

Biogenic Synthesis of Silver Nanoparticles using Leaves of *Crinum asiaticum* Linn.

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Abstract: In this study, we report the phytofabrication of silver nanoparticles using the leaf broth of *Crinum asiaticum* Linn. (Family: Amaryllidaceae). The leaf broth was added to 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 into silver nanoparticles. The reaction medium changed its colour from pale yellow to dark brown observed the incubation period. It indicates the formation of silver nanoparticles. The synthesized silver nanoparticles were characterized by UV-Visible spectroscopy, X-ray diffraction patterns (XRD), Fourier Transform Infrared Spectroscopy (FT-IR), Scanning Electron Microscopy (SEM) with Energy Dispersive X-ray (EDX) patterns and Transmission Electron Microscopy (TEM). This type of phyto-mediated synthesis appears to be cost effective, eco-friendly and an alternative to conventional physical and chemical methods for the synthesis of silver nanoparticles.

Keywords: *Crinum asiaticum*, Leaf broth, Silver nanoparticles, Reaction medium.

1. Introduction

Several approaches have been used for the synthesis of silver nanoparticles such as chemical [1], physical [2] and biological methods [3,4,5]. Among these methods, the biological synthesis of nanoparticles using microorganisms [6], enzyme [7], seaweeds [8,9] and higher plants [10,11] have been suggested as possible eco-friendly alternatives to chemical and physical methods [12]. Now-a-days plants have been used in the synthesis of metal nanoparticles such as silver [13], Gold [14], Palladium [15] and they have a pivotal role in the production of good quality and quantity of nanoparticles within few hours [16,17]. Moreover, plants are found to have higher rate of reduction of metal ions when compared to microorganisms [18,11]. At present, many researchers achieved the green synthesis of stable silver nanoparticles using various plants [19,20,13,21,22,11]. In the recent past, there are several reports pertaining to the synthesis of silver nanoparticles using various natural products like green tea (*Camellia sinensis*) [23], neem leaf broth (*Azadirachta indica*) [24], *Aleo vera* plant extract [25], latex of *Jatropha curcas* [26] and *Odina wodier* leaf extract [27]. Green nanotechnology is very safe as it utilizes non-toxic chemicals and simple solvent water for synthesizing nanoparticles [28,29,30,4,17]. This modern technique of green nanotechnology has facilitated the production of smaller nanoparticles with low toxicity to human and greater efficacy against bacteria [31,32,33,17]. In that line, the present study is aimed to synthesize the silver nanoparticles in an eco-friendly approach using the leaves of *Crinum asiaticum*, and characterize them in terms of their size, shape and distribution.

2. Materials and Methods

All the reagents used in the present study were obtained from Himedia Laboratories Pvt. Ltd., (Mumbai, India). *Crinum asiaticum* (Family: Amaryllidaceae), is a stout herb with bulbous root stock, lanceolate leaves, large flowers in umbels, subtended by two spatheaceous bracts, salver shaped perianth, six stamens, three-celled ovary, many ovules in each cells, filiform style, minute stigma and large and sub-

globose fruit [34]. Fresh and healthy leaves of *Crinum asiaticum* were collected from the Botanical garden of Ayya Nadar Janaki Ammal College, Sivakasi, Tamil Nadu, India.



Figure 1: *Crinum asiaticum* Leaves

The collected leaf samples were thoroughly washed with tap water followed by distilled water to remove the surface contaminants and dried for 48 hours under shade. The dried leaves were ground to make fine powder using mortar and pestle and sieved using 20 mesh sieve to get uniform size range. For the preparation of leaf broth, the sieved leaf powder of *Crinum asiaticum* (10g) was added to 100ml of distilled water and boiled at 70°C for ten minutes. The freshly prepared leaf broth (10 ml) was re-suspended in 190 ml of aqueous solution of silver nitrate and this mixture is used as reaction medium. This reaction medium was kept in an Incubator cum shaker (ORBITEK-MODEL) with 250 rpm at 27°C for 24 hrs. From these reaction media a small aliquot of the samples was collected separately to characterize the silver nanoparticles that were synthesized during the above reaction. The characterization was performed through the following analyses: UV-Visible spectroscopy (UV-Vis), Fourier Transform Infra-Red Spectroscopy (FTIR), X-ray diffraction (XRD) analysis, Scanning Electron Microscopy (SEM), Energy Dispersive X-ray analysis (EDX) and Transmission Electron Microscopy (TEM).

