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SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES USING *ARISTOLOCHIA BRACTEOLATA* AND ITS ANTIBACTERIAL EFFICACY

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ABSTRACT: In recent years, nanoparticles have emerged as novel agent and have been used in all fields of science. In the present study, the silver nanoparticles are synthesized rapidly, cost-effectively and in eco-friendly manner by using the leaves of *Aristolochia bracteolata*. In this protocol, when silver nitrate ($AgNO_3$) was mixed with the aqueous leaf extract, bioreduction of Ag^+ to Ag^0 was observed by the colour change from watery yellow to reddish brown. After assessing the formation of silver nanoparticles with the help of UV-VIS spectroscopy, they were characterized by using Scanning Electron Microscopy (SEM), X-Ray Diffraction Spectroscopy (XRD) and Fourier Transform Infrared Spectroscopy (FTIR). These green synthesized silver nanoparticles were tested for their antibacterial activity using the disc diffusion method against the test cultures of gram-positive *Staphylococcus aureus, Bacillus subtilis* and gram-negative *Pseudomonas aeruginosa, Proteus vulgaris* bacterial strains. The zone of inhibition obtained revealed that the novel silver nanoparticles exhibited a tremendous potential antibacterial activity against all the four multidrug-resistant bacterial strains and has potent application in medical and pharmaceutical industries.

Keywords: *Aristolochia bracteolata*, silver nanoparticles, UV-VIS Spectroscopy, SEM, XRD, FTIR

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1.INTRODUCTION

Nanotechnology have grown day by day casting its influence on almost all spheres of human life generating novel materials known as nanoparticles in the nanometer range between 1 to 100 nm dimensions. It shows completely new or improved physical, chemical, catalytic, biological properties based on its specific characteristics such as size, shape and their morphology. These properties have drawn the interest of researchers to use them in modern industries, biomedical and pharmaceutical applications [1-4]. Recently, metallic nanoparticles like gold, silver and platinum are well recognized of which silver nanoparticles (AgNPs) emerged as an arch product, are broadly used in shampoos, soaps, detergents, cosmetics, toothpastes and medical and pharmaceutical products. Hence they are directly encountered by human systems [5, 6]. Silver has been a safe and non-toxic antibacterial agent used for centuries to prevent and treat infection as early as 69 BC. It has long been recognized as having an inhibitory effect towards many bacterial strains and microorganisms commonly present in medical and industrial processes [7-9]. Antibacterial activity of silver containing materials can be applied in medicine for reduction of infections on the burn treatment [10], prevention of bacteria colonization on catheters [11], elimination of microorganisms on textile fabrics [12], as well as disinfection in water treatment [13].A vast number of methods viz. chemical reduction of metals in aqueous solutions with or without thermal deposition, use of stabilizers in organic solvents, chemical reduction and photo-reduction, microwave assisted processes have been widely applied to synthesize nanoparticles [14-18]. In general, physical and chemical methods of synthesis are effective but expensive, resulting in wastage of energy and toxicity to the environment. Hence, biological methods of synthesis employing bacteria [19, 20], yeast [21], fungi [22, 23] or plant extracts [24-30]as reducing agents are mostly employed. This method of using plant extracts is more desirable rather than microorganisms because they exclude the process of culturing and maintaining those microorganisms. Moreover, this method is cost-effective, environmentally friendly and single-step method of green synthesis of nanoparticles. Medicinal plants are noted for their healing or disease-curing potential over centuries. Plants are largely being exploited for the synthesis of nanoparticles as they are easily available, safe and easy to handle and they possess versatile metabolites, which play vital roles in the reduction of metals. Moreover, unlike synthetic drugs, plant metabolites have an enormous therapeutic potential to heal various infectious diseases without side effects [31]. There is a great deal of scrutinity and support for the search for new and useful drugs from higher plants in developing countries. *Aristolochiabracteolata*belongs to the family *Aristolochiaceae* (locally known as kiramar (in Hindi) and Adutinnapalai (in Tamil) in India). It, a widespread weed in cultivated fields, is traditionally being used as a gastric stimulant and in the treatment of cancer, dysentery, lung inflammation and snake bites [32]. Its roots and leaves are bitter and antihelmintic in nature. Hence, an attempt has been made to synthesize and

characterize silver nanoparticles using *Aristolochia bracteolata* leaf extract and to evaluate its antibacterial efficacy.

