



## GREEN SYNTHESIS OF SILVER NANOPARTICLES USING SEED EXTRACT OF *ARGYREIA NERVOSA*

REBECCA THOMBRE<sup>1\*</sup>, FENALI PAREKH<sup>1</sup> AND NEETA PATIL<sup>2</sup>

<sup>1</sup>Department of Biotechnology, <sup>2</sup>Department of Botany, Modern College, Shivajinagar, Pune- 411005, Maharashtra, India.  
\*Assistant Professor, Department of Biotechnology, Modern College, Shivajinagar, Pune-411005.India.

### ABSTRACT

A simple method for synthesis of biologically stabilized silver nanoparticles was developed using the seed extract of *Argyria nervosa*. The nanoparticles synthesized using *A. nervosa* were characterized by UV-Vis spectrophotometry, X-Ray Diffraction, SEM- EDS and FTIR. The antibacterial and antifungal activity of silver nanoparticles was assessed against *Staphylococcus aureus*, *Bacillus subtilis*, *Aspergillus niger*, *Escherichia coli* and *Pichia pastoris*. The silver nanoparticles synthesized thus demonstrated potent antagonistic activity against bacteria and fungi which possess potential applications in medicine and pharmaceutical fields.

**KEY WORDS:** Green synthesis, *Argyria nervosa* , Silver nanoparticles, Antagonistic activity.



**REBECCA THOMBRE**

Assistant Professor, Department of Biotechnology,  
Modern College, Shivajinagar, Pune-411005. India.

## INTRODUCTION

Metallic nanoparticles like gold, platinum, selenium and silver nanoparticles have various applications in fields of medicine, defense and drug synthesis. Green synthesis is one of the most eco- friendly techniques for the synthesis of nanoparticles. This kind of a synthesis includes usage of plant or microbes as a source. Among this, plants are considered as a best suited option as they can be made available easily for large scale biosynthesis of nanoparticles. Nanoparticles synthesized by plants are more stable and faster. Synthesis of silver nanoparticles from *Euphorbia hirta* L<sup>1</sup> and ginger<sup>2</sup> have already been reported. As the nanoparticles are synthesized from plants, these nanoparticles seem to incorporate the plant peptides as capping agents during synthesis, which stabilizes the nanoparticles as well as enhances their antagonistic properties<sup>3</sup>. In this study we have synthesized silver nanoparticles using *Argyrea nervosa* (Burm. f.) Bojer (Convolvulaceae) which is a shrub with woody stem found mainly West Ghats, India. Seeds of *Argyrea nervosa* are known to possess hypotension, spasmolytic and anti-inflammatory activity. Phytochemical analysis of the plant extracts reveals the presence of triterpenoids, flavanoids, steroids and lipids while seeds are rich in Argyreoside<sup>4</sup>.

## MATERIALS AND METHODS

### (i) Plant material

The seeds of *Argyrea nervosa* were collected from University of Pune Campus, Pune, India. They were authenticated by Botanical Survey of India, Pune (Voucher No: RSZARN3). The seeds were thoroughly washed with autoclaved distilled water and crushed. The powder was further used preparation of 10 g/L aqueous extract. This extract was filtered and stored at 4°C until further use.

### (ii) Chemicals

Silver nitrate (AgNO<sub>3</sub>) was purchased from Fisher Scientific, USA. Nutrient agar was purchased from Himedia, India. Autoclaved

distilled water was used throughout the experiment. All other chemicals were of analytical grade.

### (iii) Synthesis of nanoparticles

A modification of the method described by Thombre *et al.* (2013) was used for synthesis of nanoparticles. 10ml of freshly prepared 10mM AgNO<sub>3</sub> solution was added to 10ml of fresh seed extract. The reaction mixture was incubated for 30 min or till color change to dark brown was observed. The nanoparticles were then synthesized by drying at 80° C<sup>5</sup>.

### (iv) Characterization of nanoparticles

The synthesised nanoparticles were characterized using UV-Vis Spectroscopy (Schimadzu UV 1600) over a range of 250-700 nm. The topography of the nanoparticles was studied by subjecting them to SEM (Scanning Electron Microscope) analysis. The nanoparticles were subjected to EDS (Energy Dispersive spectroscopy) to analyze their chemical properties. FTIR (Fourier Transform Infrared Spectroscopy) was performed to obtain wide spectrum of nanoparticles over a narrow range. This method gives us information about plant peptides that have coated the particles during synthesis procedure. The XRD (X-Ray Diffraction Analysis) was performed to note the size of the obtained nanoparticle.

