

SYNTHESIS OF SILVER NANOPARTICLES USING A NOVEL STEM EXTRACT OF *ANABASIS LACHNANTHA* and THEIR INSECTICIDAL, ANTIOXIDANT, and ANTIMICROBIAL EFFECTS

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<https://doi.org/10.55251/jmbfs.12686>

ARTICLE INFO

Received 16. 4. 2025
Revised 7. 5. 2025
Accepted 9. 5. 2025
Published 1. 6. 2025

Regular article

OPEN ACCESS

ABSTRACT

Biogenic fabrication of metallic nanoparticles is an emerging discipline for the production of nanoproducts that exert potent antioxidant and antibacterial activity, and do not suffer from the limitations inherent in physicochemical synthesis methods. A sustainable and environmentally beneficial method is the synthesis of green nanoparticles using various plant extracts. This study developed a direct method for synthesizing silver nanoparticles (AgNPs) using methanolic, ethanolic, and aqueous extracts of *Anabasis lachnantha* Aellen and Rech., which served as both reduction and capping agents. AgNPs production was optimized by using different pH levels (ranging from 0 to 14), temperature variations (25°C to 95°C), and the addition of 1 mM salt solutions (0.2 to 1 mL). The UV-Vis spectroscopy data showed that AgNPs synthesized from methanolic extract had maximum absorbance at pH 8-9, at 55°C, and at 0.2 mL NaCl concentration. In UV-spectroscopy analysis, a maximum peak is observed at approximately 450 nm, which is favorable for the biosynthesis of AgNPs. Fourier transform infrared spectroscopy (FT-IR) confirmed the involvement of proteases, flavonoids, amides, and aldehydes in the reduction process of AgNPs. Scanning electron microscopy (SEM) analysis of synthesized silver nanoparticles was primarily spherical. Energy dispersive x-ray spectroscopy (EDS) analysis of methanol, ethanol, and aqueous AgNPs revealed the existence of Ag nanoparticles at 3 Kve. The insecticidal activity of AgNPs was tested against *Tribolium castaneum* at various concentrations (50, 100, and 200 µg.mL⁻¹), and the results showed superior efficacy compared to the plant extracts alone. Phytotoxic activity was also assessed at these same doses, revealing that AgNPs exhibited significantly higher phytotoxicity than the extracts. Antioxidant potential, measured through 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, indicated that AgNPs possessed greater antioxidant capacity than the plant extracts. Furthermore, biosynthesized AgNPs demonstrated potent antibacterial activity against *Pseudomonas aeruginosa* and strong antifungal activity against *Aspergillus niger*. The study concludes that AgNPs synthesized from *A. lachnantha* stem extracts have significant potential for use in pharmaceutical applications and commercial medicinal products.

Keywords: Antimicrobial activity; Antioxidant properties; *Anabasis lachnantha*; Antinsecticidal activity; Green synthesis; Phytotoxicity; Silver nanoparticles (AgNPs)

INTRODUCTION

Nanotechnology has revolutionized numerous scientific fields by enabling the manipulation of materials at the atomic and molecular levels, resulting in enhanced properties compared to their bulk counterparts. Particles having a size up to 100 nm are stated as nanoparticles (Abdelrhim *et al.*, 2021; Rashid *et al.*, 2024; Elnaggar *et al.*, 2025). Nanoparticles have different sizes, forms, and dimensions in addition to their chemical components. Because the world is undergoing hazardous destruction resulting from the interaction of modern techniques and businesses, an enormous amount of harmful chemicals and gases are being released (Ihsan *et al.*, 2023; Hayat *et al.*, 2025). It is critical to comprehend the knowledge existing in natural environments that leads to advancement in nanoparticle fabrication processes (Mogazy *et al.*, 2022; Al-Mokadem *et al.*, 2023). Nanoparticles are perfect for producing organic compounds due to their unique properties. Biological molecules go through a highly ordered assembly process that has been proven to be both successful and environmentally benign to make them suitable for metal nanoparticle biogenesis (Ullah *et al.*, 2023).

Nanoparticles are small particles, and they have acquired a lot of attention in recent years. Nanoparticles' increased capabilities, which are related to their small size, are the main reason why they can be used in several sectors (Younes *et al.*, 2020; El-Ansary *et al.*, 2023). Among the various nanomaterials, silver nanoparticles (AgNPs) have emerged as one of the most widely studied due to their exceptional physicochemical and biological properties, including antimicrobial, antioxidant, anti-inflammatory, and insecticidal activities, making them valuable in biomedical devices, drug delivery systems, agricultural pest control, and water purification

(Zhang *et al.*, 2016; Mohamed *et al.*, 2021; Abdel-Kader *et al.*, 2023). Traditional methods for synthesizing AgNPs, such as chemical reduction, photochemical reduction, and electrochemical techniques, typically involve hazardous chemicals like sodium borohydride and hydrazine as reducing and stabilizing agents, raising concerns regarding environmental and health risks (Prabhu and Poulouse, 2012; El-Moneim *et al.*, 2021). The disposal of these toxic reagents can lead to environmental contamination, further emphasizing the need for more sustainable methods.

In recent years, green nanotechnology, which emphasizes eco-friendly and sustainable synthesis approaches, has gained considerable attention (Mittal *et al.*, 2013; Haris *et al.*, 2023; Pathan *et al.*, 2025). The green synthesis of silver nanoparticles using plant extracts offers a promising alternative, as it is cost-effective, non-toxic, and environmentally sustainable (Ahmed *et al.*, 2016; Haq *et al.*, 2024; Aliero *et al.*, 2025). Plants are rich sources of bioactive compounds such as flavonoids, alkaloids, terpenoids, phenolic acids, and proteins, which act not only as reducing agents for silver ions (Ag⁺) but also as stabilizing and capping agents, preventing nanoparticle aggregation (Singh *et al.*, 2016). This biogenic route to nanoparticle synthesis aligns with the principles of green chemistry, reducing the need for hazardous chemicals while producing stable nanoparticles with enhanced biological activities.

The research indicates that species from the *Anabasis* genus are well-recognized for their abundance of bioactive compounds, such as flavonoids, alkaloids, and phenolic acids (Sofy *et al.*, 2022; Akhelova *et al.*, 2023). These compounds exhibit diverse pharmacological properties, including significant antioxidant, anti-inflammatory, and antimicrobial activities (Saboon *et al.*, 2019; Al-Khayri *et al.*,

2022). Studies focusing on similar species, such as *Anabasis articulata*, have provided substantial evidence of these bioactivities, making the genus a valuable subject for ongoing pharmacological and phytochemical investigations (Shegebayev et al., 2023).

To date, no systematic studies have explored the potential of *A. lachnantha* for the green synthesis of AgNPs, providing a novel avenue for investigation. Previous studies have demonstrated that plant-mediated AgNPs exhibit enhanced biological activities, including antimicrobial and insecticidal properties. AgNPs synthesized using plant extracts have shown broad-spectrum antimicrobial efficacy against Gram-positive and Gram-negative bacteria, fungi, and multidrug-resistant strains (Saxena et al., 2010; El-Ansary et al., 2023). In agricultural applications, AgNPs have proven effective against phytopathogens and insect pests, providing a sustainable solution for crop protection and food preservation (Prasad et al., 2017).

This study aimed to develop a green, eco-friendly method for synthesizing silver nanoparticles (AgNPs) using methanolic, ethanolic, and aqueous stem extracts of *A. lachnantha*. No report on the utilization of *A. lachnantha* in the biosynthesis of silver nanoparticles. The objective of our study was to highlight the potential of AgNPs derived from *A. lachnantha* as a resource for developing antimicrobial agents and agricultural nanomaterials, showcasing significant promise for both pharmaceutical and commercial uses.

MATERIAL AND METHODS

Plant collection and identification

Fresh specimens of *A. lachnantha* were gathered from Khyber Pakhtunkhwa, Pakistan. The plant species was authenticated based on the descriptions in the Flora of Pakistan. After collection, the samples were thoroughly rinsed with tap water to eliminate impurities and then left to air dry in a shaded area for approximately 7-8 days. Once completely dried, the plant material was finely ground using a mechanical grinder. The resulting powder was stored in a cool, dry, and dark environment to prevent moisture exposure and later used for extraction procedures in the laboratory (Das et al., 2010).