2. MATERIALS AND METHODS

2.1 Collection and identification of plant material

Aristolochia bracteolata was harvested from their natural habitat, situated in the outskirts of Tiruchirappalli district. The leaves were identified and authenticated at the Rapinant Herbium of St. Joseph's College (Autonomous), Tiruchirapalli. The leaves were washed with distilled water and air dried for 10 days on a clean sheet. Then, they were kept in a hot air oven at 60^0 C for 24-48 hours. The leaves were then ground to a fine powder. Then the aqueous extract of the plant material were prepared, evaporated to dryness and then stored for further use [33].

2.2 Synthesis of Silver nanoparticles

1 mM silver nitrate was added to 0.5 g of the leaf extracts to make up to a final solution of 200 ml and centrifuged for 25 minutes at 18,000 rpm. Pellets were collected and stored at 4º C. The supernatant was heated at 50º to 95º C [8].

2.3 UV-VIS Spectra Analysis

The reduction of pure Ag^+ ion was monitored by measuring the UV-VIS spectrum of the reaction medium at 5 hours after diluting a small aliquot of 10 ml of the sample with 1 ml of deionized water. UV-VIS spectral analysis was done by using a Perkin Elmer Lambda-35 spectrophotometer.

2.4 SEM Analysis

Scanning Electron Microscopic (SEM) analysis was done using Hitachi S-4500 Scanning Electron Microscope.Preparation of thin films of the sample on a carbon coated copper grid was done by just dropping a very less amount of the sample on the grid, excess solution was removed with the help of blotting paper and then, the film on the SEM grid was allowed to dry by using mercury lamp for 5 min.

2.5 XRD Analysis

X-ray diffraction (XRD) measurements of the bio-reduced silver nanoparticles were done on an X'Pert Pro P Analytial X-ray diffractometer, operating at 40 kV voltage and 30 mA current with Cu Kα radiation in a θ - 2θ configuration. The crystallite domain size was calculated from the width of the XRD peaks, using the Scherrer formula. $D= 0.94 \lambda / \beta \cos \theta$, where D is the average crystallite domain size perpendicular to the reflecting planes; λ is the X-ray wavelength; β is the full width at half maximum (FWHM), and θ is the diffraction angle.

2.6 FTIR analysis

The residual solution of 100 ml was centrifuged at 5000 rpm for 10 min after reaction and the resulting suspension was redispersed in 10 ml sterile distilled water. The centrifugation and redispersing processes were repeated three times. Finally, aqueous solutions of silver nanoparticles

were analyzed by Perkin Elmer Spectrum RX I model FTIR. The absorbance spectra were measured between 400 and 4000 cm^{-1}.

2.7 Antibacterial activity of silver nanoparticles

2.7.1 Growth and Maintenance of test microorganisms:

Bacillus subtilis (MTCC No. 441), *Pseudomonas aeruginosa* (MTCC No. 1688), *Staphylococcus aureus* (MTCC No. 99) and *Proteus vulgaris* (MTCC No. 1771) were used as test organisms. The bacteria were maintained on Nutrient Broth (NB) and Luria Broth (LB) at 37°C.

2.7.2 Antibacterial assay:

Disc diffusion method was used to know the antibacterial activity of the synthesized silver nanoparticles [34]. Fresh overnight cultures of the test microorganisms (100 µl) were seeded into respective medium. Various concentrations of the silver nanoparticles biosynthesized using the leaf extract of *Aristolochia bracteolata* (40, 80, 120 and 160 µg/ml) were impregnated onto the sterile disks (6 mm). The impregnated disks were dried and placed on test organism seeded sterile petriplates. The antibacterial assay plates were incubated at 37°C for 24 h and the zones of inhibition were measured.