3. Results and Discussion

3.1 UV-Visible Spectrum of Silver Nanoparticles

UV-Visible spectral analysis was carried out on a Labomed (Model UV-D3200) UV-Visible spectrophotometer. The leaf extract was pale yellow in color before addition of silver nitrate and it changed to brown color within one hour (Fig. 2 inset) and it suggested the rapid formation of silver nanoparticles. Intensity of brown color was directly proportional to the incubation period and it may be due to the excitation of Surface Plasmon Resonance (SPR) variations [35,36]. The time duration for the colour change of the reaction medium was found to be varied from plant to plant. For example, *Aleo vera* [25], the leaf extracts of *Ocimum sanctum* and *Vitex negundo* [37] took 24 hours, two hours and four hours respectively.

In the present study, the SPR vibrations are found between 300-600nm, with the λ max at 465nm and the absorbance raised up to 0.36 a.u. at 24 hours incubation period (Fig. 1). The λ max of silver nanoparticles also varies from plant to plant by which they are synthesized. The λ max of silver nanoparticles synthesized using *Euphorbia hirta* was 430nm [31]; *Odina wodier* was 450nm [27]; *Merremia tridentata* was 440nm [11], while it was 380nm in case of silver nanoparticles synthesized by *Nerium indicum* [18].

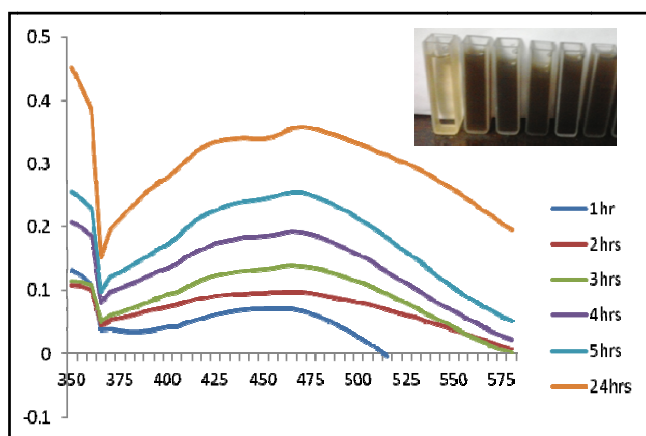


Figure 2: UV-Visible absorption spectra of silver nanoparticles synthesized by leaf broth of *Crinum asiaticum*. The inset shows the colour change of the reaction medium (left to right). A- 1h; B- 2hrs; C- 3hrs; D- 4hrs; E- 5hrs and F-24hrs

3.2 Spectrofluorimetric analysis

Figure 3 shows the Emission and Excitation spectra of silver nanoparticles synthesized using leaf broth of *Crinum asiaticum*. The excitation peak obtained at 424 nm while the emission peak was observed at 436 nm for the silver nanoparticles synthesized using leaf broth and the quantum yield (Q= Emission/ Excitation) of the silver nanoparticles

was 10.32 (Figure 3). In the spectrofluorimetric analysis of silver nanoparticles synthesized using *Nicotiana tabacum*, Prasad *et al.* (2011) [38] noticed excitation peak at 414nm and emission peak at 576nm; using leaf broth of *Tecoma stans*, Arunkumar *et al.* (2013b)[39] observed excitation peak at 430 nm and emission peak at 424 nm.

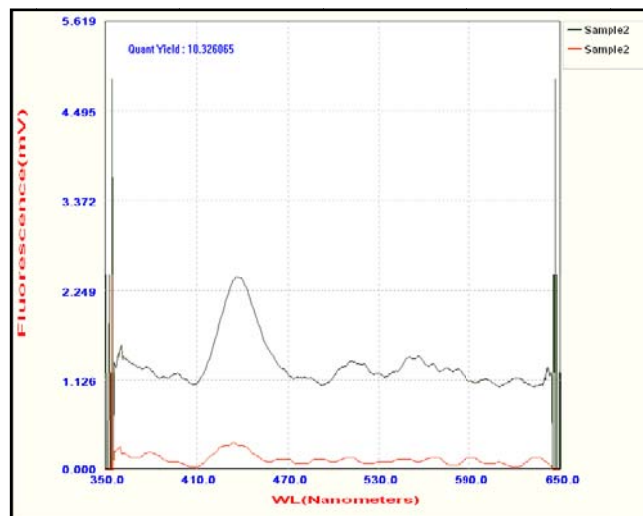


Figure 3: Spectrofluorimetric analysis of silver nanoparticles synthesized by leaf broth of *Crinum asiaticum*

