Journal of Ecobiotechnology 2012, 4(1): 54-57
ISSN: 2077-0464
Available Online: http://journal-ecobiotechnology.com/

Biogenesis of silver nanoparticles using leaf extract of *Turnera ulmifolia* Linn. and screening of their antimicrobial activity

M.S. Shekhawat*, N. Kannan and M. Manokari

K.M. Centre for Postgraduate Studies, Pondicherry-605 008, India

Abstract
Development of nontoxic and clean techniques for the synthesis of silver nanoparticles (Ag NPs) has attracted increasing attention in recent years. The biological synthesis of silver nanoparticles using *Turnera ulmifolia* plant leaf extract was studied in present investigation. The synthesized nanoparticles were confirmed by color changes from pale green to reddish brown and characterized by UV-Visible spectrophotometer. A peak between 415 nm to 425 nm was obtained on spectrometer characterized the biosynthesis of silver nanoparticles. The synthesized nanoparticles also showed antibacterial activity against four disease causing microorganisms.

Keywords: Silver nanopaticles, *Turnera ulmifolia*, UV-Visible spectrophotometer, antibacterial activity.

INTRODUCTION

Nanoparticles are usually referred as particles with a size of 1 nm to 100 nm. These particles show completely new properties depends on specific characteristics such as size, morphology, distribution etc. [1]. They exhibit larger surface area to volume ratio. As specific surface area of nanoparicles is increased, their biological effectiveness also can increase. This is relevant for catalytic reactivity and antimicrobial activity of silver nanoparticles.

Production of nanomaterials can be achieved mainly by physical, chemical and biological procedures. The physical methods involve high pressure, energy and temperature, and the chemical procedures generate a large amount of toxic byproducts. Thus, there is a need for a safe and alternate way which includes a clean, nontoxic and eco-friendly method of nanoparticle synthesis [2, 3]. Biogenic synthesis of nanoparticles provides advancement over physical and chemical methods because it is an eco-friendly and also cost effective.

Many biological organisms (prokaryotic/eukaryotic as well as unicellular/multicellular) are known to produce nanomaterials either intra or extra-cellularly [4, 5, 6]. Biosynthesis of nanoparticles of silver and gold by plants was achieved in several plants species such as Alfalfa [7], *Emblica officinalis* [8], *Carica papaya* [9], *Parthenium hysterophorus* [10], *Azadirachta indica* [11], *Capsicum annuum* [12], *Hibiscus rosasinesis* [13] and, geranium and neem [14, 15].

Biologically synthesized nanoparticles using plant extracts may have applications in various human body-contacting areas such as foods, cosmetics and medicines [16]. Silver particles have long been recognized as antimicrobial agents and having inhibitory effect on microbes present in medical and industrial practices [17]. These are mostly used in medical field as topical ointments to prevent infection against burn and open wounds [18]. Ag NPs containing materials were also employed in textile fabrics, as food additives, and in package and plastics to eliminate microorganisms. Because of such a wide range of applications, numerous methods regarding the fabrication of silver nanoparticles, as well as various silver based compounds containing ionic silver (Ag^+) have been developed [19]. In this study, reducing silver ions present in the aqueous solution of silver nitrate with the help of *T. ulmifolia* leaf extract and their antibacterial assessment was performed to produce new compounds against different multi-drug-resistant human pathogens.

Received: Nov 17, 2011; Revised: Dec 22, 2011; Accepted: Jan 24, 2012.

*Corresponding Author

M.S. Shekhawat
K.M. Centre for Postgraduate Studies, Pondicherry-605 008, India

Tel: +91-98943 89390; Fax: +91-413-2251687
Email: smahipal3@gmail.com

MATERIALS AND METHODS

Collection of plant materials and extract preparation

The plant material was collected from the coastal area of Pondicherry, India. Fresh, green and mature leaves were harvested and thoroughly washed with distilled water. The leaves were finely cut in small pieces. The plant leaf broth solution was prepared by using 5 gm of washed and cut leaves in a 250 ml Erlenmeyer flask with 50 ml of sterile distilled water and then boiling the mixture for 5 min. The herbal aqueous extract was collected in separate conical flasks by standard filtration method and stored at 4°C.

