BIOSYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL EFFECT OF PLANT-MEDIATED SILVER NANOPARTICLES FROM *ADENANTHERA PAVONINA* L.

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Abstract : Nanoparticle research is currently an area of intense scientific interest due to its wide variety of potential applications in various fields. The novel nanomaterials have been used widely for the treatment of wastewater, groundwater, surface water and other environmental products contaminated by toxic metal ions, organic and inorganic solutes. In the present investigation silver nanoparticles were synthesized from the aqueous leaf extract of *Adenanthera pavonina* through biological method. The formation of AgNPs was confirmed by the visible colour change. The maximum absorption peak of 447 nm was obtained from UV-Vis spectroscopy. FT-IR analysis revealed the presence of various phytochemical compounds. Further characterization of AgNPs was done using SEM and EDX. The size of the hexagonal shaped silver nanoparticles was found to be 4- 6 nm. Antibacterial activity of silver nanoparticles showed their potential in controlling bacteria effectively.

Key Words: AgNPs, *Adenanthera pavonina*, UV-VIS, FTIR, SEM, EDX.

I. INTRODUCTION

Nanotechnology is an important field of modern research dealing with design, synthesis, and manipulation of particle structure ranging from approximately 1-100 nm in one dimension. Nanotechnology is acquiring importance rapidly in a number of areas such as cosmetics, health care, optics, electronics, mechanics, food and feed, environmental health, biomedical sciences, space industries, chemical industries, energy science, drug-gene delivery, single electron transistors, optoelectronics, catalysis, nonlinear optical devices, light emitters and photoelectrochemical applications [1]. Silver nanoparticles are being used as good antimicrobial agents. It is a well known fact that silver ions and silver based compounds are highly toxic to microorganisms [2]. They can be synthesizajpcred by physical, chemical and biological methods. Even though physical and chemical methods are numerous in number, they are expensive and may use toxic substances. Biological method is an alternate, cost effective and feasible method to synthesize silver nanoparticles, which is carried out through microbes and plants.

The plant mediated biological synthesis of silver nanoparticles has been reported from many plants such as *Emblica officinalis* [3], *Parthenium* [4], *Aloe vera* [5], *Pisonia grandis* [6], *Jatropha curcas* [7], *Justicia genderussa* [8]. Recent investigations have explained that specially formulated metal oxide nanoparticles have excellent antibacterial activity [9], and antimicrobial formulations comprising nanoparticles could be effective bactericidal materials [10] [11].

The present work is therefore aimed to evaluate the ability of the leaves of *Adenanthera pavonina* L. in the reduction of $AgNO_3$ into silver nanoparticles (AgNPs) and to characterize the biosynthesized silver nanoparticles, using UV-Visible spectroscopy, FT-IR (Fourier Transform Infrared) spectroscopy, EDX (Energy Dispersive X-ray spectroscopy) and SEM (Scanning Electron Microscopy). The antibacterial activity of the AgNPs was analyzed using standard procedures.

II. MATERIALS AND METHODS

2.1. Plant Description

Adenanthera pavonina L. belonging to the family Fabaceae, is 6-15 m tall, a medium-sized to large deciduous tree with the trunk up to 45 cm in diameter; usually erect; dark brown to grayish outer hard bark and a soft inner pale brown bark ; leaves with 2-6 opposite pairs of pinnae, oval-oblong, bipinnate; each with 8-21 leaflets on short stalks; flowers borne in spike-like racemes, creamy yellow in colour, small sized, fragrant; petals 5, connate at base, 10 prominent stamen-bearing anthers; pods narrow and long, 15- 21 × 2.5 cm.

2.2. Collection and Extraction of Plant material

The leaves of the plant were collected from Tiruchirappalli, Tamil Nadu, a southern state in India. For the preparation of the aqueous extract, 10.0 g of green healthy leaves were thoroughly washed in tap water followed by double

distilled water thrice. After washing, they were cut into fine pieces and were boiled in 100ml of distilled water. After 15min, the aqueous extract was filtered through Whatman No.1 filter paper (42µm) to get the clear plant extract. The aqueous leaf extract was used as a reducing agent for the synthesis of silver nanoparticles.

