Green synthesis of silver nanoparticles (Ag-NPs) from *Olea dioica* Roxb., leaf extracts and its biological activity

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ABSTRACT

Introduction and Aim: The silver nanoparticles have attained a special place in the area of nanotechnology because of their different biological applications. Fabrication of nanoparticles using green synthesis is done because of its wide applications in different fields such as biomedical, medicine, agriculture and food engineering. This study is to develop an easy and eco-friendly method for the synthesis of Ag-NPs using leaf extracts of the medicinal plant.

Materials and Methods: The medicinal plants are rich sources of various medicinal properties. *Olea dioica* Roxb., leaf extract was used to investigate the effects of Ag-NPs having antibacterial activity and antioxidant capacity. The plant leaf extract contains flavonoids, alkaloids, saponins, and phenolic compounds which acts as reducing and stabilizing agents. The green synthesized silver nanoparticles were characterized by various techniques like UV-visible spectrophotometer, FTIR spectroscopy, and SEM analysis.

Results: The synthesis of silver nanoparticles from plant source, and analysis of nano particles by UV-Vis spectra, SEM and FTIR. The biological evaluations of Ag-NPs indicated an excellent inhibitory efficacy, antioxidant and antimicrobial activity for their future applications in medicine.

Conclusion: The synthesized silver nanoparticles exhibited potent antioxidant and antimicrobial activities against Gram-positive and Gram-negative bacteria. The silver (Ag-NPs) nanoparticles synthesized by the pot green synthesis method proves its potential use in various medical applications.

Keywords: Silver nanoparticles; Medicinal plants; Ag-NPs; *Olea dioica* Roxb.,

INTRODUCTION

Nanoscience is based on the manipulation of individual molecules to manufacture materials from them for implementation well below the sub-microscopic level (1). Nanobiotechnology is a well-growing technology as an interdisciplinary eco-friendly research area today and used in broad research sections such as biology, biochemistry, chemistry, physics, biomedicine, nanomedicine, and material engineering (2). It deals with various shapes and size of nanoparticles in the range of 1 to 100 nm. Materials in the nano-dimensions have a high surface to volume ratio that gives them some unique or similar properties that are varied from the same material in bulk which are helpful in various fields such as electronics, biomedical, photonics, etc., The nanoparticles are also utilized in the field of solar energy conversion, and water treatment. Among the various fine metals, silver is preferred as a nanoparticle because of its antibacterial catalytic activity and it has no toxicity towards human beings which is similar to other metals (4). Previously several methods have been used for the synthesis of Ag-NPs, which can be either, chemical, physical or biological methods.

Green synthesis of nanoparticles has been considered as one of the hopeful methods for synthesis of

nanoparticles because of their low toxicity, biocompatibility and eco-friendly nature (3). Thus, synthesis of silver nanoparticles by eco-friendly processes using the plant materials like root, leaf extract, bark, stem, fruit latex, bud is being carried out (5).

Medicinal plants have been used from ancient times to attempt cures for various diseases. The therapeutic power of traditional herbal medicines has been realized and familiar since Rigveda and Atharvaveda. Medicinal plants are potentially renewable natural resources and are generally considered to play a beneficial role in human health care (6-8). In view of this in the present study silver nanoparticles were synthesised using leaves of *Olea dioica* Roxb.

Olea dioica Roxb., is an important ethno medicinal plant medicinal plant belong to *Oleaceae* family. It grows in open evergreen forests up to 1100-1200 m and is distributed throughout the Western Ghats region of Kodagu, Dakshina Kannada, Udupi and Hassan. The plant parts such as roots, bark, and leaves are used for anti-cancer, antioxidant, and anti-AChE activity. Although vast amount of literature is available on the green synthesis of Ag-NPs, to the best of our knowledge no information is available on the synthesis of silver nano particles using the leaves of *Olea dioica* Roxb., In the present investigation a

stable and eco-friendly method has been developed for the synthesis of silver nanoparticles using methanolic extracts of *Olea dioica* Roxb. Thus, synthesised Ag-NPs were evaluated for their antimicrobial activities.

MATERIALS AND METHODS

Chemicals

Silver nitrate, (AgNO_3) was purchased from Himedia Ltd., India. All other chemicals used in this study were of analytical grade.

Preparation of plant extract

Olea dioica Roxb., leaves were collected from Pilikula Mangalore, Dakshina Kannada, Karnataka, India. Freshly collected leaves were washed 3-4 times with tap water and finally washed with distilled water, and shade dried at room temperature for 23 days and mechanically made a fine powder. Fifty

grams of powder was extracted with methanol (12 hours) using Soxhlet extractor. The extracts were evaporated at room temperature and stored in brown bottles at room temperature until screened. This methanolic extract was used for the synthesis of silver nanoparticles.

