The Bioscan 11(1): 57-60, 2016
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

PREPARATION, CHARACTERIZATION AND ANTIBACTERIAL STUDIES OF SILVER NANOPARTICLES SYNTHESIZED BY *SOLANUM NIGRUM* LEAF EXTRACT

S. RACHEL DEVA KIRUBAI[1*], B. VEDHA[1], T. PANNEERSELVAM[1] AND D. J. MUKESH KUMAR[2]
[1]PG & Research Department of Microbiology
Adhiparasakthi College of Arts and Science (Autonomous), Vellore - 632 506, INDIA
[2]CAS in Botany, University of Madras, Guindy campus, Chennai, Tamil Nadu, INDIA
e-mail: rachelanbu15@gmail.com

KEYWORDS
Solanum nigrum
Silver nanoparticles
FESEM
HRTEM
Antibacterial activity

Received on :
13.09.2015

Accepted on :
28.01.2016

***Corresponding author**

ABSTRACT
Nanotechnology is anew emerging field where it finds applications in all the aspects of life sciences. In order to meet the increasing demands of therapeutic agents, new methods are being discovered for the eco-friendly synthesis of therapeutically important silver nanoparticles. Traditionally,silver nanoparticles are synthesized using chemical techniques, which involve often toxic chemicals. As an alternative to the conventional techniques, in the present study, an eco-friendly, cost effective method was suggested for biosynthesis of silver nanoparticles using the reduction of $AgNO_3$ by the phytochemicals present in *Solanum nigrum* leaves.The colour change of the silver nitrate solution after addition of plant extract indicated the reduction of silver ions. The formation of peak around 420 nm in UV-Visible spectroscopy confirmed the presence of silver nanoparticles. The FESEM and HRTEM analysis revealed the spherical structure of the synthesized nanoparticles. The silver nanoparticles was further tested against human pathogens and was found to be highly toxic against tested bacteria.In conclusion, the silver nanoparticles synthesized using *Solanum nigrum* leaf extract showed better antibacterial activity against human pathogenic bacteria which can be employed for the treatment of bacterial infections.

INTRODUCTION

Nanotechnology is the study and design of machines on the molecular and atomic level. In regard to medicine and therapeutics, this field is emerging as a new scope for the treatment of various diseases (Furno *et al*., 2004). Several nanostructures like nanotube, nanoshell, nanocrystal and quantum dots find their way in the medical application for their size distribution and morphology. Because of their unique physical and chemical properties at nanoscale, there is a huge interest in metal nanoparticles.

Nanomaterials can also be modified structurally for better efficiency in different fields such as bioscience and medicine (Crabtree *et al*., 2003).Metallic nanoparticles are found to be most promising as they possess remarkable antibacterial properties (Asra *et al*., 2012). The nanocrystalline silver was used as an antimicrobial agent for the treatment of wounds in ancient days itself (Brunet *et al*., 2009).

A number of approaches are available for the production of metal nanoparticles such as chemical reduction, electrochemical techniques and green chemistry route (Dušan *et al*., 2011). It is claimed that nanoparticles synthesized using biological route is environmentally safer and also biocompatible (Rajkishore *et al*., 2013).Recently, it was found that aqueous silver ions can be reduced by the phytochemicals present in the aqueous extracts of plant parts to generate extremely stable silver nanoparticles. The emergence of resistance against antibiotics among the bacteria becomes the serious concern in the treatment of bacterial infections

(Rajeshwari *et al*., 2010). Apart from being environmentally friendly process, the present study holds the hypothesis; the use of *Solanum nigrum* extract might add synergistic antibacterial effect to the biosynthesized nanoparticles (Ranjana *et al*., 2013).

Against these backdrops, the present study aimed at the biosynthesis of silver nanoparticles using aqueous extract of *Solanum nigrum* leaves, its characterization and evaluation of its bactericidal effect against selected pathogenic bacterial strains. Silver nanoparticles were synthesized by short term interaction of *Solanum nigrum* extract and 0.01M $AgNO_3$ solution and have been characterized by UV spectroscopy, FESEM, HRTEM and EDAX. The antibacterial activity of the nanoparticles was also studied by disc diffusion method.

