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Phytosynthesis of silver nanoparticles using *Pterocarpus santalinus* leaf extract and their antibacterial properties

Kasi Gopinath, Shanmugam Gowri and Ayyakannu Arumugam*

Abstract

Green synthesis is one of the rapid, reliable, and best routes for the synthesis of silver nanoparticles (Ag NPs). The current study revealed that the aqueous leaf extract of *Pterocarpus santalinus*, which contains steroids, saponins, tannins, phenols, triterpenoids, flavonoids, glycosides, and glycerides, is found to be responsible for bioreduction during the synthesis of spherical Ag nanoparticles. The formed Ag NPs were characterized by ultraviolet-visible (UV-vis), Fourier transform-infrared (FT-IR), X-ray diffraction (XRD), atomic force microscopy (AFM), energy-dispersive X-ray spectroscopy (EDX), and scanning electron microscopy (SEM) analysis. UV-vis spectra of the aqueous medium containing silver nanoparticles showed a surface plasmon resonance peak at 418 nm. FT-IR analysis was performed to analyze the biomolecules responsible for the reduction of Ag NPs. XRD results confirmed the presence of silver nanoparticles with face-centered cubic structure. The EDX analysis showed the completed inorganic composition of the synthesized Ag NPs. AFM analysis exemplified the results of particle sizes (41 nm). The calculated crystallite sizes are in the range of 20 to 50 nm, and the spherical nature of the Ag NPs was ascertained by SEM. The synthesized Ag NPs exhibited good antibacterial potential against gram-positive and gram-negative bacterial strains. The zone of inhibition effect of antibacterial activity depends upon the concentration of Ag NPs.

Keywords: *Pterocarpus santalinus*; Leaf extract; Nanoparticles; Green synthesis; Antimicrobial activity

Background

The application of nanoscale materials and structures, usually ranging from 1 to 100 nm, is an emerging area of nanoscience and nanotechnology. Silver nanoparticles (Ag NPs) have rapidly increased due to their unusual optical, chemical, electronic, photo-electrochemical, catalytic, magnetic, antibacterial, and biological labeling properties [1]. Silver nanoparticles were used in broad range of applications like biomedical [2], drug delivery [3], food industries [4], agriculture [5], textile industries [6], water treatment [7] as an antioxidant [8], antimicrobial [9], anti-cancer [10], cosmetics [11], ointments [12], and larvicides [13]. Nanoparticle synthesis is usually carried out by various physical and chemical methods using various hazardous and toxic chemicals. However, green synthesis approaches of producing Ag NPs are an alternative source of conventional method and possess

excellent antimicrobial activity [1]. Recently, the green synthesis of Ag NPs has been reported using the extract of plants such as *Artocarpus heterophyllus* [14], *Sesbania grandiflora* [15], *Punica granatum* [16], *Pithecellobium dulce* [17], *Malva parviflora* [18], *Iresine herbstii* [19], *Hibiscus cannabinus* [20], *Hevea brasiliensis* [21], *Euphorbia prostrata* [22], *Cissus quadrangularis* [23-25], *Catharanthus roseus* [26], *Coccinia grandis* [27], *Ixora coccinea* [28], *Lippia citriodora* [29], *Manilkara zapota* [30], *Piper pedicellatum* [31], *Prosopis juliflora* [32], and *Semecarpus anacardium* [33].

Pterocarpus santalinus belongs to Fabaceae family, and it has been used for treatment of vomiting, treating eye diseases, mental aberrations, and ulcers. In addition, it has shown to have antipyretic, anti-inflammatory, anthelmintic, tonic, hemorrhage, dysentery, aphrodisiac, and diaphoretic activities [34]. In this present study, we have reported the green synthesis and characterization of silver nanoparticles using *P. santalinus* leaf extract. To the best

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of our knowledge, this is the first report for the synthesis of Ag NPs using *P. santalinus* leaf extract.

Results and discussion

A reduction of Ag NPs was clearly observed when *P. santalinus* leaf extract was added with AgNO₃ solution within 20 min. The colorless solution was changed to brown color which indicates the formation of silver nanoparticles.

