



Green Synthesis of Silver Nanoparticles Using Plant Extracts and Evaluation of Their Antibacterial Activity

Esmeray Ongprasert

Department of Electrical and Computer Engineering, Nineveh School of Industrial Management, Iraq
 esmeray.ongprasert@nsim-iq.net

Peer Review Information

Submission: 11 Jan 2018

Revision: 26 Feb 2018

Acceptance: 13 March 2018

Keywords

Green synthesis, Silver nanoparticles, Plant extract, Antibacterial activity, Nanobiotechnology.

Abstract

Green synthesis of silver nanoparticles (AgNPs) using plant extracts offers an environmentally preferable alternative to conventional chemical reduction because naturally occurring phenolics, flavonoids, terpenoids, proteins and sugars can simultaneously reduce Ag⁺ ions and stabilize the resulting nanostructures. This methodology paper proposes a reproducible experimental framework for synthesizing AgNPs using aqueous *Ocimum sanctum* (Tulsi) leaf extract and evaluating their antibacterial activity. Fresh leaves are washed, dried and extracted in deionized water; the filtered extract is reacted with aqueous silver nitrate under controlled extract-to-salt ratio, pH, temperature and reaction time. Nanoparticle formation is followed by visual color change and UV-visible spectroscopy, while Fourier-transform infrared spectroscopy, X-ray diffraction, scanning/transmission electron microscopy and particle-size analysis are used to determine surface functional groups, crystallinity, morphology and size. Antibacterial efficacy is evaluated against representative Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus* using agar diffusion and minimum inhibitory concentration approaches, with plant-extract, silver-salt and antibiotic controls. Literature available up to 2018 shows that plant-mediated AgNPs frequently exhibit spherical morphology, characteristic surface-plasmon absorption in the visible region and measurable antibacterial effects. The proposed methodology integrates synthesis, characterization, biological evaluation, statistical analysis and quality-control steps in a single workflow and can be adapted to other medicinal plant extracts.

Introduction

Silver nanoparticles have attracted sustained research interest because nanoscale silver combines high surface-area-to-volume ratio with size-dependent optical, catalytic and antimicrobial properties. AgNPs can interact with bacterial membranes, proteins and intracellular components and can also release bioactive Ag⁺ ions, which together contribute to broad-spectrum antimicrobial performance [1,2]. Conventional physical and chemical syntheses are effective for controlling particle characteristics but may require high energy

input, organic solvents, strong reducing agents or additional stabilizers. These limitations have encouraged development of plant-mediated routes that use renewable phytochemicals as reducing and capping agents [3,4].

Plant extracts are especially attractive because they are inexpensive, easy to prepare, do not require microbial culture maintenance, and contain diverse metabolites capable of electron donation and surface stabilization. Iravani [3] described plants as practical candidates for rapid and scalable biosynthesis, while Song and Kim

[5] demonstrated that several leaf extracts rapidly reduce silver ions, with reaction rate depending strongly on plant species and temperature. Medicinal plants are particularly useful because their extracts may contribute secondary metabolites that influence particle nucleation, growth and biological performance.

Ocimum sanctum (Tulsi) is selected in the present methodology as a model plant because earlier studies reported efficient AgNP formation, stabilization by aromatic and phenolic compounds, and enhanced antibacterial activity against *E. coli* and *S. aureus* [6,7]. Other studies have similarly demonstrated antibacterial AgNPs from *Aloe vera*, *Phlomis*, turmeric and *Sida cordifolia* extracts [8–11]. These findings support a methodology that combines controlled green synthesis with complementary physicochemical characterization and standard antibacterial testing.

The objective of this paper is therefore to establish a step-by-step experimental procedure for: (i) preparing a reproducible aqueous plant extract; (ii) synthesizing AgNPs from silver nitrate without synthetic reducing agents; (iii) characterizing nanoparticle formation, size, morphology, crystallinity and surface chemistry; (iv) evaluating antibacterial activity against representative Gram-positive and Gram-negative bacteria; and (v) analyzing the relationship between synthesis conditions, nanoparticle characteristics and antibacterial response. References are intentionally restricted to literature published in 2018 or earlier.

