Research Article

A green approach in the synthesis of silver nanoparticles using bark of *Eucalyptus globulus,* Labill

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ABSTRACT

The present study is aimed to synthesize silver nanoparticles through a green approach using the bark of *Eucalyptus globulus,* Labill (Family: *Myrtaceae*). The bark broth was prepared and resuspended in aqueous solution of silver nitrate and it is known as reaction medium. This reaction medium was kept in an incubator cum shaker with 250rpm at 27°C for 24 hours to reduce the silver nitrate in to silver nanoparticles. The colour change in the reaction medium (Pale yellow to dark brown colour) was observed during the incubation period. It indicates the synthesis of silver nanoparticles. The reaction medium was analyzed using UV-Visible Spectroscopy, Fourier Transform Infra-Red (FT-IR) Spectroscopy, X-ray diffraction (XRD) analysis, Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray (EDX) analyses and their results explain the synthesis and characterization of silver nanoparticles. Transmission Electron Microscopy (TEM) elucidates the size and shape of the synthesized silver nanoparticles. The size of the silver nanoparticles is varying between 05 and 50nm with the mean 30.5 ± 2.5nm. This type of green approach using the plant parts in the synthesis of silver nanoparticles appears to be cost effective, eco-friendly and easy alternative to physical and chemical methods which does not provoke toxic chemicals in the synthesis protocol.

Keywords: Bark broth, Bioreduction, *Eucalyptus globulus,* Reaction medium, Silver nanoparticles.

INTRODUCTION

Nanotechnology gains much attention among the researchers due to the specific characteristic properties (size, distribution and morphology) of nanomaterials.[1] The use of nanoparticles or nano structured materials is extensively studied.[2-9] Plants are used to synthesize metal nanoparticles such as silver[10], gold[11] and Palladium[12] extracelluraly. Biologically active compounds present in the plant extracts such as proteins, polysaccharides and vitamins play a major role in the reduction of silver nitrate into silver nanoparticles.[13]

The synthesis of metal nanoparticles using physical and chemical methods provokes toxic effects to human and the environment.[14] The synthesis of silver nanoparticles has been achieved using bacteria[15], fungi[16], Sea weed, *Kappaphycus alvarezii*[17] *etc*., Several reports explain the synthesis of silver nanoparticles using the leaves of higher plants such as *Helianthus annus*, *Basella alba*, *Oryza sativa, Saccharum officinarum*, *Sorghum bicolor* and *Zea mays*[18], *Citrullus colocynthis*[19], *Camellia sinensis*[20], *Chenopodium album*[21], *Enicostemma littorale* and *Rauvolfia tetraphylla*[22], *Capsicum annuum*[23], *Cinnamomum camphora*[24], *Gliricidia sepium*[25], *Carrica papaya*[26], *Murraya koenigii*[27], *Arbutus unedo*[28], *etc.* Thus, there is a growing need to develop a green protocol using the leaves of higher plants for the synthesis of silver nanoparticles which does not produce any toxic effect and that can suitably scaled up for a large scale synthesis. Hence, the present study is aimed to synthesize the silver nanoparticles in an eco-friendly manner using the barks of *Eucalyptus globules,* and characterize them in terms of their size, shape and distribution.

MATERIALS AND METHODS

All the reagents used in the present study were obtained from Himedia Laboratories Pvt.Ltd. (Mumbai, India). The barks of *Eucalyptus globulus,* Labill were collected in the Botanical Garden of Ayya Nadar Janaki Ammal College campus, Sivakasi. The collected bark samples were thoroughly washed with running tap water followed by distilled water to remove the surface contaminants. The bark samples (10g) ground to make fine powder and suspended in 100ml of distilled water at 90°C for 10 minutes to prepare the bark broth. 10ml of freshly prepared bark broth was re-suspended in 190ml of aqueous solution of silver nitrate and this mixture was used as reaction medium.[29] This reaction medium was kept in an Incubator cum shaker (ORBITEK) with 250 rpm at 30°C for 24 hours. From this reaction medium, a small aliquot of the sample was used to characterize the silver nanoparticles synthesized during the above reaction. The characterization of synthesized silver nanoparticles was performed through the following analyses: UV-Visible spectroscopy, Spectrofluorimeter, Fourier Transform Infra-Red (FT-IR) Spectroscopy, X-ray diffraction (XRD) analysis, Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray (EDX) analyses explain the synthesis of silver nanoparticles. Transmission Electron Microscopy (TEM) analyses.