Synthesis of silver nanoparticles

The methanol leaf extracts of *Olea dioica* Roxb., was used in the bio-reduction of silver ions. Five ml of leaf extract was taken in BOD bottle separately and was mixed with 95 ml of 1mM AgNO_3 solution. The silver nanoparticles synthesized were separated by centrifugation at 10,000 rpm for 10 minutes. The pellets were washed thoroughly at least three times with methanol, ethanol, and water to remove any biological contaminants. The particles were then dried and stored for further analysis (6, 12, 13).

Fig.1 Colour change in the leaf extract and silver nitrate at a different time of incubation

Flow-chart: 1 Change in colour of the solution with time when silver salt was added to leaf extract.

Characterization of silver nanoparticles UV-visible spectral analysis

The bio-reduction of silver nanoparticles was observed by UV-spectroscopy of the solution

between 200 and 700 nm using (Beckman Coulter) UV-Vis spectrophotometer (14).

Effect of different contact time on Ag-NPs concentration

The effect of different contact time on silver nanoparticles concentration in the reaction mixture at different time intervals is exhibited with a colour change of the solution. The intensity of UV-absorption peaks is gradually increased and then becomes stable because the Ag-NPs concentration is initially increased with increase in different contact time which is due to the effect of outside Plasmon character of Ag-NPs (16).

FTIR (Fourier-transform infrared spectroscopy)

For FTIR analysis, the sample was prepared by dispersing the silver nanoparticles uniformly in a matrix of KBr (potassium bromide). The characterization of functional groups on the silver nanoparticles the spectra was scanned in the range of 4000–500 cm^{-1} . The intense bands were compared with standard values to identify the functional groups (15).

SEM analysis

The sample for SEM analysis was prepared by dissolving nanoparticles in 0.1 ml of deionized water. This was placed on a glass coverslip and air-dried. The coverslip itself was used during SEM analysis (Carl Zeiss, Germany, and Model No. EVO LS 15). The images of Ag-NPs were obtained (16) in a scanning electron microscope at different nanometers (25, 50 nm).

Microbial cultures

The microbial cultures used in the present study were obtained from Department of Microbiology, Mangalore University, PG Centre, Chikka Aluvara, Kodagu, Karnataka, India. The cultures used were pathogenic bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus sp.*, *Proteus vulgaris*, *Klebsiella* and *Salmonella sp.* The pure bacterial cultures were sub-cultured on nutrient agar media. Different concentrations of silver nanoparticles in distilled water were used for antibacterial activity. The zone of bacterial inhibition was

recorded after incubating the plates for 24 hours at 37°C (9).

Antioxidant assay

DPPH assay

Antioxidant activity was determined by Brand-Williams method (11) with a slight modification. The absorbance of the reaction mixture was measured at 517 nm after incubating at room temperature in dark condition for 30 min. using ascorbic acid as a standard. The percentage of antioxidant activity was calculated using the following formula.

RESULTS

$$\% \text{ inhibition} = \frac{\text{Absorbance (Control)} - \text{Absorbance (Test)}}{\text{Absorbance (Control)}} \times 100$$

UV-visible spectroscopy

The synthesis of silver nanoparticles from plant leaf extracts

The colour changed to brown due to the addition of 1mM AgNO_3 to the plant extracts. There was no colour change observed in 0 min. After 2-4 hours, colour of the solution changes from green to brown, the UV-Vis absorption spectra were studied at 300 to 700 nm, which confirmed the presence of Ag-NPs (Fig. 2). The absorption peak of silver nanoparticles is at 438 nm.

Different contact time and T_m (temperature) affect the synthesis of nanoparticles (Graph 2) and show the UV- absorption spectra of Ag-NPs at different time in the range of 20-60 min and 20-60°C. The temperature increased the change in the colour of the solution rapidly. When the T_m of the reaction mixture is increased, an increase in the synthesis of silver nanoparticles is logical which is due to the amplification in a decreased rate of Ag^+ ions and the solution colour turning from brown to dark brown within 3 min and decrease in particle size. An increase in T_m above 40-60°C increases the absorbance peak (15).