2.3. Synthesis of silver nanoparticles

Analytical grade Silver nitrate ($AgNO_3$) was purchased from Hi media laboratories Pvt. Ltd, Mumbai, India. The aqueous silver nitrate solution of 1.0 mM was prepared with double deionized water and kept at room temperature for 24 hours. For the reduction of silver ions, 10mL of leaf extract was mixed with 90mL of 1.0mM aqueous solution of $AgNO_3$ in 250mL Erlenmeyer flask and incubated at room temperature in dark. The change in colour was observed with reference to control.

2.4. Characterization of silver nanoparticles

Change in colour of the aqueous silver nitrate solution from light yellow to yellowish brown indicates the excitation of surface plasmon resonance due to reduction of silver nitrate [12]. UV-visible spectroscopic analysis was carried out by using UV-Visible absorption spectrophotometer (Shimazdu) with a resolution of 20nm between 300 to 700nm. The reduction of silver ions into metallic silver nanoparticles was monitored by UV-Visible spectra of silver nanoparticles in aqueous solution. FT-IR (Fourier Transform Infra-red Spectroscopy) is a sensitive technique useful for identifying the silver nanoparticles. In FT-IR, the silver nanoparticles absorb light in the infra-red region of the electromagnetic spectrum. This absorption corresponds to the bonds present in the silver nanoparticles. The frequency range is measured as wave numbers typically over the range 4000-500cm^{-1}. The structure, composition, and average size of the synthesized silver nanoparticles were analyzed by Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray microanalysis spectroscopy (EDX).

2.5. Estimation of antibacterial activity

The antibacterial activity of the AgNPs synthesized from the leaf extract was studied following the method suggested by Priya Banerjee *et al*. [13]. The antibacterial activity was tested against a Gram positive bacteria (*Bacillus cereus*) and a Gram negative bacteria (*Escherichia coli*). Disc diffusion method was followed for testing AgNPs containing solution. The discs were soaked with double distilled water (negative control), leaf extract of the plant, silver nitrate solution and solution containing plant-mediated synthesized silver nanoparticles.

