Asian Journal of Chemistry; Vol. 26, No. 7 (2014), 0000-0000

ASIAN JOURNAL OF CHEMISTRY

Green Synthesis of Nanosilver Particles from Extract of *Eucalyptus citriodora* and Their Characterization

MUHAMMAD ZAHID QURESHI[1], TABASSUM BASHIR[1,*], SHAZIA KHURSHEED[1], FARAZ AHMAD[1], MUHAMMAD AYUB[2], ALAN REYNOLDS[3] and GHULAM HUSSAIN[1]

[1]Department of Chemistry, Government College University, Lahore, Pakistan
[2]Department of Microbiology, Hazara University, Mansehra, Pakistan
[3]Experimental Techniques Center, Brunel University London, UK

*Corresponding author: Tel: +92 333- 4391934; E-mail: tabassumcaa@gmail.com

(*Received*: ; *Accepted*:) AJC-0000

The primary motivation for the study to develop simple eco-friendly green synthesis of silver nanoparticles using leaf extract of *E. citriodora* as reducing and capping agent. The green synthesis process was quite fast and silver nanoparticles were formed within 0.5 h. The synthesis of silver nanoparticles was investigated by UV-visible spectroscopy, X-ray diffraction, SEM and FTIR. The developed nanoparticles demonstrated that *E. citriodora* is good source of reducing agents. UV-visible absorption spectra of the reaction medium containing silver nanoparticles showed maximum absorbance at 460 nm. FTIR analysis confirmed reduction of Ag^+ to Ag^0 atom in silver nanoparticles. The XRD pattern revealed the crystalline structure of SNPs. The SEM analysis showed the size and shape of the nanoparticles. The environmental friendly method provides simple, easy and cost effective faster synthesis of nanoparticles than chemical methods and can be used in several areas such as food, medicine and medical application, *etc*.

Key Words: Silver nanoparticles, *Eucalyptus citriodora*, Capping agents, Scanning electron microscopy, Nanotechnology.

INTRODUCTION

Nanoparticles are being viewed as fundamental building blocks of nanotechnology. The optical electrical, magnetic and catalytic properties of metal nanoparticles have been intensively studied during the last two decades because of their unique properties[1]. The development of biologically inspired experimental process for synthesis nanoparticles is evolving into an important branch of nanotechnology[2,3]. Biogenic synthesis is useful not only because of its reduced environmental impact[3,4] compared with some of the physiochemical production methods, but also because it can be used to produce large quantities of nanoparticles that are free of contamination and have a well define size and morphology[5]. Biosynthesis routes can actually provide nanoparticles of a better defined size and morphology than some of the physiochemical methods of production[6]. The antibacterial activities of silver nanoparticles are related to their size, with the smaller particles having higher activities on the basis of equivalent silver mass content[7]. Concerning the biological application of nanoparticles. It has been emphasized that methods of synthesis through biological system. There are different plant extracts have been used and reported for synthesis of gold, silver and biometallic nanoparticles[7].

In the present study, *Eucalyptus citriodora* was used for source of reducing agent. The plant is easily available in all the regions in Pakistan. *Eucalyptus* extract show various biological effects, such as antibacterial, antifungal, antihyperglycemic and antioxidant activities[8]. There are more than 500 *Eucalyptus* species, ranging from shrubs to several hundred-foot trees. *Eucalyptus* leaves and oil are utilized for medicinal and other uses, such as fragrance in perfumes. Volatile oils are derived principally from species that are rich in 1,8-cineol (eucalyptol, α-monoterpene), such as *Eucalyptus globulus* Labillardiere (blue gum), *E. smithii*, or *E. fructicetorum*. *E. globules* Labillardiere is the most common medicinal species[9].

EXPERIMENTAL

Preparation of plant extract: 10 g of fresh leaves of *E. citridora* were washed thoroughly with double-distilled water and were then cut into small pieces. These finely cut pieces were then mixed with 100 mL doubled distilled water and this mixture was kept for boiling for a period of 15 min. After cooling, it was filtered through Whatman Filter paper No. 1. Filtrate placed at 4 °C for further experiment.

