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# PLANT MEDIATED SYNTHESIS OF SILVER NANOPARTICLES AND THEIR APPLICATIONS

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## ABSTRACT

To meet the increasing demands for commercial nanoparticles new eco-friendly "green" methods of synthesis are being discovered. Plant mediated synthesis of nanoparticles offers single step, easy extracellular synthesis of nanoparticles. The biosynthesis of silver nanoparticles using Bryophytes as plant source is practiced previously. They are easy to harvest and easy to make an extract and therefore, can be used for green synthesis of nanoparticles. In this study, the plant extract was prepared in ethanol and treated with silver nitrate to obtain nanoparticles. The synthesis of nanoparticles was confirmed by change in colour from pale green to reddish brown. Further, a peak between 400nm to 440nm was obtained on UV-Vis spectrophotometer which confirmed the biosynthesis of silver nanoparticles. Presence of silver nanoparticles was observed after carrying out SE microscopy with EDS that gave a strong silver signal. Since the nanoparticles show antibacterial activity, their immobilization was also carried out.

**KEY-WORDS: Silver nanoparticles, *Anthoceros*, biosynthesis, antibacterial activity, gauze cloth.**

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## INTRODUCTION

Since the last decade, nanoparticle biosynthesis is the active area of research. The most effectively studied nanoparticles in the recent past are those made from the noble metals such as silver[1], gold[2] and platinum[3]. Nanoparticles find vast applications in various fields ranging from medical to physical fields.[4,5,6] Various strategies are employed for synthesis of silver nanoparticles[7]. Nanoparticles are synthesized by reduction in solutions[8], thermal decomposition of silver compounds[9], microwave assisted synthesis[10], and laser mediated synthesis[11] and biological reduction method[12]. The latest is the most preferred way for synthesis of nanoparticles as it offers one step, eco-friendly way of synthesis of nanoparticles. Biosynthesis of nanoparticles using plant extracts is the favourite method of green, eco-friendly production of nanoparticles and exploited to a vast extent because the plants are widely distributed, easily available, safe to handle and with a range of metabolites. The plant material used for biosynthesis of nanoparticles includes Angiospermic plants such as *Helianthus annus*, *Oryza sativa*, *Zea mays*, *Sorghum bicolor*[13], *Eucalyptus hybrida*[14], *Artocarpus heterophyllus*[15] and Gymnospermic plants such as *Cycas*[16] and many more. Biosynthesis of nanoparticles is also attempted in primitive organisms such as Fungi and Bacteria[1,18].

Bryophytes are primitive land plants and show simple organization of the plant body (thallus)[19]. However, the phytochemical work on these primitive plants shows that they possess a variety of chemicals and therefore can be used in many ways[20, 17, 21, 24]. As compared to Angiospermic plants, Bryophytes are advantageous as the interference of the biochemicals could be lower due to their simple primitive organization of body. In this paper we state simple eco-friendly, one step process of biosynthesis of silver nanoparticles using *Anthoceros* (Bryophyta- Anthocerotae) as the plant source.

There are fewer reports on incorporation of nanoparticles in some substrate or their adsorption on some surface[22, 23]. It is important to carry out such studies since the antibacterial activity of the silver nanoparticles can be exploited to a larger extent and in various fields.

## MATERIALS AND METHODS

### *Plant material and extraction process*

Fresh, green mature thalli of *Anthoceros* were used for preparation of extract. The thalli were thoroughly cleaned using water and detergent. 5g plant material was weighed and was boiled in 10 ml of 80% ethanol. The ethanolic extract thus obtained was filtered through coarse filter paper to obtain a clear extract.

### *Synthesis of nanoparticles*

0.5mM aqueous solution of silver nitrate was prepared for synthesis for silver nanoparticles. 1ml of this solution was added to 5 ml extract of the plant material and was boiled to obtain silver nanoparticles.

### *Characterization of silver nanoparticles*

**a) UV- Vis spectra analysis**

The reduction of metallic $Ag^+$ ions was monitored by measuring the UV- Vis spectrum after about 10 minutes of reaction. A small aliquot was drawn from the reaction mixture and a spectrum was taken on a wavelength from 200nm to 600nm on UV-Vis spectrophotometer (Systronics Double beam spectrophotometer 2202).

**b) SEM analysis**

For the SEM and EDS analysis the suspension of nanoparticles was dried into powder and about 1mg fine powder was used for the analysis.

SEM analysis was carried out on JEOL JSM 6360A (SEM) and using JEOL JSM 1600A fine coater for uniform coating of Platinum on the sample.