MATERIALS AND METHODS

Preparation of *Solanum nigrum* extract

The *Solanum nigrum* leaves were purchased from local market located in Chennai, India. The leaves were washed with double distilled water and were shade dried at room temperature for 7 days and blended to a fine powder. About 4 g of *Solanum nigrum* leaf powder was weighed and transferred into a 250ml beaker containing 40ml of distilled water, mixed well and boiled for 3 min and filtered through Whatmann No.1 filter paper to obtain a clear extract (Ananthan *et al*., 2014). The extract was used for the reduction of silver nitrate for the silver nanoparticles synthesis.

Synthesis of Silver nanoparticles

Silver nanoparticles were synthesized by biological reduction method using the leaf extract of *Solanum nigrum*. All the solutions of reacting materials were prepared in double distilled deionised water. 0.01M of silver nitrate solution was prepared by dissolving 0.44g of silver nitrate in 35ml of double distilled water. To this solution, 5ml of plant extract was added and kept under high speed stirring for 20-40 mins in a magnetic stirrer. The silver nanoparticles synthesis was confirmed by the colour change from pale yellow to reddish brown (Priya Banerjee *et al.*, 2014).

Characterization of Silver nanoparicle

The bioreduction of $Ag+$ ion was monitored by the UV-visible spectrophotometer (Technomp 8500 spectrometer). Further, the structural characterization of the nanoparticles was done by FESEM and energy dispersing analysis of X-ray (EDX) measurements (HITACHI SU6600) at resolution of 3 nm to 8 nm and magnification 10.3 mm x to 200,000 operating on voltage of 15kV. The films of the samples were prepared on a carbon coated grid by dropping a small amount of the sample and then allowed to dry prior to measurement. The internal morphology of the synthesized silver nanoparticles was further analysed using High Resolution Transmission Electron Microscope equipped with EDX.

Antibacterial activity

Four bacterial species *Staphylococcus aureus* MTCC 766, *Bacillus subtilis* MTCC 441, *Proteus vulgaris* MTCC 1771 and *Pseudomonas aeruginosa* MTCC 2488 were obtained from MTCC, IMTECH Chandigarh, India. The antimicrobial activities were determined by agar well diffusion method. Under aseptic conditions, 20 ml of Mueller Hinton agar (MHA) medium was poured in to pre-sterilized petridishes. After the solidification of the medium,the plates were then seeded with test bacteria suspended in peptone water. About 6 mm wells were then punched in the bacteria seeded agar plates and the wells were filled with different dilutions (varying from 2.5 to 20 μl) of silver nanoparticles from stock of 20 mg/ml. Streptomycin discs for bacteria (10 μg/disc) were used as positive control. The petridishes were incubated for 24 hours at 37°C and the diameter of zone of inhibition was measured. (Stoimenov *et al.*, 2002).

RESULTS AND DISCUSSION

UV-visible spectroscopy studies

UV-visible spectroscopy was used for detecting the signature of silver nanoparticles. UV-Visible spectroscopy is a powerful tool for the characterization of noble metal particles, which exhibits strong surface plasmon resonance absorption in the visible region and are highly sensitive to the surface modification. Further, the peak obtained between 400nm and 440nm on UV-Vis spectrometer confirmed the presence of biosynthesized silver nanoparticles(Fig. 1).Silver being a noble metal, silver nanoparticles are ideal for synthesizing and as observed, they exhibit strong surface plasmon resonance absorption in the visible region and are highly sensitive to the

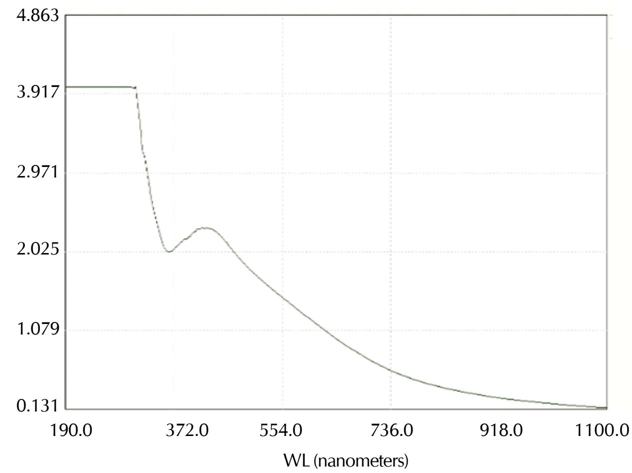

Figure 1: UV-Vis absorption spectrum of silver nanoparticles synthesized by *Solanum nigrum* plant extract

