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SYNTHESIS OF SILVER NANOPARTICLES USING *ADATHODA VASICA* AQUEOUS LEAF EXTRACT AND ITS EFFECT ON BACTERIAL ACTIVITY

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ABSTRACT

The silver nanoparticles (AgNPs) were synthesized using aqueous *Adathoda vasica* leaf extract as reducing and stabilizing agent are reported and evaluated for antibacterial activity against gram positive and gram negative bacterial species such *Salmonella typhi*, *Bacillus subtilis*. The nature of silver nanoparticles was analyzed by UV-visible spectrum, FT-IR, XRD, EDAX and TEM. The AgNPs were with an average size of 45 to 50 nm in size and mostly spherical in shape. The antibacterial activity of synthesized AgNPs was compared with that of aqueous extract of *A. vasica* by disc diffusion method. The biosynthesized AgNPs showed significantly inhibited bacterial growth against (14mm) *Salmonella typhi* and *Bacillus subtilis* (12 mm). This study revealed that aqueous leaf extract of *A.vasica* showed minimum inhibitory zone than the silver nanoparticles used. Thus AgNPs showed broad spectrum and antibacterial activity at lower concentration (1mM $AgNO_3$ in 100 ml) and may be alternative ecofriendly, nontoxic, conventional therapeutic approach in future.

KEYWORD: *A. vasica*, UV – Vis spectrophotometer, FT-IR, XRD, EDAX and TEM.

INTRODUCTION

Synthesis of metal nanoparticles and their characterization has been an emerging field of nanotechnology since the past few decades because of their unique properties and potential application in the fields of physics, chemistry, biology and medicine.[1] Nanotechnology has gained attraction in the twenty-first century and grows rapidly due to the ability to manipulate and harness properties of assemblies that are at the nanosize scale of various biomolecules.[2] Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology.[3] Sustainable initiatives have been taken to use greenchemistry approach to improve and protect environmentalissues. Development of cost effective and ecofriendly methods to synthesize nanomaterials seems to be a challenge for researchers.[4,5] Some Simpler techniques add the valuation to the synthesis productivity and operabilityof the nanoparticles. Biosynthesis of nanoparticles has received considerable attention due to the growing need to develop environmentally benign technologies in material synthesis. For instance, a great deal of effort has been put into the biosynthesis of inorganic materials, especially metal nanoparticles using microorganisms.[6,7] From recent results, researchers inspired on biological systems to develop benign nanoparticles using microorganisms, yeast and plant or plant extracts termed as green chemistry approaches.[8] Recently, plant extracts including bark, leaves, flowers and fruits have been used to synthesize the metal NPs. These biogenically synthesized NPs show more compatibility for pharmaceutical and other biomedical applications than those synthesized by chemical and physical procedures.[9,10] Here in, we report for the synthesis of silver nanoparticles, reducing the silver ions present in the solution of silver nitrate by the aqueous extract of *A. vasica* leaf. Further these biologically synthesized nanoparticles were found highly toxic against different pathogenic bacteria and fungi. Based on the literature survey it is understood that there is not much work has been done on this aspect. Therefore this study focused to carryout biomedical application of *A. vasica* using various standard techniques.

MATERIALS AND METHODS

Preparation of the extract

Fresh leaves of *Adathoda vasica* were collected from Thanjavur District, Tamil Nadu. The leaves were washed thoroughly with distilled water and shade dried at room temperature. These leaves were ground well to get fine powder. It was sieved using 50µm mesh size seiver. 10 gms of powder was taken to boil in a 100 ml of double distilled water and filtered using Whatmann No.1 filter paper (pore size 25µm).

Bio Synthesis of silver nanoparticles

1mM aqueous solution of Silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. 10 ml of *A.vasica* leaf extract was added into 90 ml of aqueous solution of 1 mM Silver nitrate and kept at room temperature for 24 hours. A change in the colour was observed indicating the formation of silver nanoparticles.

