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Green synthesis and evaluation of antibacterial activity of silver nanoparticles from *Aristolochia Bracteolata* leaf extract

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Abstract
Nanochemistry is relatively a new area of science arisen in the last decade of past century after discovery of fullerenes and nanotubes. Metal nanoparticles can be prepared by physical, chemical and biological routes; the first one is a physical approach that utilizes several methods such as evaporation/condensation and laser ablation. Various metals like copper, titanium, gold, silver and iron were used for the synthesis of nanoparticles. Among the noble metals, silver nanoparticles have become the focus of intensive research due to its wide range of applications for many sectors of life and Industry. In this work, we describe a cost effective and environment friendly technique for green synthesis of silver nanoparticles from 1mM $AgNO_3$ solution through the extract of *Aristolochia bracteolata* leaf as it is acts as a reducing as well as capping agent. Nanoparticles were characterized using UV–Vis absorption spectroscopy, FTIR. SEM analysis showed the size ranges from 8-15nm as well spherical and crystalline nature of the nanoparticles. The synthesized silver nanoparticles exhibited potential antimicrobial activity.

Keywords: nanochemistry, *aristolochia bracteolata*, silver nanoparticles, sem and anti-bacterial activity.

1. Introduction
Nanochemistry is relatively a new area of science arisen in the last decade of past century after discovery of fullerenes and nanotubes. It is introduced into more extent interdisciplinary integrated modern science now known as rapidly developing nanotechnology [1]. Nanotechnology is becoming an innovative area of increasing research and industrial interest since the1980's. Nanotechnology can be defined as the manipulation of atom by atom from the material world by the combination of engineering, chemical and biological approaches. In the past decade, considerable attention has been paid for the development of novel strategies for the synthesis of different kind of nano-objects. Most of the current strategies are usually worked by the use of physical or chemical principles to develop a variety of nano-objects with multiple applications. Main fields of nanotechnology applications range from catalysis, micro- and nano-electronics (semiconductors, single electrons transistors), non-linear optic devices, photo-electrochemistry to biomedicine, diagnostics, foods and environment, chemical analysis and others [2].
Metal nanoparticles can be prepared by physical, chemical and biological routes; the first one is a physical approach that utilizes several methods such as evaporation/condensation and laser ablation. The second one is a chemical approach in which the metal ions in solution are reduced in conditions favoring the subsequent formation of small metal clusters or aggregates [3]. Various metals like copper, titanium, gold, silver and iron were used for the synthesis of nanoparticles. Among the noble metals, silver nanoparticles have become the focus of intensive research due to its wide range of applications for many sectors of life and industry [4]. Recently, biosynthetic methods employing naturally occurring reducing agents such as polysaccharides, biological microorganism such as bacteria and fungus or plants extract, i.e. green chemistry, have emerged as a simple and viable alternative to more complex physical and chemical synthetic procedures to obtain AgNP's [5].
In the recent decades, increased development of green synthesis of nanoparticles is inevitable because of its incredible applications in all fields of science. There were numerous work have been produced based on the plant and its extract mediated synthesis of nanoparticles. AgNP's has been synthesized by using the plant broth from a wide variety of plants such as *Catharanthus roseus* [6] and *Bacopa monnieri* [7] Keeping in view, in the present study aimed to explore the novel approaches for the biosynthesis of silver nanoparticles using *Aristolochia bracteolata* leaf and also evaluate the antibacterial activity of the synthesised AgNP's.

2. Materials and Methods
2.1 Chemicals
All the experiments were conducted at room temperature. Materials used for the synthesis of silver nanoparticles were AR grade silver nitrate ($AgNO_3$) purchased from Merck, India.

2.2 Collection of Aristolochia bracteolata leaves
The *Aristolochia bracteolata* leaves were collected in January 2018 from the campus of M. R. Government Arts College, Mannargudi, Thiruvarur district, Tamil Nadu from a single herb. The leaves were identified and authenticated

by Dr. A. Muruganandam, Assistant Professor, M. R. Government Arts College, Mannargudi, Tamil Nadu. India. A Voucher specimen has been deposited at the Department of Microbiology, M. R. Government Arts College, Mannargudi, Tamil Nadu, India.

