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Synthesis of Silver Nanoparticles using Leaf Extract of *Andrographis paniculata*

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ABSTRACT

In the present investigation, an attempt was made to prepare nanoparticle by using a medicinally important plant *Andrographis paniculata*. Because now the biologically synthesized nanoparticle have been widely used in the field of medicine. Silver nitrate (AgNO₃) was used to synthesis the silver nanoparticle by using leaf extract of *Andrographis paniculata*. The synthesized silver nanoparticle from 1 mM AgNO₃ solution through the leaf extract were characterized using UV-Vis Spectrophotometry, XRD, SEM and FTIR. X-ray diffraction and SEM analysis showed the average particle size of 28 nm with cubic and hexagonal shape and it confirmed the formation of nanoparticle in the sample. The synthesized silver nanoparticle can be used for various applications due to its eco-friendliness, non toxic and compatibility for pharmaceutical and other applications.

Key words: Silver nitrate, nanoparticle, biosynthesis, leaf extract, *Andrographis paniculata*

INTRODUCTION

There is growing need to develop eco-friendly and body benign nanoparticle synthesis process without use of toxic chemicals in the synthesis of protocols to avoid adverse effects in biomedical applications (Ankamwar, 2010). Nanometal particles, especially silver, have drawn the attention of researchers (Safaepour *et al.*, 2009). Because of their extensive application in the development of new technologies in the areas of electronics, material sciences and medicine at the nanoscale (Magudapathy *et al.*, 2001). Silver nanoparticles have many applications; for example, they might be used as spectrally selective coatings for solar energy absorption and intercalation material for electrical batteries, as optical receptors, as catalysts in chemical reactions, for biolabelling and as antimicrobials (Joerger *et al.*, 2000; Panacek *et al.*, 2006). Many reports well documented on the biogenesis of silver nanoparticles using several plant extracts. The reducing property of different plant constituents may play a critical role in the reduction of Ag⁺ to silver nanoparticles (Shankar *et al.*, 2004). The use of environmentally benign materials like plant leaf extract (Parashar *et al.*, 2009), bacteria (Saifuddin *et al.*, 2009), fungi (Bhainsa and D'Souza, 2006) and enzymes (Willner *et al.*, 2007) for the synthesis of silver nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis protocol. Chemical synthesis methods lead to presence of some toxic chemical absorbed on the surface that may have adverse effect in the medical applications. Green synthesis provides advancement over chemical and physical method as it is cost

effective, environment friendly, easily scaled up for large scale synthesis and in this method there is no need to use high pressure, energy, temperature and toxic chemicals. Therefore, the objective of this present study was to synthesis the biologically active nanoparticle from the leaf extract of medicinal plant *Andrographis paniculata*.

MATERIAL AND METHODS

Plant materials: The plant *Andrographis paniculata* were collected from the campus of Rajah Serfoji Govt. College in Thanjavur (DT) Tamil Nadu during October to December 2011.

Preparation of the extract: *Andrographis paniculata* leaf were collected and used to prepare the aqueous extract. Leaf weighing 25 g were thoroughly washed in distilled water, dried, cut into fine pieces and were crushed into 100 mL sterile distilled water and filtered through Whatman No.1 filter paper (pore size 25 µm). The filtrate was further filtered through 0.6 µm sized filters.

Synthesis of silver nanoparticles: One millimole aqueous solution of silver nitrate (AgNO₃) was prepared and aqueous extract of leaf of *Andrographis paniculata* used for the synthesis of silver nanoparticles. 10 mL of *Andrographis paniculata* leaf extract was added into 90 mL of aqueous solution of 1 mM silver nitrate for reduction into Ag⁺ ions and kept at room temperature for 5 h.

UV-Vis spectra analysis: The reduction of pure Ag⁺ ions was monitored and measured in the UV-Vis spectrophotometer UV-2450 (Shimadzu).

XRD measurement: The silver nanoparticle solution thus obtained was purified by repeated centrifugation at 5000 rpm for 20 min followed by redispersion of the pellet of silver nanoparticles into 10 mL of deionized water. After freeze drying of the purified silver particles, the structure and composition were analyzed by XRD and SEM. The dried mixture of silver nanoparticles was collected for the determination of size of Ag nanoparticles. Pro X-ray diffract meter operated at a voltage of 40 kV and a current of 30 mA with Cu K α radiation in a θ -2 θ configuration. The crystallite domain size was calculated from the width of the XRD peaks, assuming that they are free from non-uniform strains, using the Scherrer formula:

$$D = \frac{0.94\lambda}{\beta \cos\theta}$$

where, D is the average crystallite domain size perpendicular to the reflecting planes, λ is the X-ray wavelength, β is the Full Width at Half Maximum (FWHM) and θ is the diffraction angle.

