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PLANT MEDIATED SYNTHESIS OF SILVER NANOPARTICLES AND EVALUATION OF BIOLOGICAL ACTIVITY

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ABSTRACT

Much work has been carried out based on the synthesis of plants and their nanoparticles mediated by extracts. Metal-bound nanoparticles are typically synthesized by wet chemical methods, where the chemicals used are quite often toxic and flammable. In this study, we describe a cost-effective and environmentally friendly technology for the green synthesis of silver nanoparticles from a 1 mM AgNO3 solution via *Sansevieria roxburghiana* leaf extract, which acts as a reducing agent as well as a styling agent. The nanoparticles were characterized by UV–Vis absorption spectroscopy and SEM analysis showed a dense polydispersed spherical shape with an average particle size range of 14 to 41 nm. The synthesized silver nanoparticles showed potential antibacterial activity against several bacteria and fungi.

Keywords: Silver nanoparticles, *Sansevieria roxburghiana*, FTIR, UV–Visible, SEM, Antimicrobial activity.

INTRODUCTION

Nanoscience is recognized as a new interdisciplinary science. Could be clarified as a comprehensive knowledge of the important properties of nanometer-sized substances[1]. The 'nano' attachment indicates 1 billion units or 10^{-9} units. It is generally accepted, from the point of view of nanoscience and nanotechnology, that the unit should be only one unit of dimension, and not another scientific unit of measurement. It is generally established that nanoparticles constitute a group of atoms of the order of 1– 100 nm. Nanoparticles exhibit completely new or improved properties depending on their distinct physical appearance, such as size, morphology and distribution[2].

Metal-bound nanoparticles can be synthesized by chemical, physical and biological pathways; a physical approach using many methods such as condensation/evaporation and laser ablation. The chemical approach to metal ions in solution is reduced under conditions favorable to the continuous development of aggregates or small metal clusters 3. Many metals such as titanium, copper, silver, gold and iron were commonly used to synthesize nanoparticles. Among precious metals, silver nanoparticles have become the center of intensive research due to their diversity of applications for various industrial sectors[4]. Currently, biosynthesis methods involving natural reducing agents such as biological microorganisms, polysaccharides such as fungi and plants, bacterial extracts or green chemistry are suitable alternatives to more complex chemical and physical synthesis procedures. It emerged as a viable alternative AgNPs[5].

In the current era, better advances in the green synthesis of nanoparticles are expected due to their incredible use in all areas of scientific disipline. Various studies have been carried out based on the intermediate synthesis of nanoparticles with plant extracts. Many plants including *Bacopa monnieri*[6], and *Catharanthus roseus*[7] used for the synthesis of AgNPs. With this in mind, this study uses *Sansevieria roxburghiana* leaf extract to explore new approaches to the biosynthesis of silver nanoparticles and evaluate their antimicrobial activity.

MATERIAL METHODS

Collection of plant materials

Sansevieria roxburghiana leaves were collected from herbs from Ariyalur, Tamil Nadu, India. The plants were identified and authenticated by Dr. S. John Britto, Botanist, Herbarium and center for molecular systematics, St. Joseph's college Trichy-Tamil Nadu. India. A Voucher specimen has been deposited at the Rapinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil nadu, India.

Preparation of leaf extract

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

Synthesis of Ag nanoparticles using leaf extracts

Five ml of *Sansevieria roxburghiana* leaf extract was added to 45 ml of 1 mM aqueous $AgNO_3$ 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].

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).

SEM analysis of silver nanoparticles

Scanning electron microscopic (SEM) analysis was done using ZEISS machine. The sample were 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.

Antimicrobial activity

Microorganisms

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

Antimicrobial assay

Antibiogram was done by disc diffusion method[9, 10] using herbal extracts. Petri plates were prepared by pouring 30 ml of Nutrient and Potato dextrose agar medium separately for bacteria and fungi. The test organism was inoculated on solidified agar plate with the assistance of micropipette and blowout and permitted to dry for 10 mints. The surfaces of media were inoculated with fungi /bacteria from a broth culture. A sterile cotton swab is immersed into a consistent microbial test suspension and used to equally inoculate the entire surface of the Nutrient and Potato dextrose agar plate. Briefly, inoculums comprising bacteria on Nutrient agar plates for bacteria and fungi for Potato dextrose media. Using sterile forceps, the sterile filter papers (6 mm diameter) containing each 30µl of plant extract, $AgNO_3$ solutions, AgNPs and Standard solution as Chloramphenicol and fluconazole were laid down on the surface of inoculated agar plate. The plates were incubated at 37°C for 24/48 h for the bacteria and fungi at room temperature (30±1). Each sample was tested in triplicates.

RESULT AND DISCUSSION

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, there by 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].

