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Research Article

Herbal derived nanomedicines against for some human pathogens

D. Amirthavarshini[1], Sarmad Moin[1], T. Uma Sankari[2] and A. Vinoth Kanna[*1]

[1]Department of Biotechnology, Thanthai Hans Roever College (Autonomous), Perambalur – 621 212, Tamil Nadu, India.
[2]Department of Molecular Biology, School of Life Sciences, Madurai Kamaraj University, Palkalai Nagar, Madurai - 625 021, Tamil Nadu, India.

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***Correspondence Info:**
A. Vinoth Kanna
Department of Biotechnology,
Thanthai Hans Roever College,
Affiliated to Bharathidasan University,
Perambalur – 621 212, Tamil Nadu, India

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Abstract

The aim of this study was to achieve the silver nanocrystals for therapeutic values. The *Aristolochia bractiata* leaves extract as a reducing substance for the biosynthesis of silver nanoparticles. UV-Vis spectra analysis, Dynamic Light Scattering Particle size analyzer, X-ray Diffraction method and Scanning electron microscopy (SEM) with X-ray spectroscopy (EDS) have been employed to characterize and confirmed the nanocrystal formation. In antimicrobial activity, the silver nanoparticles were most against some human pathogens. In bacteria, the test sample was most effective against *Salmonella typhimurium* NCIM 2501 (B5) while smaller effect was noticed from *Micrococcus luteus* NCIM 2871 (B4). In fungi, which was effective against *Trichophyton rubrum* MTCC 3272 (F4) whereas smaller effect was observed in *Cryptococcus sp.* MTCC 7076 (F2). All the microbial strains depict higher sensitivity to the higher concentration (30µL) for the test sample when compared to the positive control except few bacterial strains.

Keywords: *Aristolochia bractiata*, Biosynthesis, Silver nanoparticles, Antimicrobial activity.

1. Introduction

Natural products once served humankind as the source of all drugs, and higher plants provided most of these therapeutic Agents. Research and developments in nanotechnology are leading to acceptance of this technology in day-to-day life as it continues to provide solutions and alternatives to technological, environmental, and health challenges. Nanostructures are the matter of interest for all applications of Nanotechnology wherein shape and size of the nanoparticles (NPs) determine their characteristic property [1]. Due to the growing demand for various Nanoparticles, it is necessary to develop synthesis methods that are cost-effective and environment-friendly. The majority of the existing procedures used for nanoparticle synthesis rely upon physical and chemical methods that sometimes involve toxic and hazardous chemicals. Moreover, the specific requirement for size and shape of nanoparticles cannot be met with the physio-chemical methods. In this respect, biological methods involving microorganisms or plant extracts are more effective [2]. The integration of the principles of green chemistry to nanotechnology toward the synthesis of "green" nanoparticles is a current requirement.

Nanotechnology is an expanding area of research where we use to deal with the materials in nano-dimensions [3]. The conventional procedures for synthesizing metal nanoparticles need sophisticated and costly instruments or high-priced chemicals. Moreover, the techniques may not be environmentally safe [4]. Therefore "green" technologies for synthesis of nanoparticles are always preferred. In this dissertation a "green" method for Silver nanoparticle synthesis is described. The method is simple, convenient and eco-friendly.

In general, the size of a nanoparticle spans the range between 1 and 100 nm. Metallic nanoparticles have different physical and chemical properties from bulk metals (e.g., lower melting points, higher specific surface areas, specific optical properties, mechanical strengths, and

specific magnetizations), properties that might prove attractive in various industrial applications [5,6]. However, how a nanoparticle is viewed and is defined depends very much on the specific application.

In the recent past, many researchers followed this biological route for nanoparticle synthesis. On the basis of their observations, it was inferred that plant bodies rich in secondary metabolites, might be useful for metal nanoparticle preparation. Hence a locally available plant (*Aristolochia bractiata*), which are known to contain relatively high amount of nutrients, were selected for silver nanoparticle synthesis. It was supposed that the plant extract will act as capping agent as well [7] Silver nanoparticles were prepared from aqueous solution of silver nitrate using different volumes of plant extract.