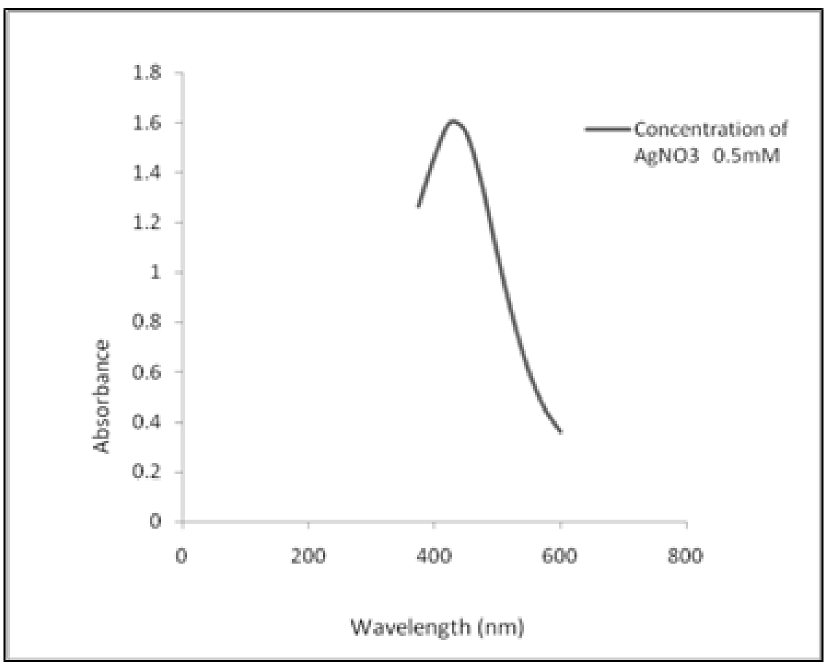
**c)EDS analysis**
EDS analysis was carried out on JEOL JED-2300 Analysis Station at accelerating voltage of 20 keV

***Incorporation of silver nanoparticles in gauze cloth***
1ml silver nanoparticle suspension was poured onto gauze cloth discs of diameter 1cm. The gauze cloth discs were dried at $35^0$C for 7 days. SEM of these discs was carried out on JEOL JSM 6360A (SEM) and using JEOL JSM 1600A fine coater for uniform coating of Platinum on the sample. Antibacterial activity of these discs was assayed using the standard disc diffusion method.

## RESULT AND DISCUSSION

Plant mediated synthesis of nanoparticles is a common practice in recent days. There are many reports of biosynthesis of silver nanoparticles using many Angiospermic plants. *Anthoceros* shows a simple organization of plant body. It inhabits *Nostoc* a blue green alga as a symbiont. Due to the simple organization of thallus the downstream processing of the nanoparticles could be easier as compared to the one done in Angiospermic plants. The symbiotic association of the blue green alga-*Nostoc* may play some role in the biosynthesis of silver nanoparticles.

