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ANTIMICROBIAL ACTIVITY OF SILVER NANOPARTICLES SYNTHESIZED USING *SOLANUM VIOLACEUM* AND THEIR CHARACTERIZATION

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Abstract

The present article reports an eco-friendly method for synthesizing Ag nanoparticles using the aqueous leaf extract of the plant *Solanum violaceum*, its characterization and its anti-bacterial, anti-fungal activities. The synthesized nanoparticles were characterized by Ultraviolet-Visible (UV-Vis) spectroscopy, showing an absorption peak at 436 nm due to the Surface Plasmon Resonance (SPR) property. The EDAX spectrum shows a strong signal at 3.02 keV, indicating that the synthesized nanoparticles are highly pure silver. High-Resolution Transmission Electron Microscopy (HR-TEM) showed the nanoparticles were spherical and triangular. The synthesized nanoparticles were less stable, which was confirmed by zeta potential. The nanoparticles thus synthesized were studied for antimicrobial activity and exhibit excellent activity against various organisms.

Keywords: *Solanum violaceum*, AgNPs, HR-TEM, Antibacterial, Antifungal, SPR peaks.

Introduction

Nanotechnology is an area of science concerned with the creation, characterization, manufacture, management and application of nanostructured materials for various purposes [1].Over the last decade, the world has witnessed an exponential increase in the application of nanoscience and nanotechnology, resulting in significant advances in developing new nanomaterials [2].All this progress in the research and development of new nanoparticle applications will immediately impact commerce and society [3].Silver is one of the most commercialized nanomaterials, with 500 tonnes of silver nanoparticles produced each year and expected to increase in the coming years [4].Various estimates of global AgNP production have been made in recent years [5].AgNP's antimicrobial activity is one of the primary reasons they are used in the formulation of surface cleaners, toys, textiles, air and water disinfection, antimicrobial catheters, antimicrobial gels, antimicrobial paints, food packaging supplies, clinical clothing and food preservation [6].AgNPs have been shown to be efficient against plant fungus in various investigations, indicating that they could be helpful in suppressing spore-producing fungal plant diseases [7].Ion sputtering, chemical reduction, sol-gel and other techniques are available to synthesize silver nanoparticles[8-11].Unfortunately, many nanoparticle synthesis methods involve using hazardous chemicals or high energy requirements, both of which are difficult and wasteful[12].Environmentally friendly 'green' processes in chemistry and chemical technologies are becoming increasingly popular and much needed due to global environmental concerns[13].Techniques for producing nanoparticles using naturally occurring reagents such as sugars, biodegradable polymers (for example, chitosan), plant extracts and microorganisms as reductants and capping agents could be appealing for nanotechnology [14-16].Greener nanoparticle synthesis provides an advantage over other methods because it is simple, one-step, cost-effective, environmentally friendly and relatively reproducible, resulting in more stable materials [17].Given the vast potential of plants as sources, this research aims to use a biological green technique for synthesizing silver nanoparticles as an alternative to conventional methods.*Solanum violaceum* belongs to the Solanaceae family, which is extensively used as vegetables and fruits.It has also been used in traditional medicine to treat asthma, dry cough, flatulence, worms and fever. Different plant parts, such as roots, fruits and seeds, are used for various treatments. In the current study, the leaves of the plant *Solanum violaceum* were used to synthesize silver nanoparticles.

Materials and Methods

Fresh and healthy leaves of *Solanum violaceum* were collected from Pechipparai, Kanniyakumari District, Tamilnadu. Silver nitrate ($AgNO_3$) analytical grade reagent from Sigma-Aldrich and deionized water are used to synthesize AgNPs from the aqueous leaf extract of the plant *Solanum violaceum*.

Preparation of aqueous leaf extract of *Solanum violaceum*

The leaves of the plant *Solanum violaceum* were well cleaned using tap water followed by double distilled water. Around 10 g of the fresh leaves were boiled with 100 mL of deionized water for 30 minutes at 60°C, and the extract was filtered using Whatman No.1 filter paper.

