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# BIO SYNTHESIS OF SILVER NANOPARTICLES FROM *ACHYRANTHES ASPERA* SEEDS EXTRACT AND ITS ANTIMICROBIAL ACTIVITY AGAINST FISH PATHOGEN

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**Abstract**

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. In the present study, the synthesis of silver nanoparticles using the seeds extract of *A. aspera* through a green route and its antibacterial activity. The synthesized silver nanoparticles were characterized by UV-VIS spectroscopy, SEM, and FTIR. The synthesized silver nanoparticles have a potent antibacterial activity against Fish pathogenic bacteria *Aeromonas hydrophila* compared to reference drug.

**Keywords:** *Achyranthes aspera,* AgNps, *Aeromonas hydrophila,* Antimicrobial Activity

## INTRODUCTION

Indian greeneries are the chief and cheap source of medicinal plants. 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 [1]. Medicinal plants have the capacity to

produce a large number of organic phytochemicals with complex structural diversity that is known as secondary metabolites. These secondary metabolites that can easily reduce silver nitrate to silver nanoparticles. Nanoparticles were synthesized through various methods such as chemical, photochemical, electromagnetic and biological techniques [2]. Synthesis of Nanoparticles through biological technique provides advancement over physical and chemical methods. Plant mediated synthesis of metal nanoparticles is gaining more importance owing to its simplicity, rapid rate of synthesis of nanoparticles of attractive and diverse morphologies and elimination of elaborate maintenance of cell cultures and eco-friendliness [3]. Silver nanoparticles (AgNPs) are presently one of the most frequently used nanomaterials in consumer products because of their proposed antimicrobial properties [4]. Silver in the form of $Ag^+$ ions has toxic effects on many pathogens, including bacteria, viruses, and fungi. Eco-friendly green synthesis with plant extracts plays a very important role in nanotechnology , without any harmful chemicals. plants provide a better platform for nanoparticle synthesis as they are free from toxic chemicals as well as provide natural capping agents [5].

In the recent past there has been a tremendous increase in the use of plant based health products in developing as well as developed countries resulting in an exponential growth of herbal products globally. One of the best plants is *A. aspera* Linn belongs to the family Amaranthaceae. It is an annual, stiff erect herb and commonly found as a weed throughout India. It is an important medicinal plants having many therapeutic uses against inflammation, microbes, odontalgic, Rheumatism, Bronchitis, skin disease and rabies. The aim of the present work to synthesize AgNPs using an eco-friendly method and evaluate the antibacterial effect.

## Material Collections

### Collection and Authentication of experimental plant:

Fresh healthy and young seeds (3 to 6 month old) of *A. aspera* L. were collected from their natural habitat of Saliyamangalm in Thanjavur district, Tamilnadu, India, and authenticated by professionals in Department of Botany, St. Joseph's College, Tiruchirappalli, India. The herbarium number of the pant (RVR001)

**Preparation of Extract:** The dried and powdered seed of *A. aspera* (500 g) in the fine powder was then extracted using soxhlet extractor. The soxhlet extraction was carried out for 3 days and the extract was collected. The excess of water was evaporated by using vacuum evaporator. The sample is evaporated to dryness under boiling water bath at $55^0$c.

### Preparation of 1mM Silver nitrate aqueous solution:

An accurately weighed 0.017g of silver nitrate was dissolved with 100ml of

double distilled water and stored in amber colour bottle until further use.

**Synthesis of silver nanoparticles:**

5ml of the seed extract of *A. aspera* was taken in the conical flask separately and placed on a magnetic stirrer with hot plate. To this 50ml of 1mM AgNO3 solution was added drop wise with constant stirring 120rpm at 50-60°C. The colour change of the solution was checked periodically. The colour change of the medium from colourless to brown after 5h was observed which indicated the formation of silver nanoparticles. It showed that aqueous silver ions could be reduced by the extract of *A. aspera* to generate extremely stable silver nanoparticles.