UV-vis spectroscopy and Fourier transform-infrared spectroscopy analysis

The mixture of leaf extract and AgNO₃ solution was subjected to ultraviolet-visible (UV-vis) spectroscopy analysis in the recorded spectra, and it showed a observable peak at 418 nm which corresponds to the wavelength of the surface plasmon resonance of Ag NPs (Figure 1). Various reports have established that the resonance peak of silver nanoparticles appears around this region [22]. Fourier transform-infrared (FT-IR) analysis was performed to identify the possible biomolecules responsible for the reduction of the Ag⁺ ions and capping of the reduced Ag NPs synthesized using *P. santalinus* leaf extract. The strong IR bands were observed at 3,382, 2,922, 2,337, 1,614, 1,384, 1,070, and 590 cm⁻¹. The bands which appeared at 3,382 and 2,922 cm⁻¹ correspond to -OH stretching and aliphatic -C-H stretching, respectively [35]. The bands at 2,337 and 1,613 cm⁻¹ are due to the CO₂ and C=C stretching, respectively. The IR bands observed at 1,384 and 1,070 cm⁻¹ may be ascribed to -C-O and -C-O-C stretching modes, respectively. The low band at 590 cm⁻¹ corresponds to C-Cl stretching. Hence, the main components such as steroids, saponins, tannins, phenols, triterpenoids, flavonoids, glycosides, and glycerides present in the leaf extract of *P. santalinus* are prime responsible for the observed reduction and

capping during the synthesis of Ag NPs. The two new strong bands recorded at 825 and 463 cm⁻¹ in the spectra of the synthesized material were assigned to C-H bending and metal (Ag), respectively. The C-H bending peak may be raised due to the reduction of AgNO₃ to Ag nanoparticles (Figure 2).

X-ray diffraction and energy dispersive X-ray spectroscopy analysis

X-ray diffraction pattern (XRD) was recorded for the synthesized Ag NPs (Figure 3). Three distinct diffraction peaks at 38.04°, 44.23°, and 64.37° were indexed with the planes (111), (200), and (220) for the face-centered cubic silver as per the JCPDS card no. 89-3722 [36]. The well-resolved and intense XRD pattern clearly showed that the Ag NPs formed by the reduction of Ag⁺ ions using *P. santalinus* leaf extract are crystalline in nature. Similar results were reported for Ag NPs in the literature [14-22]. The low intense peak at 77.34° belongs to (311) plane. Energy-dispersive X-ray spectroscopy (EDX) (Figure 4) illustrated the chemical nature of synthesized silver nanoparticles using *P. santalinus* leaf extract. The peak was obtained at the energy of 3 keV for silver, and also some of the weak peaks for C, O, Cl, Al, and Na were found. The emission energy at 3 keV indicates the reduction of silver ions to element of silver. Similarly, *C. quadrangularis* stem extract derived silver nanoparticles showed an EDX spectrum, emission energy at 3 keV for silver, and some of the weak signals from Cl, K, O, Ca, Mg, and S were observed [25].

Atomic force microscopy and scanning electron microscopy

Surface topology of the formulated silver nanoparticles was studied by atomic force microscopy (AFM) analysis (Figure 5a, b). The micrographs clearly indicate that the

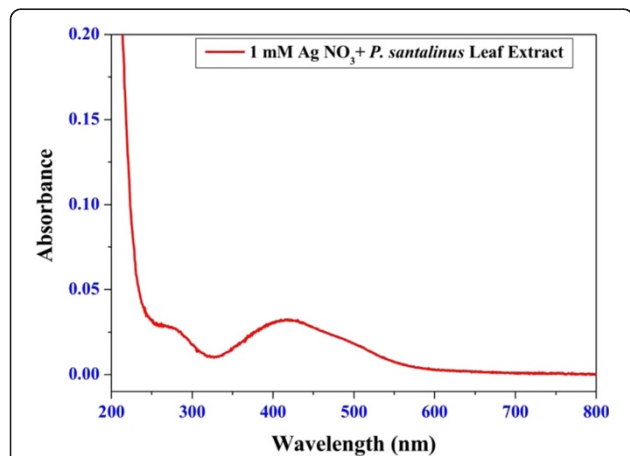


Figure 1 UV-vis spectrum of Ag NPs synthesized by *P. santalinus* leaf extract.

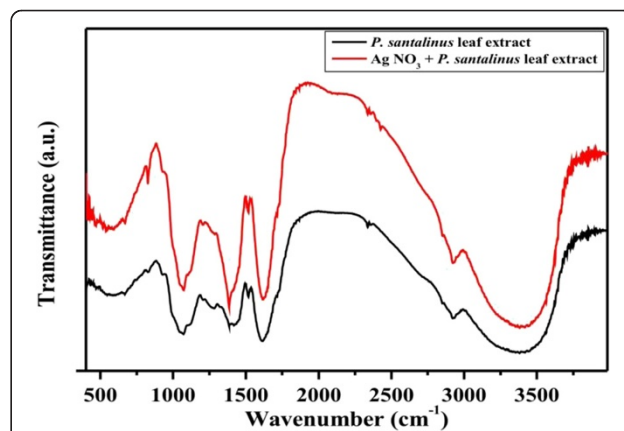


Figure 2 FT-IR spectra of silver nanoparticles synthesized using *P. santalinus* leaf extract.