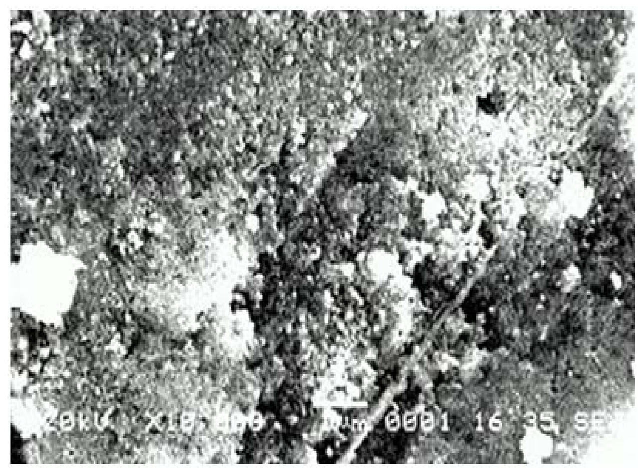

Figure 2: FE-SEM image of the synthesized silver nanoparticles

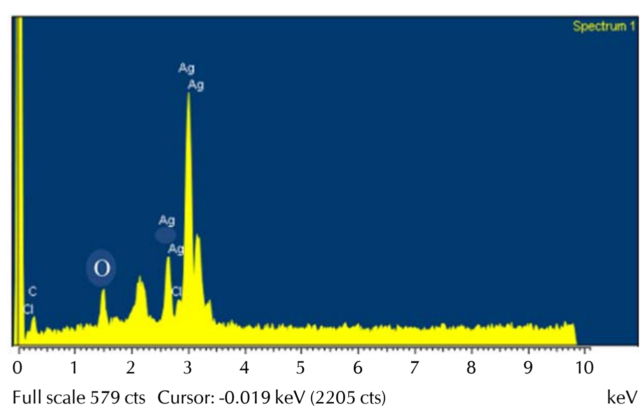

Figure 3: FESEM-EDAX spectrum of silver nanoparticles

surface modification (Ledo-Sua´rez *et al.*, 2007).

Scanning electron microscopic studies

The surface morphology and structure of the synthesized nanoparticles were examined by the FESEM. The FESEM images (Fig. 2) of the silver nanoparticles exhibited that most of the silver nanoparticles were spherical in shape and few of them