Scanning electron microscopic (SEM) analysis of silver nanoparticles: SEM analysis was done using Hitachi S-4500 SEM machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

FTIR analysis of dried biomass after bioreduction: The residual solution of 100 mL after reaction was centrifuged at 5000 rpm for 10 min and the resulting suspension was redispersed in 10 mL sterile distilled water. The centrifugation and redispersing process was repeated three times.

Thereafter, the purified suspension was freeze dried to obtain dried powder. Finally, the dried nanoparticles were analyzed by FTIR, Nicolet Avatar 660 (CECRI, Karaikudi).

RESULTS

The synthesized nanoparticles using *Andrographis paniculata* leaf extract exhibited a reddish brown color in aqueous solution due to excitation of surface plasmon vibrations. While the leaf extract of *Andrographis paniculata* mixing with the aqueous solution of the silver ion complex, it started to change the color from watery to yellowish brown due to reduction of silver ion (Fig. 1). The absorption spectrum of aqueous silver nitrate only solution exhibited maximum at about 220 nm and there was no color change. The synthesized AgNO₃ nanoparticle were detected by UV-Vis spectroscopy at various nm 200, 300, 400, 500 and 600 nm, the particle has increasingly sharp absorbance maximum peak at 280 nm and gradually decreased while nanometer increased (Fig. 2). *Andrographis paniculata* was found to exhibit very strong absorption peaks at 400 to 500 nm. Absorption spectra of Ag nanoparticles formed on the reaction mixture at different time intervals at 280 nm. The particle has increasing sharp between 1 to 5 h the gradually decreased while the time has increased. The UV-Vis spectra recorded from the reaction medium after 5 h, the light absorption was more due to presence of nanoparticle formed in the reaction media and were showed strong absorbance peaks at 280 nm (Fig. 2). Broadening of peak indicated that the particles are poly dispersed. The biosynthesized silver nanostructure by employing *Andrographis paniculata* leaf extract was further demonstrated and confirmed by the characteristic peaks observed in the XRD image (Fig. 3) and the structural view were observed under the scanning electron microscope (Fig. 4). The XRD pattern showed three intense peaks in the whole spectrum of 2θ values ranging from 10 to 80. The typical XRD pattern (Fig. 3) revealed that the sample contains a mixed phase (cubic and hexagonal) structures of silver nanoparticles (Fig. 4). The average estimated particle size of this sample was 28 nm derived from the FWHM of peak (Fig. 3). The SEM image showed the high density silver nanoparticles synthesized by the *A. paniculata* development of silver nanostructures. FTIR analysis was used for the



Fig. 1: Synthesis of nanoparticle using silver nitrate and leaf extract of *Andrographis paniculata*
a: Leaf extract, b: 1 mM AgNO₃ without leaf extract and c: 1 mM AgNO₃ with 10% leaf extract after 5 h

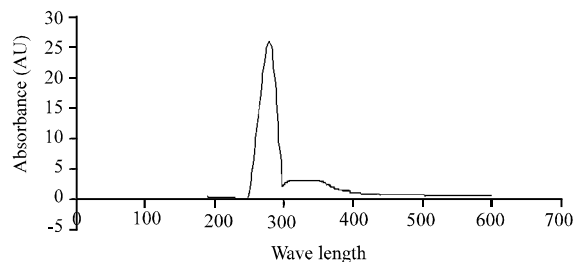


Fig. 2: UV-Vis absorption spectrum of leaf extract synthesized by treating 1 mM aqueous AgNO_3 solution with 10% *Andrographis paniculata* leaf extract after 5 h

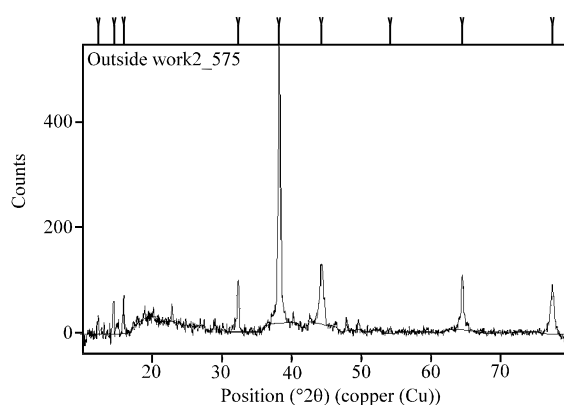


Fig. 3: XRD pattern of silver nanoparticles synthesized by treating 10% *Andrographis paniculata* with 1 mM aqueous AgNO_3 solution

