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# Green synthesis of silver nanoparticles using *Dodonaea viscosa* extract and their antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*

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[1]Department of Applied Biological Science, College of Biotechnology, Al-Nahrain University. Aljaderya, Baghdad, Iraq.
Tel.: +964-07901798097, ♥email: zeinab.sabeeh@ced.nahrainuniv.edu.iq
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**Abstract.** *Omran ZS, Mohsin RA, Al-Ani EH, Dayem TAA, Salih ZT. 2026. Green synthesis of silver nanoparticles using* Dodonaea viscosa *extract and their antibacterial activity against* Escherichia coli *and* Staphylococcus aureus*. Asian J Agric 10 (1): g100155. https://doi.org/10.13057/asianjagric/g100155.* Silver nanoparticles (AgNPs) have attracted considerable attention as alternative antimicrobial agents, and plant-mediated synthesis represents an eco-friendly approach that minimizes the use of hazardous chemicals. In this study, *Dodonaea viscosa* AgNPs were examined to evaluate its activity against *Escherichia coli* and *Staphylococcus aureus* comparing to aqueous and ethanol extract. The phytochemical profile of the extract was analyzed by GC-MS, comparative analysis of both extracts indicated that aqueous extract contained a higher relative abundance of biologically relevant polar compounds. This observation supported its selection for subsequent green synthesis and antibacterial assays. The synthesized nanoparticles were characterized using FTIR, SEM, AFM, and EDX to confirm the functional groups involved in nanoparticle capping, as well as their morphology, particle size, and elemental composition. Antibacterial activity was evaluated against Gram-negative *E. coli* and Gram-positive *S. aureus* using the agar well diffusion method. The biosynthesized AgNPs, prepared from 0.5 and 1 mM $AgNO_3$, exhibited inhibition zones ranging from 16 to 29 mm, demonstrating enhanced antibacterial activity compared with the crude aqueous and ethanolic extracts. Overall, these findings indicate that *D. viscosa*-mediated AgNPs represent a promising eco-friendly antibacterial nanomaterial with improved efficacy. Future studies should include increased biological replication, robust statistical validation, and cytotoxicity assessment on mammalian cell lines to support potential biomedical translation.

**Keywords:** AgNPs, antibacterial*, Dodonaea viscosa*, *Escherichia coli*, *Staphylococcus aureus*

## INTRODUCTION

The widespread use of medicinal plants and herbal remedies has played a central role in the treatment of human diseases for centuries, particularly in managing infectious conditions (Olanipekun 2023). Medicinal plants are rich sources of phytochemicals that contribute to natural defense mechanisms and disease prevention through antimicrobial, antioxidant, and anti-inflammatory activities (Arunprasath and Priyankadevi 2018; Boy et al. 2018; Ridhwan et al. 2025). Despite these natural resources, the global healthcare system continues to rely heavily on synthetic antibiotics. The extensive and often inappropriate use of these antibiotics has led to the rapid emergence of antimicrobial resistance (AMR), which now represents a major public health challenge worldwide. Resistant bacterial strains reduce the effectiveness of conventional therapies, increase treatment costs, and contribute to higher morbidity and mortality rates. Consequently, there is an urgent need to explore alternative antimicrobial strategies that are effective, sustainable, and environmentally safe (Patra et al. 2025).

In recent years, nanotechnology has emerged as a promising field for addressing antimicrobial resistance, particularly through the development of metal-based nanoparticles. Among these, silver nanoparticles (AgNPs) have attracted considerable attention due to their broad-spectrum antimicrobial activity and unique physicochemical properties (Dewi et al. 2023; Dhahir and Shaheen 2025). AgNPs exhibit enhanced surface area-to-volume ratios, enabling stronger interactions with microbial cell membranes and intracellular components (Zhu et al. 2025). Their antimicrobial efficacy is strongly influenced by particle size, shape, surface charge, and distribution (Teffo et al. 2010). Although conventional physical and chemical methods are commonly used for nanoparticle synthesis, these approaches often involve high energy consumption, toxic chemicals, and environmentally hazardous by-products. In contrast, green synthesis offers an eco-friendly alternative by utilizing biological resources such as plant extracts, microorganisms, and biomolecules as reducing and stabilizing agents. This approach is considered cost-effective, non-toxic, and sustainable, making it suitable for biomedical and pharmaceutical applications (Shahzadi et al. 2025).

Despite the advantages of green synthesis, controlling nanoparticle size, morphology, and monodispersity remains a significant challenge. Variations in phytochemical composition, plant age, geographic location, and extraction conditions can influence nanoparticle formation and stability (Aditya et al. 2025). Therefore, identifying medicinal plants with consistent bioactive profiles and strong reducing potential is essential for producing stable and biologically active nanoparticles. Silver nanoparticles synthesized through plant-mediated routes have demonstrated promising applications in catalysis, optics, antimicrobial formulations, and biomaterials manufacturing (Sivalingam et al. 2025). Importantly, when synthesized at controlled concentrations, AgNPs exhibit improved chemical stability, catalytic activity, and biocompatibility compared with bulk silver and silver salts (Bhuvaneswari et al. 2015). Additionally, the slow and sustained release of silver ions from nanoparticles represents a major advantage, contributing to prolonged antimicrobial action while reducing acute toxicity (In et al. 2013).

*Dodonaea viscosa* is a medicinal plant belonging to the family Sapindaceae and is commonly known as Shahs or Zaitoon Alramal (Al-Bimani and Hossain 2020). This plant has been widely used in traditional medicine and is known to contain a diverse range of bioactive compounds, including flavonoids, terpenoids, tannins, saponins, and phenolic acids (Arunprasath and Priyankadevi 2018). Several studies have reported the antibacterial potential of *D. viscosa*, particularly against Gram-positive bacteria such as *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Corynebacterium diphtheriae* (Balasubramanian et al. 2025)

In contrast, limited or no inhibitory effects have been observed against certain Gram-negative bacteria, including *Escherichia coli* and *Pseudomonas aeruginosa*, which is often attributed to differences in cell wall structure and permeability (Taranath et al. 2015). These findings highlight the potential of *D. viscosa* as a source of antimicrobial agents while also indicating the need for strategies that enhance its activity against a broader spectrum of pathogens.