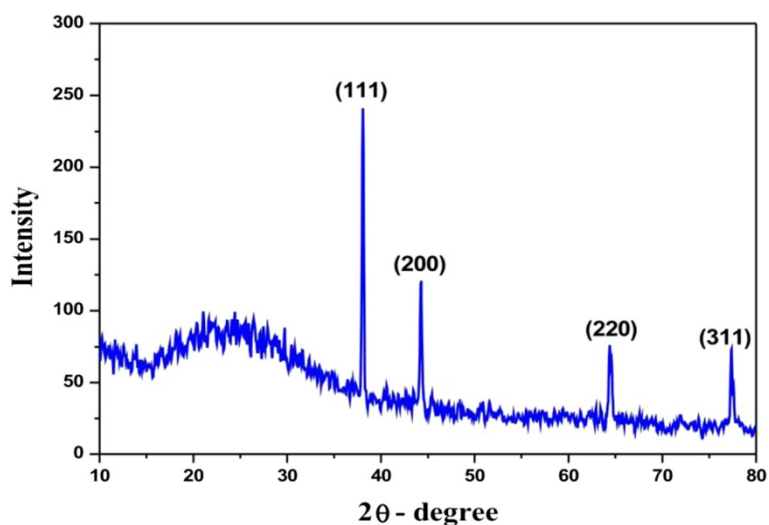


Figure 3 XRD pattern of synthesized silver nanoparticles.

formulated Ag NPs possess spherical shape and have the calculated sizes in the range of 20 to 50 nm. The scanning electron microscopy (SEM) image (Figure 6) further ascertains that the silver nanoparticles are predominantly spherical in morphology with their sizes ranging from 20 to 50 nm and have an average size of about 20 nm.

Antibacterial assay

The antibacterial assay was performed against three gram-positive and gram-negative bacterial pathogens

using green-synthesized Ag NPs. The concentration of silver nanoparticles varied between 10, 50, and 100 μL (Table 1). *Staphylococcus aureus* and *Streptococcus pneumoniae* exhibit similar zone of inhibition for all three concentrations. The most significant effect of silver nanoparticles at low concentration of 10 μL per disc against *S. dysenteriae* is to produce a 2-mm inhibition zone of gram-negative bacteria, and at the same concentration, the nanoparticles did not show any significant effect on *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Proteus vulgaris* (Figure 7). The zone of inhibition increases with

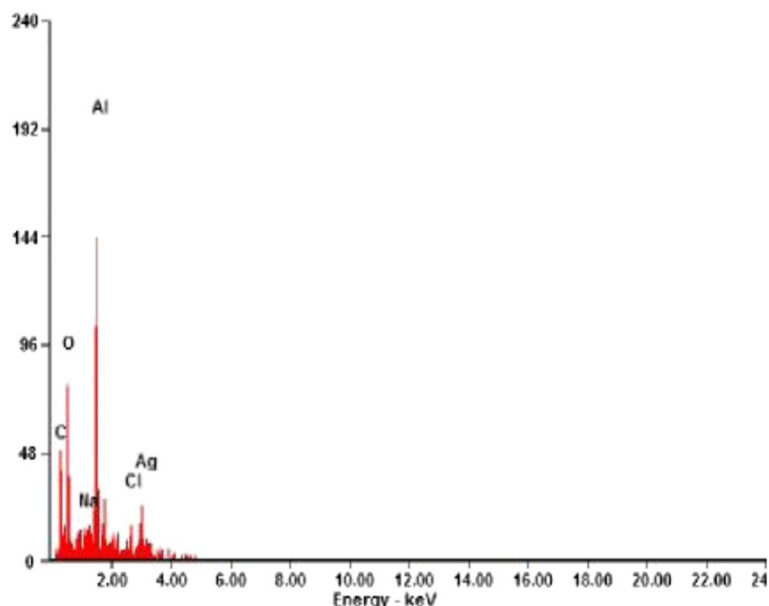


Figure 4 EDX analysis of silver nanoparticles.

