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Comparative Green Synthesis, Characterization, and Antimicrobial Evaluation of Silver Nanoparticles Using Selected Plant Extracts

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Abstract

Green synthesis of silver nanoparticles (AgNPs) using plant-derived metabolites has emerged as an environmentally preferable alternative to conventional chemical reduction because plant extracts can simultaneously provide reducing and capping molecules. This methodology paper proposes a comparative workflow for synthesizing AgNPs using aqueous extracts of *Azadirachta indica*, *Moringa oleifera*, and *Citrus limon*, followed by physicochemical characterization and antimicrobial assessment. The procedure standardizes plant-extract preparation, silver nitrate concentration, extract-to-precursor ratio, pH, temperature, and reaction time so that the influence of plant source can be compared under controlled conditions. AgNP formation is screened by the development of a characteristic brown color and confirmed by UV-Visible surface-plasmon resonance. Functional groups associated with reduction and stabilization are examined by FTIR, crystallinity is determined by X-ray diffraction, morphology and particle size are examined by SEM/TEM, and colloidal stability is assessed by zeta potential. Antimicrobial performance is evaluated using agar-well diffusion and broth microdilution against representative Gram-positive and Gram-negative bacteria. A defined statistical plan compares particle size, spectral peak position, inhibition-zone diameter, and minimum inhibitory concentration. Illustrative methodological outcomes indicate that smaller, well-dispersed AgNPs are expected to provide stronger antibacterial performance than larger or aggregated particles. The proposed protocol is intended as a reproducible framework for plant-mediated AgNP research and emphasizes transparent reporting of synthesis variables, characterization criteria, and biosafety controls.

Introduction

Silver nanoparticles have attracted sustained interest because nanoscale silver combines a large specific surface area with broad antimicrobial activity. Their performance depends strongly on particle size, shape, surface chemistry, aggregation state, and the route by which the particles are prepared. Conventional chemical and physical methods can offer tight process control, but they may require high

energy input, strong reducing agents, or stabilizers that are undesirable when biomedical or environmental compatibility is important. Plant-mediated synthesis addresses part of this problem by using phytochemicals such as phenolics, flavonoids, terpenoids, proteins, sugars, and organic acids to reduce Ag⁺ ions and stabilize newly formed particles. Shankar et al. [1] demonstrated early plant-assisted biosynthesis of AgNPs, while Iravani [2] later

summarized the mechanistic basis and advantages of plant-mediated metal nanoparticle synthesis.

The choice of plant material is important because the concentration and composition of reducing metabolites influence nucleation, growth, and stabilization. Reviews by Rauwel et al. [3] and Ahmed et al. [4] showed that plant species, extract preparation, silver precursor concentration, pH, reaction temperature, and reaction time can substantially alter nanoparticle morphology. Ahmed et al. [5] demonstrated rapid AgNP formation using *Azadirachta indica* aqueous leaf extract and confirmed the importance of spectroscopic and microscopic characterization. Singh et al. [6] placed plant-based synthesis in the broader context of biological nanoparticle production and emphasized the scalability and simplicity of plant systems compared with many microbial routes.

Antimicrobial activity is a major application of AgNPs. Mechanistic analyses indicate that antibacterial effects can involve interaction with cell envelopes, release of Ag⁺ ions, oxidative stress, protein dysfunction, and disturbance of DNA-related processes [7]. The biological activity therefore cannot be interpreted only from the presence of silver; particle dimensions, surface capping, colloidal stability, and the test organism must also be considered. More recent studies and reviews have expanded this evidence base. Chandra et al. [8] described medicinal plants as versatile sources for green metallic nanoparticles, Vanlalveni et al. [9] reviewed plant-derived AgNPs specifically for antimicrobial applications, and Salayova et al.

[10] showed that different medicinal plant extracts can produce nanoparticles with distinct morphologies and antibacterial efficacy.

