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SILVER NANOPARTICLES OF *Azadirachta indica*: BIOLOGICAL METHOD OF SYNTHESIS AND CHARACTERIZATION

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ABSTRACT

Nanotechnology is an emerging interdisciplinary field which mainly concerns with the fabrication of nanoparticles with specific requirements in terms of size, shape and controlled dispersity. Over the last few decades, the synthesis of noble metal nanoparticles have been the subject of many applied researches due to their unique chemical and physical properties. Among the nobel metals, silver nanoparticles are of great scientific interest as they have very unique and promising properties that make them highly suitable for many nanotechnological applications. A number of approaches are available for the synthesis of silver nanoparticles. Of all the methods available for nanoparticle synthesis, bioinspired synthesis of nanoparticles has become a sprouting area that has proven to be better as it offers good manipulation and control over nanoparticle growth and their stabilization. The present investigations is an attempt to synthesis silver nanoparticles from *Azadirachta indica* (neem) leaf extract and characterize them using UV–visible absorption spectroscopy, X-ray diffraction (XRD), Fourier transform spectroscopy (FTIR), Transmission electron microscopy (TEM) & Scanning electron microscopy

(SEM). In UV visible spectroscopy, a strong surface plasmon resonance band was obtained in the range of 430-435 nm indicating the rapid formation of silver nanoparticles. The TEM and SEM images showed that silver nanoparticals are roughly spherical and of uniform particle size, and the average particle size is 100 nm. Nanoparticle XRD patterns revealed three characteristic peaks corresponding to three different facets of silver; (111), (200) and (220). The FTIR spectra reflected distinctive bands which are indicative of bioorganics involving in the reduction of silver ions into silver nanoparticles. In conclusion, it has been proven that *Azadirachta indica* extract is found to be effective in synthesizing silver nanoparticles of desired characteristics to be applied in different fields.

KEYWORDS: *Azadirachta indica*, FTIR, SEM, Silver nanoparticles, TEM and XRD.

INTRODUCTION

Nanotechnology is a field that is blooming in every day, enhancing almost all aspects of human life. This has become one of the most active areas of research in modern material sciences owing to its wide spread applications in different fields such as electronic (Cui, 2001), magnetic (Tuutijarvi et al., 2009), optoelectronic (Tanabe, 2007), information storage (Zhang et al., 2010) and drug delivery (Bhumkar et al., 2007). In recent years, colloidal silver nanoparticles are widely used for its unique chemical and physical properties. The diversity and significance of silver nanoparticles have generated a massive interest in developing versatile and reliable methods to synthesize silver nanoparticles with controlled properties.

Numbers of physical and chemical methods have been reported for the synthesis of AgNPs (Darroudi, et al., 2011, Kilin et al., 2008). However, concern has been raised on the toxicity of chemical agents used in AgNPs synthesis. Hence, it is vital to develop an eco-friendly and cost effective approach for AgNPs synthesis without using toxic chemicals to the human health and environment. Biological systems such as use of plant materials provide an innovative idea for the production of nanoparticles (Bansal et al., 2011).

There is an increasing demand in utilizing the silver nanoparticles of diverse properties as the important component in various products and fields. Many studies have proven that these distinctive properties are mainly influenced by size, shape, interactions with stabilizers, media and methods of synthesis (Bahareh and Hamid, 2015). Therefore, controlled synthesis of nanoparticles with respect to their size, shape and stability is a key challenge to reach their better applied characteristics.

Silver nanoparticles are being used in numerous technologies and incorporated into a vast range of consumer products that take benefits of their desirable optical, conductive, and antibacterial properties. For example, different sized nanoparticles can be used as biological tags in different assays for quantitative detection. As anti-bacterial agents, AgNPs were applied in a vast range of applications in disinfecting medical devices and home appliances to water treatment (Bosetti et al., 2002, Cho et al., 2005, Gupta and Silver, 1998, Jain and Pradeep, 2005 and Li et al., 2008). Moreover, the textile industry has been highly benefitted with the use of AgNPs in different textile fabrics. In this direction, silver nanocomposite fibers were prepared containing silver nanoparticles incorporated inside the fabric (Yeo et al., 2003). The cotton fibers containing AgNPs exhibited high antibacterial activity against *Escherichia coli* (Yeo et al., 2003, Duran et al., 2007).

Hence, present study focused to synthesize silver nanoparticles from *Azadirachta indica* leaf extract and to characterize silver nanoparticles for future applications.