2. Materials and Methods

2.1 Preparation of plant (*Aristolochia bractiata*) extract

A large number of plant extracts has already been used to synthesize metal nanoparticles from metal salts. But the preparation methods of those plant extracts were complicated. Here we had used a very easy and effective technique to prepare the required plant extract. Relatively fresh green (*Aristolochia bractiata*) were purchased from the local market. Plant leaves (200g) were thoroughly washed by sterile water. The removed from the stalk, washed again and smashed inside a grinder. The smashed were then filtered to remove the debris. At the end, the filtered leaf extract was centrifuged at 5000 rpm for 15 minutes to obtain the liquid plant extract. The extract was preserved inside a refrigerator for future use.

2.2 Preparation of Silver Nitrate solutions

A 50 mM stock solution of $AgNO_3$ was carefully transferred in a 50-ml volumetric flask and de-ionized water was added drop-wise while swirling to dissolve the salt up to the mark. The solution was diluted as required and all the solutions were kept away from light (the containers were wrapped with brown papers) and kept in dark.

2.3 Preparation of silver nanoparticles by adding plant extract to $AgNO_3$ solutions

Before addition of plant extract to the $AgNO_3$ solution, the volume of 50 mM $AgNO_3$ solution required to attain a specific concentration (5mM, 10mM, 20mM and 25mM) of the silver salt. A set of six test tubes (Experimental 4, Control 2 – one for the leaf extract only and another for $AgNO_3$ only) were taken and marked. In five test tubes (Experimental 4, Control B), requisite volumes of AgNO3 solution and water were added one after another as shown in the table above; water (2 ml) was added in the rest tube (Control A). In five tubes (Experimental 4, Control A), 2 ml extract was poured and in the rest (Control B) only 2 ml water. The contents were mixed thoroughly and left at room temperature in dark for the formation of nanoparticles. To get a concentrated solution of the nanoparticles free from organic contents of plant leaf extract, the solution was subjected to ultracentrifugation at 30000 rpm for 12 hours. Thereafter the supernatant was aspirated out from the centrifuge tube and the loose precipitate formed at the bottom was dispersed in a small volume of deionized water. It was used in further analysis for determining various parameters like size, shape, chemical composition.

2.4 Description of Characterization Methods

For characterization of the Green silver nanoparticles following methods were necessary by means of which we can confirm the production of Silver nanoparticles; we can have an idea of its size distribution profile and surface morphology and above all, we can determine the actual particle size as well. The following instruments were used to characterize the Green silver nanoparticles that we synthesized biologically. UV-vis spectroscopy, dynamic light scattering, x-ray diffraction, scanning electron microscopy, energy dispersive x-ray spectroscopy

2.5 UV-Vis spectra analysis

A small quantity of biosynthesized Nano flakes was characterized by UV-VIS spectroscopy. After colour development, a small aliquot of the solution was absorbed between 200 and 900nm under UV-VIS spectroscopy.

2.6 Dynamic Light Scattering *Particle size analyzer*

In order to find out the particles size distribution the Ag powder was dispersed in water by horn type ultrasonic processor [Vibronics, model: VPLP1]. Then experiment was carried out in computer controlled particle size analyzer [ZETA Sizers Nano series (Malvern Instruments Nano ZS)] to find out the particles size distribution.

2.7 X-ray Diffraction method

The phase evolution of calcined powder as well as that of sintered samples was studied by X-ray diffraction technique (Philips PAN analytical, The Netherland) using Cu Ká radiation. The generator voltage and current was set at 35 KV and 25 mA respectively. The Ag samples were scanned in the 2è ranges 15 to 700C range in continuous scan mode. The scan rate was 0.04^o/sec.

2.8 Scanning electron microscopy (SEM) and X-ray spectroscopy (EDS)

In this research work, Jeol JSM-6480 LV SEM machine were used to characterize mean particle size, morphology of nanoparticles. Compositional analysis on the sample was carried out by the energy dispersive X-ray spectroscopy (EDS) attached with the SEM. The EDX analysis of Ag sample was done by the SEM (JEOLJSM

5800) machine. The EDX normally reveals the presence of phases.

