Plant mediated synthesis of silver nanoparticles using a bryophyte: *Fissidens minutus* and its anti-microbial activity.

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Abstract:

Biological methods of synthesis have paved way for the "greener synthesis" of nanoparticles. These have proven to be better methods due to slower kinetics, they offer better manipulation and control over crystal growth and their stabilization. The synthesis of silver nanoparticles by a Bryophyte, *Fissidens minutus* is reported in this paper. Aqueous Extract of *Fissidens minutus* treated with 0.5 mM silver nitrate solution produced silver nanoparticles at room temperature rapidly. Nanoparticles were characterized by means of UV–Vis spectroscopy, Scanning electron microscopy (SEM) and Energy Dispersive Spectrometry (EDS). Nanosilver has proved as a potent antibacterial agent showing its activity against both gram positive and gram negative organisms. The present study emphasizes the use of primitive plant form for the synthesis of silver nanoparticles.

Keywords: *Fissidens minutus*, Silver Nanoparticles, EDS, SEM, Antibacterial activity.

1. Introduction

The field of nanotechnology is an active area of research. The unusual physicochemical, optical and electronic properties of nanoparticles arise primarily due to the confinement of electrons within particles of dimensions smaller than the bulk electron delocalization length, process termed as Quantum Confinement. These properties of nanoparticles are considered in application such as opticoelectronics, catalysis, reprography, single electro transistor, Medicine, nonlinear optical devices and photo electrochemical for biolabelling, and as antimicrobials application.

Synthesis of silver nanoparticles is usually achieved by chemical reduction method [Guzmán Maribel G. *et.al* (2008)], thermal decomposition in organic solvent [Esumi, K., *et al*. (1990)], chemical reduction and photo reduction in reverse micelles [Petit, C. *et.al* (1999)], and radiation chemical reduction [Ahmadi, T. S. *et.al* (1996)]. Most of these methods are extremely expensive and also involve use of toxic, hazardous chemicals that pose potential environmental and biological risks. There is a growing need to develop environmentally friendly processes through Biomimetic approaches. Sometimes the synthesis of nanoparticles using plants can prove advantageous over other biological synthesis processes in terms of elaborate processes of maintaining microbial cultures [Sastry, M.*et.al* (2003)] [Sastry, M. *et al* (2004)]. This has motivated an upsurge in research on the synthesis routes that allow better control of shape and size for various nanotechnological applications.

Substantial works have already been initiated with respect to synthesis of noble metals nanoparticles using fungi like *Fusarium oxysporum* [Sastry, M. *et.al* (2003)],[Durán N. *et al* (2005)], *Penicillium* sp. [Hemath Naveen K.S et.al (2010)], bacteria such as *Bacillus subtilis* (Natarajan K,et.al (2010)] and plants such as *Eucalyptus hybrida* [Dubey M et.al (2009)], *Coriandrum Sativum* [Sathyavathi R. et.al (2010)], *Cycas* [Jha A.K. & K.

Prasad ,(2010)] , *Helianthus annus* , *Basella alba*, *Oryza sativa*, *Zea mays*, *Sorghum bicolor* [Arangasamy L. & Munusamy Vivekanandan (2008)],and in *Azadirachta Indica* [Shankar S.S et.al (2004)]

In the Present studies we describe an eco-friendly approach toward silver nanoparticle biosynthesis. To our best knowledge with all the possible referencing, we state that it is the first study that uses Bryophytes as plant source for synthesis of silver nanoparticles. *Fissidens minutus* belongs to Class Musci, Order Fissidentales, and Family Fissidentaceae. Typically 1.5-2 mm high, they commonly grow close together in clumps or mats in damp or shady locations. 8-12 pairs of leafy appendages which are 0.8-1 mm long, 0.3 mm broad, oblong lingulate and sporophyte is not seen [Dabhade G.T (1998)]. It holds significant value due to the ample abundance of the plant and easy, rapid, energy efficient, green and economically scalable room temperature protocol for the synthesis.