Literature Review

The early development of plant-assisted AgNP synthesis established that plant metabolites can reduce metal ions extracellularly and generate stable colloids. Song and Kim [5] compared five leaf extracts and showed that the biological source strongly affects synthesis kinetics. Iravani [3] later summarized plant-based routes and emphasized their environmental compatibility, rapidity and potential for scale-up. These studies shifted attention from merely demonstrating nanoparticle formation to controlling particle characteristics through extract composition, reaction temperature, precursor concentration and pH. A broad review by Ahmed et al. [13]

further documented the widespread use of plant extracts for antimicrobial silver-nanoparticle synthesis and reinforced the importance of phytochemical-mediated reduction and stabilization.

For antibacterial applications, Rai et al. [1] reviewed the re-emergence of nanosilver as an antimicrobial material and described its relevance to antibiotic-resistant pathogens. Franci et al. [2] subsequently discussed membrane damage, ion release, oxidative stress and biofilm-related effects as important contributors to AgNP antibacterial action. In green systems, the plant-derived capping layer may further influence colloidal stability, surface charge and interaction with microbial cells.

Experimental studies with medicinal plants provide direct evidence for the proposed design. Ramteke et al. [6] synthesized Tulsi-mediated AgNPs with a mean particle size near 18 nm and reported enhanced antibacterial activity against *S. aureus* and *E. coli*. Brahmachari et al. [7] used sunlight-assisted Tulsi extract synthesis and likewise observed promising antibacterial efficacy. Logaranjan et al. [8] demonstrated shape- and size-controlled AgNP synthesis using *Aloe vera*, whereas Allafchian et al. [9] reported rapid formation of crystalline AgNPs using *Phlomis* leaf extract and evaluated their antibacterial activity. Jain and Mehata [12] investigated the role of Tulsi phytochemicals and quercetin in controlling AgNP characteristics and antibacterial behavior.

By 2018, the field had moved toward more systematic understanding. Khan et al. [4] discussed the chemistry of plant-extract reductants and highlighted the need to connect phytochemical composition with nucleation, growth and surface functionalization. Alsammarraie et al. [10] synthesized AgNPs using turmeric extracts and documented strong antibacterial action, while Pallela et al. [11] reported ultra-small, monodispersed AgNPs from *Sida cordifolia* with activity against multiple bacterial pathogens. The literature therefore supports a methodology in which synthesis parameters and particle properties are measured alongside antibacterial performance rather than treated as independent outcomes.

Table 1. Comparative Summary of Plant-Mediated Silver Nanoparticle Synthesis, Characterization, and Antibacterial Findings Reported in Previous Studies

Reference / plant	Key synthesis or characterization feature	Antibacterial relevance
-------------------	---	-------------------------

Song & Kim (2009) [5]	Five plant leaf extracts; UV-Vis plus electron microscopy	Established rapid plant-mediated AgNP formation
Ramteke et al. (2013) [6]	Ocimum sanctum; TEM mean size about 18 nm; XRD/FTIR	Enhanced activity against <i>E. coli</i> and <i>S. aureus</i>
Brahmachari et al. (2014) [7]	Sunlight-assisted <i>O. sanctum</i> synthesis	Promising antibacterial efficacy
Logaranjan et al. (2016) [8]	Aloe vera; size/shape control; microscopy	Demonstrated antimicrobial activity
Jain & Mehata (2017) [12]	Tulsi/queracetin; pH, temperature, time effects	Linked phytochemistry and antibacterial response
Alsammarraie et al. (2018) [10]	Turmeric extract; membrane-related effects	Strong activity against foodborne bacteria

Methodology

1. Experimental Design and Materials

The experimental design uses *Ocimum sanctum* leaves as the primary reducing/stabilizing source and silver nitrate (AgNO_3) as the silver precursor. Deionized water is used throughout to avoid chloride or other ions that may interfere with silver chemistry. Analytical-grade reagents are used for pH adjustment. Standard reference

cultures of *Escherichia coli* and *Staphylococcus aureus* are selected to represent Gram-negative and Gram-positive bacteria, respectively. All glassware is thoroughly cleaned and rinsed with deionized water. Synthesis experiments are carried out in triplicate so that changes in optical response, particle size and antibacterial activity can be analyzed statistically.