RESULTS AND DISCUSSION

UV-Visible Spectrum of silver nanoparticles

The aqueous silver nitrate was reduced into silver nanoparticles due to the exposure of bark broth of *Eucalyptus globulus*. The bark broth was pale yellow in colour before the addition of aqueous silver nitrate. After the exposure of the bark broth to aqueous silver nitrate, it started to turn brown colour within five minutes and became dark brown within 24 hrs of incubation period (Figure 1 inset). It indicates the reduction of silver nitrate into silver nanoparticles. The time taken for the colour change varies from the substrate to substrate. For instance, twelve hours and three hours periods were taken in case of leaf broths of *Turnera ulmifolia*[30] and *Eucalyptus* hybrida[31] respectively. In the present study the SPR vibrations observed between 300 to 600 nm. The absorbance of the reaction medium was noted in the intervals of 5min, 30min, 1, 2, 4 and 24 h. They showed the λ_{max} at 440 nm with the raise in absorbance up to 0.85.a.u. (Figure 1). Similarly the silver nanoparticles synthesized by *Glycyrrhiza glabra* root extract had the SPR bands that are broad with λ_{max} at 440 nm and the raise in absorbance which explains the increased production of silver nanoparticles.[32] In extracellular biosynthesis of silver nanoparticles using a mycorrhizal mushroom, *Tricholoma crassum* had the SPR bands with the λ_{max} at 440 nm.[33]

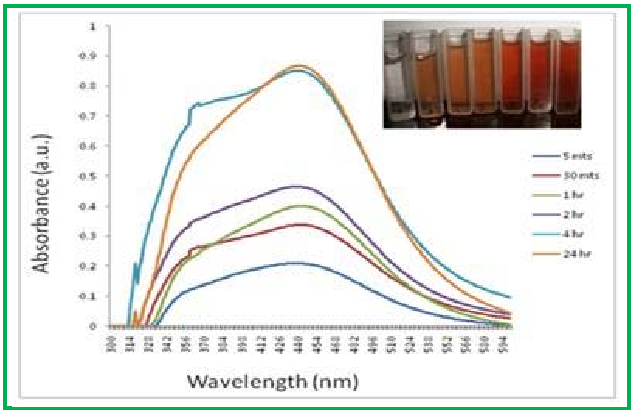

Figure 1: UV –Visible absorption spectra of silver nanoparticles synthesized by bark broth of *Eucalyptus globulus,* Labill. The inset shows the colour change of the reaction medium (left to right). A-Control; B-5 minutes; C-30 minutes; D-1 hr; E-2 hr; F-4 hr; G-24hr.

Spectrofluorimetric analysis

Figure 2 shows the respective emission and excitation spectra of silver nanoparticles synthesized using bark broth of *Eucalyptus globulus*. The excitation peak was formed at 430 nm while the emission peak was observed at 450 nm in silver nanoparticles synthesized using bark broth. The excitation peak at 430 nm and emission peak at 450 nm well correlate with the absorption maxima (λ max) recorded with UV-Vis spectrum. In the spectrofluorimetric analysis of silver nanoparticles synthesized using *Nicotiana tabacum*, Prasad *et al*.[34] noticed excitation peak at 414nm and emission peak at 576nm. The quantum yield obtained for the reaction medium with *Eucalyptus globulus* bark is 1.0.

FTIR Spectroscopic analysis

Figure 3 shows the FTIR Spectrum of silver nanoparticles synthesized using the bark broth of *Eucalyptus globulus*. FTIR bands observed are 605, 653, 752, 806, 1112, 1195, 1336, 1398, 1452, 1593, 1668, 2108, 2268, 2682, 2883, 2975, 3193 and 3313cm^{-1}. The bands at 1593 cm^{-1} and 1398- 827 cm^{-1} correspond to N-H bond of primary and secondary amides and –C-N-stretching vibration of amides or –C-O- stretching of alcohols, ether, carboxylic acids and anhydrides.[35] The peaks at 1397 cm^{-1} and 652.93 cm^{-1} indicate the presence of alkyl halide groups. The broad absorption peak at 1114 cm^{-1} indicates the presence of alcoholic groups.[36] The band at 1452 cm^{-1} corresponds to deprotenated carboxylic acid group. The barks of *E. globulus* contain simple phenolics (such as gallic acid, ellagic acid, protocatechuic acid) and their derivatives[37], flavonoids[38] and more complex polyphenolic compounds such as proanthocyanidins (condensed tannins).[39] These compounds which are present in the bark broth may be responsible for the reduction of silver nitrate into silver nanoparticles.

