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# Biobase synthesis of silver nanoparticles using leaf extract of *Calotropis procera*

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**ABSTRACT**

**Development of biologically inspired experimental process for the synthesis of nanoparticles is an important branch of nanotechnology. Biosynthesis of nanoparticles using plant extracts is currently under exploitation. In view, the present work on synthesis of silver (Ag) nanoparticles using biomolecules from leaf extract Calotropis procera was investigated. The complete reduction of silver ions was noticed after 48 h of reaction at 300C under shaking condition. Synthesized nanoparticles were characterized using UV-Vis spectroscopy, XRD and SEM. The silver nanoparticles were predominately spherical in shape and polydispersed in nature with an average size of 55 nm.**

**Key words:** Synthesis, Ag nanoparticles, Biomolecules, *Calotropis procera*

The nanoparticles of a wide range of materials can be prepared by numerous methods like chemical, physical and biological methods. The chemical and physical methods may successfully produce pure, well-defined nanoparticles, they are quite expensive and potentially dangerous to the environment (Shankar *et al.*, 2003). Therefore presently, there is a growing need to synthesize the nanoparticles in a cost effective and safe way. The exploitation of biological systems emerged as a novel method for the synthesis of nanoparticles in this regard. The use of biological resources like microorganisms and plants or their extracts for synthesis of nanoparticles offers numerous benefits and compatibility for many applications. Bioinspired synthesis of nanoparticles provides advancement over chemical and physical methods as it is a cost effective and environment friendly and in this method there is no need to use high pressure, energy, temperature and toxic chemicals during the synthesis of nanoparticles (Parashar *et al.*, 2009).

In biosynthesis methods, the use of microorganisms in the deliberate and controlled synthesis of nanoparticles is a relatively new and exciting area of research with considerable potential for development. While, microorganisms such as bacteria, fungi and actinomycetes continue to be investigated in metal nanoparticle synthesis, the use of parts of whole plants in nanoparticle synthesis methodologies is an exciting possibility that is relatively unexplored and underexploited[1]. Using the plants for synthesis of nanoparticles could be advantageous over other environmentally benign biological processes by eliminating the elaborate process of maintaining cell cultures. Hence, in this present study we investigated the synthesis of silver nanoparticles through biological method using leaf extract of medicinal plant *Calotropis procera*.

## MATERIALS AND METHODS

The fresh and young leaf samples of *Calotropis procera* was washed thoroughly with double distilled water (to remove any dust particles that could interfere with formation of nanoparticles) and surface sterilized with 0.1 per cent $HgCl_2$ for 2 to 3 min under the hood of laminar air flow. Twenty gram of sterilized leaf samples were taken and cut into small pieces. The finely cut leaves were placed in a 500 ml Erlenmeyer flask containing 100 ml of sterile double distilled water. Then, the mixture was boiled for 5 min (process of boiling leads to rupture of the cell walls in leaves and thus, release of inter cellular material into solution). After boiling, the mixture was cooled and filtered.

Bioreduction of metal ions in solution was done through addition of leaf extract into the metal

solution having known concentration. Silver nitrate ($AgNO_3$) was used as precursor for synthesizing the silver nanoparticles. Five ml of leaf extract was added to 100 ml of 1 mM $AgNO_3$ aqueous solution in conical flask of 250 ml content at room temperature. After that the flask was put into shaker (150 rpm) at $30^0$ C and reaction was carried out for a period of 48 h. The colour changes of reaction mixtures (metal ion solution and leaf extract) was recorded through visual observation.

Characterization of silver nanoparticles was done by using UV-Vis spectroscopy, X-Ray Diffraction (XRD) and Scanning Electron Microscopy (SEM).

**Determination of crystallite size**

The average crystallite size of silver was calculated from the width of the XRD peak using the Scherrer's formula,

$$D = k\lambda / \beta\cos\theta$$

Where,

*D* - Average crystallite domain size perpendicular to reflection planes

K - Constant

$\lambda$ - X-ray Wavelength

$\beta$ - Angular FWHM of the XRD peak at the diffraction angle

$\theta$ - Diffraction angle

**Fourier Transform Infrared Spectroscopy (FT-IR)**

FT-IR measurement of sample was performed using Nicolet Avatar Model FT-IR spectrophotometer in a diffuse reflectance mode at a resolution of 4 $cm^{-1}$ in KBr pellets.