2.3 Preparation of leaf extract
The dried powdered of *Aristolochia bracteolata* was well with mortar and pestle to make a powder. Twenty grams of *Aristolochia bracteolata* powder was mixed into one hundred mille liter of deionized water and therefore the mixture was boiled for ten minutes. Once cooling the leaf extract was filtered with Whatman No. one paper. The filtrate was hold on at 4°C for synthesis of nanoparticles.

2.4 Synthesis of Ag nanoparticles using leaf extracts
Silver nanoparticles synthesis by using five ml extract of *Aristolochia bracteolata* was added to fourty five ml of 1 mM of aqueous silver nitrate solution in a 250ml Erlenmeyer flask. The conical flask was then incubated in the dark room at 5hrs. The without leaves extract maintained as control setup. The obtained silver nanoparticle solution was purified by repeated centrifugation at 10,000 rpm for 15 min. The obtained freeze dried Ag nanoparticles were used for characterization [8].

2.5 UV-Vis and FTIR Spectra analysis
The reduction of pure Ag^+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium for 5 hours after diluting a small aliquot of the sample into distilled water. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer UV-2450 (Shimadzu). The resulting pellet is dissolved in deionized water and filtered through whatman filter paper No: 42. An aliquot of this filtrate containing silver nanoparticles were used for Fourier transmission Infrared spectroscopy (FTIR).

2.6 SEM analysis of silver nanoparticles
Scanning electron microscopic (SEM) analysis was done using ZEISS machine. Thin films of the sample were prepared 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 placing it under a mercury lamp for 5 min.

2.7 Antibacterial activity
Antibacterial activity was determined using disc diffusion method [9, 10]. The overnight inoculated bacterial cultures were spread over the freshly prepared Muller-Hinton agar plates. Petri plates were prepared by pouring 30 ml of Nutrient agar medium for bacteria. A sterile cotton swab is dipped into standardized bacterial strains of *Escherichia coli, Klebsiella pneumonia, Proteus mirabilis, Pseudomonas aeruginos, Proteus vulgaris* and *Staphylococcus aureus* were spread on solidified nutrient agar plate and allowed to dry for 10 minutes. Using sterile forceps, the sterile filter papers (6 mm diameter) containing the sample solutions (30µl) and standard (30µl) were laid down on the surface of inoculated agar plate. The plates were incubated at 37°C for 24 h for the bacteria (30±1). Each sample was tested in triplicates. The Chloramphenicol (Bacteria) disc (Reference discs) also kept on the same plate incubated at 37 ◦C for 24 h and after incubation the zone of inhibition was measured.

3. Results and Discussion
3.1 Synthesis of silver nanoparticles
The synthesis of silver nanoparticles through leaf extracts were carried out. Leaf extract is used as reducing agent for its distinctive properties with 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 also well known that silver nanoparticles exhibit yellowish - brown colour in aqueous solution due to excitation of surface plasmoo vibrations in silver nanoparticles The appearance of yellowish-brown colour (Plate.1) in the reaction vessels suggest the formation of silver nanoparticles (SNPs) [11].

$AgNO_3$ = 1 mM $AgNO_3$ without *Aristolochia bracteolata* extract. AgNPs = 1 mM $AgNO_3$ with *Aristolochia bracteolata* leaf extract after 5 hrs of incubation (Brown colour)

Plate 1: Formation of brown colour after addition of $AgNO_3$ indicate synthesis of AgNPs in the process of reduction of Ag^+ to Ag nanoparticles and control ($AgNO_3$)