The integration of nanotechnology with traditional medicinal plants represents a promising strategy to overcome the limitations of crude plant extracts. The concept of green synthesis of metal nanoparticles was first introduced by Raveendran et al. (2003), who demonstrated an environmentally friendly approach for producing metal nanoparticles using natural reducing agents. Since then, extensive research has focused on plant-mediated synthesis of nanoparticles using various botanical sources (Mittal et al. 2013). Silver nanoparticles synthesized using extracts of *Solanum tricobatum*, *Syzygium cumini*, *Centella asiatica*, *Citrus sinensis*, *Azadirachta indica*, and *Crocus sativus* showed better antimicrobial activity compared with their corresponding crude extracts (Logeswari et al. 2013; Verma and Mehata 2016). These studies suggest that phytochemicals not only facilitate nanoparticle formation but may also synergistically enhance biological activity.

Despite the growing body of literature on green-synthesized silver nanoparticles, studies focusing on *D. viscosa* as a biological resource for AgNP fabrication remain limited, particularly under controlled experimental conditions (Maliszewska and Sadowski 2009). Therefore, the present study aimed to synthesize silver nanoparticles using the aqueous leaf extract of *D. viscosa* characterized the physicochemical properties of synthesized nanoparticles, and evaluate their antibacterial activity against, specifically *E. coli* and *S. aureus* bacteria. This work seeks to contribute to the development of eco-friendly antimicrobial nanomaterials derived from medicinal plants and to provide insights into their potential applications in combating bacterial infections.

## MATERIALS AND METHODS

### Extraction of *Dodonaea viscosa* plant

Fresh green leaves of *D. viscosa* were collected from the gardens of Al-Nahrain University, Baghdad, Iraq. The collected leaves were thoroughly washed under running tap water to remove dust and debris, followed by rinsing with distilled water. The leaves were then dried in an electric oven at 45°C until a constant weight was achieved. After drying, the leaves were ground into a coarse powder using a mechanical grinder.

A total of 100 g of powdered leaves was obtained, from which 50 g was used for extraction. The powdered material was soaked separately in 250 mL of analytical-grade ethanol and sterile distilled water and left to macerate for 24 hrs. at room temperature with occasional shaking. After extraction, the mixtures were filtered using Whatman filter paper to remove plant residues. The resulting filtrates were concentrated under reduced pressure using a rotary evaporator at 50°C for 1 hr. The concentrated extracts were then further dried in an electric oven at 45°C to obtain the final crude extract powders. The dried aqueous extract was stored in airtight containers at 4°C until further use.

The dried *D. viscosa* leaf extract was reconstituted in the corresponding solvent to prepare a stock solution at a ratio of 1:5 (extract: solvent), yielding a final concentration of 20% (w/v). From this stock solution, working concentrations of (25, 50, 75, and 100%) were prepared by serial dilution. All prepared solutions were sterilized by filtration through a 0.45 µm Millipore membrane filter under aseptic conditions prior to use in subsequent experiments.

### Phytochemicals analysis

Phytochemical profiling of *D. viscosa* aqueous leaf extract was performed using Gas Chromatography-Mass Spectrometry (GC-MS) at Ibn Albeetar Laboratories, Ministry of Industry and Minerals, Baghdad, Iraq. The analysis was carried out using an Agilent Technologies gas chromatograph (Model 7820A) coupled with a mass selective detector (Model 5977E, USA).

Chromatographic separation was achieved on an HP-5MS capillary column (30 m × 0.25 mm internal diameter, 0.25 µm film thickness). Helium (99.999% purity) was used as the carrier gas at a constant flow rate of 1.0 mL/min. The injector temperature was maintained at

250°C, and 1 µL of the sample was injected in split mode with a split ratio of 10:1.

The oven temperature program was set as follows: the initial oven temperature was 60°C and held for 2 min., then increased at a rate of 10°C/min to 280°C, followed by a final hold at 280°C for 10 min., resulting in a total run time of approximately 34 min.

The mass spectrometer was operated in Electron Ionization (EI) mode at 70 eV. The ion source temperature was set at 230°C, the quadrupole temperature was maintained at 150 °C, and the transfer line temperature was kept at 280°C. Mass spectra were acquired in full scan mode over a mass-to-charge (m/z) range of 40-550.

Phytochemical constituents were tentatively identified by comparing the obtained mass spectra with those in the Wiley and NIST/EPA/NIH mass spectral libraries based on spectral similarity and retention time matching. Based on the GC-MS profiling, the aqueous extract was selected for the green synthesis of silver nanoparticles in subsequent experiments.

**Green synthesis of silver nanoparticles**

Silver nanoparticles (AgNPs) were synthesized using the aqueous leaf extract of *D. viscosa* as a biological reducing and stabilizing agent. Aqueous silver nitrate ($AgNO_3$) solutions with concentrations of 0.5 mM and 1.0 mM were freshly prepared by dissolving 0.0849 g and 0.1699 g of $AgNO_3$, respectively, in 1 L of distilled water. For each synthesis reaction, the plant extract was mixed with the corresponding $AgNO_3$ solution at a volume ratio of 1:9 (extract: $AgNO_3$), where 10 mL of the aqueous extract was added to 90 mL of $AgNO_3$ solution. The reaction mixtures were initially incubated at 45°C for 15 min. and subsequently heated under continuous stirring at 90°C for 60 min. The pH of the reaction mixtures was adjusted to 10.0 using NaOH and $H_3PO_4$ solutions while monitoring with a calibrated pH meter. The formation of AgNPs was monitored visually by the appearance of a dark brown color, indicating surface plasmon resonance. The same synthesis conditions were applied for both $AgNO_3$ concentrations to allow direct comparison of nanoparticle formation and properties (Daniel et al. 2013).