Graph 1: UV-Vis spectra of silver nanoparticles at different time intervals

Graph 2: Absorption spectra of silver nanoparticles at 1mM concentration. The above figure shows an increase of absorption intensity as a function of Ag-NP concentration

FTIR

FTIR measurement was used to find out the interaction of silver ions with the bioactive components present in the plant leaf extracts that are responsible for stabilizing of Ag-NPs. The absorption

bands in the FTIR spectrum indicate the occurrence of functional groups in the silver nanoparticles (Fig. 2). FTIR results show the band in the range of 3500-500 cm^{-1} ; different band range represents different functional groups (Table 1).

Fig. 2: FTIR spectrum of Ag-NPs

Table 1: FTIR peak values and functional groups of Ag-NPs

Extracts	Peak Value	Functional Group	Functional Group Name	Vibrations
Synthesis of nanoparticles	639.20	C-I	Haloalkane	Stretch
	970-799	C-C	Alkane	-
	1121.21	C-F	Haloalkane	Bending
	2921-2852	C-H	Alkane	Stretch
	1463-1382	C-O, C-F	Alcohols, ethers, esters, and haloalkane	Bend out- of- plane
	1633.57	C=C	Alkene	-
	1721.07	C=O	Acid, saturated	-
	2921-2852	C-H	Alkane	Stretch
	3449.61	O-H	alcohols and Phenols	Stretch

SEM

The morphology of silver nanoparticles was examined using SEM and observed the reduction of AgNO_3 with plant leaf extract. The SEM results also

depicted the morphology and size details of Ag-NPs with high-density. An assembly of spherical and uniform Ag-NPs were observed (Fig. 3).

Fig. 3: SEM image of the biosynthesized silver nanoparticles

Antimicrobial activities

Silver nanoparticles were tested for antibacterial activity against both Gram-positive and Gram-negative pathogenic bacteria. Green synthesized silver nanoparticles exhibited good antibacterial

activity against the pathogenic bacteria (Table 2 and Fig. 4). The silver nanoparticles synthesized using methanolic leaf extract showed good antibacterial activity compared to those synthesised using water extract of leaf.

Fig. 4: The methanol (M) and water (W) extracts of Ag-NPs solution at (15 μ L) different concentration

Table 2: Zone of inhibition (in mm)

Sl. no	Name of the Bacteria	Methanol extract	Water extract
1	<i>E. coli</i>	4 mm	2 mm
2	<i>S. epidermis</i>	2 mm	1 mm
3	<i>B. subtilis</i>	3 mm	1 mm

Antioxidant activity

The antioxidant activity of silver nanoparticles was evaluated by DPPH radical scavenging method. A

potent antioxidant activity was observed, and the activity was increased in a dose dependent manner (Graph 3).

Graph 4: AgNPs at different concentrations and compared with positive control

DISCUSSION

Green synthesis of nanoparticles has been considered as one of the hopeful methods for synthesis of nanoparticles because of their low toxicity, biocompatibility and eco-friendly nature (3, 17). The colour of silver nanoparticles changed to brown due to the addition of 1mM AgNO₃ to the plant extracts. The absorption peak of silver nanoparticles is at 438 nm.

UV-vis spectra were used to observe the effect of contact time and optimum temperature on Ag-NPs concentration in the reaction mixture at altered optimum intervals of the time period. The solution was observed for colour change (Fig. 1 and Flow chart 1). The intensity of UV-vis absorption peaks was increased gradually and then became constant because the concentration of silver nanoparticles was initially increased with increase in temperature and different contact time. Different contact time was obtained for this experiment between 40-60 minutes (Graph) which showed a very broad absorbance peak showing an increasing particle size (10, 18).

FTIR spectrum showed different peak positions at 639.20, 961.20, 1121, 1522.04, 1646.15, 1739.98,

2359.98, 2927, 3369 cm⁻¹. The similarities between the spectra with some marginal shifts in peak position clearly indicates the presence of the residual plant extract in the sample as a capping agent to the silver nanoparticles. The peak located at the different range and functional groups of each peak (Fig. 3 & Table 1). Antimicrobial activity of Ag-NPs was carried out against pathogenic bacteria (Fig. 4 & Table 2). The synthesized Ag-NPs exhibited excellent antibacterial activity against both Gram-positive and Gram negative such as *S. aureus* and *E. coli* bacteria (19, 20). The nanoparticles are also utilized in the field of solar energy conversion, and water treatment. Further, in biomedical application, silver nano particles are being used for anticancer, anti-inflammatory activity.

CONCLUSION

The green synthesis of Ag-NPs from *Olea dioica* Roxb., leaf extract is simple, cost-effective, fast, environment-friendly and the plant leaf phytochemical acts as the reducing agent. The silver (Ag-NPs) nanoparticles synthesized by the one-pot green synthesis method are proved to be potential reagents in various medical and industrial applications.

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