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Phyto Mediated Eco-Friendly Synthesis Of Nanoparticles And Evaluation Of Antibacterial And Antifungal Activities

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ABSTRACT
The synthesis of plant-mediated nanoparticles is becoming more and more popular because of its efficiency, low toxicity, environmental friendliness, and quick processing times. A lot of research has been done using extracts to facilitate the synthesis of plants and their nanoparticles. The wide variety of plants used in this investigation of plant-based nano-synthesis is examined, emphasizing their distinct phytochemical compositions and characteristics. The most common approach for creating metal-bound nanoparticles is wet chemical synthesis, which frequently uses hazardous and combustible compounds. Here, we present an economical and eco-friendly method for producing silver nanoparticles from a 1 mM $AgNO_3$ solution using SRLE, which serves as both a reducing and a styling agent. UV-Vis absorption spectroscopy and SEM analysis revealed that the nanoparticles had a dense, polydispersed spherical form and ranged in average particle size from 14 to 41 nm. Potential antibacterial action against a variety of bacteria and fungi was demonstrated by the produced silver nanoparticles.

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

INTRODUCTION

It is acknowledged that nanoscience is a new multidisciplinary field of study. could be defined as a thorough understanding of the crucial characteristics of materials with a nanoscale size [1]. One billion units, or 10^{-9} units, are indicated by the "nano" appendage. From the perspective of nanoscience and nanotechnology, it is widely acknowledged that the unit should be one dimension and not another scientific unit of measurement. It is widely accepted that atoms with sizes ranging from 1 to 100 nm make up nanoparticles. Depending on their unique physical characteristics, including size, shape, and distribution, nanoparticles can have entirely new or enhanced features [2].

There are three approaches to create metal-bound nanoparticles: chemically, physically, and biologically. The physical method involves a variety of techniques, including laser ablation and condensation/evaporation. Conditions conducive to the ongoing formation of aggregates or tiny metal clusters decrease the chemical approach to metal ions in solution [3]. A wide range of metals, including iron, copper, silver, titanium, and gold, were frequently utilized to create nanoparticles. Because of their numerous uses in a wide range of industries, silver nanoparticles have emerged as the focus of extensive research among precious metals [4]. More complicated chemical and physical synthesis processes can currently be replaced with biosynthesis techniques that use natural reducing agents like biological microbes, polysaccharides like fungus and plants, bacterial extracts, or green chemistry [5]. Given its amazing applications in every field of science, greater advancements in the environmentally friendly production of nanoparticles are anticipated in the modern day. On the basis of the intermediate synthesis of nanoparticles using plant extracts, numerous investigations have been conducted. Numerous plants, such as *Bacopa monnieri* [6] and *Catharanthus roseus* [7], are employed to synthesize AgNPs. This study employs SRLE to assess the antibacterial activity of silver nanoparticles and investigate novel methods for their manufacture.

MATERIAL METHODS

Collection of plant materials

The leaves of *Sansevieria roxburghiana* were gathered from herbs in Ariyalur, Tamil Nadu, India. Dr. S. John Britto, a botanist at St. Joseph's College in Trichy, Tamil Nadu, India, who works in the Herbarium and Center for Molecular Systematics, identified and verified the plants. At St. Josephs College's Rapinat Herbarium in Thiruchirappalli, Tamil Nadu, India, a voucher specimen has been placed.

Preparation of leaf extract

To prepare a leaf powder, the dried leaves were thoroughly ground using a mortar and pestle. 100 milliliters of deionized water were combined with 20 grams of SRL powder, and the liquid was then heated for ten minutes. After chilling, Whatman No. 1 filter paper was used to filter the leaf extract. The extract was stored for future research at 4°C.

Synthesis of Ag nanoparticles using leaf extracts

45 ml of 1 mM aqueous AgNO3 solution and 5 ml of SRLE were combined in a 250 ml Erlenmeyer flask, and the mixture was allowed to sit at room temperature for five hours in the dark. A control setup was likewise kept, but without leaf extract. Through repeated centrifugation at 10,000 rpm for 15 minutes and re-dispersion of the pellet in de-ionized water, the resulting Ag nanoparticle solution was purified. The AgNPs were then dried in preparation for SEM examination [8].

UV-Vis and FTIR Spectra analysis

Five hours after diluting a tiny aliquot of the sample in distilled water, the UV-Vis spectra of the reaction medium was measured in order to evaluate the reduction of silver ions. Deionized water is used to dissolve the produced pellet, which is then filtered via Whatman filter paper No. 42. Fourier transmission infrared spectroscopy (FTIR) uses this filtrate that contains silver nanoparticles.