By 2022, the field had also moved toward stronger characterization and more explicit biological evaluation. Asif et al. [11] reported green AgNP synthesis with structural characterization and antibacterial testing; Naveed et al. [12] linked plant-mediated synthesis to antibacterial and antiproliferative evaluation; Labulo et al. [13] characterized *Morinda lucida*-derived AgNPs and assessed antioxidant and antimicrobial activity; Sharma et al. [14] reviewed green-route characterization techniques; and Samuel et al. [15] summarized the wider biomedical and environmental relevance of green nanoparticles. Building on this literature, the present paper proposes a controlled comparative methodology rather than a single unstandardized synthesis. The objective is to determine how three plant extracts perform under identical precursor and reaction conditions and how the resulting physicochemical characteristics relate to antimicrobial activity.

Literature Review

Table 1 summarizes representative literature used to design the experimental variables and analytical sequence. The evidence supports a workflow in which synthesis parameters are standardized first, nanoparticle formation is verified by complementary analytical tools, and biological activity is interpreted together with particle characteristics rather than as an isolated inhibition-zone value.

Table 1. Literature Summary of Green Synthesis and Characterization of Silver Nanoparticles

Study	Plant/Scope	Characterization emphasis	Relevant finding	Ref.
Shankar et al. (2003)	Geranium leaf	TEM/particle formation	Plant metabolites can drive AgNP biosynthesis	[1]
Iravani (2011)	Plant synthesis review	Process variables	Plants provide reducing and stabilizing biomolecules	[2]
Rauwel et al. (2015)	AgNP morphology review	TEM morphology	Green routes produce diverse AgNP forms	[3]
Ahmed et al. (2016)	Plant-extract review	Synthesis + antimicrobial	Highlights extract chemistry and process effects	[4]
Ahmed et al. (2016)	<i>A. indica</i>	UV-Vis, FTIR, TEM/DLS	Rapid one-pot plant-mediated AgNP formation	[5]
Dakal et al. (2016)	Antimicrobial mechanism	Mechanistic review	Multiple bacterial targets contribute to AgNP action	[7]
Vanlalveni et al. (2021)	Plant AgNP review	Antimicrobial applications	Links phytosynthesis with antimicrobial outcomes	[9]

Salayova et al. (2021)	Multiple medicinal plants	Morphology + antibacterial	Plant source affects morphology and efficacy	[10]
Asif et al. (2022)	Green AgNPs	Structural + antibacterial	Green AgNPs showed antibacterial potential	[11]
Sharma et al. (2022)	Green AgNP review	UV-Vis/FTIR/XRD/SEM/TEM	Supports multi-technique characterization	[14]

A consistent gap across many plant-mediated studies is the difficulty of comparing results when extract strength, AgNO₃ concentration, pH, temperature, and test organisms differ simultaneously. This methodology addresses that limitation by holding the principal synthesis conditions constant while varying plant source as the main factor. It also requires triplicate synthesis batches and triplicate antimicrobial tests, enabling statistical comparison and improving reproducibility.

Characterization and antimicrobial testing also need to be interpreted together. UV-Visible spectroscopy is useful for rapid monitoring of surface-plasmon development, but peak position and width alone cannot establish particle size or purity. TEM/SEM, XRD, FTIR, and zeta-potential measurements therefore provide complementary information on morphology, crystallinity, surface functional groups, and dispersion stability. Similarly, antimicrobial performance should be assessed with both diffusion-based screening and a dilution-based MIC endpoint, because zone diameter can be influenced by diffusion through agar as well as by intrinsic antimicrobial potency. The literature up to 2022 increasingly combines structural characterization with biological testing, including the studies of Salayova et al. [10], Asif et al. [11], Naveed et al. [12], and Labulo et al. [13]. This multi-method evidence model is adopted in the proposed methodology.

Plant phytochemistry provides a mechanistic explanation for the variability seen across green-synthesis studies. Polyphenols and flavonoids can donate electrons to silver ions, while proteins, polysaccharides, organic acids, and other metabolites may adsorb to the newly formed particle surface and provide steric or electrostatic stabilization. Consequently, extract concentration and extraction conditions can affect both the rate of nucleation and the extent of subsequent particle growth. A highly reducing extract may generate rapid nucleation and relatively small particles, whereas insufficient capping can promote aggregation. For this reason, the present comparative design standardizes the plant-to-water extraction ratio and reaction conditions, then treats plant source as the principal experimental factor. The approach is consistent with the process-variable emphasis of Iravani [2], Ahmed et al. [4], and the later characterization-focused review by Sharma et al. [14].