Synthesis of silver nanoparticles: 1 mM aqueous solution of silver nitrate ($AgNO_3$) were prepared and used for the synthesis of silver nanoparticles. 10 mL of extract were taken and 100 mL of $AgNO_3$ solution was added to it. The colour change from pale green to dark brown due to surface plasmon resonance. This occurs due to the collective oscillation of the conduction electrons confined to metallic nanoparticles. They were incubated at room temperature for 24 h. The colourchange indicate the synthesis of silver nanoparticles. UV-visible spectra showed strong SPR band at 460 nm and thus indicating the formation of silver nanoparticles The silver nanoparticles (AgNPs) obtained by *E. citriodora* leaves extract were centrifuged at 13,000 rpm for 25 min and subsequently dispersed in sterile distilled water to get rid of any uncoordinated biological materials.

Analysis of bioreduced silver nanoparticles

UV-Visible spectroscopy:UV-Visible spectroscopic analysis was carried out on Shimadzu UV 1700. Cuvette of path length 10 mm was used. The measurements were carried out as a function of reaction time at room temperature.

X-ray diffractometry: XRD measurements were recorded on PANalyticalX'Pert PRO X-ray diffractometer. For XRD measurements, the AgNPs were dried in oven at 60 °C and such dried powder was further analyzed on XRD for their phase structure and exact material identification. The CuK_α radiation (λ = 1.582 Å) was selected and the diffractogram was obtained in the 2θ range of 20-80°.

Fourier transform infrared (FTIR) spectroscopy: The binding properties of AgNPs synthesized by *E. citriodora* leaf extract were investigated by FTIR analysis. FTIR measurements were taken on MIDAC 2000M series. Dried and powdered AgNPs were palleted with potassium bromide (KBr) (1:10 proportion). The spectra were recorded in the wavenumber range of 4000-450 cm^{-1} and analyzed by subtracting the spectrum of pure KBr.

SEM analysis: Scanning electron microscopic (SEM) analysis was done using JSM-6480 SEM machine. Thin films of synthesized and stabilized silver nanoparticles were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid and sample was analyzed for morphology and size of the silver nanoparticles.

RESULTS AND DISCUSSION

U.V-Visiblespectroscopy: The formation of AgNPs was observed upon the colour change of the leaf extract of *Eucalyptus citriodora* from transparent yellow into brown, as shown in Fig. 1, due to the coherentoscillation of electrons at the surface of nanoparticles, resulting insurface plasmon resonance[9]. The colour change into brown was noted within 20 min and the colour intensity increased significantly with increasing the $AgNO_3$ concentration at a fixed volume of leaf extract of *E. citriodora*. The UV-visible spectrophotometry was also used to confirm the formation of the AgNPs as shown in Fig. 1. From Fig. 1, the SPR band steadily increased in intensity with a prominent peak at about 460 nm at 1 mM concentration. The change of colour and intensity of the SPR band might be due to the variation in concentration, size and shape of the resulting AgNPs[11].

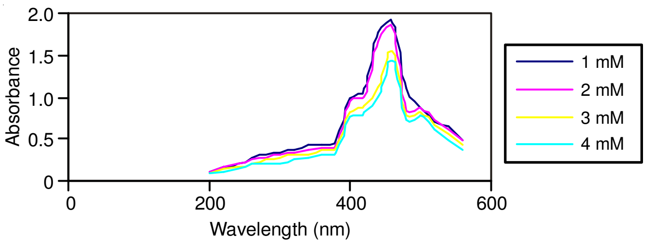

Fig. 1. UV-Visible spectroscopy of silver nanoparticles

XRD: The results of the XRD analysis showed 2θ intense values with various degree (31.769°, 37.605°, 43.83°, 64.07° and 77.20°) these results are corresponds to (101), (111), (200), (220) and (311) Bragg's reflection based silver nanoparticles[12] (Fig. 2).

