *P. suffruticosa*-Ag-NPs

Simulated XRD pattern of silver nanoparticles synthesized using *P. suffruticosa* plant extract showing characteristic diffraction peaks at $2\theta \approx 38^\circ$, 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, primarily, indicated a low level of aggregation, spherical shape of nanoparticles, and distribution of uniform particles with a range of particle size of 30 nm to 98 nm. The *A. glauca* and *P. suffruticosa*-AgNPs were mainly spherical in their shapes; the overall average diameter of the *A. glauca* -AgNPs was approximately equal, around 70.9 +/-13.6 nm, with smooth surfaces and evenly spread (SEM/TEM) (Fig. 8 A), and *P. suffruticosa*-AgNPs were slightly larger, about 95 +/-3.5 nm, which is correlated with the reduction and capping effects of the root extract (Fig. 8 B).

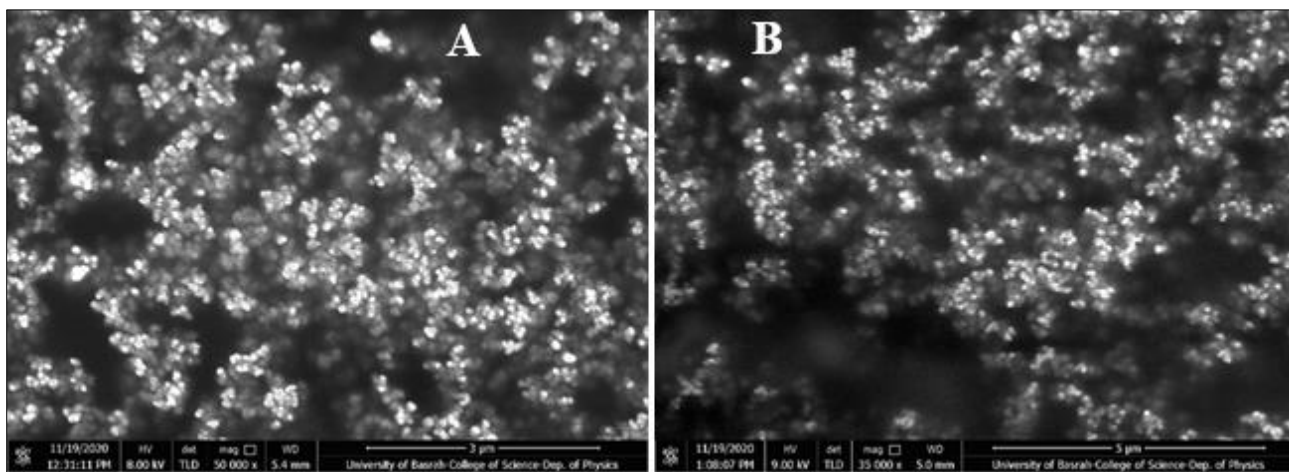


Fig. 8. A) SEM of *A. glauca*-Ag-NPs. B) SEM of *P. suffruticosa*-Ag-NPs

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

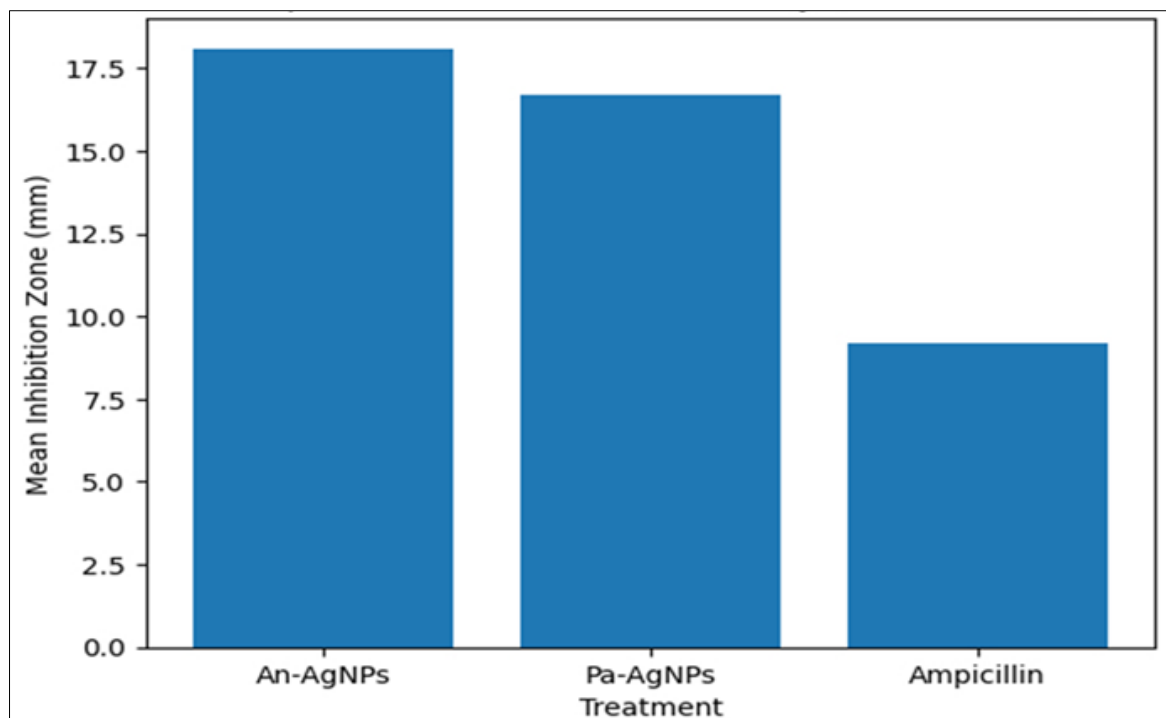


Fig. 9: Using ANOVA analysis, the antimicrobial activity of *A. glauca*-Ag-NPs and *P. suffruticosa*-Ag-NPs was compared

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. As an example, *A. glauca*-AgNPs study verified that the conversion of plant extracts into a nanoparticle form are indeed characterized by an enormous improvement of the antibacterial action relative to the crude extracts [17]. Likewise, recent studies of *P. suffruticosa* silver nanoparticles proved a high level of inhibition against the growth of microbes and biofilms formation, which highlights their high biological activity [18]. Besides, current studies have shown the improvements in AgNPs that are green-synthesized because of their minute size, a high surface area, and synergistic effect of phytochemicals that act as reducing and stabilizing agents [19, 20].