MATERIALS AND METHODS

PREPARATION OF *Azadirachta Indica* (NEEM) LEAF EXTRACT

Fresh leaves of *Azadirachta indica* (neem) free from diseases were collected and washed thoroughly 2-3 times with tap water followed by sterile distilled water. Aqueous extract of neem was prepared by taking 2.5 g of finely cut *Azadirachta indica* leaves in a 500-mL Erlenmeyer flask with 100 mL of sterile distilled water and boiled for 2 min. The mixture was then filtered using whatman filter papers (De Silva et al., 2013).

SYNTHESIS OF SILVER NANOPARTICLES

Aqueous solution of 0.001 M silver nitrate was prepared to synthesize silver nanoparticles. Silver nanoparticle solution was synthesized by mixing *Azadirachta indica* (Neem) leaf extract with 0.001 M silver nitrate at 1:8 ratio (Kumari et al., 2014, Vivehananthan et al., 2015). Mixture was then incubated for 15 hrs at room temperature. AgNPs dispersed in the solution were pelleted down by centrifugation at 12000 rpm for 15 min. Silver nanoparticles were recovered by adding 100% acetone to the pellets and air dried for 2 days.

CHARACTERIZATION OF SILVER NANOPARTICLES

Nanoparticles were characterized by their size, morphology, and surface charge, using techniques such as UV- visible spectroscopy, SEM, TEM, XRD and FTIR.

UV-VISIBLE SPECTROSCOPY

Samples were subjected to optical measurements carried out by UV-vis spectrophotometer between 200 to 800 nm. The UV-visible spectroscopic measurements were performed on a Shimadzu dual-beam spectrophotometer (model U-1800) with a resolution of 1 nm.

SCANNING ELECTRON MICROSCOPY (SEM)

Silver nanoparticles were allowed to dry completely and ground well. Fixation was performed by incubation in a solution of a buffered chemical fixative gluteraldehyde. The dry specimen was mounted on a specimen stub using an adhesive epoxy resin or electrically conductive double-sided adhesive tape and sputter coated with gold palladium alloy before examination in the microscope.

TRANSMISSION ELECTRON MICROSCOPY (TEM) MEASUREMENTS

The 80 kV Ultra High Resolution Transmission Electron Microscope (JEOL model 1200 EX) was used to perform TEM analysis of biosynthesized silver nanoparticles which were prepared for analysis by drop-coating nanoparticles solution on carbon-coated copper TEM grids (400 μm × 40 μm mesh size). Samples were dried and kept under vacuum in desiccators before loading on to a specimen holder.

X- RAY DIFFRACTION (XRD)

The XRD analysis was conducted to determine the structural properties of crystalline silver nanoparticles with an XRD-6000 X-ray diffractometer (Shimadzu, Japan) operated at a voltage of 40 kV and a current of 30 Ma.

FOURIER TRANSFORM SPECTROSCOPY (FTIR)

FTIR analysis of dried powder of nanoparticles was done after bioreduction of silver ions into silver nanoparticles to confirm that bioreduction of $Ag+$ ions to silver nanoparticles. Silver nanoparticles were ground with dry potassium bromide. Powder was filled in a 2 mm internal diameter micro-cup and loaded onto FTIR set at 26^0C + or – 10^0C. The samples were scanned using infra-red in the range of 400-4000 cm^{-1} using FTIR spectrometer.

RESULTS AND DISCUSSION

BIONANOPARTICLES

Transformation of silver ions into silver nanoparticles during exposure to the neem leaf extract was initially followed by color change from pale yellow to dark yellowish brown color in aqueous solution as shown in Fig. 1. This could be attributed to the excitation of surface plasmon vibrations which is typical of silver nanoparticles (Ahmad et al. 2003).

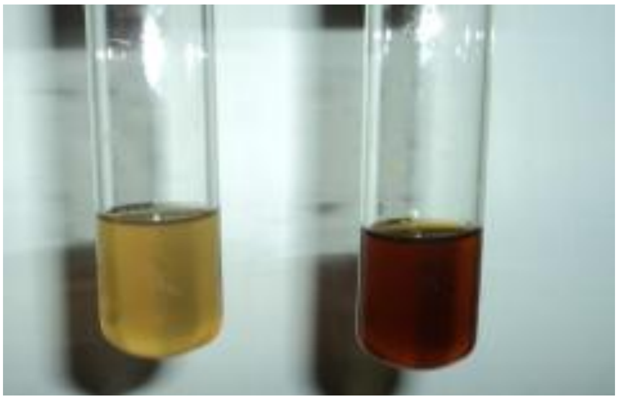

Fig.1. Neem leaf extract before (left) and after (right) addition of 0.001M silver nitrate solution

