

Synthesis and Evaluation of Antibacterial Activity of Silver Nanoparticles from *Lantana Camara* Leaf Extract

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ARTICLE DETAILS

Article History

Published Online: 10 December 2018

Keywords

Lantana camara, Silver nanoparticles, UV-Visible, FTIR, SEM, Antibacterial activity

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ABSTRACT

There were many work have been made based on the plant and its extract mediated synthesis of nanoparticles. Metal related nanoparticles are usually synthesized by wet chemical methods, where the chemicals used are quite often toxic and flammable. In this work, we describe a cost effective and environment friendly technique for green synthesis of silver nanoparticles from 1mM AgNO₃ solution through the extract of *Lantana camara* leaf as it is acts as a reducing as well as capping agent. Nanoparticles were characterized using UV-Vis absorption spectroscopy and SEM analysis showed the average particle size range 15-40nm with higher density polydispersed spherical in shape. The synthesized silver nanoparticles exhibited potential antibacterial activity against some bacteria such as *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* represented respectively.

1. *P.americana* classification

Nanoscience has been recognized as a new interdisciplinary science. It can be definite as a whole knowledge on important properties of nano-size substances [1]. The attach 'nano' designates one billionth or 10⁹ units. It is commonly believed in the perspective of nanoscience and nanotechnologies, the units should only be those of dimensions, rather than of any other unit of scientific measurement. It is generally established that nanoparticles are groups of atoms in the range of 1–100 nm size. Nanoparticles display completely new or enhanced properties based on definite physical appearance such as size, morphology and distribution [2].

Metal related nanoparticles can be synthesized by chemical, physical and biological routes; the physical approach that uses numerous methods such as condensation/evaporation and laser ablation. The chemical approach in the metal ions in solution is reduced in conditions favoring the successive development of aggregates or small metal clusters [3]. Many metals like titanium, copper, silver, gold and iron were commonly used for the synthesis of nanoparticle. Among the noble metals, silver nanoparticles have become the focus of intensive research due to its varied ranges of application for various sectors of industry [4]. Currently, biosynthetic methods engaging naturally occurring reducing agents such as biological microorganism, polysaccharides such as fungus or plants and bacteria extract, i.e. green chemistry, have appeared as a modest and feasible alternative to more complex chemical and physical synthetic procedures to obtain AgNPs [5].

In the current eras, better progress of green synthesis of nanoparticles is expected because of its unbelievable usage in all fields of science disipline. There were various work have been created based on the plant extract intervened synthesis of nanoparticles. Many plants including *Bacopa monnieri* [6],

and *Catharanthus roseus* [7] used for the synthesis of AgNPs Keeping in view, in the present study to explore the novel approaches for the biosynthesis of silver nanoparticles using *Lantana camara* leaf and evaluate the antibacterial activity.

2. Materials and Methods

2.1. Chemicals

All the experiments were conducted at room temperature. Chemicals used for the production of silver nanoparticles are Analytical grade silver nitrate (AgNO₃) purchased from Merck, India.

2.2. Collection of plant materials

The *Lantana camara* leaves were collected in January 2015 from Alangottai, Mannargudi taluka, Tamil Nadu from a single herb. A Voucher specimen (EB001) has been deposited at the Rapinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil Nadu, India.

2.3. Preparation of leaf extract

The dried leafs were pulverized well with mortar and pestle to make a powder. 20 grams of *Lantana camara* leaves powder was mixed into 100 ml of deionized water and the mixture was boiled for 10 min. TThe leaf extract was filtered with Whatman No. 1 filter paper after cooling. The extract was kept at 4 C for further study.

2.4. Synthesis of Ag nanoparticles using leaf extracts

Five ml of *Lantana camara* leaf extract was added to 45 ml of 1 mM aqueous AgNO₃ solution in a 250 ml Erlenmeyer flask and incubated in the dark at 5hrs at room temperature. A control setup as without leaf extract also maintained. The Ag nanoparticle solution thus achieved was purified by repeated centrifugation at 10,000 rpm for 15 min followed by re-dispersion of the pellet in de-ionized water. Then the AgNPs were dried for using SEM analysis [8].