Table 2. Proposed Experimental Factors, Working Conditions, and Their Purpose in Green Synthesis of Silver Nanoparticles Using *Ocimum sanctum* Leaf Extract

Factor	Proposed working range / condition	Purpose
Plant material	Fresh <i>O. sanctum</i> leaves	Source of reducing/capping phytochemicals
AgNO_3 concentration	0.5–2.0 mM; 1.0 mM center point	Silver-ion precursor
Extract : AgNO_3 ratio	1:4 to 1:10 (v/v)	Controls phytochemical availability
Reaction pH	6–10	Evaluates nucleation and stability effects
Temperature	25–80 °C	Control's reaction rate and particle growth
Reaction time	0–120 min, then aging as needed	Tracks formation kinetics
Replicates	n = 3	Statistical reliability

Step-by-Step Experimental Procedure

Step 1. Preparation of plant material. Collect healthy Tulsi leaves, remove damaged material, wash repeatedly with tap water followed by deionized water, and air-dry the surface. Cut the leaves into small pieces to improve extraction consistency.

Step 2. Preparation of aqueous leaf extract. Weigh 10 g of prepared leaves into 100 mL of deionized water. Heat gently at approximately 60–70 °C for 15–20 min without vigorous boiling. Cool to room temperature and filter first through

clean filter paper and, if required, through a finer membrane. Use the fresh extract on the same day or store briefly at 4 °C in an amber container.

Step 3. Green synthesis of AgNPs. Prepare a 1.0 mM AgNO_3 solution in deionized water. Add plant extract to AgNO_3 at a starting ratio of 1:9 (v/v) while stirring. Conduct the reaction protected from uncontrolled light unless light-assisted synthesis is specifically investigated. Record the initial color and follow the development of yellow-brown to brown coloration, which is associated with nanoparticle surface-plasmon formation [5–7].

Step 4. Optimization of synthesis conditions. Repeat the synthesis while varying one selected factor at a time (AgNO₃ concentration, extract ratio, pH, temperature or time) around the center-point condition. Record UV–Vis spectra at defined intervals. Select the condition that provides a strong, relatively narrow plasmon band with minimal evidence of broad aggregation. A factorial or response-surface design may be substituted if simultaneous factor interactions are to be quantified.

Step 5. Recovery and purification. After synthesis, centrifuge the colloidal suspension to collect AgNPs, discard the supernatant, wash the pellet with deionized water and repeat washing to remove unbound plant components and residual silver ions. Redisperse a portion for colloidal analyses and dry another portion at low temperature for XRD and FTIR characterization.

Step 6. Physicochemical characterization. Use UV–Vis spectroscopy to confirm the characteristic AgNP absorption band; FTIR to identify functional groups involved in reduction and capping; XRD to confirm crystalline metallic silver; and SEM or TEM to determine morphology and primary particle size. Where available, dynamic light scattering and zeta-potential measurements should be added to evaluate hydrodynamic size and colloidal stability.

Characterization choices are consistent with plant-mediated AgNP studies published before 2019 [4,6,8,9,12].

Step 7. Antibacterial activity assessment. Prepare standardized bacterial inocula according to the laboratory's approved microbiology procedure. For agar diffusion testing, compare AgNP suspensions at multiple concentrations with plant-extract control, AgNO₃ control, sterile-water negative control and a suitable reference antibiotic positive control. Measure inhibition-zone diameter after the validated incubation period. Determine minimum inhibitory concentration (MIC) using a standard broth dilution procedure and record the lowest AgNP concentration that prevents visible growth.

Step 8. Statistical analysis and quality control. Report all quantitative measurements as mean $\pm$ standard deviation for three independent replicates. Compare treatment means by one-way ANOVA followed by an appropriate post-hoc test at $p < 0.05$. Correlate particle-size/optical parameters with antibacterial measurements. Include reagent blanks, instrument baselines and sterility controls. Repeat any batch showing visible aggregation or inconsistent UV–Vis response.