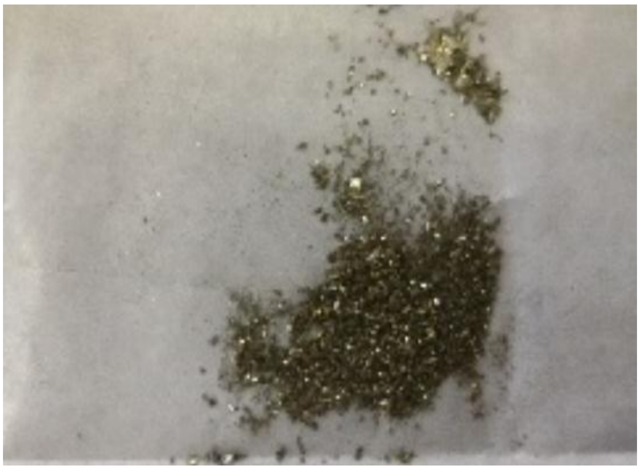

Fig.2. Dried silver nanoparticles (AgNPs)

UV-VIS SPECTRAL ANALYSIS

The results obtained for UV- visible spectrum for the silver nanoparticle prepared from neem leaf extract showed that the maximum absorption was in the range of 430-435 nm as shown in Fig.3. UV absorption spectrum of metal nanoparticles is dominated by the surface plasmon resonance (SPR) which is the collective oscillations of the conduction electrons confined to metallic nanoparticles (Tripathy et al., 2010). In the present investigation, the formation of silver nanoparticles was confirmed using this SPR phenomenon in the UV visible spectroscopy indicating the potential of neem leaf extract in producing silver nanoparticles.

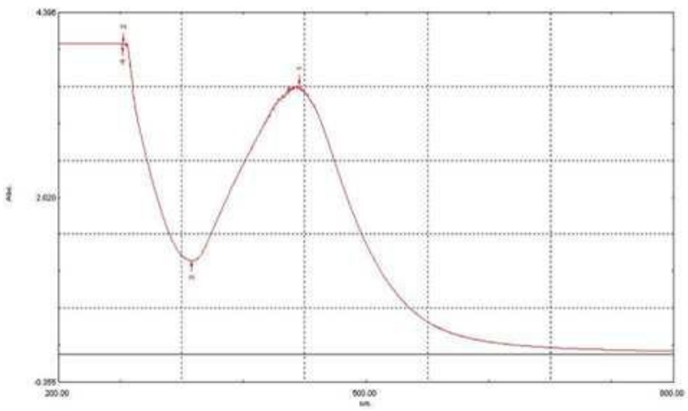

Fig.3. UV-visible spectra recorded from the 0.001 M aqueous silver nitrate 2.5% neem leaf extract reaction

SCANNING ELECTRON MICROSCOPY (SEM) ANALYSIS

SEM image as shown in Fig. 4 indicated that silver nanoparticles are relatively spherical. It was also shown that AgNPs were found to be in the range of 100 nm and appeared to be assembled on to the surface due to the interactions such as hydrogen bond and electrostatic interactions between the bio-organic capping molecules bound to the Ag nanoparticles.

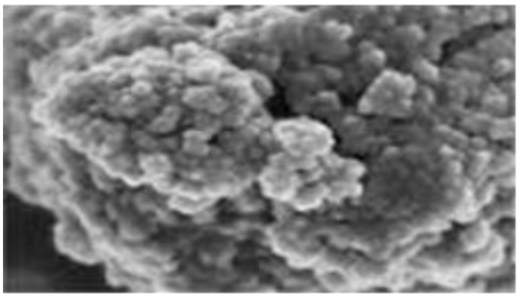

Fig.4. SEM micrograph of biosynthesized nanoparticles

TRANSMISSION ELECTRONE MICROSCOPY (TEM)

TEM micrographs of the synthesized Ag nanoparticles are presented in Fig.5. It was observed that most of the Ag nanoparticles are spherical in shape and uniform in size. The estimated size of the nanoparticles was also proven to be 100 nm.