Preparation of ethanolic, methanolic, and aqueous extracts

To prepare the ethanolic extract, 100 g of powdered *A. lachnantha* stem was placed in a flask, and 500 mL of ethanol was added. The mixture was shaken continuously for seven days. Afterward, the solution was filtered, and the ethanol was evaporated using a water bath set at $75 \pm 3^\circ\text{C}$. The dried ethanolic extract was then utilized as a reducing agent for synthesizing nanoparticles (Leon et al., 2013). For the methanolic extract, 50 g of powdered stem was combined with 250 mL of methanol and subjected to the same shaking process for seven days. Following filtration, the solvent was evaporated at $64 \pm 3^\circ\text{C}$ using a water bath, resulting in a dried methanolic extract, which also served as a reducing agent during nanoparticle synthesis (Rodríguez-León et al., 2013). The aqueous extract was prepared by mixing 4 g of powdered stem with 100 mL of distilled water, boiling the mixture at 50°C for 15 minutes. A 1 mL sample of the resulting broth was added dropwise into a 50 mL solution of 0.1 mM AgNO_3 , with constant stirring at $50\text{--}60^\circ\text{C}$, producing a color change, indicating the reduction of silver ions (Saxena et al., 2010).

Biosynthesis of AgNPs

A standard ethanolic extract ($200 \text{ g}\cdot\text{mL}^{-1}$) was combined with a 1 mM silver nitrate solution in different ratios (1:1, 1:2, 1:3, 1:4, 1:5). The mixture was continuously stirred for 24 hours using a magnetic stirrer. After this period, the appearance of a brown color indicated the reduction of silver ions and the formation of silver nanoparticles (AgNPs), a process facilitated by the plant's phytometabolites (Kokila et al., 2016). This method was similarly applied to the methanolic and aqueous extracts of *A. lachnantha*. The resulting AgNPs were characterized using UV-visible spectroscopy (UV-1800 Shimadzu UV spectrophotometer). The samples were then centrifuged at 14,800 rpm for 10 minutes, and the resulting pellet was resuspended in distilled water to remove any soluble components. The sample was then stored for 24 hours for further use (Lima et al., 2024).

AgNPs optimization

Effects of pH, temperature, and salt

The pH of the *A. lachnantha* stem extract was adjusted using NaOH and HCl to investigate its influence on the synthesis of silver nanoparticles (AgNPs). pH values ranging from 1 to 12 were evaluated, with absorbance readings taken using UV-visible spectroscopy (Ahmad et al., 2013). Fourteen vials, each containing 3 mL of the nanoparticle solution, were exposed to various temperatures (35, 45, 55, 65, 75, 85, and 95°C) in a water bath for 10 minutes, followed by absorbance measurements using UV-visible spectrometry (Ahmad et al., 2019). To examine the effect of salt on nanoparticle synthesis, a 1 mM NaCl solution was prepared by dissolving 1.168 g of NaCl in 20 mL of distilled water. Different volumes of the

salt solution (0.2, 0.4, 0.6, 0.8, and 1.0 mL) were added to the stem nanoparticle solution, and absorbance was measured using UV-visible spectroscopy.

Characterization of silver nanoparticles

UV-Visible Spectroscopy

The silver nanoparticles' UV-visible spectrum of *A. lachnantha* stem extract and silver nitrate solution was validated. The absorbency of the manufactured silver nanoparticles was tested using pure water as a blank. The synthesis of AgNPs was observed using the UV-1602 twin beam UV-Vis Spectrometry, which has a resolution of 1 nm. Using wavelength ranges of 300-800nm, the spectrum absorbency of AgNPs produced using reaction mixtures containing stem extract and silver nitrate solution in varying proportions (1:1, 1:2, 1:3, 1:4, and 1:5) was assessed (Padalia et al., 2015).

Fourier Transform Infrared Spectroscopy (FT-IR)

The silver nanoparticles were dried thoroughly in a vacuum drier at 37°C , after which it was mixed with KBr and molded into test pellets using a mechanical pellet press. Using a "Perkin-Elmer spectrophotometer FT-IR SPECTRUM ONE" with a 4 cm^{-1} resolution and a range of 4000 cm^{-1} , FT-IR spectroscopy was carried out on the materials. The stem extract of *A. lachnantha* was subjected to FT-IR spectrometry using the same approach as the nanoparticles of *A. lachnantha* (Acay et al., 2019).

Scanning electron microscopy (SEM)

A. lachnantha was used to synthesize AgNPs from the stem extract of the plant, which was vacuum-dried at 350°C . A sample of manufactured Ag nanoparticles was made by electroplating a very small amount of Ag onto a carbon-coated SEM grid, letting it dry, and then coating it with Ag using an Auto fine coating technique (Spi-module sputter coater). This sample was then examined under a FE-SEM to determine its morphological characteristics (Mofolo et al., 2020).

Spectroscopy of energy dispersion (EDS)

Energy dispersive X-ray spectroscopy was used to determine the existence of basic Ag in silver nanoparticles. For this, a particulate solution was centrifuged at 14800 rpm for 15 minutes, followed by vacuum drying at 35°C . The powdered mass was then subjected to EDX analysis using thermal Scanning electron microscope equipment (Mofolo et al., 2020).

Biological activities of AgNPs

Insecticidal activity

The insecticidal potential of silver nanoparticles and plant extracts versus *Tribolium castenium* was compared. Silver nanoparticles and plant extracts were combined in distilled water to make a stock solution ($1 \text{ mg}\cdot\text{mL}^{-1}$). From the stock solution, three concentrations (50, 100, and $200 \mu\text{g}\cdot\text{mL}^{-1}$), each with two samples, were created. The filter paper was cut to fit the Petri dish size and inserted into the plates. All sample concentrations were put on filter paper. Only distilled water was used as a control. All plates were stored for 24 hours to allow for complete evaporation. 30 healthy insects were selected from the pharmacognosy laboratory and distributed to all plates with the aid of a clean brush after the liquid had completely evaporated. After incubation, the insects were inspected at 24, 48, and 72 hours. The following formula was used to compute the mortality percentage (Dastagir and Hussain, 2013):

$$\text{Mortality rate (\%)} = 100 \frac{\text{Number of insects in control}}{\text{Number of live insects in test}} \times 100$$

Phytotoxic assay

Phytotoxic activity was assessed using *Lemna aequinoctialis* as a test plant to examine the phytotoxic potential of AgNPs produced by reducing and capping *A. lachnantha* stem extracts. The experiment was conducted in Petri dishes with three replicates in three distinct concentrations (50, 100, and $200 \mu\text{g}\cdot\text{mL}^{-1}$). The Petri dishes were autoclaved for 24 hours at 121°C . In distilled water, a stock solution ($1 \text{ mg}\cdot\text{mL}^{-1}$) of each sample (leaf and bulb extracts, as well as their silver nanoparticles) was created. E-medium was made for *Lemna* plant growth, and 15 mL of it was applied to each Petri dish. Each sterilized pot received 10 healthy plants (each with three fronds). The positive and negative controls were standard herbicide (Atrazine 10%) and E-medium alone, respectively. At 28°C , all plants were incubated for seven days. The number of fronds per plate was counted after the incubation period (Sultana et al., 2019). The following formula was used to calculate the percent growth inhibition in comparison to the negative control:

$$\text{Inhibition (\%)} = \frac{\text{Number of fronds in test}}{\text{Number of fronds in control}} \times 100$$

Antioxidant assay

The bleaching action on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) (dark purple solution) was used to test the reducing potential of both plant extracts and their nanoparticles produced. Ascorbic acid was identified as the leading antioxidant. Then, different concentrations of samples (plant extracts and silver nanoparticles) were made, like 50, 100, and 200 $\mu\text{g}\cdot\text{mL}^{-1}$, and in each concentration, 1 mL of plant extract and 2 mL DPPH solution were mixed in test tubes. The mixture was thoroughly mixed before being left to hatch for one hour in the dark at room temperature. Ascorbic acid was employed as a reference standard, and the stock solution was made by dissolving it in distilled water at the same concentration (50, 100, and 200 $\mu\text{g}\cdot\text{mL}^{-1}$). Then, using a blank of methanolic solution as a reference, the absorbance of each sample was determined in a UV spectrophotometer at 517 nm. The antioxidant potential was calculated using the following formula (Khan et al., 2020; Uddin et al., 2012):

$$\text{Antioxidant activity (\%)} = \frac{\text{Control absorbance} - \text{Test absorbance}}{\text{Control absorbance}} \times 100$$

Antibacterial assay

The bacterium strain *Pseudomonas aeruginosa* was received from the Department of Biotechnology, AWKUM. The strain was washed thoroughly in the nutrient broth after being incubated for 12 hours. The antibacterial efficacy of the AgNPs and plant extracts against *Pseudomonas aeruginosa* was evaluated using the disc diffusion method. Four discs were placed on a medium, each holding a different quantity of silver nanoparticles and plant extracts. Antibiotic and distilled water served as the +ve and -ve controls, respectively. The plates were then incubated at 25 °C for 24 hours. Inhibition zones were measured on a regular scale to evaluate the antibacterial potency (Keshari et al., 2020).