**Physical and optical characterization of AgNPs**

The physicochemical characterization of biosynthesized silver nanoparticles (AgNPs) was performed using Fourier-Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM), and Energy-Dispersive X-ray spectroscopy (EDX) to comprehensively evaluate functional groups, morphology, particle size, and elemental composition. FTIR analysis was conducted to identify the biomolecules involved in the reduction and stabilization of AgNPs. The spectra revealed characteristic absorption bands corresponding to hydroxyl (-OH), amine (-NH), carbonyl (C=O), and other functional groups, confirming the involvement of plant-derived phytochemicals in nanoparticle capping and stabilization.

To obtain accurate measurements of individual nanoparticle size, AFM analysis was performed. AFM images demonstrated a more uniform distribution of discrete nanoparticles with significantly smaller dimensions compared to SEM observations. Therefore, AFM-derived measurements were considered representative of the actual size of individual AgNPs, while SEM was primarily used for morphological assessment and visualization of aggregation. This complementary use of SEM and AFM resolves the apparent discrepancy in particle size reporting. The elemental composition of the synthesized nanoparticles was further confirmed by EDX analysis.

**Antibacterial activity assessment**

The antibacterial activity of biosynthesized silver nanoparticles (AgNPs) derived from the aqueous leaf extract of *D. viscosa* was evaluated against pathogenic Gram-negative *E. coli* and Gram-positive *S. aureus* using the agar well diffusion method (Daniel et al. 2013). Pure bacterial cultures were collected from Alyarmook local hospital, *E. coli* from urine (urinary tract infection) and *S. aureus* from (skin infection) and were subculture in nutrient broth and incubated at 37°C until reaching logarithmic growth phase. The bacterial suspensions were adjusted to a turbidity equivalent to 0.5 McFarland standard ($1.5 \times 10^8$ CFU/mL) to ensure uniform inoculum density. Mueller-Hinton Agar (MHA) medium was prepared, sterilized, poured into sterile Petri plates, and allowed to solidify to obtain a uniform agar layer with a thickness of approximately 2-3 mm.

Each standardized bacterial suspension was evenly inoculated onto the agar surface using a sterile cotton swab to ensure uniform distribution. Wells of 6 mm diameter were aseptically punched into the agar using a sterile micropipette tip. A volume of 10 µL of each test solution was added to the corresponding wells. The tested treatments included *D. viscosa* aqueous extract, AgNPs suspensions synthesized using two stock solutions which was millimolar concentrations 0.5 mM and 1.0 mM $AgNO_3$, a negative control consisting of the corresponding solvent, $AgNO_3$ solutions alone at matching concentrations (0.5 mM and 1.0 mM) as an additional control, and a standard antibiotic disc/solution (e.g., ciprofloxacin) used as a reference positive control. All test solutions were sterilized by filtration through a 0.45 µm Millipore membrane filter prior to application.

Plates were incubated at 37°C for 24hrs., after which antibacterial activity was assessed by measuring the diameter of the clear zones of inhibition surrounding each well. The inhibition zones were measured in millimeters (mm) using a calibrated ruler and recorded.

Each treatment was tested against each bacterial strain in triplicate (n = 3), with three independent plates prepared per treatment. Then the mean inhibition zone diameter was calculated.

**Statistical analysis**

All antibacterial assays were performed in triplicate (n = 3), and the results were expressed as mean ± Standard Deviation (SD). Statistical analysis was conducted using one-way Analysis of Variance (ANOVA) to evaluate

significant differences among the tested treatments. When statistically significant differences were detected, the Least Significant Difference (LSD) test was applied as a post hoc comparison to determine differences between individual group means. A p-value of $p < 0.05$ was considered statistically significant. All statistical analysis were performed using appropriate statistical software.

## RESULTS AND DISCUSSION

### Phytochemical analysis

The GC-MS analysis was performed to characterize the phytochemical composition of *D. viscosa* leaf extracts. The aqueous extract showed a phytochemical profile dominated by sugars and carbohydrate derivatives, fatty acids and their methyl esters, and minor phenolic compounds. Prominent constituents included methylated sugars such as 4-O-methylmannose, as well as fatty acid derivatives including hexadecanoic acid and octadecenoic acid methyl esters. In contrast, the ethanolic extract exhibited a more complex chromatographic profile enriched with lipophilic compounds, including fatty acid esters, hydrocarbons, and terpenoid-related constituents, particularly within the retention time range of 25-28 min.

Comparative analysis of both extracts indicated that aqueous extract contained a higher relative abundance of biologically relevant polar compounds. This observation supported its selection for subsequent green synthesis and antibacterial assays.

The complete GC-MS profiles of the aqueous and ethanolic extracts are provided as Figures 1 and 2 and Table 1. All compound identifications were based on library matching and should therefore be considered putative.

### Nanoparticle characterization

The formation of silver nanoparticles (AgNPs) was initially indicated by a visible color change of the reaction mixture from colorless to dark brown (Figure 3), which is attributed to the excitation of Surface Plasmon Resonance (SPR) resulting from the reduction of $Ag^{+}$ ions to $Ag^{0}$ nanoparticles.

FTIR was employed to identify the functional groups involved in the reduction, capping, and stabilization of the biosynthesized AgNPs. The FTIR spectra of AgNPs synthesized at 0.5 mM and 1 mM concentrations exhibited several characteristic absorption bands (Figure 4). The broad band observed around 3261 $cm^{-1}$ corresponds to O-H stretching vibrations, indicating the presence of hydroxyl groups, likely derived from phenolic or alcoholic compounds. Peaks detected in the range of 2935 $cm^{-1}$ were associated with C-H stretching of aliphatic groups. The absorption bands at approximately 1712 $cm^{-1}$ and 1607 $cm^{-1}$ correspond to C=O stretching of carbonyl groups and C=C or amide-related vibrations, respectively. Bands observed at 1367 $cm^{-1}$ and 1040 $cm^{-1}$ were attributed to C-N stretching of amines and C-O stretching vibrations of alcohols or carboxylic acids. Lower-frequency bands below 900 $cm^{-1}$ may be related to metal-oxygen interactions, supporting the involvement of plant biomolecules in nanoparticle stabilization. These results suggest that multiple functional groups contributed to the capping and stabilization of the synthesized AgNPs.