Fig.5. TEM micrograph of biosynthesized nanoparticles

X-RAY DIFFRACTION (XRD) ANALYSIS

From the Fig. 6, it is noted that three XRD peaks appear at 66°, 26° and 20° due to reflections from (111), (200), and (220) planes of silver respectively. In addition to the peaks, representative of different faces of silver nanocrystals, additional, and unassigned, peak in vicinity of the characteristic peaks of silver (22.5°) were observed. These peaks might have resulted from some bioorganic compounds or protein (s) present in the neem leaf extract as reported in Tripathy et al., 2010.

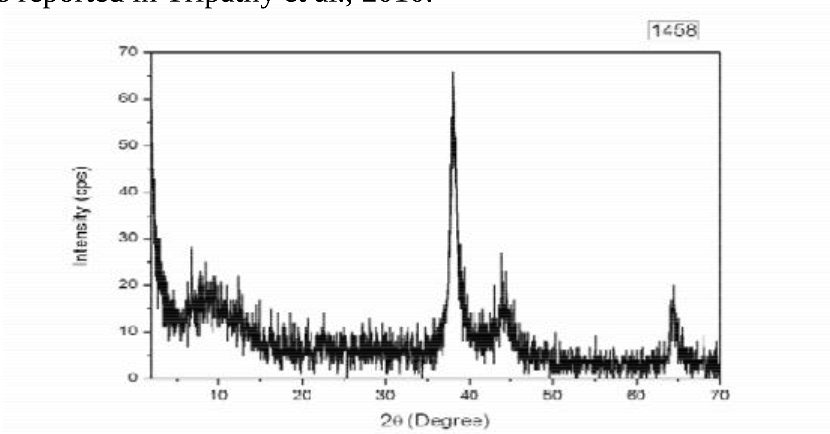

Fig.6. XRD pattern of silver nanoparticles synthesized using neem leaf extract

FOURIER TRANSFORM SPECTROSCOPY (FTIR) OF AgNPs

FTIR measurement was carried out to identify the possible biomolecules responsible for capping and efficient stabilization of Ag nanoparticle from neem leaf extract. FTIR spectrum analysis of silver nanoparticles showed the presence of active functional groups in the synthesized silver nanoparticles as shown in Fig.7. The peaks observed at 3419.52 and 2920.28 correspond to O–H and C–H bond respectively. The assignment at 1642.28 corresponds to C=C group. The peak at 1163, 1113 might be contributed by the acyl and phenyl groups respectively. From the analysis of FTIR studies, it has been proven that carboxyl (-C=O), hydroxyl (-OH) and carbon-hydrogen (C–H) bonds were present and those may mainly involve in fabrication of silver nanoparticles.

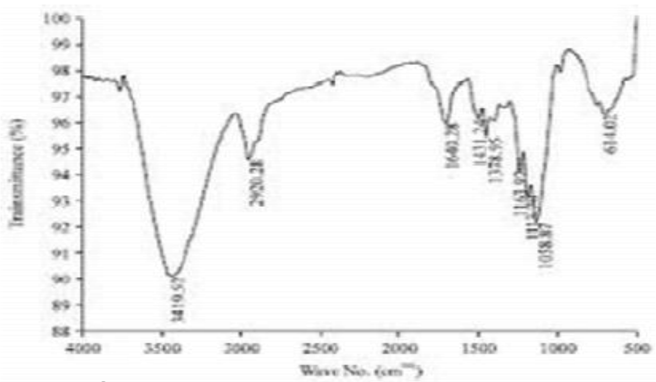

Fig.7. FTIR spectra of silver nanoparticles synthesized by neem leaf extract

CONCLUSIONS

In conclusion, it is shown that silver nanoparticle synthesized from the leaf extract of neem showed a promising results after characterizing by UV-visible spectroscopy, XRD, TEM, SEM and FTIR. Green synthesis of silver nanoparticles was confirmed by colour change which was detected quantitatively by UV visible spectroscopy at 435 nm. Further characterizations with TEM and SEM revealed the spherical AgNPs of particle size 100 nm.

The XRD pattern of AgNPs indicated the presence of the particles of silver nanocrystals. FTIR showed the structure, respective bands of the synthesized nanoparticles and the stretch of bonds. However, the ultimate use of the silver nanoparticles strongly depends on their properties such as shape and size of the particles along with the size distribution. Therefore, further studies could be carried out by investigating the various activities of nanoparticles of the plant extract of *Azadirachta indica*.

