

Comparative Study on Synthesis, Optimization and Physicochemical Characterization of Silver Nanoparticles Using Leaf, Stem and Root Extracts of *Andrographis paniculata*

Sharika Farhana

Islamic University

Kamrun Nahar

Islamic University

Nashiru Billa

Qatar University

Md. Ashraful Alam

Bangladesh Council of Scientific and Industrial Research

Sharif Md. Al-Reza

`sharif@acce.iu.ac.bd`

Islamic University

Article

Keywords: *Andrographis paniculata*, Silver nanoparticles (AgNPs), Green synthesis, Organ-specific phytochemicals, Synthesis optimization, Physicochemical characterization

Posted Date: September 24th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10822573/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Green synthesis of silver nanoparticles (AgNPs) using different organs of the same plant offers a sustainable strategy for modulating nanoparticle properties while promoting whole-plant utilization. Here, AgNPs were synthesized separately using aqueous leaf, stem, and root extracts of *Andrographis paniculata*, and synthesis conditions were systematically optimized. Nanoparticle formation was confirmed by UV–Visible spectroscopy, showing characteristic surface plasmon resonance peaks at 428, 434, and 431 nm for leaf-, stem-, and root-derived AgNPs, respectively. Optimal synthesis conditions varied among plant organs, particularly for reaction time, temperature, extract concentration, extract-to-AgNO₃ ratio, and pH, while 0.01 M AgNO₃ was optimal for all three systems. FTIR analysis indicated the involvement of plant-derived functional groups in nanoparticle reduction and stabilization, whereas XRD and SAED confirmed the face-centred cubic crystalline structure of metallic silver. FE-TEM revealed predominantly spherical nanoparticles with mean sizes of approximately 7, 11, and 10 nm for leaf, stem, and root, respectively. DLS showed hydrodynamic diameters of 77–78 nm with PDI values below 0.30. These findings demonstrate that plant-organ selection significantly influences AgNPs synthesis conditions and physicochemical characteristics.

1. Introduction

Silver nanoparticles (AgNPs) remain among the most extensively investigated nanomaterials because of their unique optical, catalytic, antimicrobial and surface-dependent physicochemical properties, making them valuable for sensing, environmental, catalytic and biomedical applications. However, nanoparticle performance depends strongly on synthesis conditions, as the reaction environment directly influences nucleation, crystal growth, morphology, crystallinity and colloidal stability. Consequently, current research is focused not only on producing AgNPs but also on developing synthesis strategies that are reproducible, scalable, environmentally sustainable and capable of precisely controlling nanoparticle characteristics [1, 2].

Conventional physical methods (e.g., ball milling, laser ablation, flame pyrolysis, and inert-gas condensation) and chemical methods (e.g., sol–gel, polyol synthesis, microemulsion and chemical vapour deposition) often require expensive instrumentation, high energy input, multiple processing steps, and hazardous reducing agents such as sodium borohydride and hydrazine, together with organic solvents [3–6]. These approaches may generate toxic by-products and increase environmental and occupational risks. Consequently, green chemistry has shifted attention toward biological resources—including plants, fungi, bacteria and algae - as renewable, low-cost and environmentally benign alternatives for nanoparticle synthesis [7, 8].

Among biological approaches, plant-mediated synthesis has emerged as one of the most attractive methods because it is rapid, cost-effective, scalable and does not require sterile culture conditions or sophisticated equipment [3, 9, 10]. Numerous medicinal plants, including *Cinnamomum tamala*, *Clerodendrum viscosum* and *Citrus sinensis*, have been successfully used for the green synthesis of

silver nanoparticles, demonstrating that plant phytochemicals can effectively reduce Ag^+ ions while simultaneously stabilizing the resulting nanoparticles through natural capping mechanisms [11–13]. However, these studies investigated only a single plant organ. To date, no systematic comparison of leaf-, stem- and root-mediated AgNP synthesis from *Andrographis paniculata* under identical reaction conditions has been reported. Plant extracts contain diverse phytochemicals—including phenolics, flavonoids, terpenoids, alkaloids, saponins, steroids, proteins and carbohydrates—that simultaneously reduce Ag^+ ions and stabilize the resulting nanoparticles through functional groups such as $-\text{OH}$, $-\text{NH}$, $-\text{COOH}$ and $-\text{CHO}$ [14–16]. This dual reducing and capping function contributes to the excellent stability, reproducibility and biocompatibility of green-synthesized AgNPs compared with chemically synthesized counterparts [3, 17, 18].

Importantly, phytochemical composition varies not only among plant species but also among different organs of the same plant. Leaves, stems, roots, flowers, fruits and seeds differ considerably in the type and concentration of secondary metabolites because of their distinct physiological roles and developmental processes [8, 19, 21]. As a result, extracts from different plant organs can produce AgNPs with different synthesis efficiencies, reaction kinetics, particle sizes, morphologies, crystallinities and colloidal properties. Despite its importance, this organ-dependent variation remains relatively underexplored in green nanotechnology [8, 20].

The characteristics of biosynthesized AgNPs are also strongly influenced by reaction parameters, including pH, temperature, reaction time, plant extract concentration, metal precursor concentration and extract-to-precursor ratio [14, 21, 22]. These variables regulate nucleation and crystal growth, ultimately determining nanoparticle size distribution, stability and reproducibility. Therefore, systematic optimization of synthesis conditions is essential for obtaining AgNPs with consistent and application-specific properties [14, 16, 23]. *Andrographis paniculata* Wall. ex Nees (Acanthaceae), commonly known as the "King of Bitters," is a medicinal plant widely distributed throughout South and Southeast Asia [26–28]. The plant is rich in diterpenoid lactones, particularly andrographolide and its analogues, together with flavonoids, polyphenols, xanthenes and noriridoids which collectively contribute to its diverse pharmacological activities [27–30]. These phytochemicals exhibit diverse biological activities, including anti-inflammatory, antimicrobial, anticancer, antidiabetic, hepatoprotective and immunomodulatory effects, while the plant also possesses a favourable safety profile, making it an attractive candidate for green nanotechnology applications [25, 27, 28, 31].

Accordingly, this study presents a systematic comparative investigation of AgNP biosynthesis using aqueous leaf, stem, and root extracts of *A. paniculata*. The effects of reaction time, temperature, pH, extract concentration, AgNO_3 concentration and extract-to- AgNO_3 ratio were optimized separately for each plant organ, and the synthesized nanoparticles were characterized by UV–Visible spectroscopy, FTIR, XRD, FE-SEM, FE-TEM and DLS analyses. We hypothesized that (i) plant-organ selection significantly influences AgNPs synthesis efficiency and optimum reaction conditions, and (ii) organ-specific phytochemical variation results in measurable differences in nanoparticle physicochemical properties. To our knowledge, this is the first systematic comparison of aqueous leaf-, stem- and root-

mediated synthesis of AgNPs from *A. paniculata* under identical experimental conditions. The findings demonstrate that plant-organ selection is an effective strategy for tailoring the synthesis conditions and physicochemical properties of green-synthesized silver nanoparticles.

2. Materials and Methods

2.1. Chemicals and Reagents

Silver nitrate was purchased from Merck, Germany. Deionized water was used throughout the experiments for the preparation of plant extracts and silver nitrate solutions. Whatman No. 1 filter paper (Cytiva, UK) was used for filtration of the plant extracts. All chemicals and reagents used in this study were of analytical grade and were used as received without further purification.

2.2. Preparation of Plant Extracts

Fresh leaves, stems and roots of *Andrographis paniculata* were collected from Jahangirnagar University, Dhaka, Bangladesh (voucher specimen number: 45932). Plant materials were washed thoroughly with deionized water, air-dried and ground separately into fine powder. One gram of each powdered sample was extracted with 100 mL deionized water at 80°C for 5 min. After cooling to room temperature, the extracts were filtered through Whatman No. 1 filter paper and stored at 4°C until use.

2.3. Green Synthesis of Silver Nanoparticles

Silver nanoparticles (AgNPs) were synthesized separately using the aqueous leaf, stem and root extracts of *Andrographis paniculata*. Briefly, 80 mL of 0.01 M silver nitrate (AgNO_3) solution was mixed with 20 mL of each plant extract under continuous magnetic stirring. The synthesis reactions were carried out at the desired reaction temperatures (e.g., 20–80°C) according to the experimental design. The reduction of Ag^+ ions and the formation of AgNPs were initially confirmed by the gradual colour change of the reaction mixture from colourless to pale yellow and finally to dark brown, indicating the excitation of surface plasmon resonance (SPR) due to nanoparticle formation. Following synthesis, the AgNPs were recovered by centrifugation at 10,000 rpm for 15 min and washed several times with distilled water followed by acetone to remove unreacted biomolecules and other water-soluble impurities. The purified nanoparticles were freeze-dried (lyophilized) and stored in airtight containers under dry conditions for subsequent physicochemical characterization and biological studies. The biosynthesis procedure was adapted and modified from previously reported aqueous plant extract-mediated AgNPs synthesis methods [11, 13, 32].

2.4. Optimization of Synthesis Parameters

The synthesis conditions were optimized separately for the leaf, stem and root extracts by investigating the effects of reaction temperature (20–80°C), reaction time (5–270 min), plant extract concentration (20–80 g L⁻¹), AgNO_3 concentration (0.001–0.015 M), extract-to- AgNO_3 volume ratio (1:1–2:3) and reaction pH (2–11). The pH was adjusted using 0.1 N HCl or 0.1 N NaOH. Each parameter was varied

individually while keeping all other reaction conditions constant. Nanoparticle formation was monitored by visual colour change and UV–Visible spectroscopy and the optimum conditions were selected based on the maximum intensity and stability of the characteristic surface plasmon resonance (SPR) band.