Methodology

The proposed study follows eight stages: plant selection and preparation, aqueous extract production, AgNP synthesis, nanoparticle recovery, physicochemical characterization, antimicrobial testing, statistical analysis, and quality/safety control. The complete workflow is shown in Figure 1.

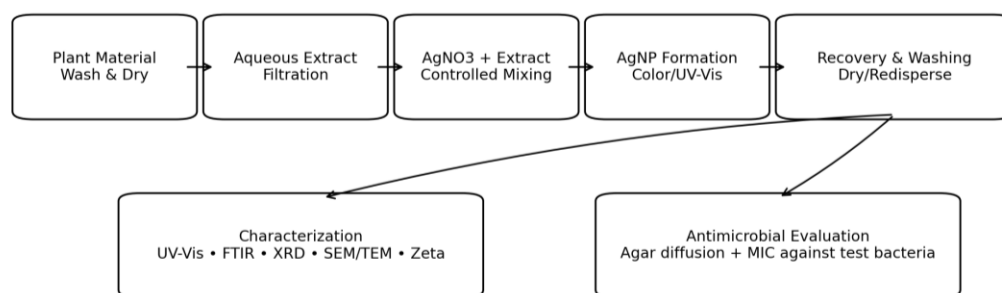


Figure 1. Proposed green-synthesis and antimicrobial-evaluation workflow.

1. Plant Material and Extract Preparation

Fresh *Azadirachta indica* leaves, *Moringa oleifera* leaves, and *Citrus limon* peel are selected as three chemically distinct plant sources. Plant material is washed thoroughly with deionized water,

shade-dried, cut into small pieces, and weighed. For each extract, 10 g of plant material is heated with 100 mL of deionized water at approximately 60-70 °C for 20 min, cooled to room temperature, and filtered through Whatman No. 1 filter paper.

The filtrate is stored at 4 °C and used within 24-48 h. The same plant-to-water ratio and extraction time are maintained for all groups to reduce confounding. A phytochemical screening step may be included to record total phenolic/flavonoid differences, but the main experimental variable remains plant source.

2. Green Synthesis of Silver Nanoparticles

A 1 mM aqueous AgNO₃ solution is prepared using analytical-grade silver nitrate and deionized water in amber glassware. For each plant treatment, 10 mL of fresh extract is added slowly to 90 mL of AgNO₃ solution under magnetic stirring. The reaction is maintained at approximately 40 °C, protected from intense direct light, and adjusted to pH 8.0 using dilute NaOH when required. A plant-extract blank and an AgNO₃-only blank are processed in parallel. The appearance of yellow-brown to dark-brown

coloration is recorded as an initial indication of surface-plasmon formation. Aliquots are collected at 0, 15, 30, 60, 120 min and 24 h for UV-Visible analysis. The reaction is considered substantially complete when the principal absorbance peak stabilizes in wavelength and intensity.

3. Recovery and Purification

Following synthesis, the dispersion is centrifuged at approximately 10,000-12,000 rpm for 15 min. The supernatant is removed and the pellet is washed three times with deionized water to reduce unbound plant metabolites and residual ions. A portion is redispersed in sterile deionized water for zeta-potential and antimicrobial testing; the remainder is dried at low temperature for XRD and FTIR analysis. All treatments are prepared in three independent synthesis batches.