3. RESULTS AND DISCUSSION

3.1 Synthesis of Silver Nanoparticles:

As the leaf extract of *Aristolochia bracteolata* (Figure 1) was mixed with the aqueous solution of silver nitrate, reduction of Ag^+ ions into Ag^0 particles occurred. The colour change from watery yellow to reddish brown colour indicated the formation of silver nanoparticles (Figure 2).

3.2 UV-VIS Spectral Analysis:

UV-VIS spectra of the colloidal solution of silver nanoparticles were recorded as a function of time. Absorption spectra of the silver nanoparticles produced in the reaction media at 10 min had the absorbance peak at 428 nm and the broadening of the peak elucidated that the particles were polydispersed (Figure 3).

3.3 SEM Analysis:

SEM analysis provided further insight into the features of the silver nanoparticles. The SEM image of the drop coated films of the silver nanoparticles depicted relatively cubic and hexagonal shaped nanoparticles in the diameter range of 54-106 nm (Figure 4).

3.4 XRD Analysis:

The silver nanostructure biosynthesized by employing the leaf extract of *Aristolochia bracteolata* was further validated and verified by the distinct peaks observed in XRD analysis. The XRD pattern divulged three intense peaks in the entire spectrum of 2θ value ranging from 20 to 70. The peaks at 2θ values of 38.10, 46.28 and 57.52 corresponding to (111), (200), (312) respectively, for silver were observed Table. (1). Average size of the particles synthesized was 9 nm with the size ranging

between 54-106 nm. The average estimated particle size was 9 nm as derived from the FWHM of the peak corresponding to 111 planes (Figure 5).

3.5 FTIR analysis:

FTIR absorption spectrum after the reduction of Ag ions is shown in Figure 6.The peaks witnessed for *Aristolochiabracteolata*stabilized silver nanoparticles were at 3413.33, 2972.74, 1637.93 and 680.52 cm^{-1}, respectively. The absorbance band at 1637.93 cm^{-1} denoted stretching vibrations for –C C–C O, –C–C– [(in-ring) aromatic], C–O (esters, ethers) and C–O (polyols).

3.6 Antibacterial activity of Silver Nanoparticles:

The antimicrobial activity of *Aristolochia bracteolata* mediated silver nanoparticles was evaluated against the selected pathogenic gram-positive *Staphylococcus aureus, Bacillus subtilis* and gram-negative *Pseudomonas aeruginosa, Proteus vulgaris* bacterial strains by standard disc diffusion method. Silver nanoparticles revealed higher antibacterial activity against all the strains (Table. 2). The antibacterial activity of Ag nanoparticles at 40 µg/ml concentration showed minimum inhibition zones for all the bacterial strains. The inhibition zone was 7.53 mm for *Staphylococcus aureus*, 8.37 mm for *Bacillus subtilis*, 8.73 mm for *Pseudomonas aeruginosa*, and 7.37 mm for *Proteus vulgaris* respectively (Figure 7a, 7b, 7c and 7d). *Pseudomonas aeruginosa* showed highest activity against 40 µg/ml concentration. The antibacterial activity of Ag nanoparticles at 80 µg/ml concentration showed intermediate inhibition zones against *Staphylococcus aureus* (9 mm), *Bacillus subtilis* (10.80 mm), *Pseudomonas aeruginosa* (9.63 mm), and *Proteus vulgaris* (8.60 mm) respectively (Figure 7a, 7b, 7c and 7d). The antibacterial activity of Ag nanoparticles at 120 µg/ml concentration showed increased inhibition zone against *Staphylococcus aureus* (10.27 mm) *Bacillus subtilis* (14.80 mm) followed by *Pseudomonas aeruginosa* (12.6 mm) and *Proteus vulgaris* (11.53 mm). Ag nanoparticles revealed highest antibacterial activity at 160µg/ml concentration against *Staphylococcus aureus* (11.23 mm) *Bacillus subtilis* (16.27 mm) followed by *Pseudomonas aeruginosa* (14.03 mm) and *Proteus vulgaris* (15.73 mm) respectively (Figure 7a, 7b, 7c and 7d). The highest activity was against gram positive *Bacillus subtilis* at 160 µg/ml concentration and lowest activity was against gram negative *Proteus vulgaris* at 40 µg/ml concentration.