Characterization of synthesized silver nanoparticles

UV-Vis spectral analysis was done by using Shimadzu Elico UV-visible spectrophotometer (SL – 210 double beam, Japan). One millilitre of the sample was pipetted into a test tube and subsequently analyzed at room temperature. Dynamic light scattering (Spectroscatter 201) was used to determine the average size of synthesized silver nanoparticles. FT - IR spectra were recorded on Perkin Elmer 1750 FTIR Spectrophotometer. The particle size and surface morphology was analyzed using Transmission electron microscopy (TEM), operated at an accelerated voltage of 120 kV. Photoluminescence studies were evaluated by using eclipse Fluorescence spectrophotometer (agilent technologies).

Antibacterial assay

The antibacterial assays were done on human pathogenic *Salmonella typhi* G ($-^{ve}$) and *Bacillus subtilis* G ($+^{ve}$) by using standard disc diffusion method followed Kirby Bauer.[15] Mackonkey broth (HiMedia) medium was used to sub culture bacteria and was incubated at 37^0C for 24 h**.**

RESULT AND DISCUSSION

Bio synthesis of silver nanoparticles using *A. vasica* aqueous leaf extract can be easily monitored from the change in colour of reaction mixture. The colour of the reaction mixture was changed to reddish brown from yellowish colour after addition of silver nitrate in 24 hours (Fig. 1).

UV-Visual spectrum analysis

The formation of silver nanoparticles was recognized by UV-Vis spectrum (Fig. 2). As the extract was mixed in the aqueous solution of the silver ion complex, it started to change the colour from colorless to yellowish brown due to reduction of silver ion which indicates the formation of SNPs. The AgNP's synthesized from aqueous extracts of *A.vasica* showed the optical peak at 410 nm with the absorption of 3.14. The highest range of peaks indicated that the particles are polydispersed. The frequency and width of the surface plasmon absorption depends on the size and shape of the metal nanoparticles as well as on the dielectric constant of the metal itself and the surrounding medium.

(a) (b)

Figure 1 (a) 1mM $AgNO_3$ without leaf extract and (b) 1mM $AgNO_3$ with extract.

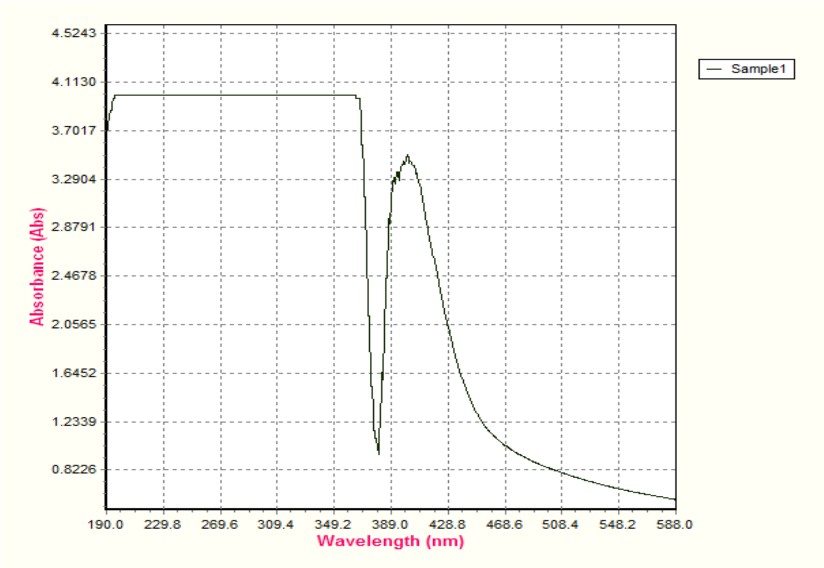

Figure: 2. UV-Vis absorption spectrum of Adathoda leaf extract.