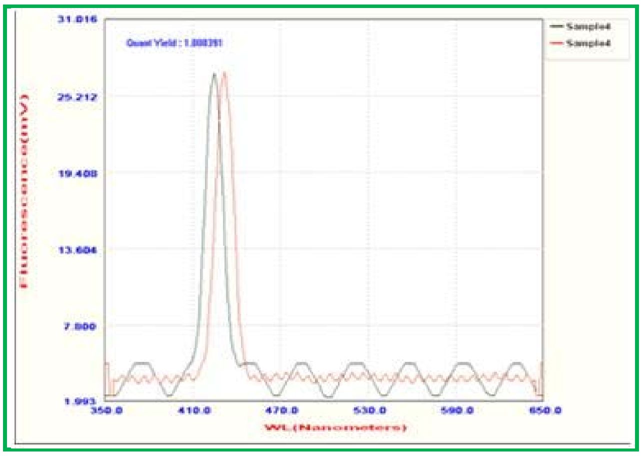

Figure 2: Spectrofluorimetric analysis of silver nanoparticles synthesized by bark broth of Eucalyptus *globulus,* Labill.

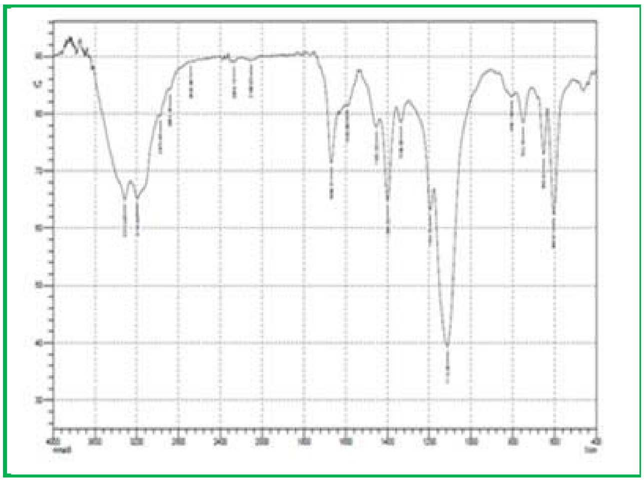

Figure 3: FTIR spectrum of synthesized silver nanoparticles using bark aqueous broth with silver nitrate of *Eucalyptus globulus,* Labill.

XRD analysis

Figure 4 shows the XRD pattern of silver nanoparticles synthesized using *Eucalyptus globules* bark broth. Studies on XRD pattern revealed four intense peaks of 2θ values

27.7°(226), 32.1°(264), 38.0° (111) and 44.2° (220). A comparison of our XRD spectrum with the standard one, confirmed that the obtained silver nanoparticles were in the form of nano crystals and face centered cubic structures.[40,41] The mean size of silver nanoparticles were calculated using the Scherrer formula $D=0.94\lambda/\beta\cos\theta$, 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.[42,43] The average size of silver nanoparticles obtained is 30.5nm.

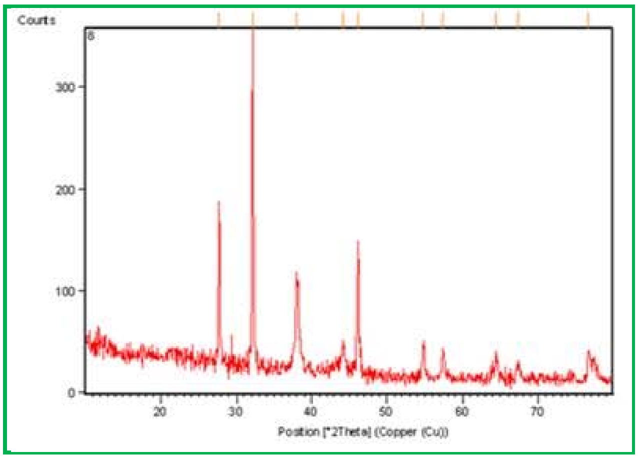

Figure 4: XRD pattern of silver nanoparticles formed after reaction of bark broth of *Eucalyptus globulus,* Labill.

