e- ISSN 0976 - 3651
Print ISSN 2229 - 7480

International Journal of Biological & Pharmaceutical Research
Journal homepage: www.ijbpr.com

IJBPR

PHYTOFABRICATION OF SILVER NANOPARTICLES FROM A NOVEL PLANT SOURCE AND ITS APPLICATION

***Kulkarni AP, Srivastava AA, Nagalgaon RK, Zunjarrao RS**

*Department of Botany, Modern College of Arts, Science and Commerce, Pune-411005.

ABSTRACT

To meet the increasing demands for commercial nanoparticles new eco-friendly "green" methods of synthesis are being discovered. Biological methods of synthesis have paved way for the "greener synthesis" of nanoparticles and these have proven to be better methods. Due to slower kinetics, they offer better manipulation and control over crystal growth and their stabilization. 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 alcoholic plant extract was treated with silver nitrate to obtain nanoparticles. The synthesis of nanoparticles was confirmed by change in colour from 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. Furthermore, these nanoparticles were incorporated in the gauze cloth and its antibacterial activity is tested. This work demonstrates the possible use of biologically synthesized silver nanoparticles by its incorporation in gauze cloth leading them to medical application.

Keywords: Silver nanoparticles, *Riccia*, biosynthesis, SEM with EDS.

INTRODUCTION

Since the last decade, Nanoparticle biosynthesis is the active area of research. Nanotechnology is a field that is burgeoning day by day making an impact in all spheres of human life. The most effectively studied nanoparticles in the recent past are those made from the noble metals such as silver (Nelson Durán et al., 2005), gold (Balprasad Ankamwar, 2010) and platinum (Deng QY et al., 2009). Nanoparticles find vast applications in various fields ranging from medical to physical fields (Sathyavathi R et al., 2010; Henley SJ et al., 2006; David D Evanoff Jr et al., 2005). In the present scenario Nanoscale materials have emerged up as novel antimicrobial agents owing to their high surface area to volume ratio and its unique chemical and physical properties.

Various strategies are employed for synthesis of silver nanoparticles (Thabet M Tolaymat et al., 2010). Nanoparticles are synthesized by reduction in solutions (Maribel G Guzmán et al., 2008), thermal decomposition of silver compounds (Navaladian S et al., 2007), microwave assisted synthesis (Sreeram KJ et al., 2008), and laser mediated synthesis (Reza Zamiri et al., 2011) and biological reduction method (Murali Sastry et al., 2003). 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

Corresponding Author

Kulkarni AP
E-mail: apkulk@rediffmail.com

Angiospermic plants such as *Helianthus annus*, *Oryza sativa*, *Zea mays*, *Sorghum bicolor* (Arangasamy Leela et al., 2008), *Eucalyptus hybrid* (Manish Dubey et al., 2009), *Artocarpus heterophyllus* (Thirumurugan A et al., 2010) and Gymnospermic plants such as *Cycas* (Anal K Jha et al., 2010) and many more. Biosynthesis of nanoparticles is also attempted in primitive organisms such as Fungi and Bacteria (Nelson Durán et al., 2005; Kannan Natarajan et al., 2010).

Bryophytes are primitive land plants and show simple organization of the plant body (thallus) (Crandall-Stotler et al., 1980). However, the phytochemical work on these primitive plants shows that they possess a variety of chemicals and therefore can be used in many ways (Yoshinori Asakawa, 2007). In this paper we state simple eco-friendly, one step process of biosynthesis of silver nanoparticles using *Riccia* (Bryophyta- Hepataceae) as the plant source. Riccia is a genus of liverworts in the order Marchantiales**.**

MATERIALS AND METHODS

Plant material and extraction process

Fresh, green mature thalli of *Riccia* were used for preparation of extract. The thalli were thoroughly cleaned using water and detergent. 1g plant material was weighed and was crushed in 10 ml of ethanol. The aqueous extract thus obtained was filtered through coarse filter paper to obtain a clear extract.

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. The plant extract with the substrate (i.e. silver nitrate solution) were kept at 25 ^{0} C on a shaker at 150 rpm in dark.

Characterization of silver nanoparticles

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).

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.

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 Nanoparticle suspension was poured on the gauze cloth discs (diameter 1cm) and these discs were dried at 40^{0}C for 7 days. SEM of these discs was carried out on JEOL JED-2300 Analysis Station at accelerating voltage of 20 keV. Antibacterial activity of the nanoparticles embedded gauze cloth.