2.9 Antimicrobial activity of *Aristolochia bractiata* derived silver nanoparticles

2.9.1 Microorganisms

The cultures were obtained from MTCC, Chandigarh and NCIM, Pune, India. Microbial strains *Aeromonas liquefaciens* MTCC 2645 (B1), *Enterococcus faecalis* MTCC 439 (B2), *Klebsiella pneumonia* NCIM 2883 (B3), *Micrococcus luteus* NCIM 2871 (B4), *Salmonella typhimurium* NCIM 2501 (B5), *Vibrio cholerae* MTCC 3906 (B6), *Candida albicans* MTCC 1637 (F1), *Cryptococcus sp*. MTCC 7076 (F2), *Microsporum canis* MTCC 3270 (F3), *Trichophyton rubrum* MTCC 3272 (F4). were tested for antimicrobial sensitivity using the disc diffusion method [8]. This method was used to evaluate in vitro antibacterial and antifungal activity of test sample against certain human pathogenic microorganisms on muller hinton agar (MHA) and potato dextrose agar (PDA), respectively. A sterile cotton swab was used to inoculate the standardized bacterial suspension (test culture suspensions prepared in sterile 0.85% saline matching an optical density of 0.5 McFarland standards corresponding to 108 CFU/ml) on surface of agar plate rotating the plate every 60° to ensure homogeneous growth. The 15 and 30 µL of test solutions were poured in each disc, separately. One separate disc was used for control study by taking sterile triple distilled water (without test sample). The plates were incubated at 37±1°C for 24–48 h (for bacteria) and 25 ±1°C for 48-72 h (for fungus). After incubation, the zone of inhibition was measured with ruler/HiAntibiotic ZoneScale-C. The assays were performed in triplicate and the average values are presented. Methicillin – 10mcg (for bacteria) and Itraconazole – 10mcg (for fungus) was used as positive control. All the media, standard discs and HiAntibiotic ZoneScale-C were purchased from Hi-Media (Mumbai, India).

3. Result and Discussion

3.1 Green syntheses of silver nanoparticles using plant extracts

The reduction of silver ions to silver nanoparticles by this extract was completed within 10 min. The extracellular silver nanoparticles syntheses by aqueous leaf extract validate quick, simple, economical process comparable to chemical and microbial methods. The use of plants as the production assembly of silver nanoparticles has drawn attention, because of its rapid, eco-friendly, non-pathogenic, economical protocol and providing a single step technique for the biosynthetic processes [9]. The reduction and stabilization of silver ions by combination of biomolecules such as proteins, amino acids, enzymes, polysaccharides, alkaloids, tannins, phenolic, saponins, terpinoids and vitamins which are already established in the plant extracts having medicinal values and are environmental benign, yet chemically complex structures [10].

3.2 Characterization of Silver Nanoparticles

3.2.1 UV-Vis Spectroscopy

After addition of plant (*Aristolochia bractiata*) extract to the aqueous solution of AgNO3 of different concentrations, the mixture showed a gradual change in colour at room temperature with time from yellowish to wine-red and the colour intensified after 48 hours. The colour is characteristic of the surface plasmon resonance (SPR) of silver nanoparticles. The control sets showed no change in colour under the same experimental conditions. The reduction of silver ion to silver nanoparticle was reflected in spectral data obtained by using a UV-Vis spectrophotometer. It shows an absorbance peak around 450 nm for all four samples (Figure 1), which is specific for silver nanoparticles. The resulting infusion is then filtered thoroughly until no insoluble material appeared in the broth [11]. To 10^3 M AgNO3 solution, on addition of few mL of plant extract follow the reduction of pure Ag(I) ions to Ag (0) which can be monitored by measuring the UV–visible spectra of the solution at regular intervals [12].

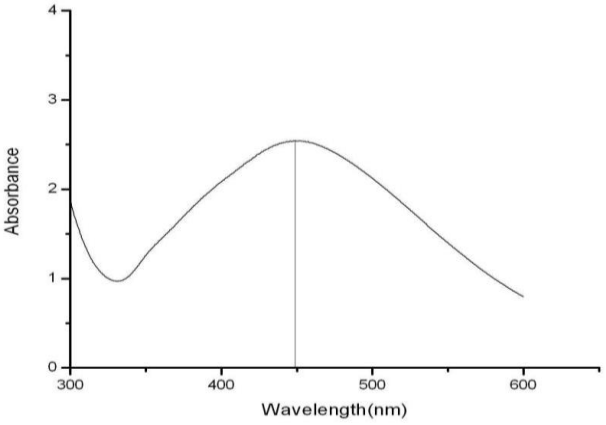


Figure 1: The absorbance spectrum of silver nanoparticles showing maximum absorbance near 450nm

3.2.2 Dynamic Light Scattering Analysis

Dynamic light scattering or Photon Correlation Spectroscopy is a technique used in material physics for determining the size distribution profile of nanoparticles in suspension or polymers in solution. Light scattering technique is used here to determine the size distribution profile of nanoparticles present in the final solution after ultracentrifugation. The study revealed that the average particle size of Ag nanoparticles ranges within 2-60 nm with average size of approximately 44 nm as shown in the Figure 2.

