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Plant mediated synthesis of silver nanoparticles - tapping the unexploited sources

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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. Bryophytes show simple organization of the plant body and abundant during rainy season. They are easy to harvest and easy to make an extract. In this study, the plant extract was prepared in water and ethanol and treated with different concentrations of 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 spectrometer 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. Silver nanoparticles also showed antibacterial activity against four disease causing microorganisms.

Keywords: silver nanoparticles, *Anthoceros*, biosynthesis, SEM with EDS, antibacterial activity.

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], 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, 17].

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]. In this paper we state simple eco-friendly, one step process of biosynthesis of silver nanoparticles using *Anthoceros* (Bryophyta- Anthocerotae) as the plant source.

MATERIALS AND METHODS

1.1 *Plant material and extraction process*

Fresh, green mature thalli of *Anthoceros* were collected from Mahabaleshwar (Mahrashtra, India) and used for preparation of extract. The thalli were thoroughly cleaned using water and detergent. 5g plant material was weighed and was crushed in 10 ml of distilled water. The aqueous extract thus obtained was filtered through coarse filter paper to obtain a clear extract. Alcoholic extracts were also prepared using 70% alcohol. The procedure for preparation of alcoholic extract was same to that for preparation of aqueous extract.

1.2 *Synthesis of nanoparticles*

1mM 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 to obtain silver nanoparticles. Same protocol was followed for synthesis of nanoparticles in ethanol extract.

The plant extract with the substrate (i.e. silver nitrate procured from Sigma Aldrich) were kept at 25^0C on a shaker at 150 rpm in dark.

Different concentrations of silver nitrate were used to standardize the optimum concentration of silver nitrate for synthesis of silver nanoparticles. The concentrations ranged from 0.5mM, 1mM, 2mM, 3mM and 5mM of silver nitrate.

1.3 *Characterization of silver nanoparticles*

1.3.1 UV- Vis spectra analysis

The reduction of metallic Ag+ ions was monitored by measuring the UV- Vis spectrum after about 16 hours 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).

1.3.2 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.

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

1.4 *Antibacterial activity*
Antibacterial activity was assayed using standard well diffusion method against human pathogenic bacteria *Escherichia coli*, *Pseudmonas aeroginosa*, *Bacillus subtilis* and *Klebsiella pneumoniae*. Nutrient Agar (NA) was prepared for cultivation of the bacteria. 100µl of fresh overnight grown cultures of the bacteria were spread on Nutrient Agar containing Petri plates. With a sterile borer 1mm holes were punched in the medium. 100 µl of the solution containing nanoparticles was inoculated in this hole and the plates were incubated at 37^0C overnight. The next day, zone of inhibition in the bacterial mat was measured.

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. However, after extensive literature survey and to the best of our knowledge, we report the biosynthesis of silver nanoparticles from a Bryophyte for the first time. *Anthoceros* shows simple organization of plant body.

Fig.1 Variation in concentration of silver

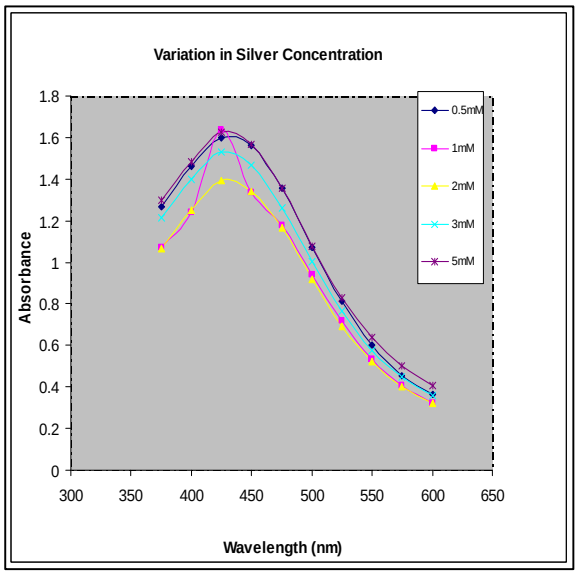


Fig.2 Spectra for variation in concentration of silver

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.