Antibacterial activity was assayed using standard disc diffusion method against human pathogenic bacterium *Pseudomonas aeruginosa*. Nutrient Agar (NA) was prepared for cultivation of the bacteria. 100µl of fresh overnight grown cultures of the bacterium was spread on Nutrient Agar containing Petri plates. The discs incorporated with nanoparticles were placed on the medium. Plates were incubated at 37^{0}C 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. Biosynthesis of silver nanoparticles is also tried in Bryophytes earlier (Kulkarni *et al.*, 2011; Srivastava *et al.*, 2011.). However, after extensive literature survey and to the best of our knowledge, we report the biosynthesis of silver nanoparticles from *Riccia* sp. for the first time.

Preparation of extract

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

Fig. 1. UV-Vis spectrum of silver nanoparticles

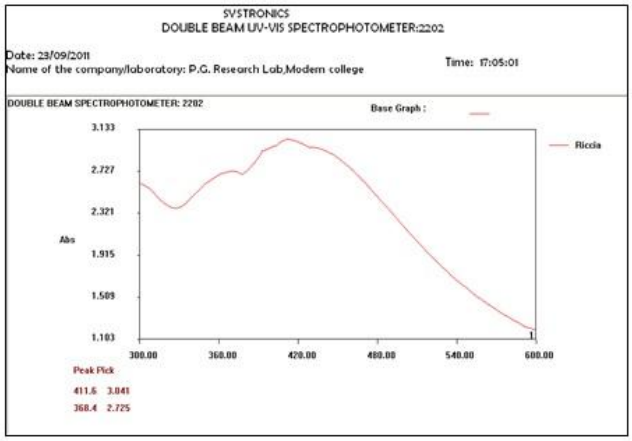


Fig. 2. Change in colour of the extract after synthesis of nanoparticles

Characterization of silver nanoparticles

There was a visible colour change after the substrate was provided to the plant extract. Initially the plant extract was green. Upon providing the silver salt, it turned red (Fig. 2). The presence of nanoparticles was confirmed by obtaining a spectrum in visible range of 200nm to 600nm. A typical peak at 411.6nm was obtained due to the surface plasmon resonance of silver nanoparticles. (Fig.1).

The presence of nanoparticles was confirmed by carrying out SEM (Fig.4) 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.3) 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.

Incorporation of the silver nanoparticles in gauze cloth

Silver nanoparticles were incorporated in the gauze cloth by pouring the nanoparticle suspension on the gauze cloth discs and drying them. There was a colour change in the gauze cloth from white to brownish red after drying. The incorporation of the nanoparticles was also monitored by carrying out SEM of the gauze cloth discs. (Fig. 5)The antibacterial activity of these discs was tested against a bacterium *Pseudomonas aeruginosa* causing skin infections. A clear zone was obtained just below the discs showing inhibition of the bacterium due to silver nanoparticles. These studies indicate that the nanoparticles can be used for incorporating in various substrates and can be applied accordingly.