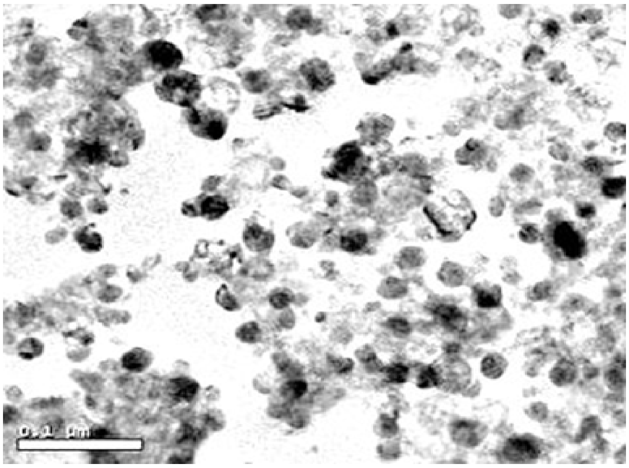

Figure 4: HR-TEM image of silver nanoparticles

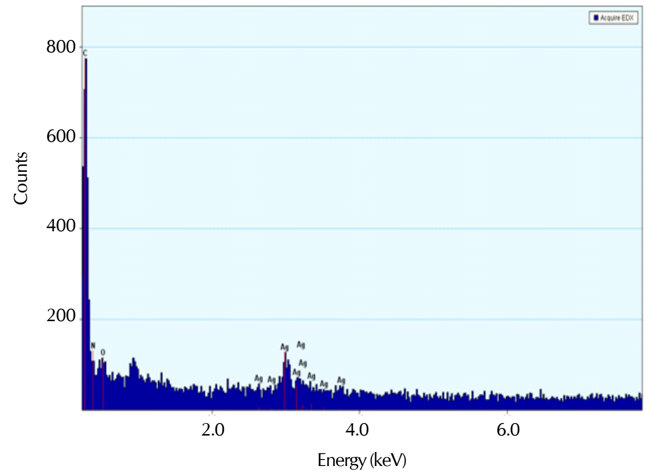

Figure 5: HRTEM-EDX of silver nanoparticles

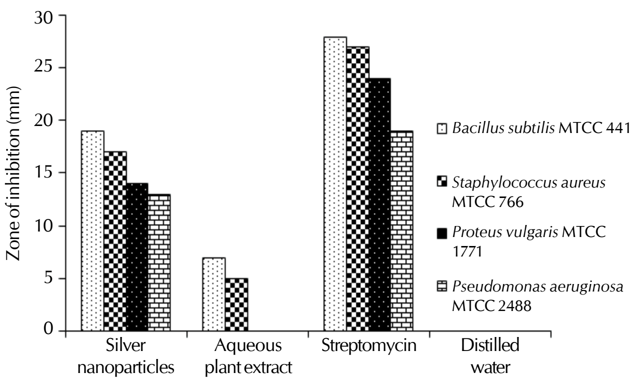

Figure 6: Antimicrobial activity of biologically synthesized silver nanoparticles

were hexagonal. After carrying out FESEM microscopy, the presence of silver nanoparticles was confirmed by EDX which gave a strong silver signal(Fig. 3). HRTEM confirmed the presence of silver nanostructures and gave clear image of nanoparticles(Fig. 4). It is also observed that the shape was spherical.Beyond SEM, FE-SEM and HRTEM provides us the clear structural variation in nanoparticles among the same clusters (Ajitha *et al*., 2013). The EDX profile (Fig. 5) of the silver nanoparticles obtained through FESEM and HRTEM indicated that the sample contains silver in maximum concentration whereas carbon and oxygen in minimum concentrations, which might occurred due to the presence of impurities (Raffi *et al*., 2008).

Antibacterial Studies

Agar well diffusion method was used to study the antibacterial activity of the silver nanoparticles against the selected pathogens by measuring the zone of inhibition. In this method, wells of 6 mm diameter were loaded with the different concentrations of solutions. In the present study, the biosynthesized AgNPs showed excellent antibacterial activity against tested pathogenic bacterial strains of. Our results showed that AgNPs synthesized from *S. nigrum* possess discrete antibacterial activity against bacteria at different concentrations of 25-200 μg/mL (Fig. 6).The results were in conformity with other published works of Singhal *et al*. (2011), Prasad *et al*. (2011). The inhibitory action of silver has been recognized in ancient days and applied as therapeutic agent for the prevention of wound infections (Silver and Phung, 1996; Sondi and Sondi, 2004). The Nano crystalline silver shows the most effective inhibitory action with a rapid inhibition rate (Kim *et al*., 2007). In the present study *S. nigrum* was taken for synthesis of AgNPs because of its medicinal values. Various studies have been done by many researchers which confirm that *S. nigrum* was found to be good antibacterial agent against pathogenic and non-pathogenic organisms.

REFERENCES

Ajitha, B., Divya, A., Harish, G. S. and Sreedhara Reddy, P. 2013. The Influence of Silver Precursor Concentration on Size of Silver Nanoparticles Grown by Soft Chemical Route. *Research J. Physical Sciences*. **1(7):** 11-14.

Ananthan, Padmashree, Gopal Kumar Sharma, Anil Dutt Semwal. and Chitra shekarachar Mahesh 2014. Antioxygenic Activity of *Solanum nigrum* L. Leaves in Sunflower Oil Model System and Its Thermal Stability. *Food and Nutrition Sciences*. **5:** 1022-1029.

Asra Praveen, Aashis, S. Roy and Srinath, Rao 2012. Biosynthesis and characterisation of silver nanoparticles from *Cassia auriculata* leaf extract and in vitro evaluation of antimicrobial activity. *Int J. Applied Biol and Pharma Tech*. **3(2):** 222-228.

Brunet, L., Lyon, D. Y., Hotze, E. M., Alvarez, P. J. J., Wiesner, M. 2009. Comparative photoactivity and antibacterial properties of C60 fullerenes and titanium dioxide nanoparticles. *Environ. Sci. Technol*. **43:** 4355-4360.