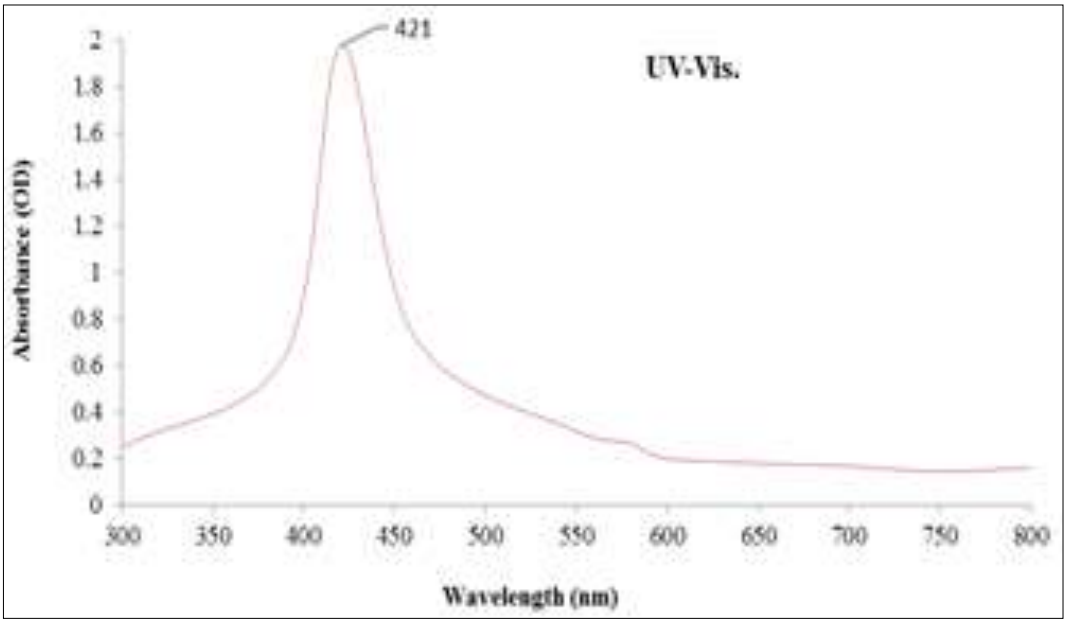


Fig 1: UV-Vis absorption spectrum of silver nanoparticles synthesized by treating 1mM aqueous $AgNO_3$ solution with *Aristolochia bracteolata* leaf extract after 5 hrs.

FTIR is an important tool which enables us to understand the involvement of functional groups in the interactions between metal particles and biomolecules. In the present work, FTIR spectra was used for the identification of biomolecules responsible for capping and stabilizing the silver nanoparticles. The FTIR spectra of the *Aristolochia bracteolata* is given in the Fig 2 and Table 1. FTIR spectrum of *Aristolochia bracteolata* extract shows peak at 3304.30, 2775.70,1651.23,1405.37, 1313.70 and 674.69. The band appeared at about 688.87 cm^{-1} can be assigned the aromatic rings. The strong broad band appearing at 3366.11 cm^{-1}can be associated to the stretching vibrations of alcoholic and phenolic O–H. Therefore, from the results of FTIR analyses of extract mediated synthesized of silver nanoparticles it can be concluded that some of the biological molecules of leaf extract such as alkaloids, phenols, flavonoids, amino acids, glycosides, and tannins are responsible for the biotransformation of silver ions to silver nanoparticles and its stabilization in aqueous medium. This result was good agreement with the earlier reports [13, 14].