FT-IR Analysis

The resuts obtained for the FTIR spectrum of AgNPs showed the highest peak (3305 cm^{-1}) and the lowest peak (480 cm^{-1}). Whereas moderate peak values were observed in between 1217 cm^{-1} to 1635 cm^{-1}. This peak revealed that it could be due to the presence of the different functional biomolecules may be on the surface of nanoparticles same trend of results were also observed by.[13, 14] From the above results it is understood that the presence of the O-H and N-H functional and repressible of group may influence and also responsible for the formation of silver nanoparticles and its stabilization.

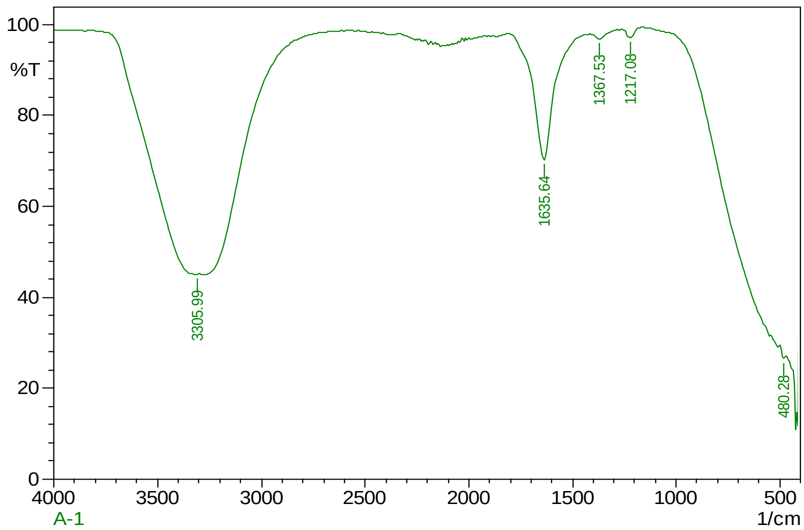

Figure: 3 FTIR spectra of bio-reduced silver nanoparticles. The peak in the graph represents the different functional groups responsible for the stabilization of AgNPs.

XRD Analysis

Analysis of a nanoparticles using X- ray differaction confirmed the crystalline nature of the biosynthesized particles (Fig. 4) shows the X- Ray diffracted image of silver nanoprticles. The diffracted intensities were recorded from 20^{θ} to 80 $^{\theta}$ 2 theta angles. The diffraction pattern showed peaks at 27.82, 32.50, 38.35 and 46.50 correspond to silver nanoparticles confirming the existence silver planes in the sample. The Braggs reflections were observed at 2θ = 27.82, 32.50, 38.50 and 46.01. Hence XRD pattern thus clearly shows the formation of silver nanoparticles. Average size of the particles synthesized was 36.3 nm with cubic and hexagonal shapes. The obtained results illustrate that the silver ions had indeed been reduced to AgO by *A. vasica* plant extract under reaction conditions.