2.5. UV-Vis and FTIR Spectra analysis

The reduction of Ag^+ ions was observed by determining the UV-Vis spectrum of the reaction medium at 5 hours after diluting a small aliquot of the sample into distilled water. The formed pellet is dissolved using deionized water and filtered through whatman filter paper No: 42. This filtrate containing silver nanoparticles are used for Fourier transmission Infrared spectroscopy (FTIR).

2.6. SEM analysis of silver nanoparticles

Scanning electron microscopic (SEM) analysis was done using ZEISS machine. The sample was prepared as thin films on a carbon coated copper grid by just dropping a very small amount of the sample on the grid. Extra solution was removed using a blotting paper and then the films on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

2.7. Antibacterial activity

2.7.1. Microorganisms

Escherichia coli (Gram negative), *Staphylococcus aureus* (Gram positive) and *Bacillus subtilis* (Gram positive) were the microorganisms used and they were obtained from Microbial type culture collection (MTCC) at the institute of Microbial Technology (IMTECH), Chandigarh, India.

2.7.2. Antimicrobial assay

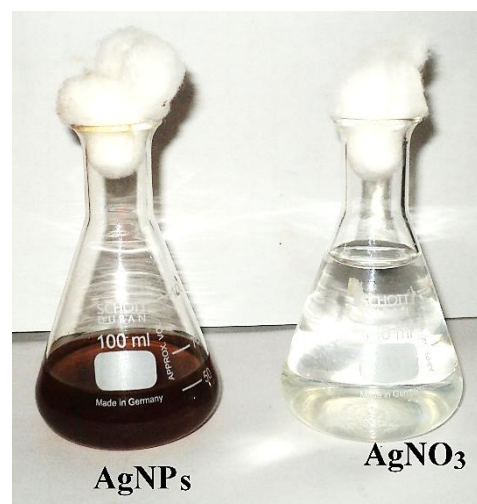
Antibiogram was done by disc diffusion method [9, 10] using herbal extracts. Petri plates were prepared by pouring 30 ml of NA medium for bacteria. The test organism was inoculated on solidified agar plate with the assistance of micropipette and blowout and permitted to dry for 10 mins. The surfaces of media were inoculated with fungi /bacteria from a broth culture. A sterile cotton swab is immersed into a consistent bacterial test suspension and used to equally inoculate the entire surface of the Nutrient agar plate. Briefly, inoculums comprising *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* on Nutrient agar plates for bacteria. Using sterile forceps, the sterile filter papers (6 mm diameter) containing each 30 μ l of plant extract, AgNO_3 solutions, AgNPs and Standard solution as Chloramphenicol were laid down on the surface of inoculated agar plate. The plates were incubated at 37 $^\circ\text{C}$ for 24 h for the bacteria and at room temperature (30 \pm 1). Each sample was tested in triplicate.

3. Results and Discussion

3.1. Synthesis of silver nanoparticles

The synthesis of silver nanoparticles through leaf extracts were carried out. Leaf extract is used as reducing agent as distinctive properties catalytic and chemical stability. Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications, makes this method potentially exciting for the large-scale synthesis of other inorganic materials (nanomaterials). The aqueous silver ions when exposed to herbal extracts were reduced in solution, thereby leading to the formation of silver hydrosol. The time duration of change in colour varies from plant to plant. The phytochemicals present in the leaf extract were considered responsible for the reduction of silver ions. It is well known that silver nanoparticles exhibit yellowish - brown colour in aqueous solution due to excitation of surface plasmon

vibrations in silver nanoparticles. The appearances of yellowish-brown colour (Fig. 1) in the reaction vessels suggest the formation of silver nanoparticles (SNPs) [11].