**Table 1.** Major compounds identified by GC-MS in *Dodonaea viscosa* leaf extracts

| Extracts | RT (min) | Putative compounds | Chemical classes |
|---|---|---|---|
| Aqueous | 12.77 | Phenol, 2,4-bis(1,1-dimethylethyl) | Phenolic |
| Aqueous | 16.4 | 4-O-Methylmannose | Carbohydrate |
| Aqueous | 18.58 | Hexadecanoic acid, methyl ester | Fatty acid ester |
| Ethanolic | 20.78 | Octadecenoic acid, methyl ester | Fatty acid ester |
| Ethanolic | 26.74 | Isomyocorene | Terpenoid-related |

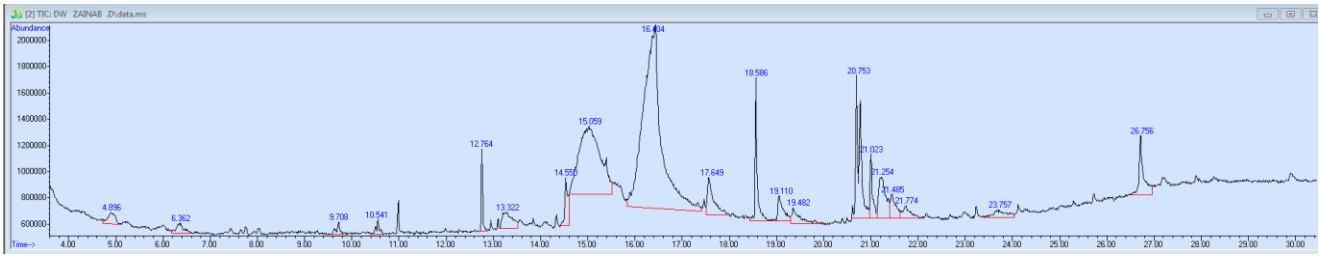


**Figure 1.** GC-mass analysis for *Dodonia viscosa* aqueous extract

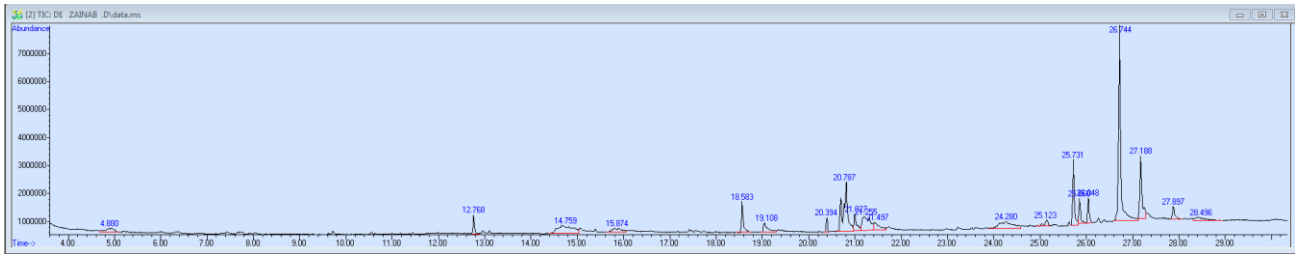


**Figure 2.** GC-mass analysis for *Dodonia viscosa* ethanolic extract

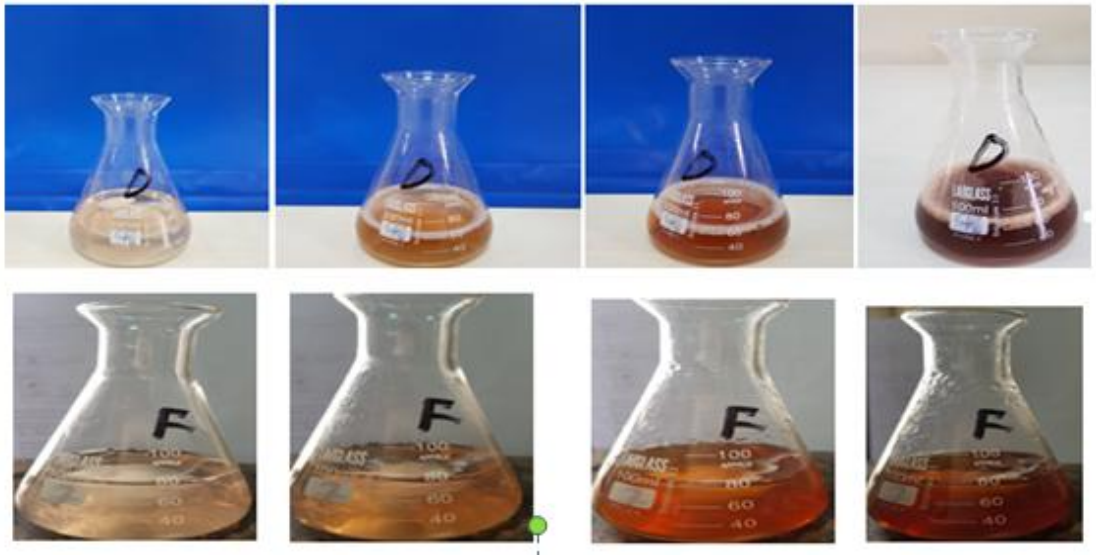
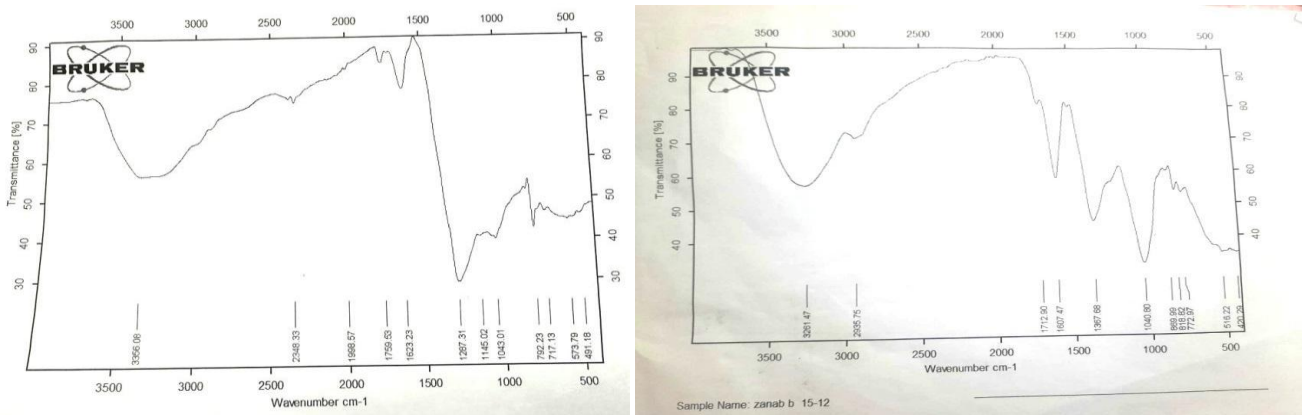