2. Material and Methods

2.1 Plant Material and synthesis

Fissidens minutus was collected in Mahabaleshwar, Maharashtra, a region in Western Ghats of India. Freshly collected mosses were thoroughly washed, cleaned. 1 gram thallus was used for extraction in distilled deionized water (Aqueous Extraction) as well as in 70% ethanol (Alcoholic Extraction). The plant material was boiled at 80^0 c for 10 minutes. The plant material was finely macerated and filtered. Silver Nitrate was procured from Sigma Aldrich. 1 ml of 0.5 mM Silver Nitrate was added to 10 ml of filtrate and incubated on rotary shaker for an hour. The change in colour was observed with reference to control.

2.2 Optimization of silver nitrate Concentration

The concentration of silver nitrate is an important parameter. The varying concentration ranging from 0.5mM, 1 mM, 2mM, 3 mM and 5 mM Silver Nitrate were used as substrate.

2.3 UV-Visible Spectrometric analysis

The Sample was analyzed through Spectrum studies (300-600nm) with Systronics Double Beam UV-Visible Spectrophotometer 2202.

2.4 SEM and EDS Studies

Characterization of silver nanoparticle synthesized using *Fissidens minutus* were determined by two independent techniques, Scanning Electron Microscope (SEM) and Energy Dispersive Spectroscopy (EDS). Silver nanoparticles were coated with Platinum coating by means of Jeol JSM 1600 fine coater and then analyzed on Jeol JSM 6360A Scanning Electron Microscope for a detailed examination of Silver Nanoparticles morphology.

The presence of elemental silver signal was confirmed in the sample with EDS Studies using Jeol JED-2300 analysis station.

2.5 Antibacterial studies

The Efficiency of silver nanoparticles was tested on four laboratory strains Test Organisms, *Escherichia coli, Bacillus cereus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* by Well Diffusion Assay. The cultures were maintained on Nutrient agar. The microbial test organisms were grown at 37°C for 24 hours.

3 Results and Discussions

3.1 Biosynthesis

Synthesis of silver nanoparticles from silver nitrate is one of the most widely used methods for the synthesis of silver colloids. During the biosynthesis using the extract the color of the reaction medium changed rapidly from light greenish to dark yellowish brown (Fig.1A) due to Surface Plasmon Resonance. This occurs due to the collective oscillations of the conduction electrons confined to metallic nanoparticles. This results in strong light scattering, by an electric field at a wavelength where resonance occurs resulting in appearance of strong absorbance bands. Throughout the study the formation of silver nanoparticles was confirmed with this characteristic. It has been found that the maximum peak is observed in 0.5 mM Silver Nitrate. (Fig. 1B and 1C) therefore the optimum concentration was found to be 0.5 mM Silver Nitrate.

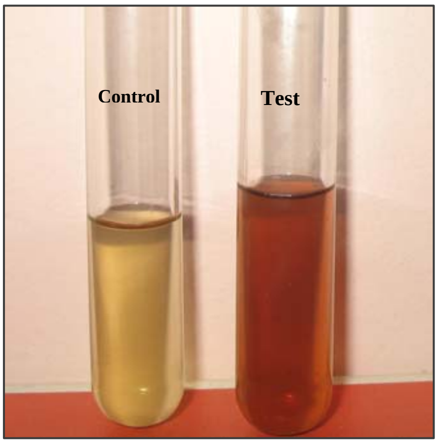

Fig 1A Color change after synthesis of Nanoparticles

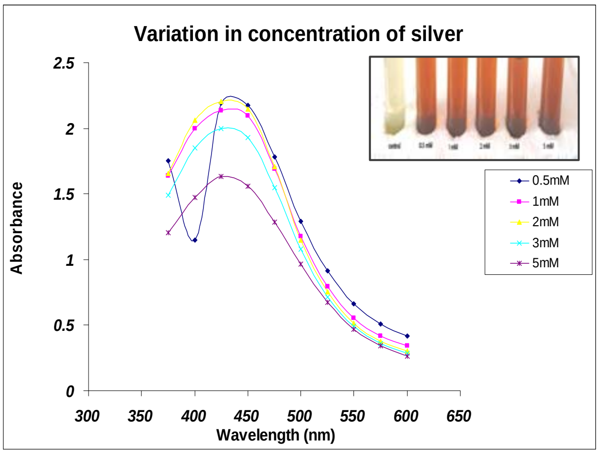

Fig.1B Spectral peaks at variable concentration of Silver Nitrate
Inset: Variation in silver concentration