SEM and EDX analysis

Figure 5 shows the SEM image of silver nanoparticles at 20,000X magnification. The SEM image shows the silver nanoparticles with different shapes such as relatively spherical, hexagonal and cubic with a diameter range of 30-50 nm. The spherical shaped silver nanoparticles were obtained with the size ranged from 40 to 50nm using the leaf extract of *Euphorbia hirta.*[44] The silver nanoparticles synthesized using *Datura metel* were spherical shaped with a diameter ranged from 20 to 35 nm.[45] Similar results were obtained in the synthesis of silver nanoparticles using the leaf broth of *Merremia tridentata.*[46] Figure 6 shows EDX spectrum of silver nanoparticles synthesized using *Eucalyptus globulus,* bark broth. A strong silver signal obtained in the EDX spectrum (65.11%) assures the significant presence of elemental silver. The EDX Spectrum of Ag along with week signals of O, Mg, Al, Si, Cl, and K as the mixed components in the reaction medium. The feeble signal may possibly due to the biomolecules that are bound to the surface of silver nanoparticles.[7] The C and K signals were likely due to X-ray emission from carbohydrates/proteins and enzymes that are present in the cell wall of the biomass.[47]

TEM analysis

TEM analysis reveals the size and shape of biosynthesized silver nanoparticles (Figure 7a). The silver nanoparticles obtained are well structured with almost spherical in shape. Some of them are hexagonal and cubic in nature. Figure 7b shows the distribution pattern of silver nanoparticles which is varying between 05 and 50 nm with the mean 30.5 ± 2.5nm. Krishnaraj *et al.*[48] synthesized silver nanoparticles with the size ranged between 20 and 30nm using *Acalypha indica.* The spherical shaped nanoparticles of size 05 - 20nm were obtained using *Sesuvium portulacastrum.*[49] In Soap nuts the formation of polydispersed nanoparticles of size 06-35nm and the average size of the nanoparticles was found to be 18 nm.[50]

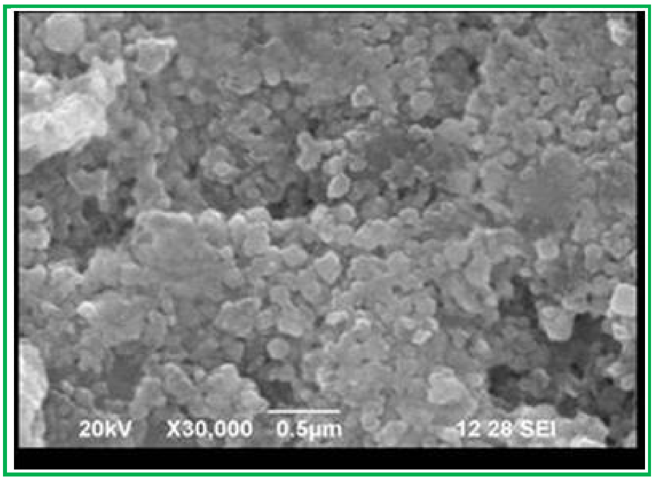

Figure 5: SEM images of silver nanoparticles synthesized from the *Eucalyptus globulus,* Labill bark broth.