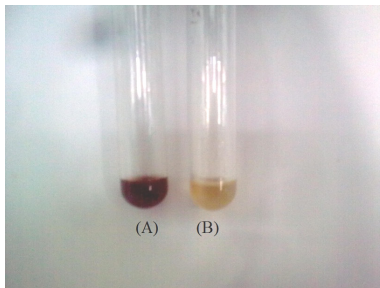
### (v) Antagonistic activity of silver nanoparticles against bacteria and fungi

The antagonistic activity of silver nanoparticles was studied against bacteria, yeast and fungi by using the agar well diffusion method<sup>6</sup>. Sterile nutrient agar plates were prepared and incubated to check overnight sterility. 0.1ml of test bacterial, yeast and fungal cultures were spread on the Nutrient agar plates. Using a cork borer 6mm wells were prepared on agar plates. To these wells 20µl of nanoparticle solution of 40mg/ml concentration was added. The plates were incubated at 37°C overnight. After 24 hours, the zone of inhibition was measured.

## RESULTS AND DISCUSSION

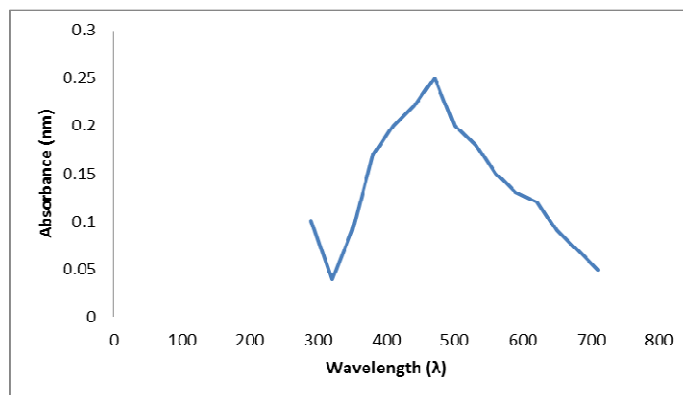
### *(i) Synthesis and characterization of silver nanoparticles*

Aqueous seed extract of *Argyria nervosa* acts as a reducing agent as stated by Balaprasad [5] which reduces metallic silver to nanosilver and hence the color change was obtained (Fig.1).



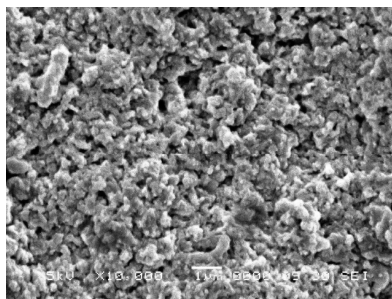
**Figure 1**  
***Color change due to synthesis of silver nanoparticles:***  
***(A) Aqueous seed extract of *Argyria nervosa*,***  
***(B) Aqueous extract and silver nitrate after incubation.***

The synthesized nanoparticles were characterized using SEM, XRD, FTIR, EDS and UV Vis spectroscopy analysis. The reduction of silver ions to nanosilver was monitored and confirmed using UV spectra. After the color change was obtained a small aliquot of sample was diluted with distilled water and subjected to UV analysis. The characteristic peak value for silver nanoparticles is between 400-570nm. (Fig. 2)



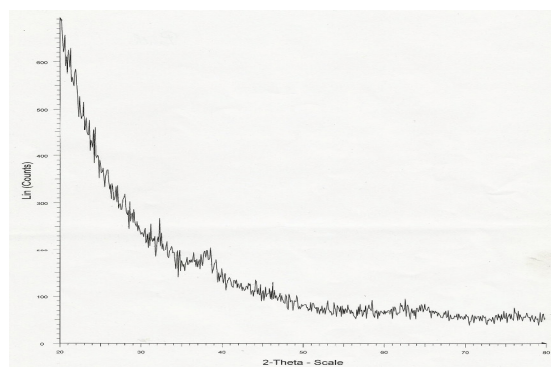
**Figure 2**  
***UV Vis spectra of nanoparticles produced by extract of *Argyria nervosa*.***

The spectrum of the sample was obtained for wavelength range from 270nm to 570nm. The  $\lambda_{max}$  of the nanoparticles was observed at 470nm. This is because of a phenomenon called Surface Plasmon Resonance (SPR) exhibited by silver nanoparticles. The silver nanoparticles oscillate when exposed to electromagnetic radiation and this oscillation gives a typical peak value<sup>7</sup>. The SEM image of the nanoparticles represents the topography of the particles is shown in Fig.3