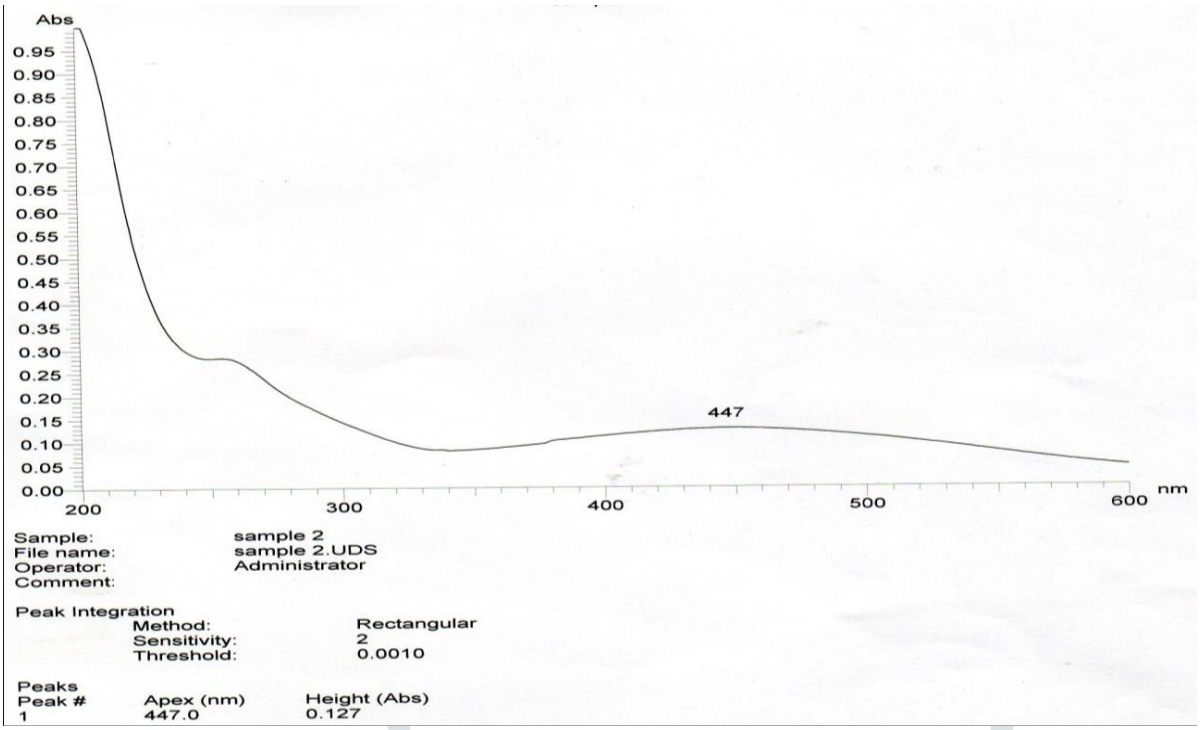
III. RESULTS AND DISCUSSION

3.1. Visual observation and UV-Visible spectroscopic analysis

The color was changed in the leaf extract when reacted with 1mM $AgNO_3$ from pale yellow to brown and dark brown within 24 hours (Fig. 1). These attained the maximum intensity after 12 hrs, with increasing intensity during the period of incubation indicative of the formation of silver nanoparticles. UV-Visible spectroscopic measurement was showed strong absorption peak at 447 nm in aqueous leaf extract of *Adenanthera pavonina* (Fig. 2).

Fig. 1: Colour change recorded at different time intervals after AgNO3 was exposed to *Adenanthera pavonina* L. leaf extract.

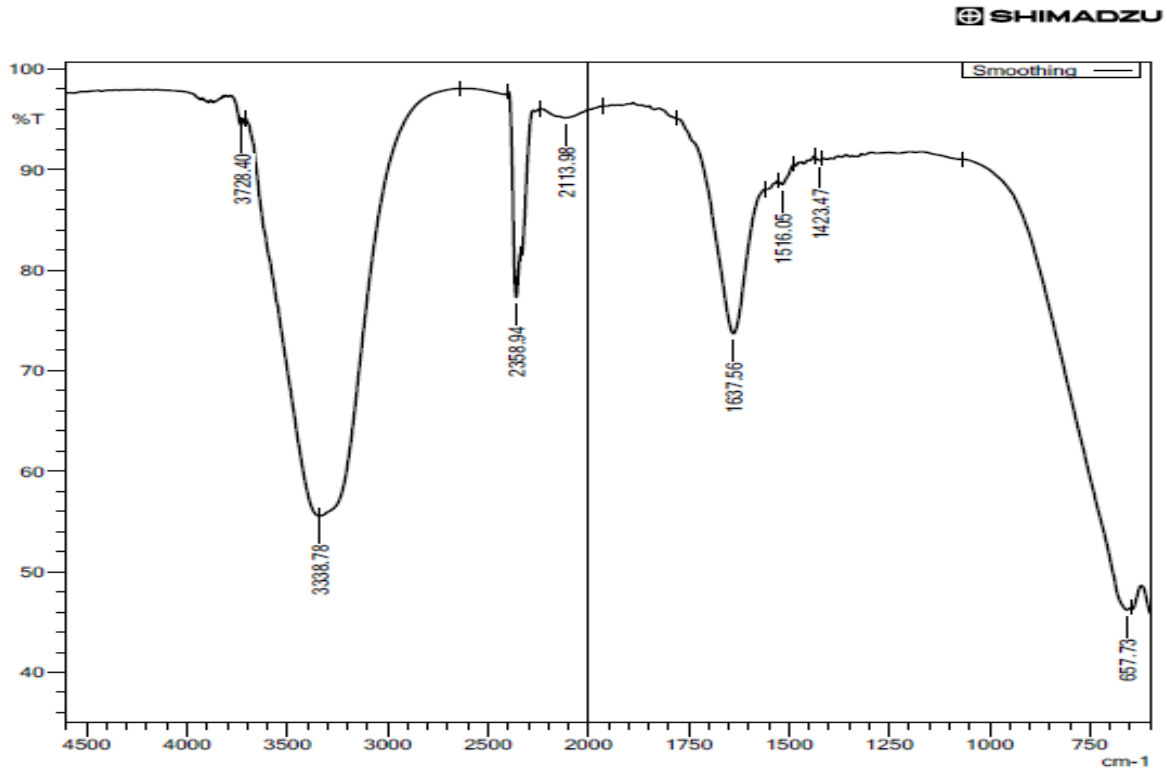
Fig. 2: UV-absorption spectrum recorded from *Adenanthera pavonina* leaf extract mediated AgNPs.