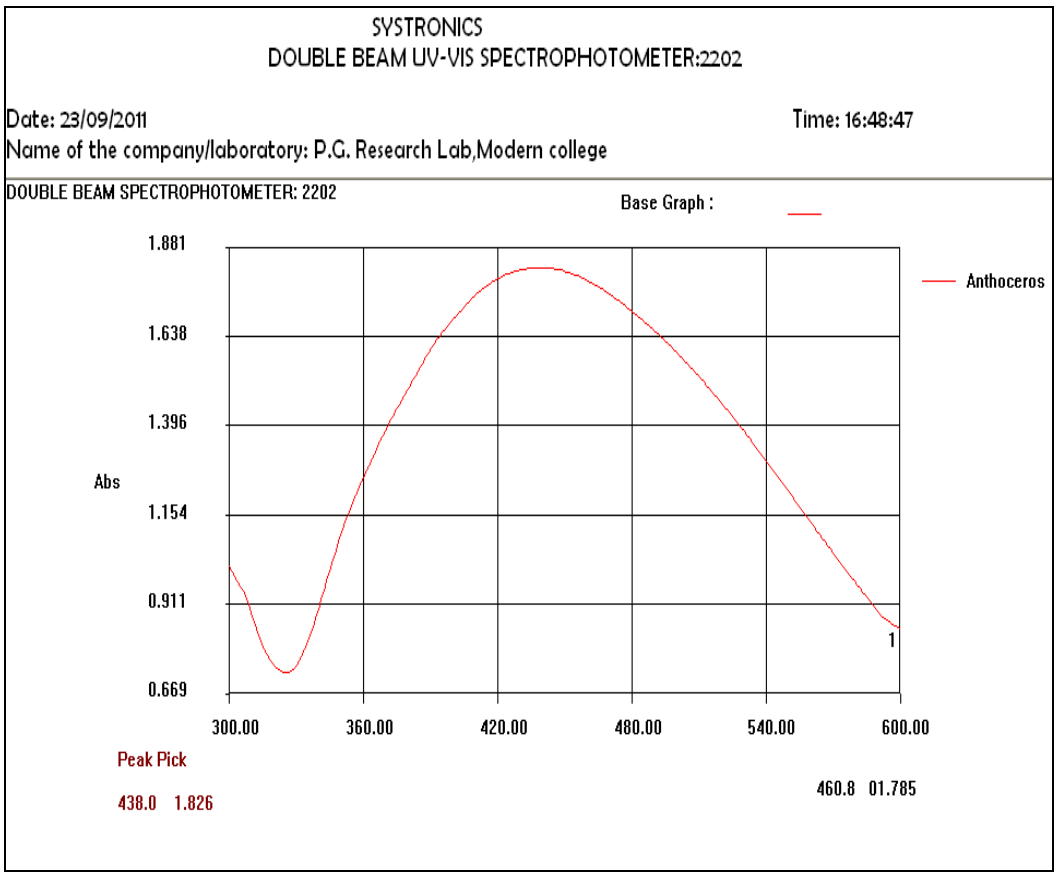


Fig. 3 UV-Vis spectrum of silver nanoparticles

2.1 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 aqueous as well as ethanolic extract. Extract of the thalli shows light

2.2 Concentration of silver nitrate

Optimum concentration for synthesis of nanoparticles was standardized using different concentrations of silver nitrate. The optimum concentration suitable for nanoparticles synthesis found to be 1mM (Fig.1 and Fig. 2). The observation also shows that higher concentration of the salt may prove to be inhibitory for the synthesis of nanoparticles.

2.3 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.3) The ethanolic extract also showed a colour change from light green to red (Fig. 4a). Further, upon subjecting to the spectrum in visible range, a peak at 425nm was obtained showing presence of silver nanoparticles (Fig. 4b).

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.