PE:Plant extract; **$AgNO_3$**: 1 mM $AgNO_3$ without *Sansevieria roxburghiana* leaf extract. **AgNPs**:1 mM $AgNO_3$ with *Sansevieria roxburghiana* leaf extract after 5 hrs of incubation (Brown colour)

Figure 1 Synthesis of $AgNO_3$ and control ($AgNO_3$)

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 *Sansevieria roxburghiana* leaf extract at the peaks observed at 420 nm indicate the presence of silver nanoparticles which is synthesized by *Sansevieria roxburghiana* leaf 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].

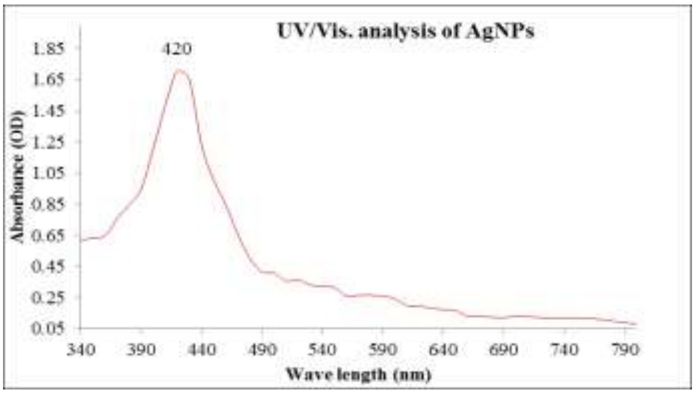


Figure 2 UV-Vis absorption spectrum of silver nanoparticles synthesized by *Sansevieria roxburghiana* leaf extract after 5 hrs.

FTIR analysis of silver nanoparticles

FTIR is an commonly used 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 *Sansevieria roxburghiana* leaf extract is given in the Fig 3. FTIR spectrum of *Sansevieria roxburghiana* leaf extract shows peak at 3438.29, 1637.52 and 668.39. The band peak at about 1637 cm^{-1} can be assigned for aromatic rings. The strong broad band appearing at 3438 cm^{-1}can be associated to the stretching vibrations of alcoholic and phenolic O–H. At 668cm^{-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 results agreement with earlier reports[13].

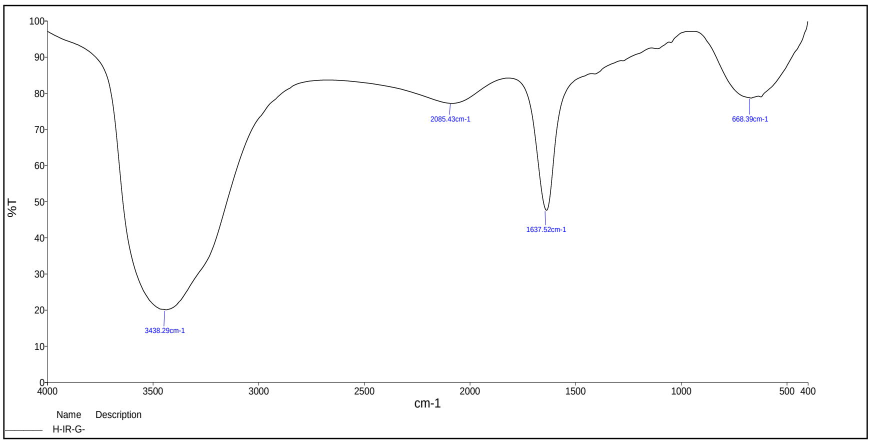


Figure 3 FTIR analysis of silver nanoparticles synthesized by treating 1mM aqueous $AgNO_3$ solution with *Sansevieria roxburghiana* leaf extract.

Table 1: FTIR analysis of silver nanoparticles synthesized by treating 1mM aqueous $AgNO_3$ solution with SRLE

S. No	Peak	Bond	Functional group
	3438.29	O-H stretch, H-bonded	Alcohols, phenols
	1637.52	N-H bend	1° amines
	668.39	C-Br stretch	Alkyl halides

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 presences 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 14.23 to

41.56 nm. Similar result of the silver nanoparticles size was reported by using *Coccinia grandis* leaf extract[8], and by using *Allophylus serratus* Leaf[14].

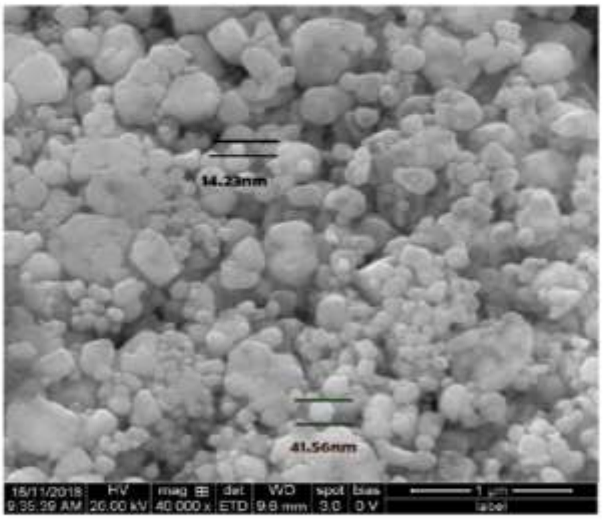


Figure 4 High resolution scanning electron microscopic (SEM) image of silver nanoparticles (AgNPs). Polydispersed (Cluster) AgNPs ranged between 14.23 to 41.56nm.