Table 2. Experimental Design and Controlled Parameters for Plant-Mediated AgNP Synthesis

Variable	Proposed setting	Controlled purpose	Recorded output
Plant extract	Neem / Moringa / lemon peel	Primary independent factor	Extract ID and batch
AgNO ₃ concentration	1 mM	Equal precursor availability	Initial silver concentration
Extract:AgNO ₃ volume	10:90 mL	Comparable reducing input	Final reaction volume
pH	8.0 ± 0.2	Controls nucleation/reduction	Final pH
Temperature	40 ± 2 °C	Control's reaction kinetics	Reaction temperature
Reaction time	Up to 24 h	Tracks completion	UV-Vis peak versus time
Replicates	n = 3 independent batches	Reproducibility	Mean ± SD

4. Physicochemical Characterization

UV-Visible spectroscopy is performed from 300 to 700 nm to identify the AgNP surface-plasmon resonance, commonly observed in the visible region. FTIR spectra of dried extract and AgNP samples are recorded to identify shifts in O-H, C=O, C-O, N-H, and related bands associated with phytochemical binding. XRD is used to confirm crystalline metallic silver and to identify the characteristic face-centered-cubic reflections. SEM provides surface morphology and aggregation information, while TEM is preferred for direct particle-size and shape measurement. At least 100 particles per treatment are measured from representative TEM micrographs to calculate mean diameter and size distribution. EDX may be used to verify elemental silver, and zeta potential is measured to assess colloidal stability. The use of multiple complementary methods follows current green-AgNP characterization recommendations [14].

5. Antimicrobial Evaluation

Antimicrobial testing is conducted using authenticated reference strains, for example *Staphylococcus aureus* ATCC 25923 as a Gram-positive organism and *Escherichia coli* ATCC 25922 as a Gram-negative organism. All microbiological work should be performed only in an appropriately equipped laboratory under institutional biosafety procedures. Agar-well diffusion provides a screening measure of inhibition-zone diameter. Sterile AgNP dispersions are tested at equal silver-equivalent concentrations, alongside plant-extract blanks, AgNO₃ control, sterile-water negative control, and a standard antibiotic positive control. For quantitative comparison, broth microdilution is used to determine minimum inhibitory concentration (MIC) across a two-fold dilution series. Growth/no-growth endpoints are recorded after the validated incubation period specified by the laboratory standard operating

procedure. The study does not interpret inhibition zones alone because diffusion depends on particle dispersion and matrix effects.

6. Statistical Analysis and Performance Criteria

For each plant treatment, UV-Vis peak wavelength, particle size, zeta potential, inhibition-zone diameter, and MIC are reported as mean $\pm$ standard deviation from independent replicates. One-way ANOVA is used to test the

effect of plant source on continuous outcomes, followed by an appropriate post-hoc comparison when the overall test is significant ($p < 0.05$). Pearson or Spearman correlation is used to examine whether particle size or zeta potential is associated with antimicrobial performance. A successful synthesis is defined by a reproducible plasmon peak, crystalline silver confirmation by XRD, nanoscale morphology by TEM/SEM, and antimicrobial activity that is greater than the corresponding plant-extract blank.

Table 2. Evaluation Criteria for Physicochemical Characterization and Antibacterial Activity of AgNPs

Outcome	Method	Acceptance criterion	Interpretation
AgNP formation	UV-Vis	Stable visible SPR peak	Confirms nanoparticle optical response
Crystallinity	XRD	FCC Ag reflections present	Confirms crystalline metallic silver
Size/morphology	TEM/SEM	Predominantly nanoscale, limited aggregation	Relates structure to activity
Surface chemistry	FTIR	Band shifts versus plant extract	Indicates capping/reducing groups
Colloidal stability	Zeta potential	Higher absolute potential preferred	Lower aggregation tendency
Antibacterial activity	Zone + MIC	Activity > extract blank	Confirms AgNP-associated effect

Results

Table 4 presents an illustrative data pattern showing how outcomes from the proposed methodology would be reported. These numerical values are methodological examples

for analysis and formatting; they are not presented as measurements from an actual experiment. Actual results must be replaced with laboratory data generated using the stated protocol.