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REFERENCES

1. Ahmad A, Mukherjee P, Senapati S, Mandal D, Khan MI, Kumar R, Sastry M. Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. Colloids Surf B Biointerfaces, 2003; 28: 313–318.
2. Bansal V, Ramanathan R, Bhargava SK. Fungus-mediated biological approaches towards green synthesis of oxide nanomaterials. Australian Jornal of Chemistry, 2011; 64: 279-293.
3. Bhumkar DR, Joshi HM, Sastry M, Pokharkar VB. Chitosan reduced gold nanoparticles as novel carriers for transmucosal delivery of insulin. Pharmaceutical Research, 2007; 24: 1415–1426.
4. Bosetti M, Masse A, Tobin E, Cannas M. Silver coated materials for external fixation devices: in vitro biocompatibility and genotoxicity. Biomaterials, 2002; 23 (3): 887.
5. Cho M, Chung H, Choi W, Yoon J. Different inactivation behaviors of MS-2 phage and *Escherichia coli* in TiO_2 photocatalytic disinfection. Applied and

Environmental Microbiology, 2005; 71 (1): 270.

6. Cui Y, Lieber CM. Functional Nanoscale Electronic Devices Assembled Using Silicon Nanowire Building Blocks. Science, 2001; 291: 851-853.
7. Darroudi M, Ahmad MB, Zak AK, Zamiri R, Hakimi M. Fabrication and characterization of gelatin stabilized silver nanoparticles under UV-light. International Journal of Molecular Science, 2011; 12: 6346-6356.
8. De Silva WH, Vivehananthan K, Fernando CAN. Green Synthesis of Silver Nanoparticles using *Azadirachta indica* (Neem) Leaf Extract. Proceeding of 12th Agricultural Research Symposium, 30-31 August, 2013. Wayamba University of Sri Lanka, 2013; 6-10.
9. Duran N, Marcato PD, De Souza GIH, Alves OL, Esposito E. Antibacterial effect of silver nanoparticles produced by fungal process on textile fabrics and their effluent treatment. Journal of Biomedical Nanotechnology, 2007; 3 (2): 203-208.
10. Gupta A, Silver S. Silver as a biocide: Will resistance become a problem?. Nature Biotechnology, 1998; 16 (10): 888.
11. Jain P, Pradeep T. Potential of silver nanoparticle-coated polyurethane foam as an antibacterial water filter. Biotechnology and Bioengineering, 2005; 90 (1): 59-63
12. Khodashenas B, Ghorbani HR. Synthesis of silver nanoparticles with different shapes, Arabian Journal of Chemistry, Forthcoming, 2015.
13. Kilin DS, Prezhdo OV, Xia YN. Shape-controlled synthesis of silver nanoparticles: Ab in vitro study of preferential surface coordination with citric acid. Chemical Physical Letters, 2008; 458: 113-116.
14. Kumari AHVV, Vivehananthan K, Fernando CAN and Srirathan B. Synthesis and Antimicrobial Effects of Silver Nanoparticles of *Azadirachta indica* (Neem) Leaf Extract. Proceedings of the 13th Agricultural Research Symposium, Wayamba University of Sri Lanka, 2014; 205-209.
15. Li Q, Mahendra S, Lyon DY, Brunet L, Liga MV, Li D Alvarez, PJJ. Antimicrobial nanomaterials for water disinfection and microbial control: Potential applications and implications. Water Research, 2008; 42 (18): 4591-4602.
16. Tanabe K. Optical radiation efficiencies of metal nanoparticles for optoelectronic applications. Materials Letters, 2007; 61 (23,24): 4573–4575.
17. Tripathy A, Raichur AM, Chandrasekaran N, Prathna TC, Mukherjee A. Process variables in biomimetic synthesis of silver nanoparticles by aqueous extract of *Azadirachta indica* (Neem) leaves. Journal of Nanoparticle Research, 2010; 2: 237–246

18. Tuutijarvi T, Lu J, Sillanpaa M, Chen G. As (V) adsorption on maghemite nanoparticles. J. Hazard Mater, 2009; 166 (2-3): 1415-20.
19. Vivehananthan K, Kumari AHVV, De Silva WH, Fernando CAN. Synthesis and Evaluation of Antimicrobial Property of Bionanoparticles. Research Journal of Applied Sciences, 2015; 10 (2): 75-80.
20. Yeo SY, Lee HJ, Jeong SH. Preparation of nanocomposite fibers for permanent antibacterial effect. Journal of Materials Science, 2003; 38 (10): 2143-2147.
21. Zhang H, Liu Y, Sun S. Synthesis and Assembly of Magnetic Nanoparticles for Information and Energy Storage Applications. Frontiers in Physics. China, 2010; 5: 347.