3.2. FT-IR spectroscopic analysis

The FT-IR spectrum of purified and dried silver nanoparticles (Fig. 3) showed absorbance peak at 657, 1423, 1516, 1637, 2113, 2358, 3338 and 3721 cm^{-1}. It indicates that the stretching vibrations such as C-H of aromatic rings, C-H bend and C=O, C=N and C=C double bonds, C≡C and C≡N triple bonds; and O-H, N-Hand C-H single bonds. It suggests that terpenoids, flavonoids, ketones, aldehydes, amides and carboxylic acids could be the phytochemical compounds responsible for the formation and stabilization of silver nanoparticles The fabrication of silver nanoparticles using the leaf extract of *Adenanthera pavonina* L. indicated the role of carboxyl (-C=O), hydroxyl (-OH) and amine (-NH) in the synthesis of silver nanoparticles [14] [15].

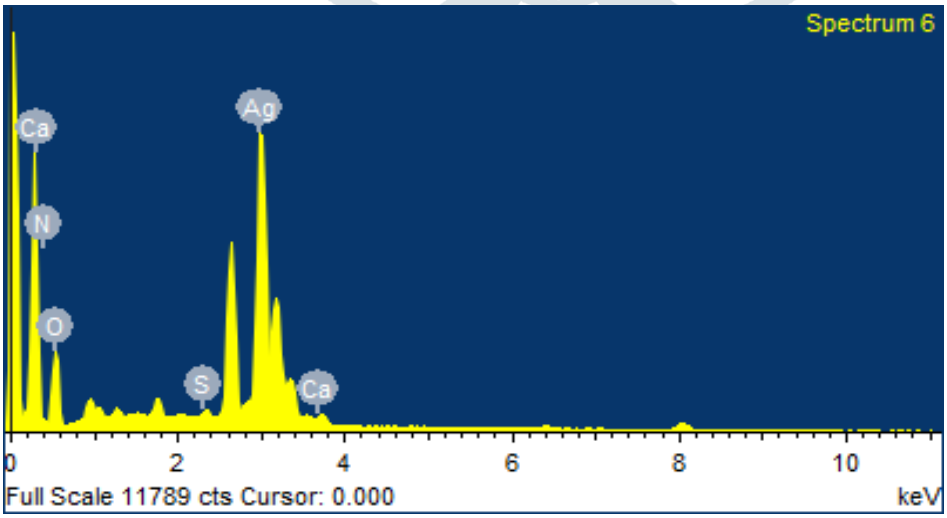
Fig. 3: FT-IR spectrum of *Adenanthera pavonina* leaf extract mediated AgNPs



3.3. EDX and SEM analysis

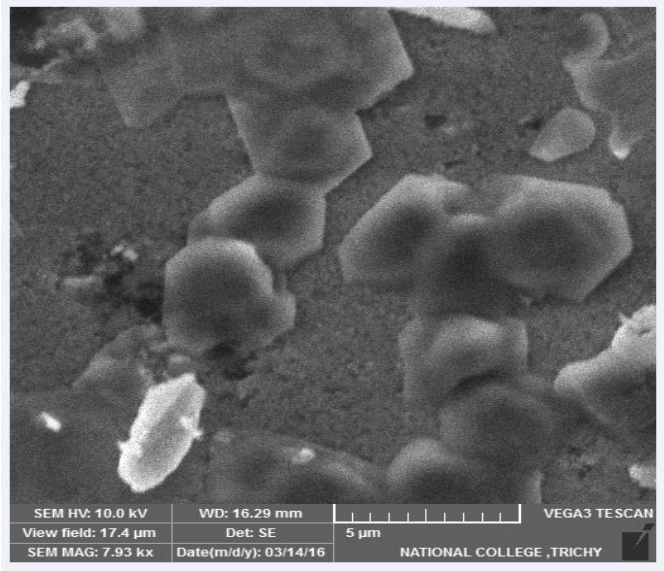
The presence of elemental silver can be observed from the EDX spectrum (Fig. 4) of biosynthesized silver nanoparticles. A strong elemental silver signal along with week Cl, O, Si and unknown elements also recorded suggests that the EDS profile shows a strong silver signal along with weak oxygen and carbon peaks, which may have originated from the biomolecules bound to the surface of the AgNPs. It has been reported that nanoparticles synthesized using plant extracts are surrounded by a thin layer of some capping organic material from the plant leaf broth and are, thus, stable in solution for a longer period after synthesis [12]. This is another advantage of nanoparticles synthesized using plant extracts over those synthesized using chemical methods. Metallic silver nanoparticles generally show typical absorption peak approximately at 3 KeV due to SPR.