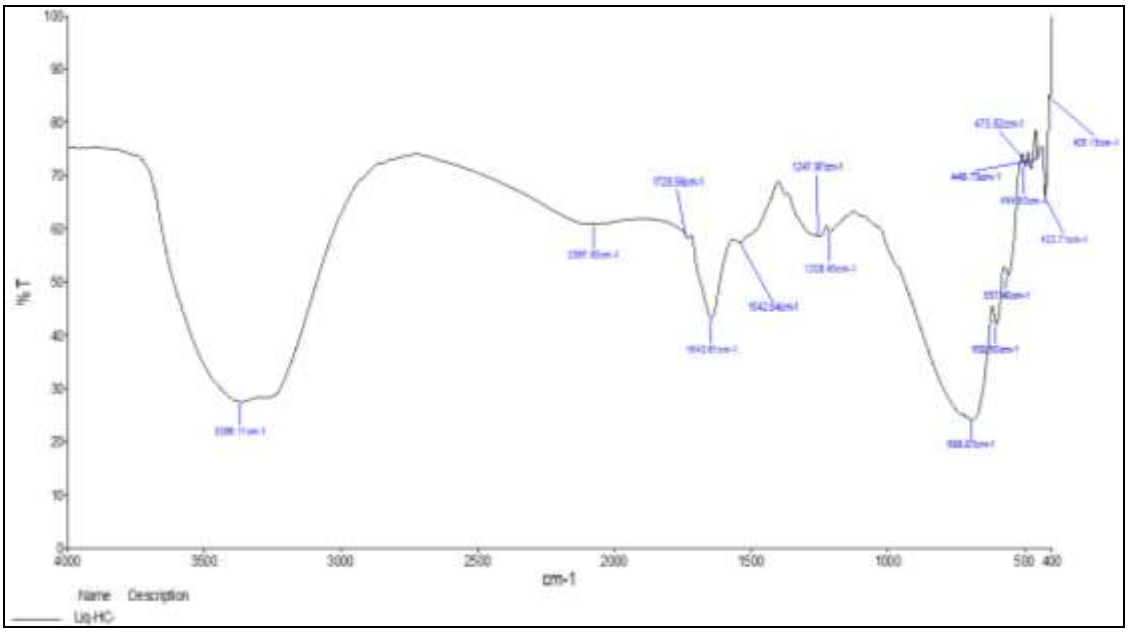


Fig 2: FTIR analysis of silver nanoparticles synthesized by treating 1mM aqueous $AgNO_3$ solution with *Aristolochia bracteolata* extract.

Table 1: FTIR analysis of silver nanoparticles synthesized by treating 1mM aqueous $AgNO_3$ solution with *istolochia bracteolata* extract.

S. No.	Peak Values	Functional groups
1	3366.11	Alcohols, phenols
2	1728.56	Carboxylic acids, aldehydes,
3	1643.61	Alkenes
4	1247.97	Aliphatic amines
5	1208.40	Alcohols, Carboxylic acids,
6	688.87	Aromatics

3.3 Scanning Electron Microscope (SEM)

The surface morphology, size and shape of the silver nanoparticles were analyzed by scanning and transmission electron microscope. Plate 2 shows the SEM image of silver nanoparticles synthesized from leaf extract. The SEM images show individual silver nanoparticles which are of 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 8 to 15 nm. Similar result of the silver nanoparticles size was reported by using *Aristolochia bracteolata* leaf extract [15].