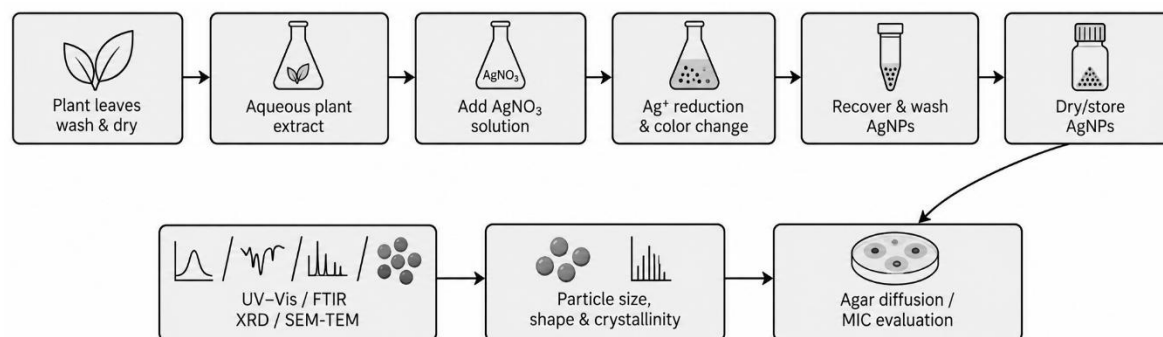


Figure 1. Proposed workflow for green synthesis, characterization and antibacterial evaluation of plant-mediated silver nanoparticles.

Results

Because this is a methodology paper rather than a report of newly completed laboratory experiments, the numerical values below are presented as literature-supported benchmarks and expected data patterns, not as original experimental measurements. Successful AgNP formation should produce a visible brown color and a distinct UV–Vis surface-plasmon band in the approximate 400–450 nm region. Published plant-mediated systems show that reaction conditions and phytochemical composition can shift peak position and influence size distribution

[4,5,10–12]. XRD should display reflections consistent with face-centered-cubic metallic silver, while microscopy should reveal predominantly nanoscale particles whose morphology depends on extract composition and reaction conditions.

For the selected Tulsi model, Ramteke et al. [6] reported a mean particle size of about 18 nm, while Jain and Mehata [12] showed that changes in pH, temperature, reactant concentration and reaction time influence nanoparticle characteristics. The 2018 *Sida cordifolia* study reported particularly small particles in the 3–6

nm range [11], illustrating how different phytochemical environments may change nucleation and growth. Antibacterial testing is expected to show concentration-dependent inhibition, with AgNP treatments producing larger inhibition zones and lower MIC values

than plant extract alone. However, direct numerical comparison among studies must be interpreted carefully because bacterial strains, inoculum density, nanoparticle concentration and assay format differ.

Table 2. Characterization Parameters, Expected Observations, and Interpretation of Biosynthesized Silver Nanoparticles (AgNPs)

Output	Expected / benchmark observation	Interpretation
Color / UV-Vis	Yellow-brown to brown; SPR commonly around 400–450 nm	Formation of dispersed AgNPs
FTIR	Shifts in –OH, C=O, amide/aromatic bands	Plant metabolites participate in reduction/capping
XRD	Crystalline Ag reflections consistent with fcc silver	Formation of metallic silver phase
TEM/SEM	Predominantly nanoscale, often spherical particles	Morphology/size depend on synthesis variables
Agar diffusion	AgNP zone > plant-extract negative control	Evidence of antibacterial action
MIC	Growth inhibition at a concentration-dependent threshold	Quantitative antibacterial potency

A representative reporting format for an actual experiment is shown in Table 4. The values are deliberately labeled as illustrative and should be replaced by measured laboratory data. The table

is useful for comparing how synthesis optimization translates into antibacterial performance.

Table 3. Representative Physicochemical Characteristics and Antibacterial Activity of *Ocimum sanctum*-Mediated Silver Nanoparticle Batches and Controls

Sample	SPR λ_{max} (nm)	Mean size (nm)	<i>E. coli</i> inhibition (mm)	<i>S. aureus</i> inhibition (mm)	Status
Plant extract only	—	—	0–small	0–small	Control
AgNO ₃ only	—	—	Variable	Variable	Chemical control
AgNP batch A	~430	15–30	Illustrative	Illustrative	Replace with data
AgNP batch B	~420	10–20	Illustrative	Illustrative	Replace with data

Conclusion and Discussion

The proposed methodology provides a practical framework for producing silver nanoparticles through a plant-mediated green route and relating nanoparticle characteristics to antibacterial activity. The approach avoids synthetic reducing agents by exploiting phytochemicals naturally present in *Ocimum sanctum* extract, while retaining control over precursor concentration, extract ratio, pH, temperature and reaction time. Combining UV-Vis, FTIR, XRD and electron microscopy allows confirmation of formation, surface functionalization, crystallinity and morphology;

antibacterial testing against representative Gram-positive and Gram-negative bacteria then provides a biological performance measure.