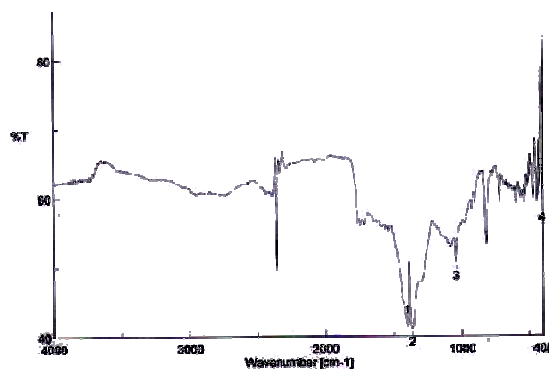
**Figure 3**  
***SEM image of silver nanoparticles produced by Argyria nervosa extract.***

The SEM image suggests the presence of roughly spherical silver nanoparticles. The incidence of X-rays on the powdered nanoparticles gives a particular pattern which helps to characterize the nanoparticles as shown in the XRD graph (Fig 4).



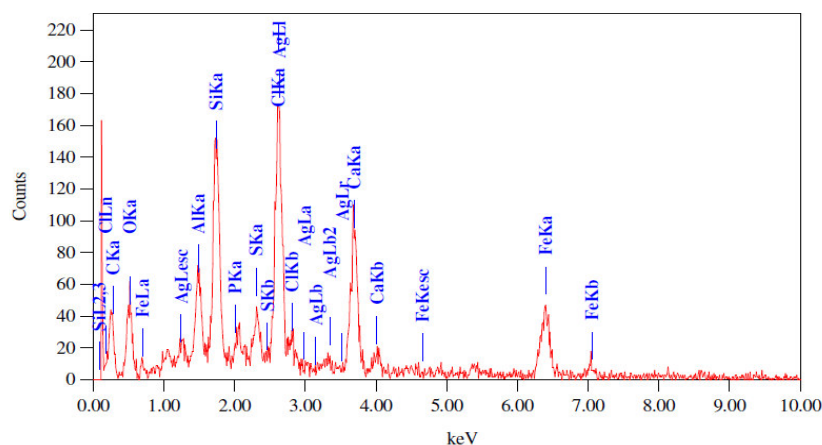
**Figure 4**  
***XRD pattern of the silver nanoparticles produced by extract of Argyria nervosa.***

XRD (manual mode) was used to characterize the AgNp. The 2 $\theta$  angle is converted to the diameter using the Scherrer formula ( $D_p = K\lambda / \beta_{1/2} \cos\theta$ ). The size of silver nanoparticles synthesised by green synthesis was estimated to be around 20-50 nm. FTIR analysis also gives a set of peak values unique for the sample along with information of the plant peptides that are present in the sample as the plant extract acts as a reducing agent (Fig.5). FTIR analysis is used to confirm the presence of plant peptides visible due to the bending produced by amide bonds<sup>8</sup>.



**Figure 5**  
***FTIR spectra of nanoparticles synthesized by extract of Argyria nervosa.***

The FTIR clearly indicates the presence of proteins as evidenced by the above figure 5. The elemental composition of nanoparticles was studied using EDS. The EDS image of nanoparticle sample is shown in fig. 6.



**Figure 6**

**EDS analysis of produced nanoparticles by *Argyria nervosa* extract.**

### (ii) Antagonistic activity of silver nanoparticles against bacteria and fungi

The antimicrobial activity of silver nanoparticles was studied against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli*, while antifungal activity was studied against *Aspergillus niger* and *Pichia pastoris* (Table 1).

**Table 1**

**Antagonistic activity of silver nanoparticles against bacteria and fungi**

Organism	Zone of inhibition (mm)
<i>Escherichia coli</i>	16
<i>Staphylococcus aureus</i>	14
<i>Bacillus subtilis</i>	17
<i>Pichia pastoris</i>	12
<i>Aspergillus niger</i>	17

Biological synthesis of silver nanoparticles is an alternative to chemical synthesis and it used the reducing properties of biological products for synthesis of silver nitrate to nanosilver<sup>9</sup>. Biological synthesis of nanoparticles has been previously reported using neem, tamarind and aloe vera extracts<sup>10, 11, 12</sup>. However there are not reports on synthesis of silver nanoparticles using an aqueous seed extract of *Argyria nervosa*. The phytochemical in the seed reduce the silver salts and not only produce silver nanoparticles but also stabilize it by capping the nanoparticles with the plant peptides. The antimicrobial activity of the nanoparticles is thus enhanced due to the presence of plant proteins and phytochemical.