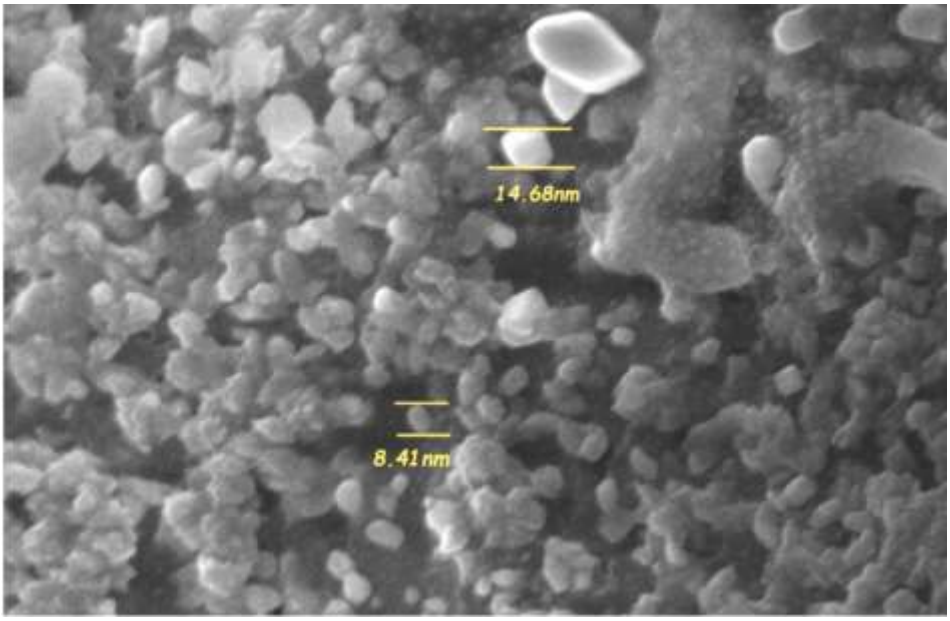


Plate 2: High resolution scanning electron microscopic (SEM) image of silver nanoparticles (AgNPs). Polydispersed (Cluster) AgNPs ranged between 8–15nm.