Antifungal assay

The antifungal activity of nanoparticles and plant extracts (stem) at various concentrations (50, 100, and 200 $\mu\text{g}\cdot\text{mL}^{-1}$) against *Aspergillus niger* was assessed using the agar well diffusion method. Clotrimazole (a common antifungal medication) and purified water were used as positive controls. The plates were incubated at 25 °C for 3 days, after which the inhibition zones were measured (Asad et al., 2022).

Statistical analysis

All data were expressed as the standard error of the mean (n=3) and analyzed using one-way ANOVA followed by Dunnett's test for comparisons. Graphs were generated using Origin 7.5 software.

RESULTS and DISCUSSION

Synthesis of Silver Nanoparticles

AgNPs were synthesized using the methanolic extract from the stem of *A. lachnantha*, which acted as a reducing agent in the AgNO_3 solution. When the methanolic stem extract was combined with a 1 mM AgNO_3 solution, a noticeable color change from green to light brown occurred within 10-15 minutes, gradually darkening to brown over 50-60 minutes. This color shift indicates the reduction of Ag^+ ions to AgNPs, a process similar to that observed by Alruqi et al. (2018). Likewise, AgNPs were produced by mixing the ethanolic stem extract of *A. lachnantha* with a 1 mM AgNO_3 solution. The gradual color change from green to dark brown within 50-60 minutes confirmed the reduction of silver ions, consistent with the findings of Asad et al. (2022). The aqueous extract followed the same synthesis process, with the extract added to the 1mM AgNO_3 solution. A color change from yellow to dark brown after 55-60 minutes confirmed the reduction of silver ions. Similar results were reported by Mallikarjuna et al. (2011), Jain et al. (2019), and Mofolo et al. (2020), who highlighted the role of plant phytochemicals, such as proteins, alkaloids, phenols, and terpenes, in nanoparticle formation (Abbasi et al., 2017).

AgNPs optimization

Effect of pH

pH plays a critical role in controlling the size and morphology of AgNPs. Using methanolic, ethanolic, and aqueous extracts of *A. lachnantha*, the effect of varying pH (1–12) was examined. The synthesis of AgNPs utilizing plant extract is impacted by pH. Changes in pH influence the charges of organic atoms in plant extracts. The charges of biomolecules influence the production of silver nanoparticles because the silver ion is a cation (Marciniak et al., 2020). Due to the large concentration of hydroxyl (OH) ions on the surface of the nanoparticles in alkaline pH, the colloidal solution was controlled by the opposite charge, which reduced the formation and decreased the size of the nanoparticles. However, at low pH values (acidic), large-sized nanoparticles were produced because of AgNPs accumulation. A large variety of functional groups were available for Ag ion

interaction at higher pH levels, which made it easier to fabricate many small-sized nanoparticles (Anigol et al., 2017).

The UV-Vis spectroscopy data showed that AgNPs synthesized from methanolic extract had maximum absorbance at pH 8-9, suggesting that basic conditions promote AgNPs formation (Fig 1A). This is consistent with the stabilization of capping proteins at higher pH levels. Similarly, AgNPs synthesized using ethanolic extract exhibited optimal absorbance at pH 11–12 (Fig 1B), whereas for aqueous extract, the highest absorbance was observed under acidic conditions (pH 1-2) (Fig 1C). This suggests that different extracts require specific pH ranges for effective nanoparticle synthesis. These results align with previous studies, including those by Yuan et al. (2017) and Anigol et al. (2017), which showed that the pH value impacts the size and stability of AgNPs. As pH increases, smaller spherical nanoparticles are typically formed due to the enhanced reduction rate of silver ions, while lower pH values yield larger, irregular particles (Alqadi et al., 2014). The UV-Vis spectrum can be used to study how particle size changes with variations in certain parameters, such as temperature, concentration, or pH of the medium. Alterations in any of these parameters can lead to changes in particle size. A reduction in particle size, from larger to smaller, is indicated by a blue shift, while an increase or agglomeration of particle size is represented by a red shift (Akhter et al., 2024).

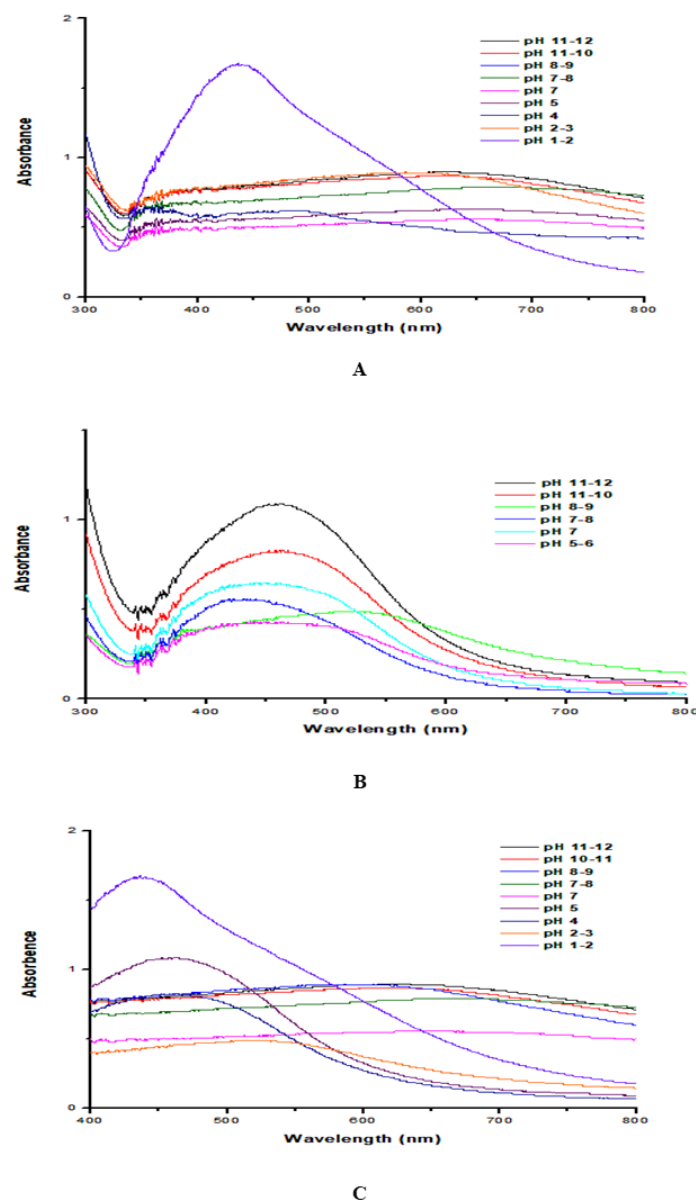


Figure 1 UV-Spectrometry of AgNPs pH effect utilizing methanol (A), ethanol (B), and aqueous (C) extract of *A. lachnantha* stem.

