ANTIBACTERIAL ACTIVITY OF SILVER NANO PARTICLES USING LEAF EXTRACT ARISTOLOCHIA BRACTETA

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ABSTRACT

Nanoparticles have promising action in variety of areas and fields. Green synthesis of nanoparticles is novel way to synthesis nanoparticles by using biological source. It is gaining attention due to its cost effective, eco-friendly and large scale production possibilities. The aqueous extract of fresh leaves of Aristolochia Bracteta was used for synthesis of silver nanoparticles. Biosynthesis of AgNps from the leaf extract was carried out and characterisation of the synthesized AgNps was done using antibacterial and antifungal activity. The Ag - Nps exhibit good antibacterial and antifungal activities. New materials with outstanding electrical, optical, magnetic and mechanical properties are rapidly being developed for use in information technology, medicine, bio-engineering, and energy and environmental applications.

Key words: Nanomaterial, Antibacterial and Antifungal activity

1. INTRODUCTION

Nanoscience primarily deals with the synthesis, characterization, exploration, and exploitation of nanostructures materials. These materials are characterized by at least one dimension in the nanometre range. A nanometre (nm) is one billionth of a meter or 10^{-9} m. One nanometre is approximately the length equivalent to 10 hydrogen or 5 silicon atoms aligned in a line [1]. The processing, structure and properties of materials with grain size in the tens to several hundreds of nanometre range are research areas of considerable interest over the past years. A revolution in materials science and engineering is taking place as researchers find ways to pattern and characterize materials at the nanometre length scale [2].

Nanomaterials are cornerstones of nanoscience and nanotechnology. Nanostructure science and technology is a broad and interdisciplinary area of research and development activity that has been growing explosively worldwide in the past few years [3]. It is already having a significant commercial impact, which will assuredly increase in the future. Nanoscale materials are defined as a set of substances where at least one dimension is less than approximately 100 nanometers. This plant has wonderful medicinal uses and every part of this plant is used to treat all bacterial skin infections, skin diseases, worm killer and fever. The studies on the plant mediated biosynthesis of Ag - NPs using Aristolochia Bracteta leaf extract as reducing and stabilizing agent, which were characterized using Antibacterial and antifungal activities [4].

2. MATERIALS AND METHODS

2.1 Preparation of Leaf Extract

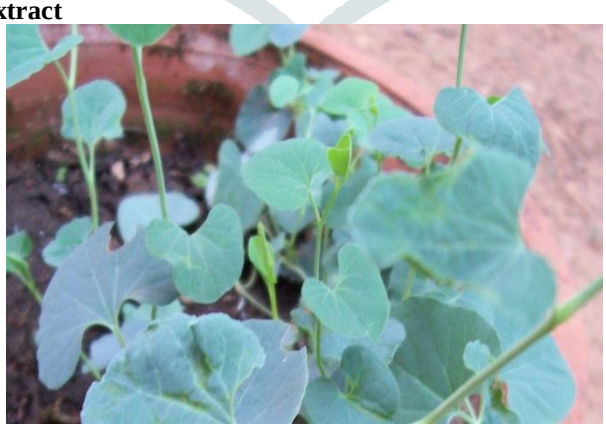

Fig2.1. Leaf of Aristolochia Bracteta

Fresh leaves of Aristolochia Bracteta (aadu theenda paalai) were collected from Madathupatti, Gonurvillage, Meetur talk, Salem district, Tamil Nadu, India. The leaves were thoroughly washed with tap water and double distilled water. The cleaned leaves were dried with absorbent paper at room temperature for few days. Then 30 grams of leaves were taken and finely grinded into powder with the help of mortor and dispensed with 100 ml of distilled water and it is stirred in stirrer for 25 minutes. Then, the extract of the leaves were collected in beakers by standard filtration method using whatman no 1 filter paper and then it is used for nanoparticle synthesis [5].

2.2 Preparation of Silver nanoparticles

1M of Silver nitrate solution was prepared by dissolving 1.698 grams of Silver nitrate in 75ml of distilled water and stirred for 30 minutes. Then 25ml of leave extract was added to the Silver nitrate solution drop by drop and stirring was continued for another one hour till nanoparticles were formed. The observed colour change from smoky white to dark mud brown indicated the formation of silver nanoparticles. The coloured silver nanoparticles solution was centrifuged at 10,000 rpm for 5 minutes, the surpentent liquid was decanted. The resulting suspension was redispersed in 5ml of distilled water and centrifugation process was repeated for three times. There after purified suspension was dried by keeping it in hot water bath and grinded into fine nano powders using a mortor. These resulting powdered nano particles were used for characterisation of Silver nano particles [6].