3.3 FT-IR Spectroscopic Analysis

FT-IR measurements (using Shimadzu FT-IR spectrophotometer through KBR pellet method) identified the biomolecules in the leaf broth of *Crinum asiaticum*, which are responsible for reduction and providing stability to the silver nanoparticles as capping agents. Fig. 4 shows the FT-IR spectrum of synthesized silver nanoparticles and it reveals the presence of different functional groups. The absorption bands observed in the region of 400-4000 cm^{-1} are 605.61 cm^{-1} , 1336.58 cm^{-1} , 1670.24 cm^{-1} and 2975.96 cm^{-1} correspond to -C-H bend (alkenes), 808.12 cm^{-1} corresponds to -C=C- stretch (alkenes), 1112.85 cm^{-1} corresponds to -C (triple bond) C- stretch (alkynes), 653.82 cm^{-1} , 1398.30 cm^{-1} and 2883.38 cm^{-1} correspond to C-N stretch (aliphatic amines), 752.19 cm^{-1} , 1454.23 cm^{-1} and 2106.12 cm^{-1} correspond to C-N stretch (aromatics amines), 3313.48 cm^{-1} corresponds to N-H stretch (amines), 1195.78 cm^{-1} corresponds to C (triple bond) N stretch (nitriles), 2266.20 cm^{-1} and 3193.90 cm^{-1} correspond to O-H stretch (carboxylic acids). Jain *et al.* (2009) [40] reported that polyols were mainly responsible for the reduction of Ag ions, whereby they themselves got oxidized to unsaturated carbonyl groups leading to a broad peak at 1650 cm^{-1} (for reduction of Ag). The FTIR spectrum band, bioreduction of Ag⁺ ions may be due to the major involvement of polyphenols in the biosynthesis process of silver nanoparticles [41].

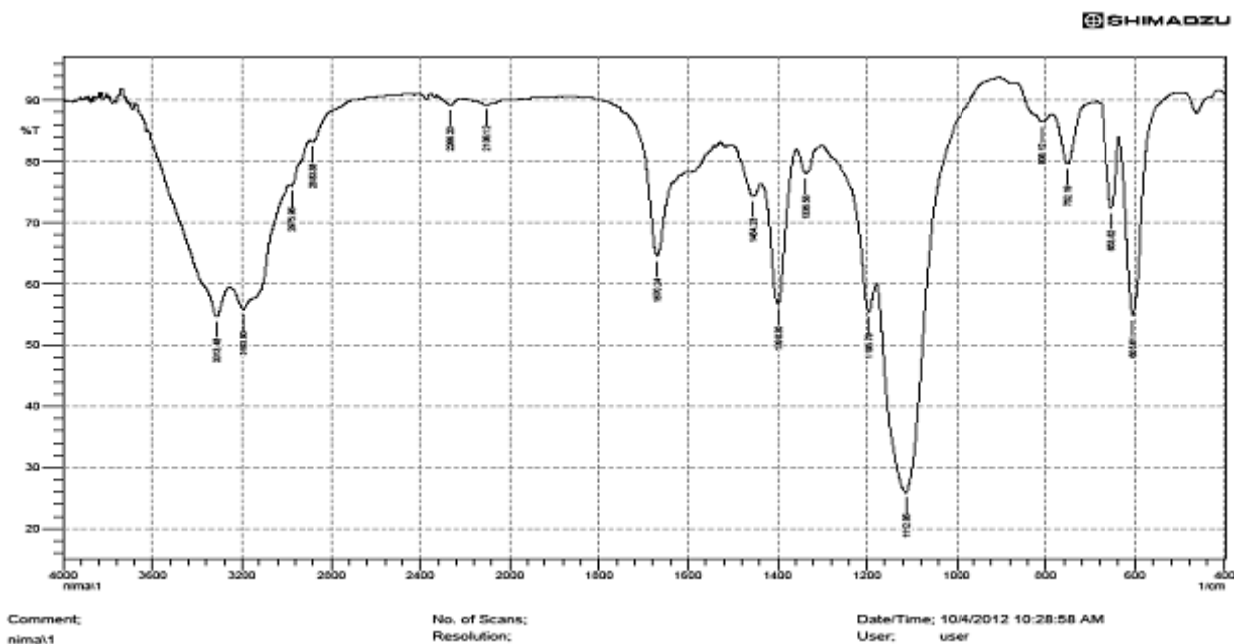


Figure 4: FTIR spectrum of synthesized silver nanoparticles using leaf aqueous broth of *Crinum asiaticum* with silver nitrate.