Fig.4a Ethanol extraction for silver nanoparticles

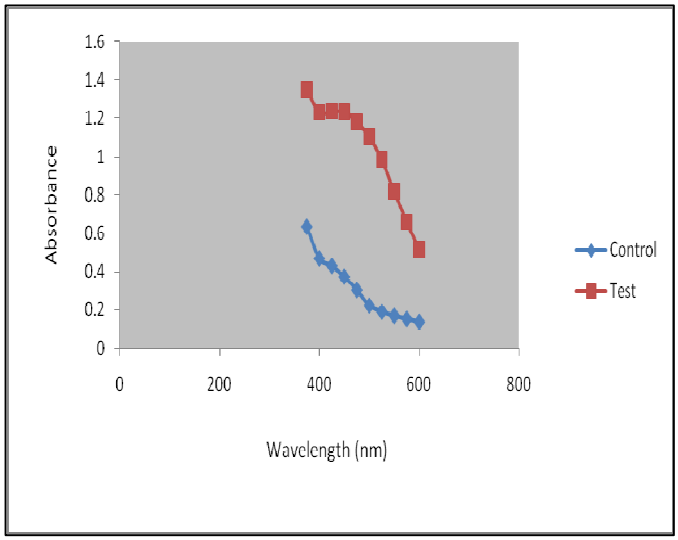


Fig. 4b Spectrum of the silver nanoparticles after ethanol extraction

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.5) 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. The presence of nanoparticles was confirmed by carrying out SEM (Fig.6) that showed cuboidal and triangular shaped nanoparticles of size approximately 20-50 nm.

Fig.5 EDX spectrum of silver nanoparticles

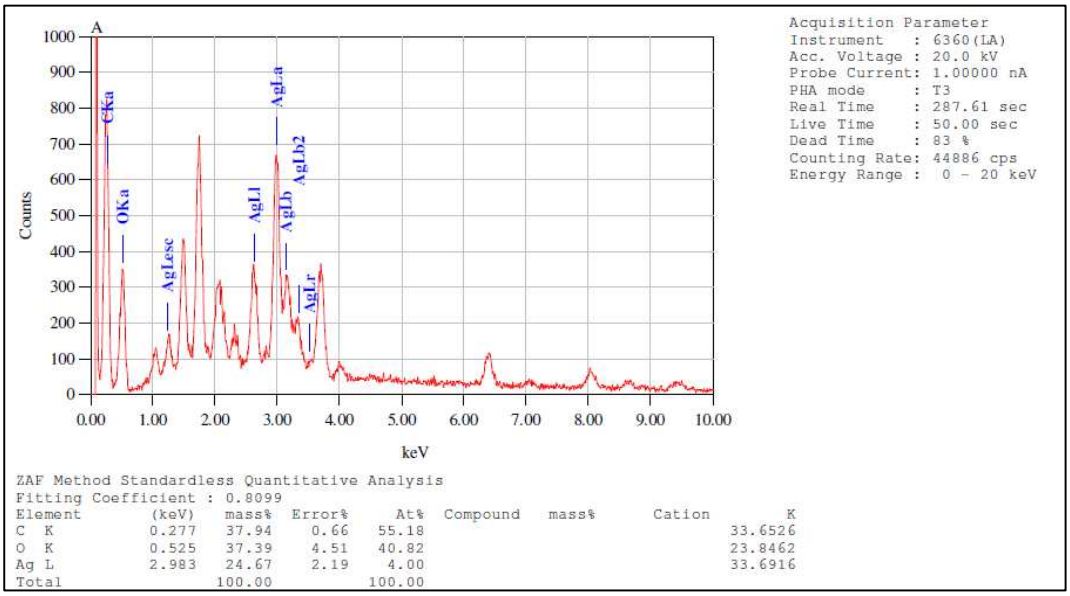
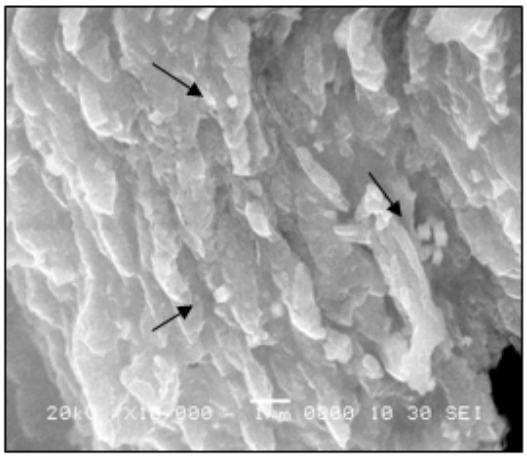