Figure 1. *Aristolochia bracteolata* leaves

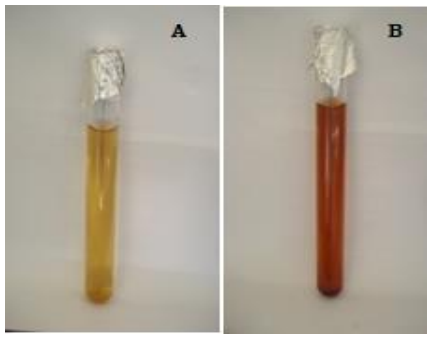

Figure 2. Colour change before (A) and after (B) the process of reduction of Ag^{+} to Ag nanoparticles

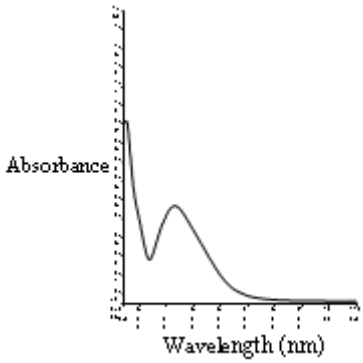

Figure 3. UV- VIS absorption spectra of Ag nanoparticles synthesized from *Aristolochia bracteolata* leaf extract with 1mM aqueous silver nitrate solution

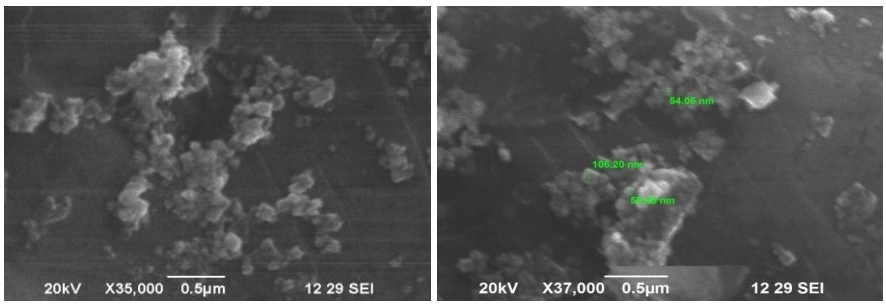

Figure 4. SEM image of silver nanoparticles formed by *Aristolochia bracteolata* leaf extract with1mM aqueous silver nitrate solution.

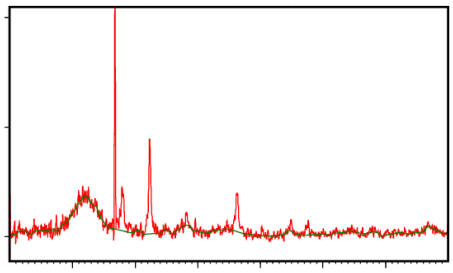

Figure 5. XRD pattern of silver nanoparticles synthesized by treating *Aristolochia bracteolata* leaf extract with 1mM aqueous silver nitrate solution.

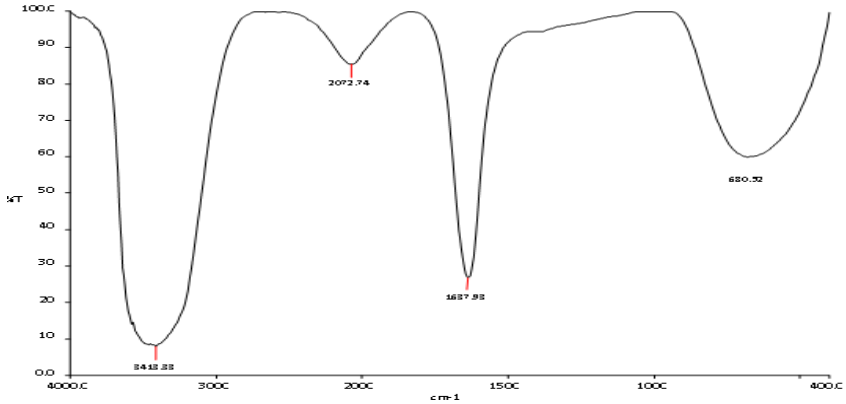

Figure 6. FTIR spectra of silver nanoparticles synthesized by treating *Aristolochia bracteolata* leaf extract with 1 mM aqueous silver nitrate solution.