3.4 XRD Analysis

Crystalline metallic silver nanoparticles were examined by X-ray diffractometer Shimadzu (XRD 6000). Fig. 5 shows the X-ray diffraction pattern obtained for the synthesized silver nanoparticles using *Crinum asiaticum* leaf extract. XRD pattern showed four distinct diffraction peaks at $2\theta = 27.7^\circ$, 32.1° , 38.0° and 46.1° which indexed planes (111) (200) (220) and (311) of the face - centered cubic structure of silver. The XRD pattern clearly shows that the silver nanoparticles are crystalline nature. The Full Width at Half Maximum (FWHM) values was measured for 111, 200, 220 and 311 planes of reflection and they were used to calculate the size of nanoparticles following the Debye-Scherrer equation. The average size of the silver nanoparticles obtained is 30nm.

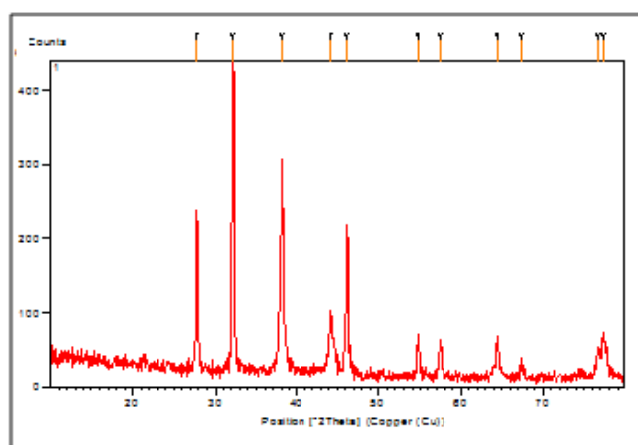


Figure 5: XRD pattern of silver nanoparticles formed after reaction of leaf broth of *Crinum asiaticum*.

3.5 SEM and EDAX analysis

SEM images provided the information about the morphology and size of the biologically synthesized silver nanoparticles. The obtained silver nanoparticles are

uniformly spherical in shape and the diameter of synthesized nanoparticles was measured as 20-30 nm (~) and uniform spherical shape (Fig. 6). Similarly, the spherical shaped silver nanoparticles with a diameter ranging from 30 to 40nm were synthesized using *Boswellia ovalifoliolata* [22]; ~ 5-30nm using the leaf broth of *Odina wodier* [27]; 30-50 nm using the bark of *Eucalyptus globulus* [42] and 30-50nm using *Merremia tridendata* [11].

The EDAX result shows a large peak of silver that confirms its presence in the suspension (Fig. 7) and synthesized silver nanoparticles are crystalline in nature. The EDAX results provide chemical analysis of field of view and as well as the spot analysis of minute particles and confirms the presence of specific elements [43].

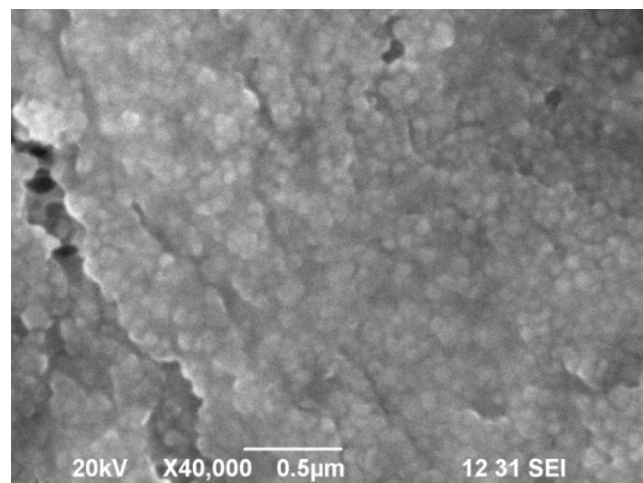


Figure 6: SEM images of silver nanoparticles synthesized from the *Crinum asiaticum* leaf broth