Synthesis of AgNPs using aqueous leaf extract of *Solanum violaceum*

50 mL of the prepared aqueous leaf extract was added to 50 mL of 1 mM $AgNO_3$ solution in a conical flask. Then the mixture was kept on a hot plate with a magnetic stirrer at 80°C for 45 minutes. Then the mixture was allowed to cool overnight. The products were collected by centrifugation, washed thrice with deionized water and further used for characterization studies and applications.

Characterization of synthesized AgNPs

The green synthesized AgNPs were monitored by a JASCO V-650 spectrophotometer within the range 200-800 nm. Jeol/JEM 2100 high-resolution transmission electron microscope (HR-TEM) was used to study the morphology and size of the nanoparticles. Zeta sizer nano series (Malvern) was used to find the stability of the nanoparticles.

Anti-bacterial activity

The sample-loaded disc was placed on the surface of the Mueller-Hinton medium and the plates were kept for incubation at 37°C for 24 hours. At the end of incubation, inhibition zones were examined around the disc and measured with a transparent ruler in millimetres.

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Anti-fungal activity

Antibiotic susceptibility tests were determined by the agar disc diffusion (Kirby–Bauer) method. First, up to 40 µl of each extract concentration was introduced into the sterile discs using sterile pipettes. The disc was then placed on the surface of the SDA (Sabouraud Dextrose Agar) medium, and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 22°C for 48 hours. At the end of incubation, inhibition zones were examined around the disc and measured with a transparent ruler in millimetres.

Results and Discussions

The present study shows the formations of AgNPs using the aqueous leaf extract of the plant *Solanum violaceum*. The results show that the formation of AgNPs occurred within 45 minutes of the reaction time at 80°C. Initially, there is no change in the reaction medium. However, after 15 minutes, the reduction started with slight turbidity. The reaction mixture turns reddish brown in colour with continuous stirring and heating. The colour is due to the surface plasmon resonance of Ag nanoparticles.

Characterization of AgNPs synthesized using *Solanum violaceum* leaf extracts

UV-Visible Spectroscopy

The formation of AgNPs was confirmed by UV-Visible spectroscopy. The respective Surface Plasmon Resonance peaks were recorded between 200 – 800 nm. A distinct absorption peak was obtained at 436 nm (Fig.1).

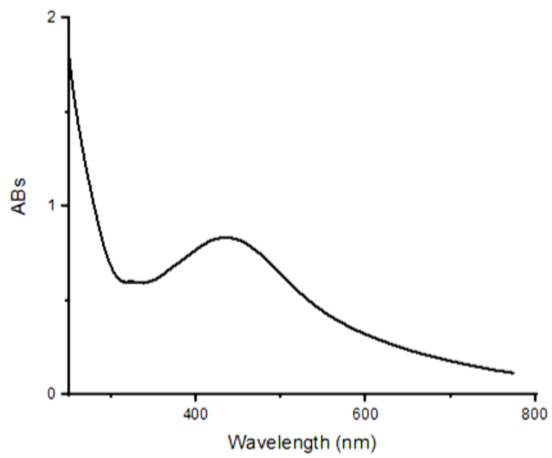

Figure 1: UV-Visible spectrum of AgNPs from *Solanum violaceum*

EDAX Analysis

Energy Dispersive X-ray (EDAX) Spectroscopy shows a strong metal signal for Ag, which confirms the presence of pure Ag metal as the major constituents. In the present investigation, the synthesized AgNPs show strong absorption at around 3.02 keV (Fig.2) with 85.47% silver and 14.53% carbon (Table.1).