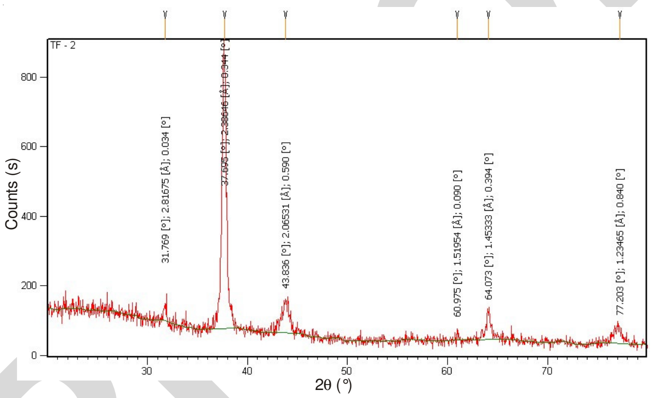

Fig. 2. XRD pattern of silver nanoparticle synthesis by leaf extract of *E. citriodora*

FTIR: The results of the FTIR used to identify the possible bio molecules responsible for the stabilization of the synthesized silver nanoparticles. The prominent peaks of the FTIR results are showing the correspond values to the alcohol, phenol group (O-H stretching-3424), amides group (N-H stretching-3357), carboxylic group (O-H stretch-3280) alkenes, aromatics (C-H 3094), alkanes (C-H 2884), aliphatic saturated aldehydes (C=O 1729), unsaturated aldehyde (C=O 1667) and aromatic (C-C 1586). The observed peaks are considered as major functional groups in different chemical classes such as triterpenoids, flavonoids and polyphenols[13]. Hence, the terpenoids are proved to have good potential activity to convert the aldehyde groups to carboxylic acids in the metal ions. Further, amide groups are also responsible for the presence of the enzymes and these enzymes are responsible for the reduction synthesis and stabilization of the metal ions, further, polyphenols are also proved to have potential reducing agent in the synthesis of the silver nanoparticles[13-15].

SEM: According to SEM analysis the silver nanoparticles were spherical in shape with varied particle size in nm. The larger silver particles may be due to the aggregation of the smaller ones (Fig. 3).

Conclusion

The present study demonstrated the extracellular biosynthesis of an isotropic silver nanoparticles using the leaf extract of *E. citriodora*. We found that the leaves of *E. citriodora* can be a good source of synthesis of silver nanoparticles. The

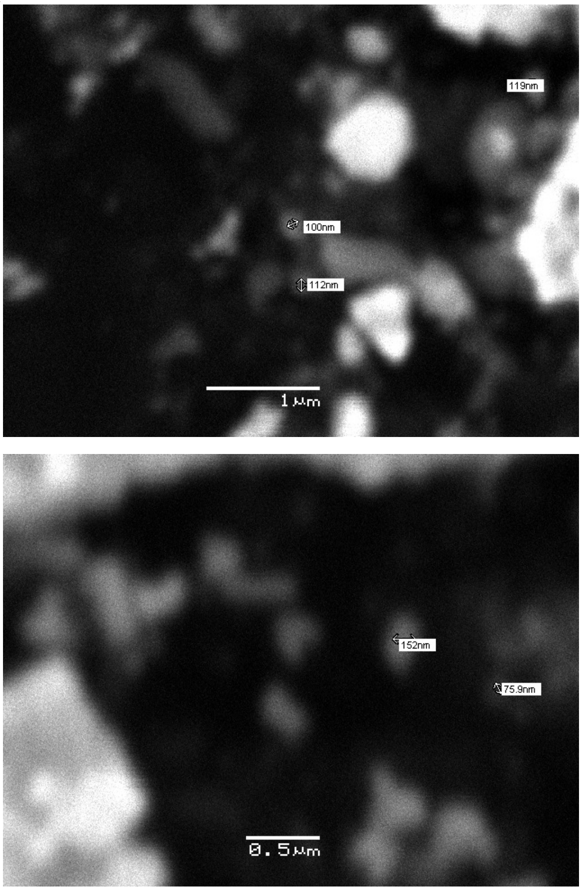

Fig. 3. SEM Micrograph of silver nanoparticles from *E. citriodora*

formation of AgNPs was well studied. The AgNPs characterization and morphology was studied with UV-Visible spectroscopy, XRD and SEM techniques. The FTIR examination of the samples confirms the involvement of enzymes and amino groups in the reduction and stabilization of the AgNPs. This procedure is easy, cost-effective, energy saving and environment friendly. It can scaled up for large scale synthesis of silver nano-particles.

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