



**INTERNATIONAL JOURNAL OF
PHARMACEUTICAL SCIENCES**
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: <https://www.ijpsjournal.com>



Review Article

Green Synthesis of Silver Nanoparticles Using Plant Extracts: A Review

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ARTICLE INFO

Published: 24 Oct 2026

Keywords:

Green synthesis, Silver nanoparticles, Plant extract, Neem, Tulsi, Antibacterial activity, Surface plasmon resonance

DOI:

10.5281/zenodo.23609735

ABSTRACT

The present review focuses on the green synthesis of silver nanoparticles using plant extracts. Silver nanoparticles are widely studied because of their strong antimicrobial activity and their use in dressings, coatings and other biomedical products, but the physical and chemical methods of their synthesis need costly equipment or toxic chemicals. In green synthesis, the phytochemicals of plant extracts, such as polyphenols, flavonoids, terpenoids and proteins, reduce silver ions to silver nanoparticles and also cap and stabilise them, in a simple, low-cost and environmentally friendly one-step process. The review describes the methods of synthesis, the mechanism of green synthesis, the factors affecting it, the profile of Neem (*Azadirachta indica*) and Tulsi (*Ocimum sanctum*) leaves and of silver nitrate, the procedure of synthesis, the techniques of characterisation, the evaluation of the antibacterial activity by the agar well diffusion method, and the important reports on the subject. The review indicates that plant extracts can be effectively used for the preparation of silver nanoparticles, which show surface plasmon resonance at about 400 to 450 nm and antibacterial activity, while further work on safety, characterisation and stability is required.

INTRODUCTION

Nanotechnology deals with the design, production and use of materials whose dimensions lie in the nanometre range, generally between 1 and 100 nm. At this size a material has a very large surface area compared with its volume, and its optical, electrical, chemical and biological properties can differ greatly from those of the same material in bulk form. Because of these properties, nanoparticles are being used in medicine, drug

delivery, diagnostics, cosmetics, textiles, food packaging and environmental treatment.[1,2]

Among the metal nanoparticles, silver nanoparticles (AgNPs) are the most widely studied because of their strong antimicrobial activity. They have been reported to act against bacteria, fungi and some viruses, and they are used in wound dressings, coatings for medical devices, water purification and personal care products. [3,4] Their antibacterial action is explained by several

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.



mechanisms: attachment to the bacterial cell wall and membrane, release of silver ions, interaction with sulphur-containing proteins and enzymes, and generation of reactive oxygen species.[4,5,6,7]

Silver nanoparticles also show a characteristic colour, because the free electrons on the particle surface oscillate with the incident light (surface plasmon resonance), which gives an absorption band in the visible region and makes the particles easy to detect by UV-visible spectroscopy.[8]

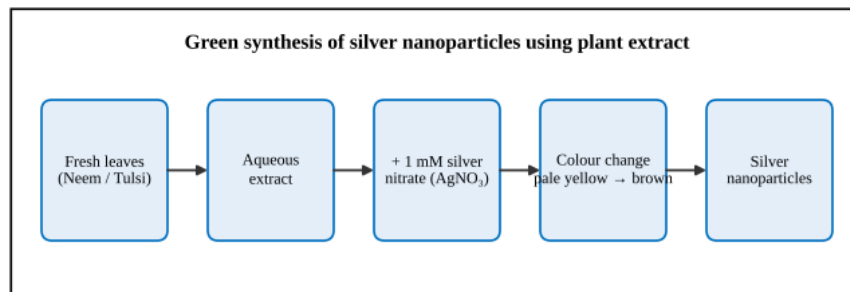


Figure 1: Green Synthesis of Silver Nanoparticles Using Plant Extract

Methods of Synthesis of Nanoparticles

Nanoparticles can be prepared by physical, chemical and biological methods, each of which has its own

advantages and drawbacks.[1,2] The methods are compared in the table below.

