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Research Article

SYNTHESIS OF SILVER NANOPARTICLES FROM *ANDROGRAPHIS PANICULATA* AND EVALUATION OF THEIR ANTIBACTERIAL ACTIVITY

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ABSTRACT

Objective: *Andrographis paniculata* is one of the most important antibacterial effects. The result proved *A. paniculata* has antibacterial activity. The objective of this study was to the synthesis of silver nanoparticles (AgNPs) from *A. paniculata* and evaluates antibacterial method.

Methods: Synthesis of AgNPs from *A. paniculata* leaves was done using 1 mM AgNO3 solution and incubates 24 h at room temperature. Characterization of synthesized NPs was done by ultraviolet-visible absorption spectroscopy, Fourier transform-infrared analysis, scanning electron microscopy analysis, and antibacterial activity.

Results: In this result, synthesized AgNPs from leaf extract of *A. paniculata* showed potential antibacterial activity with various human pathogenic bacteria. Antibacterial activity of the AgNPs was performed by a disk diffusion method. The highest antibacterial activity of AgNPs synthesized by *A. paniculata* leaf was found against *Salmonella typhi* (30 mm). The AgNPs synthesized in this process were found to have efficient antibacterial activity against human pathogenic bacteria.

Conclusion: In totality, the AgNPs prepared are safe to be discharged in the environment and possibly utilized in processes of pollution remediation. AgNPs may also be efficiently utilized in agricultural research to obtain better health of crop plants as shown by our study. The study concluded that the AgNPs from *A. paniculata* leaf extract have potential antibacterial activity.

Keywords: Silver nanoparticles, Scanning electron microscopy analysis, Ultraviolet-visible spectra, Fourier transform-infrared analysis, Antibacterial activity.

INTRODUCTION

Indian greeneries are the chief and cheap source of medicinal plants and plant products. From centuries till date, these medicinal plants have been extensively utilized in Ayurveda. Recently, many such plants have been gaining importance due to their unique constituents and their versatile applicability in various developing fields of research and development. Nanobiotechnology is presently one of the most dynamic disciplines of research in contemporary material science, whereby plants and different plant products are finding an imperative use in the synthesis of nanoparticles (NPs). In general, particles with a size <100 nm are referred to as NPs. Entirely novel and enhanced characteristics such as size, distribution, and morphology have been revealed by these particles in comparison to the larger particles of the mass material that they have been prepared from Wildenberg [1]. NPs of noble metals such as gold, silver, and platinum are well recognized to have significant applications in electronics, magnetic, optoelectronics, and information storage [2-5]. One such important member of the noble metal NPs is silver NPs (AgNPs). They are also broadly applied in shampoos, soaps, detergents, cosmetics, toothpastes, and medical and pharmaceutical products and are hence directly encountered by human systems [6,7].

Earlier, the antibacterial properties of silver and silver nitrate were well incorporated in the field of medical science. Furthermore, the medicinal importance of innumerable plants and plant parts was known. However, the plant-mediated silver nanoproduct is a relatively newer concept. Nanobiotechnology and their derived products are unique not only in their treatment methodology but also due to their uniqueness in particle size, physical, chemical, biochemical properties, and broad range of application as well. This current emerging field of nanobiotechnology is at the primary stage of development due to lack of implementation of innovative techniques in large industrial scale and yet has to be improved with the modern technologies. Hence, there is a need to design an economic, commercially feasible as well environmentally sustainable route of synthesis of AgNPs to meet its growing demand in diverse sector. Various approaches available for the synthesis of silver NPs include chemical [8], electrochemical [9], radiation [10], photochemical methods [11] and Langmuir-Blodgett [12,13], and biological techniques [14]. In this race of AgNP preparation, plant-mediated green biomimetic synthesis of AgNP is considered a widely acceptable technology for rapid production of AgNPs for successfully meeting the excessive need and current market demand and resulting in a reduction in the employment or generation of hazardous substances to human health and the environment. Studies have shown that Alfalfa roots can absorb Ag (0) from agar medium and are able to transport it to the plant shoot in the same state of oxidation [15]. Existing literature also reports successful synthesis of AgNPs through a green route where the reducing and capping agent selected was the latex obtained from *Andrographis paniculata* [16]. The antimicrobial effects of leaf extract of these *A. paniculata* plants and their respective biologically synthesized AgNPs were evaluated by disc diffusion method. Discovering these new biological sources for synthesis of AgNPs are more advantageous than contemporary physical or chemical procedures as these sources are abundantly available, cost-effective, and conveniently utilizable. Results obtained from analysis of antibacterial property of these AgNPs ensure that they are safe to be discharged in the environment and hence fit to be applied for pollution remediation.