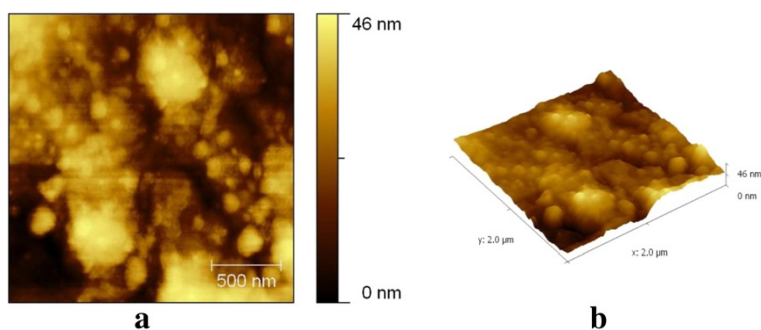


Figure 5 AFM image of synthesized silver nanoparticles (a) 2D image, (b) 3D image.

increasing concentration of silver nanoparticles as the Ag NPs bind with cytoplasmic membrane and killed the bacterial cell. Electrostatic attraction of silver nanoparticles causes damage of bacterial cell membrane to the formation of pits on the surface, and these structural changes take place due to cell expiration [37]. The prokaryotic bacteria have a mesosome cell organelle, and they are present in the inside of plasma membrane. It produced more enzymes as well as major function of cellular respiration, DNA replication, cell division, and increased the surface area of the bacterial cell membrane. Ag NPs interfere with the bacterial cell membrane and bind with mesosome cell organelle and after that reduce the mesosomal function and increase the ROS generation. Ag NPs interact with thiol groups in protein which induced

the inactivation of the bacterial protein synthesis as well as DNA replication [38]. Similarly, oxygen associates with silver and reacts with the sulfhydryl ($-S-H$) groups on cell wall to remove the hydrogen atoms (as water), causing the sulfur atoms to form an R-S-S-R bond, blocked the respiration, and causing the lethal effect of bacterial cells [39]. Ag NPs naturally interact with the membrane of bacteria and disrupt the membrane integrity; silver ions bind to sulfur, oxygen, and nitrogen of essential biological molecules and inhibit bacterial growth [40].

Conclusions

The present work indicates the green-synthesized Ag NPs using *P. santalinus* leaf extract. The AFM and SEM images suggested that the particles are spherical shaped

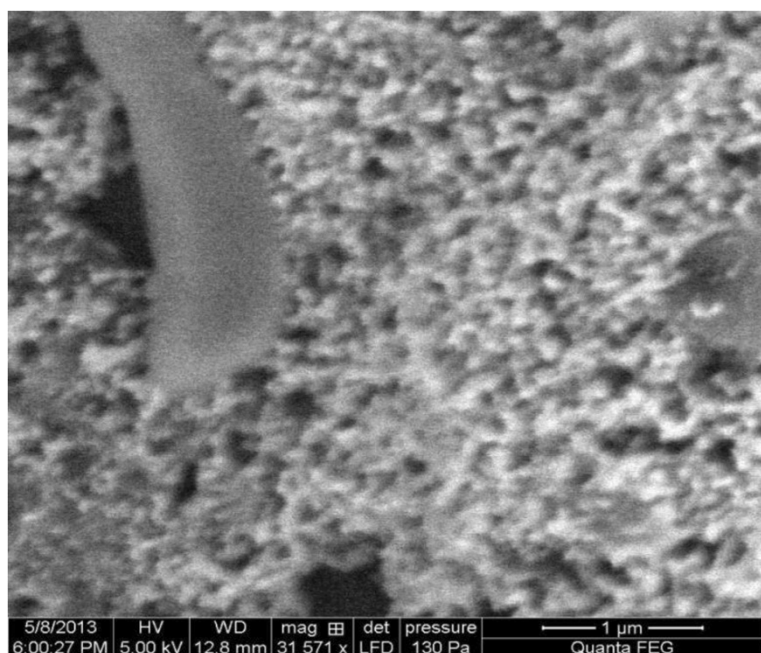


Figure 6 SEM image of Ag NPs using the leaf extract of *P. santalinus*.

Table 1 Antibacterial activity of silver nanoparticles at various concentrations against gram-positive and gram-negative bacteria

Concentration of silver nanoparticles	Zone of inhibition (in mm)					
	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus pneumoniae</i>	<i>Pseudomonas aeruginosa</i>	<i>Proteus vulgaris</i>	<i>Shigella dysenteriae</i>
10 μ L	0	1	1	0	0	2
50 μ L	1	1	1	1	2	2
100 μ L	2	3	3	3	2	3

with average size of 20 nm. The antimicrobial activity depends upon the concentration of Ag NPs to produce the most significant effects against the gram-positive and gram-negative bacteria. This green-synthesized method is rapid, facile, convenient, less time consuming, environmentally safe, and can be applied in a variety of existing applications. This plant leaf extract compounds can be extended to the synthesis of other metal and metal oxide nanoparticles.