Table: Methods of Synthesis of Silver Nanoparticles[1,2,9]

Sr. No.	Method	Examples	Limitations
1	Physical	Evaporation-condensation, laser ablation, ball milling	Need costly equipment and high energy
2	Chemical	Reduction of a silver salt with sodium borohydride, citrate or other reducing agents, with a stabiliser	Use of toxic chemicals; hazardous waste
3	Biological (green)	Reduction by plant extracts, bacteria, fungi or algae	Variable composition of the biological material; control of size needs study

Green Synthesis Using Plant Extracts

Green synthesis uses biological materials in place of hazardous chemicals. Plant extracts are particularly convenient because they are easily available, safe to handle and need no special culture conditions, which makes the process faster and simpler than synthesis with microorganisms.[1,2,10,11] Plant extracts contain polyphenols, flavonoids, terpenoids, alkaloids, proteins and sugars. These phytochemicals reduce the silver ions (Ag^+) of silver nitrate to neutral silver atoms (Ag^0), and the same or other molecules from the extract

adsorb on the particle surface and act as capping and stabilising agents that prevent aggregation.[1,2,12]

The process can be pictured in three steps: first the silver ions are reduced by the phytochemicals and the atoms collect to form small nuclei; then the nuclei grow into nanoparticles; and finally the particles are capped by plant molecules and become stable in suspension. The first visible sign is a change of colour of the reaction mixture from pale yellow or colourless to yellowish-brown or dark brown. The method is simple, inexpensive, mostly one-step, can be done at room temperature and is environmentally friendly.[1,10]

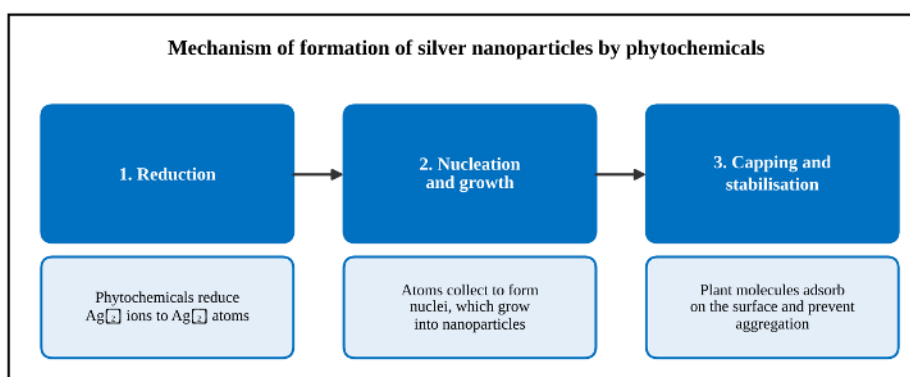


Figure 2: Mechanism of Formation of Silver Nanoparticles by Plant Phytochemicals

Factors Affecting the Synthesis

Table: Factors Affecting the Green Synthesis of Silver Nanoparticles[2,12]

Sr. No.	Factor	Effect
1	Type and concentration of extract	Decides which and how much reducing and capping phytochemical is available
2	Concentration of silver nitrate	Too low gives few particles; too high may cause aggregation and a precipitate
3	Ratio of extract to silver nitrate	Affects the rate of reduction, the size and the stability
4	Temperature	Higher temperature generally speeds up the reaction
5	pH	Changes the reducing power of the phytochemicals and the charge on the particles
6	Reaction time	Colour and absorption intensity increase until the reaction is complete
7	Light	Silver salts are light-sensitive; reactions are usually protected from strong light

Profile of Materials

The plants, the silver salt and the nanoparticles are described here, because their properties decide how the synthesis is carried out. Neem and Tulsi are two easily

available plants that have been used for the green synthesis of silver nanoparticles.[13,14]

Neem (*Azadirachta indica*)

Table: Profile of Neem[13,15]