AgNO_3 = 1 mM AgNO_3 without *Lantana camara* extract.
AgNPs = 1 mM AgNO_3 with *Lantana camara* leaf extract after 5 hrs of incubation (Brown colour)

Fig. 1 Formation of brown colour after addition of AgNO_3 indicate synthesis of AgNPs in the process of reduction of Ag^+ to Ag nanoparticles and control (AgNO_3)

3.2. UV-Vis and FTIR Spectra analysis

It is commonly predictable that UV-Vis spectroscopy could be used to study size and shape-controlled nanoparticles in aqueous suspensions. Fig. 2 illustrate the UV-Vis spectrum noted from the reaction medium next 5 hours. The UV-vis spectra of the reaction mixture of silver nitrate solution with *Lantana camara* leaf extract at the peaks observed at 420 nm indicate the presence of silver nanoparticles which is synthesized by *Lantana camara* extract, the peak was raised due to the effect of surface plasmon resonance of electrons in the reaction mixture and the broadening of peak indicated that the particles are polydispersed. Appearance of this peak assigned to a surface plasmon, is well-documented for various metal nanoparticles with size ranging from 2 nm to 100 nm [12].

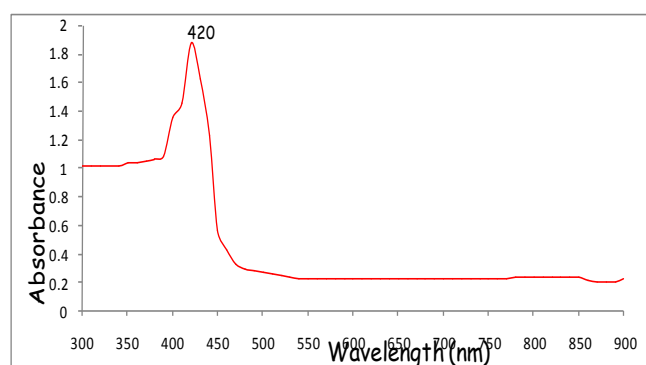


Fig. 2 UV-Vis absorption spectrum of silver nanoparticles synthesized by treating 1mM aqueous AgNO_3 solution with *Lantana camara* leaf extract after 5 hrs.

FTIR is a commonly used method to identify the functional groups in the interactions between metal particles and biomolecules. In the present work, the identification of biomolecules responsible for capping and stabilizing the silver nanoparticles using FTIR spectrum. The FTIR spectra of the *Lantana camara* is given in the Fig 3. FTIR spectrum of *Lantana camara* extract shows peaks at 690, 1045, 1079, 1385, 2082 and 3436. The band peak at about 1637 cm^{-1} can be assigned for aromatic rings. The strong broad band appearing at 3436 cm^{-1} can be associated to the stretching

vibrations of alcoholic and phenolic O–H. At 1079 cm^{-1} a peak is observed that could be for plant ascribed to multiplet C–O group. Therefore, from the results of FTIR analyses of extract mediated synthesized silver nanoparticles it can be concluded that some of the biological molecules of leaf extract such as flavonoids, phenols, alkaloids, glycosides, amino acids and tannins are accountable for transformation of silver ions to silver nanoparticles and its stabilization in aqueous medium. This result agrees with earlier reports [13].