Effect of Temperature

Temperature also significantly influences the rate of AgNP synthesis. The effect of temperature variations (25°C–95°C) was assessed by measuring the UV absorbance of the synthesized nanoparticles. With increasing temperatures, a gradual increase in the rate of silver NPs synthesis and plasmon resonance

absorbance has occurred. The rise in absorbance shows an increase in nanoparticle silver NPs size. Methanolic extract-based AgNPs showed maximum absorbance at 55°C, which declined at higher temperatures due to the denaturation of proteins and other biomolecules (Fig 2A). Similarly, AgNPs synthesized from ethanolic extract peaked at 65°C (Fig 2 B), while aqueous extract-based AgNPs demonstrated optimal synthesis at 55°C (Fig 2C). These findings indicate that 55 °C is the most suitable temperature for AgNPs synthesis using *A. lachnantha* stem extracts. Similar results were reported by Birla et al. (2013), who demonstrated an increase in the size of AgNPs with increasing temperature. Additionally, studies by Ghojavand et al. (2020) and Rakib-Uz-Zaman et al. (2022) confirmed that temperature plays a crucial role in optimizing nanoparticle size and shape.

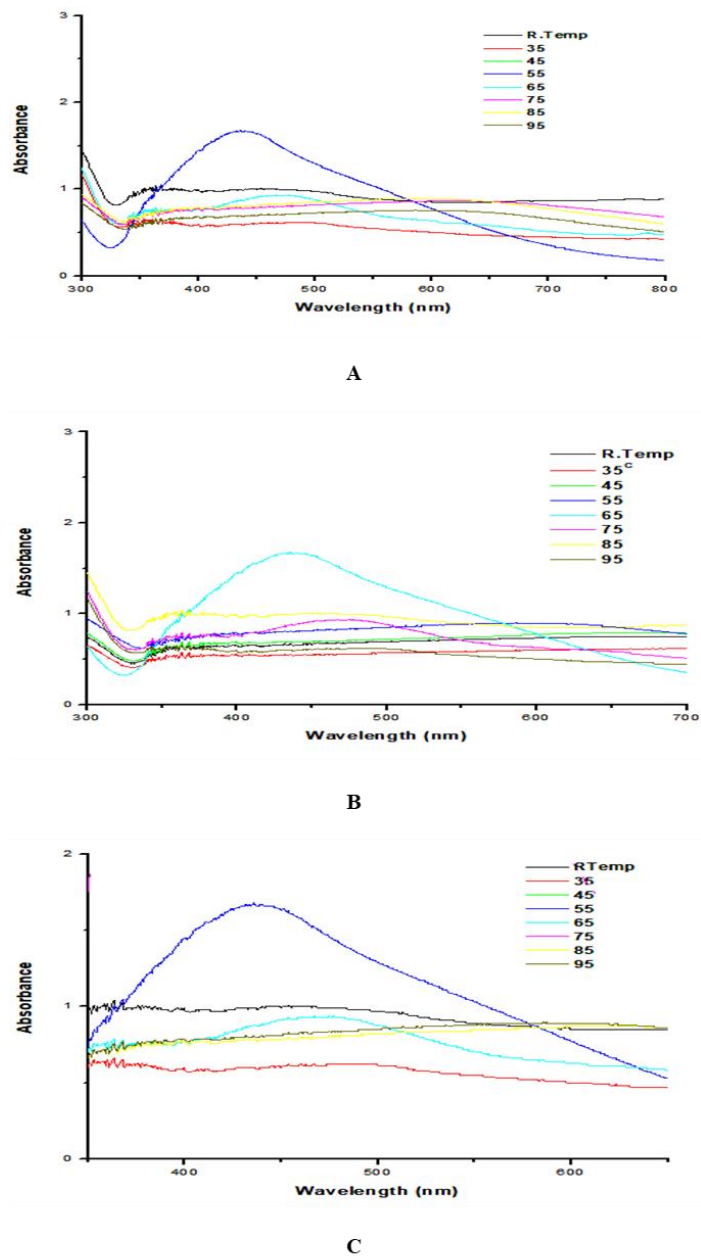


Figure 2 UV-Spectroscopy of AgNPs temperature effect using methanol (A), ethanol (B), and aqueous (C) extract of *A. lachnantha* stem

Effect of NaCl Concentrations

The effect of NaCl concentration on AgNP synthesis was examined by introducing varying volumes (0.2 to 1 mL) of a 1 mM NaCl solution into the reaction mixture. Methanolic, ethanolic, and aqueous extracts of *A. lachnantha* produced AgNPs with the highest absorbance at 0.2 mL NaCl concentration, as shown by UV spectroscopy (Figs 3A- C). As NaCl concentration increased, absorbance gradually decreased, likely due to the aggregation of silver nanoparticles caused by excess Cl⁻ ions. This is consistent with findings by Reduan et al. (2016) and Ahmed et al. (2022), who reported that NaCl promotes the growth of larger nanoparticles by facilitating aggregation. Higher concentrations of NaCl lead to reduced AgNPs

formation as the reaction slows due to the electrostatic interactions between Cl⁻ ions and the nanoparticle surface.

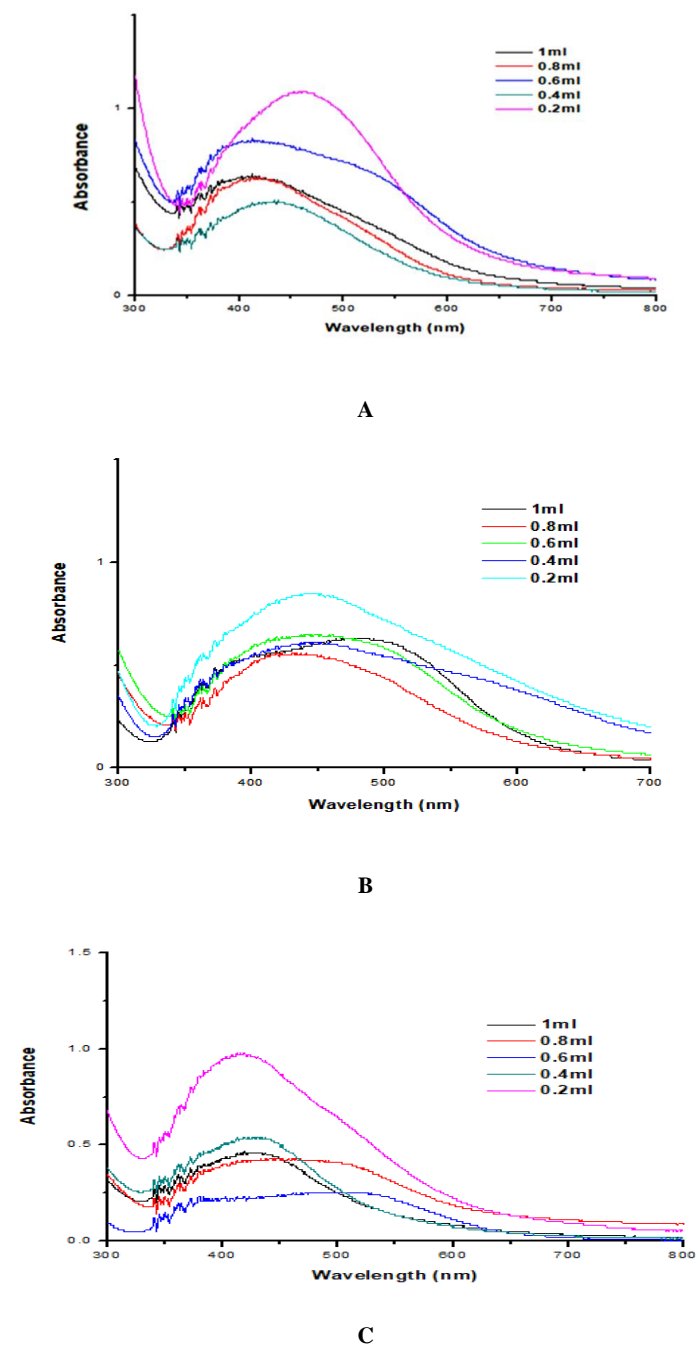


Figure 3 UV-Spectroscopy of AgNPs NaCl effect using methanol (A), ethanol (B) and aqueous (C) extract of *A. lachnantha* stem.