METHODS

Plant collection

Fresh, mature leaves of *A. paniculata* leaf were collected from Mannargudi, Thiruvarur (Dt), Tamil Nadu, India.

Preparation of plant extract

The collected plant leaves were washed thrice in sterile distilled water to remove adhering soil particles and salts. The washed samples were shade dried for 1 week at room temperature. The leaves were cut into small pieces and grained into powder. The pure plant extract was

prepared by adding 10 g of plant powder into 100 ml of distilled water and boiled for 5 min. The boiled extract was filtered through Whatman No.1 filter paper, and the supernatant was used.

Synthesis of AgNP

In the typically synthesis process of AgNPs, add 10 ml of pure plant extract sample into the 90 ml of 1 mM of silver nitrate solution in 250 ml of conical flask. The reaction mixture was kept at room temperature under mechanically stirring. The color change was noted and the NPs formation was monitored.

Ultraviolet (UV) spectroscopy analysis of AgNPs

Synthesis of AgNPs solution with leaf extract may be easily observed by UV-visible (UV-vis) spectroscopy. The reduction of the Ag+ ions in solution was monitored by periodic sampling of aqueous component and measuring the UV spectra of the solution. UV spectra of these aliquots were monitored as a function of reaction on a spectrophotometer (UV-1800 series).

Fourier transform-infrared (FTIR) analysis of AgNPs

Possible functional group involved in the synthesis and stabilization of AgNPs was studied by FTIR spectroscopy. The FTIR was recorded in the range of 500–4500/cm; the various modes of vibrations were identified and assigned to determine the different functional groups present in the leaf extract of *A. paniculata*.

Scanning electron microscopy (SEM)

SEM analysis was done using JEOL JSM-6610 LV SEM machine. Thin films of the sample were prepared on a carbon-coated platinum grid by adjusting the dropping of a very small amount of the sample on the grid; extra solution was removed using a blotting paper. The film on the SEM grid was dried under a mercury lamp for 5 min. The thin film on grid was examined using scanning electron microscope.

Antibacterial assay

Disc preparation

The 6 mm (diameter) discs were prepared from Whatman No.1 filter paper. The discs were sterilized by autoclave at 121°C. After the sterilization, the moisture discs were dried on hot air oven at 50°C. Then, various plant extract discs and control discs were prepared.

Collection of test microorganisms

The bacterial strains of *Escherichia coli, Staphylococcus aureus, Enterobacter aerogenes, Vibrio cholera, Streptococcus pyogenes,* and *Salmonella typhi* were obtained from microbial type culture collection centre, Chandigarh.

Assay of antibacterial activity

Antibacterial activity test was carried out following the modification of the method originally described by Bauer *et al*. [17]. Muller–Hinton agar was prepared and autoclaved at 15 lbs pressure for 20 min and cooled to 45°C. The cooled media was poured into sterile Petri plates and allowed for solidification. The plates with media were seeded with the respective microbial suspension using sterile swab. The various solvents extract prepared discs individually were placed on the each Petri plates and also placed control and standard (clindamycin [10 µg] for bacteria) discs. The plates were incubated at 37°C for 24 h. After incubation period, the diameter of the zone formed around the disc was measured and expressed in mm.

RESULTS

Silver ions were reduced to AgNP when added to *A. paniculata* plant powders. It was observed that color of the solution turned to dark brown after 24 h of the reaction which indicated the formation of AgNPs.

UV-vis spectroscopy analysis

UV-vis spectra of synthesized AgNPs leaf extract of *A. paniculata*. The UV absorption spectra obtained for in *A. paniculata* leaf-derived AgNPs. A characteristic peak at 395.1 nm wavelength is clearly observed, confirming the formation of AgNPs is shown in Fig. 1.