Fig. 3. EDS spectrum of silver nanoparticles

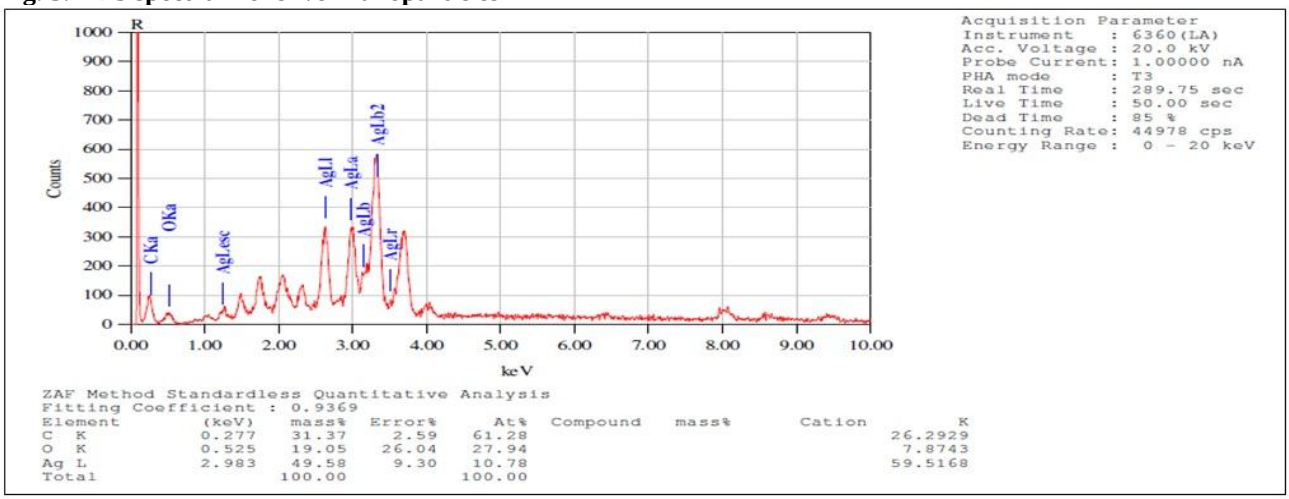


Fig.4 SEM image of the silver nanoparticles

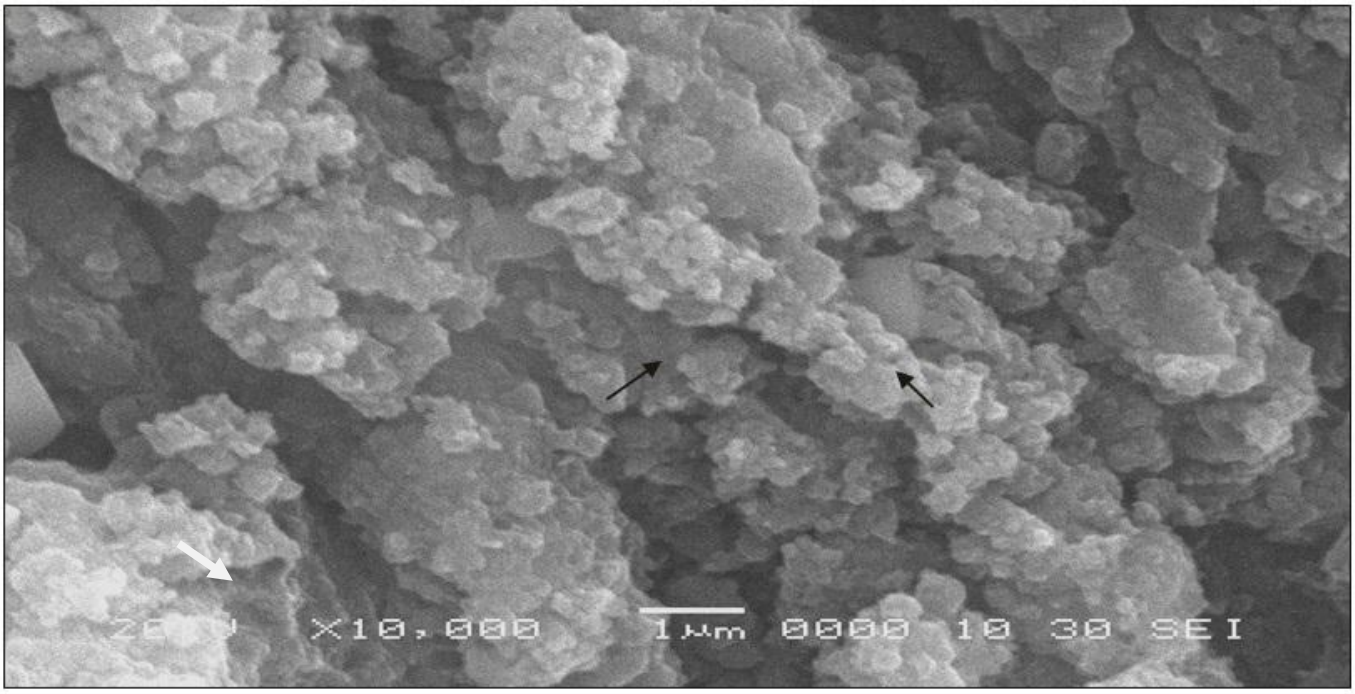


Fig. 5 SEM of the gauze cloth disc

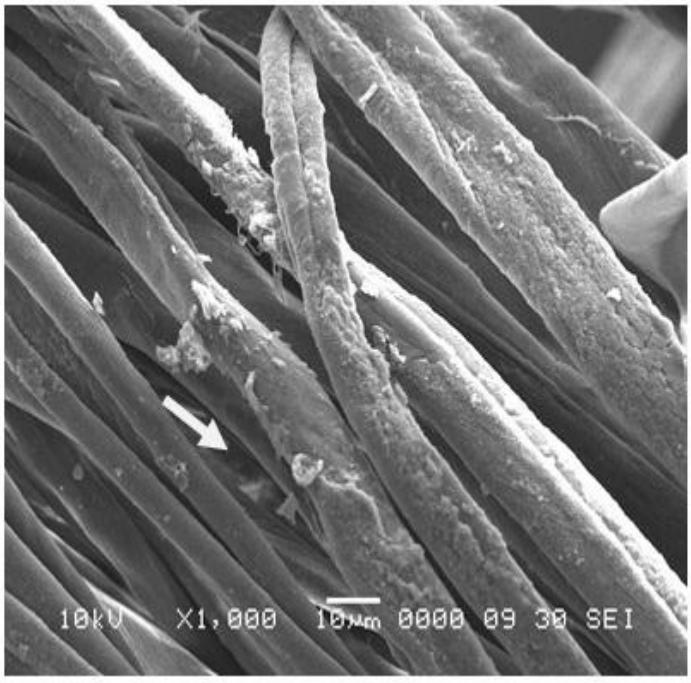
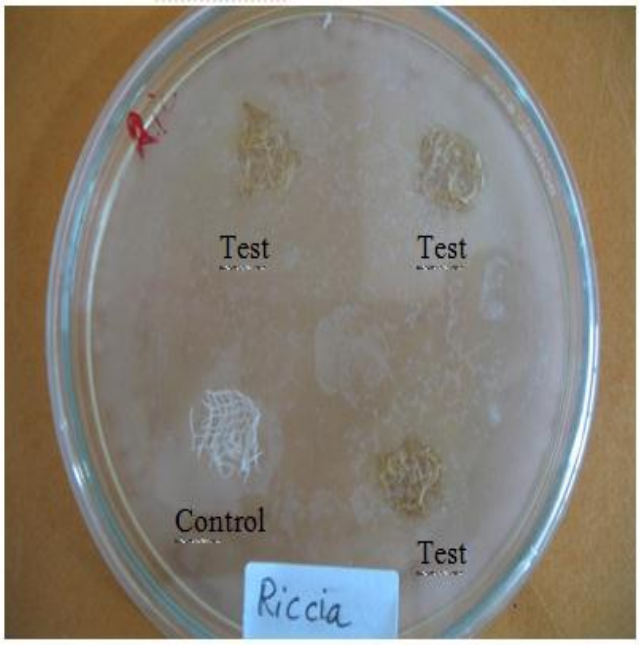


Fig. 6 Antibacterial activity of gauze cloth discs against *Pseudomonas aeruginosa*



CONCLUSION

Riccia proves to be a novel source for biosynthesis of silver nanoparticles. Due to the simple organization of thallus, the extraction and synthesis of nanoparticles is a facile process. The gauze incorporated nanoparticles show antibacterial activity and therefore can be used on a large scale to avoid bacterial infections especially in case of burns and skin problems. Thus it is proven from this study that the silver nanoparticles synthesized from *Riccia* seem to be promising and effective antibacterial agent.

REFERENCES

Anal K Jhaa, Prasad K. Green Synthesis of Silver Nanoparticles Using *Cycas* Leaf. *International Journal of Green Nanotechnology: Physics and Chemistry*. 2010; 2(2): 110-117.

Arangasamy Leela and Munusamy Vivekanandan. Tapping the unexploited plant resources for the synthesis of silver nanoparticles. *African Journal of Biotechnology*. 2008; 7(17): 3162-3165.

Balprasad Ankamwar. Biosynthesis of Gold Nanoparticles (Green-Gold) Using Leaf Extract of Terminalia Catappa. *E-Journal of Chemistry*, 2010; 7(4): 1334-1339.

Crandall-Stotler, Barbara. Morphogenetic designs and a theory of bryophyte origins and divergence. *BioScience*. 1980; 30: 580-585.

David D Evanoff Jr. and George Chumanov. Synthesis and Optical Properties of Silver Nanoparticles and Arrays. *ChemPhysChem*. 2005; 6: 1221-1231.