2.5. Characterization of Silver Nanoparticles

The synthesized silver nanoparticles (AgNPs) obtained from the leaf, stem and root extracts of *Andrographis paniculata* were characterized to confirm nanoparticle formation and evaluate their optical properties, functional groups, crystallinity, morphology, elemental composition, particle size and colloidal stability.

The formation of AgNPs was initially confirmed by the characteristic colour change of the reaction mixture from pale yellow to dark brown and subsequently by UV–Visible spectroscopy using a UV–Visible spectrophotometer (U-2900, Shimadzu, Japan). Absorption spectra were recorded over the wavelength range of 300–600 nm to identify the characteristic surface plasmon resonance (SPR) bands of AgNPs.

Fourier-transform infrared (FTIR) spectra were recorded using an FTIR-8400S spectrophotometer (Shimadzu, Japan) over the range of 4000–600 cm^{-1} to identify the functional groups involved in the reduction and stabilization of AgNPs.

The crystalline structure and phase purity of the synthesized AgNPs were determined using an X-ray diffractometer (SmartLab SE, Rigaku, Japan). The diffraction patterns were indexed according to the standard face-centred cubic (fcc) crystal structure of metallic silver.

The morphology and surface characteristics of the synthesized AgNPs were examined using a FE-SEM (field-emission scanning electron microscope) (Sigma 300 VP, Carl Zeiss, Germany) operated at an accelerating voltage of 5 kV. Freeze-dried nanoparticle powders were mounted on conductive carbon tape, sputter-coated with a thin gold layer, and imaged at 30,000 $\times$ and 90,000 $\times$ magnifications to evaluate particle morphology and size distribution.

Particle morphology, size, and crystallinity were further investigated using field-emission transmission electron microscopy (JEM-200F, JEOL, Japan) operated at an accelerating voltage of 200 kV. A dilute nanoparticle suspension was deposited onto carbon-coated copper grids and air-dried before analysis. FE-TEM micrographs were acquired at a magnification of 100,000 $\times$ to determine nanoparticle morphology and particle size, while selected area electron diffraction (SAED) patterns were recorded to confirm the crystalline nature of the synthesized nanoparticles.

The hydrodynamic particle size, size distribution, and polydispersity index (PDI) of the colloidal AgNPs were measured using a Zeta sizer Ultra (Malvern Panalytical Ltd., UK) by dynamic light scattering (DLS), providing information on nanoparticle dispersion and colloidal stability.

2.6. Phytochemical Screening

Qualitative phytochemical screening was carried out separately on the aqueous leaf, stem, and root extracts of *Andrographis paniculata* to identify the major classes of bioactive secondary metabolites involved in the biosynthesis of silver nanoparticles. The extracts were analyzed for the presence of alkaloids (Dragendorff's test), terpenoids (Liebermann–Burchard test), phenolic compounds (lead acetate test), saponins (foam test), flavonoids (alkaline reagent test), tannins (ferric chloride test), steroids (Liebermann–Burchard test) and carbohydrates (Molisch's test) using standard qualitative phytochemical procedures described in the literature [31, 33, 34].

2.7. Use of AI-assisted tools

ChatGPT (OpenAI) was used for language editing and manuscript organization; all scientific content was reviewed and verified by the authors.

3. Results and Discussion

3.1. Visual Observation

The formation of AgNPs using aqueous leaf, stem, and root extracts of *A. paniculata* was initially indicated by a progressive colour change from pale yellow to dark brown following addition of AgNO₃ (Supplementary Fig. S1). The colour intensity increased with reaction time and remained unchanged after 24 h, indicating completion of the reduction process. The characteristic brown colour is attributed to localized surface plasmon resonance of metallic AgNPs. Differences in colour intensity among the three extracts may reflect organ-dependent variations in phytochemical composition and reduction efficiency.

3.2. Phytochemical Screening of *Andrographis paniculata* Extracts

Qualitative phytochemical screening detected terpenoids, saponins, flavonoids, alkaloids, steroids, and carbohydrates in leaf, stem, and root extracts, whereas tannins were absent (Supplementary Table S1). These metabolites can participate in Ag⁺ reduction and nanoparticle stabilization. Although the three organs showed similar phytochemical classes, differences in metabolite abundance may contribute to the observed organ-dependent variations in AgNP synthesis and physicochemical properties.

3.3. Comparative Optimization of Green Synthesis Parameters

AgNPs synthesis using aqueous leaf, stem, and root extracts of *Andrographis paniculata* was optimized by varying reaction time, temperature, extract concentration, AgNO₃ concentration, extract-to-AgNO₃ ratio, and pH (Fig. 1). Differences in optimum conditions among the three organs indicate that organ-specific phytochemical composition influences AgNPs formation, consistent with previous plant-mediated synthesis studies [11–13].

3.3.1. Effect of Reaction Time

SPR absorbance increased progressively with reaction time (Fig. 1A), with characteristic bands at approximately 429, 420, and 432 nm for leaf-, stem-, and root-mediated AgNPs, respectively. Maximum SPR intensity occurred at 40 min for leaf and stem and 50 min for root, indicating slower reduction kinetics for the root extract, likely reflecting differences in phytochemical composition [11–13].

3.3.2. Effect of Reaction Temperature

Temperature markedly influenced AgNP formation (Fig. 1B). Maximum SPR absorbance was observed at 70°C for leaf, 60°C for stem, and 80°C for root. Lower temperatures resulted in reduced nanoparticle formation, whereas temperatures beyond the optimum decreased SPR intensity, potentially because of particle aggregation or degradation of thermolabile reducing and stabilizing phytochemicals [2, 18, 20].

3.3.3. Effect of Plant Extract Concentration

Increasing extract concentration enhanced SPR absorbance up to an optimum, consistent with greater availability of phytochemicals involved in Ag⁺ reduction and nanoparticle stabilization [11–13, 33, 35, 36]. Maximum absorbance occurred at 50 g/L for leaf and 60 g/L for stem and root extracts (Fig. 1C).

3.3.4. Effect of Silver Nitrate Concentration

SPR absorbance increased with AgNO₃ concentration up to 0.01 M for all three extracts (Fig. 1D). At 0.015 M, absorbance declined slightly, possibly because the available phytochemicals were insufficient to stabilize the increased number of nanoparticles, promoting aggregation [37, 38]. Thus, 0.01 M AgNO₃ was optimal for all three plant organs.

3.3.5. Effect of Extract-to-Silver Nitrate Ratio

The extract-to-AgNO₃ ratio significantly affected nanoparticle formation (Fig. 1E). Maximum SPR absorbance was obtained at 1:1 for leaf and stem extracts and 1:2 for root extract, indicating organ-dependent differences in the balance between reducing phytochemicals and available Ag⁺ ions.

3.3.6. Effect of pH

AgNPs formation increased under alkaline conditions (Fig. 1F), whereas comparatively low SPR absorbance was observed at acidic pH, likely because of reduced ionization and reducing activity of plant phytochemicals [11–13, 39, 40]. Maximum absorbance occurred at pH 9 for leaf and stem and pH 11 for root, demonstrating an organ-dependent response to reaction pH.

3.4. UV–Visible Absorption Spectroscopy

UV–Visible spectroscopy was employed to confirm the formation of AgNPs synthesized using the aqueous leaf, stem and root extracts of *Andrographis paniculata*. The biosynthesized AgNPs exhibited a characteristic reddish-brown colour due to the excitation of localized surface plasmon resonance (SPR).

Typically, SPR bands appearing within the wavelength range of 400–450 nm is considered indicative of the reduction of Ag⁺ ions to metallic Ag⁰ nanoparticles [41]. As shown in Fig. 2, the UV–Visible spectra of the synthesized AgNPs displayed distinct absorption peaks at 428 nm, 434 nm and 431 nm for the leaf-, stem- and root-mediated AgNPs, respectively, confirming the successful biosynthesis of silver nanoparticles. Among the three extracts, the leaf-derived AgNPs exhibited the highest absorbance, followed by the stem and root extracts, suggesting a higher nanoparticle yield that may be attributed to the greater reducing and stabilizing capacity of the phytochemicals present in the leaf extract. The slight variation in SPR peak position among the three samples indicates differences in particle size and surface characteristics arising from the distinct phytochemical compositions of the leaf, stem and root extracts. The SPR bands observed in this study are consistent with previous reports of plant-mediated AgNP synthesis using *C. tamala*, *C. viscosum*, and *C. sinensis*, confirming successful nanoparticle formation [11–13].

3.5. Fourier Transform Infrared (FTIR) Analysis

Fourier Transform Infrared (FTIR) spectroscopy was employed to identify the functional groups involved in the reduction and stabilization of AgNPs synthesized using the leaf, stem, and root extracts of *Andrographis paniculata* [41]. The FTIR spectra of the synthesized AgNPs exhibited characteristic absorption bands corresponding to hydroxyl/amine, aliphatic, carbonyl/aromatic, amine and carbohydrate functional groups (Fig. 3). The major absorption bands and their assignments are summarized in Supplementary Table S2.

The FTIR spectra of the leaf-, stem-, and root-derived AgNPs exhibited similar functional group patterns, indicating that comparable classes of phytochemicals participated in nanoparticle biosynthesis. These observations are consistent with the qualitative phytochemical screening (Supplementary Table S1), which confirmed the presence of terpenoids, flavonoids, alkaloids, saponins, steroids and carbohydrates in all three plant extracts. The identified functional groups suggest that these phytochemicals acted as reducing agents by facilitating the conversion of Ag⁺ ions into metallic Ag⁰ nanoparticles and subsequently adsorbed onto the nanoparticle surface as natural capping agents, thereby improving colloidal stability and preventing particle aggregation [43, 44].