3. CHARACTERIZATION TECHNIQUE

3.1 Antimicrobial Activity

The antibacterial activity of pure leaf extract and silver nanoparticles were tested for the agar - well diffusion method. The agar medium was used to cultivate bacteria. Fresh over night of culture of inoculums (100 µL) of each culture was spread on to Mueller Hinton Agar plates. Sterile paper disc of 5mm diameter containing 30 µg/mL silver nanoparticles along with standard antibiotic (30 µg/mL) containing disc was placed in each plate as control. The plates were incubated at 37°c overnight. Next day the inhibition zones around the disc were measured [7].

(i) Preparation of nutrigent agar

Define volume of peptone (0.6%), yeast extract (0.15%) and di-pottasium di-hydrogen phosphate 15 psi for 20 minute(0.36%) was dissolved in distilled water and PH value must be to 7.2. This solution is sterilized by autoclaving at 15 psi for 20 minutes.

(ii) Preparation of peptone Water

Definite volume of peptones (0.6%), yeast extract (0.3%) and the beef extract (0.13%) are dissolved in distilled water and PH adjusted to 7.2 and sterilized by autoclaving.

(iii) Preparation of subculture

One day prior to the testing, inoculations of the above bacterial cultures are made in the nutrient agar and incubated at 37°c for 18-24 hrs.

(iv) Sterilization

Sterilization of the medium, tubes, borer, etc. are done by the autoclaving at 151bs/inch for 20 minutes. The glass wares like syringes, petri dishes, pipettes, and empty test tubes are sterilized by dry heat in an oven at temperature of 160°c for 1 hr.

(v) Preparation of Agar Plates

The petri dishes which measured around 32 cm diameters and 2 cm thickness are selected after sterilizing by dry heat in an oven. Base layer is obtained by pouring around 20-30ml of Muller Hinton agar solution to obtain a thickness of 4mm. It is then kept for solidification. The overnight grown subculture is taken in define volumes of peptone water and incubated at 37°c at least for 2-4 hrs prior plating. After incubation with the help if cotton swap, the organisms are streaked on petri dish containing base layer medium [8].

3.2 Experimental Procedure

Agar well diffusion method is followed to determine an antimicrobial activity, nutrient agar (NA) and potato dextrose agar (PDA) plates are swapped (sterile cotton swabs) with eight hour old-broth culture of respective bacteria and fungi. Wells (10mm diameter and about 2cm a part) are made in each of these plates using sterile cork borer. Stock solution is prepared at a concentration of 1 mg/ml in plant extract. Viz methanol, ethanol, petroleum ether and water about 100 of different concentrations of plant solvent extracts are added sterie syringe into the wells and allowed to diffuse at room temperature for 2 hours. Control experiment comprising inoculums without plant extract are setup. The plates are incubated at 370˚c for 18-24 hrs for bacterial pathogens and 280˚c for 48 hrs fungal pathogens. The diameter of the inhibition zone (mm) is measured and the activity index is also calculated [9].

4. RESULT AND DISCUSSION

4.1 Phytochemical Screening:

The phytochemical analysis extracts of ***Aristolochia bracteata*** revealed the presence of alkaloids, tannins, saponins, flavinoids, glycosides, carbohydrates, Steroids compounds and proteins. The presence of various phytochemicals was observed.

4.2 Antibacterial activity:

The bactericidal activity of the biosynthesized Silver nanoparticles using leaf extract Aristolochia Bracteta has potential antibacterial activity against both Gram- negative and Gram-positive human pathogens. AgNps displayed antibacterial activity against Gram positive and Gram negative bacteria with varying

degrees, as suggested by the diameter of inhibition zone. The result of antibacterial was shown in table 4.1. Zone of inhibition was observed with the crude leaf extract (Aristolochia Bracteta) and different concentrations of Silver nano particles (10 µL, 25 µL, 50 µL, 75 µL and 100 µL) [10, 11]. Against two gram positive bacteria (S.aureus and B.Subtilis) , two gram negative bacteria (S.typhi and E.Coli) and two fungi (A.flavus and Trichoderna SP). The antibacterial activities of Silver nanoparticles at high concentration were found to be more than at lower concentration. Whereas the antibacterial activity Leaf extract was found to be nil even at high concentration.