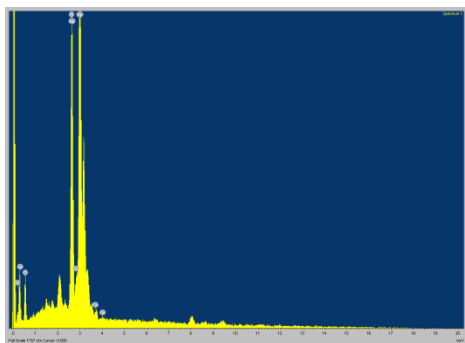


Figure 7: EDX images of silver nanoparticles synthesized from the *Crinum asiaticum*

3.6 TEM analysis

Transmission electron microscopy (TEM) analysis of the sample was carried out using Philips-Techno 10 instrument operated at an acceleration voltage of 200KV with resolution of 0.3nm. The typical bright-field TEM micrographs of the synthesized silver nanoparticles suggest that the particles are mostly spherical in shape (Fig. 8a). The size distribution of silver nanoparticles ranges between 10 and 60 with the mean 30 ± 3.68 nm (Fig. 8b). However, the size of the silver nanoparticles synthesized using the leaf broth of *Tecoma stans* was found to range 5 to 30 nm (~) with the mean 15 ± 6.99 nm [39] and the mean size of the silver nanoparticles synthesized using the bark of *Eucalyptus globules* was 30.5 ± 2.5 nm [42].

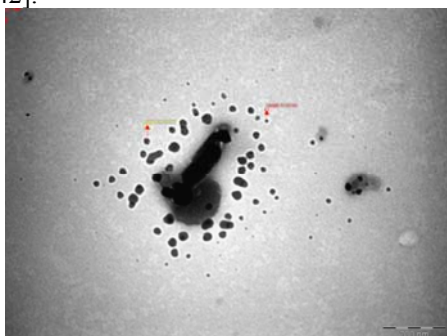


Figure 8a: TEM image of silver nanoparticles synthesized from the *Crinum asiaticum* leaf

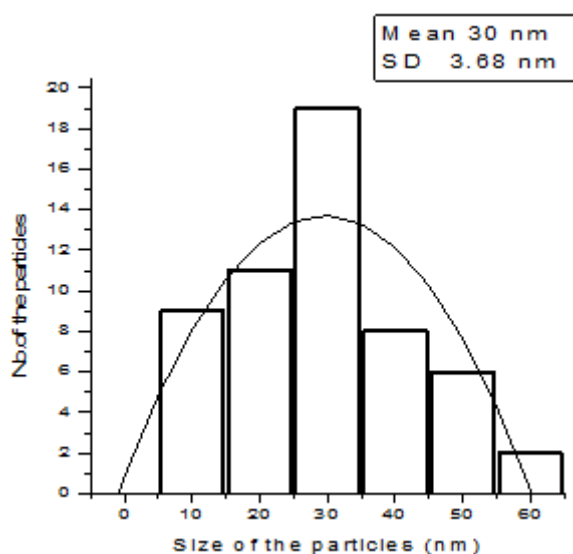


Figure 8b: Histogram of silver nanoparticles synthesized from the *Crinum asiaticum* leaf

4. Conclusion

We achieved the rapid reduction of silver nitrate into silver nanoparticles. The reaction medium changed its color from pale yellow to dark brown within 24 hours of incubation period. The UV-Visible spectrum of the reaction medium has λ_{max} at 465 nm. The Emission and Excitation spectra from spectrofluorometric study were found at 424 and 436 nm. The FT-IR spectrum showed the bands at 605.61, 653.82, 752.19, 808.12, 1112.85, 1195.78, 1336.58, 1398.30, 1454.23, 1670.24, 2106.12, 2266.20, 2883.38, 2975.96, 3193.90 and 3313.48 cm^{-1} it may be ascribed to the reduction of silver nitrate into silver nanoparticles. The SEM image shows the synthesized particles, which ranged in size from 20-30 nm and were spherical in shape. The strong silver peak obtained from the EDX spectrum confirms the significant presence of elemental silver. The XRD and TEM analyses determine the average size of the nanoparticles is 30 ± 3.68 nm respectively. The rapid, eco-friendly and biological synthesis of silver nanoparticles using leaf broth of *Crinum asiaticum* provides a good quality and quantity of silver nanoparticles.

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