Slight variations in band intensity among the three samples indicate differences in the relative abundance of phytochemicals in the leaf, stem and root extracts, which may contribute to the observed variations in nanoparticle formation efficiency and physicochemical properties. Similar FTIR peak shifts corresponding to O–H, C = O, C–H and other functional groups have previously been reported, indicating that polyphenols, flavonoids and other phytochemicals act as both reducing and stabilizing agents during AgNPs [11–13, 42]. Overall, the FTIR results confirm the active involvement of plant-derived biomolecules in the green synthesis and stabilization of AgNPs.

3.6. X-ray diffraction (XRD) analysis

X-ray diffraction (XRD) analysis was performed to determine the phase composition, crystallinity and purity of the silver nanoparticles (AgNPs) synthesized using the leaf, stem, and root extracts of *Andrographis paniculata*. As illustrated in Fig. 4, all three AgNPs samples exhibited well-defined diffraction peaks at approximately 38.34°, 44.57°, 64.98°, 78.11°, and 82.32°, corresponding to the (111), (200), (220), (311) and (222) crystallographic planes, respectively. These characteristic diffraction peaks are in good agreement with the standard face-centred cubic (fcc) crystal structure of metallic silver (JCPDS Card No. 04-0783), confirming the successful biosynthesis of crystalline AgNPs [45].

The diffraction profiles of the leaf-, stem- and root-mediated AgNPs displayed the characteristic reflections of face-centred cubic metallic silver. Although the relative intensities of the diffraction peaks varied among the three samples, their positions remained essentially unchanged, indicating that all three plant organs produced AgNPs with the same crystalline phase. The observed differences in relative peak intensity may be associated with variations in crystallite orientation, nucleation and crystal growth caused by differences in the phytochemical composition of the leaf, stem and root extracts. Similar fcc diffraction patterns have been reported for plant-mediated AgNPs synthesized using *Cinnamomum tamala*, *Clerodendrum viscosum* and *Citrus sinensis* extracts [11–13].

The average crystallite size of the silver nanoparticles was calculated from the XRD peaks, using the Debye-Scherrer formula [43]

$$D = 0.94\lambda / \beta \cos\theta$$

where D is the average crystallite size, λ is the X-ray wavelength, β is the full width at half maximum (FWHM) of the diffraction peak, and θ is the Bragg diffraction angle.

The average crystallite size of the biosynthesized AgNPs was calculated to be approximately 35 nm for leaf, 37 nm for stem and 31 nm for root confirming the nanocrystalline nature of the particles. This result is in good agreement with the particle size obtained from DLS and microscopic analyses, further supporting the successful green synthesis of crystalline silver nanoparticles.

Overall, the XRD results demonstrate that leaf, stem, and root extracts of *A. paniculata* effectively facilitated the formation of highly crystalline, phase-pure AgNPs with a face-centered cubic structure, highlighting the suitability of these plant extracts as eco-friendly reducing and stabilizing agents for nanoparticle synthesis.

3.7. Field Emission Scanning Electron Microscopy (FE-SEM) Analysis

Field Emission Scanning Electron Microscopy (FE-SEM) was employed to investigate the surface morphology, particle size and distribution [47] of silver nanoparticles (AgNPs) synthesized using the leaf, stem, and root extracts of *Andrographis paniculata*. Representative low- and high-magnification FESEM micrographs are presented in Fig. 5. The low-magnification images (A1–C1) revealed a dense

distribution of nanoparticles with slight agglomeration, whereas the corresponding high-magnification images (A2–C2) clearly illustrated the morphology and particle dimensions of the synthesized AgNPs.

The FESEM analysis confirmed that AgNPs synthesized from all three plant extracts were predominantly spherical with a relatively uniform distribution. The average particle sizes were approximately 19 nm (12–35 nm) for the leaf 27 nm (20–42 nm) for the stem and 23 nm (17–38 nm) for the root-derived AgNPs. These findings demonstrate that all synthesized nanoparticles were well within the nanoscale range (< 100 nm). Among the three plant extracts, the leaf extract produced the smallest average particle size, whereas the stem extract generated relatively larger nanoparticles, with the root extract yielding particles of intermediate size.

Although slight agglomeration was observed in all samples, the nanoparticles remained well dispersed over the surface. Such aggregation is commonly observed in biosynthesized AgNPs because of their high surface energy and the drying process during FE-SEM sample preparation. Nevertheless, the individual nanoparticles retained their spherical morphology, indicating that the phytochemicals present in *Andrographis paniculata* effectively regulated nanoparticle nucleation and growth while simultaneously acting as natural reducing and capping agents. This interpretation is consistent with the phytochemical screening and FTIR analyses, which confirmed the presence of flavonoids, terpenoids, alkaloids, saponins, steroids, and carbohydrates responsible for the reduction of Ag⁺ ions and stabilization of the synthesized nanoparticles.

The differences in average particle size among the leaf-, stem- and root-mediated AgNPs can be attributed to variations in the composition and concentration of bioactive metabolites present in different plant tissues, which influence the nucleation rate and crystal growth during nanoparticle synthesis. The comparatively smaller average particle size of the leaf-derived AgNPs may have contributed to the stronger surface plasmon resonance observed in the UV–Visible spectra, although the absorbance intensity is also influenced by nanoparticle concentration and dispersion. Furthermore, the FE-SEM observations corroborate the XRD results, confirming the crystalline nature of the synthesized AgNPs. The particle sizes measured by FE-SEM were considerably smaller than the hydrodynamic diameters obtained from DLS analysis because DLS measures nanoparticles together with the surrounding hydration layer and adsorbed phytochemical corona rather than the metallic core alone.

Overall, the FE-SEM analysis confirms the successful green synthesis of predominantly spherical AgNPs with nanoscale dimensions and relatively narrow particle-size distributions, demonstrating that the leaf, stem and root extracts of *Andrographis paniculata* are effective reducing and stabilizing agents for the production of well-defined silver nanoparticles. These findings are consistent with previous studies reporting that plant-mediated AgNPs generally exhibit predominantly spherical morphology with particle sizes ranging from approximately 10 to 60 nm, depending on the phytochemical composition of the plant extract and synthesis conditions [9, 11–13, 24].

3.8. Field Emission Transmission Electron Microscopy (FE-TEM) Analysis

Field Emission Transmission Electron Microscopy (FE-TEM) was employed to investigate the morphology, particle size, and crystallinity of silver nanoparticles (AgNPs) synthesized using the leaf, stem, and root extracts of *Andrographis paniculata*. Owing to its high spatial resolution, FE-TEM provides direct visualization of nanoparticle morphology and enables accurate estimation of particle size while confirming the structural characteristics of biosynthesized nanomaterials [48]. Representative TEM micrographs and Selected Area Electron Diffraction (SAED) patterns are presented in Fig. 6.

The TEM micrographs (A1–C3) revealed that AgNPs synthesized from all three plant extracts were predominantly spherical and well dispersed, although slight aggregation was observed in some regions because of particle–particle interactions during sample preparation. The nanoparticles exhibited nanoscale dimensions with relatively narrow size distributions. The average particle sizes were approximately 7 nm (5–15 nm) for the leaf-, 11 nm (6–19 nm) for the stem- and 10 nm (7–16 nm) for the root-derived AgNPs. Among the three samples, the leaf extract produced the smallest nanoparticles, whereas the stem-derived AgNPs exhibited slightly larger particle sizes, with the root-derived AgNPs showing intermediate dimensions. These variations are likely associated with differences in the concentration and composition of bioactive phytochemicals present in each plant part, which influence the nucleation and growth processes during nanoparticle biosynthesis.

The Selected Area Electron Diffraction (SAED) patterns (A4, B4, and C4) displayed a series of well-defined concentric diffraction rings indexed to the (111), (200), (220), (311) and (222) crystallographic planes of metallic silver. These diffraction rings are characteristic of the face-centred cubic (fcc) crystal structure of elemental silver and are consistent with previously reported SAED patterns of biosynthesized AgNPs [45, 46]. The presence of distinct diffraction rings confirms the highly crystalline nature of the synthesized nanoparticles.

The SAED results are in excellent agreement with the XRD analysis, where identical diffraction planes corresponding to the fcc structure of metallic silver were observed. Furthermore, the particle sizes determined by FE-TEM were smaller than those obtained from FE-SEM and DLS analyses. This difference is expected because TEM measures the actual metallic nanoparticle core, whereas FE-SEM measures surface morphology and DLS determines the hydrodynamic diameter, which includes the surrounding layer of adsorbed phytochemicals and solvent molecules. The agreement among FE-TEM, XRD, FE-SEM and DLS analyses confirms the successful green synthesis of crystalline, spherical, and stable AgNPs using the leaf, stem, and root extracts of *A. paniculata*.

Overall, the FE-TEM observations demonstrate that all three plant extracts effectively produced uniformly distributed, highly crystalline AgNPs. The comparatively smaller particle size observed for the leaf-derived nanoparticles is consistent with the stronger UV–Visible absorption and suggests more efficient reduction of Ag^+ ions, further supporting the role of plant-derived phytochemicals as reducing and stabilizing agents during nanoparticle formation.

3.9. Dynamic Light Scattering (DLS) analysis

Dynamic Light Scattering (DLS) analysis was performed to determine the hydrodynamic particle size and polydispersity index (PDI) of silver nanoparticles (AgNPs) synthesized using the leaf, stem, and root extracts of *Andrographis paniculata*. DLS measures the hydrodynamic diameter of nanoparticles dispersed in a liquid medium, which includes the metallic core together with the surrounding hydration layer and adsorbed biomolecules [49, 51]. The hydrodynamic size distribution and PDI values of the synthesized AgNPs are presented in Fig. 7.