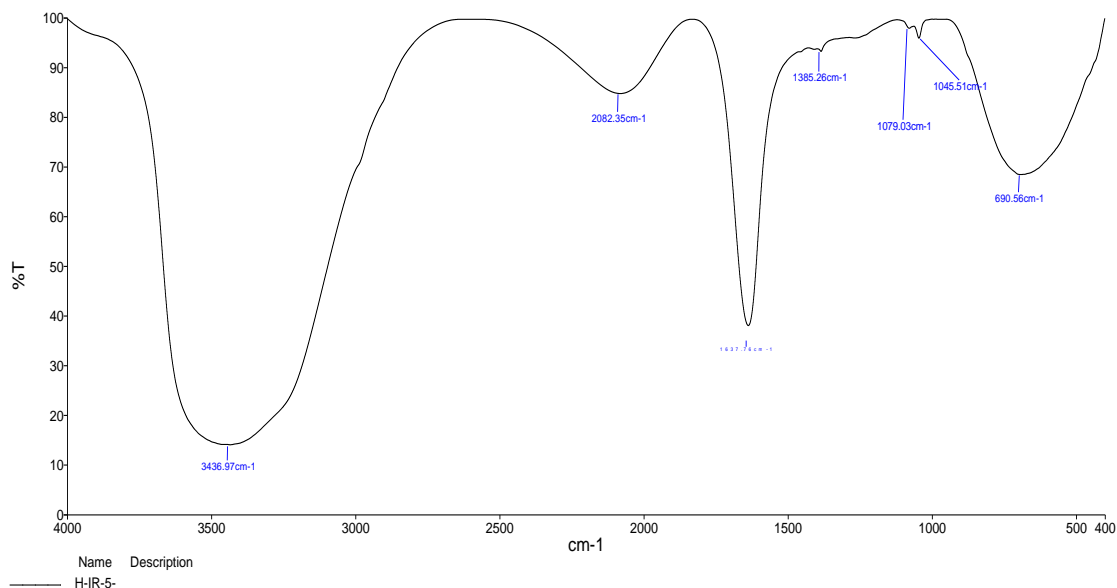


Fig. 3 FTIR analysis of silver nanoparticles synthesized by treating 1mM aqueous AgNO_3 solution with *Lantana camara* extract.

3.3. Scanning Electron Microscope (SEM)

The surface morphology, size and shape of the silver nanoparticles were analyzed by Scanning Electron Microscope. Fig. 4 shows the SEM image of silver nanoparticles synthesized from leaf extract. The SEM images show individual silver nanoparticles which are higher density polydispersed spherical in shape as well as number of aggregates with no defined morphology. The presence of

biomolecules in the leaf extract has resulted in the synthesis of spherical silver nanoparticles and the aggregation may be due to the presence of secondary metabolites in the leaf extract. The SEM image shows the size of the silver nanoparticles ranging from 15 to 40 nm. Similar result of the silver nanoparticles size was reported by using *Coccinia grandis* leaf extract [8], and by using *Allophylus serratus* Leaf [14].

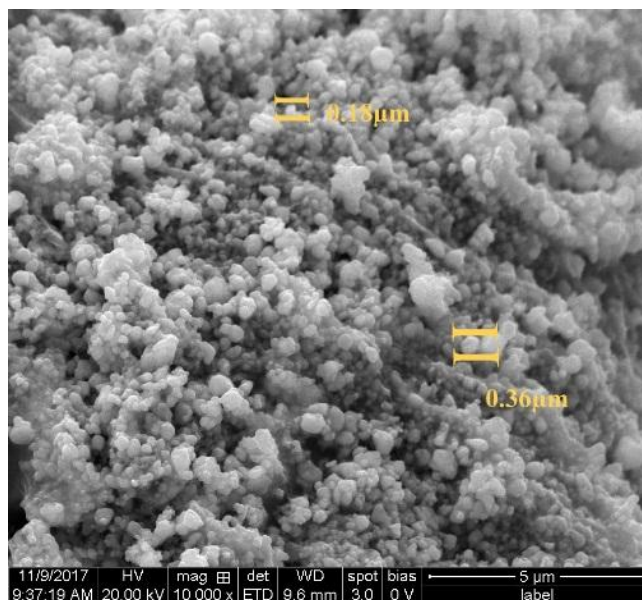


Fig. 4 High resolution scanning electron microscopic (SEM) image of silver nanoparticles (AgNPs). Polydispersed (Cluster) AgNPs ranged between 15–40nm.