Attribute	Details
Biological source	Fresh or dried leaves of <i>Azadirachta indica</i> A. Juss.
Family	Meliaceae
Common names	Neem, Kadunimb
Part used	Leaves
Main constituents	Limonoids (such as azadirachtin, nimbin), flavonoids, polyphenols, tannins, glycosides
Known uses	Traditional antibacterial, antifungal, anti-inflammatory and skin uses
Relevance	Neem leaf broth has been used to reduce silver and gold ions to nanoparticles

Tulsi (*Ocimum sanctum*)

Table: Profile of Tulsi[14,15]

Attribute	Details
Biological source	Fresh or dried leaves of <i>Ocimum sanctum</i> L. (also called <i>Ocimum tenuiflorum</i>)
Family	Lamiaceae



Attribute	Details
Common names	Tulsi, Holy basil
Part used	Leaves
Main constituents	Volatile oil rich in eugenol, flavonoids, phenolic acids such as rosmarinic acid, ursolic acid
Known uses	Traditional use for cough, fever and as an antimicrobial and adaptogen
Relevance	Tulsi leaf extract has been used to prepare silver nanoparticles with antimicrobial activity

Silver Nitrate and Silver Nanoparticles

Table: Profile of Silver Nitrate

Attribute	Details
Formula and molecular weight	AgNO ₃ ; 169.87 g/mol
Description	Colourless crystalline solid; dissolves freely in water
Behaviour	Light-sensitive; darkens on exposure to light and organic matter; strong oxidising agent
Role	Source of silver ions (Ag ⁺), which are reduced to silver atoms (Ag ⁰)
Handling	Stains skin and clothing black; irritant to eyes and skin; silver waste must be collected, not drained

The weight of silver nitrate needed for a 1 mM solution is worked out as follows: one mole is 169.87 g, so 1 mM (0.001 mole per litre) needs 0.16987 g per litre, which is about 0.017 g in 100 mL.

Silver nanoparticles are particles of metallic silver, usually 1 to 100 nm in size. In suspension they appear yellow to brown, depending on the size, shape and concentration, and they show a surface plasmon

resonance absorption band, which for spherical silver particles generally lies in the region of about 400 to 450 nm.[8] Their antibacterial action makes them useful for dressings, coatings and other biomedical uses.[3,4]

Role of Plant Phytochemicals

Table: Role of Phytochemicals in the Green Synthesis[1,2,12]

Sr. No.	Phytochemical class	Likely role
1	Polyphenols and flavonoids	Donate electrons and reduce Ag ⁺ to Ag ⁰ ; also adsorb on the particle surface
2	Terpenoids and essential-oil components	Reduction and capping
3	Alkaloids and glycosides	Possible reducing and stabilising action
4	Proteins and amino acids	Capping and stabilising the particles
5	Reducing sugars	Reduction of silver ions

Method of Synthesis of Silver Nanoparticles Using Plant Extract

The procedure generally followed for the synthesis of silver nanoparticles with a leaf extract is described step by step below.[1,2,12]

Step 1: Collection and Washing of Plant Material

Fresh, healthy leaves are collected, authenticated by a botanist or pharmacognosist, and washed thoroughly with tap water and then with distilled water to remove dust.[15]

Step 2: Preparation of Plant Extract

About 10 g of finely chopped leaves are boiled in 100 mL of distilled water for 10 to 15 minutes. The mixture is cooled to room temperature and filtered through Whatman No. 1 filter paper, and the filtrate is used as the aqueous extract, preferably fresh.[13,14]



Step 3: Preparation of Silver Nitrate Solution

A 1 mM solution of silver nitrate is prepared by dissolving about 0.017 g of silver nitrate in 100 mL of distilled water. The solution is kept in an amber-coloured bottle, because it is light-sensitive, and is prepared fresh for each experiment.