Characterization of AgNPs

UV-Spectroscopic Analysis

UV-visible spectroscopy is an essential tool for determining the formation and stability of silver nanoparticles. In this study, AgNPs synthesized from methanolic, ethanolic, and aqueous extracts of *A. lachnantha* were analyzed at different extract-to-silver nitrate ratios (1:1, 1:2, 1:3, 1:4, 1:5, and 1:6). Methanolic extract-based AgNPs exhibited maximum absorbance at a 1:4 ratio (Fig. 4A), while ethanolic extract-based AgNPs peaked at a 1:5 ratio around 400-500 nm (Fig 4B). Aqueous extract-based AgNPs also showed a peak absorbance at 1:5 around 400 nm (Fig 4C). The UV-Vis results align with those reported by Ghojavand et al. (2020) and Moodley et al. (2018), who demonstrated that AgNPs synthesized using plant extracts exhibit distinct peaks around 440-450 nm. This confirms the successful synthesis of AgNPs with similar characteristics across different extraction methods.

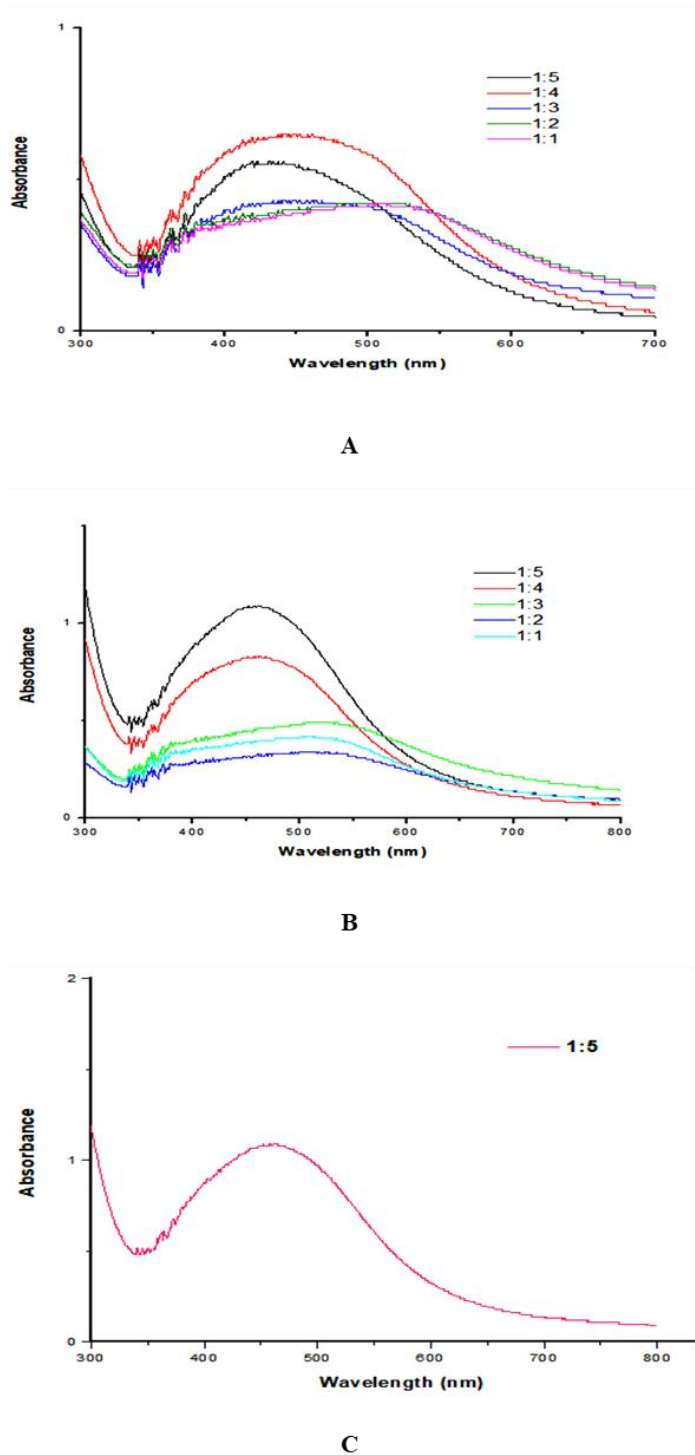


Figure 4 UV-Spectrometry of AgNPs using methanol (A), ethanol (B), and aqueous (c) extract of *A. lachnantha* stem.

Fourier transform infrared spectroscopy (FT-IR)

FT-IR analysis was conducted on dried powder samples of ethanolic, methanolic, and aqueous crude extracts and their respective synthesized AgNPs within the wavelength range of 500–4000 cm^{-1} . This analysis aimed to identify the potential interactions between silver and biological compounds that may be responsible for the biosynthesis and stabilization (as reducing agents) of AgNPs. In the FT-IR spectra of methanol-based AgNPs, absorbance bands were observed at 2847.8 cm^{-1} , corresponding to the C-H stretch (aldehydes), 2919.7 cm^{-1} for C-H stretch (alkanes), 1605.1 cm^{-1} for C=C stretch (aromatic compounds), 1377.5 cm^{-1} for NO_2 stretch (nitro compounds), and 1026.2 cm^{-1} for C-F bands (alkyl halides) (Fig 5A). The FT-IR spectra of ethanol-based AgNPs showed peaks at 2915.6 cm^{-1} (C-H stretch for alkanes), 1605.3 cm^{-1} (C=C stretch for alkenes), and 3518.2 cm^{-1} (N-H stretch for amines) (Fig. 5B). Aqueous-derived AgNPs exhibited bands at 3203.6 cm^{-1} (C-H stretch for aromatic compounds), 1726.9 cm^{-1} and 1571.6 cm^{-1} (C=O stretch for aldehydes), and 1823.1 cm^{-1} (C=O stretch for anhydrides) (Fig 5C).

These findings align with those of **Asad et al. (2021)**, who observed similar spectra in AgNPs synthesized using *Amaryllis vittata* L. and **Mallikarjuna et al. (2011)**, who used *Ocimum* leaf extract. The presence of functional groups like terpenoids, flavonoids, aldehydes, and carboxylic acids is responsible for nanoparticle stabilization (**Ankamwar et al., 2005**).

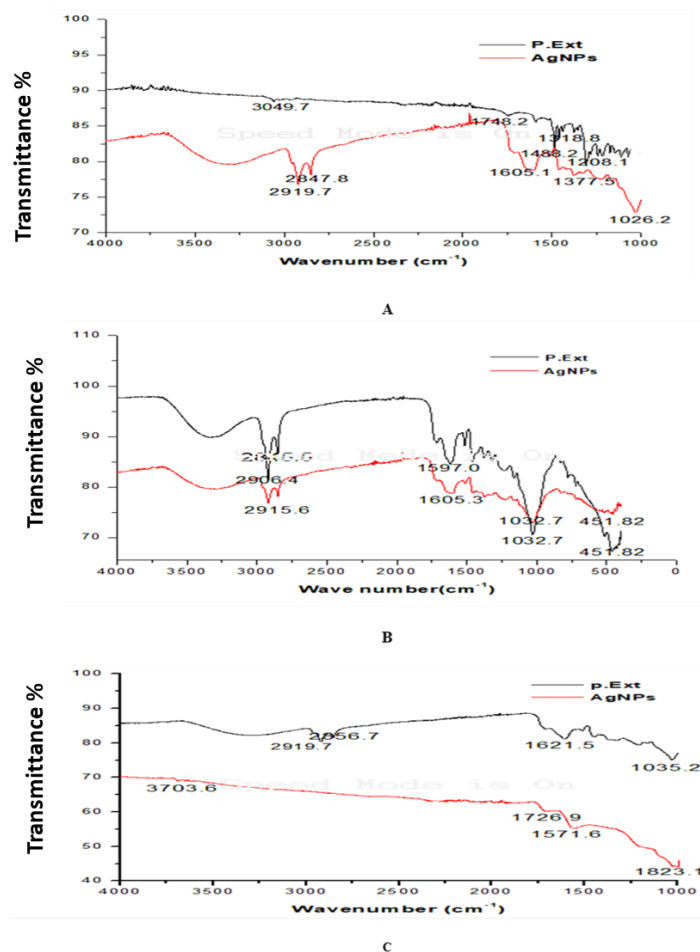
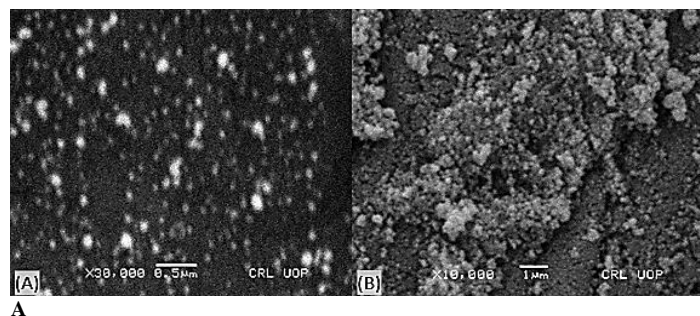


Figure 5 FT-IR spectra of AgNPs powder synthesized using methanol (A), ethanol (B), and aqueous (C) extract of *A. lachnantha* stem.