3.4. Antimicrobial activity

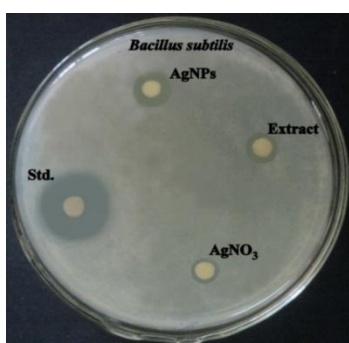
The SNPs of *Lantana camara* shows highest antibacterial activity was observed against *E. coli*, *S. aureus* and *Bacillus subtilis*. The inhibitory activities in culture media of the Ag nanoparticles reported in Table 1 were comparable with standard antimicrobiotic viz. chloramphenicol. In this study, silver nanoparticles exhibited antimicrobial activity against *E.*

coli (plate1) that was similar to that found by [15]. The inhibitory result of Ag nanoparticles was mild in *S. aureus* and *Bacillus subtilis* (plate1) as related with other microorganisms; these results suggest that the antimicrobial effects of Ag nanoparticles may be associated with characteristics of certain bacterial species.

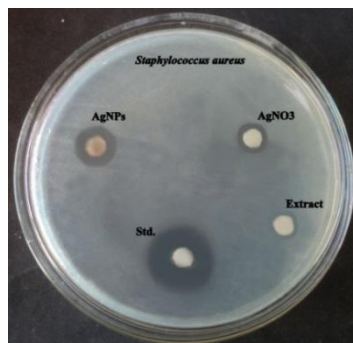
Table 1: Anti-bacterial activity of AgNPs, AgNO₃ and *Lantana camara* extract

Samples	Doses	<i>Escherichia coli</i> (mm)	<i>Staphylococcus aureus</i> (mm)	<i>Bacillus subtilis</i> (mm)
AgNO ₃	30µl/ml	1.58±0.11	1.76±0.12	1.68±0.11
<i>Lantana camara</i>	30µl/ml	1.06±0.07	0.82±0.05	0.75±0.05
AgNPs	30µl/ml	3.28±0.22	2.30±0.16	2.11±0.14
Standard (chloramphenicol)	30µl/ml	6.29±0.44	5.78±0.40	5.71±0.39

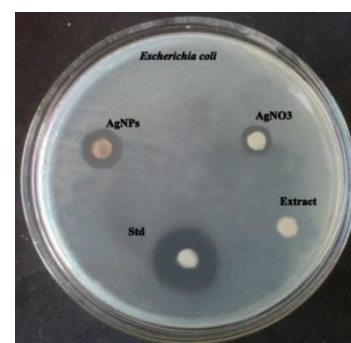
Values were expressed as Mean ± SD for triplicate.



Escherichia coli



Staphylococcus aureus



Bacillus subtilis

AgNO₃ = Silver Nitrate; AgNPs = Silver Nanoparticles

Plate 1: shows the Antibacterial activity of of AgNPs, AgNO₃ and *Lantana camara* extract

Silver has been identified to have a disinfecting agency and has been establish in claims ranging from traditional medicines to culinary items. Moreover, several salts of silver and their derivatives are commercially manufactured as antimicrobial agents [16]. In small concentrations, silver is safe for human cells, but lethal for bacteria and viruses [17]. Reduction of the particle size of the materials is an efficient and reliable tool for improving their biocompatibility that can be achieved using nanotechnology.

4. Conclusion

Medicinal plants have therapeutically important compounds in their diverse parts. The synthesis of nanoparticles using plants depends on the nature of plant such as its phytochemical content, special adaptation, and medicinal importance.

In this study, we investigated eco-friendly and cost-effective green synthesis of silver nanoparticles using leaf extract of medicinal plant *Lantana camara*. Water soluble organic compounds present in the leaf extract was mainly responsible for synthesis of silver nanoparticles by reducing silver ions to nanosized silver particles. The UV-visible spectroscopy, FTIR and SEM studies of the synthesized silver nanoparticles elucidated that the silver nanoparticles were crystalline in nature, spherical in shape with size ranging between 15 and 40nm and stable. The synthesized silver nanoparticles exhibited antibacterial activity. This finding suggests that the synthesis of AgNPs using *Lantana camara* leaf extract could be a good source for developing green nano-medicine for the management of antibacterial activity.

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