Wavelength (nm)	Absorbance (AU)
395.1	3.983761183
1020.6	0.018017777

FTIR spectroscopy analysis

FTIR analysis was used for the characterization of silver nanoparticles. FTIR absorption spectra of synthesis of silver nanoparticles are shown in Fig. 2. The absorption bands in Fig. 3 are observed in the region of 4000–500/cm is showed peaks at of 3447.64, 2080.06, 1637.89, 1398.83, 1078.78, and 668.26/cm. The FTIR analysis spectrum showed sharp absorbance between 500 and 4000/cm.

This represents the different functional groups of absorbed biomolecules on the surface of nanoparticles. It indicates the influence of organic

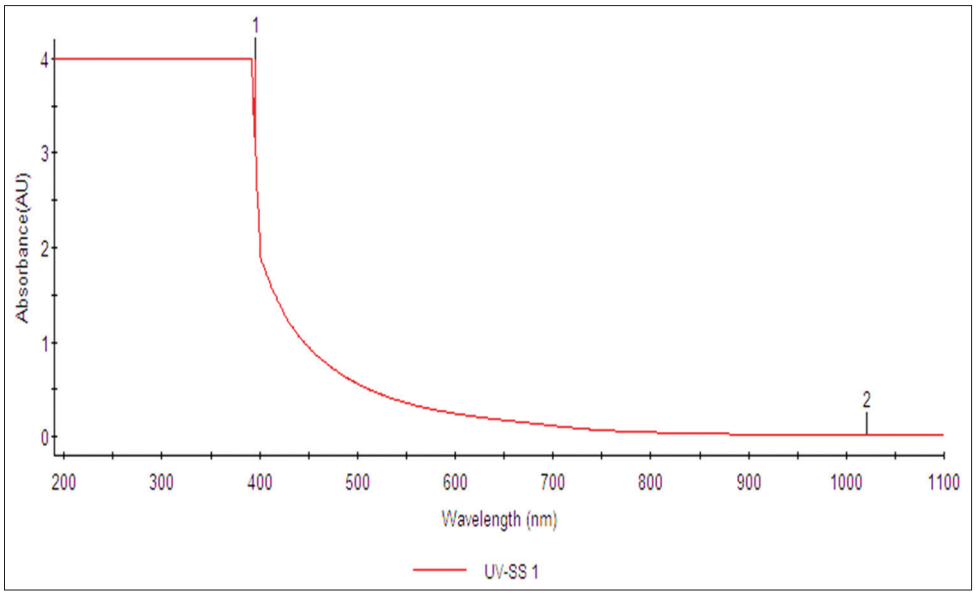

Fig. 1: Ultraviolet-visible peak value of synthesis on silver nanoparticles of leaf extract of Andrographis paniculata

moieties on the formation of silver nanoparticles and its stabilization. The absorption peak at around 668.26/cm can be assigned as alkyl halide C-Cl stretching, likewise, the peak at 1078.78/cm as ether C-O stretching, 1398.83/cm as alkane -C-H bending, 1637.89/cm as alkene C=C stretching, 2080.06/cm as alkyne -ↄ- stretching, and 3447.64/cm as amine N-H stretching..

Group frequency/cm of the sample	Bond	Functional assignment
3447.64/cm	N-H stretching	Amine
2080.06/cm	-ↄↄ- stretching	Alkyne
1637.89/cm	C=C stretching	Alkene
1398.83/cm	-C-H bending	Alkane
1078.78/cm	C-O stretching	Ether
668.26/cm	C-Cl stretching	Alkyl Halide

SEM analysis

Fig. 3 shows representative SEM images recorded of the AgNPs synthesized by *A. paniculata* leaf extract. The structural view of AgNPs synthesized from *A. paniculata* plant extract was observed under scanning electron microscope (Fig. 3).

Antibacterial activity

The zones of inhibition for *A. paniculata* on bacterial species are showing in Plate-1a, Table 1. Antibacterial activity of AgNPs on bacterial strains the zone of inhibition increases with increase in dose. The antibacterial activities of the AgNPs synthesized from the *A. paniculata* were effectively accessed against one Gram-positive bacteria (*S. aureus*) and five Gram-negative bacteria (*E. coli, E. aerogenes, V. cholerae*, *S. pyogenes,* and *S. typhi*).

The zone of inhibition for *A. paniculata* on bacterial species is shown in Table 1. In Plate-1b, the antibacterial activity of AgNPs on six bacterial strains shows clear increase in inhibition zones with increase dose. The tabular column-1 indicates that the human pathogen *S. typhi* shows the highest antibacterial activity. The pathogen *S. aureus* indicates the lowest activity.