**Figure 3.** The color change of solution indicating the interaction of nanomaterial salts with the extract over time, AgNPs 1mM (labeled D) and 0.5mM (labeled F)




**A** **B**

**Figure 4.** FTIR spectrum of biosynthesized AgNPs 1 mM (A) and 0.5 mM (B) using *Dodonia viscosa* aqueous extract

Scanning Electron Microscopy (SEM) analysis revealed that the synthesized AgNPs exhibited irregular and aggregated morphologies with a cheese-like appearance, while a limited number of particles appeared individually dispersed (Figure 5). Such aggregation may be commonly observed in biosynthesized nanoparticles due to the presence of organic capping agents. The SEM analysis was employed to examine the surface morphology and aggregation behavior of the synthesized nanoparticles. The SEM micrographs (Figures 5.A-B) showed that the AgNPs were predominantly spherical to quasi-spherical in shape, with some degree of anisotropy and aggregation. The apparent particle sizes observed in SEM images ranged from approximately 20 to 100 nm. This wider size range is attributed to particle clustering and aggregation effects that commonly occur during sample preparation and drying for SEM analysis, leading to an overestimation of particle dimensions.

Atomic Force Microscopy (AFM) was used to quantitatively evaluate the surface topography and particle size of the synthesized AgNPs. The two-dimensional and three-dimensional AFM images demonstrated relatively uniform particle distribution. The average particle size of AgNPs synthesized at 1 mM was approximately 8.47 nm (Figure 6.A), whereas those synthesized at 0.5 mM showed a smaller average size of approximately 7.12 nm (Figure 6.B). These results indicate that precursor concentration influences nanoparticle size, with lower $AgNO_3$ concentration favoring the formation of smaller nanoparticles.

Energy-Dispersive X-ray (EDX) analysis confirmed the elemental composition of the synthesized nanoparticles, showing a dominant silver signal, which verifies the successful reduction of $AgNO_3$ to AgNPs in both concentrations (Figures 7.A and B). Minor signals corresponding to other elements are likely derived from biomolecules present in the plant extract that remained attached to the nanoparticle surface. which displayed a strong characteristic signal corresponding to elemental silver, indicating the successful reduction of $Ag^+$ ions to

metallic $Ag^0$. Minor signals corresponding to other elements were attributed to phytochemical residues from the plant extract acting as capping agents.

**Antibacterial activity**

The inhibitory zone of three replicates, diameter was measured and tabulated (Table 2). The present investigation reveals that AgNPs synthesized by leaf extract of *D. viscosa* for both concentrations (1 and 0.5) mM increased the antibacterial activity against *E. coli* and *S. aureus* comparing to aqueous extract. The antibacterial activity of aqueous extract tested against Gram-negative *E. coli* showed inhibition zones of 15, 19, 22 and 27mm for concentration of (25, 50, 75 and 100%) respectively and 17, 19,20 and 27 mm for concentration of (25, 50, 75 and 100%) against Gram-positive *S. aureus* (Figure 8).

The antibacterial activity of AgNPs at concentration of 0.5mM tested against Gram-negative *E. coli* showed synergistic inhibition zone as shown in (Table 3) recorded (17, 21, 23 and 28 mm) for concentrations of (25, 50, 75 and 100%) while (18, 20, 23 and 25mm) for concentration of (25, 50, 75 and 100) % against Gram-positive *S. aureus* (Figure 9).

The antibacterial activity of AgNPs at concentration of 1mM tested against Gram-negative *E. coli* showed synergistic inhibition zone of 16, 19, 22 and 27 mm for concentrations of (25, 50, 75 and 100%) and (22,24, 26 and 29mm) for concentration of (25, 50, 75 and 100 %) respectively against Gram-positive *S. aureus* (Figure 10). The results showed that 100% concentration of both extracts was found to be effective in inhibiting the growth of *E. coli* and *S. aureus.*