Fig. 4: EDX image from *Adenanthera pavonina* mediated AgNPs



SEM image (Fig. 5) shows the surface morphology of the silver nanoparticles. The particles obtained were mostly hexagonal in nature. The size of the hexagonal shaped silver nanoparticles that were synthesized using leaf broth of *Adenanthera pavonina* L. were found to be 4- 6 nm.

Fig.5: SEM image of *Adenanthera pavonina* leaf extract reduced AgNPs.

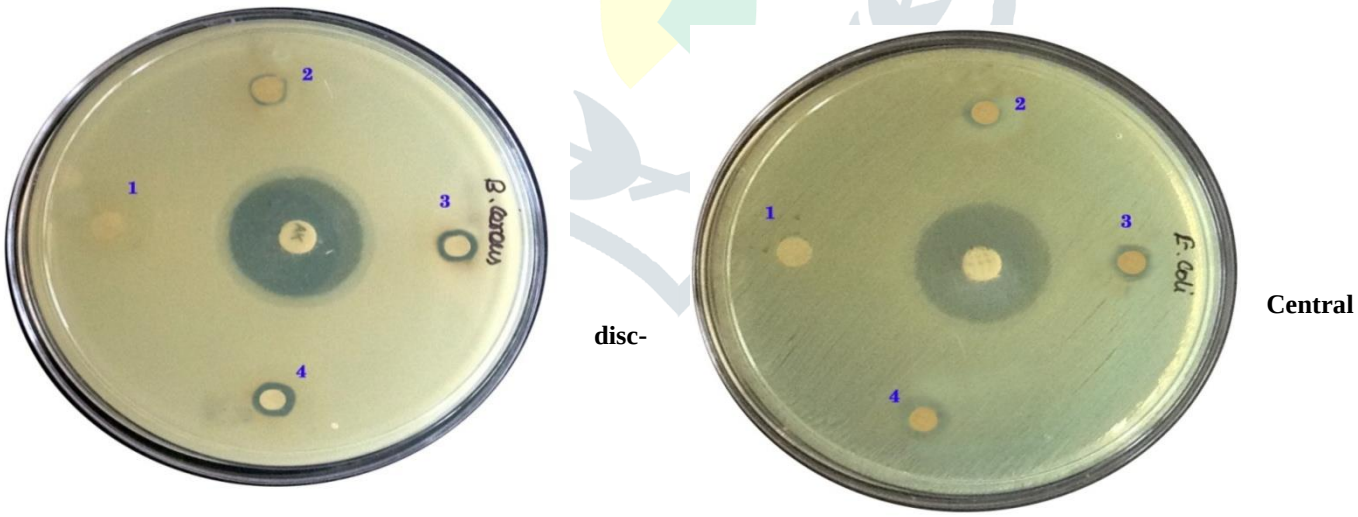


3.4. Estimation of antibacterial activity

In this study, the antibacterial property of biosynthesized AgNPs was investigated by growing *Bacillus cereus* and *E. coli* colonies on nutrient agar plates supplemented with AgNPs. Maximum antibacterial activity was exhibited by the AgNPs as indicated from the inhibition zones (Fig. 6). Results obtained in previous studies [16] also support the antibacterial potential of AgNPs. The zone of bacterial inhibition by AgNPs prepared from leaf extract showed that silver nanoparticles had inhibitory effect for both gram positive (4 mm) as well as gram negative bacteria (3mm). Such results have been already reported by Priya Banerjee *et al*. [13] with AgNPs synthesized using different plant extracts. Also, in comparison to AgNPs, antibacterial activity exhibited by plant extracts and $AgNO_3$ were not much significant. No zone of inhibition was obtained in case of negative control.

Fig.6 Antibacterial Activity of Silver nanoparticles synthesized using *Adenanthera pavonina* leaf extract

A: *Bacillus cereus* **B:** *E.coli*



Central disc- Amoxicillin disc (positive control);**1** –disc soaked in deionized water (negative control); **2** –disc soaked in crude leaf extract;**3** – disc soaked in $AgNO_3$;**4** –disc soaked in AgNPs

IV. CONCLUSION

The ability to synthesize the silver nanoparticles using the aqueous leaf extract of *Adenanthera pavonina* was demonstrated in the present study. The color change indicated the presence of silver nanoparticles and it was confirmed through UV-Visible spectroscopy, EDX and SEM analysis. The antibacterial activity of the synthesized AgNPs was also confirmed by the experimental evidences. Further studies could be carried out in future to use this plant as the phytostimulant to synthesize silver nanoparticles and to explore their commercial utilization.

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