The DLS results demonstrated that all three AgNP suspensions exhibited a narrow particle size distribution with average hydrodynamic diameters of 77 nm for the leaf-, 78 nm for the stem- and 76 nm for the root-derived AgNPs. The close agreement among these values indicates that the three plant extracts produced nanoparticles with comparable colloidal characteristics despite differences in their phytochemical compositions. The slightly larger hydrodynamic diameters obtained by DLS compared with those measured by FE-TEM and FE-SEM are expected because DLS measures the hydrated particle together with the surface-bound phytochemicals and solvent molecules rather than the metallic nanoparticle core alone [48].

The corresponding PDI values were 0.212 for leaf AgNPs, 0.235 for stem AgNPs, and 0.267 for root AgNPs. All PDI values were below 0.30, indicating a relatively narrow particle size distribution and good dispersion stability. In general, lower PDI values represent greater particle-size uniformity, whereas higher values indicate broader size distributions and a greater tendency toward aggregation. Nanoparticles with PDI values below 0.50 are generally considered suitable for biomedical and drug-delivery applications because they exhibit acceptable colloidal stability and limited aggregation [50]. Among the synthesized AgNPs, the leaf-derived nanoparticles exhibited the lowest PDI, suggesting the highest degree of monodispersity and colloidal stability, while the root-derived AgNPs showed a slightly broader size distribution but remained within the acceptable range.

The DLS findings are consistent with the FE-SEM and FE-TEM observations, which revealed predominantly spherical nanoparticles with relatively uniform size distributions. The slightly larger hydrodynamic sizes observed by DLS further confirm the presence of phytochemical molecules adsorbed on the nanoparticle surface, as demonstrated by the FTIR analysis. These biomolecules function as natural capping agents, improving colloidal stability by preventing excessive particle aggregation. Overall, the DLS results demonstrate that the leaf-, stem- and root-mediated AgNPs possess comparable nanoscale hydrodynamic diameters, low polydispersity, and good colloidal stability, confirming the effectiveness of *A. paniculata* extracts as reducing and stabilizing agents for the green synthesis of silver nanoparticles.

3.10. Comparative Analysis of Organ-Dependent AgNP Synthesis

This comparative investigation (Table 1) demonstrates that the biosynthesis of AgNPs is governed not only by plant species but also by organ-specific phytochemical composition. Although leaf, stem, and

root extracts contained similar classes of phytochemicals, their different physiological functions within the plant are reflected in variations in metabolite abundance, which ultimately influence reduction efficiency, nucleation rate, crystal growth, and nanoparticle stabilization. Consequently, each plant organ required distinct optimum synthesis conditions and produced nanoparticles with different physicochemical characteristics. These findings confirm that plant-organ selection is an important experimental variable that should be considered during the design and optimization of green nanoparticle synthesis.

Overall, the leaf extract exhibited the most favourable characteristics for AgNPs production by generating nanoparticles with the highest SPR intensity, the smallest particle size, and the lowest PDI, indicating efficient reduction and excellent colloidal stability. In contrast, stem- and root-derived nanoparticles required different reaction conditions and displayed comparatively larger particle sizes while maintaining similar crystallinity, morphology, and structural integrity. The close agreement among UV–Visible spectroscopy, FTIR, XRD, FE-SEM, FE-TEM, SAED and DLS analyses provides strong evidence that all three plant organs successfully produced stable, crystalline AgNPs while demonstrating that organ-dependent phytochemical variation can be exploited as a simple, sustainable, and reproducible strategy for tailoring nanoparticle properties for future biomedical, catalytic, sensing, and environmental applications.

Table 1

Comparative summary of optimization conditions and physicochemical characteristics of silver nanoparticles synthesized using *Andrographis paniculata* leaf, stem, and root extracts.

Parameter	Leaf AgNPs	Stem AgNPs	Root AgNPs
Major phytochemicals	Terpenoids, flavonoids, alkaloids, saponins, steroids, carbohydrates	Same	Same
Optimum reaction time	40 min	40 min	50 min
Optimum temperature	70°C	60°C	80°C
Optimum extract concentration	50 g/L	60 g/L	60 g/L
Optimum AgNO ₃ concentration	0.01 M	0.01 M	0.01 M
Optimum extract:AgNO ₃ ratio	1:1	1:1	1:2
Optimum pH	9	9	11
SPR peak (UV–Vis)	428 nm	434 nm	431 nm
FTIR functional groups	O–H/N–H, C–H, C = O/C = C, C–N/C–O, C–O	Comparable	Comparable
Crystal structure (XRD)	Face-centred cubic (fcc) Ag	Same	Same
Major XRD planes	(111), (200), (220), (311), (222)	Same	Same
Average crystallite size (XRD)	35 nm	37 nm	31 nm
Average FE-SEM particle size	~ 19 nm	~ 27 nm	~ 23 nm
Average FE-TEM particle size	~ 7 nm	~ 11 nm	~ 10 nm
Hydrodynamic diameter (DLS)	77 nm	78 nm	76 nm
Polydispersity index (PDI)	0.212	0.235	0.267
Morphology (FE-SEM/FE-TEM)	Predominantly spherical	Predominantly spherical	Predominantly spherical
Colloidal stability	Excellent	Good	Good

Parameter	Leaf AgNPs	Stem AgNPs	Root AgNPs
Overall synthesis performance	Highest	Moderate	Moderate
Distinctive characteristic	Smallest particles, lowest PDI and highest SPR intensity	Largest particle size	Highest optimum pH and temperature

4. Conclusion

This study provides a systematic comparative evaluation of silver nanoparticles (AgNPs) biosynthesis using aqueous leaf, stem, and root extracts of *Andrographis paniculata* under identical experimental conditions. Although all three plant organs successfully mediated the formation of crystalline and predominantly spherical AgNPs, clear organ-dependent differences were observed in synthesis kinetics, optimum reaction conditions, particle dimensions, and size-distribution characteristics. These findings demonstrate that plant-organ selection represents a critical experimental variable in nanoparticle synthesis and can substantially influence the resulting physicochemical properties.

Among the investigated plant organs, the leaf extract exhibited the most favourable overall synthesis performance, producing AgNPs with the highest surface plasmon resonance intensity, the smallest particle dimensions, and the lowest polydispersity index. In contrast, stem- and root-mediated synthesis required distinct reaction conditions and generated comparatively larger nanoparticles while retaining the characteristic face-centred cubic structure of metallic silver. The complementary results obtained from UV–Visible spectroscopy, FTIR, XRD, FE-SEM, FE-TEM/SAED, and DLS collectively demonstrate that organ-specific phytochemical environments influence Ag⁺ reduction, nanoparticle nucleation and growth, and the resulting physicochemical characteristics.

Overall, this work establishes plant-organ selection as a simple, sustainable, and experimentally accessible strategy for tuning the properties of green-synthesized AgNPs without introducing additional chemical reducing or capping agents. By linking organ-dependent phytochemical variation with synthesis optimization and nanoparticle characteristics, this study provides a useful framework for the rational development of plant-mediated nanomaterials and supports future application-specific investigations of AgNPs in biomedical, antimicrobial, catalytic, sensing, and environmental systems.

Declarations

Competing Interests

The authors declare no competing interests.

Funding

This research received no external funding.

Author Contribution

Sharika Farhana: Conceptualization, methodology, investigation, data curation, formal analysis, validation, visualization, project execution, writing-original draft, and writing-review & editing. Conducted the experimental work, analysed and interpreted the data, and prepared all figures and tables. Sharif Md. Al-Reza: Conceptualization, supervision, project administration, scientific guidance, validation, and writing—review & editing. Kamrun Nahar: Methodology, scientific consultation, technical guidance, validation, and writing-review & editing. Nashiru Billa: Conceptualization, scientific consultation, validation, interpretation of nanoparticle characterization data, and writing-review & editing. Md. Ashraful Alam: XRD analysis, technical support, data validation, and writing-review & editing.

Acknowledgement

The authors gratefully acknowledge the Chemical Research Division and the Institute of Glass and Ceramic Research and Testing Division, Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh, for providing laboratory facilities and instrumental support. The authors also thank the Department of Applied Chemistry and Chemical Engineering, Islamic University, Kushtia, Bangladesh, for its academic support throughout this research. The authors sincerely acknowledge Jashore University of Science and Technology, Jashore, Bangladesh, for providing the field-emission scanning electron microscopy (FE-SEM) analysis facilities, and Kyung Hee University, Seoul, Republic of Korea, for performing the field-emission transmission electron microscopy (FE-TEM) analysis.

Data Availability

All data supporting the findings of this study are available within the paper and its Supplementary Information.

References

1. Nathanael, K., Cheng, S., Kovalchuk, N. M., Arcucci, R. & Simmons, M. J. H. Optimization of microfluidic synthesis of silver nanoparticles: A generic approach using machine learning. *Chem. Eng. Res. Des.* **193**, 65–74. <https://doi.org/10.1016/j.cherd.2023.03.007> (2023).
2. Okka, E. Z. et al. Green synthesis and the formation kinetics of silver nanoparticles in aqueous *Inula viscosa* extract. *Optik* **294**, 171487. <https://doi.org/10.1016/j.ijleo.2023.171487> (2023).
3. Arshad, F. et al. Bioinspired and green synthesis of silver nanoparticles for medical applications: A green perspective. *Appl. Biochem. Biotechnol.* **196**, 3636–3669. <https://doi.org/10.1007/s12010-023-04719-z> (2024).