Scanning electron microscopy (SEM)

SEM analysis was conducted to investigate the morphological characteristics of AgNPs synthesized from methanolic, ethanolic, and aqueous extracts of *A. lachnantha* stem. SEM images revealed that most of the AgNPs were spherical, although some irregular particles were also observed. Aggregation of nanoparticles was detected, which may be attributed to the evaporation of solvent during sample preparation (Figs 6A-C). Similar results were reported by **Vijayakumar et al. (2013)** in their study on AgNP synthesis using *Boerhaavia diffusa* extract and **Thombre et al. (2012)**, who analyzed AgNPs synthesized using *Artocarpus heterophyllus* extract. The presence of rod-shaped and rectangular nanoparticles was also observed in other studies (**Ansari et al., 2018; Yarrappagaari et al., 2020**).



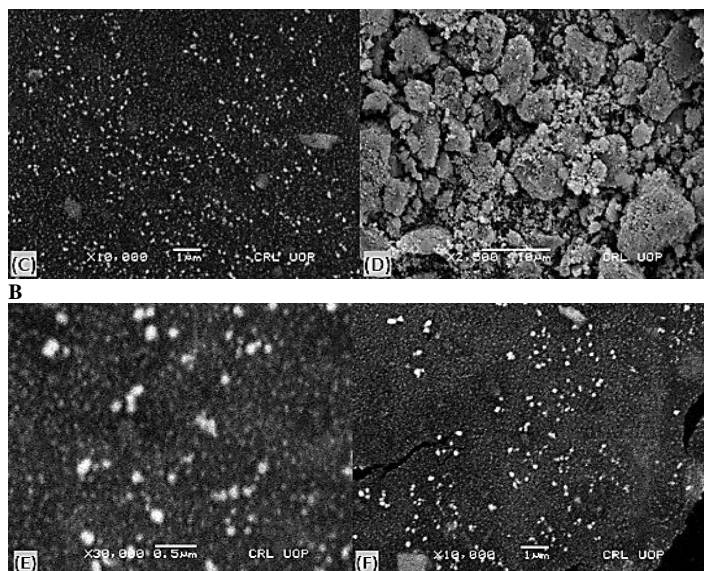


Figure 6 SEM analysis micrographs (A) methanol-AgNPs, (B) ethanol-AgNPs, and aqueous-AgNPs.

Energy dispersive X-ray spectroscopy (EDS)

The elemental composition of methanol, ethanol, and aqueous-based AgNPs was determined using EDS analysis. The presence of silver (Ag) was confirmed by a strong signal at 3 keV in all samples, with additional signals corresponding to elements such as K, C, Al, Si, O, S, Fe, Mg, Cl, and P (Figs 7A- C). These additional signals likely originate from stabilizing molecules in the plant extracts. **Mofolo et al. (2020)** reported similar findings, confirming the presence of Ag, C, N, and O in nanoparticles synthesized using *Pechuel-oeschea leubnitzia*. **Vijayakumar et al. (2013)** and **Ravichandran et al. (2016)** also used EDS to confirm the synthesis of AgNPs, which exhibited strong silver peaks along with other trace elements.

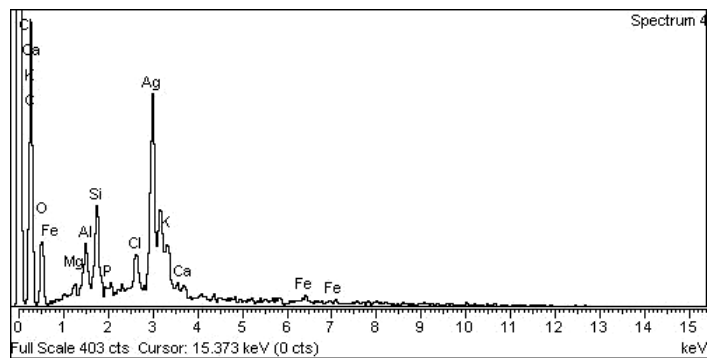
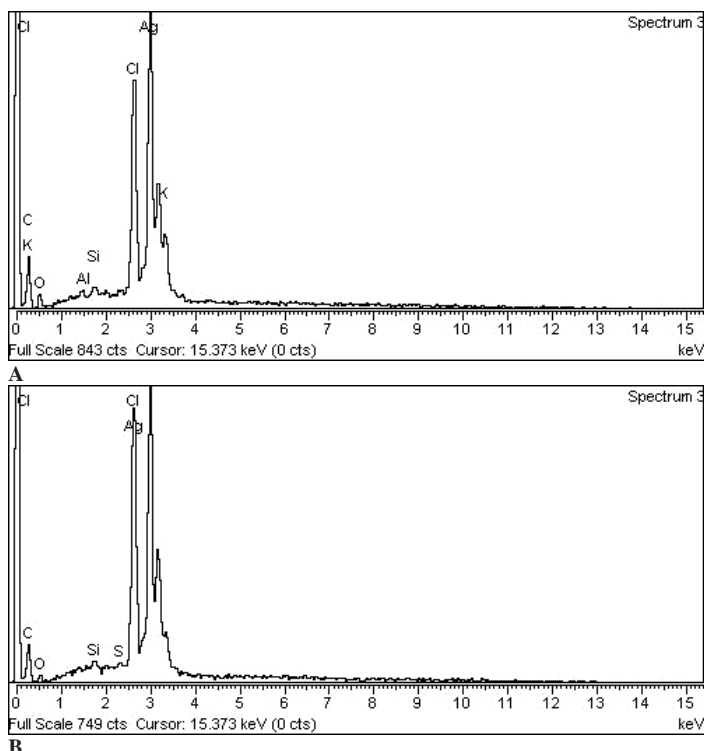


Figure 7 EDS analysis of methanol (A), ethanol (B), and aqueous-AgNPs (C).

Biological Activities of AgNPs

Insecticidal Activity

The insecticidal activity of AgNPs synthesized from methanolic, ethanolic, and aqueous extracts of *A. lachnantha* stem was tested against *Tribolium castaneum*. Methanol-based AgNPs showed mortality rates of 45% at 50 $\mu\text{g.mL}^{-1}$, 49% at 100 $\mu\text{g.mL}^{-1}$, and 60% at 200 $\mu\text{g.mL}^{-1}$. Ethanolic AgNPs exhibited mortality rates of 47%, 55%, and 47% at 50, 100, and 200 $\mu\text{g.mL}^{-1}$, respectively, while aqueous-based AgNPs showed 55%, 60%, and 65% mortality at 50, 100, and 200 $\mu\text{g.mL}^{-1}$, respectively (Fig 8). These results demonstrate that AgNPs are significantly more effective than crude extracts in terms of insecticidal potential. **Vadlapudi and Amanchy (2017)** and **Ahmad et al. (2022)** similarly reported the insecticidal potential of AgNPs, with mortality rates of up to 55.2% in *Tribolium castaneum*. AgNPs cause dehydration of insect integument and, with the help of abrasion and sorption, cause damage to the cuticle layer by destroying the protective wax present in the cuticle. It was also revealed that blockage of the trachea and spiracles of insects occurs due to the impairment of the alimentary canal on exposure to AgNPs, which leads to mortality, as documented by **Asad et al. (2022)**. The first mechanism suggests that the permeability of insect membranes decreases after exposure to AgNPs. This is due to the ability of the small nanoparticles to easily cross cellular barriers, allowing for interaction with genes related to membrane function. The second proposed mechanism indicates that AgNPs can exert genetic effects on insects by penetrating the exoskeleton and binding to sulfur or phosphorus atoms in proteins. This binding may interfere with translation and transcription processes, ultimately affecting gene expression (**Martinez-Cisterna et al., 2024**).

