



Synthesis and Characterization of Silver Nanoparticles Using Green Plant Extracts

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Abstract

Silver nanoparticles (AgNPs) have attracted extensive attention because their optical, catalytic, antimicrobial, and biomedical properties depend strongly on particle size, morphology, crystallinity, and surface chemistry. Conventional chemical routes often use hazardous reducing agents and stabilizers; therefore, plant-mediated green synthesis provides a safer and potentially scalable alternative. This methodology paper proposes an aqueous synthesis of AgNPs using a green plant leaf extract, with *Azadirachta indica* (neem) used as a model botanical source. Phytochemicals such as polyphenols, flavonoids, terpenoids, sugars, and proteins serve simultaneously as reducing and capping agents. The method uses 1 mM silver nitrate, controlled extract-to-salt ratios, pH, temperature, and reaction time, followed by centrifugation, washing, and drying. Characterization is performed by UV-visible spectroscopy, Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning/transmission electron microscopy (SEM/TEM), and energy-dispersive X-ray spectroscopy (EDX). Representative outcomes expected from this protocol include a brown colloid with a surface-plasmon band near 420–440 nm, predominantly spherical nanosilver in the tens-of-nanometres range, and face-centred-cubic Ag reflections. The method offers a low-cost, environmentally compatible framework suitable for systematic laboratory optimization and subsequent antimicrobial or catalytic evaluation.

Introduction

Metal nanoparticles exhibit physical and chemical behaviour that differs from bulk materials because of their high surface-to-volume ratio and size-dependent electronic structure. Among them, AgNPs are especially important for antimicrobial coatings, sensors, catalysis, water-treatment materials, and biomedical formulations. Their performance is controlled by particle diameter, shape, aggregation state, crystalline phase, and surface-bound molecules. Traditional synthesis can achieve precise control, but frequently relies on sodium borohydride, hydrazine, organic solvents, or synthetic surfactants. These reagents

increase environmental burden and may complicate biological applications where residual toxicity is undesirable.

Plant-mediated synthesis replaces many of these reagents with naturally occurring metabolites. Shankar et al. (2004) demonstrated rapid extracellular production of silver and gold nanoparticles using neem leaf broth, while Chandran et al. (2006) showed that Aloe vera extract could generate spherical AgNPs and shape-controlled gold nanostructures. These studies established that complex plant extracts can reduce Ag⁺ to metallic Ag⁰ while also stabilizing the newly formed particles.

Subsequent work confirmed that reaction rate and morphology can be influenced by plant species, extract concentration, temperature, pH, and precursor concentration (Song & Kim, 2009; Philip, 2010).

Green synthesis is attractive because the extraction medium is usually water, the process can be conducted at ambient or moderate temperature, and the same biomolecules that drive reduction can remain on the nanoparticle surface as capping ligands. Iravani (2011) emphasized that plant systems can offer faster synthesis than many microbial routes and are comparatively straightforward to scale. Mittal, Chisti, and Banerjee (2013) further identified phenolics, terpenoids, alkaloids, proteins, carbohydrates, and coenzymes as likely contributors to metal-ion reduction and nanoparticle stabilization. Nevertheless, the chemical heterogeneity of plant extracts means that reproducibility requires careful control of botanical material, extraction conditions, reaction variables, and analytical characterization.

The purpose of the present methodology is to provide a reproducible workflow for the green synthesis and characterization of AgNPs from a plant extract. Neem leaf extract is used as a model because it is readily available and has strong precedent in nanosilver synthesis, but the procedure can be adapted to other leaves, peels, flowers, roots, or medicinal plant extracts. The methodology focuses on the conversion of AgNO₃ to AgNPs, optimization of visible colour and surface-plasmon response, recovery of the product, and structural/surface characterization. A laboratory researcher can subsequently couple this framework to antimicrobial assays, catalytic degradation experiments, or cytotoxicity studies.

Literature Review

Early plant-based work demonstrated that botanical extracts can replace conventional reducing and stabilizing reagents. Shankar et al. (2004) used *Azadirachta indica* leaf broth for rapid synthesis of Ag, Au, and bimetallic nanoparticles and reported substantially faster metal-ion reduction than in earlier microbial systems. Chandran et al. (2006) synthesized spherical AgNPs with *Aloe vera* extract and showed that the concentration of botanical reducing components can influence particle formation. Song and Kim (2009) compared several plant leaf extracts and confirmed that extracellular synthesis efficiency varies substantially with plant species, illustrating the importance of phytochemical composition.