**Anti-Bacterial study:**

Antibacterial activity study of AgNPs was determined using well method. *A. hydrophila* was isolated from haemorrhagic septicemia diseased fish. The overnight inoculated bacterial cultures were spread over the freshly prepared Muller-Hinton agar plates. The 6 mm sterile discs (Himedia) were kept on at centre of plate of AgNPs (30 µl) was poured on disc. The Chloromphenical disc (reference disc) also kept on the plate incubated at 37 ◦C for 24 h and after incubation the zone of inhibition was measured.

**Characterization techniques:**

The techniques used for AgNPs characterization were as followed.

**UV Visible spectrum**

The resulting AgNPs is dissolved in deionized water and filtered through whatman filter paper No: 42. Silver nanoparticles are characrterized by UV-Vis spectrophotometer (Systronic). The bioreduction is monitored in the UV absorption spectrometer from 340 to 700 nm range.

**SEM analysis of silver nanoparticles**

Scanning electron microscopic (SEM) analysis was done using JSM 6701F – 6701 machine (Japan). 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 putting it under a mercury lamp for 5 min.

**Fourier transmission Infrared spectroscopy**

The pellet is dissolved in deionized water and filtered through whatman filter paper No: 42. An aliquot of this filtrate containing silver nanoparticles are used for Fourier transmission Infrared spectroscopy (FTIR). FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks in ranging from 400-4000 cm-1 and their functional groups.

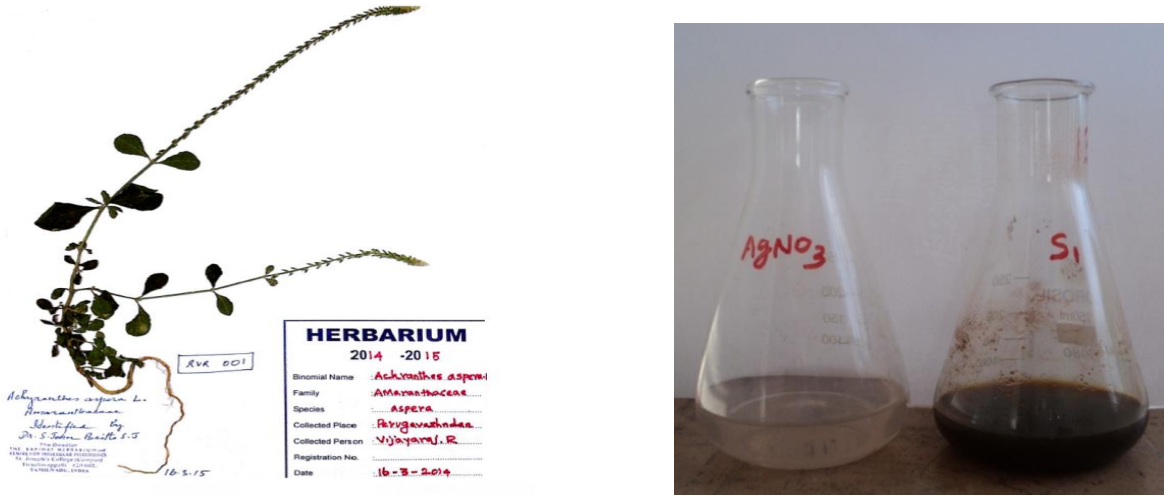


Fig 1 Biosynthesis of silvernano particle from plant extract

$AgNO_3$= 1mM $AgNO_3$without extract. AgNPs (S1)=1 mM $AgNO_3$ with ***A.aspera*** extract after 5 h of incubation (Brown colour).