Synthesis and characterization of Ag NPs

1mM aqueous solution of Silver nitrate (Himedia, Mumbai) was prepared for synthesis of silver nanoparticles. For the synthesis of Ag NPs, two boiling tubes were taken, one containing 10 ml of 1mM $AgNO_3$ solution as control and the second containing 9 ml of 1mM Silver nitrate solution and 1 ml of plant leaf extracts as test solution. These were incubated at room temperature for 1-2 hours. The color change of the leaf extracts from pale yellow to dark brown was checked periodically. The brown color formation indicates that the Silver nanoparticles were synthesized from the herbs and they were centrifuged at 5000 rpm for 15 minutes in order to obtain the pellet which is used for further study. Supernatant is discarded and the pellet is dissolved in deionised water. The silver nanoparticules

were confirmed by color changes and qualitatively characterized by UV-Visible spectrophotometer.

UV- Vis spectra analysis

The reduction of metallic Ag^+ ions was monitored by measuring the UV-Vis spectrum after about 12 hours of reaction. A small aliquot was drawn from the reaction mixture and a spectrum was taken on a wavelength from 300 nm to 700 nm on UV-Vis spectrophotometer (Systronics Double beam spectrophotometer 2202).

Antibacterial activity

Antibacterial activities of plant extract-mediated silver nanoparticles were assayed using standard well-diffusion method. The test bacteria (human pathogenic bacteria) such as *Escherichia coli*, *Pseudomonas aeroginosa*, *Staphylococcus aureus* and *Enterococcus faecalis* were included in this study to assess the susceptibility pattern of the nanoparticles. 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 1 mm holes were punched in the medium. 100 µl of the solution containing nanoparticles was inoculated in this hole and the plates were incubated at 37ºC for 24-48 hours for observing zone of inhibition.

RESULTS AND DISCUSSION

Synthesis and characterization of Ag NPs

Extracts from plants may act as reducing and capping agents in silver nanoparticles synthesis. The reduction of Ag^+ ions by combinations of biomolecules found in these extracts (e.g. enzymes / proteins, amino acids, polysaccharides, vitamins etc.) is environmentally benign, yet chemically complex [20]. The extract of lower plants (algae) was also used to synthesize Ag NPs at room temperature. Proteins in the extract provide dual function of Ag^+ reduction and shape control in the nanoparticle synthesis. The carboxyl groups in aspartic and/or glutamine residues and the hydroxyl groups in tyrosine residues of the proteins were suggested to be responsible for the Ag^+ ion reduction [21].

It is well known that silver nanoparticles exhibit dark brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles [22]. The appearances of yellowish-brown color in the reaction vessels suggest the formation of silver nanoparticles (SNPs) [14]. The green synthesis of silver nanoparticles through leaf extract of *T. ulmifolia* was carried out in present investigation. The color was changed in the cell free extract when challenged with 1mM $AgNO_3$ from pale yellow to dark brown (Fig. 1A) in 15 min and attained maximum intensity after 12 hrs with intensity increasing during the period of incubation indicative of the formation of silver nanoparticle. Control (without silver ions) showed no change in color of the cell filtrates when incubated under same conditions (Fig. 1C).

The important aspect of Ag NPs is that their optical properties depend upon the particle size and shape. These optical properties are dominated by the collective oscillation of conduction electrons resulting from the interaction with electro-magnetic radiation. When light absorbance capacity of sol-gel medium is increased, size of nanoparticle is also increased, and when peak height for UV-Vis absorption (nm) is increased, then concentration of nanoparticles is increased [23].

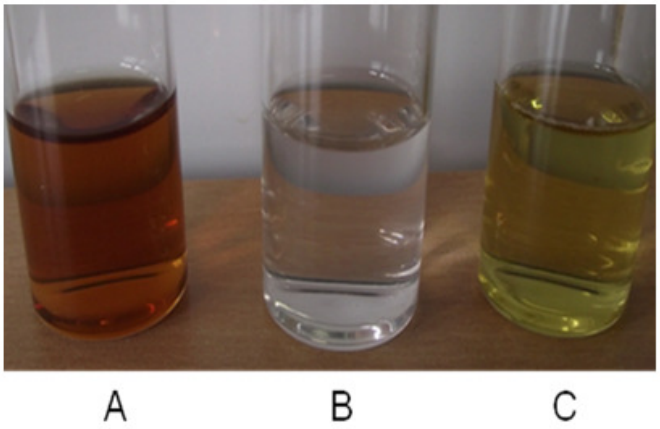

Fig 1. Synthesis of Ag NPs using *T. ulmifolia* leaf extracts, A. Solution containing Ag NPs B. 1mM $AgNO_3$ solution and C. Filtered leaf extract.