4. Darr, J. A., Zhang, J., Makwana, N. M. & Weng, X. Continuous hydrothermal synthesis of inorganic nanoparticles: Applications and future directions. *Chem. Rev.* **117**, 11125–11238. <https://doi.org/10.1021/acs.chemrev.6b00417> (2017).
5. Ijaz, I., Gilani, E., Nazir, A. & Bukhari, A. Detail review on chemical, physical and green synthesis, classification, characterizations and applications of nanoparticles. *Green. Chem. Lett. Rev.* **13**, 223–245. <https://doi.org/10.1080/17518253.2020.1802517> (2020).
6. Salgado, P., Mártire, D. O. & Vidal, G. Eucalyptus extracts-mediated synthesis of metallic and metal oxide nanoparticles: Current status and perspectives. *Mater. Res. Express.* **6**, 082006. <https://doi.org/10.1088/2053-1591/ab254c> (2019).
7. Kaabipour, S. & Hemmati, S. A review on the green and sustainable synthesis of silver nanoparticles and one-dimensional silver nanostructures. *Beilstein J. Nanotechnol.* **12**, 102–136. <https://doi.org/10.3762/bjnano.12.9> (2021).
8. Osman, A. I. et al. Synthesis of green nanoparticles for energy, biomedical, environmental, agricultural, and food applications: A review. *Environ. Chem. Lett.* **22**, 841–887. <https://doi.org/10.1007/s10311-023-01682-3> (2024).
9. Jafarzadeh, S. et al. Green synthesis of nanomaterials for smart biopolymer packaging: Challenges and outlooks. *J. Nanostruct. Chem.* **14**, 113–136. <https://doi.org/10.1007/s40097-023-00527-3> (2024).
10. Salem, S. S. A mini review on green nanotechnology and its development in biological effects. *Arch. Microbiol.* **205**, 128. <https://doi.org/10.1007/s00203-023-03467-2> (2023).
11. Nahar, K., Aziz, S., Bashar, M. S., Haque, M. A. & Al-Reza, S. M. Synthesis and characterization of silver nanoparticles from *Cinnamomum tamala* leaf extract and its antibacterial potential. *Int. J. Nano Dimens.* **11**, 88–98 (2020).
12. Nahar, K., Yang, D. C., Rupa, E. J., Khatun, M. & Al-Reza, S. M. Eco-friendly synthesis of silver nanoparticles from *Clerodendrum viscosum* leaf extract and its antibacterial potential. *Nanomed. Res. J.* **5**, 276–287. <https://doi.org/10.22034/nmrj.2020.03.008> (2020).
13. Nahar, K., Rahaman, M. H., Khan, G. M. A., Islam, M. K. & Al-Reza, S. M. Green synthesis of silver nanoparticles from *Citrus sinensis* peel extract and its antibacterial potential. *Asian J. Green. Chem.* **5**, 135–150 (2021).
14. Thomas, S. et al. Plant-based synthesis, characterization approaches, applications and toxicity of silver nanoparticles: A comprehensive review. *J. Biotechnol.* **394**, 135–149. <https://doi.org/10.1016/j.jbiotec.2024.08.009> (2024).
15. Zuhrotun, A., Oktaviani, D. J. & Hasanah, A. N. Biosynthesis of gold and silver nanoparticles using phytochemical compounds. *Molecules* **28**, 3240. <https://doi.org/10.3390/molecules28073240> (2023).
16. Dhir, R. et al. Plant-mediated synthesis of silver nanoparticles: Unlocking their pharmacological potential—a comprehensive review. *Front. Bioeng. Biotechnol.* **11**, 1324805. <https://doi.org/10.3389/fbioe.2023.1324805> (2024).

17. Dubey, R. K., Shukla, S. & Hussain, Z. Green synthesis of silver nanoparticles: A sustainable approach with diverse applications. *Chin. J. Appl. Physiol.* **39**, e20230007. <https://doi.org/10.62958/j.cjap.2023.007> (2023).
18. Keat, C. L., Aziz, A., Eid, A. M. & Elmarzugli, N. A. Biosynthesis of nanoparticles and silver nanoparticles. *Bioresour Bioprocess.* **2**, 47. <https://doi.org/10.1186/s40643-015-0076-2> (2015).
19. Fu, X. et al. Specialized structures and developmental mechanisms of secondary metabolite-synthesizing organs and tissues in medicinal plants. *J. Integr. Plant. Biol.* **68**, 926–941. <https://doi.org/10.1111/jipb.70133> (2026).
20. Patil, S. & Chandrasekaran, R. Biogenic nanoparticles: A comprehensive perspective in synthesis, characterization, application and its challenges. *J. Genet. Eng. Biotechnol.* **18**, 67. <https://doi.org/10.1186/s43141-020-00081-3> (2020).
21. El Shafey, A. M. Green synthesis of metal and metal oxide nanoparticles from plant leaf extracts and their applications: A review. *Green. Process. Synth.* **9**, 304–339. <https://doi.org/10.1515/gps-2020-0031> (2020).
22. Álvarez-Chimal, R. & Arenas-Alatorre, J. Á. Green synthesis of nanoparticles: A biological approach. In *Advances in Green Chemistry: Prevention–Assurance–Sustainability (P-A-S) Approach* (ed Shah, K. J.) (IntechOpen, London, <https://doi.org/10.5772/intechopen.1002203>. (2023).
23. Akintelu, S. A., Bo, Y. & Folorunso, A. S. A review on synthesis, optimization, mechanism, characterization, and antibacterial application of silver nanoparticles synthesized from plants. *J. Chem.* 3189043 (2020). (2020). <https://doi.org/10.1155/2020/3189043>
24. Eker, F., Akdaşçi, E., Duman, H., Bechelany, M. & Karav, S. Green synthesis of silver nanoparticles using plant extracts: A comprehensive review of physicochemical properties and multifunctional applications. *Int. J. Mol. Sci.* **26**, 6222. <https://doi.org/10.3390/ijms26136222> (2025).
25. Farhana, S. et al. Chemical composition of fixed oil and in vitro antimicrobial activity of *Andrographis paniculata* root. *J. King Saud Univ. Sci.* **34**, 101921. <https://doi.org/10.1016/j.jksus.2022.101921> (2022).
26. Chauhan, E. S., Sharma, K. & Bist, R. *Andrographis paniculata*: A review of its phytochemistry and pharmacological activities. *Res. J. Pharm. Technol.* **12**, 891–900. <https://doi.org/10.5958/0974-360X.2019.00153.7> (2019).
27. Okhuarobo, A. et al. Harnessing the medicinal properties of *Andrographis paniculata* for diseases and beyond: A review of its phytochemistry and pharmacology. *Asian Pac. J. Trop. Dis.* **4**, 213–222. [https://doi.org/10.1016/S2222-1808\(14\)60509-0](https://doi.org/10.1016/S2222-1808(14)60509-0) (2014).
28. Gonde, D. P., Bhole, B. K., Kakad, K. S. & *Andrographolide* diterpenoid constituent of *Andrographis paniculata*: Review on botany, phytochemistry, molecular docking analysis, and pharmacology. *Ann. Pharm. Fr.* **82**, 15–43. <https://doi.org/10.1016/j.pharma.2023.10.001> (2024).
29. Nooreen, Z. et al. An insight into ethanopharmacology, phytochemistry, and pharmacological properties of genus *Andrographis*. *Curr. Org. Chem.* **29**, 519–568. <https://doi.org/10.2174/0113852728327331240905094331> (2025).

30. Avula, B. et al. Phytochemical analysis of phenolics and diterpene lactones in *Andrographis paniculata* plant samples and dietary supplements. *J. Pharm. Biomed. Anal.* **262**, 116866. <https://doi.org/10.1016/j.jpba.2025.116866> (2025).
31. Farhana, S., Aziz, S., Banu, S. A. & Al-Reza, S. M. UV & FT-IR spectroscopic analysis of *Andrographis paniculata* ethanolic leaf extract. *Res. J. Life Sci. Bioinform Pharm. Chem. Sci.* **5**, 12–21. <https://doi.org/10.26479/2019.0504.02> (2019).
32. Arunachalam, R. et al. Phytosynthesis of silver nanoparticles using *Coccinia grandis* leaf extract and its application in the photocatalytic degradation. *Colloids Surf. B Biointerfaces.* **94**, 226–230. <https://doi.org/10.1016/j.colsurfb.2012.01.040> (2012).
33. Parekh, J. & Chanda, S. In vitro antimicrobial activity and phytochemical analysis of some Indian medicinal plants. *Turk. J. Biol.* **31**, 53–58 (2007).
34. Guruvaiah, P., Arunachalam, A. & Velan, L. P. T. Evaluation of phytochemical constituents and antioxidant activities of successive solvent extracts of leaves of *Indigofera caerulea* Roxb. using various in vitro antioxidant assay systems. *Asian Pac. J. Trop. Dis.* **2**, S118–S123. [https://doi.org/10.1016/S2222-1808\(12\)60132-2](https://doi.org/10.1016/S2222-1808(12)60132-2) (2012).
35. Ibrahim, H. M. M. Green synthesis and characterization of silver nanoparticles using banana peel extract and their antimicrobial activity against representative microorganisms. *J. Radiat. Res. Appl. Sci.* **8**, 265–275. <https://doi.org/10.1016/j.jrras.2015.01.007> (2015).
36. Kokila, T., Ramesh, P. S. & Geetha, D. Biosynthesis of silver nanoparticles from Cavendish banana peel extract and its antibacterial and free radical scavenging assay: A novel biological approach. *Appl. Nanosci.* **5**, 911–920. <https://doi.org/10.1007/s13204-015-0401-2> (2015).
37. Rastogi, L. & Arunachalam, J. Green synthetic route for the size-controlled synthesis of biocompatible gold nanoparticles using aqueous extract of garlic (*Allium sativum*). *Adv. Mater. Lett.* **4**, 548–555. <https://doi.org/10.5185/amlett.2012.11456> (2013).
38. Zaki, S., Elkady, M. F., Farag, S. & Abd-El-Haleem, D. Determination of the effective origin source for nanosilver particles produced by *Escherichia coli* strain S78 and its application as an antimicrobial agent. *Mater. Res. Bull.* **47**, 4286–4290. <https://doi.org/10.1016/j.materresbull.2012.08.042> (2012).
39. Safekordi, A. A., Attar, H., Ghorbani, H. R., Sorkhabadi, S. & Rezayat, M. Optimization of silver nanoparticles production by *Escherichia coli* bacterium (DH5α) and the study of reaction kinetics. *Asian J. Chem.* **23**, 5111–5118 (2011).
40. Sathishkumar, M. et al. *Cinnamomum zeylanicum* bark extract and powder-mediated green synthesis of nanocrystalline silver particles and their bactericidal activity. *Colloids Surf. B Biointerfaces.* **73**, 332–338. <https://doi.org/10.1016/j.colsurfb.2009.06.005> (2009).
41. Ahmed, S., Ahmad, M., Swami, B. L. & Ikram, S. A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise. *J. Adv. Res.* **7**, 17–28. <https://doi.org/10.1016/j.jare.2015.02.007> (2016).
42. Padalia, H., Moteriya, P. & Chanda, S. Green synthesis of silver nanoparticles from marigold flower and its synergistic antimicrobial potential. *Arab. J. Chem.* **8**, 732–741.