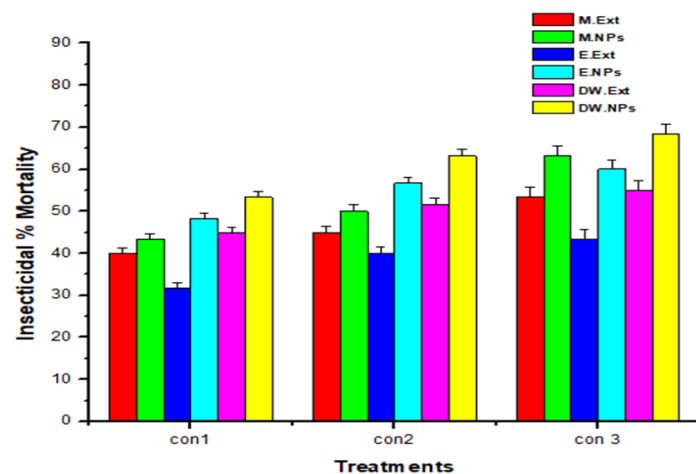


Figure 8 Insecticidal activity of methanolic, ethanolic, and aqueous-based AgNPs and plant extract of *A. lachnantha*. The values are the means of three replicates with standard deviation (\pm SD). Concentration1: 50 $\mu\text{g.mL}^{-1}$, Concentration 2: 100 $\mu\text{g.mL}^{-1}$, and Concentration 3: 200 $\mu\text{g.mL}^{-1}$.

Phytotoxic Assay

The phytotoxic activity of AgNPs synthesized from *A. lachnantha* stem extracts was evaluated using *Lemna aquinoctialis* at different concentrations (50, 100, and 200 $\mu\text{g.mL}^{-1}$). Atrazine (a common herbicide) demonstrated non-significantly above 90% of inhibition at 200 $\mu\text{g.mL}^{-1}$. Ethanolic AgNPs exhibited the highest inhibition rate at 78% at 200 $\mu\text{g.mL}^{-1}$, followed by methanolic AgNPs (70%) and aqueous AgNPs (67%) (Fig 9). These results are consistent with previous findings by **Sultana et al. (2019)**, who observed that AgNPs synthesized from *Malcolmia cabulica* exhibited up to 85% inhibition of *Lemna minor* growth. The phytotoxicity

of AgNPs could potentially be linked to their ability to interfere with photosynthesis and pigment aggregation in plant cells, causing photosynthetic disruption (Dewez et al., 2018). *Lemna aquiculinalis* was repressed by Ag nanoparticles and plant extract at each aim. This outcome is alluded to biological unsettling influence brought about by AgNPs to the Lemna plant. Because of the energizers released by AgNPs pigment aggregation, Lemna's photosynthetic disintegration was disturbed because of the increase in biomass. As a result, these energizers made by AgNPs could be combined with herbicides to eliminate pathogenic strains that damage crops and increase agricultural yields (Dewez et al., 2018; Sultana et al., 2019; Asad et al., 2022).

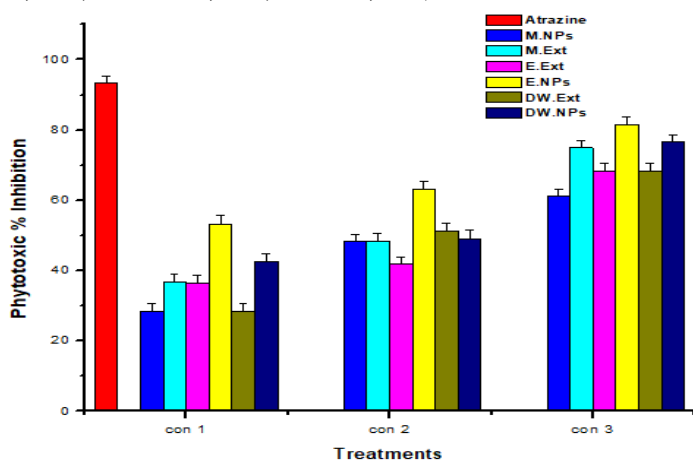


Figure 9 Phytotoxic assay of methanolic, ethanolic, and aqueous-based AgNPs and stem extracts of *A. lachnantha* as compared to Atrazine. The values are the means of three replicates with standard deviation (\pm SD). Con1: 50 $\mu\text{g.mL}^{-1}$, Con2: 100 $\mu\text{g.mL}^{-1}$, and Con3: 200 $\mu\text{g.mL}^{-1}$.

Antioxidant Assay

The antioxidant activity of AgNPs was evaluated using the DPPH method. AgNPs derived from methanolic, ethanolic, and aqueous extracts showed 70%, 72%, and 80% inhibition at 200 $\mu\text{g.mL}^{-1}$, respectively, in comparison to 88% for ascorbic acid (Fig 10). These results indicate significant antioxidant potential for AgNPs, corroborating findings by Asad et al. (2022), who reported strong free radical scavenging activity for AgNPs synthesized from *Amaryllis vittata*. Other studies, including those by Keshari et al. (2020) and Saad et al. (2021), have similarly demonstrated the potent antioxidant potential of bicapped AgNPs, which can be attributed to the presence of bioactive molecules on the nanoparticle surface. The antioxidant potential of AgNPs is revealed due to the presence of bioactive molecules (functional groups) on the surface of these nanoparticles, causing the reduction and capping of silver ions. These functional groups play an active role in neutralizing free radicals documented by Keshari et al. (2020).

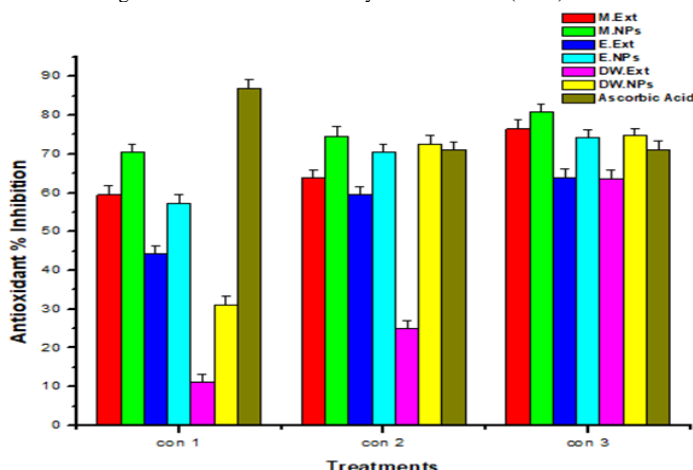


Figure 10 Antioxidant assay of methanolic, ethanolic, and aqueous-based AgNPs and stem extracts as compared to Ascorbic acid. The values are the means of three replicates with standard deviation (\pm SD). Con1: 50 $\mu\text{g.mL}^{-1}$, Con2: 100 $\mu\text{g.mL}^{-1}$, and Con3: 200 $\mu\text{g.mL}^{-1}$.

Antibacterial Assay

The antibacterial activity of synthesized AgNPs was tested against *Pseudomonas aeruginosa* using the disc diffusion method. Methanolic AgNPs exhibited a significant inhibition zone of 16 mm, while ethanolic and aqueous AgNPs showed

zones of 14 mm and 17 mm, respectively (Fig 11). These results were comparable to the antibiotic streptomycin, which had a zone of inhibition of 19 mm. Studies by Yuan et al. (2017) and Saxena et al. (2010) support these findings, highlighting the strong antibacterial potential of AgNPs, particularly against Gram-negative bacteria like *P. aeruginosa* and *E. coli*. This result is supported by the reason provided by Balamanikandan et al. (2015) and Yin et al. (2020), that silver nanoparticles can continuously release silver ions, which are effective in killing microbes. This process occurs due to the electrostatic attraction of silver ions to sulfur-containing proteins, enabling the ions to attach to the cell wall and cytoplasmic membrane of bacteria. Once attached, these ions can increase the permeability of the cytoplasmic membrane, leading to the disruption of the bacterial envelope. When free silver ions are taken up by the cells, they can deactivate respiratory enzymes. This deactivation generates reactive oxygen species (ROS) while interrupting the production of adenosine triphosphate (ATP). Reactive oxygen species play a key role in damaging the cell membrane and modifying deoxyribonucleic acid (DNA). Since sulfur and phosphorus are crucial components of DNA, the interaction between silver ions and these elements can interfere with DNA replication and cell reproduction, potentially leading to the death of the microorganisms. Additionally, silver ions can inhibit protein synthesis by denaturing ribosomes found in the cytoplasm. According to Park et al. (2014), damage to bacterial cells by the penetration of cell walls increases cell permeability, leading to cell death.