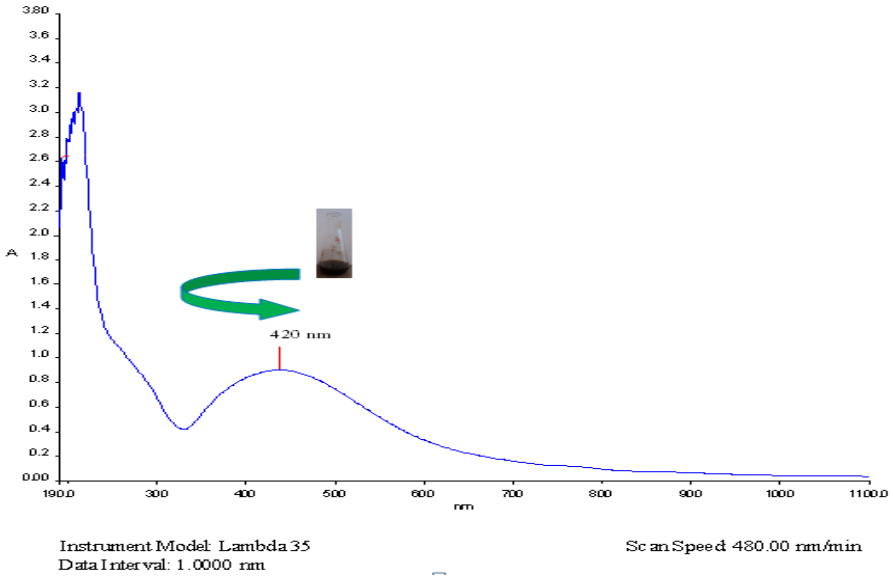


Fig 2: UV-Vis absorption spectrum of nanoparticles

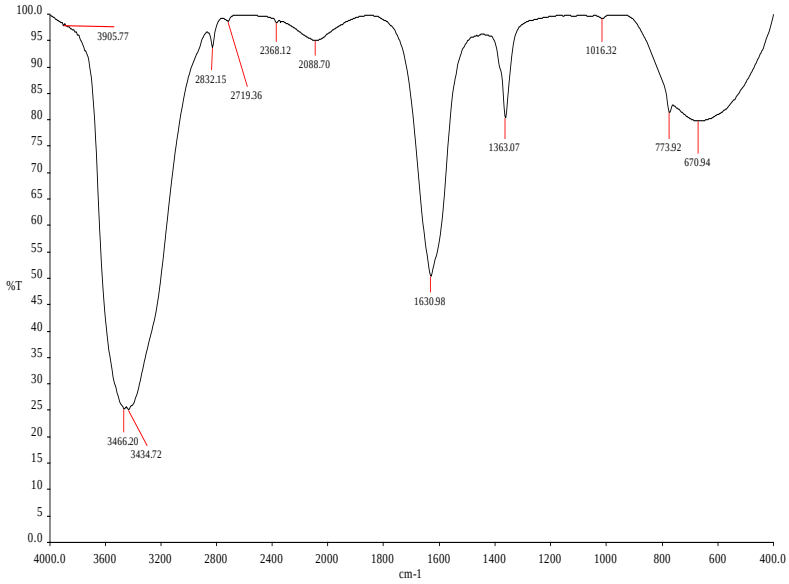


Fig 3: FTIR analysis of silver silvernano particles

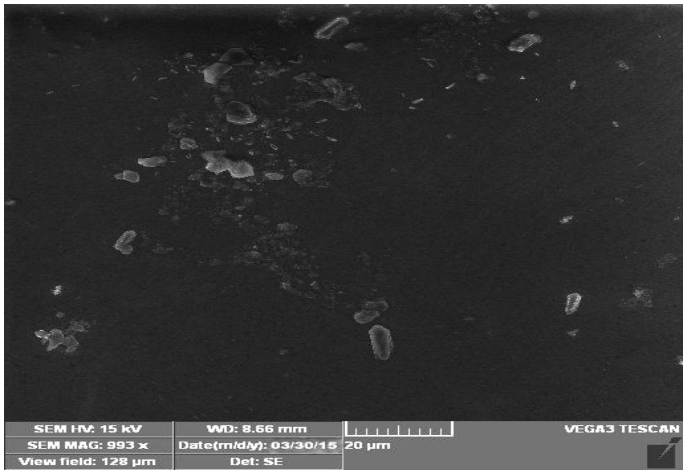


Fig 4: Image of silvernano particles in SEM

Table.1 Antibacterial Activity Of AgNPS and *Achyranthes aspera* Seed Extract

| SAMPLE | CONCENTRATIONS | *A. hydrophila* (mm) |
|---|---|---|
| Plant extract | 30µl | 8 ± 0.10 |
| AgNPs | 30µl | 10 ± 0.09 |
| Standard (Chloromphenical) | 30µl | 9 ± 0.04 |