Methods

Synthesis of silver nanoparticles using *P. santalinus* leaf extract

Fresh *P. santalinus* plant leaves were collected from Karaikudi (Tamil Nadu, India) and used to retrieve their extraction. First, the leaves were cleaned with tap water, followed by distilled water and then finely cut into small pieces. Ten grams of finely cut leaves was added with 100 mL of double distilled water and boiled at 50°C to 60°C for 5 min. The obtained extraction was filtered using Whatman No. 1 filter paper, and the filtrate was collected in 250-mL Erlenmeyer flask and stored at room temperature for further usage. Then, 1 mL of *P. santalinus* leaf extract was added to 100 mL of 1 mM AgNO₃

solution at room temperature, and the reduction of Ag NPs was clearly observed within the next 20 min.

Characterization

The synthesized silver nanoparticles were subjected to UV-vis analysis in the wavelength range of 350 to 800 nm using Shimadzu spectrophotometer (model UV-1800, Shimadzu, Kyoto, Japan) operating at a resolution of 1 nm. Also, FT-IR analysis was carried out in the range of 400 to 4,000 cm⁻¹. XRD pattern was recorded using Cu K α radiation ($\lambda = 1.54060 \text{ \AA}$) with nickel monochromator in the range of 2θ from 10° to 80°. The average crystallite size of the synthesized Ag NPs was calculated using Scherrer's formula $D = 0.9\lambda/\beta\cos\theta$. EDX analysis for a thin film of the sample prepared on a aluminum foil (1 cm \times 1 cm) by dropping 100 μ L of the sample on the foil and allowed to dry for 30 min for further use. AFM analysis for a thin-film sample was prepared on a glass slide (1 cm \times 1 cm) by dropping 100 μ L of the sample on the slide and allowed to dry for 30 min. The slides were then scanned with AFM (APE Research-model no: A100SGS). The AFM characterization was carried out in ambient temperature in non-contact mode using silicon nitrite tips with varying

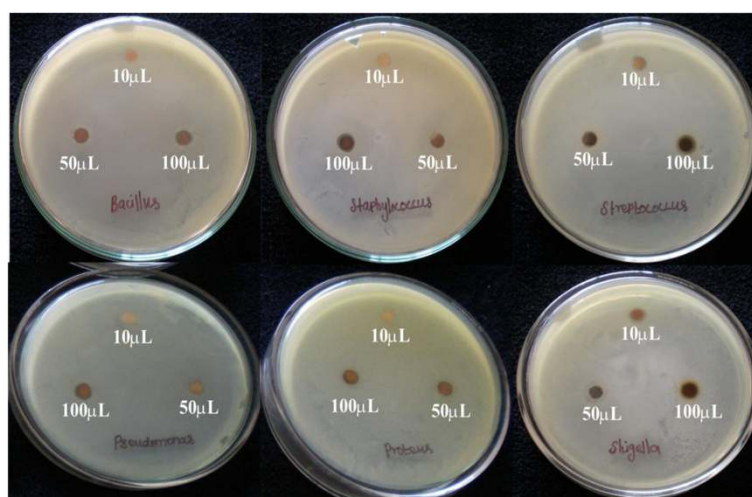


Figure 7 Antibacterial activity of Ag NPs at different concentrations against gram-positive and gram-negative bacteria.

resonance frequencies. The morphology of the synthesized Ag NPs was examined by SEM.

Antibacterial activity of Ag NPs

The minimum inhibitory concentration of the green-synthesized Ag NPs was examined using three gram-positive (*B. subtilis*, *S. aureus*, *S. pneumoniae*) and three gram-negative bacteria (*P. aeruginosa*, *P. vulgaris*, *S. dysenteriae*) were assayed by disk diffusion method. These six bacteria were grown in nutrient broth medium for 24 h. Approximately 20 mL of molten and cooled nutrient agar was poured into the petri dishes. The six tested organisms were swapped over the nutrient agar medium, and the disks containing silver nanoparticles were kept over the medium using sterile forceps. The silver nanoparticle-loaded disks were prepared at different concentrations of 10, 50, and 100 µL/6-mm disc. The plates were incubated for 24 h at 37°C, and the inhibition zone diameters were measured.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

KG and SG carried out the nanoparticles synthesis, characterization, and antimicrobial activity. AA carried out the manuscript preparation. All authors read and approved the final manuscript.

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