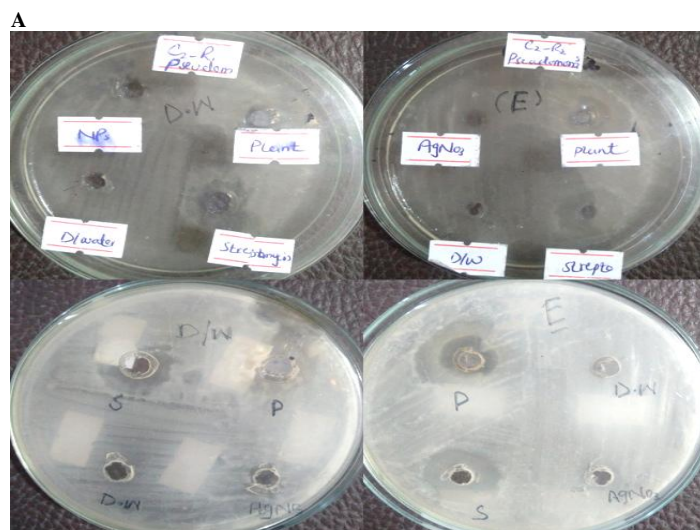
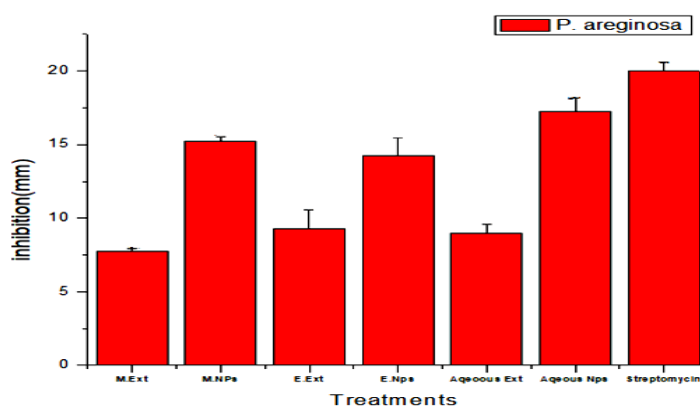
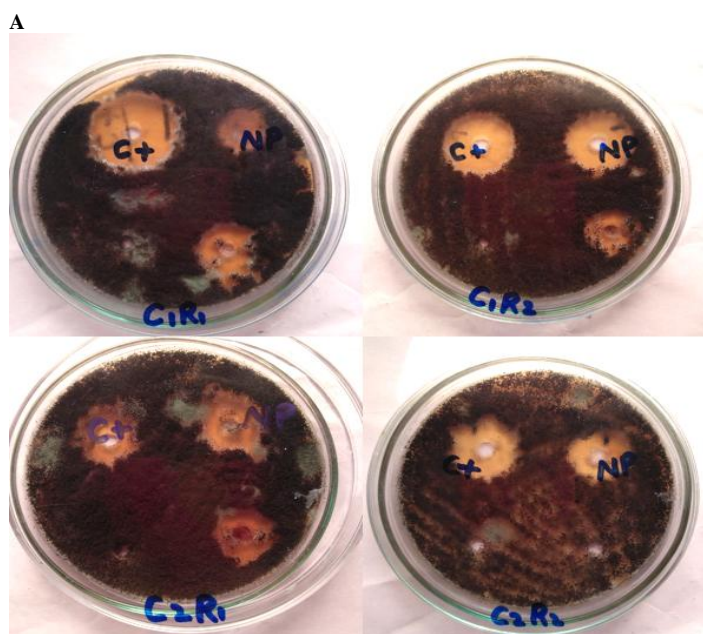
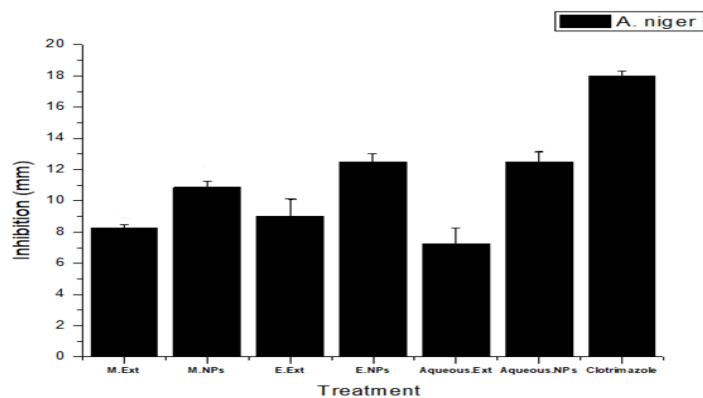


Figure 11 Antibacterial assay of methanolic, ethanolic, and aqueous-based AgNPs and stem extracts as compared to streptomycin.

Antifungal Assay

The antifungal activity of AgNPs was assessed against *Aspergillus niger* using the well diffusion method. Methanolic AgNPs exhibited a significant inhibition zone of 11 mm, while ethanolic and aqueous AgNPs showed zones of 13 mm and 12 mm, respectively (Fig 12). These results were comparable to clotrimazole, a standard antifungal drug with an inhibition zone of 18 mm. Balamanikandan et al. (2015) and Rouhani et al. (2013) similarly reported the antifungal potential of biosynthesized AgNPs against *Aspergillus* species, demonstrating that AgNPs have considerable efficacy as antifungal agents. This is due to the large surface area to volume ratio of these silver nanoparticles because as the surface area to volume increases, the interaction of nanoparticles to fungal cells increases, which causes damage to the structure of fungal cells by inactivating protein and condensing DNA molecules, leading to cell death (Balamanikandan et al., 2015). It was determined that silver nanoparticles, in general, disturb cell wall

permeability by attaching to the cell wall and affecting the respiration process of the cell. It was also revealed that these nanoparticles destroy cell structure by interacting with phosphorus and sulfur-containing compounds, i.e., protein and DNA (Asad et al., 2022). AgNPs significantly damaged the cellular components of the cell walls of pathogenic fungi. They degraded the structure and integrity of the cell membranes, causing both spores and mycelia to become wrinkled and depressed. This led to a reduction in oxygen consumption during mycelial respiration and inactivation of key enzymes that affect the respiratory chain. As a result, bound lipids and enzymes triggered cell lysis. Ultimately, the cells twisted and expanded until they withered and died. The electrical conductivity analysis revealed that AgNPs treatment increased cell membrane permeability and destroyed the integrity of the cell membranes of four pathogens. This resulted in excess water entering the cells, causing an increase in cell volume due to osmotic pressure, which ultimately led to the exudation of cellular contents (Li et al., 2022).



B Figure 12 Antifungal assay of methanolic, ethanolic, and aqueous-based AgNPs and stem extracts as compared to clotrimazole

CONCLUSION

This study successfully demonstrated the green synthesis of AgNPs using methanolic, ethanolic, and aqueous stem extracts of *A. lachnantha* Aellen and Rech. This environmentally sustainable approach offers an efficient method for fabricating AgNPs. The bio-fabricated AgNPs displayed strong phytotoxic and insecticidal activity, indicating their potential application in agricultural nanotechnology. The enhanced insecticidal properties of these nanomaterials against phytopathogens can significantly contribute to improved crop protection and food preservation. Furthermore, antioxidant assays demonstrated that the AgNPs synthesized from methanol, ethanol, and aqueous extracts exhibit remarkable free radical-scavenging activity, highlighting their potential as effective antioxidants. Additionally, antimicrobial assays confirmed that the synthesized AgNPs possess strong antibacterial and antifungal properties, making them effective against a wide range of pathogenic microbes. These findings suggest that *A. lachnantha* is a valuable resource for the rapid and eco-friendly synthesis of silver nanoparticles, which have considerable potential for use as antimicrobial agents. These findings encourage researchers to focus on using stem

extracts of *A. lachnantha* Aellen and Rech for synthesizing various nanoparticles, opening new applications.

Acknowledgments: Not applicable.

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