Step 4: Mixing of Extract and Silver Nitrate

Ninety millilitres of the silver nitrate solution are taken in a clean conical flask, and 10 mL of the plant extract (a ratio of about 1:9) are added slowly with constant stirring on a magnetic stirrer. The mixture is kept at room temperature or warmed gently to about 60 °C and protected from strong light.

Step 5: Observation of Colour Change

A change in colour from pale yellow or colourless to yellowish-brown or dark brown shows the reduction of silver ions and the formation of nanoparticles. It usually occurs within minutes to a few hours. Two controls are run in parallel: silver nitrate without extract, and extract without silver nitrate, and neither should show the brown colour.

Step 6: UV-Visible Spectroscopic Analysis

The reaction mixture is diluted suitably and scanned between 300 and 700 nm. The appearance of an absorption band at about 400 to 450 nm indicates the surface plasmon resonance of the silver nanoparticles. [8] A sharp, narrow band suggests small and uniform particles, whereas a broad band or a band at a longer wavelength suggests larger or aggregated particles.

Step 7: Optimisation of Reaction Conditions

One variable is changed at a time, while the others are kept constant, and the effect is judged from the colour and from the intensity and position of the UV-visible peak (see the table below).[2,12]

Step 8: Recovery and Purification

The reaction mixture is centrifuged at about 10,000 rpm for 15 minutes, the supernatant is discarded and the pellet is resuspended in distilled water and centrifuged again. The washing is repeated two or three times to remove free extract and unreacted silver ions.

Step 9: Drying and Storage

The final pellet is dried at about 50 to 60 °C in an oven, powdered gently and stored in a closed, light-protected container.

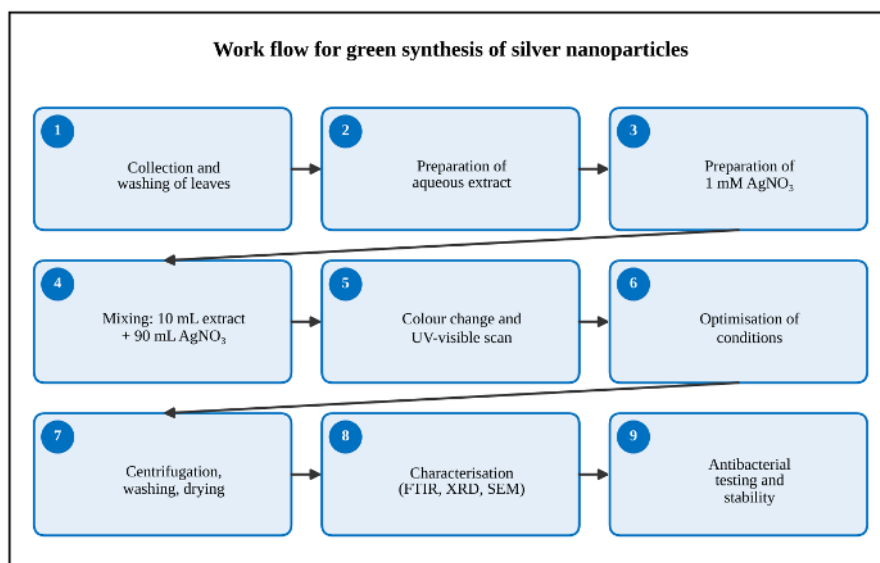


Figure 3: Work Flow for Green Synthesis of Silver Nanoparticles

Table: Variables Studied for the Optimisation of the Synthesis[2,12]

Sr. No.	Variable	Levels commonly studied	Reference value
1	Ratio of extract to silver nitrate (v/v)	1:9, 2:8, 3:7	1:9
2	Silver nitrate concentration	0.5 mM, 1 mM, 2 mM	1 mM
3	Temperature	Room temperature, about 60 °C	Room temperature
4	pH of the mixture	About 4, 7 and 9	Natural pH
5	Reaction time	0, 15, 30, 60 minutes and up to 24 hours	Until the colour is stable