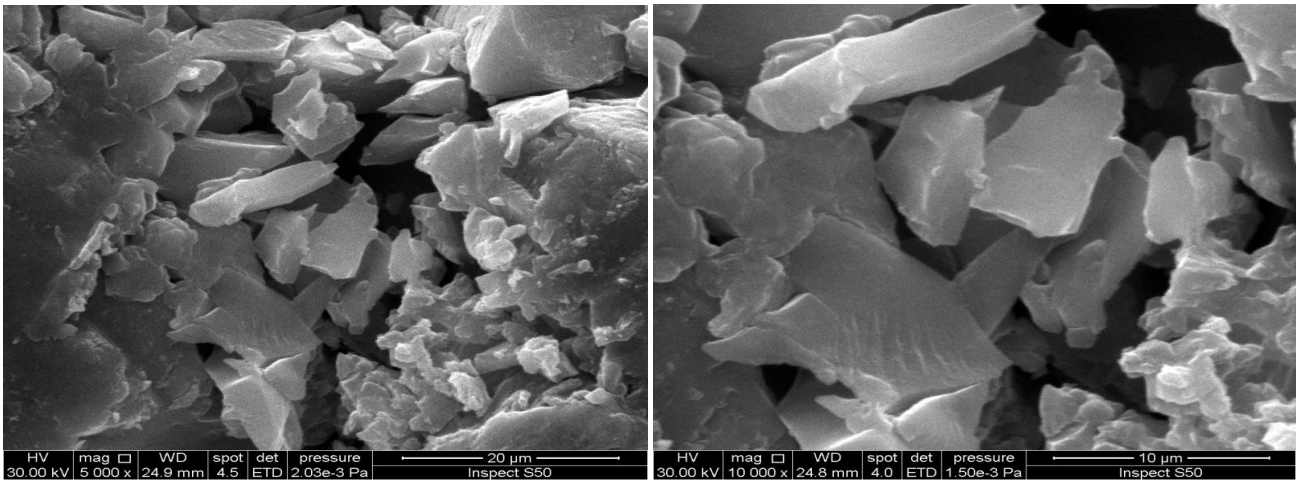



**Figure 5.** Field emission SEM analysis of AgNPs 1 mM (A) and 0.5 mM (B) synthesized from *Dodonia viscosa* aqueous extract

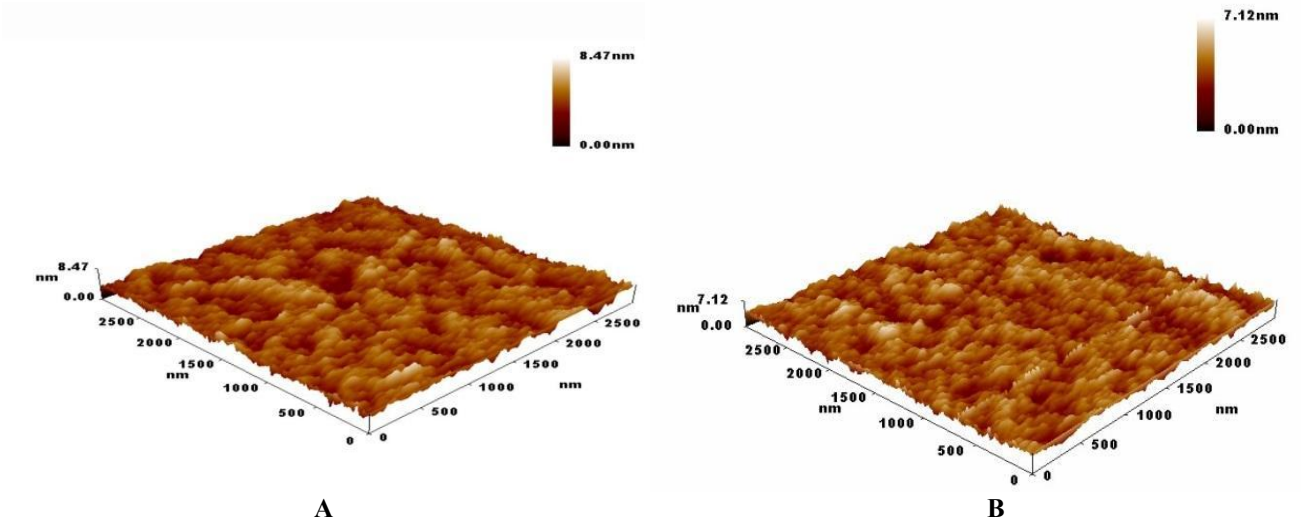



**Figure 6.** AFM assay of 1 mM (A) and 0.5 mM (B) AgNPs synthesized using *Dodonia viscosa* leaves aqueous extract two dimensional image

**Table 2.** Inhibition zones (mm) of *D. viscosa* aqueous extract against *E.coli* (gram -ve ) and *S. aureus* (gram +ve) (Mean ± SD, n = 3)

| **Concentrations of *Dodonia viscosa* aqueous extract** | **Bacteria** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | ***E. coli* (gram -ve)** | | | | ***S. aureus* (Gram +ve)** | | | |
| | **25%** | **50%** | **75%** | **100%** | **25%** | **50%** | **75%** | **100%** |
| Inhibition zones (mm) | 15.0±0.58 | 19.0±0.58 | 22.0±0.58 | 27.0±0.58 | 17.0±0.58 | 19.0±0.58 | 20.0±0.58 | 27.0±0.58 |

**Table 3.** Inhibition zones (mm) of *D.viscosa* extract AgNPs (0.5 and 1 mM) against *E. coli* (Gram -ve) *S. aureus* (Gram +ve) (Mean ± SD, n = 3)

| **Concentrations %** | ***E. coli* (Gram -ve)** | | | | ***S. aureus* (Gram +ve)** | | | |
|---|---|---|---|---|---|---|---|---|
| | **25%** | **50%** | **75%** | **100%** | **25%** | **50%** | **75%** | **100%** |
| AgNPs 0.5 mM | 17.0±0.58 | 21.0±0.58 | 23.0±0.58 | 28.0±0.00 | 18.0±0.58 | 20.0±0.58 | 23.0±0.58 | 25.0±0.00 |
| AgNPs 1 mM | 16.0±0.58 | 19.0±0.58 | 22.0±0.58 | 27.0±0.00 | 22.0±0.58 | 24.0±0.58 | 26.0±0.58 | 29.0±0.00 |