- <https://doi.org/10.1016/j.arabjc.2014.11.015> (2015).
43. Pant, G., Nayak, N. & Gyana Prasuna, R. Enhancement of antidandruff activity of shampoo by biosynthesized silver nanoparticles from *Solanum trilobatum* plant leaf. *Appl. Nanosci.* **3**, 431–439. <https://doi.org/10.1007/s13204-012-0164-y> (2013).
44. Roopan, S. M. et al. Low-cost and eco-friendly phytosynthesis of silver nanoparticles using *Cocos nucifera* coir extract and its larvicidal activity. *Ind. Crops Prod.* **43**, 631–635. <https://doi.org/10.1016/j.indcrop.2012.08.013> (2013).
45. Chanda, S. Silver nanoparticles (medicinal plants mediated): A new generation of antimicrobials to combat microbial pathogens—a review. In *Microbial Pathogens and Strategies for Combating Them: Science, Technology and Education* (ed. Méndez-Vilas, A.) 1314–1323 Formatex Research Center, Badajoz, Spain, (2013).
46. Ajitha, B., Reddy, Y. A. K. & Reddy, P. S. Biogenic nano-scale silver particles by *Tephrosia purpurea* leaf extract and their inborn antimicrobial activity. *Spectrochim Acta Mol. Biomol. Spectrosc.* **121**, 164–172. <https://doi.org/10.1016/j.saa.2013.10.077> (2014).
47. Fatema, S., Shirsat, M., Farooqui, M. & Pathan, M. A. Biosynthesis of silver nanoparticles using aqueous extract of *Saraca asoca* leaves, its characterization and antimicrobial activity. *Int. J. Nano Dimens.* **10**, 163–168 (2019).
48. Kanniah, P. et al. Green synthesis of multifaceted silver nanoparticles using the flower extract of *Aerva lanata* and evaluation of its biological and environmental applications. *ChemistrySelect* **5**, 2322–2331. <https://doi.org/10.1002/slct.201903228> (2020).
49. Murdock, R. C., Braydich-Stolle, L., Schrand, A. M., Schlager, J. J. & Hussain, S. M. Characterization of nanomaterial dispersion in solution prior to in vitro exposure using dynamic light scattering technique. *Toxicol. Sci.* **101**, 239–253. <https://doi.org/10.1093/toxsci/kfm240> (2008).
50. Danaei, M. et al. Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. *Pharmaceutics* **10**, 57. <https://doi.org/10.3390/pharmaceutics10020057> (2018).
51. International Organization for Standardization. *ISO 22412:2017 Particle size analysis-Dynamic light scattering (DLS)* (ISO, 2017).

Figures

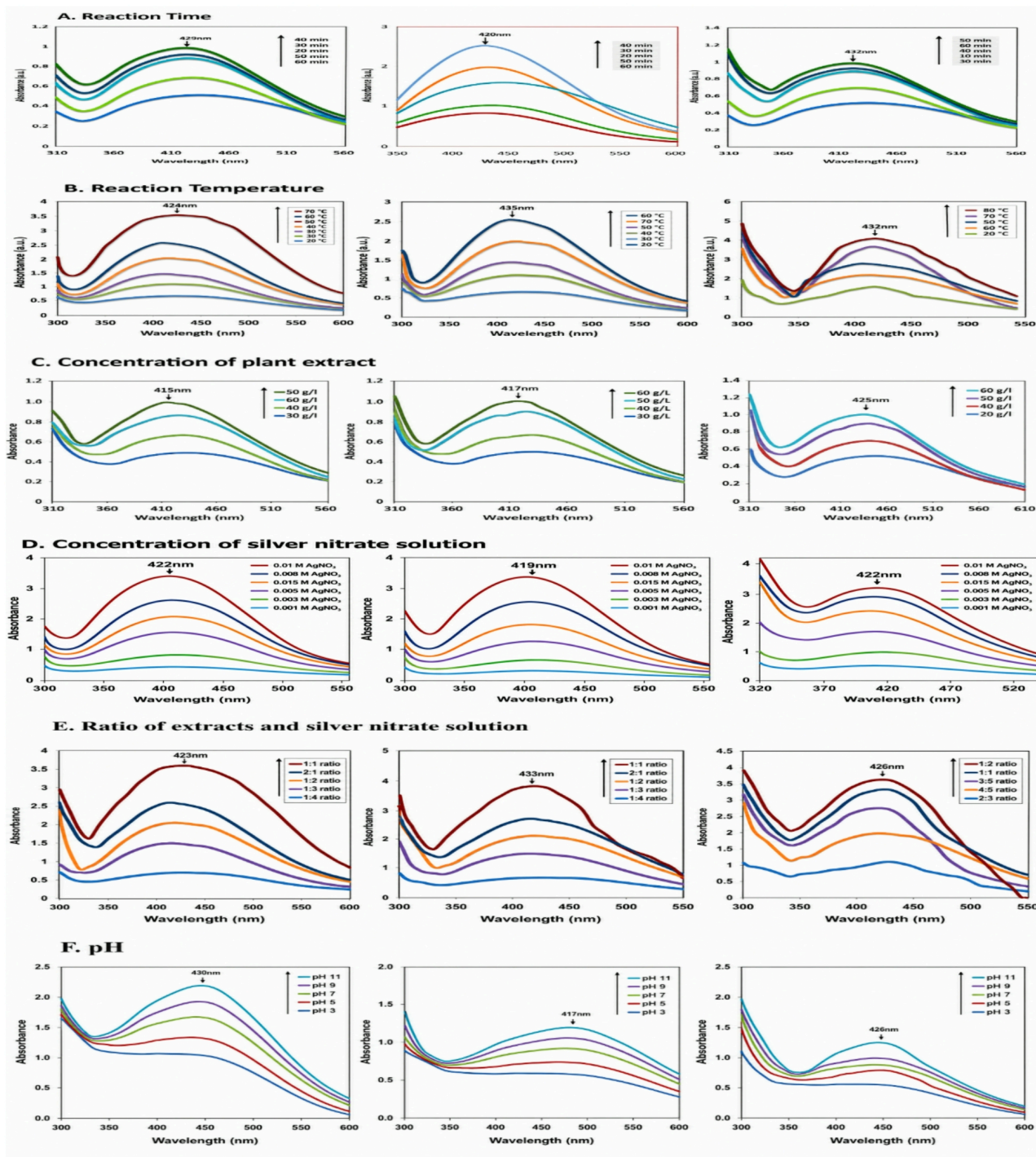


Figure 1

The optimum synthesis conditions were identified from the maximum intensity and stability of the characteristic surface plasmon resonance (SPR) bands for each plant organ. (A) Reaction time, (B) reaction temperature, (C) plant extract concentration, (D) AgNO_3 concentration, (E) extract-to- AgNO_3 ratio, and (F) reaction pH. The characteristic surface plasmon resonance (SPR) bands were used to determine the optimum synthesis conditions for each plant extract.