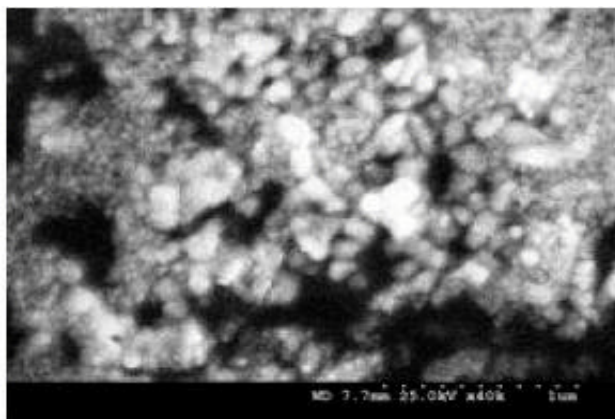


Fig. 4: SEM micrograph of silver nanoparticles

characterization of nanoparticles. FTIR absorption spectra of water soluble extract before and after reduction of Ag ions are shown in Fig. 5. Absorbance bands in Fig. 5a (before bioreduction) are observed in the region of $500\text{-}3500\text{ cm}^{-1}$ are 3354.04, 2925.22, 2858.42, 1650.24, 1541.04, 1450.97,

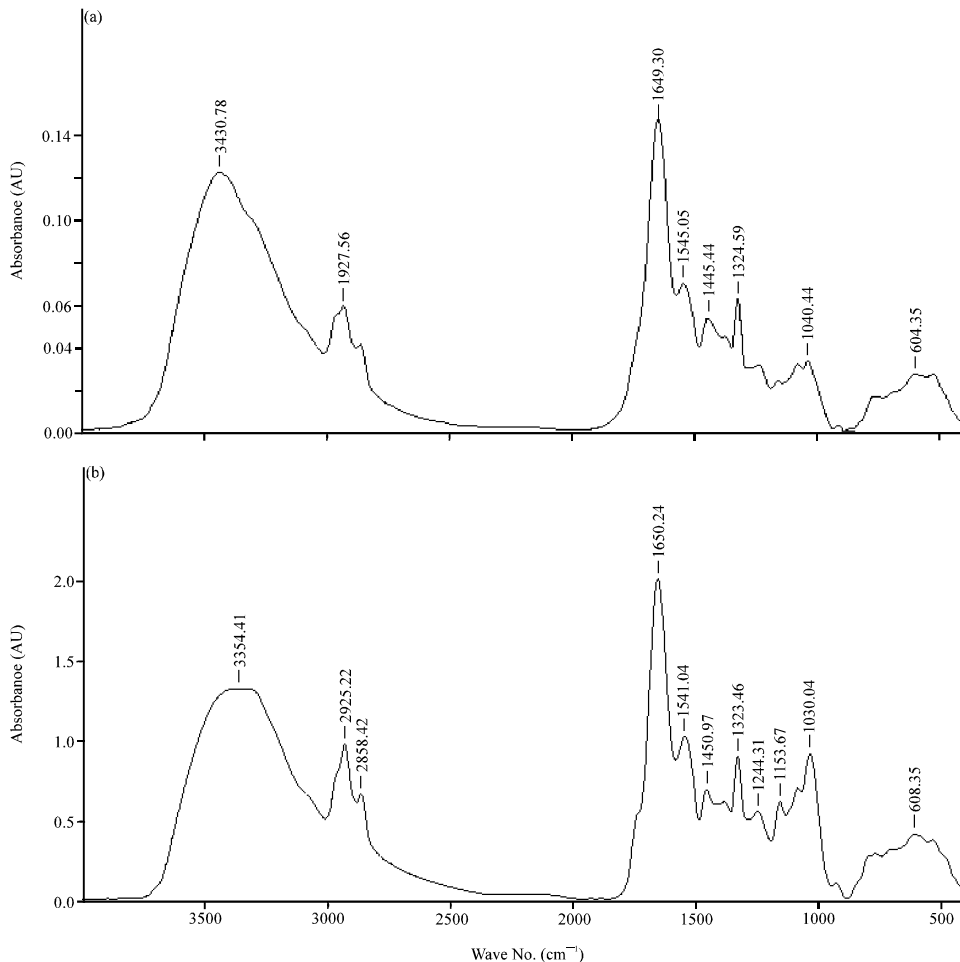


Fig. 5(a-b): FTIR graph of (a) Leaf extract and (b) Nanoparticles synthesized in 10% leaf extract solution

1323.46, 1244.31, 1153.67, 1030.04, 608.35 cm^{-1} . These absorbance bands are known to be associated with the stretching vibrations for -C C-C O, -C C- [(in-ring) aromatic], -C-C- [(in-ring) aromatic], C-O (esters, ethers) and C-O (polyols), respectively. In particular, the 1244 cm^{-1} band arises most probably from the C-O group of polyols such as hydroxyflavones and catechins. The total disappearance of this band after the bioreduction (Fig. 5b).