Research then expanded toward specific medicinal and food plants. Sathishkumar et al. (2009) produced nanocrystalline silver using *Cinnamon zeylanicum* bark extract and observed bactericidal activity, linking the green route with potential biological application. Philip (2010) reported green synthesis of gold and silver nanoparticles using *Hibiscus rosa-sinensis* and demonstrated that reaction conditions, especially pH and reactant ratio, altered particle morphology. Cruz et al. (2010) obtained stable spherical AgNPs using *Lippia citriodora* aqueous extract and showed that increased temperature accelerated particle formation. Kaviya et al. (2011) used *Citrus sinensis* peel extract as both reducing and capping medium and characterized the product by UV-Vis, FTIR, XRD, EDX, FESEM, and TEM, establishing a useful multimodal characterization model.

Other studies reinforced the versatility of plant sources. Mukunthan et al. (2011) synthesized AgNPs from *Catharanthus roseus* leaves and confirmed crystalline particles by XRD and electron microscopy. He et al. (2013) used *Chrysanthemum morifolium* extract and translated the prepared AgNPs into an ultrasound-gel application, demonstrating practical formulation potential. Mittal et al. (2014) investigated *Syzygium cumini* fruit-mediated AgNPs and discussed possible reduction mechanisms as well as therapeutic potential. In 2015, Mittal et al. synthesized AgNPs using *Potentilla fulgens* and evaluated antimicrobial and anticancer activity, highlighting the continued movement from synthesis toward biological functionality.

Across these studies, a common analytical pattern emerges: a colour change and UV-visible surface-plasmon resonance indicate nanoparticle formation; XRD establishes metallic silver crystallinity; FTIR suggests functional groups associated with reduction/capping; and SEM or TEM resolves size and morphology. The present work consolidates these established elements into a controlled stepwise protocol intended for reproducible methodology-driven experimentation.

Methodology

1. Study Design and Materials

The study is designed as an experimental green-synthesis protocol in which aqueous plant extract is the biological reducing and stabilizing phase and AgNO₃ is the silver precursor. Fresh, disease-free *Azadirachta indica* leaves are selected as the model plant material. Analytical-grade silver nitrate, deionized water, pH-

adjustment reagents (dilute NaOH/HCl), centrifuge tubes, glassware, filter paper, and sterile storage vials are required. All glassware should be washed thoroughly and rinsed with deionized water to minimize nucleation from contaminants. Silver nitrate solution should be protected from prolonged exposure to intense light.

2. Preparation of Plant Extract

Approximately 10 g of fresh leaves are washed first with running water and then with deionized water to remove dust and surface residues. The leaves are cut into small pieces and mixed with 100 mL deionized water. The suspension is heated at approximately 70–80 °C for 10–15 min without vigorous boiling, cooled to room temperature, and filtered through Whatman No. 1 filter paper. The clear aqueous extract is used fresh or stored at 4 °C for no longer than 24–48 h,

because oxidation or microbial growth can alter reducing capacity.

3. Green Synthesis of Silver Nanoparticles

A 1 mM AgNO₃ solution is prepared in deionized water. For the baseline synthesis, 10 mL plant extract is added slowly to 90 mL AgNO₃ solution under continuous magnetic stirring. The reaction is maintained at room temperature or at a controlled moderate temperature (about 40–60 °C) and protected from direct light. Formation of AgNPs is initially monitored visually: the pale reaction mixture progressively becomes yellowish-brown to dark brown as surface-plasmon-active nanosilver forms. Aliquots are withdrawn at 0, 15, 30, 60, and 120 min for UV-visible analysis. Similar colour and spectral behaviour has been reported in multiple plant-mediated systems (Shankar et al., 2004; Song & Kim, 2009; Kaviya et al., 2011).