SEM analysis of silver nanoparticles

ZEISS was used to do scanning electron microscopy (SEM) examination. A very tiny amount of the sample was dropped onto a carbon-coated copper grid to create thin coatings over the grid. After blotting off excess solution using a blotting paper, the films on the SEM grid were left to dry for five minutes under a mercury lamp.

ANTIMICROBIAL ACTIVITY

Microorganisms

Microbial type culture collection (MTCC) at the Institute of Microbial Technology (IMTECH), Chandigarh, India, provided the bacteria used, which included Gram-positive *Staphylococcus aureus*, Gram-negative *Escherichia coli*, Gram-negative *Bacillus subtilis*, and fungi *Candida albicans* and *Aspergillus flavus*.

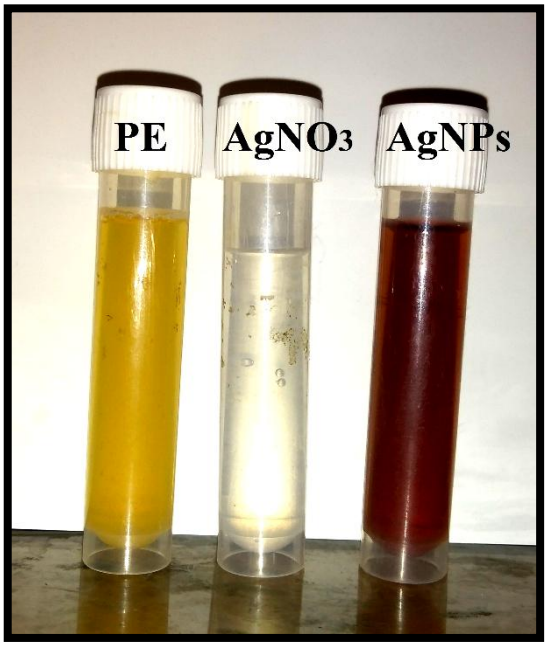
Antimicrobial assay

Using herbal extracts, the disc diffusion method9, 10 was used to perform the antibiogram. 30 milliliters of nutrient and potato dextrose agar medium were added to each petri plate individually for the bacteria and fungi. Using a micropipette and blowout, the test organism was seeded onto a hardened agar plate and allowed to dry for ten minutes. Fungi and bacteria from a broth culture were used to inoculate the media surfaces. To evenly inoculate the whole surface of the Nutrient and Potato dextrose agar plate, a sterile cotton swab is submerged in a uniform microbiological test suspension. In short, bacterial inoculums on nutrient agar plates for bacteria and fungi for potato dextrose media. On the surface of the inoculated agar plate, sterile filter papers (6 mm in diameter) containing 30µl of plant extract, AgNO3 solutions, AgNPs, and Standard solution as fluconazole and chloramphenicol were placed using sterile forceps. For 24 or 48 hours, the plates were incubated at 37°C for the bacteria and fungi at room temperature (30±1). Every sample was examined three times.

RESULT AND DISCUSSION

Synthesis of silver nanoparticles

Leaf extracts were used in the production of silver nanoparticles. Because of its unique catalytic and chemical stability, leaf extract is employed as a reducing agent. This approach has the potential to be interesting for the large-scale synthesis of various inorganic materials (nanomaterials) due to the applicability of such environmentally friendly nanoparticles in antibacterial, wound healing, and other medical and electronic applications. Silver hydrosol was created as a result of the aqueous silver ions being reduced in solution by herbal extracts. The length of time it takes for a plant to change color varies. The phytochemicals found in the leaf extract were thought to be in charge of the silver ion reduction. The activation of surface plasmon vibrations in silver nanoparticles is known to cause them to appear yellowish-brown in aqueous solution. The reaction channels' yellowish-brown hues (Fig. 1) point to the creation of silver nanoparticles (SNPs) [11].



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

Figure 1 $AgNO_3$ Synthesis and control

Spectra analysis (UV-Vis and FTIR)

The use of UV-Vis spectroscopy to investigate size- and shape-controlled nanoparticles in aqueous solutions is widely expected. Figure 2 shows the UV-Vis spectra recorded over the following five hours from the reaction liquid. The presence of silver nanoparticles produced by SRLE is indicated by the UV–vis spectra of the reaction mixture of silver nitrate solution with SRLE at the peaks observed at 420 nm. The surface plasmon resonance of the electrons in the reaction mixture caused the peak to rise, and the broadening of the peak suggested that the particles are polydispersed. This peak's appearance, attributed to a surface plasmon, is well-documented for a variety of metal nanoparticles ranging in size from 2 nm to 100 nm [12].