Preliminary Phytochemical Screening of the Extract **Table: Phytochemical Tests for the Plant Extract[15]**

Sr. No.	Constituent	Test	Positive indication
1	Phenolic compounds and tannins	5% ferric chloride test; lead acetate test	Blue-black or green colour; white precipitate
2	Flavonoids	Shinoda test; alkaline reagent test	Pink to magenta colour; yellow colour fading on acid
3	Alkaloids	Mayer's, Dragendorff's and Wagner's tests	Cream, orange to reddish-brown precipitate
4	Saponins	Foam (froth) test	Persistent foam
5	Terpenoids	Salkowski test	Reddish-brown ring at the interface
6	Carbohydrates	Molisch's test	Purple ring at the junction
7	Proteins	Biuret test	Violet colour

Characterisation of Silver Nanoparticles

The nanoparticles formed are confirmed and characterised by one or more of the techniques given below. UV-visible spectroscopy is the simplest and is

available in most college laboratories, while the other techniques need special facilities.[2,12]

Table: Techniques Used for the Characterisation of Silver Nanoparticles[2,8]

Sr. No.	Technique	Information obtained
1	Visual observation	Colour change, formation of a precipitate (simplest first indication)
2	UV-visible spectroscopy	Surface plasmon resonance peak at about 400 to 450 nm
3	FTIR spectroscopy	Functional groups of the extract compounds present on the particle surface
4	X-ray diffraction (XRD)	Crystalline nature of the silver; estimate of crystallite size
5	SEM or TEM	Shape and size of the particles
6	Particle size and zeta potential	Average size, distribution and surface charge (stability)

Evaluation of Antibacterial Activity

The antibacterial activity of the nanoparticles is commonly tested by the agar well diffusion method.[16,17] Sterile nutrient agar plates are seeded with a standardised suspension of a Gram-positive organism (for example *Staphylococcus aureus*) and a Gram-negative organism (for example *Escherichia coli*). Wells of

about 6 mm are cut with a sterile borer and filled with a fixed volume of the test samples, the plates are incubated at 37 °C for 24 hours, and the diameter of the zone of inhibition is measured in millimetres. Each test is done in triplicate.

Table: Arrangement of Wells in the Agar Well Diffusion Test[16,17]

Sr. No.	Well	Content
1	Test	Silver nanoparticle suspension made with the plant extract
2	Control 1	Plant extract alone
3	Control 2	1 mM silver nitrate solution
4	Negative control	Distilled water
5	Positive control	A standard antibiotic of known activity



The stability of the nanoparticle suspension is checked by storing it in amber bottles at room temperature and in a refrigerator, and by recording the colour, any sedimentation and the UV-visible spectrum at fixed intervals. A stable suspension keeps its colour and absorption peak, whereas aggregation shows as loss of colour, a shifted or broadened peak or a deposit.

Review of Literature

Iravani (2011) reviewed the green synthesis of metal nanoparticles using plants, compared plant-based synthesis with physical and chemical methods, and discussed the role of plant metabolites in reducing metal ions and stabilising the particles.[1]

Mittal et al. (2013) reviewed the synthesis of metallic nanoparticles using plant extract and described the possible mechanisms of formation, the plant parts used, the factors that control the size and shape, and the applications of the nanoparticles.[2] **Kumar and Yadav (2009)** reviewed the plant-mediated synthesis of silver and gold nanoparticles and their applications.[10]

Ahmed et al. (2016) reviewed the plant extract mediated synthesis of silver nanoparticles for antimicrobial applications, and summarised the plants used, the methods of characterisation and the antimicrobial activity of the nanoparticles.[12] **Makarov et al. (2014)** and **Kharissova et al. (2013)** reviewed the green synthesis of metal nanoparticles using plants and the greener approaches to nanoparticle synthesis.[9,11]