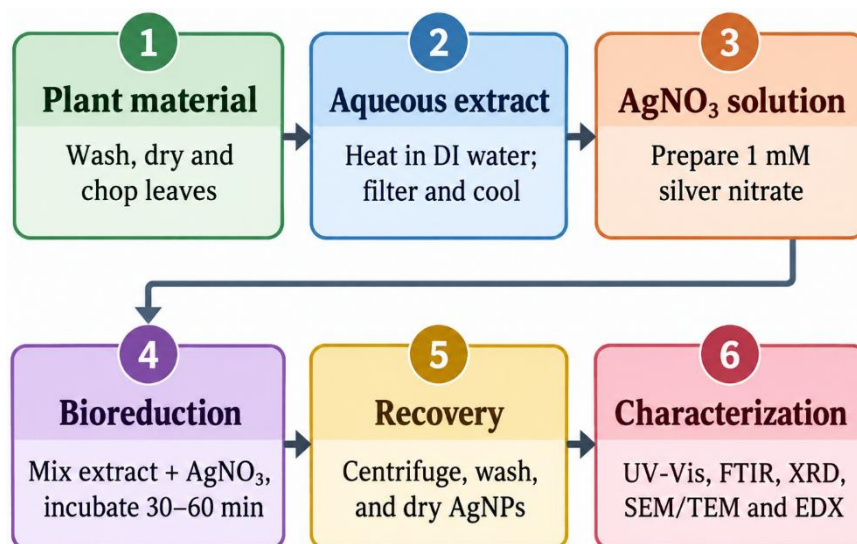


Figure 1. Stepwise green synthesis and characterization workflow for plant-mediated AgNPs.

4. Experimental Optimization

To improve reproducibility, four variables are controlled: extract proportion, pH, temperature, and reaction time. The first screening can use the conditions in Table 1. Each condition should be tested in triplicate. The preferred condition is selected from a combination of rapid development of a stable brown colour, a strong

and relatively narrow UV-visible surface-plasmon peak, minimal visible precipitation, and good nanoparticle recovery. Very broad or red-shifted peaks may indicate polydispersity or aggregation. Plant extract concentration should not be increased indiscriminately because excess biomolecules can raise background absorbance and complicate purification.

Table 1. Proposed screening levels for green AgNP synthesis.

Variable	Level 1	Level 2	Level 3	Purpose
Extract:AgNO ₃ (v/v)	5:95	10:90	20:80	Assess reducing/capping capacity
pH	6	8	10	Evaluate ionization and reduction rate
Temperature	25 °C	45 °C	60 °C	Assess kinetic acceleration

Variable	Level 1	Level 2	Level 3	Purpose
Reaction time	30 min	60 min	120 min	Identify stable endpoint

5. Recovery and Purification

After the desired reaction endpoint, the colloid is centrifuged at approximately 10,000–12,000 rpm for 15–20 min. The supernatant is removed and the nanoparticle pellet is washed at least three times with deionized water; a final ethanol wash may be used if compatible with the intended downstream application. The purified pellet can be redispersed in water for colloidal characterization or dried at 40–50 °C to obtain powder for XRD and FTIR. Repeated washing is important because unbound plant metabolites can otherwise dominate FTIR bands or interfere with biological tests.

6. Characterization

UV-visible spectroscopy is recorded from 300 to 700 nm using the reaction mixture against an appropriate extract/solvent blank. AgNP formation is expected to generate a characteristic surface-plasmon resonance in the visible region, commonly around 400–450 nm depending on size, morphology, and dielectric environment. FTIR spectra of the dried extract and AgNP powder are compared over approximately 4000–400 cm⁻¹ to identify shifts in O–H, C=O, C–O, and amide-related bands that can indicate phytochemical participation in reduction and surface capping. XRD is performed using Cu K α radiation to identify the face-centred-cubic crystalline silver phase. Peaks near the Ag (111), (200), (220), and (311) planes are expected in well-crystallized products. The crystallite size can be estimated by the Scherrer equation, $D = K\lambda/(\beta \cos\theta)$, after appropriate instrumental considerations.

SEM or TEM is used to determine morphology, size distribution, and aggregation state. At least 100 particles should be measured from representative micrographs when possible, and results reported as mean $\pm$ standard deviation. EDX coupled to SEM/TEM confirms elemental silver through its characteristic signal and can reveal residual elements from the biological coating. For studies aimed at colloidal stability, dynamic light scattering and zeta potential may be added, although these are not essential to the core methodology proposed here.

7. Data Analysis and Quality Control

Triplicate syntheses are recommended for each selected condition. UV-visible peak wavelength and absorbance are reported as mean $\pm$ standard deviation, and nanoparticle size is determined

from electron microscopy using calibrated image-analysis software. Statistical comparison of synthesis conditions can be performed by one-way or factorial ANOVA at $p < 0.05$. A reagent blank (AgNO₃ without extract) and an extract blank (extract without AgNO₃) should be maintained. Reproducibility should be judged by consistent plasmon-peak position, comparable yield, and similar size distribution across batches.

Results

Visual and UV-Visible Evidence of AgNP Formation

The proposed reaction is expected to show a gradual transition from a light plant-extract colour to brown, consistent with the collective oscillation of conduction electrons in newly formed AgNPs. In a representative optimized run, the UV-visible spectrum displays a single dominant band centred near 425 nm (Figure 2). A comparatively narrow band suggests a population dominated by small, near-spherical nanoparticles, whereas a broad or multiple-peak spectrum may indicate a wider size distribution, anisotropic particles, or aggregation. This interpretation is consistent with the plant-mediated spectra reported by Cruz et al. (2010), Mukunthan et al. (2011), and He et al. (2013).