These nanoparticles are able to cause cell membrane disruption, production of reactive oxygen species as well as block crucial processes in the cell, which is more effective than traditional antibiotics in certain instances. 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 $\mu\text{g/mL}$, and for *P. suffruticosa*-AgNPs was 32.5 $\mu\text{g/mL}$ (mean \pm SD, $n=3$). Both were significantly lower than controls ($p<0.01$). The IC_{50} value was approximately 35 $\mu\text{g/mL}$, 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 ($\mu\text{g/mL}$)	Cell viability (%)	
	<i>A. glauca</i> -AgNPs	<i>P. suffruticosa</i> -AgNPs
5	92 \pm 3	90 \pm 2
10	81 \pm 2	85 \pm 3
25	63 \pm 3	65 \pm 2
50	42 \pm 2	45 \pm 3
100	19 \pm 2	25 \pm 2

An inhibitory concentration of (30-60) $\mu\text{g/mL}$ is generally half-maximal (IC_{50}) which means that it has the potential to offer anticancer ability. Although *A. glauca* and *P. suffruticosa* extracts both produce bioactive AgNPs, *P. suffruticosa*-AgNPs tend to exhibit a greater level of inhibitory (antimicrobial and anticancer) activity, potentially because of variations in the phytochemical composition as well as capping efficacy [13].

CONCLUSION

Silver nanoparticles green-synthesized with the extracts of roots of *A. glauca* and *P. suffruticosa* have considerable antimicrobial and anticancer activities and can be linked to the structures and morphological features. These AgNPs, which are delivered through plants, are the future of biomedical applications of AgNPs and additional in vivo investigations and mechanistic interpretation are required. These findings indicate that AgNPs through plants hold a great possibility in biomedical research such as antimicrobial and cancer therapy. In-vivo and clinical investigations are still suggested.

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