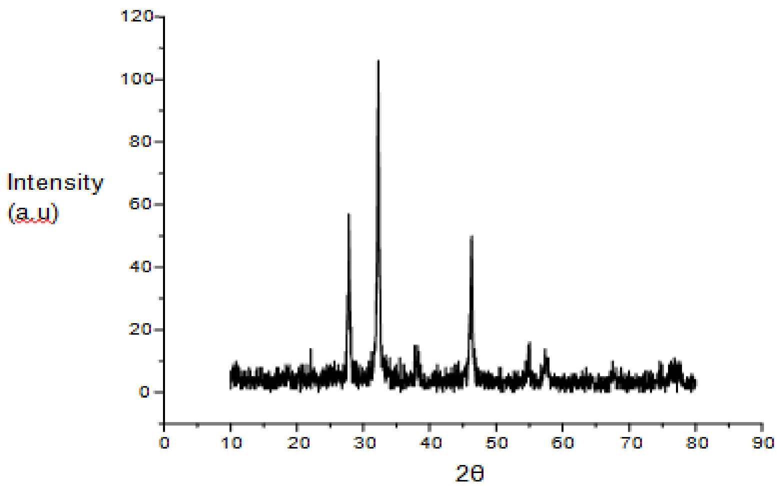

Figure: 4. XRD pattern of Silver nanoparticles synthesized by leaf extract

TEM analysis

Transmission electron microscopy (TEM) has been used to identify the size, shape and morphology of biosynthesized nanoparticles. It reveals that the silver nanoparticles are well dispersed and predominantly spherical in shape, while some of the NPs were found to be irregular in shape as shown in (Fig. 5). The nanoparticles are homogeneous and spherical which confirms to the shape of SPR band in the UV- visible spectrum. The size of the biosynthesized SNPs obtained the TEM micrograph showed the range from 10nm to 50 nm diameter, generated by using leaf extract of *A. vasica*.

EDX analysis

In fig (5a) a standard EXD spectrum recorded on the examined sample is shown. In the margin area of the spectrum clearly shows peak spectrum located between 0 to 0.9 KV and is the middle part of the presented spectrum one can clearly see 3 peaks located between at 2kV and 4 kV in the right part of the spectrum at 8 kV clearly comes from the capper. The maxima are directly related to the silver characteristic line. The copper and silver spots in the examined samples confirm the presence of stabilizations. The spectrum obtained during EDX studies were used for carrying out quantitative analysis, which proved high silver content in the examined sample.

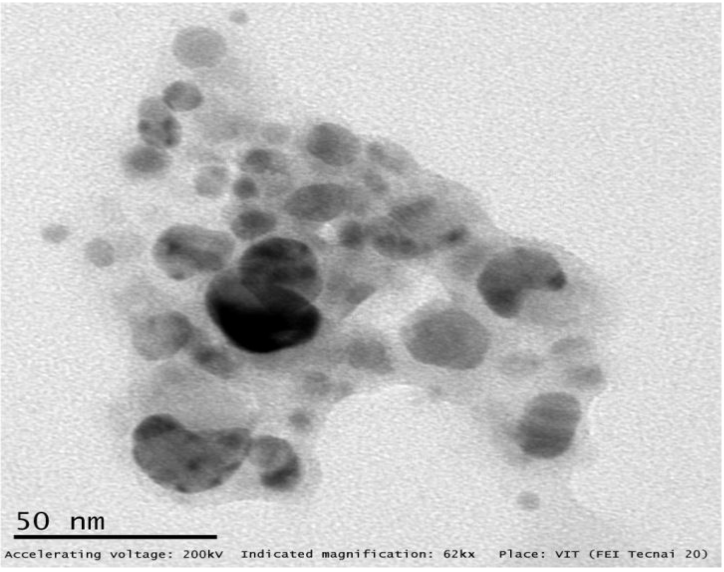

Figure: 5. TEM microscope of silver nanoparticles synthesized using *A.vasica* extract