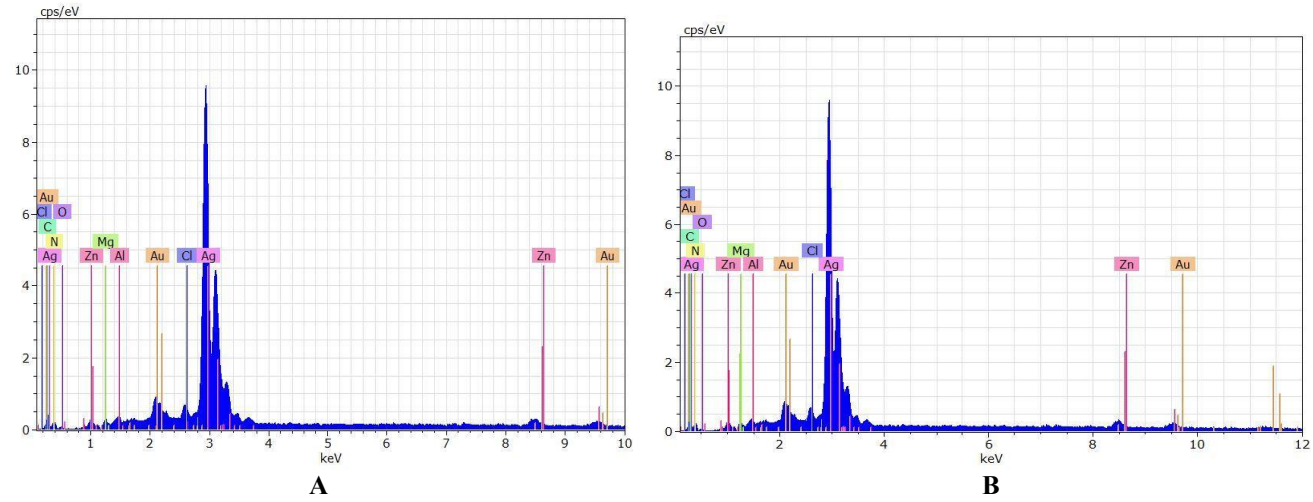



**Figure 7.** EDX image of AgNPs 0.5 mM (A) and 1 mM (B) synthesized using *Dodonia viscosa* aqueous leaves extract

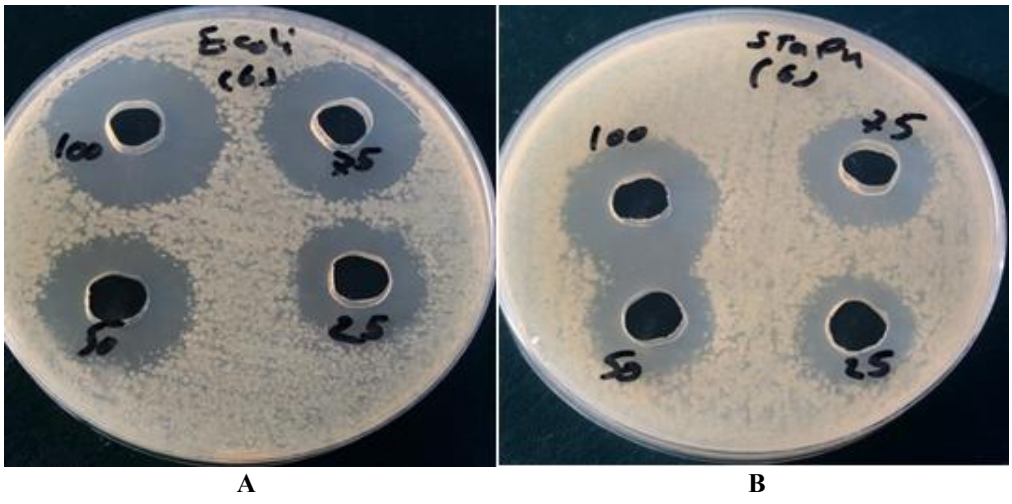



**Figure 8.** *Dodonia viscosa* aqueous extract effect on inhibition zone of A. *E. coli* and B. *S. aureus*

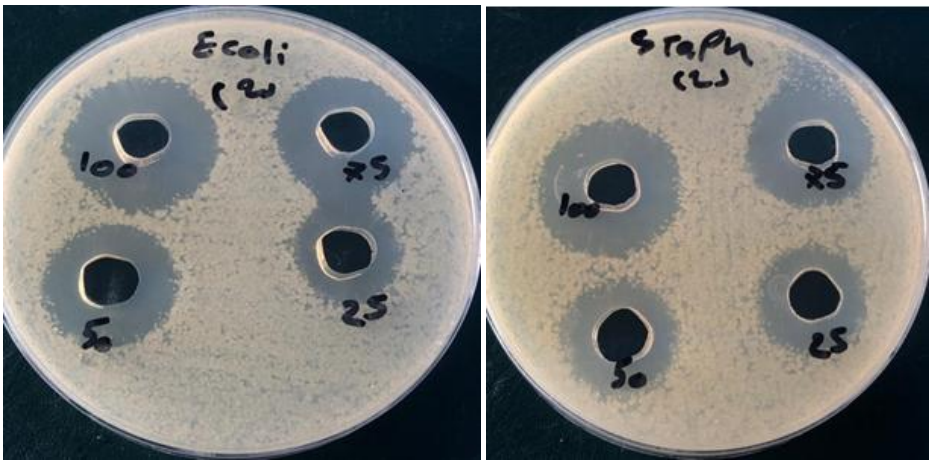
## Discussion

The effectiveness of *D. viscosa* against multidrug-resistant bacteria has garnered attention. The aim of the present study was to evaluate the efficacy of *D. viscosa* plant against *E. coli* and *S. aureus.* This study demonstrated that *D. viscosa* leaf extract can serve as an effective biological reducing and stabilizing agent for the green synthesis of silver nanoparticles (AgNPs). The observed

color change and subsequent characterization analysis confirmed successful nanoparticle formation, supporting previous findings that plant-derived phytochemicals facilitate $Ag^+$ reduction and nanoparticle stabilization (Ahn et al. 2019; Fahimirad et al. 2019).