DISCUSSION

Silver nanoparticle was synthesized from the leaf of *Andrographis paniculata*. Rao and Savithramma (2011) revealed the synthesis nanoparticles from leaf of *Svensonia hyderabadensis* and leaves of *Allium cepa* by Saxena *et al.* (2010) and *Clerodendrum inerme* by Farooqui *et al.* (2010) and in *Argemone mexicana* (Khandelwal *et al.*, 2010). Leaf extract of *Andrographis paniculata* while mixing with the aqueous solution of the silver ion complex, it started to change the color from watery to yellowish brown. Which indicated the formation of silver nanoparticles and this might be due to the reduction of silver ion to form the nanoparticles. Similarly, Elumalai *et al.* (2010) observed that the reduction of silver ion in Ag nitrate exposed plant extract followed by color

change and Rao and Savithamma (2011) also reported that the *Svensonia hyderabadensis* solution of the silver ion complex started to change the colour from yellow to dark brown due to reduction of silver ions. Silver nanoparticles exhibited yellowish brown colour in aqueous solution due to excitation of surface plasmon vibrations (Shankar *et al.*, 2004). Chen *et al.* (2002) observed the intensity of color development in the reaction mixture of different plants such as in *Helianthus annuus* (sunflower), *Basella alba* (spinach) and *Saccharum officinarum* (sugarcane). Further, who stated that the synthesized AgNO_3 nanoparticle were detected by UV-Vis spectroscopy at various nanometers and the particle has increasingly sharp absorbance and the peak was maximum at 280 nm and then it was gradually decreased while nanometer increased. *Andrographis paniculata* was also found to exhibit very strong absorption peaks at 400 to 500 nm. and the absorption spectrum of aqueous silver nitrate only solution exhibited maximum at about 220 nm and there was no color change. Rao and Savithamma (2011) reported that the UV-Vis spectrophotometric measurements were showed strong absorption peak at 300 to 400 nm.

Chen *et al.* (2002) suggested that the difference in the morphology of the synthesized nanoparticle might be a reason for the variations in the absorption peaks.

The biosynthesized silver nanostructure by employing *Andrographis paniculata* leaf extract was further demonstrated and confirmed by the characteristic peaks observed in the XRD image and the structural view were observed under the scanning electron microscope. The average estimated particle size of this sample was 28 nm derived from the FWHM of peak. The SEM image showed the high density silver nanoparticles synthesized by the *A. paniculata* development of silver nanostructures. FTIR analysis was used for the characterization of the extract and the resulting nanoparticles. FTIR absorption spectra of water soluble extract before and After reduction of Ag ions. Absorbance bands are observed in the region of $500\text{-}3500\text{ cm}^{-1}$. Saifuddin *et al.* (2009) observed the FTIR measurements to identify the possible biomolecules responsible for capping and efficient stabilization of the metal nanoparticles synthesized in leaf broth. The beaks near 3354.04 , 2925.22 and 2858.42 cm^{-1} assigned to OH stretching and aldehydic C-H stretching, respectively (Jain *et al.*, 2009). The total disappearance of this band after the bioreduction may be due to the fact that the polyols are mainly responsible for the reduction of Ag ions, where by they themselves get oxidized to unsaturated carbonyl groups leading to a broad peak at 1650.24 cm^{-1} . The weaker band at 1244.31 cm^{-1} corresponds to amide I arising due to carbonyl stretch in proteins (Sathyavathi *et al.*, 2010). The result of all earlier research strongly support the need of preparation of silver nanoparticle from the medicinally important medicinal plants for various medicinal uses.

CONCLUSION

Synthesis of silver nanoparticle from the leaf of *Andrographis paniculata* was confirmed by the colour changes from yellow to dark brown. Which indicated the formation of silver nanoparticles. Therefore, the growing need of developing a eco-friendly nanoparticle synthesis is possible and it can be used for various biomedical applications to avoided the adverse effects chemically synthesized nanoparticle in the filed of medical applications.

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