Fig: 4.1 Antibacterial activities for Silver nano particles

Table 4.1: Antibacterial activity for Silver Nanoparticles

S.No	Sample	Concentration	G+ve		G-ve	
1	**Positive Control**	10µL	**S.aureus**	**B.subtilis**	**S.typhi**	**E.coli**
2	**Silver Nano particles**	25µL	-	3	-	**NA**
3	**Silver Nano particles**	50µL	5	4	-	**NA**
4	**Silver Nano particles**	75µL	6	6	5	**NA**
5	**Silver Nano particles**	100µL	7	7	6	**NA**

Table 4.2: Antibacterial activity for Aristolochia Bracteata

S.No	Sample	Concentration	G+ve		G-ve	
1	**Positive Control**	10µL	**S.aureus**	**B.subtilis**	**S.typhi**	**E.coli**
2	**Aristolochia Bracteata**	25µL	NA	NA	NA	NA
3	**Aristolochia Bracteata**	50µL	NA	NA	NA	NA
4	**Aristolochia Bracteata**	75µL	NA	NA	NA	NA
5	**Aristolochia Bracteata**	100µL	NA	NA	NA	NA

Fig.4.2 Antibacterial activity for Aristolochia Bracteta leaf extract

4.3 Antifungal activity

. The results of antifungal activity were shown in table 4.3.The Antifungal activity of AgNps is greater only at lower concentration when compared with the leaf extract. But at high concentration the Antifungal activity is found to be low when compared with the Silver nanoparticles. These data confirms the concentration level of AgNps in inhibiting zone of fungi [11,12,13].

Fig 4.3. Antifungal activity for Silver nanoparticles

Table: 4.3 Antifungal activities for Silver nanoparticles

S.No	Sample	Concentration	Aspergillus flavus	Trichoderma sp
1	**Positive Control**	20µL	**Tetracycline**	
2	**Silver Nano particles**	25µL	NA	NA
3	**Silver Nano particles**	50µL	4	NA
4	**Silver Nano particles**	75µL	5	NA
5	**Silver Nano particles**	100µL	6	NA

Table: 4.4 Antifungal activity for Aristolochia Bracteata

S.No	Sample	Concentration	Aspergillus flavus	Trichoderma sp
1	**Positive Control**	20µL	**Tetracycline**	
2	**Aristolochia Bracteata**	25µL	NA	NA
3	**Aristolochia Bracteata**	50Ml	NA	NA
4	**Aristolochia Bracteata**	75Ml	8	7
5	**Aristolochia Bracteata**	100µL	12	9

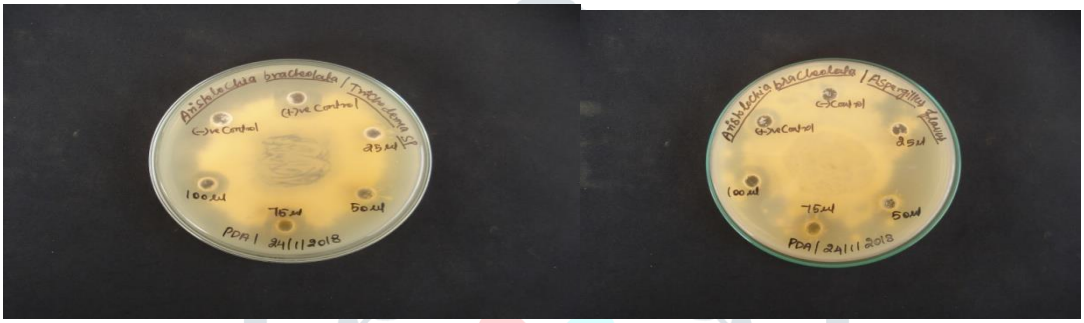

Fig 4.4. Antifungal activity for Aristolochia Bracteta leaf extract

5. CONCLUSION

The biological synthesis of Silver nanoparticles using **Aristolochia Bracteata** provides a simple and efficient route for the synthesis of nanoparticles. The green chemistry approach towards the synthesis of silver nanoparticles has many advantages such as economic, viability etc. The colour change from smoky white to mud brown after addition of leaf extract to aqueous solution of silver nitrate indicated the formation of Silver nanoparticles. The Ag-Nps were characterized with antibacterial and antifungal activities, it confirms the presence of different Phytochemical Screening.

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