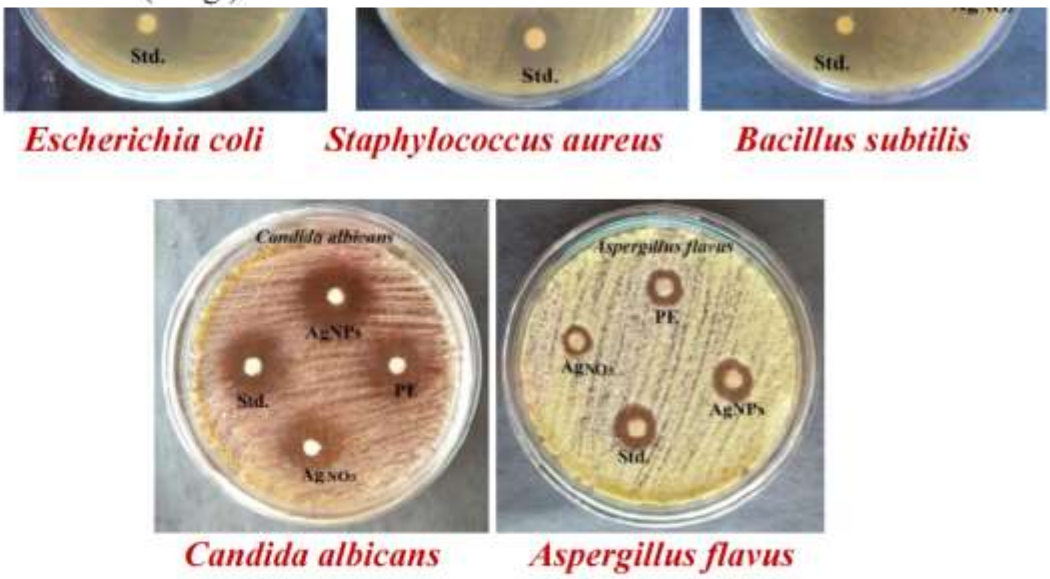
Antimicrobial activity

The SNPs of *Sansevieria roxburghiana* leaf extract shows highest antimicrobial activity was observed against *E. coli*, *S. aureus*, *Bacillus subtilis*, *Candida albicans* and *Aspergillus flavus*. The inhibitory activities in culture media of the Ag nanoparticles reported in Table 2 were comparable with standard antimicrobiotic viz. chloramphenicol for bacteria and Fluconazole for fungal. 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 microbial species.

Table 2: Anti-microbial activity of AgNPs, $AgNO_3$ and *S. roxburghiana* extract

Microbial Strains	Dose (30 µl)			Std. (30 µl)
	$AgNO_3$	PE	AgNPs	
Bacterial strains				
***Escherichia coli* (mm)**	4.85 ± 0.33	5.90 ± 0.41	9.45 ± 0.66	11.35 ± 0.79
***Bacillus subtilis* (mm)**	3.80 ± 0.26	4.60 ± 0.32	8.50 ± 0.59	10.25 ± 0.71
***Staphylococcus aureus* (mm)**	4.10 ± 0.28	5.25 ± 0.36	9.30 ± 0.65	11.05 ± 0.77
Fungal strains				
***Candida albicans* (mm)**	3.10 ± 0.21	4.05 ± 0.28	7.25 ± 0.50	10.05 ± 0.70
***Aspergillus flavus* (mm)**	2.65 ± 0.18	3.15 ± 0.22	5.85 ± 0.40	9.40 ± 0.65

Values were expressed as Mean ± SD for triplicates.Std. Chloramphenicol (Bacteria) Fluconazole (Fungi).



$AgNO_3$ = Silver Nitrate; AgNPs = Silver Nanoparticles

Plate 1: Shows the Antimicrobial activity of of AgNPs, $AgNO_3$ and SRLE

Silver has been identified to have a disinfecting agent 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.

Conclusion

The synthesized silver nanoparticles exhibited crystalline in nature, spherical in shape with size ranging between 14 to 41 nm and stable and have antimicrobial activity. This finding suggests that the synthesis of AgNPs using *Sansevieria roxburghiana* leaf extract could be a good source for developing green nano-medicine for the management of antimicrobial activity.

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