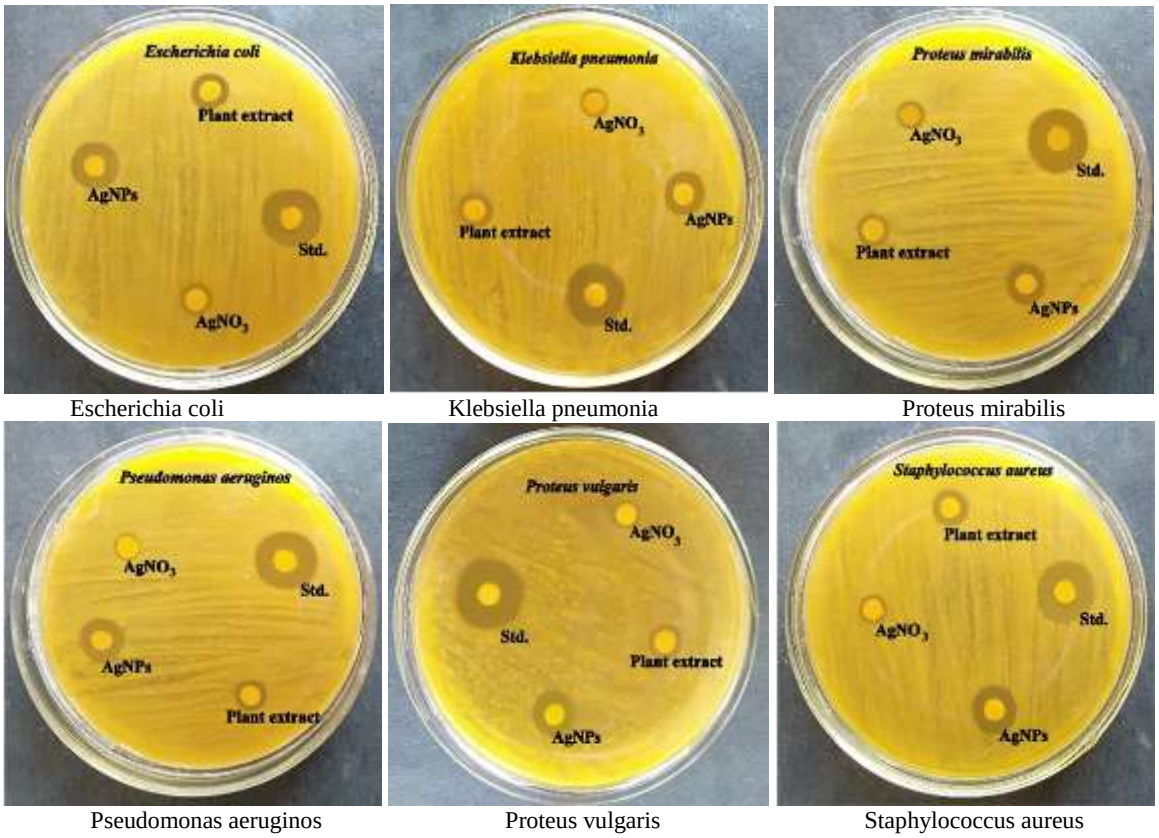
3.4 Antibacterial activity

The AgNP's of *Aristolochia bracteolata* leaf shows highest antibacterial activity was observed against *E. coli, K. pneumoniae, P. mirabilis, Ps. aeruginos, P. vulgaris* and *St. aureus*. The inhibitory activities in culture media of the Ag nanoparticles reported in table 2 and plate 3 were comparable with standard antimicrobiotic *viz.* Chloramphenicol. When Ag nanoparticles were tested they effectively inhibited microbial growth. The results show that Ag nanoparticles having antimicrobial activity against *E. coli* that was similar as observed by Sondi and Salopek-Sondi [16]. The silver nanoparticles attached to the surface of the cell membrane troubling the permeability and respiration functions followed by dysfunction of metabolic pathways including, Ag+ can interact with nucleic acids they preferentially interact with the bases in the DNA rather than with the phosphate functional groups. Thereby, inhibiting the cell division and also damaged the cell envelope and cellular contents of the bacteria [17]. However, the interaction between the components of outer cell membrane and AgNPs is not understood. Itis well established that Ag^{+} and Ag^{-} ions founded compounds have strong antimicrobial activity; several researchers are keen in using other inorganic nanoparticles as antibacterial agents [18, 9]. In another study, the mechanism of silver nanoparticles against bacterial cells due to the shape and size of the bacterial cells increased, and the cytoplasmic membrane, contents, and outer cell layers exhibited structural abnormalities [20].

Table 2: Antibacterial activity of *Aristolochia bracteolate* extract and AgNPs

Bacterial Strains	$AgNO_3$ (30µl)	Plant extract (30µl)	AgNPs (30µl)	Std. (30µl)
Escherichia coli (mm)	0.90±0.06	2.00±0.14	4.50±0.31	7.10±0.49
Klebsiella pneumonia (mm)	0.50±0.03	0.80±0.05	2.90±0.20	6.70±0.46
Proteus mirabilis (mm)	0.30±0.02	0.70±0.04	2.70±0.18	6.50±0.45
Pseudomonas aeruginos (mm)	0.60±0.04	1.10±0.07	3.50±0.24	6.80±0.47
Proteus vulgaris (mm)	0.20±0.01	0.50±0.03	2.50±0.17	6.30±0.44
Staphylococcus aureus (mm)	0.70±0.04	1.60±0.11	4.20±0.29	7.00±0.49

Values were expressed as Mean ± SD Bacterial standard: Chloramphenicol



Escherichia coli | Klebsiella pneumonia | Proteus mirabilis

Pseudomonas aeruginos | Proteus vulgaris | Staphylococcus aureus

Plate 3: shows the antibacterial activity of Aristolochia bracteolata AgNPs

4. Conclusion

Medicinal plants have medically important compounds in their different parts. The synthesis of nanoparticles using plants depends on the nature of plant such as its phyto-chemical content, special adaptation, and medicinal importance. In this study, we investigated eco-friendly and cost-effective green synthesis of silver nanoparticles using leaf extract of medicinal plant Aristolochia bracteolata. Water soluble organic compounds present in the leaf extract was mainly responsible for synthesis of silver nanoparticles by reducing silver ions to nanosized silver particles. The UV-visible spectroscopy, FTIR and SEM studies of the synthesized silver nanoparticles elucidated that the silver nanoparticles were crystalline in nature, spherical in shape with size ranging between 8 and 15nm and stable. The synthesized silver nanoparticles exhibited antimicrobial activity. This finding suggests that the synthesised AgNPs using Aristolochia bracteolata leaf extract could be a good source for developing green nano-medicine for the infectious diseases.

5. References

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