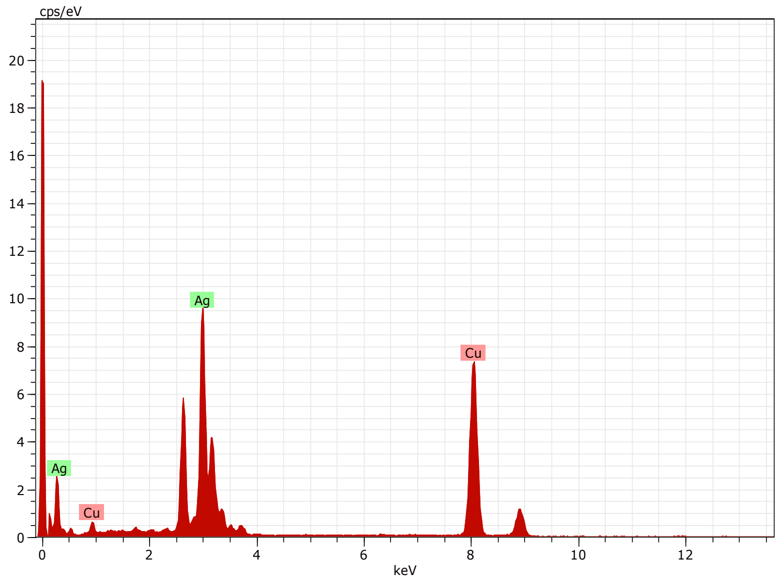

Figure: 5a. EDAX spectra of Silver Nanoparticles

Antibacterial assay

Antibacterial potential of biosynthesized silver nanoparticles using *A. vasica* leaves extract was evaluated against the microorganisms following disc diffusion method are represented in (Table 1and fig. 6). That could be due to the different mechanism by which the silver nanoparticle inhibits cell wall synthesis by interference with cell wall synthesis, inhibition of protein synthesis, interference with nucleic acid synthesis and inhibition of a metabolic pathway.[11,12] It has been proven that the antibacterial property of silver nanoparticles is size dependent. Followed by based on the observation from this assay SNPs showed more significant clear zone of inhibition against S*almonella typhi* following by *Bacillus subtilis* than that of the normal *A. vasica* leaf extract. Zone of inhibition was found to be in the range of 14 mm for tested *Salmonella typhi* and 12 mm for *Bacillus subtilis*. Whereas for aqueous leaf extract 10 mm for S*almonella typhi* and 8 mm for *Bacillus subtilis*. However, as compared to *B. subtilis* maximum inhibition was observed in the s. typhi. The result shows that SNPS synthesized from leaf extract of *A. vasica* revealed potential antibacterial activity against *S. typhi*. The zone of inhibition of streptomycin was compared with silver nanoparticles. It was observed that zone of inhibition in the control plate showed maximum zone of inhibition.

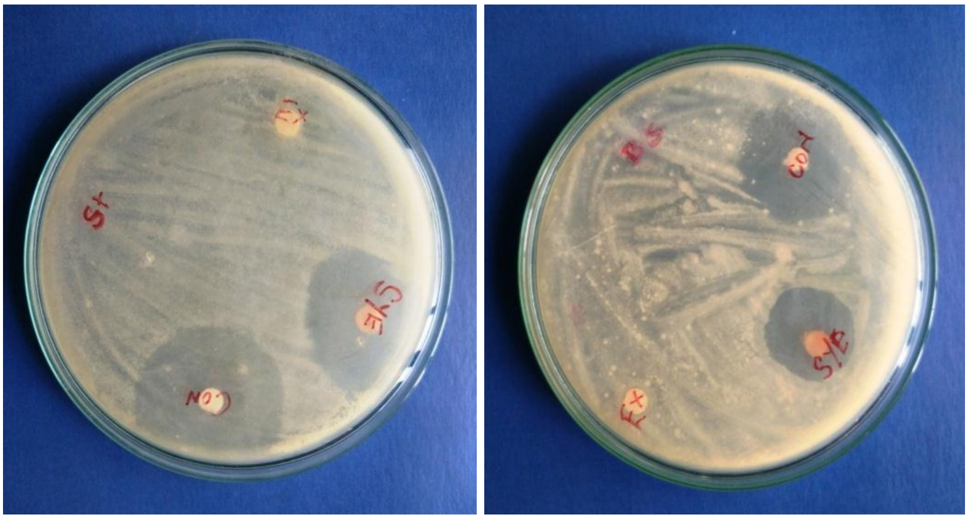

Fig.6. A- *Salmonella typhi* **B- *Bacillus subtilis***

Table: 1 Antibacterial activity by disc diffusion method

Bacterial stains	Extract (50µl)	Silver nanoparticles (50µl)	Control (streptomycin) (20µl)
Salmonella typhi	10 mm	14 mm	20 mm
Bacillus subtillis	8 mm	12 mm	18 mm

CONCLUSION

The result showed that *A. vasica* plant plays an important role in reduction and stabilization of silver to silver nanoparticles. Further these biosynthesized silver nanoparticles showed excellent antibacterial activity. This study explores the potential use of nanoparticles as alternative medicine. Therefore, further studies are needed to fully characterize the toxicity and the mechanisms involved anticancer activity of these particles.

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