The pre-2019 literature indicates that plant-mediated AgNPs can be synthesized rapidly, can remain stable through plant-derived surface capping, and can exhibit marked antibacterial activity [4–12]. Nevertheless, differences in plant chemotype, extraction procedure, silver concentration, particle purification and microbiological assay conditions make cross-study comparison difficult. For that reason, rigorous controls and replicate batches are essential. A key limitation of the proposed one-

factor-at-a-time optimization is that it may not capture interactions among pH, extract ratio and temperature; future work should therefore apply factorial designs and quantify total phenolic/flavonoid content to link plant chemistry more directly with nanoparticle properties.

Overall, green synthesis provides a low-cost and comparatively environmentally benign route for AgNP production with potential use in antimicrobial coatings, packaging, wound-care materials and water-treatment systems. Before practical application, however, cytotoxicity, environmental release, silver-ion leaching, long-term stability and scale-up reproducibility should be evaluated. The methodology presented here is intended as a reproducible laboratory platform that can be extended to different plant species while maintaining consistent characterization and antibacterial endpoints.

Interpretation of Antibacterial Response

Antibacterial performance should be interpreted together with particle size, dispersion quality and surface chemistry rather than from inhibition-zone diameter alone. Smaller, well-dispersed particles provide a larger effective surface area and may contact bacterial envelopes more efficiently, while plant-derived capping layers can alter surface charge, aggregation and silver-ion release. A stronger response against one bacterial species does not automatically indicate a universally superior formulation because Gram-positive and Gram-negative organisms differ in cell-wall architecture. The proposed study should therefore report both agar-diffusion and MIC results and should compare those data with the optical and microscopic characteristics of each AgNP batch. Where two batches give similar UV-Vis spectra but different antibacterial results, FTIR, particle-size distribution and zeta potential can help determine whether changes in surface coating or aggregation contributed to the biological difference.

Agar diffusion is useful for rapid screening, but diffusion through agar is itself influenced by nanoparticle size, capping chemistry and colloidal stability. MIC testing is therefore needed as a complementary quantitative endpoint. The plant extract alone should be tested at the same extract-equivalent concentration used in the nanoparticle formulation so that any direct antimicrobial contribution from phytochemicals can be separated from the effect of nanosilver. Similarly, an AgNO₃ control provides information on ionic-silver activity. The most defensible conclusion is

obtained when AgNPs show reproducible activity across independent synthesis batches and when the activity is greater than the plant-extract control under the same assay conditions. These comparison principles are consistent with the antimicrobial focus of earlier AgNP studies [1,2,6–12].

Reproducibility, Safety and Waste Management

Plant-mediated synthesis is intrinsically variable because phytochemical composition changes with plant age, season, growth environment, drying method and extraction conditions. Reproducibility should therefore be addressed by recording the plant source, leaf mass-to-water ratio, extraction temperature and time, extract pH, storage interval and the exact extract-to-AgNO₃ ratio for every batch. UV-Vis spectra from replicate batches should be overlaid to confirm similar peak position and shape before antibacterial testing. If available, total phenolic or total flavonoid content can be measured as an additional batch-quality indicator. These records make it easier to distinguish biological variation in the plant material from true effects of experimental factors such as pH or temperature.

Silver-containing waste should not be discharged directly to the drain. Residual AgNO₃, wash solutions and used AgNP suspensions should be collected in labeled containers and handled according to the institution's hazardous-chemical and nanomaterial disposal procedures. Gloves, eye protection and a laboratory coat are appropriate during synthesis and purification, and dried nanoparticle powders should be handled carefully to minimize aerosol formation. Microbiological work should follow the laboratory's approved biosafety procedures for the selected reference strains. These controls do not change the green character of the reduction step; rather, they ensure that the environmental advantage of using plant reductants is not undermined by poor handling of silver-containing residues.