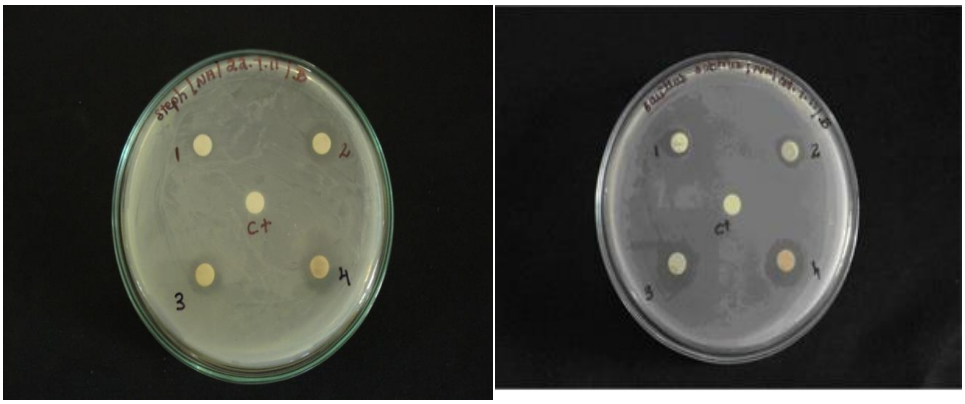

Figure 7a. The antibacterial activity of silver nanoparticles synthesized by *Aristolochia bracteolata* against *Staphylococcus aureus*; Figure 7b. *Bacillus subtilis*

Figure 7c. The antibacterial activity of silver nanoparticles synthesized by *Aristolochia bracteolata* against *Pseudomonas aeruginosa*; Figure. 7d. *Proteus vulgaris*

Ct –Negative Control; 1 – 40 µg AgNPs; 2 – 80 µg AgNPs; 3 – 120µg AgNPs; 4 – 160 µg AgNPs

Table 1: XRD pattern of silver nanoparticles synthesized by treating *Aristolochia bracteolata* leaves extract with 1 mM aqueous AgNO3 solution.

S. No.	2θ value	Plane	Element	Phase
1.	38.10	111	Ag	Cubic
2.	46.28	200	Ag	Hexagonal
3.	57.52	312	Ag	Hexagonal

Table 2: Antibacterial activity and zone of inhibition (mm) against the bacterial strains using disc diffusion method. (Mean ± S.D; n= 3)

S.NO.	Type	Bacterial Strains	Zone of Inhibition in (mm)			
			1(40 µg)	2 (80 µg)	3(120 µg)	4(160µg)
1.	*Gram positive Bacteria*	*Staphylococcus aureus*	7.53 ± 0.25	9 ± 0.36	10.27 ± 0.40	11.23 ± 0.86
2.		*Bacillus subtilis*	8.37 ± 0.25	10.80 ± 0.60	14.80 ± 0.46	16.27 ± 0.42
3.	*Gram negative Bacteria*	*Pseudomonas aeruginosa*	8.73 ± 0.25	9.63 ± 0.59	12.6 ± 0.65	14.03 ± 0.29
4.		*Proteus vulgaris*	7.37± 0.31	8.60 ± 0.53	11.53 ± 1.0	15.73 ± 0.45