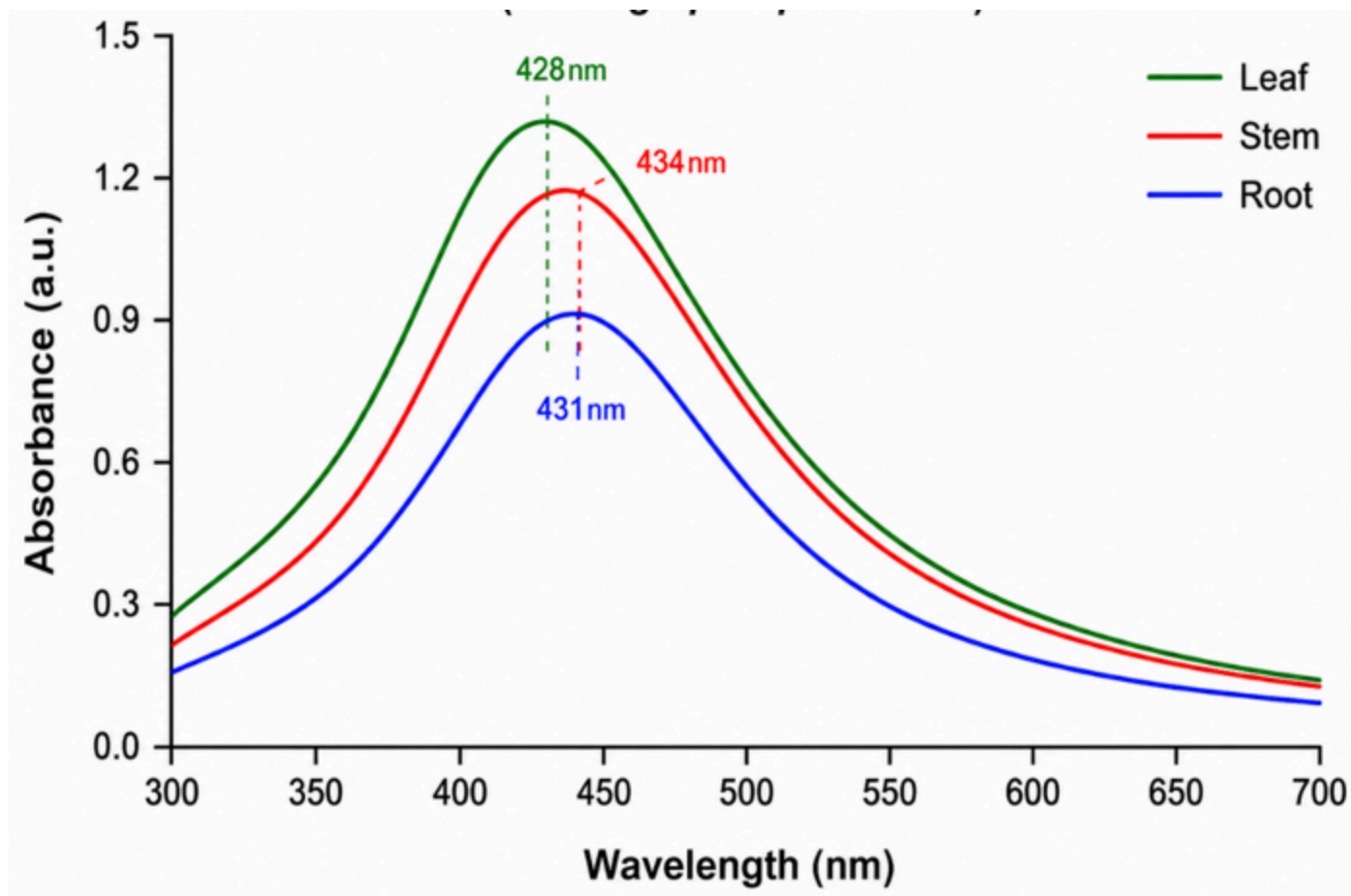


Figure 2

Comparative UV-Visible absorption spectra of silver nanoparticles (AgNPs) synthesized using aqueous leaf, stem and root extracts of *Andrographis paniculata*

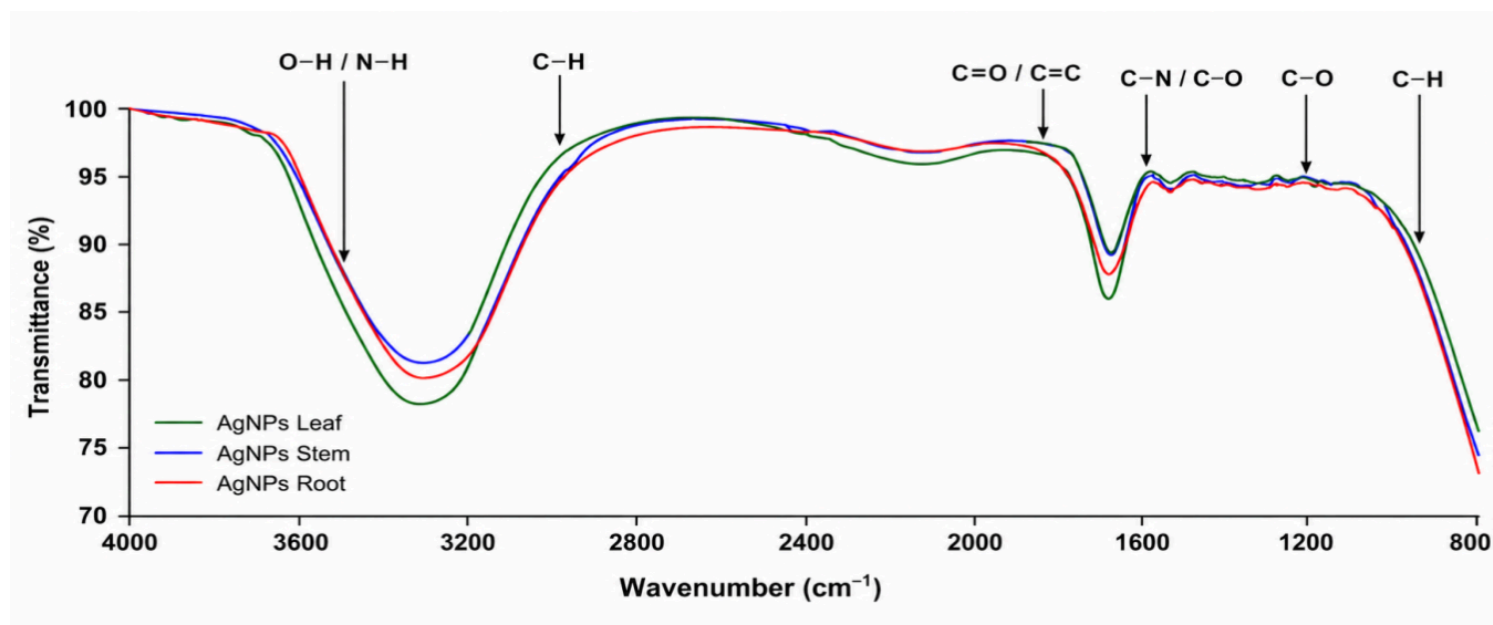


Figure 3

FTIR spectra of AgNPs synthesized using the leaf, stem and root extracts of *Andrographis paniculata*.

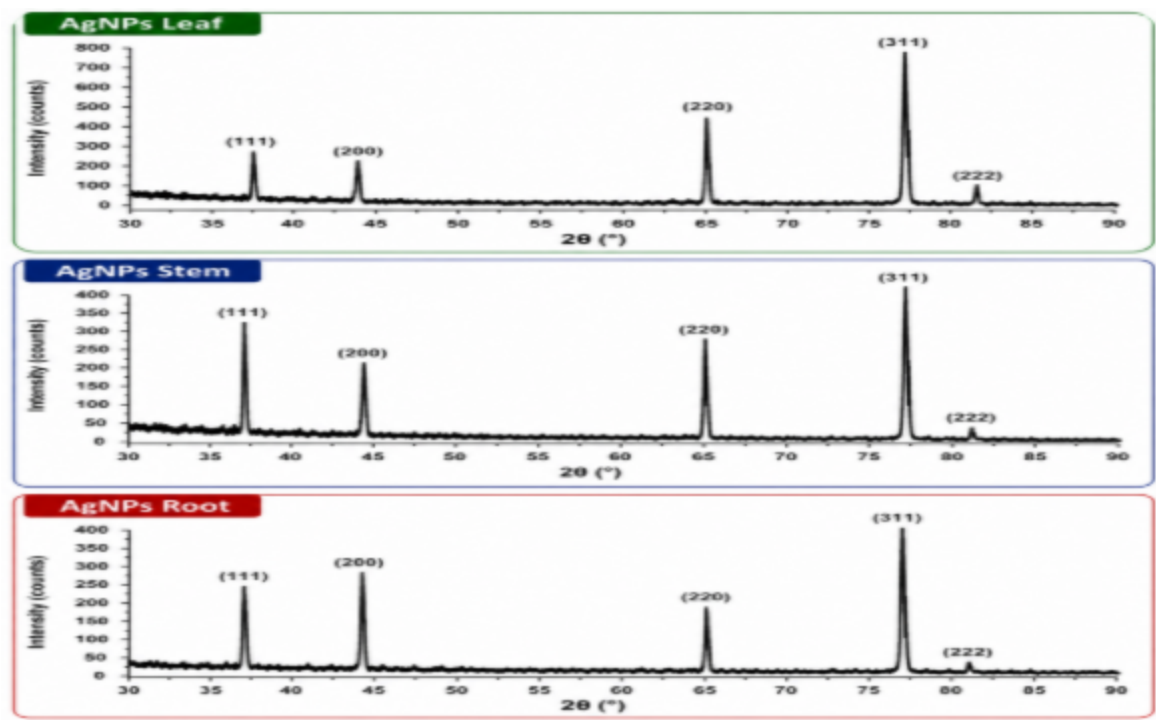


Figure 4

XRD analysis of AgNPs synthesized using the leaf, stem and root extracts of *Andrographis paniculata*