Values were expressed as Mean ± SD. AgNPs = Silver Nanoparticles

## Results:

### Photochemical analysis:

The preliminary phytochemical screening results of *A.aspera* showed various bioactive secondary metabolites constituents such as alkaloids, flavonoids, steroids, cardiac gylcosides, reducing sugars, anthraquinones, saponins, tannins and terpenoids.

### UV-Vis Spectra analysis:

The reduction of silver is confirmed in the samples by visual observation. The sample exhibited dark brown. This colour variation may be attributed to excitation of surface plasmon vibration in silver nanoparticles. After 24 hrs incubation in dark room condition, the light coloured reaction mixtures turned into dark brown for indicating silver nanoparticle formation. (Fig.2).In the present result, the Surface Plasmon Resonance (SPR) of AgNPs produced a peak at 420nm, which suggests the dispersal of silver nanoparticles.

### SEM analysis of silver nanoparticles

SEM analysis shows uniformly distributed silver nanoparticles on the surfaces of the cells (Figure 3) SEM analysis reveals individual spherical polydispersed AgNPs as well as number of aggregates, which were irregular in shape. The size of the silver nanoparticles was found to be 5-50 nm, with an average size of 20 nm. The larger silver particles may be due to the aggregation of the smaller ones.

### Antibacterial activity

The antibacterial activities of normal and silver nanoparticle synthesized *A.aspera* seeds extract were carried out by agar well diffusion method. The maximum zone of inhibition against *A.hydrophila* (8mm) on normal leaf extract. The synthesized silver nanoparticles were tested against selected bacterial pathogens. The maximum zone of inhibition against *A.hydrophila* is (10mm). The minimum inhibitory activity against *A.hydrophila* is 8 mm by normal leaf extract. The silver nanoparticles showed maximum zone of inhibition against fish bacterial pathogen *A.hydrophila* than normal plant extract. (Table.1)

## Discussion

Nanotechnology, the creation of new objects in nanoscale dimensions, is a cutting edge technology having important applications in modern biomedical research [6]. Bioinspired synthesis of nanoparticles provides advancement over chemical and physical methods as it is a cost effective and environment friendly and in this method there is no need to use high pressure, energy, temperature and toxic chemicals [7]. The green chemistry approach was reported for the use of plant extract is an efficient route for the synthesis of pure nanomaterials [8].

The color change was noted by visual observation in the bottles that contained AgNO3 solution with extract. The color of the AgNO3/extract solution changed from colorless to light yellow and, eventually, to dark brown. (Fig.1) This color change indicates the formation of AgNP in the solution. Extract without AgNO3 did not show any color changes. The successful biosynthesis of the nanoparticles was

further confirmed by using ultraviolet-visible spectroscopy (UV-vis), Fourier transform infrared spectroscopy (FTIR) and Scaning electron microscopy (SEM).

UV-Vis spectroscopic technique is one of the simplest techniques to identify the formation and stability of the silver nano-particles [9]. UV–vis absorption spectroscopy technique can be used to investigate protein–ligand complex formation and explore the structural changes in proteins [10]. It is a method (indirect) to examine the bioreduction of AgNPs from aqueous AgNO3 solution. In the present exhibit that the UV-Vis spectrum of silver nanoparticles synthesized from seed of *A.aspera* have strong absorbance peaks at 420 nm (Fig-2) and the broadening of peaks indicated that the particles are poly-dispersed. Almost all similar results were observed in [11-13].