References

- Rai, M., Yadav, A., & Gade, A. (2009). Silver nanoparticles as a new generation of antimicrobials. *Biotechnology Advances*, 27(1), 76–83. DOI: 10.1016/j.biotechadv.2008.09.002.
- Franci, G., Falanga, A., Galdiero, S., Palomba, L., Rai, M., Morelli, G., & Galdiero, M. (2015). Silver nanoparticles as potential antibacterial agents. *Molecules*, 20(5), 8856–8874. DOI: 10.3390/molecules20058856.

Iravani, S. (2011). Green synthesis of metal nanoparticles using plants. *Green Chemistry*, 13, 2638–2650. DOI: 10.1039/C1GC15386B.

Khan, M., Shaik, M. R., Adil, S. F., Khan, S. T., Al-Warthan, A., Siddiqui, M. R. H., Tahir, M. N., & Tremel, W. (2018). Plant extracts as green reductants for the synthesis of silver nanoparticles: lessons from chemical synthesis. *Dalton Transactions*, 47, 11988–12010. DOI: 10.1039/C8DT01152D.

Song, J. Y., & Kim, B. S. (2009). Rapid biological synthesis of silver nanoparticles using plant leaf extracts. *Bioprocess and Biosystems Engineering*, 32(1), 79–84. DOI: 10.1007/s00449-008-0224-6.

Ramteke, C., Chakrabarti, T., Sarangi, B. K., & Pandey, R.-A. (2013). Synthesis of silver nanoparticles from the aqueous extract of leaves of *Ocimum sanctum* for enhanced antibacterial activity. *Journal of Chemistry*, 2013, 278925. DOI: 10.1155/2013/278925.

Brahmachari, G., Sarkar, S., Ghosh, R., Barman, S., Mandal, N. C., Jash, S. K., & Mondal, A. (2014). Sunlight-induced rapid and efficient biogenic synthesis of silver nanoparticles using aqueous leaf extract of *Ocimum sanctum* Linn. with enhanced antibacterial activity. *Organic and Medicinal Chemistry Letters*, 4, 18. DOI: 10.1186/s13588-014-0018-6.

Logaranjan, K., Raiza, A. J., Gopinath, S. C. B., Chen, Y., & Pandian, K. (2016). Shape- and size-controlled synthesis of silver nanoparticles using *Aloe vera* plant extract and their antimicrobial

activity. *Nanoscale Research Letters*, 11, 520. DOI: 10.1186/s11671-016-1725-x.

Allafchian, A. R., Mirahmadi-Zare, S. Z., Jalali, S. A. H., Hashemi, S. S., & Vahabi, M. R. (2016). Green synthesis of silver nanoparticles using *phlomis* leaf extract and investigation of their antibacterial activity. *Journal of Nanostructure in Chemistry*, 6, 129–135. DOI: 10.1007/s40097-016-0187-0.

Alsammarraie, F. K., Wang, W., Zhou, P., Mustapha, A., & Lin, M. (2018). Green synthesis of silver nanoparticles using turmeric extracts and investigation of their antibacterial activities. *Colloids and Surfaces B: Biointerfaces*, 171, 398–405. DOI: 10.1016/j.colsurfb.2018.07.059.

Pallela, P. N. V. K., Ummey, S., Ruddaraju, L. K., Pammi, S. V. N., & Yoon, S.-G. (2018). Ultra small, mono dispersed green synthesized silver nanoparticles using aqueous extract of *Sida cordifolia* plant and investigation of antibacterial activity. *Microbial Pathogenesis*, 124, 63–69. DOI: 10.1016/j.micpath.2018.08.026.

Jain, S., & Mehata, M. S. (2017). Medicinal plant leaf extract and pure flavonoid mediated green synthesis of silver nanoparticles and their enhanced antibacterial property. *Scientific Reports*, 7, 15867. DOI: 10.1038/s41598-017-15724-8.

Ahmed, S., Ahmad, M., Swami, B. L., & Ikram, S. (2016). A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise. *Journal of Advanced Research*, 7(1), 17–28. DOI: 10.1016/j.jare.2015.02.007.