**Figure 1**
***UV-Visible Spectrum of Silver Nanoparticles***

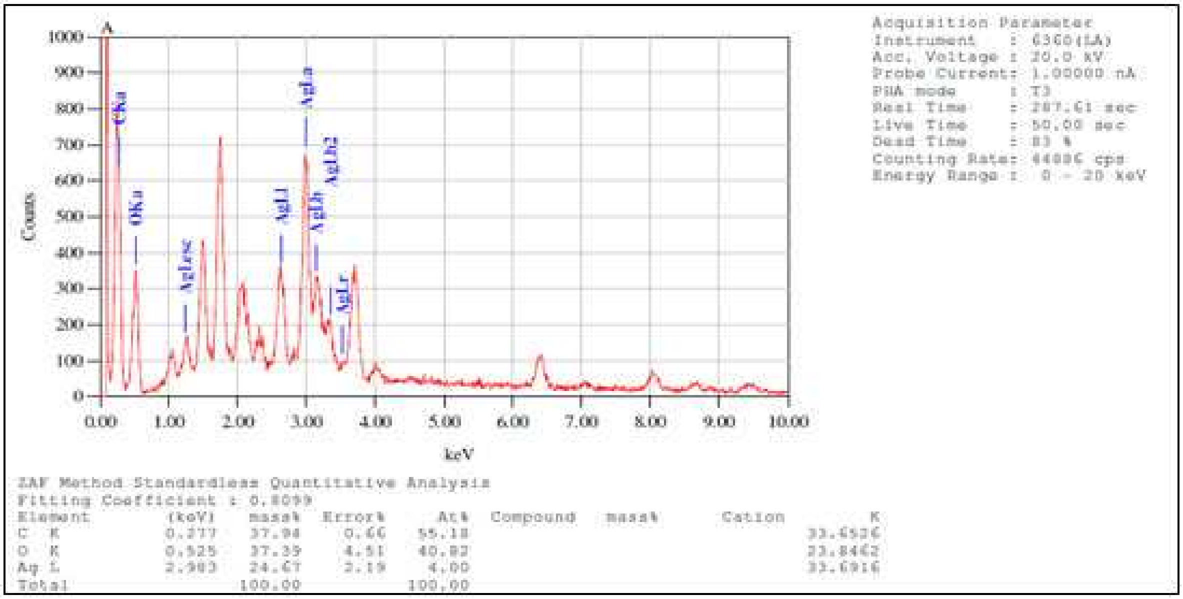



***Preparation of extract***
The thalli of *Anthoceros* grow on damp soil. When removed from the substratum, they are full of soil and hence they are to be washed thoroughly. *Anthoceros* shows simple organization of thallus and therefore it is easy to prepare extract. Extract of the thalli shows light green colour.

***Characterization of silver nanoparticles***
There was a visible colour change after the substrate was provided to the plant extract. Initially the plant extract was light green. Upon providing the silver salt, it turned red. The presence of nanoparticles was confirmed by obtaining a spectrum in visible range of 200nm to 600nm. A typical peak at 438nm was

obtained due to the surface plasmon resonance of silver nanoparticles. (Fig.1) The ethanolic extract also showed a colour change from light green to red. Further, upon subjecting to the spectrum in visible range, a peak at 425nm was obtained showing presence of silver nanoparticles.

**Figure 2**
***EDS spectrum of silver nanoparticles***




The presence of nanoparticles was confirmed by carrying out SEM (Fig.3) that showed cuboidal and triangular shaped nanoparticles of size approximately 20-50 nm. During EDS Analysis, the specimen is bombarded with an electron beam inside the scanning electron microscope. The bombarding electrons collide with the specimen atoms' own electrons, knocking some of them off in the process. A position vacated by an ejected inner shell electron is eventually occupied by a higher-energy electron from an outer shell. To be able to do so, however, the transferring outer electron must give up some of its energy by emitting an X-ray. The amount of energy released by the transferring electron depends on which shell it is transferring from, as well as which shell it is transferring to. Furthermore, the atom of every element releases X-rays with unique amounts of energy during the transferring process. Thus, by measuring the amounts of energy present in the X-rays being released by a specimen during electron beam bombardment, the identity of the atom from which the X-ray was emitted can be established. The EDS spectrum (Fig.2) showed high for silver signals. The vertical axis shows the counts of the X- ray and the horizontal axis shows energy in keV. The strong signals of silver correspond to the peaks in the graph confirming presence of silver Nanoparticles.

**Figure 3**
***SEM image of silver nanoparticles***

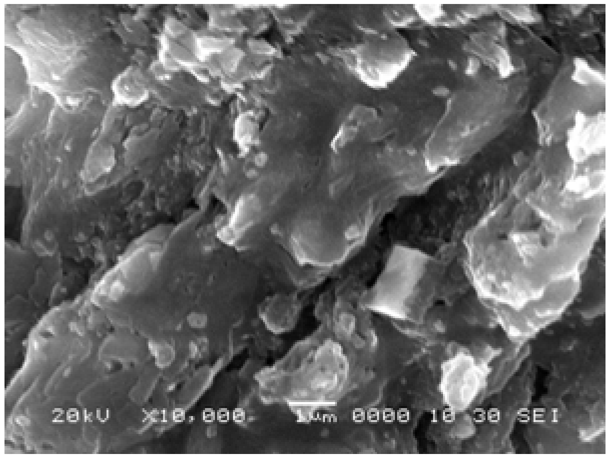
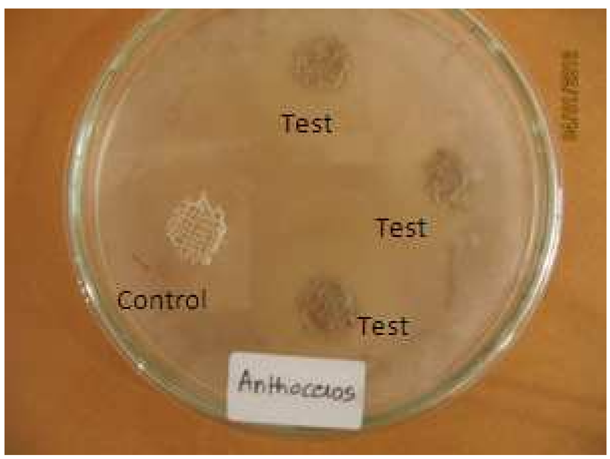