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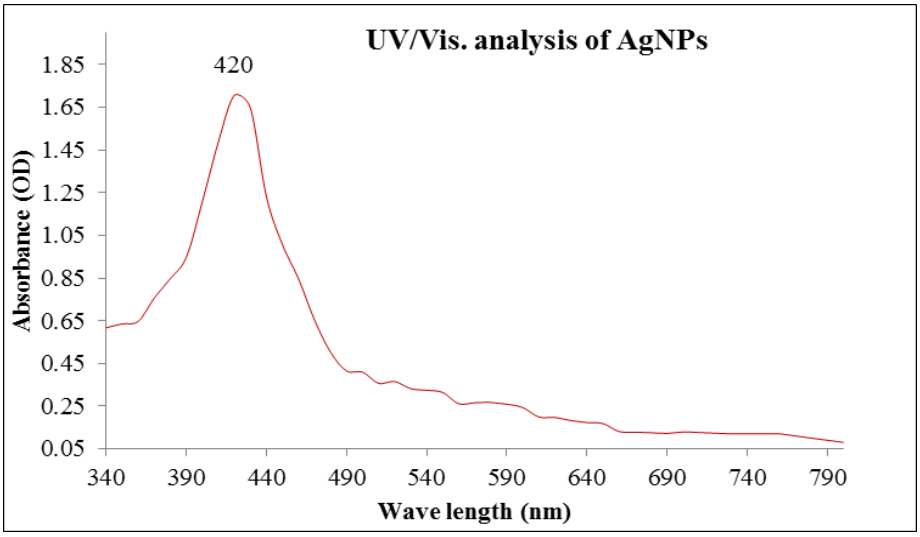

Figure 2 UV-Vis absorption spectrum of silver nanoparticles synthesized by SRLE after 5 hrs.

FTIR spectrum analysis of silver nanoparticles

When metal particles and biomolecules interact, FTIR is frequently employed to determine the functional groups involved. The current study uses the FTIR spectrum to identify the biomolecules that stabilize and cap the silver nanoparticles. Figure 3 displays the SRLE's FTIR spectrum. The SRLE's FTIR spectrum peaks at 3438.29, 1637.52 and 668.39. It is possible to attribute the band peak at roughly 1637cm^{-1} to aromatic rings. The stretching vibrations of phenolic and alcoholic O-H can be linked to the strong broad band that appears at 3438 cm^{-1}. A peak that may belong to the plant's multiplet C-O group is seen at 668 cm-1. The conversion of silver ions into silver nanoparticles and their stabilization in an aqueous medium are thus attributed to certain biological molecules of leaf extract, including flavonoids, phenols, alkaloids, glycosides, amino acids, and tannins, according to the results of FTIR analyses of extract-mediated synthesized silver nanoparticles. These findings are consistent with previous reports [13].