The synthesis of Ag NPs had been confirmed by measuring the UV-Vis spectrum of the reaction media. The UV-Vis spectrum of colloidal solutions of Ag NPs synthesized from *T. ulmifolia* leaf extract have absorbance peaks at 415-425 nm regions (Fig. 2), which are identical to the characteristics UV-visible spectrum of metallic silver. The weak absorption peak at shorter wave lengths was due to the presence of several organic compounds which were known to interact with silver ions. The time duration of change in color varies from plant to plant. *Boswellia ovalifoliolata* synthesized silver nanopartcles within 10 min whereas *Shorea tumbuggaia* and *Svensonia hyderobadensis* took 15 min to synthesize nanoparticles [24].

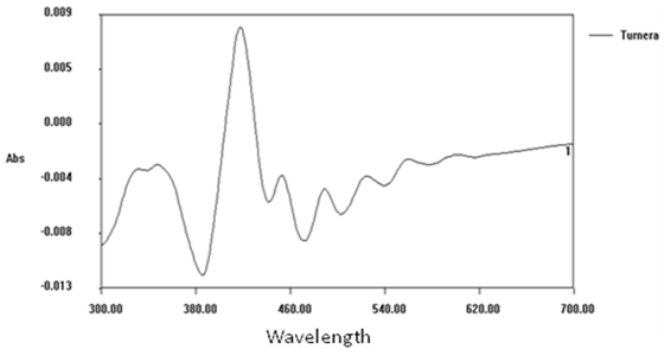

Fig 2. UV-Vis spectrum of silver nanoparticles synthesized using *T. ulmifolia*.

Antimicrobial activity of silver nanoparticles

The biologically synthesized silver nanoparticles using medicinal plants were found to be highly toxic against different pathogenic bacteria. Silver nanoparticles are very effective against micro-organisms because of their enormously high surface area. They do not remain nano size when come in contact with normal environmental fluids such as water. They agglomerate to form much larger and then these are more effective. Several studies proposed that Ag NPs may attach to the surface of the cell membrane disturbing permeability and respiration functions of the cell. Smaller Ag NPs having the large surface area available for interaction would give more bactericidal effect than the larger Ag NPs [25]. It is also

possible that Ag NPs not only interact with the surface of membrane, but can also penetrate inside the bacteria [26].

The use of plant extracts is effective against various microorganism including plant pathogens [27]. The Ag NPs of *Turnera ulmifolia* showed highest antibacterial activity against *Escherichia coli* and *Pseudmonas aeroginosa* followed by *Staphyllococcus aureus* and *Enterococcus faecalis* as shown in Fig. 3.

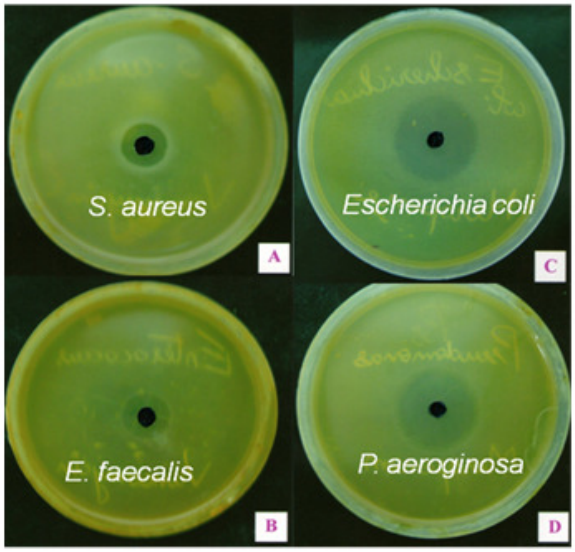

Fig 3. Antibacterial activity of Ag NPs against, A. *S. aureus* B. *E. faecalis* C. *E. coli* and D. *P. aeroginosa*.