3.2 Characterization

The Ag nanoparticles were synthesized using aqueous extract of *Fissidens minutus.* Absorption spectra of silver nanoparticles were recorded, as described by the Fig.2 In metal nanoparticles, the conduction band and valence band lies very close to each other in which electrons move freely. These free electrons give rise to a surface plasma resonance absorption band, occurring due to collective oscillation of electrons in resonance with light wave [5].

The absorption spectra of yellowish brown silver nanoparticle solution showed a Surface Plasmon Resonance with a peak at 412.8 nm.

Further, the synthesis of Silver Nanoparticles was also carried out by ethanolic extraction. The presence of nanoparticles was confirmed by spectral studies (Fig.3).

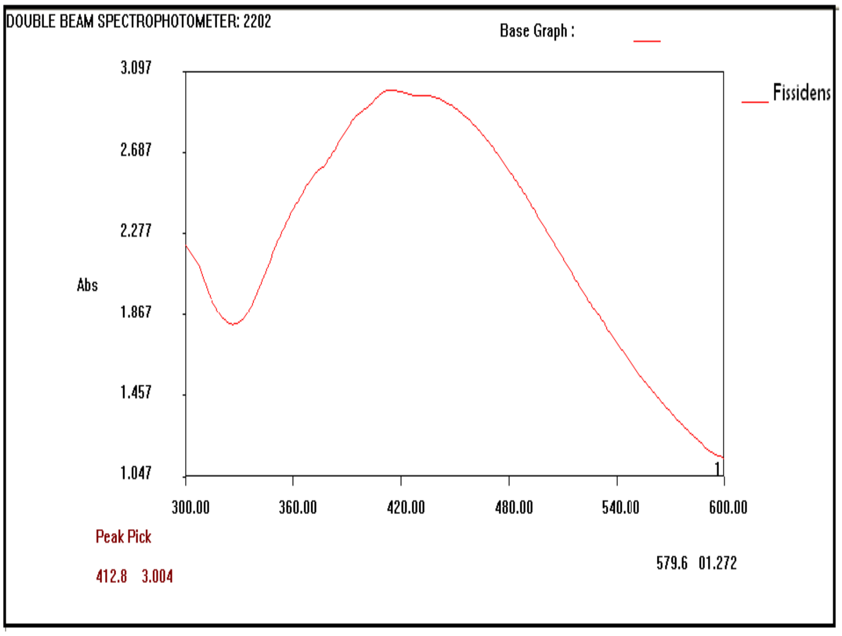

Fig. 2 Absorption Spectra via Aqueous Extraction

The SEM image shows Spherical Silver Nanoparticles (Figure. 4A).The SEM image shows silver nanoparticles aggregates.