Biological synthesis of silver nanoparticles using plant extracts is gaining enormous attention in modern nanotechnology. The present study involved the synthesis of silver nanoparticles via reduction of silver nitrate mediated by the leaf extract of *Aristolochia bracteolata*. This approach is explicitly to be cost effective and viable alternative to the conventional methods of synthesis of silver nanoparticles [35].Silver nanoparticles display yellowish brown colour in aqueous solution as a result of excitation of surface plasmon vibrations in silver nanoparticles [36]. However, the time taken for the colour change to yellowish brown varies from plant to plant. For instance, *Boswelliaovalifoliolata* has been reported tosynthesize silver nanoparticles within 10 min. However, *Shoreatum buggaia* and *Svensonia hyderobadensis* extracts have taken 15 min to synthesize nanoparticles [37].In the present study, yellowish brown colour, which is indicative of synthesis of silver nanoparticles, was achieved within 10 min. Thus, the results of the present investigation revealed that the potential weed *Aristolochia bracteolata* in cultivated fields is a good candidate for silver nanoparticle synthesis. Synthesis and stability of silver nanoparticles in aqueous colloidal solution were further confirmed using UV-Vis spectral analysis. It is well known that UV-Vis spectroscopy could be exploited to study size and shape controlled nanoparticles in aqueous suspensions [38]. The silver surface plasmon resonance band occurred at 428 nm and the intensity of the band increased steadily as a function of time of reaction without any shift in the peak wavelength. Similarly, the silver nanoparticles synthesized using *Euphorbia hirta* leaves have been reported to contain the absorbance peak at 430 nm [39].SEM analysis revealed the formation of relatively spherical shaped nanoparticles in the diameter range of 54-106 nm. Similar phenomenon was reported in literature dealing with the synthesis of silver nanoparticles mediated by the plant extracts of *Aloevera* [25] and *Emblicaofficinalis* [40]. The SEM image exposing the high density silver nanoparticles further affirmed the development of silver nanostructures mediated by the leaf extractof *Aristolochia bracteolata*. X-ray diffraction studies were performed to establish the crystalline nature of the nanoparticles, and the XRD pattern revealed cubic structure of silver [41]. XRD patterns were evaluated to ascertain peak intensity, position and full width at half maximum (FWHM) data and the mean particle size was calculated using Scherrer's formula. A comparison of the obtained XRD spectrum with the standard deciphered that the silver nanoparticles formed were of mixed phase (cubic and hexagonal) structures. The estimated mean size of the particle was 9 nm and it agreed well with the literature [42]. Thus, it was apparent that the silver nanoparticles produced by the reduction of silver nitrate using the leaf extract of *Aristolochia bracteolata* were cubic and crystalline in nature. FTIR measurements were performed to determine the probable biomolecules liable for the stabilization of the newly synthesized silver nanoparticles. The band at 3412 cm^{-1} indicated O-H stretching, H-bonded alcohols and phenols. The peak at 2972 cm-^{1} corresponded to O-H stretch carboxylic acids. The band at 1652 cm^{-1} could be assigned to N-H

bending of primary amines. The peak at 1637.93 cm^{-1} denoting (-COO-) of carboxylate ions could be responsible for stabilizing the silver nanoparticles. The leaves of *Aristolochia bracteolata* contain ceryl alcohol, β-sitosterol, and aristolochic acid [43].FTIR analysis demonstrated that the polyols were chiefly responsible for the reduction of Ag ions, whereby they themselves get oxidized to unsaturated carbonyl groups, ending up in a broad peak at 1637 cm^{-1} (for reduction of Ag). This implies that the biological molecules could execute dual functions of synthesis and stabilization of silver nanoparticles in aqueous medium [44].Silver nanoparticles adhere to the cell wall and can alter the permeability of the cell membrane causing the death of the cell [45]. The free radicals generated by the silver nanoparticles may also damage the cell membrane and create pores, which can eventually end up in cell death. It has also been put forth that silver ions may be released by the nanoparticles and these ions can interact with the thiol groups of most of the vital enzymes and inactivate them [46]. The bacterial cells will also take up silver ions, which hamper various functions of the cell and destroy the cells.

4. CONCLUSION

The leaf extract of *Aristolochia bracteolata* is capable of producing highly stable silver nanoparticles in solution without any impurity. In this process, silver nanoparticles were prepared in less time and in low cost. Hence, further study in this plant would give new green paths in the development of controlled shape and size silver nanoparticles. Moreover this method would be potentially exciting for the large-scale synthesis of silver nanoparticles in bactericidal applications. The present study confirmed that silver nanoparticles of *Aristolochia bracteolata* are capable of rendering high antibacterial efficacy and hence has a great potential in the preparation of drugs for bacterial diseases.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The author confirms that the data supporting the findings of this research are available within the article.

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CONFLICT OF INTEREST

Authors have no conflict of interest.

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