The Ag NPs synthesized from plant species are toxic to multi-drug resistant microorganisms. Similar observation was found in *Allium cepa* [28], *Argimone mexicana* [29], *Artocarpus heterophyllus* [22].

T. ulmifolia has been used in traditional medicine but so for this plant has not been tested for Ag NPs synthesis and their antimicrobial activity. The present work confirmed the medicinal values of this plant and also revealed a simple, rapid and economical route to synthesis of Ag NPs, and their capability of rendering the antimicrobial efficacy. Moreover, the synthesized Ag NPs enhance the therapeutic efficacy and strengthen the medicinal values of *Turnera ulmifolia*.

REFERENCES

[1] Baker, C., A. Pradhan, L. Pakstis, D.J. Pochan and S.I. Shah 2005. Synthesis and antibacterial properties of silver nanoparticles. *J. Nanosci. Nanotechnol.* 5:244–9.

[2] Goodsell, D.S. 2004. Bionanotechnology: Lessons from Nature. John Wiley & Sons Inc. Publication.

[3] Mukherjee, P., A. Ahmad, D. Mandal, S. Senapati, S.R. Sainkar, M.I. Khan, R. Parishcha, P. V. Ajaykumar, M. Alam, R. Kumar and M. Sastry. 2001. Fungus mediated synthesis of silver nanoparticles and their immobilization in the mycelia matrix: A novel biological approach to nanaoparticle synthesis. *Nano Lett.* 1: 515-519.

[4] Dickson, D.P.E. 1999. Nanostructured magnetism in living systems. *J. Magn. Mign. Mater.,* 203: 46- 49.

[5] Ahmad, A., S. Senapati, M.I. Khan, R. Kumar and M. Sastry. 2003. Extracellular biosynthesis of monodisperse gold nanoparticles by a novel Extremophilic actinomycete, *Thermomonospora* sp. *Langmuir,* 19: 3550-3553.

[6] Duran, N.P.D., O.L. Marcato, G.I. Alves, E. Souza, Esposito. 2005. Mechanistic aspects of biosynthesis of silver nanaoparticles by several *Fusarium oxysporum* strains. *J. Nanobiotechnol.* 3: 8-14.

[7] Gardea-Torresdey, J.L., J.L. Gomez, J. Peralta-Videa, J.G. Parsons, H.E. Troiani and Jose-Yacaman. 2003. Synthesis of Gold Nanotriangles and Silver Nanoparticles using *Aloe vera* plant extract. *Langmuir,* 13: 13-57.

[8] Ankamwar, B.D., M. Choudhary and S. Murali. 2005. Gold nanotraigles biologically synthesized using tamarind leaf extract and potential application in vapor sensing. Synth. React. Inorg. Metal-Org. *Nanometal Chem.* 35:19.

[9] Jain, D., K.D. Hemant, S. Kachhawaha and S.L. Kothari. 2009. Synthesis of plant mediated silver nanoparticles using papaya fruit extract and evaluation of their anti microbial activities. *Digest J. Nanomater. and Biostruct.* 4: 723.

[10] Ankamwar, B.D., A. Chinmay, A. Absar and S. Murali. 2005. Biosynthesis of Gold and Silver Nanoparticles using *Emblica officinalis* fruit extract, their phase transfer and transmetallation in an organism solution. *J. Nanosci. Nanotechnol.* 10: 1665.

[11] Shankar, S.S., A. Rai, A. Ahmad and M. Sastry. 2004a. 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.

[12] Mukherjee, P., M. Roy, B. Mandal, G. Dey, P.Mukherjee and J. Ghatak. 2008. Green synthesis of highly stabilized nanocrystalline silver particles by a non-pathogenic and agriculturally important fungus *Trichoderma asperellum*. *Nanotech.* 19: 75103.

[13] Harekrishna, B., K. B. Dipak, G. Sahoo, P. Sarkar, P. Sankar and A. Misra. 2009. Green synthesis of silver nanoparticles using *Jatropha curcas*. Colloids and Surfaces A. *Physiocochem. Eng. Aspects,* 339: 134.