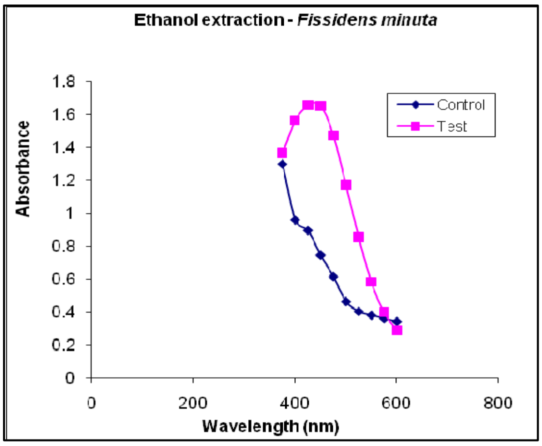

Fig. 3 Absorption spectra via ethanolic extraction

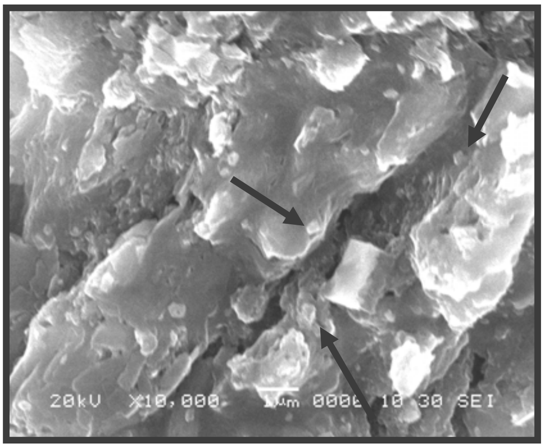

Fig. 4A SEM Image of Silver Nanoparticles

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.

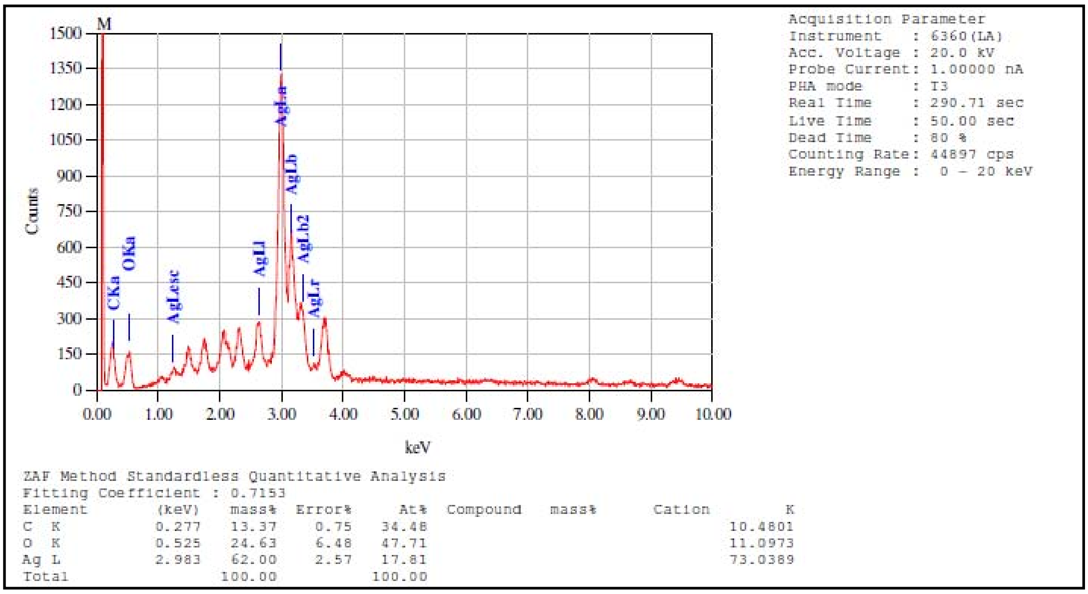

Fig. 4B EDS spectrum of Silver Nanoparticles

The vertical axis shows the amount of X-ray counts while horizontal axis shows energy emitted in keV. The EDS spectrum (Figure.3B) observed a strong signal from the silver atoms in the nanoparticles and weak signal from Carbon and Oxygen. These weak signals are from the plant organic constituents.

3.3 Antimicrobial activities

It is well known that silver ions and nanoparticles are highly toxic to microorganisms. Silver nanoparticles have been known to have inhibitory and bactericidal effects and thus we extend its application as an antibacterial agent. The Antibacterial activity is estimated by the zone of inhibition.