The silver nanoparticles are cubical, rectangular, triangular and spherical in shape with uniform distribu tion. However, on most occasions, agglomeration of the particles was observed probably due to the presence of a weak capping agent which moderately stabilize the nanoparticles [14]. The measured sizes of the agglomerated nanoparticles were in the range 287.5–293.2 nm. However, the average size of an individual particle is estimated to be 70 nm. In the present study SEM analysis provides the morphology and size details of the nanoparticles. The high density AgNPs synthesised by the plant extract of A.aspera confirms the presence of AgNPs of size ranging from 20-35nm. Particle size, size distribution and shape of silver nano particles are the important parameters that govern the properties and hence it has wide applications in medicinal fields.(Fig.4)

The Fourier transform infrared spectroscopy (FTIR) measurement was studied to identify the possible biomolecules responsible as capping and reducing agent for the AgNP synthesized by extract. FTIR gives the information about functional group present in the synthesized silver nanoparticles for understanding their transformation from simple inorganic AgNo3 to elemental silver. Our study suggested that the FTIR analysis confirmed that the bioreduction of silver ions to AgNPs is due to the reduction by capping material of the plant extract. The FTIR spectra of the sample given in the fig 3. shows the presence of silver nanoparticles, with peaks at 3452cm-1, 2086cm1, 1640cm-1 and 638 cm-1 indicates the functional group of the plant component involved in the reduction and stabilization of AgNPs. The transmittance attributes O-H stretch, C=C bond and C-H revealed that the water soluble heterocyclic components, polyols and certain proteins present in the extract are involved in the reduction of silver nitrate. These absorbance peaks are related to flavonoids, proteins and carbohydrates.

Nanoparticles are now considered a viable alternative to antibiotics and seem to have a high potential to solve the problem of the emergence of bacterial multidrug resistance [15]. In particular, silver nanoparticles (AgNPs) have attracted much attention in the scientific field [16]. Silver has long been

known to exhibit a strong toxic activity towards a wide range of microorganisms, for this reason silver based compounds have been extensively used in many bactericidal applications. [17]. The SNPs synthesized from plant species are toxic to multi-drug resistant microorganisms. It shows that they have great potential in biomedical applications. AgNps synthesized using *Alternanthera sessilis* (Linn.) extract showed antimicrobial and antioxidant activities [18]. AgNps biosynthesized from ethanolic extracts of *Phytolacca decandra*, *Gelsemium sempervirens*, *Hydrastis* canadensis and *Thuja occidentalis* showed differences in their level of anticancer and antibacterial potentials [11]. In the present study, antibacterial activities of normal and silver nanoparticle synthesized *A.aspera* extract were carried out by agar well diffusion method. Synthesized silver nanoparticles were tested against selected fish bacterial pathogen *A.hydrophila*. The silver nanoparticles maximum zone of inhibition against bacterial fish pathogen compare to normal leaf extract. The results indicated that silver nanoparticles have good antibacterial activity against bacterial *A.hydrophila*. Moreover the synthesized silver nanoparticles enhance the therapeutic efficacy and strengthen the medicinal values of *A.aspera*.

## Conclusion

The present study concluded that the seed extract of *A.aspera* can be used as an excellent source for synthesizing the silver nanoparticles. Green synthesis of nanoparticles can be ecofriendly involved in the many applications of clinical and biomedical sectors. *A.aspera* seed extract produced silver nanoparticles have been used in various applications for human being. Further the above silver nanoparticles revealed to possess an effective antibacterial property against. Fish pathogenic bacteria *A.hydrophila* .Silver nanoparticles synthesized via green rout were highly toxic to pathogenic bacteria, hence has a great potential in biomedical application and a potent antibacterial effect .This green method resulted many advantages such as ecofriendly, low cost and large scale synthesis of silver nanoparticles.

## Conflict of interest statement

We declare that we have no conflict of interest.

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