[14] Shankar, S.S., A. Rai, A. Ahmad and M. Sastry. 2005. Controlling the optical properties of lemongrass extract synthesized gold nanotriangles and potential application in infrared-absorbing optical coatings, *Chem. Mater*. 17; 566-572.

[15] Shankar, S.S., A. Rai, B. Ankamwar, A. Singh, A. Ahmad and M. Sastry. 2004b. Biological synthesis of triangular gold nanoparticles. *Nat. Mater.* 3:482.

[16] Parashar, V., R. Parashar, B. Sharma and A.C. Pandey. 2009. *Digest J. Nanomat. Biostruct.* 4(1): 45-50.

[17] Jose, R.M., L.E. Jose, C. Alejandra, H. Katherine, B.K. Juan, T.R. Jose and J.Y. Miguel. 2005. The bactericidal effect of silver nanoparticles. *Nanotech.* 16: 2346-2353.

[18] Lok, C.N., C.M. Ho, R. Chen, Q.Y. He, W.Y. Yu and H. Sun. 2007. Silver nanoparticles: partial oxidation and antibacterial activities. *J. Biol. Inorg. Chem.* 12: 527-34.

[19] David, E., E.K. Elumalai, T.N.V.K.V. Prasad, V. Kambala and P.C. Nagajyothi. 2010. Green synthesis of silver nanoparticle

using *Euphorbia hirta* L. and their antifungal activities. *Archives Appl. Sc. Res.* 2(6): 76-81.

[20] Collera-Zuniga, O., F.G. Jimenez and R.M. Gordillo. 2005. Comparative study of carotenoid composition in three Mexican varieties of *Capsicum annuum* L. *Food Chem.* 90: 109-114.

[21] Xie, J., J.Y. Lee, D.I.C. Wang and Y.P. Ting. 2007. Silver Nanoplates: From Biological to Biomimetic Synthesis", *A. C. S. Nano.* 1: 429-439.

[22] Thirumurgan, A., N.A. Tomy, R.J. Ganesh and R.S. Gobikrishnan. 2010. Biological reduction of silver nanoparticles using plant leaf extracts and its effect an increased antimicrobial activity against clinically isolated organism. *De. Phar. Chem.* 2, 279-284.

[23] Mondal, A.K., S. Mondal, S. Samanta and S. Mallick. 2011. Synthesis of Ecofriendly Silver Nanoparticle from Plant Latex used as an Important Taxonomic Tool for Phylogenetic Interrelationship. *Adv. Biores.* 2(1): 122-133.

[24] Savithramma, N., M.L. Rao, K. Rukmini and P.S. Devi. 2011. Antimicrobial activity of Silver Nanoparticles synthesized by using Medicinal Plants. *Int. J. ChemTech Res.* 3(3): 1394-1402.

[25] Kvitek, L., A. Panacek, J. Soukupova, M. Kolar, R. Vecerova, R. Prucek, M. Holecova and R. Zboril 2008. Effect of Surfactants and Polymers on Stability and Antibacterial Activity of Silver Nanoparticles (NPs). *J. Phys. Chem.* 112: 5825-5834.

[26] Morones, J.R., J.L. Elechiguerra, A. Camacho, K. Holt, J.B. Kouri, J.T. Ramirez and M.J. Yacaman. 2005. The bactericidal effect of silver nanoparticles. *Nanotech.* 16:2346-2353.

[27] Mishra, Y.K., S. Mohapatra, D. Kabiraj, B. Mohanta, N.P. Lalla, J.C. Pivin and D.K. Avasthi. 2007. Synthesis and characterization of Ag nanoparticles in silica matrix by atom beam Sputtering. *Scr. Mater*. 56: 629-632.

[28] Saxena, A., R.M. Tripathi and R.P. Singh. 2010. Biological Synthesis of silver nanoparticles by using Onion (*Allium cepa*) extract and their antibacterial activity. *Digest. J. Nanomat. Biostruct.* 5:427-432.

[29] Khandelwal, N., A. Singh, D. Jain, M.K. Upadhyay and H.N. Verma 2010. Green synthesis of silver nanoparticles using *Argimone mexicana* leaf extract and Evaluation of their antimicrobial activities. *Digest. J. Nanomater. Biostruct.* 5: 483-489.