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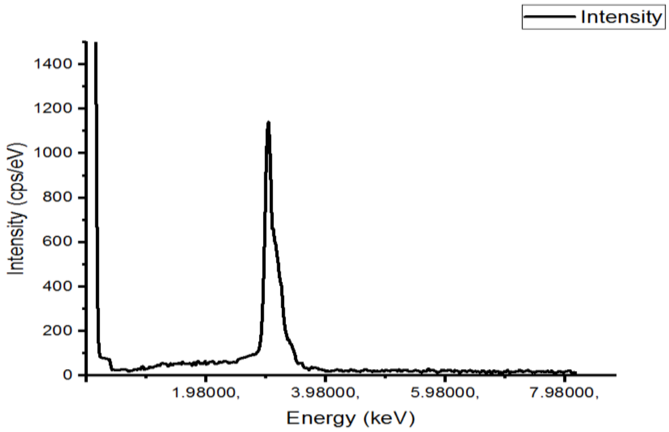

Figure 2: EDAX spectrum of AgNPs from *Solanum violaceum*

Table 1: Elemental Composition of AgNPs from *Solanum violaceum*

Sl.No	Elements	Weight %	Atomic %
1.	C	1.92	14.53
2.	Ag	98.08	85.47

High-Resolution Transmission Electron Microscopy (HR-TEM)

High-Resolution Transmission Electron Microscopy (HR-TEM) has been used to identify the nanoparticle's size, shape and morphology. The synthesized AgNPs were spherical, triangular and polydispersed with little agglomeration (Fig.3).

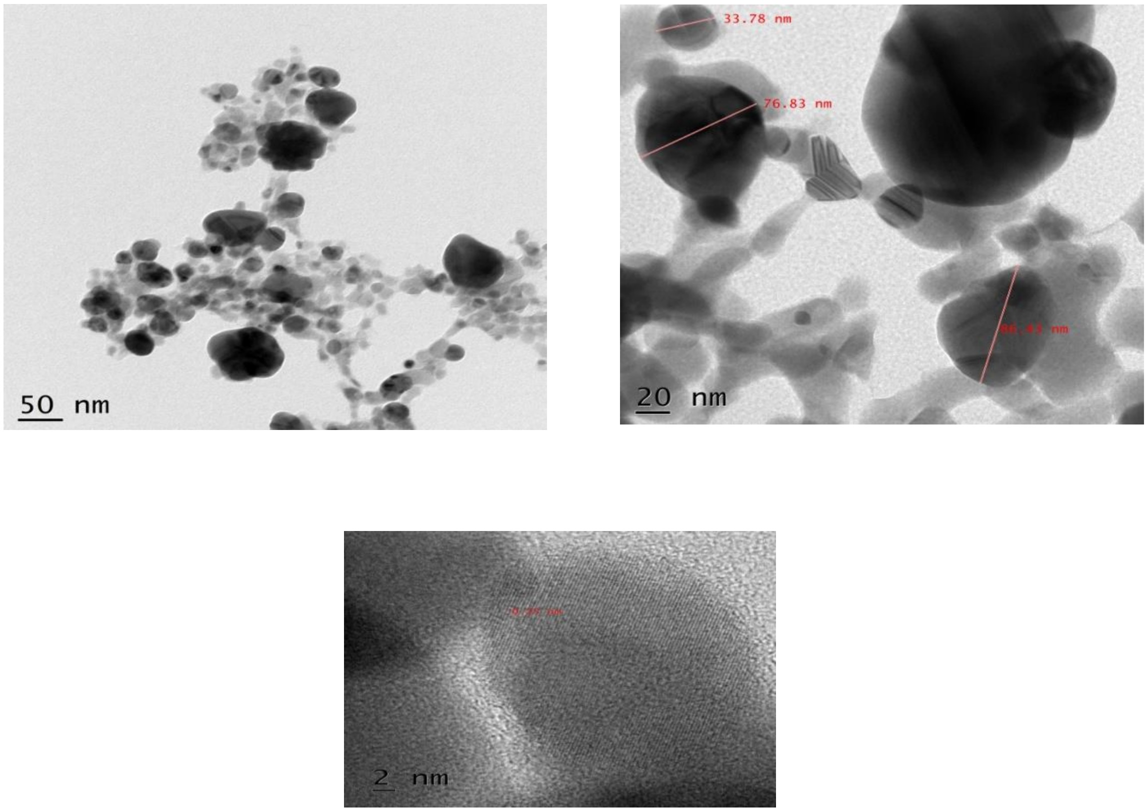

Figure 3: HR-TEM images of AgNPs from *Solanum violaceum*

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Zeta Potential

Zeta potential was used to determine the surface potential of synthesized AgNPs solutions and their stability. The zeta value of synthesized AgNPs was found at -20.5 mV (Fig.4). This value shows that the generated AgNPs were less stable in the colloidal solution.