## CONCLUSION

In this present study the synthesis of silver nanoparticles was synthesized by biological method using *Argyria nervosa* seed extract which acts as a reducing agent to reduce silver metal to nanosize. The synthesized silver nanoparticles were subjected to analysis such as SEM, UV Vis Spectroscopy, XRD, EDS and FTIR in order to characterize them. The antagonistic activity of silver nanoparticles was studied and they showed effective activity against gram negative, gram positive bacteria as well as against fungi. Thus this study proves to be an effective and economical method to

produce silver nanoparticles that show in vitro antagonistic action.

## ACKNOWLEDGEMENT

We thank Board of College and University Development (BCUD), University of Pune for financial support. We are grateful to the Principal, Modern College for providing facilities

for the research work. We thank Physics Department and Chemistry Department, University of Pune for help in analysis of samples.

## Disclosure policy

The authors declare no conflict of interest or competing interest.

## REFERENCES

1. Elumalai E, Prasad T, Kambala V , Nagajyothi P ,David E. Green synthesis of silver nanoparticle using *Euphorbia hirta* L and their antifungal activities. Archives of Applied Science Research. 2 (6):76-81, (2010).
2. Singh C, Sharma V, Naik P, Vikas K, Harvinder S. Green biogenic approach for synthesis of gold and silver nanoparticles using *Zingiber officinale*. Digest Journal of Nanomaterials and Biostructures. 6( 2),535-542, (2011).
3. Ankanna S, Prasad TNVKV, Elumalai EK and Savithramma N, Production of biogenic silver nanoparticles using *Boswellia ovalifoliolata* stem bark, Digest Journal of Nanomaterials and Biostructures ,5 (2): 369 – 372, (2010).
4. Krishnaveni, A and Santh Rani Thaakur, Pharmacognostical and Preliminary Phytochemical Studies of *Argyrea nervosa* (Burm. f.) Bojer, Ethnobotanical Leaflets, 12: 1176-83. (2008).
5. Thombre RS, Mehta S, Mohite J, Jaisinghani P. Synthesis of silver nanoparticles and its cytotoxic effect on THP-1 cancer cell line. International Journal of Pharma and Biosciences. 4(1):184-192, (2013).
6. Thombre RS, Jagtap R, Patil NM, Evaluation of phytoconstituents, antibacterial, antioxidant and cytotoxic activity of *Vitex negundo* L. and *Tabernaemontana divaricata* L. . International Journal of Pharma and Biosciences.4(1): 389-396, (2013).
7. Smitha SL, Nissamudeen KM, Philip D and Gopchandran KG, Studies on surface Plasmon resonance and photoluminescence silver nanoparticles, Spectrochim. Acta A, 71 (1): 186–190, (2008).
8. Jing A, Wang D, Luo Q and Yuan X, Antimicrobial active silver nanoparticles and Silver/polystyrene core-shell nanoparticles prepared in room-temperature ionic liquid, Materials Science and Engineering C, 29(6) :1984–1989, (2009).
9. Gardea-Torresdey, J. L.; Gomez, E.Peralta-Videa, J. R.; Parsons, J. G.;Troiani, H.; Jose-Yacaman, M. Alfalfa sprouts: A natural source for the synthesis of silver nanoparticles. *Langmuir*, 19: 1357-1361, (2003).
10. Shivshankar, S.; Rai, A.; Ahmad, A.;Sastry, M. Rapid synthesis of Au, Ag,and bimetallic Au core–Ag shell nanoparticles using Neem (*Azadirachta indica*) leaf broth. *J. Colloid Interface Sci.*, 275: 496-502, (2004).
11. Ankamwar, B.; Chaudhary, M.; Sastry,M. Gold nanotriangles biologically synthesized using tamarind leaf extract and potential application in vapor sensing. *Synth. React. Inorg. Metal-Org. Nanometal. Chem.*, 35: 19-26, (2005).
12. Prathap, S. C.; Chaudhary, M.; Pasricha,R.; Ahmad, A.; Sastry, M. Synthesis of gold nanotriangles and silver nanoparticles using *Aloe vera* plant extract. *Biotechnol. Prog.*, 22: 577-583,(2006).