DISCUSSION

The present study used in the synthesis of AgNPs leaf extract of *A. paniculata* for the studied their effect on antibacterial activity.

The reduction of Ag^+ to Ag° nanoparticles by *A. paniculata* leaf extract was measured using UV-vis spectrophotometry. It was observed that the color changes from yellow to dark brown. It indicates the formation of AgNPs, and this might be due to the reduction of silver ion to form the NPs. Similarly, Elumalai *et al*. [18] observed that the reduction of Ag^+ ion AgNO3 exposed plant extract followed by color change and [19] also reported that the Svensonia hyderabadensis solution of silver ion complex started to change the color from yellow to dark brown due to the reduction of silver ions.

The color transformation of *A. paniculata* extract treated silver nitrate might be due to vibrations in surface plasmon of silver [20]. The strong broad peak located at 395.1 nm indicates the reduction of Ag+ ions which further confirmed the formation of AgNPs. It is corroborated to the findings of Vilchis-Nestor *et al*. [21], who have reported that the noble metal silver displays characteristic absorbance at around 402.5 nm. It has been suggested that polyol components are mainly responsible for the reduction of silver ions [22]. Thus, the study suggests that leaf extract of *A. paniculata* might reduce Ag^+ to Ag°.

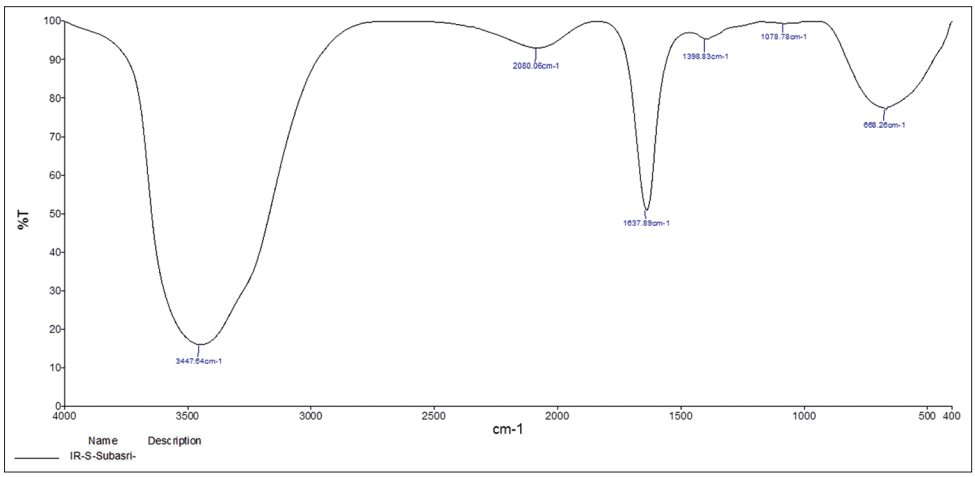

Fig. 2: Fourier transform-infrared peak value of synthesis on silver nanoparticles of leaf extract of *Andrographis paniculata*

Table 1: Antibacterial activity of *A. paniculata* AgNPs on human pathogenic bacterial strain

S. No	Bacterial species	Zone of inhibition (mm in diameter) at different concentration				
		Control	Standard 10 μg/ml	Concentration of AgNPs		
				5 μg/ml	10 g/ml	15 μg/ml
1	*E. coli*	-	22	15	18	19
2	*E. aerogenes*	-	28	23	24	25
3	*V. cholerae*	-	25	20	22	23
4	*S. aureus*	-	24	6	6	7
5	*S. pyogenes*	-	22	18	19	21
6	*S. typhi*	-	28	28	29	30

AgNPs: Silver nanoparticles, *A. paniculata: Andrographis paniculata, E. coli: Escherichia coli, S. aureus: Staphylococcus aureus, E. aerogenes: Enterobacter aerogenes, V. cholera: Vibrio cholera, S. pyogenes: Streptococcus pyogenes, S. typhi: Salmonella typhi*