## RESULTS AND DISCUSSION

Synthesis of silver nanoparticles occurred during the exposure of *Calotropis procera* leaf extract to 1 mM aqueous $AgNO_3$ solution. The complete reduction of silver ions was observed after 48 h of reaction at $30^0$ C under shaking condition. The colour change in the reaction mixture was observed during the incubation period, because the formation of silver nanoparticles was able to produce the particular colour due to their specific properties. The appearance of dark yellowish-brown colour was a clear indication of the formation of silver nanoparticles in the reaction mixture (Fig.1). The colour exhibited by metallic nanoparticles is due to the coherent excitation of all the "free" electrons within the conduction band, leading to an in phase oscillation, which is known as Surface Plasmon Resonance (Akanna *et al.*, 2010). The frequency and width of the surface plasmon absorption depends on the size and shape of the metal nanoparticles as well as on the dielectric constant of the metal itself and the surrounding medium (Dubey *et al.*, 2009).

(a) 1 mM AgNO3 solution (b) Leaf extract (c) Leaf extract + $AgNO_3$ after 48 h of reaction

**Fig 1.** Visual observation

UV-Vis spectroscopy analysis showed that the absorbance band of silver nanoparticles synthesized using *Calotropis procera* leaf extract centered at 440 nm and steadily increased in intensity as a function of time of reaction without any shift in the peak wavelength (Fig.2). The silver nanoparticles synthesized by the different plant leaf extracts shown the strong absorbance between 420 to 450 nm in UV-Vis spectral analysis (Roy and Barik, 2010).

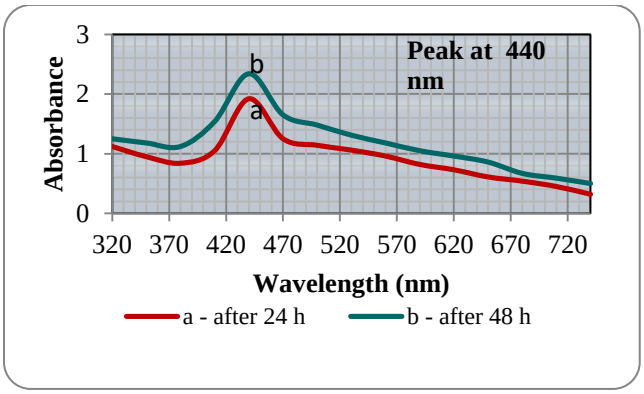


**Fig 2**. UV-Vis spectrum of silver nanoparticles

The XRD pattern obtained for silver nanoparticles showed a characteristic peak near the 2θ value of $38.04^0$ (Fig.3). Crystallite size of silver nanoparticles as estimated from the Full width at half maximum (FWHM) of the (111) peak using the Scherrer's formula exhibited an average particle size of around 55 nm. A Bragg reflection corresponding to the (111) sets of lattice planes was observed, which may be indexed based on the face-centered cubic (fcc) structure of silver (Dubey *et al.*, 2009). The XRD pattern thus clearly shows that the silver nanoparticles are crystalline in nature. The sharpening of the peaks clearly indicates that the particles are nanoregime. The size of the nanoparticles varied based on the FWHM of the peak. The FWHM values of peak get increased, the size of the nanoparticles get decreased. The line broadening of the X-ray diffraction peak is primarily due to the small particle size (Chudasama *et al.*, 2010). In addition to the Bragg peak representative of fcc silver nanocrystals, additional and yet unassigned peaks were also observed suggesting that the crystallization of bio-organic phase occurs on the surface of the silver nanoparticles (Sathyavathi *et al.*, 2010). SEM image showed individual silver particles as well as a number of aggregates. The morphology of the silver nanoparticles was predominately spherical and aggregated into larger irregular structure with no well-defined morphology observed in the micrograph (Fig.4).