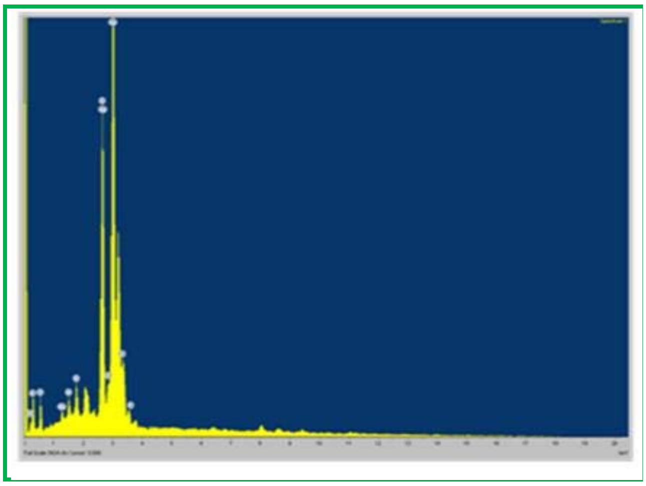

Figure 6: EDX images of silver nanoparticles synthesized from the *Eucalyptus globulus,* Labill

CONCLUSION

We successfully synthesized silver nanoparticles with different sizes and shapes through an environmentally friendly manner using *Eucalyptus globulus* bark broth. Interestingly, the reaction medium changed its colour from pale yellow to dark brown within 24 h. It indicates the formation of silver nanoparticles in the reaction medium. FTIR analysis shows the stretching vibrations of amides and – C- O- stretching vibrations of alhocols, ether, and carboxlic acid *etc*.

The biomolecules present in the bark broth may reduce silver ions into silver nanoparticles. Further, XRD, SEM, EDX and TEM analyses confirmed the significant presence of elemental silver with an average size 30.5 ±2.5nm. This method of synthesis of silver nanoparticles is an eco-friendly approach and an easy alternative to physical and chemical methods.

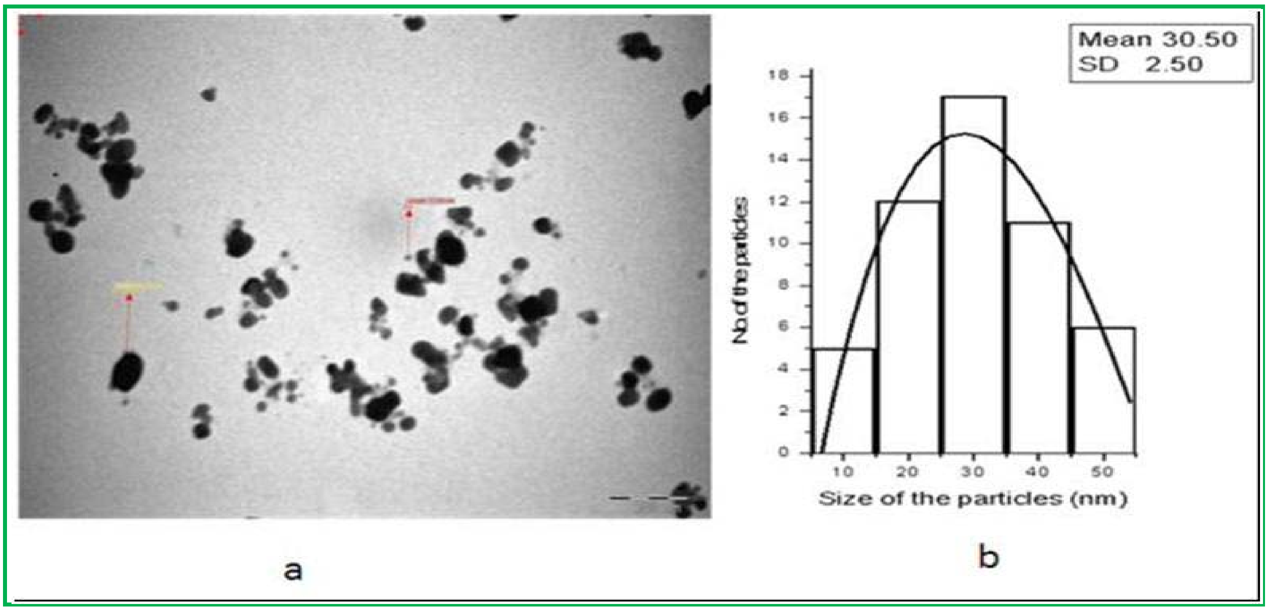

Figure 7: a) TEM images of silver nanoparticles synthesized from the *Eucalyptus globulus,* Labill. b) Size distribution of silver nanoparticles synthesized from the *Eucalyptus globulus,* Labill measured by TEM analysis.

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