Deng QY, Yang B, Wang JF, Whiteley CG, Wang XN**.** Biological synthesis of platinum nanoparticles with apoferritin**.** *Biotechnol Lett*. 2009; 31(10): 1505-9.

Henley SJ, Carey JD, Silva SRP. Silver-nanoparticle-decorated carbon nanoscaffolds: Application as a sensing platform. *Applied Physics Letters*. 2006; 89.

Kannan Natarajan, Subbalaxmi Selvaraj, Ramchandra Murty V. Microbial production of silver nanoparticles. *Digest Journal of Nanomaterials and Biostructures*. 2010; 5(1): 135-140.

Kulkarni AP, Srivastava AA, Harpale PM, Zunjarrao RS. Plant mediated synthesis of silver nanoparticles- tapping the unexploited resources. *J. Nat. Prod. Plant Resources*. 2011; 1 (4): 100-107.

Manish Dubey, Seema Bhaduria, Kushwah BS. Green synthesis of nanosilver particles from extract of Eucalyptus hybrida (Safeda) leaf. *Digest Journal of Nanomaterials and Biostructures*. 2009; 4(3): 537-543.

Maribel G Guzmán, Jean Dille, Stephan Godet. Synthesis of silver nanoparticles by chemical reduction method and their antibacterial activity. *World Academy of Science, Engineering and Technology*. 2008; 43.

Murali Sastry, Absar Ahmad, Islam Khan M, Rajiv Kumar. Biosynthesis of metal nanoparticles using fungi and actinomycete. *Current Science*. 2003; 85: 2.

Navaladian S, Viswanathan B, Viswanath RP, Varadarajan TK. Thermal decomposition as route for silver nanoparticles. Nanoscale Res Lett. 2007; 2: 44-48.

Nelson Durán, Priscyla D Marcato, Oswaldo L Alves, Gabriel IH De'Souza and Elisa Esposito. Mechanistic aspects of biosynthesis of silver nanoparticles by several *Fusarium oxysporum* strains. *Journal of Nanobiotechnology*. 2005; 3: 8.

Nelson Durán, Priscyla D Marcato, Oswaldo L Alves, Gabriel IH De Souza and Elisa Esposito. Mechanistic aspects of biosynthesis of silver nanoparticles by several *Fusarium oxysporum* strains. *Journal of Nanobiotechnology*. 2005; 3: 8.

Reza Zamiri, Azmi Zakaria, Hossein Abbastabar, Majid Darroudi, Mohd Shahril Husin, Mohd Adzir Mahdi. Laser-fabricated castor oil- capped silver nanoparticles. *International Journal of Nanomedicine*. 2011; 6: 565-568.

Sathyavathi R, Balamurali Krishna M, Venugopal Rao S, Saritha R and Narayana Rao D. Biosynthesis of Silver Nanoparticles Using Coriandrum Sativum Leaf Extract and Their Application in Nonlinear Optics. *Advanced Science Letters*. 2010; 3: 138-143.

Sreeram KJ, Nidhin M, Nair BU. Microwave assisted template synthesis of silver nanoparticles. *Bull. Mater. Sci*., 2008; 31(7): 937-942.

Srivastava AA, Kulkarni AP, Harpale PM, Zunjarrao RS. Plant mediated synthesis of silver nanoparticles using a bryophyte: Fissidens minutus and its anti-microbial activity. *International Journal of Engineering Science and Technology*. 2011; 3 (12): 8342-8347.

Thabet M Tolaymat, Amro M El Badawy, Ash Genaidy, Kirk G Scheckel, Todd P Luxton, Makram Suidan. An evidence-based environmental perspective of manufactured silver nanoparticle in syntheses and applications: A systematic review and critical appraisal of peer-reviewed scientific papers. *Science of the Total Environment*. 2010; 408: 999-1006.

Thirumurugan A, Neethu Anns Tomy, Jai ganesh R, Gobikrishnan S. Biological reduction of silver nanoparticles using plant leaf extracts and its effect on increased antimicrobial activity against clinically isolated organism. *Der Pharma Chemica*. 2010; 2(6): 279-284.

Yoshinori Asakawa. Biologically active compounds from bryophytes. *Pure Appl. Chem*., 2007; 79(4): 557-580.