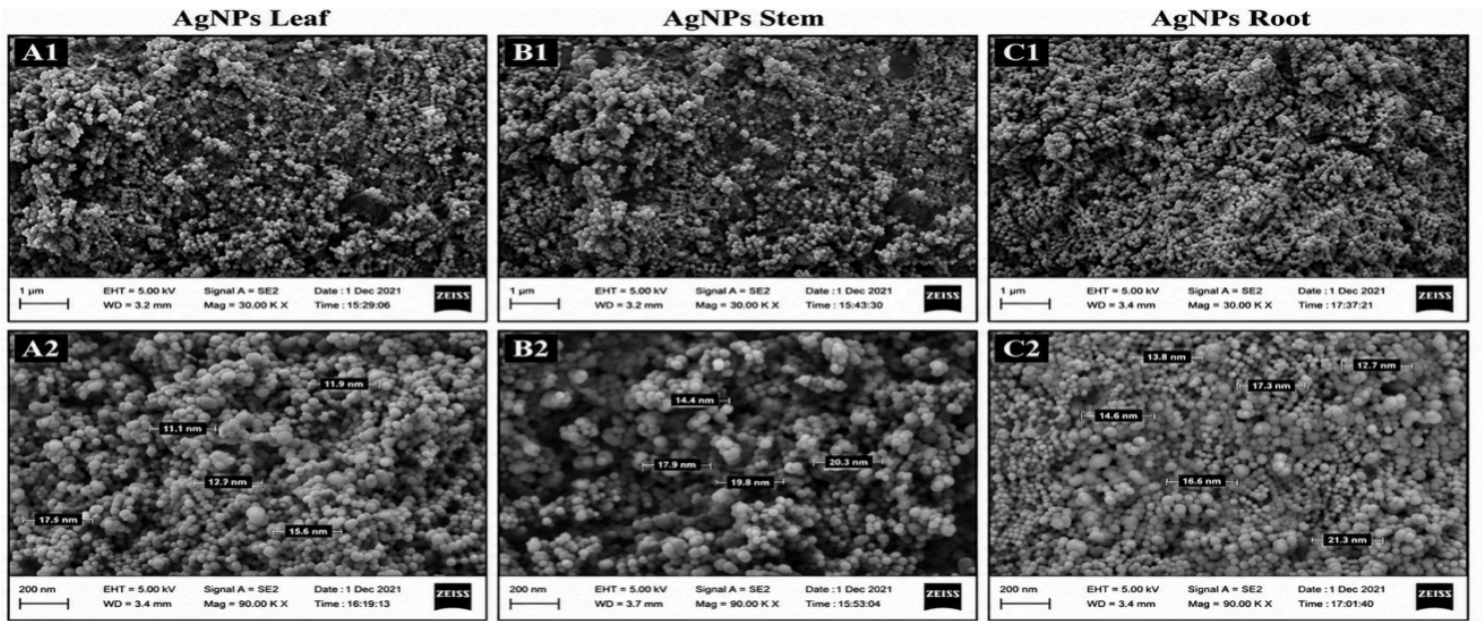


Figure 5

FE-SEM micrographs of silver nanoparticles synthesized using *Andrographis paniculata* leaf (A1–A2), stem (B1–B2), and root (C1–C2) extracts. Images were recorded at 30,000 $\times$ magnification (A1–C1; scale bar = 1 μ m) and 90,000 $\times$ magnification (A2–C2; scale bar = 200 nm).

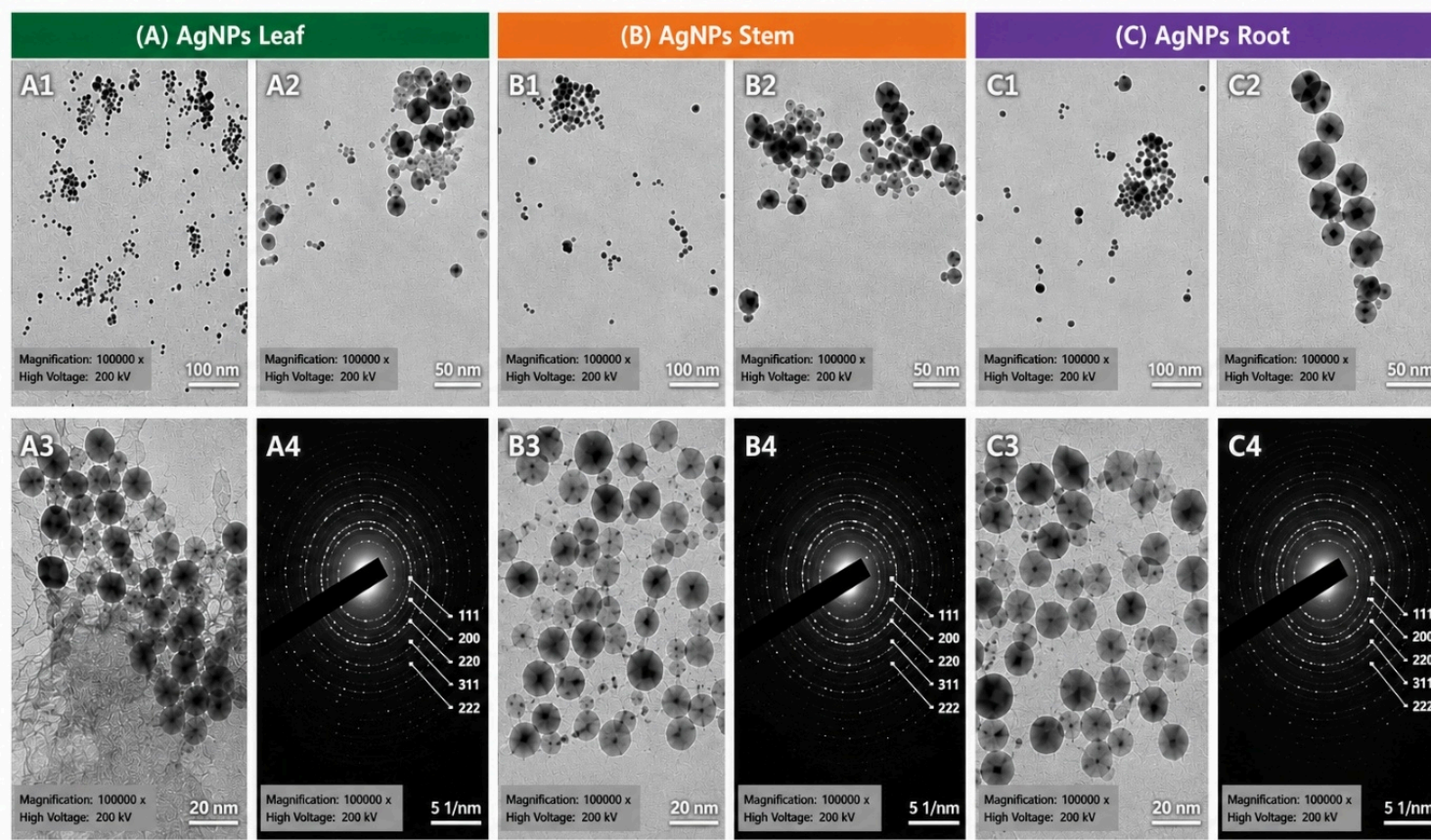


Figure 6

FE-TEM images and SAED patterns of silver nanoparticles (AgNPs) synthesized using aqueous leaf (A1–A4), stem (B1–B4), and root (C1–C4) extracts of *Andrographis paniculata*. All TEM images were recorded at 100,000 $\times$ magnification and an accelerating voltage of 200 kV. Scale bars: 100 nm (A1, B1, C1), 50 nm (A2, B2, C2), 20 nm (A3, B3, C3), and 5.1 nm $^{-1}$ (A4, B4, C4).

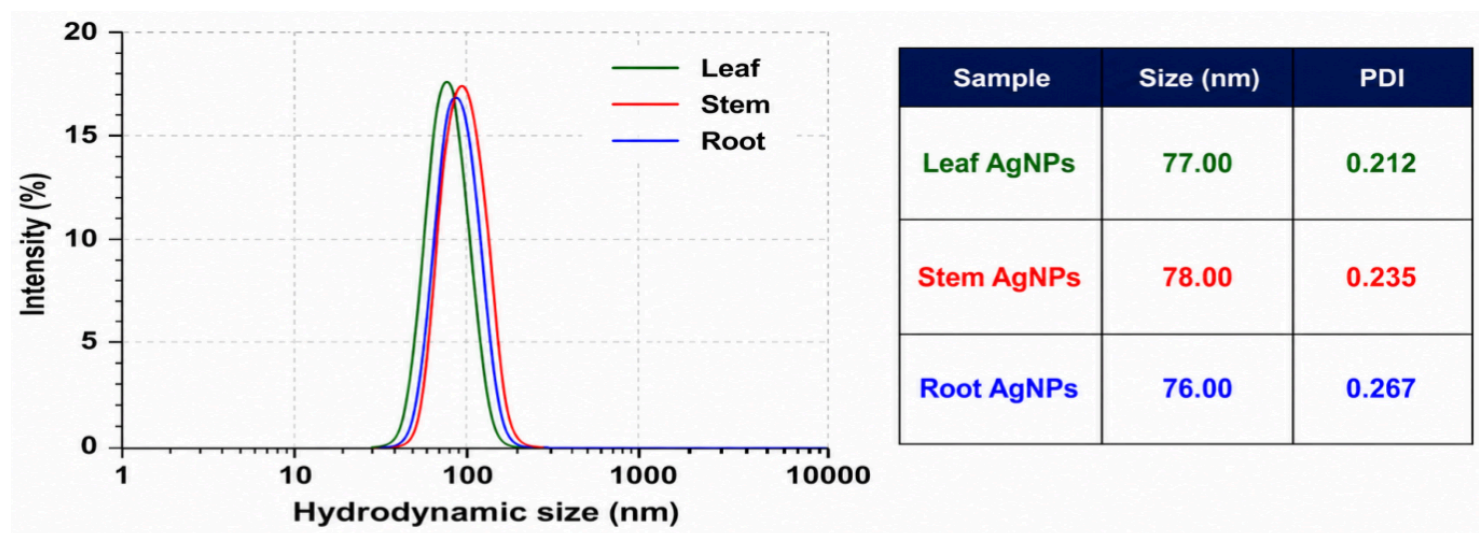


Figure 7

Dynamic Light Scattering (DLS) analysis of silver nanoparticles synthesized using *Andrographis paniculata* leaf, stem and root extracts.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryInformation.pdf](#)