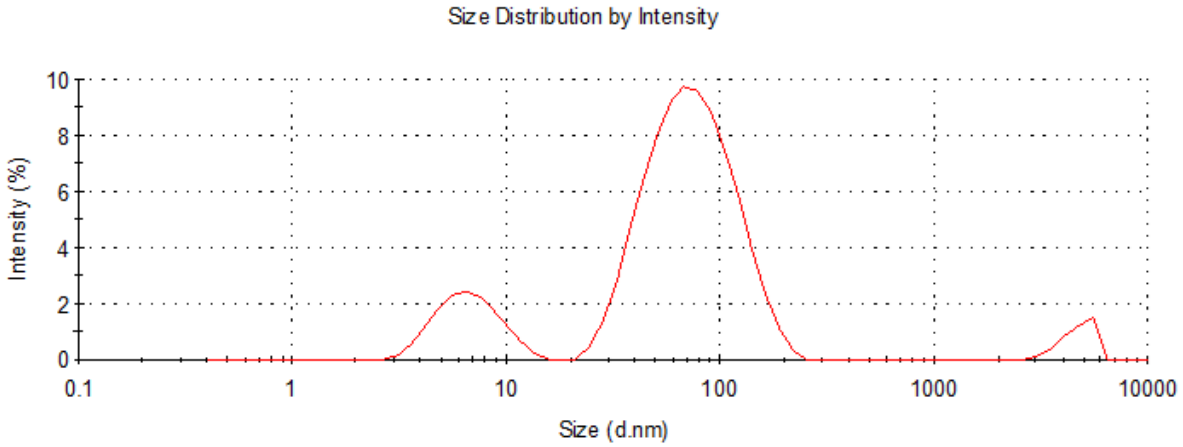


Figure 2: The DLS curve shows the average particle size as 44nm

3.2.3 X-Ray Diffraction

The suspension of silver nanoparticles was dried inside a vacuum chamber for 24 hours so that a small amount of dry silver nanoparticles can be obtained for X-ray diffraction (XRD) analysis. The XRD curve (Figure 3) confirmed that the nanoparticles are nothing but silver. Interpretation of this XRD pattern reveals the existence of diffraction lines at low angles (5° to 75°). The silver nanoparticles showed the two peaks of silver at 2θ = 38° and 44° that can be assigned to the (111) and (200) facets of silver, respectively, which go very well with the values manipulated for face centered cubic structure of silver nano-crystals (according to JCPDS: File No. 4-783). The relatively high levels of the steroids, sapogenins, carbohydrates and flavonoids act as reducing agents and phyto-constituents as the capping agents which provide stability to silver nanoparticles. The synthesized nanoparticles found to be of average size around 7–17 nm and are of spherical shaped. These nanoparticles were found to have a crystalline structure with face cantered cubic geometry as studied by XRD method. By using tea as a capping agent, 20–90 nm silver nanoparticles were synthesized with crystalline structure. Reaction temperature and the dosage of the tea extract showed an effect on the production efficiency and formation rate of nanoparticles [13].