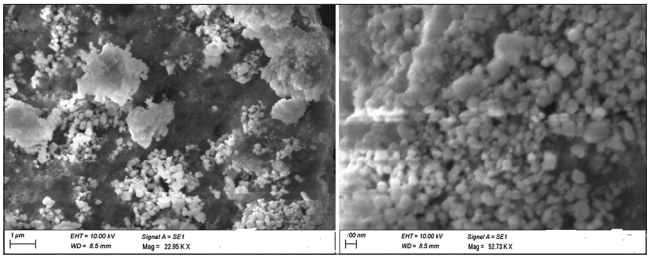

Fig. 3: Scanning electron microscopy image of synthesis on silver nanoparticles from leaf extract of Andrographis paniculata

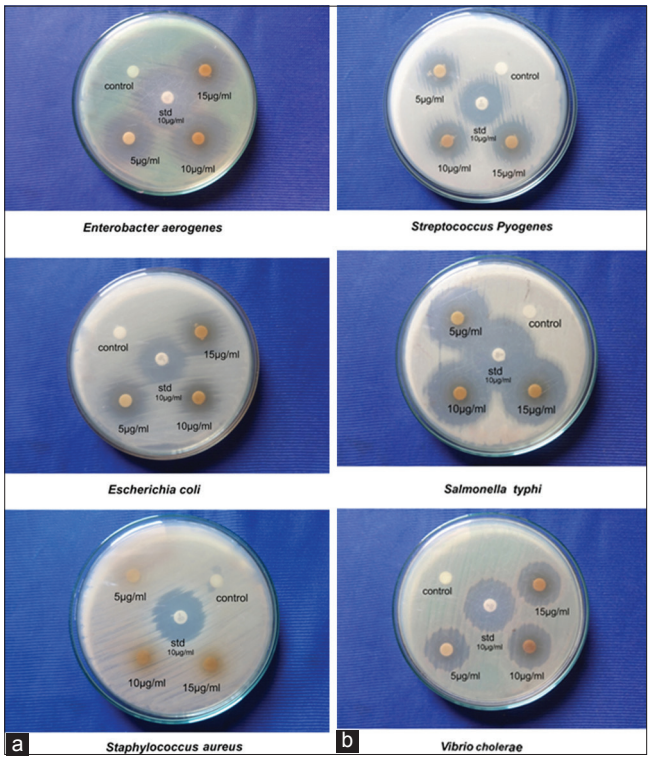

Plate 1: (a and b) Antibacterial activity of silver nanoparticles on human pathogenic bacterial strains

The functional groups of compounds adsorbed on the AgNPs were identified using FTIR studies. The peak near in FTIR 33,592,103 and 1640/cm assigned to alcohol O-H stretching, H-bonded, alkyne -ɔɔ- stretching, and alkene C=C stretching, respectively [23,24]. The total disappearance of this band after the bioreduction may be because the polyols are mainly responsible for the reduction of Ag ions. Thus, the result suggests that AgNPs might be capped by water-soluble secondary plant metabolites.

The synthesized AgNPs using *A. paniculata* were well distributed as aggregates in solution. The green synthesis of noble NPs using plant or fruit extracts and bioorganisms leads to the formation of crystalline NPs with variety of shapes and sizes ranging from 1 to 100 nm. Interestingly, the size of TAP reduced AgNPs was found to range from 24 to 90 nm under SEM observation. It has been suggested that the size of NPs is mainly determined by various factors such as the nature of plant extract and its concentration, metal salt, pH, temperature, extent of reaction time, and mixing ratio of plant extract and metal salt [25]. The *A. paniculata* AgNPs exhibited excellent antibacterial activity. The antibacterial property of *A. paniculata* AgNPs has been investigated against *S. typhi, S. aureus, E. aerogenes, V. cholerae, S. pyogenes,* and *E. coli*. It has been proven that the antibacterial property of *A. paniculata* AgNPs is concentration dependent. The antibacterial activity is found to be predominant in human pathogen *S. typhi* in comparison with other strains.

CONCLUSION

The synthesis and characterization of *A. paniculata* leaf extract of AgNPs was done and confirmed by UV-vis spectroscopy, FTIR, SEM techniques, and testing for their antimicrobial activity. Result denoted *A. paniculata* leaf extract to be a better-reducing agents FTIR analysis released the efficient capping and stabilization properties of these AgNPs. Besides, they also have good antibacterial activity against different microorganism of AgNPs. It is confirmed that can be rendered high antibacterial efficiency. Biologically synthesized NPs that are found to be exceptionally stable and also minimize toxicity and cost.

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