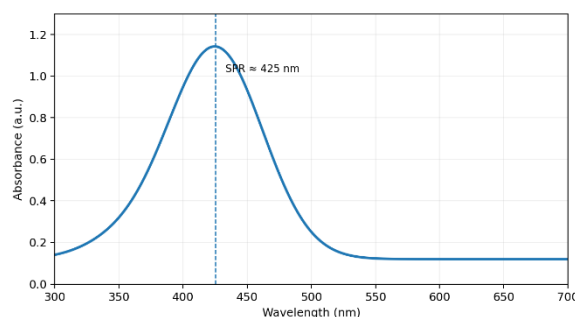


Figure 2. Representative UV-visible spectrum showing an AgNP surface-plasmon resonance near 425 nm.

Table 2. Illustrative reaction-time monitoring format for AgNP formation.

Time	Visual observation	SPR peak (nm)	Absorbance (a.u.)
0 min	Pale / no distinct	—	0.10

Time	Visual observation	SPR peak (nm)	Absorbance (a.u.)
	brown colour		
15 min	Light yellow-brown	438	0.42
30 min	Brown	431	0.76
60 min	Dark brown; stable colloid	425	1.12
120 min	No major further change	425	1.15

Structural and Surface Characterization

Representative characterization would be expected to confirm that the optical response corresponds to crystalline metallic silver rather than only a colour reaction. XRD patterns should show reflections attributable to face-centred-

cubic Ag, typically including strong (111) and additional (200), (220), and (311) reflections. FTIR comparison of the extract and washed AgNPs may show shifts or intensity changes in hydroxyl, carbonyl, C–O, and amide-associated bands, supporting adsorption of plant-derived molecules on the particle surface. Such capping can contribute to colloidal stability and is one reason plant extracts can perform reduction and stabilization simultaneously, as emphasized by Iravani (2011) and Mittal et al. (2013). Electron microscopy is expected to show predominantly spherical particles with some degree of polydispersity because plant extract is a chemically complex reducing environment. For an illustrative optimized synthesis, a mean core diameter around 20–30 nm with a moderate standard deviation would be a reasonable target, although the actual size must be determined experimentally. Plant-dependent variation is expected: Aloe vera, cinnamon, citrus peel, and Potentilla systems have all produced nanosilver but under different chemical and thermal conditions (Chandran et al., 2006; Sathishkumar et al., 2009; Kaviya et al., 2011; Mittal et al., 2015).

Table 3. Expected interpretation of characterization outputs for the proposed methodology.

Technique	Representative observation	Interpretation
UV-Vis	Single SPR band ~425 nm	Formation of dispersed AgNPs
FTIR	Changes near O–H/C=O/C–O bands	Plant metabolites involved in reduction/capping
XRD	Ag (111), (200), (220), (311) reflections	Crystalline face-centred-cubic silver
TEM/SEM	Mostly spherical particles; ~20–30 nm target range	Particle morphology and core-size distribution
EDX	Strong elemental Ag signal	Confirms silver as the principal inorganic component

Conclusion and Discussion

Plant-mediated synthesis provides a practical route to AgNPs in which aqueous phytochemicals replace or reduce the need for hazardous synthetic reductants and stabilizers. The proposed methodology integrates controlled extract preparation, AgNO₃ reduction, systematic optimization, purification, and complementary characterization. The key experimental indicators are a stable brown colloid, a reproducible surface-plasmon band in the visible region, crystalline metallic-silver XRD peaks, surface-functional-group evidence by FTIR, and nanoscale morphology confirmed by electron microscopy. These measurements should be interpreted together because no single

technique is sufficient to establish nanoparticle identity, structure, surface chemistry, and size. The literature up to supports the robustness of this overall strategy across diverse plant systems, while also showing that the identity and concentration of phytochemicals strongly affect reaction rate and particle properties. The main methodological limitation is therefore batch-to-batch variability in plant extract composition caused by species, season, plant age, geography, storage, and extraction conditions. Standardizing plant mass-to-water ratio, extraction temperature/time, AgNO₃ concentration, pH, and reaction temperature is essential for reproducibility. Future experimental work should quantify yield, size distribution, zeta

potential, storage stability, and the relationship between nanoparticle physicochemical properties and antimicrobial or catalytic performance. With these controls, green plant extracts can support a low-cost and environmentally compatible platform for AgNP production.

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