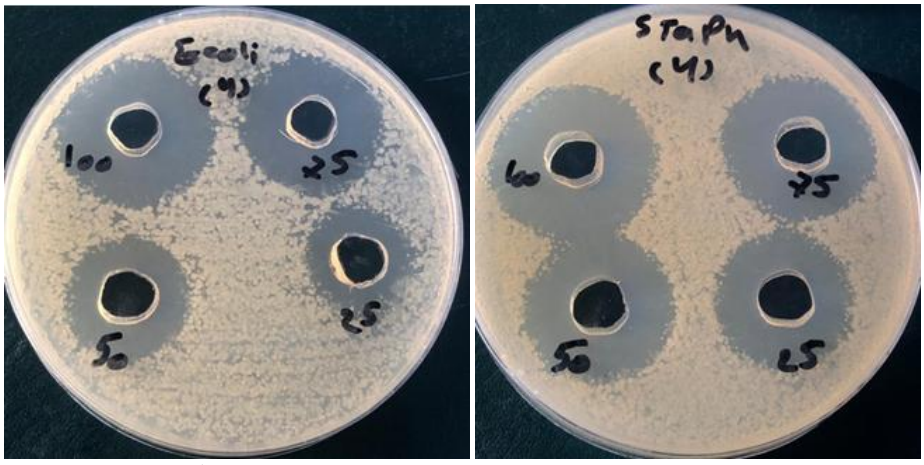
The antibacterial results revealed that biosynthesized AgNPs exhibited markedly higher inhibitory activity against *E. coli* and *S. aureus* compared with the aqueous plant extract. This enhanced activity is consistent with earlier reports indicating that phytochemicals such as flavonoids, terpenoids, and saponins contribute to nanoparticle stability and bioactivity (Ahn et al. 2019; Fahimirad et al. 2019). Ramamurthy et al. (2013) assessed the antibacterial activity of *D. viscosa* extracts against *E. coli* strains resistant to commonly used antibiotics. They found that *D. viscosa* extracts not only inhibited the growth of these resistant strains but also enhanced the effectiveness of certain antibiotics, supporting the potential role of *D. viscosa* in addressing antibiotic resistance. GC-MS analysis in the present study identified phenolic acids and fatty acid esters, which may participate in electron donation during $Ag^+$ reduction and form a protective capping layer that limits nanoparticle aggregation (Dibrov et al. 2002). The antibacterial mechanism of AgNPs is widely attributed to the release of silver ions, which interact with negatively charged bacterial cell walls, increase membrane permeability, and disrupt intracellular processes (Sondi and Salopek-Sondi 2004; More et al. 2023). Additionally, AgNP-induced reactive oxygen species generation may further contribute to bacterial cell damage and ATP depletion (Aditya et al. 2025).

The aqueous extract showed a phytochemical profile dominated by sugars and carbohydrate derivatives, fatty acids and their methyl esters, and minor phenolic compounds. Prominent constituents included methylated sugars such as 4-O-methylmannose, as well as fatty acid derivatives including hexadecanoic acid and octadecenoic acid methyl esters. These chemical classes have been widely associated with antimicrobial-related biological activities, Previous studies have reported that *D. viscosa* contains bioactive diterpenes with antibacterial properties (Cao et al. 2009; Al-Bimani and Hossain 2020; Sagara et al. 2021). The observed activity of the aqueous extract is therefore likely attributable to the combined contribution of these classes rather than to individual compounds.




**A** **B**

**Figure 9.** *Dodonia viscosa* AgNPs (0.5 mM) inhibition zone of A. *E. coli* and B. *S. aureus*




**A** **B**

**Figure 10.** *Dodonia viscosa* AgNPs (1 mM) inhibition zone of A. *E. coli* and B. *S. aureus*

The presence of such compounds may enhance the antibacterial performance of AgNPs through synergistic interactions between silver and plant-derived metabolites (Lok et al. 2006; Ramamurthy et al. 2013). A clear concentration-dependent antibacterial effect was observed, where increased AgNP concentration resulted in larger inhibition zones. Similar dose-dependent inhibition patterns were reported for AgNPs synthesized using *Iresine herbstii* leaf extract (Dipankar and Murugan 2012). The stronger antibacterial activity against *S. aureus* compared with *E. coli* may be attributed to differences in cell wall structure, particularly the thicker peptidoglycan layer in Gram-positive bacteria, which facilitates nanoparticle interaction (Sondi and Salopek-Sondi 2004).

The small particle size achieved by green synthesis according to AFM analysis (7-8.47 nm) is a critical factor contributing to antibacterial efficacy, as smaller nanoparticles possess a higher surface area-to-volume ratio, enhancing bacterial membrane interaction (Aditya et al. 2025). FTIR analysis further supported the involvement of hydroxyl, amine, and carbonyl functional groups in nanoparticle capping and stabilization, this finding is consistent with reports that plant metabolites act as natural stabilizing agents (Daniel et al. 2013; Fahimirad et al. 2019).

The findings of the presence study revealed that green synthesized AgNPs extract showed higher antibacterial activity than simple plant extract. A similar study has also been reported by (Al-Ani et al. 2025). However, consistent with earlier reports emphasizing nanoparticle safety considerations, further cytotoxicity and biocompatibility studies are required before biomedical applications can be fully realized (Totaro and Rambaldini 2009; Fahimirad et al. 2019).

In conclusion, this study AgNPs was successfully achieved using *D. viscosa* aqueous leaf extract. The aqueous extract showed a phytochemical profile dominated chemical classes (hexadecanoic acid and octadecenoic acid methyl esters) widely associated with antimicrobial-related biological activities. These chemical classes have been widely associated with antimicrobial-related biological activities. In contrast, ethanolic extract exhibited a more complex chromatographic profile enriched with lipophilic compounds, including fatty acid esters, hydrocarbons, and terpenoid-related constituents. The synthesized AgNPs showed nanoscale size, stable characteristics, and enhanced antibacterial activity against *E. coli* and *S. aureus* compared to the aqueous extract. Further studies are required to evaluate cytotoxicity, biocompatibility, stability, and activity against multidrug-resistant bacteria to support their potential biomedical applications.

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