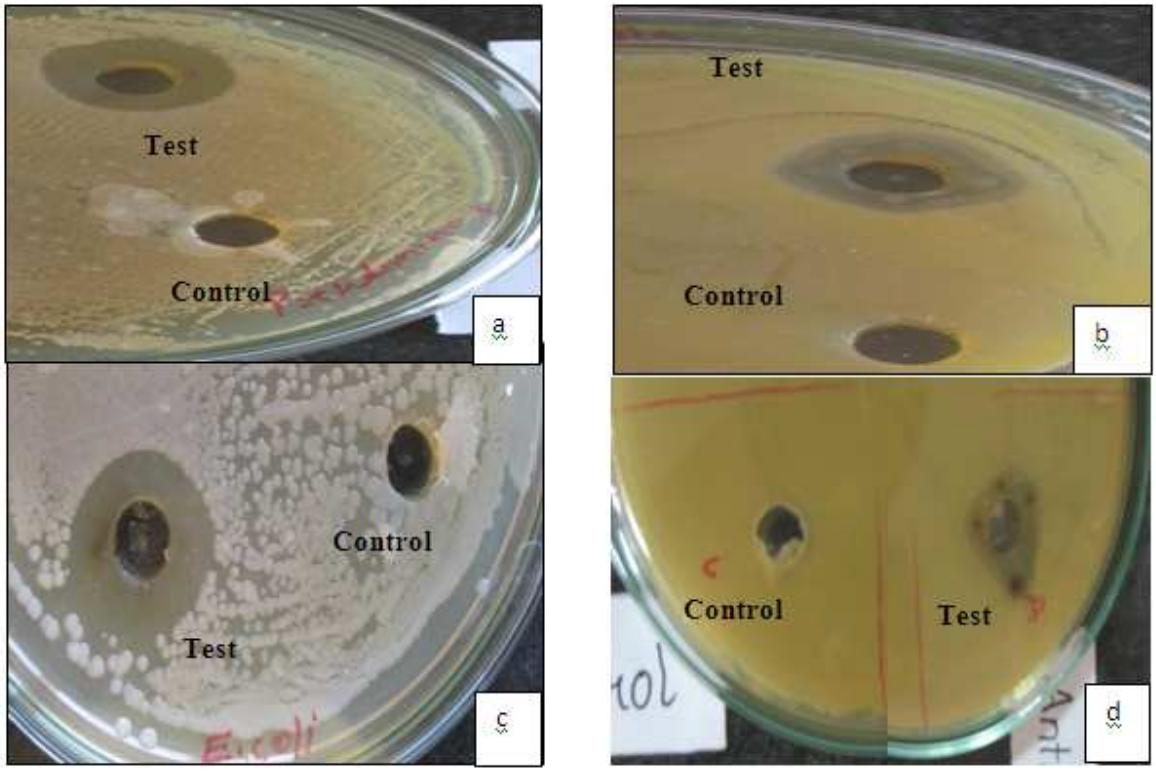
Fig.6 SEM image of silver nanoparticles



2.4 Antibacterial activity

The antibacterial activity of silver nanoparticles is reported to a large extent. The silver nanoparticles obtained from *Anthoceros* also show antibacterial activity against four strains of laboratory pathogens viz. *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Bacillus subtilis*. The zone of inhibition measured is summarized in Table no. 1. From the table, it is evident that the nanoparticles synthesized are good candidates their usage as and/or in antibacterial drugs.

Fig. 7 Antibacterial activity of silver nanoparticles against a) *P. aeruginosa* b) *K. pneumoniae* c) *E. coli* and d) *B. subtilis*



Mechanism of action of silver nanoparticles as antibacterial agents is not very well known but it seems that the nanoparticles interfere in the respiratory metabolism of the organisms and therefore, show antibacterial activity.

Table 1 Antibacterial activity of the silver nanoparticles

Name of the organism	Zone of inhibition (mm)
Pseudomonas aeruginosa	12
Escherichia coli	11
Bacillus subtilis	9
Klebsiella pneumoniae	8

CONCLUSION

We describe a simple environmentally benign method of synthesis of silver nanoparticles from a novel primitive plant source. This method can be further used for industrial production of nanoparticles at room temperature and with a single step. Since the nanoparticles thus synthesized show antibacterial activity, they can be used in various fields such as paint industry, pharmaceutical industry and so on.

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