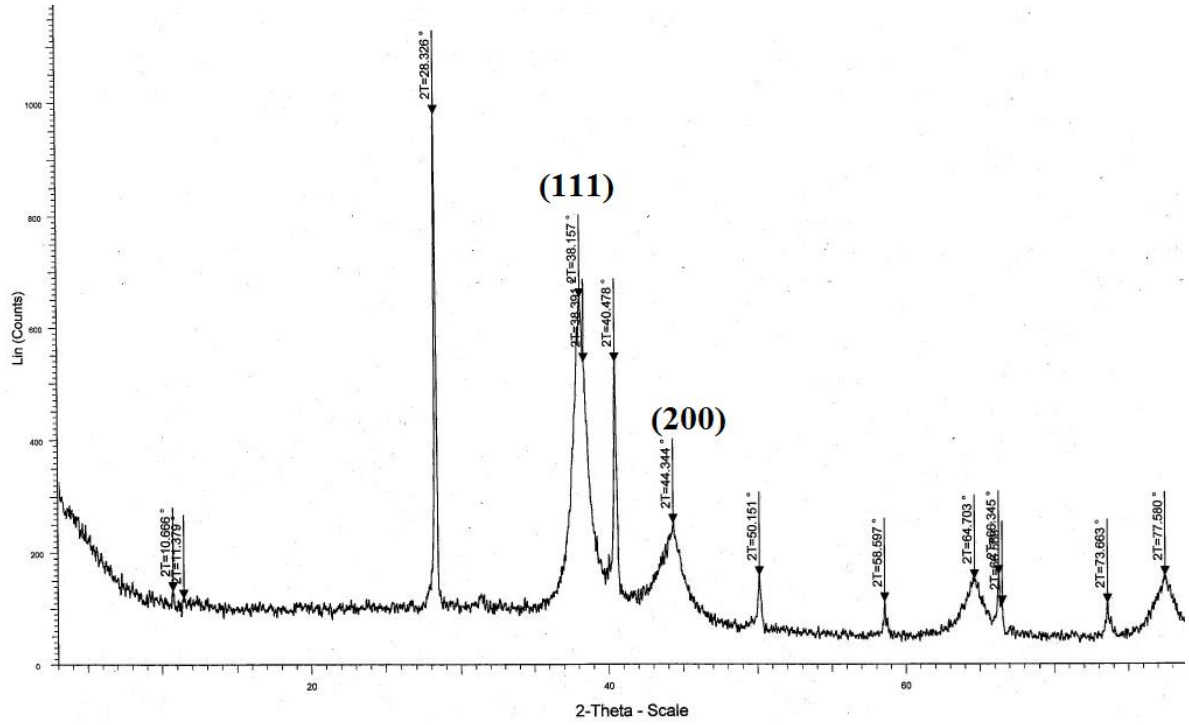


Figure 3: The XRD pattern shows two peaks assigned for (111) and (200) planes of silver

3.2.4 Scanning Electron Microscopy

Scanning Electron Microscopy is done for revealing the surface morphology of particles. Here, the bead for the SEM analysis was prepared by placing a drop of the silver nano-particle suspension on the carbon tape attached to the head of cylindrical bead and it was dried inside a vacuum dryer for a couple of hours. The particles on the top of the bead were scanned by Scanning Electron Microscope and the following image (Figure 4) was obtained. The size of spherical shaped silver nanoparticles is ranging from 5 to 20 nm, as evident by SEM. With increasing intensity of extract during the period of incubation, silver nanoparticles showed gradual change in colour of the extracts to yellowish brown with callus extract of the salt marsh plant, *Sesuvium portulacastrum* L. [14].

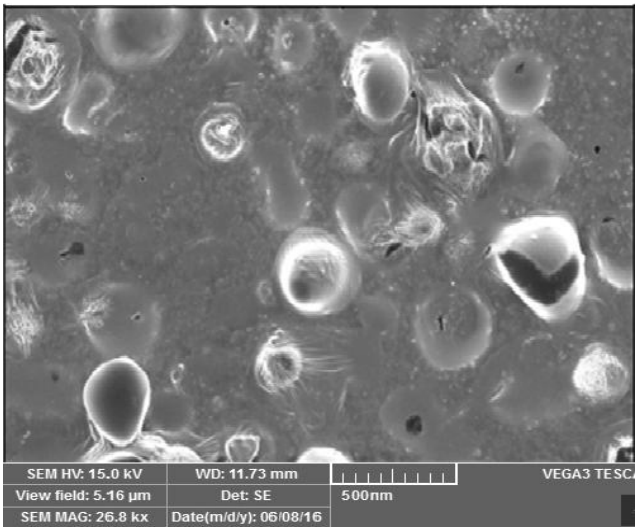


Figure 4: SEM image showing surface morphology of the silver nanoparticles

3.2.5 Energy Dispersive X-ray Spectroscopy

Energy Dispersive X-ray Spectroscopy or EDX is a technique that is mainly used to identify the presence of different elements in a sample. It is necessary to verify the presence of desired element in a sample. In the present study, this technique was used to verify the presence of Ag and the curve (Figure 5) showed a small peak of the element along with those of C and O.

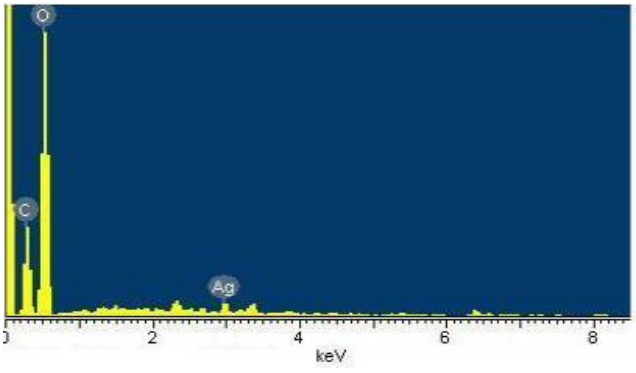


Figure 5: EDX curve of Ag-nanoparticles showing the presence of Ag and other elements (C and O).