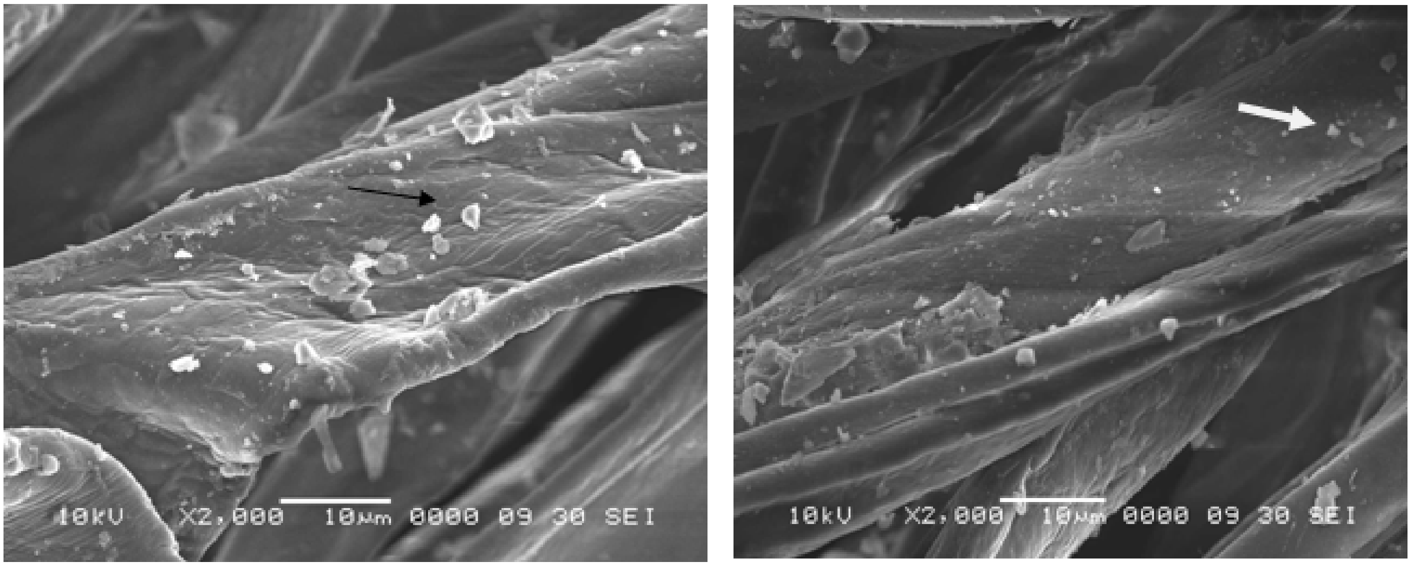

**Figure. 4**
***Gauze discs incorporated with silver nanoparticles and their antibacterial activity against Pseudomonas aeruginosa***




***Incorporation of silver nanoparticles in gauze cloth and their antibacterial activity***

Silver nanoparticles also show antibacterial activity against disease causing microorganisms[21]. Therefore, their incorporation in gauze cloth was carried out to study the antibacterial activity in immobilized condition. The suspension of silver nanoparticles when poured on the gauze cloth and dried, the colour of gauze cloth changed from white to reddish brown. SEM of the gauze cloth showed presence of nanoparticles on the adsorbed on the gauze cloth (Fig. 5). Antibacterial activity of such gauze cloth discs showed zone of inhibition against *Pseudomonas aeruginosa* below the gauze cloth (Fig. 4). The incorporation of nanoparticles in cotton cloth is carried out using the silver nanoparticles synthesized from *Azadirachta indica*[22]. However, to the best of our knowledge, the incorporation of silver nanoparticles on gauze cloth has not yet been carried out.

**Figure 5**
***SEM photos of the gauze cloth incorporated with silver Nanoparticles***




## CONCLUSION

Alcoholic extraction of the plant material provides a best and faster method of biosynthesis of silver nanoparticles. Furthermore, antibacterial activity of the silver nanoparticles can be exploited by incorporating them into some substrate such as gauze cloth and can be used to a larger extent to avoid the skin infections or other type of infections.

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