Table3. Characterization and Antibacterial Performance of AgNPs Synthesized from Different Plant Sources

Plant source	SPR peak (nm)	TEM size (nm)	Zeta (mV)	S. aureus zone (mm)	E. coli zone (mm)	MIC range ($\mu\text{g/mL}$)
A. indica	421 $\pm$ 2	18.6 $\pm$ 4.1	-27.4 $\pm$ 2.3	18.7 $\pm$ 1.2	16.9 $\pm$ 1.0	15.6-31.3
M. oleifera	426 $\pm$ 3	24.8 $\pm$ 5.6	-22.1 $\pm$ 1.8	16.5 $\pm$ 0.9	14.8 $\pm$ 1.1	31.3-62.5
C. limon peel	418 $\pm$ 2	16.9 $\pm$ 3.8	-30.2 $\pm$ 2.0	20.1 $\pm$ 1.0	18.4 $\pm$ 0.8	15.6-31.3
Plant blanks	No Ag SPR	Not applicable	Not applicable	6-9	5-8	>250

The illustrative pattern demonstrates the type of relationship expected from a controlled comparison: treatments yielding smaller, better-dispersed particles may show larger inhibition zones and lower MIC values. Such an interpretation is consistent with the broader evidence that AgNP antimicrobial behavior depends on physicochemical properties and multiple cell-targeting mechanisms [7]. The example also emphasizes the importance of comparing AgNPs with the plant extract alone, because phytochemicals may themselves possess antibacterial activity. Therefore, a larger inhibition zone for the nanoparticle dispersion relative to the corresponding extract blank is

required before activity is attributed principally to the synthesized AgNPs.

A second result expected from the protocol is convergence between characterization methods. A stable UV-Vis surface-plasmon peak supports formation, but it is not sufficient by itself. XRD should confirm crystalline silver, TEM should demonstrate nanoscale dimensions, FTIR should show the participation of plant-derived functional groups, and zeta-potential measurements should provide an indication of dispersion stability. This multi-method approach reflects the characterization framework discussed by Sharma et al. [14] and the plant-

mediated synthesis literature reviewed by Ahmed et al. [4] and Vanlalveni et al. [9].

The methodology also provides a direct basis for comparing plant sources. Prior studies show that different plants can generate different morphologies and antimicrobial responses [10]-[13]. If statistically significant differences are observed, they can be linked to extract composition and nanoparticle characteristics rather than to uncontrolled changes in precursor concentration or reaction temperature. This is the principal methodological advantage of the proposed comparative design.

Conclusion and Discussion

This methodology paper presents a reproducible framework for the green synthesis and characterization of silver nanoparticles using plant extracts for antimicrobial applications. The design uses three plant sources under standardized AgNO₃ concentration, extract ratio, pH, temperature, and reaction time, followed by complementary characterization through UV-Vis, FTIR, XRD, SEM/TEM, and zeta potential. Antibacterial performance is evaluated by both agar diffusion and MIC determination with appropriate positive, negative, precursor, and plant-extract controls. Replicate synthesis and statistical analysis are included so that conclusions are based on reproducibility rather than single-batch observations. The literature up to 2022 supports the feasibility of this approach. Early plant-assisted synthesis demonstrated that biological molecules can reduce and stabilize silver nanoparticles [1], while subsequent reviews established plant extracts as practical green synthesis systems [2]-[4]. Specific studies with *A. indica* and other medicinal plants showed that plant chemistry can produce stable AgNPs with measurable antimicrobial activity [5], [10]-[13]. Mechanistic evidence indicates that the biological action of AgNPs arises from several interacting processes and depends on particle characteristics [7]. Accordingly, the proposed method deliberately connects antimicrobial results with particle size, crystallinity, surface chemistry, and dispersion stability rather than treating antimicrobial testing as an independent endpoint. The main strengths of the proposed workflow are low-cost aqueous processing, reduced reliance on harsh chemical reducing agents, explicit process controls, and a direct comparison among plant sources. Important limitations are natural variability in phytochemical composition, possible batch-to-batch differences in extract strength, aggregation during storage, and the challenge of translating in-vitro antimicrobial activity into safe biomedical use. Green synthesis does not

automatically imply nontoxicity; therefore, any future therapeutic or consumer application would require cytotoxicity, dose-response, stability, and environmental-safety studies. Future work should replace the illustrative data with experimentally measured results, quantify extract phytochemistry, optimize pH and extract ratio using factorial design, and evaluate whether the best-performing formulation retains antimicrobial activity during storage.

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