Crabtree, J. H., Burchette, R. J ., Siddiqi, R. A., Huen, I. T., Handott, L. L. and Fishman, A. 2003. The efficacy of silver-ion implanted catheters in reducing peritoneal dialysis-related infections. *Perit Dial Int*. **23(4):** 368-74.

Dragieva, I., Stoeva, S., Stoimenov, P., Pavlikianov, E. and Klabunde, K. 1999. Complex formation in solutions for chemical synthesis of nanoscaled particles prepared by borohydride reduction process. *Nanostruct Mater*. **12:** 267-270.

Dušan Zvekiæ, Vladimir, V. Srdiæ., Maja A. Karaman, Milan and Matavulj, N. 2011. Antimicrobial properties of ZnO nanoparticles incorporated in polyurethane varnish. *Processing and Application of*

Ceramics. **5(1):** 41-45.

Furno, F., Morley, K. S., Wong, B., Sharp, B. L., Arnold, P. L., Howdle, S. M. *et al*. 2004. Silver nanoparticles and polymeric medical devices: a new approach to prevention of *infection. J. Antimicrob Chemother.* **54:** 1019-1024.

Sondi, I. and Sondi, B. S. 2004. Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria. *J. Colloid and Interface Science.* **275**: 177-182.

Kim, J. S., Kuk, E., Yu, K. N., Kim, J. 1. H., Park, S. J., Lee, H. J., Kim, S. H., Park, Y. K., Park, Y. H., Hwang, C. Y., Kim, Y. K., Lee, Y. S., Jeong, D. H. and Cho, M. H. 2007. Antimicrobial effects of silver nanoparticles. Nanomedicine: *Nanotechnol. Biol. Medic.* **3:** 95-101.

Ledo-Sua´rez, A., Rivas, J., Rodrý´guez-Abreu, C.F., Rodrý´guez, M.J., Pastor, E., Herna´ndez-Creus, A., Oseroff, S. B., Lo´pez-Quintela, M. A. 2007. Facile synthesis of stable subnanosized silver clusters in microemulsions. *Angew ChemInt Ed.* **46(46):** 8823-8827.

Parasad, K. S., Pathak, D., Patel, A., Dalwadi, P., Prasad, R., Patel, P. and Selvaraj, K. 2011. Biogenic synthesis of silver nanoparticles using *Nicotianatobaccum* leaf extract and study of their antimicrobial effect. *Afr. J. Biotechnol.***10:** 8122-813.

Priya, B., Mantosh, S., Aniruddha Mukhopahayay and Papita, D., 2014. Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: synthesis, characterization, antimicrobial property and toxicity analysis. *Bioresources and Bioprocessing.* pp. 1-3.

Raffi, M., Hussain, F., Bhatti, T. M., Akhter, J. I., Hameed, A., Hasan, M. M. 2008. Antibacterial Characterization of Silver Nanoparticles against *E: Coli* ATCC-15224. *J. Mater. Sci. Technol.* **24(2):** 192-196.

Rajeshwari, H., Nagaveni, S., Ajay Oli, KelmaniChandrakanth, R. 2010. Multiple antibiotic resistance and ESBL producing *Klebsiellapneumoniae* isolated from clinical urine samples samples. *The Bioscan*. **5(1):** 89-91.

Rajkishore, S. K., Subramanian, K. S., Natarajan, N., Gunasekaran, K. 2013. Nanotoxicity at various trophic levels: A review. *The Bioscan*. **8(3):** 975-982.

Ranjana Chakrabarty, Acharya, G. C. and Sarma,T. C. 2013. Effect of Fungicides, Trichoderma and Plant extracts on mycelial growth of *Thielaviopsisparadoxa*, under *in vitro* condition. *The Bioscan*. **8(1):** 55-58.

Silver, S. and Phung, L. T. 1996. Bacterial heavy metal resistance: new surprises. *Annu. Rev. Microbiol*. **50:** 753-789.

Singhal, G., Bhavesh, R., Kasariya, K., Sharma, A.R., Singh, R.P. 2011. Biosynthesis of silver nanoparticles using *Ocimum sanctum* (Tulsi) leaf extract and screening its antimicrobial activity. *J. Nanopart Res.***13:** 2981-2988.

Stoimenov, P. K., Klinger, R. L., Marchin, G. L. and Klabunde, K.J. 2002. Metal oxide nanoparticles as bactericidal agents. *Langmuir,* **18:** 6679-6686.