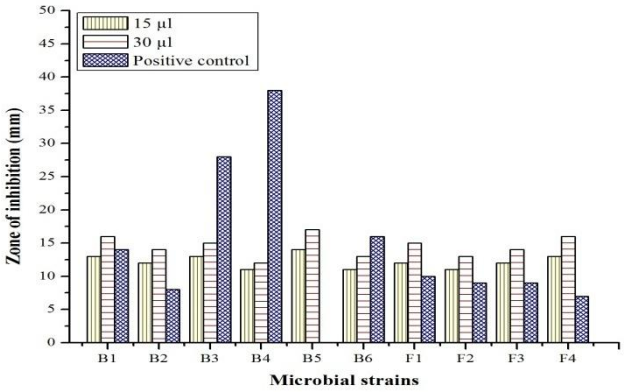
3.3 Antibacterial and Antifungal screening

The antimicrobial activity of test sample was examined with various pathogenic microorganisms using the (measure the inhibition zone) disc diffusion test. The results of the antimicrobial activities are summarized. The two tested concentrations such as 15 and 30 µL /disc produce zone of inhibition on MHA and PDA plates for bacteria and fungi, respectively. In the present study, higher (30 µL/disc) concentration of sample got greater sensitivity than (15 µL/disc) lower concentration in all the tested microorganisms. In this study, all the pathogens were fairly affected and nil effect was not observed in the test samples. In bacteria, the test sample was most effective against *Salmonella typhimurium* NCIM 2501 (B5) while smaller effect was noticed from *Micrococcus luteus* NCIM 2871 (B4). In fungi, which was effective against *Trichophyton rubrum* MTCC 3272 (F4) whereas smaller effect was observed in *Cryptococcus sp.* MTCC 7076 (F2) (Graph 1). All the microbial strains depict higher sensitivity to the higher concentration (30 µL) for the test sample when compared to the positive control except B3, B4 and B6 (Plate 1). There is no antimicrobial activity in solution devoid of sample used as a vehicle control (sterile triple distilled water), reflecting that antimicrobial activity was directly related to the sample. These silver nanoparticles exhibit antibacterial activity against *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumonia* and *Enterococcus faecal* [15]. *Acorus calamus* was also used for the synthesis of silver nanoparticles to evaluate its antioxidant, antibacterial as well as anticancer effects [16]. The dried fruit body extract of the plant, *Tribulus terrestris* L. was mixed with silver nitrate in order to synthesize silver nanoparticles [17].

The spherical shaped silver nanoparticles having size in range of 16–28 nm were achieved using this extract with antibacterial property observed by Kirby-Bauer method against multi-drug resistant bacteria such as *Streptococcus pyogens, Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli* and *Staphylococcus aureus* [18]. A silver nanoparticle of size 22 nm was synthesized using extracts of the tree *Cocous nucifera* in ethyl acetate and methanol (in ratio of EA: M40:60). It showed significant antimicrobial activity against human bacterial pathogens, viz. *Salmonella paratyphi, Klebsiella pneumoniae, Bacillus subtilis* and *Pseudomonas aeruginosa* [18,20]. A stable and spherical shaped silver nanoparticle was synthesized using extract of *Abutilon indicum*. These nanoparticles show high antimicrobial activities against *S. typhi, E. coli, S. aureus* and *B. subtilis* microorganisms [21].

At the end of this antimicrobial screening test, it is confirmed that the biologically synthesized Silver Nanoparticles (SNP) possess effective antimicrobial

property. Therefore, applications of SNPs can cover a large domain of medical, leather and food technologies.



Graph 1: Antimicrobial activity of biologically synthesized Silver nanoparticles by Aristolochia bractiata

4. Conclusion

The objective set for this study had been to prepare metal nanoparticles in a simple, cost-effective and eco-friendly way unlike chemical procedures. We used the extract of plant (*Aristolochia bractiata*) as a reducing and capping agent. By this method of preparation, the problems of environmental pollution were avoided. We successfully characterized the biologically synthesized Ag-nanoparticles, which had an average size of 18-20 nm. These nanoparticles showed antimicrobial activity against some human pathogens.

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