Shankar et al. (2004) reported the rapid synthesis of gold, silver and bimetallic gold-core silver-shell nanoparticles using neem (*Azadirachta indica*) leaf broth, which showed that simple neem leaf broth can reduce metal ions.[13] **Singhal et al. (2011)** prepared silver nanoparticles using *Ocimum sanctum* (Tulsi) leaf extract and screened their antimicrobial activity.[14]

Rai et al. (2009) described silver nanoparticles as a new generation of antimicrobials, and discussed their activity against bacteria and fungi, their mode of action and their potential biomedical uses.[3] **Morones et al. (2005)** studied the bactericidal effect of silver nanoparticles, and **Sondi and Salopek-Sondi (2004)** used *Escherichia coli* as a model Gram-negative bacterium to study the antimicrobial effect of silver nanoparticles; **Feng et al. (2000)** studied the

mechanism of the antibacterial effect of silver ions on *E. coli* and *S. aureus*. [5,6,7]

Prabhu and Poulouse (2012) reviewed the mechanism of the antimicrobial action, the methods of synthesis, the medical applications and the toxicity effects of silver nanoparticles, and are a reminder that safety must be considered together with activity.[4] **Mulvaney (1996)** described the surface plasmon spectroscopy of nanosized metal particles, which is the basis of the use of UV-visible spectroscopy for the detection of silver nanoparticles.[8]

Balouiri et al. (2016) reviewed the methods for the in vitro evaluation of antimicrobial activity, including agar diffusion, and **Bauer et al. (1966)** described the standardised single disk method for antibiotic susceptibility testing, which is the basis of diffusion tests in which the zone of inhibition is measured.[16,17]

Research gap

Many plants have been used to make silver nanoparticles, but the reaction conditions, the extract strength and the methods of testing differ between studies, so the plants cannot easily be compared with each other. Neem and Tulsi have each been reported separately. [13,14] Studies that run both plants through the same simple procedure, vary the main reaction conditions systematically and test the product against the same organisms are few.

Safety and Waste Disposal

Silver nitrate stains skin and clothing and irritates the eyes and skin, so gloves, a laboratory coat and safety glasses should be worn. Silver-containing solutions and nanoparticles should not be poured into the drain, and should be collected in a labelled container for heavy-metal waste. Bacterial cultures must be handled with aseptic technique, and used plates and cultures must be autoclaved before disposal. Since the toxicity of silver nanoparticles has been reported,[4] nanoparticles prepared in a student laboratory are meant for laboratory study only and should not be used on humans or animals.



CONCLUSION

Green synthesis of silver nanoparticles with plant extracts such as Neem and Tulsi is a simple, low-cost, one-step and environmentally friendly alternative to the physical and chemical methods. The phytochemicals of the extract act as both reducing and capping agents, and the formation of the nanoparticles can be easily followed by the colour change and by the surface plasmon resonance band at about 400 to 450 nm in the UV-visible spectrum. The extract-to-silver nitrate ratio, the silver nitrate concentration, the temperature, the pH and the reaction time are the main variables that decide the quality of the product.

The nanoparticles show antibacterial activity, which can be tested by the agar well diffusion method, and they have potential uses in dressings, coatings and other biomedical products. However, the reaction conditions and the testing methods differ between studies, the size, shape and crystallinity need special facilities for confirmation, and the toxicity of the nanoparticles must be studied before any use in humans. Further work with other plants, more organisms, determination of the minimum inhibitory concentration, cytotoxicity testing and long-term stability studies is required.

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HOW TO CITE: Ramankit Jaiswal, Mahesh Padghan, Kshitij Shirsat, Kunal Khandeshe, Manoj Dakhore, Krushna Sharma, K. Biyani, Green Synthesis of Silver Nanoparticles Using Plant Extracts: A Review, Int. J. of Pharm. Sci., 2026, Vol 4, Issue 10, 3404-3412. <https://doi.org/10.5281/zenodo.23609735>