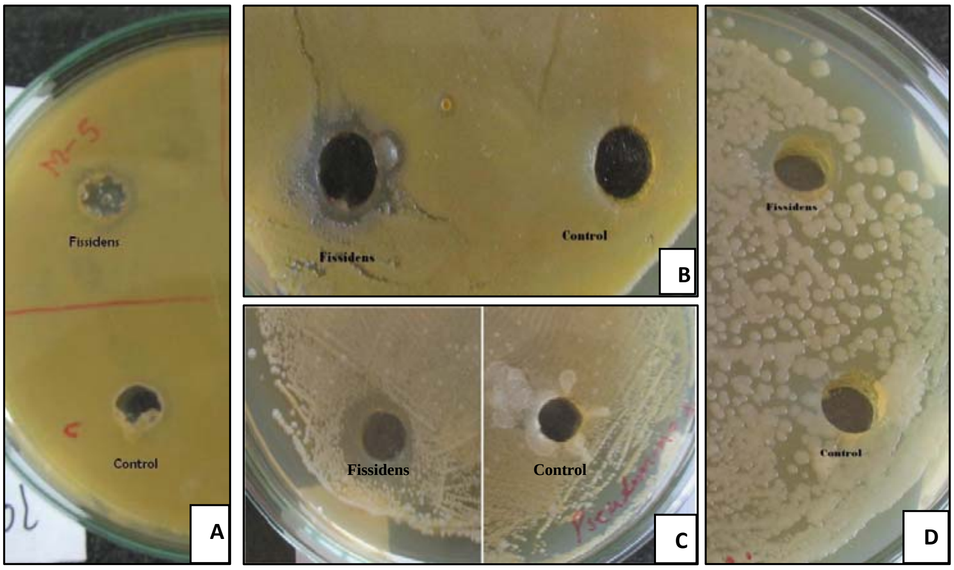

Fig. 5 Zone of Inhibition against (A) *Bacillus subtilis* (B) Klebsiella *pneumoniae* (C) Pseudomonas *aeruginosa* and (D) Escherichia *coli*

The mechanism of the bactericidal effect of silver and silver nanoparticles remains to be understood.
Several studies propose that silver nanoparticles may attach to the surface of the cell membrane disturbing permeability and respiratory function of the cell. It is also possible that silver nanoparticles not only interact with the surface of membrane, but can also penetrate inside the bacteria.

Mean Width of Zone Of Inhibition (mm) by silver nanoparticles	
Escherichia coli	6
Bacillus subtilis	4.1
Pseudomonas aeruginosa	8
Klebsiella pneumoniae	6.3

Table 1 Zone of Inhibition against the test organisms

It may be observed that silver nanoparticles have comparatively higher anti-bacterial activity against gram negative organism than gram positive, probably due to thinner peptidoglycan layer and presence of porins.

During recent times, Nanoparticles have gained importance in the field of Biomedicine. The most important and distinct property of nanoparticles is that they exhibit larger surface area to volume ratio. Specific surface area is relevant for catalytic reactivity and other related properties such as antimicrobial activity in silver nanoparticles. As specific area of nanoparticles increased, their biological effectiveness can increase due to the increase in surface energy.

4 Conclusion

Rapid and Green Synthetic method using extracts from a moss has shown a potential in silver nanoparticles synthesis. *F. minutus* was found to exhibit strong potential for rapid reduction of silver ions. The bio-reduction of silver nitrate into silver nanoparticles via the aqueous extraction and organic Extraction from *F. minutus* is reported in this paper. The characterization of Silver Nanoparticles is achieved with UV-Visible Spectroscopy, SEM and EDS. Studies carried out by SEM and EDS enabled not only a detailed examination of silver powder morphology, but also its chemical composition analysis. However, they appeared insufficient for distribution analysis of single particle sizes.

The plant extract containing Silver Nanoparticles showed its potential as Antimicrobial. They may thus be combined with antibiotic agent to enhance its bactericidal activities.

The advantage of using plants for the synthesis of nanoparticles is that they are easily available, safe to handle and possess a broad variability of metabolites that may aid in reduction. Moreover, these particles have innumerable applications. The works adds to the confirmation of previous reports on biosynthesis of Nano metals using plant extracts.

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