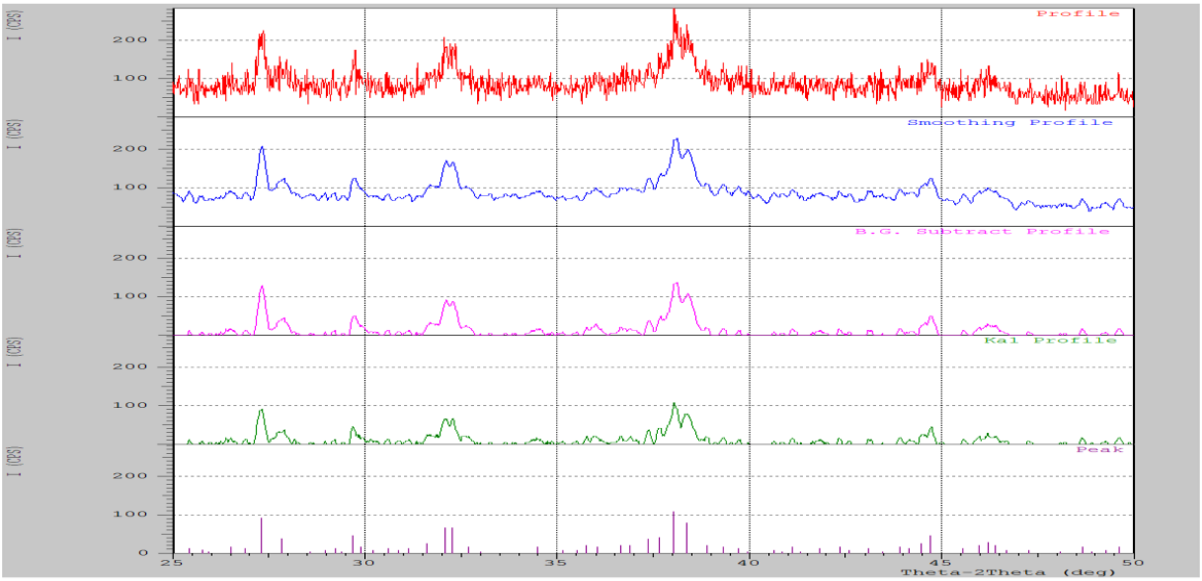


**Fig 3.** XRD pattern of silver nanoparticles

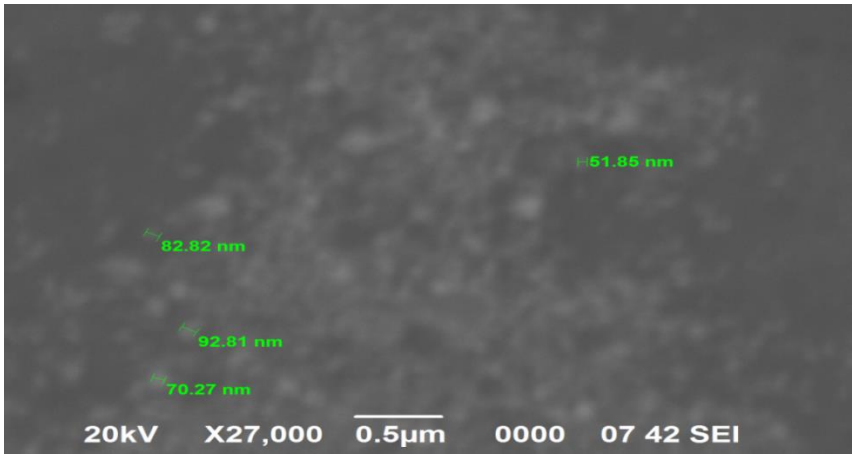


**Fig 4.** SEM image of silver nanoparticles

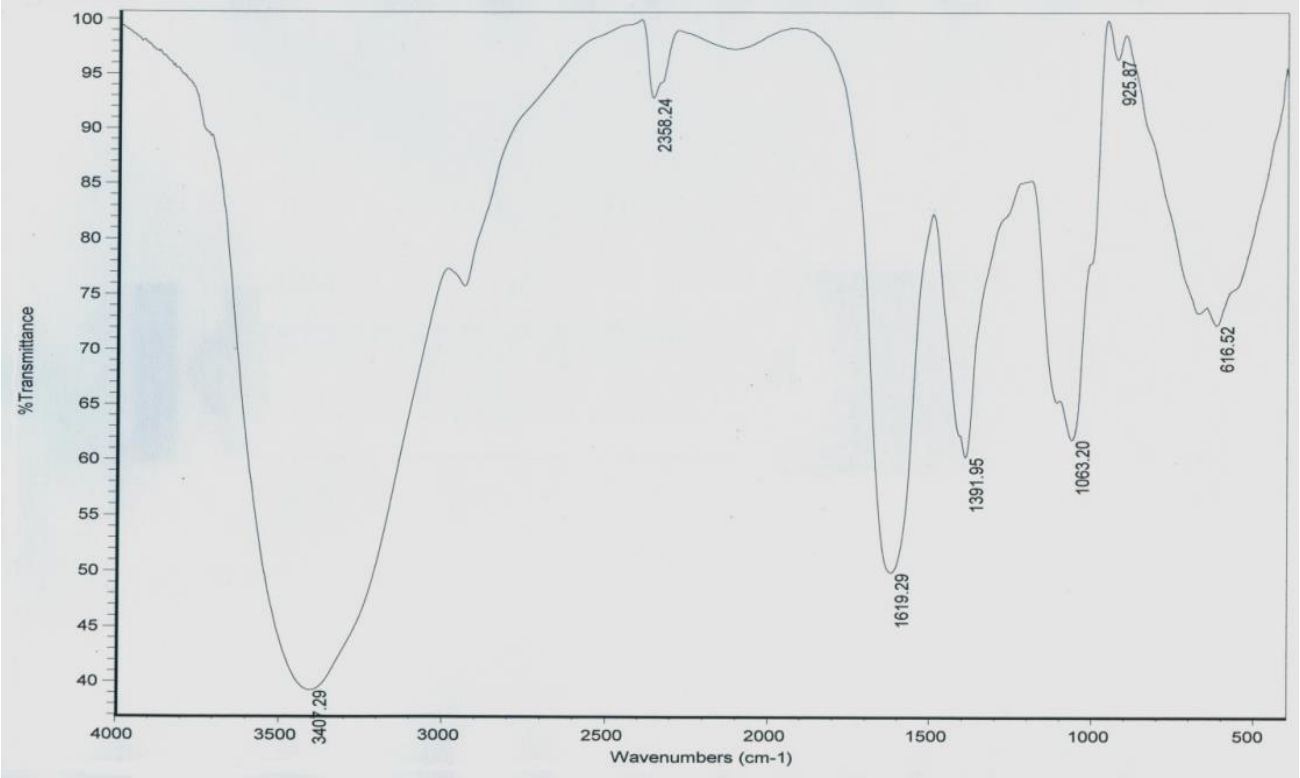


**Fig 5.** FT-IR spectrum

The SEM image nanoparticles were not in direct contact even within the aggregates, indicating stabilization of the nanoparticles by a capping agent (proteins secreted by plant leaf extract). The presence of secondary materials capping with the silver nanoparticles may be assigned to bio-organic compounds from leaf extract (Rajesh *et al.*, 2009). FT-IR measurements were carried out to identify the possible biomolecules responsible for the reduction of silver ions and capping of the bioreduced silver nanoparticles synthesized by the leaf extract of *Calotropis procera.* The wavenumber or frequency ($cm^{-1}$) of absorption band or peak assigned to the type of vibration, intensity and functional groups of the silver nanoparticles synthesized using *Calotropis procera* leaf extract are shown in fig. 5. Different functional groups were involved in reduction of silver ions to silver nanoparticles. The peaks in the region of 3407, 1619, 1391 and 1063 $cm^{-1}$ assigned to the O-H (hydroxyl group) - Alcohol and Phenol, N-H (bend) - Primary and Secondary Amides, C-H( bend) - Alkanes and C-N (stretch) – Amines / C-O (stretch) – Alcohols, Ethers, Carboxylic acids, Esters, Anhydrides, respectively. FT-IR analysis revealed that the different biomolecules and proteins from the extract of *Calotropis procera* had a strong ability to bind metal indicating that the proteins could possibly form a layer covering the metal nanoparticles (*i.e.*, capping of silver nanoparticles) to prevent agglomeration and thereby stabilize the medium. This suggested that the biological molecules could possibly perform dual functions of formation and stabilization of silver nanoparticles in the aqueous medium (Sathyavathi *et al.*, 2010).

## CONCLUSION

The present study suggests that leaf extract of *Calotropis procera* capable of producing silver nanoparticles extracellularly and the synthesized nanoparticles are quite stable in solution. The synthetic methods based on naturally occurring biomaterials provide an alternative means for obtaining the nanoparticles. The use of plants in synthesis of nanoparticles is quite novel and this biological approach have many advantages such as, ease with which the process can be scaled up, economic viability and safe way to produce nanoparticles.

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