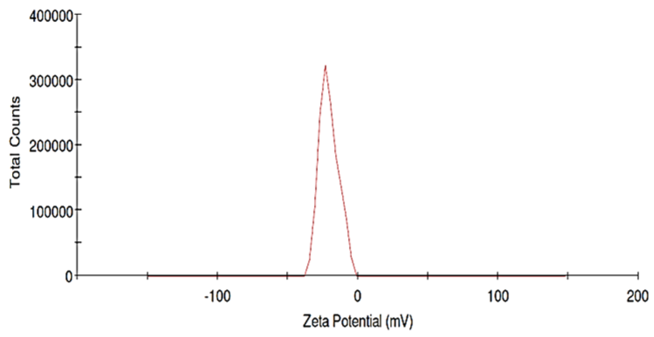

Figure 4: Zeta potential of AgNPs from *Solanum violaceum*

Biological Applications

Anti-bacterial activity of AgNPs synthesized using *Solanum violaceum* extract

The anti-bacterial activity of synthesized AgNPs was studied with various gram-positive and gram-negative bacteria (Table.2). The results show that the synthesized AgNPs have perfect anti-bacterial activity against all the bacteria studied. A maximum zone of inhibition of 14 mm was obtained for *Klebsiella pneumonia* and 13 mm for *Pseudomonas aeruginosa*, *E. coli* and *Staphylococcus aureus*, respectively.

Table 2: The anti-bacterial activity of AgNPs using *Solanum violaceum*

Sl.No	Organism	Control (mm)	AgNP (mm)
1.	*Klebsiella pneumoniae*	21	14
2.	*Pseudomonas aeruginosa*	20	13
3.	*Proteus mirabilis*	21	11
4.	*Proteus vulgaris*	20	11
5.	*Lactobacillus salivarius*	23	12
6.	*Bacillus subtilis*	22	11
7.	*Enterococcus faecalis*	22	11
8.	*Streptococcus mutans*	18	12
9.	*Escherichia coli*	15	13
10.	*Staphylococcus aureus*	22	13

Anti-fungal activity of AgNPs synthesized using *Solanum violaceum* extract

The anti-fungal activity of synthesized AgNPs was studied for *Candida albicans*, *Aspergillus flavus* and *Aspergillus niger*. A maximum zone of inhibition of 15 mm was

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obtained for *Candida albicans* and *Aspergillus flavus*, whereas no zone of inhibition was obtained for *Aspergillus niger*.

Table 3: The anti-fungal activity of AgNPs synthesized using Solanum violaceum

Sl.No	Organism	Control (mm)	AgNP (mm)
1.	*Candida albicans*	18	15
2.	*Aspergillus flavus*	16	15
3.	*Aspergillus niger*	15	-

Conclusion:

A simple method for the synthesis of AgNPs using the aqueous leaf extract of the plant *Solanum violaceum* was reported in the current study. The Ag nanoparticles were synthesized within 45 minutes. The zeta potential value obtained at - 20.5 mV indicates that the nanoparticles synthesized were less stable. The HR-TEM images confirm that the nanoparticles were spherical and triangular, possibly agglomerated. The EDAX results show that the nanoparticles were highly pure in nature. The synthesized nanoparticles were checked for anti-bacterial and anti-fungal activities. The result shows that the nanoparticles synthesized using the aqueous leaf extract of the plant *Solanum violaceum* can be used as a potential antimicrobial drug.

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