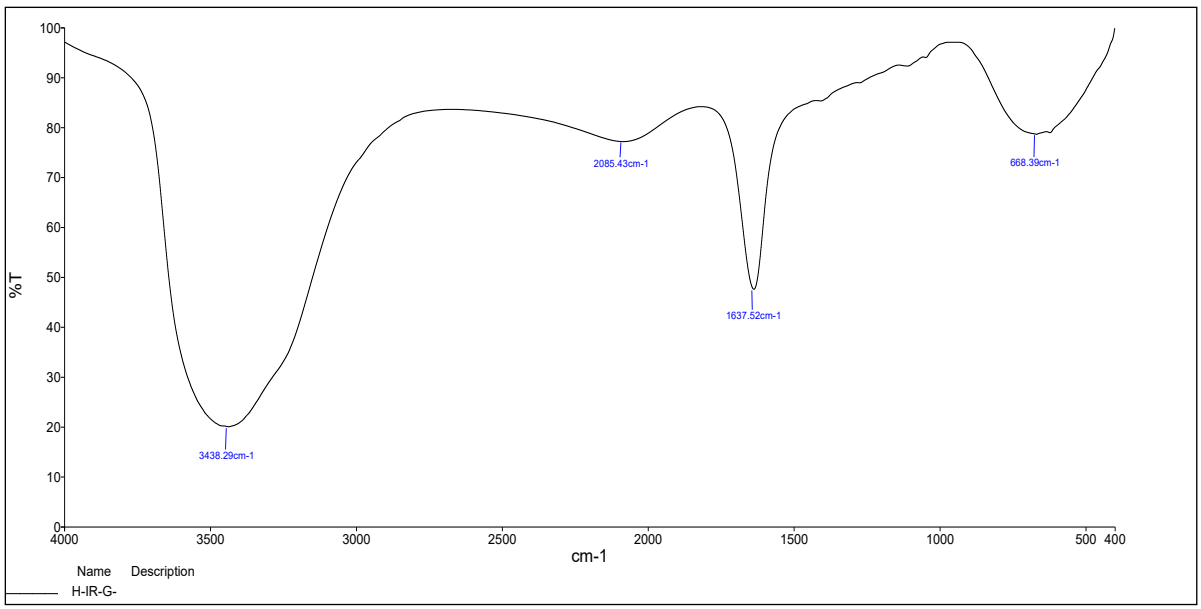

Figure 3 Silver nanoparticles produced by treating a 1 mM aqueous $AgNO_3$ solution with SRLE were subjected to FTIR analysis.

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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)

A scanning electron microscope was used to examine the silver nanoparticles' size, shape, and surface morphology. Figure 4 displays the SEM picture of leaf extract-derived silver nanoparticles. In addition to numerous aggregates with an unclear morphology, the SEM images display individual silver nanoparticles with a higher density that are polydispersed and spherical in shape. Secondary metabolites in the leaf extract may be the cause of the aggregation, and the presence of biomolecules in the extract has led to the creation of spherical silver nanoparticles. According to the SEM image, the silver nanoparticles' sizes range from 14.23 to 41.56 nm. *Coccinia grandis* leaf extract [8] and *Allophylus serratus* leaf extract gave similar results regarding the size of the silver nanoparticles [14].

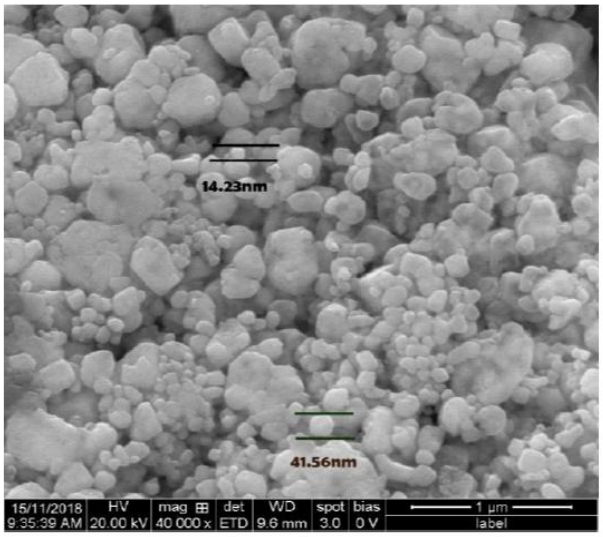

Figure 4 High resolution scanning electron microscopic (SEM) image of silver nanoparticles (AgNPs). Polydispersed (Cluster) AgNPs ranged between 14.23 to 41.56nm.

Antimicrobial Activity

The strongest antibacterial efficacy was seen in the SRLE SNPs against *Aspergillus flavus, Bacillus subtilis, E. coli, S. aureus*, and *Candida albicans*. The antimicrobial properties of the Ag nanoparticles shown in Table 2 were similar to those of common antimicrobials, such as fluconazole for fungi and chloramphenicol for bacteria, in culture medium. Silver nanoparticles had antibacterial action against *E. coli* (Plate 1) in this investigation that was comparable to that discovered by [15]. In *S. aureus* and *Bacillus subtilis* (Plate 1), the inhibitory effect of Ag nanoparticles was low compared to other bacteria; these findings imply that the antimicrobial properties of Ag nanoparticles may be linked to traits of certain microbial species.It has been determined that silver has a cleaning effect and has been used in everything from ancient remedies

to food products. Additionally, a number of silver salts and their derivatives are produced commercially as antibacterial agents [16]. Silver is harmless for human cells at low doses, but it kills bacteria and viruses [17]. One effective and dependable method for enhancing the materials' biocompatibility is to reduce their particle size, which can be accomplished with nanotechnology.

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).

CONCLUSION

The produced silver nanoparticles were stable, spherical, crystalline, and had antibacterial properties. Their sizes ranged from 14 to 41 nm. According to this research, the production of AgNPs using SRLE may be a useful tool for creating green nanomedicine for controlling antibacterial activity.

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