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## SYNTHESIS OF PLANT- MEDIATED SILVER (Ag) NANOPARTICLES USING COLEUS AROMATICUS

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### **ABSTRACT**

*Biosynthesis of nanoparticles by plant extracts is currently under exploitation. Use of plants for synthesis of nanoparticles could be advantageous over other environmentally benign biological processes as this eliminates the elaborate process of maintaining cell culture. The present study deals with the synthesis of silver nanoparticles using the leaf extract of Coleus aromaticus. The formation of silver nanoparticles was characterized through UV-Visible spectroscopy, X-Ray Diffraction (XRD) and Scanning Electron Microscopy (SEM). The complete reduction of silver ions was observed after 48 h of reaction at 30<sup>o</sup> C under shaking condition. The colour changes in reaction mixture (pale yellow to dark brown colour) was observed during the incubation period, because of the formation of silver nanoparticles in the reaction mixture enables to produce particular colour due to their specific properties (Surface Plasmon Resonance). The synthesized silver nanoparticles were predominately spherical in shape, polydispersed and ranged in size from 50 to 70 nm. The approach of plant-mediated synthesis appears to be cost efficient, eco-friendly and easy alternative to conventional methods of nanoparticles synthesis.*

**Key words:** Biosynthesis, Ag nanoparticles, *Coleus aromaticus*, XRD, SEM,

## Introduction

Nanoparticles are being viewed as fundamental building blocks of nanotechnology. An important aspect of nanotechnology concerns the development of experimental processes for the synthesis of nanoparticles of different sizes, shape and controlled dispersity. With the development of new chemical or physical methods, the concern for environmental contaminations are also heightened as the chemical procedures involved in the synthesis of nanoparticles generate a large amount of hazardous byproducts. Thus, there is a need for green chemistry that includes a clean, non toxic and environment friendly method of nanoparticles synthesis (Mukherjee *et al.*, 2001). As a result, researchers in the field of nanoparticles synthesis and assembly have turned to biological system of inspiration.

Biosynthesis of nanoparticles by plant extracts is currently under exploitation. Use of plants for synthesis of nanoparticles could be advantageous over other environmentally benign biological processes as this eliminates the elaborate process of maintaining cell culture. Biosynthetic processes for nanoparticles would be more useful if nanoparticles were produced extracellularly using plants or their extracts in a controlled manner according to their size, shape and dispersity (Kumar and Yadav, 2008). The aqueous silver nitrate solution, after reacting with geranium (*Pelargonium graveolens*) leaf extract, led to rapid formation of highly stable, crystalline silver nanoparticles (16 to 40 nm) (Shankar *et al.*, 2003). Silver nanoparticles were synthesized by treating silver ions with *Capsicum annuum* L. leaf extract, the crystalline phase of the nanoparticles changed from polycrystalline to single crystalline and their size increased with increasing reaction time. Five hours reaction time led to spherical and polycrystalline shaped nanoparticles ( $10 \pm 2$  nm) (Li *et al.*, 2007). Hence, in this present study we investigated the synthesis of silver nanoparticles through biological method using leaf extract of *Coleus aromaticus*.

### Experiment details

#### a. Preparation of leaf extract

The fresh and young leaf samples of *Coleus aromaticus* was collected, washed thoroughly with sterile double distilled water (DDW) and surface sterilized with 0.1 % HgCl<sub>2</sub> for 2 - 3 min under the hood of laminar air flow. Twenty gram of sterilized leaf samples were taken and cut into small pieces. Finely cut leaves were placed in a 500 ml Erlenmeyer flask containing

100 ml of sterile DDW. After that the mixture was boiled for 5 min and filtered. The extract was stored in 4 °C.

### **b. Synthesis of silver nanoparticles**

Silver nitrate was used as precursor in the synthesis of silver nanoparticles. Five ml of leaf extract was added to 100 ml of 1 mM AgNO<sub>3</sub> (99.99 %) aqueous solution in conical flask of 250 ml content at room temperature. The flask was thereafter put into shaker (150 rpm) at 30<sup>0</sup> C and reaction was carried out for a period of 48 h.

### **c. UV-Visible spectroscopy analysis**

The colour change in reaction mixture (metal ion solution + leaf extract) was recorded through visual observation. The bioreduction of silver ions in aqueous solution was monitored by periodic sampling of aliquots (1 ml) and subsequently measuring UV-vis spectra of the solution. UV-Vis spectra of these aliquots were monitored as a function of time of reaction on Elico UV-Vis spectrophotometer (model S3-159) operated at a resolution of 1 nm.

### **d. XRD measurement**

The sample was drop-coated onto aluminum plate by just dropping a small amount of sample on the plate frequently, allowed to dry and finally thick coat of sample was prepared. The XRD measurement was performed on a Shimadzu, model LabX-XRD-6000 instrument operated at a voltage of 20 to 30 keV and a current of 30 mA with Cu K  $\alpha$  radiation with a wavelength of 1.5418 Å.

### **e. Determination of crystalline size**

Average crystallite size of silver was calculated using the Scherrer's formula,

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

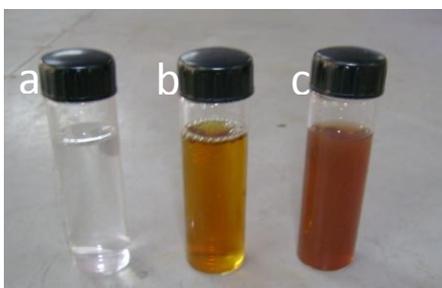
D- Average crystallite size: K- Constant:  $\lambda$ - X-ray Wavelength:  $\beta$ - Angular FWHM of the XRD peak at the diffraction angle:  $\theta$ - Diffraction angle.

### **f. SEM analysis**

The thin film of the sample was prepared on a small aluminum plate by just dropping a very small amount of the sample on the plate, extra solution were removed using a blotting paper and then the film on the plate was allowed to dry overnight. The SEM analysis was performed on a JEOL, model JSM-6390 instrument operated at an accelerating voltage of 20 keV and counting time of 100 s.

## Result and Discussion

The extracellular synthesis of silver nanoparticles occurred during the exposure of *Coleus aromaticus* leaf extract to 1 mM aqueous silver nitrate solution. The complete reduction of silver ions was observed after 48 h of reaction at 30<sup>0</sup> C under shaking condition. The colour change in reaction mixture was observed during the incubation period, because the formation of silver nanoparticles is able to produce the particular colour in the reaction mixtures due to their specific properties. The appearance of dark yellowish-brown colour is 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-SPR (Akanna *et al.*, 2010)



**Fig 1. Optical photograph of (a) 1 mM AgNO<sub>3</sub> solution (b) Leaf extract (c) Leaf extract + AgNO<sub>3</sub> after 48 h of reaction**

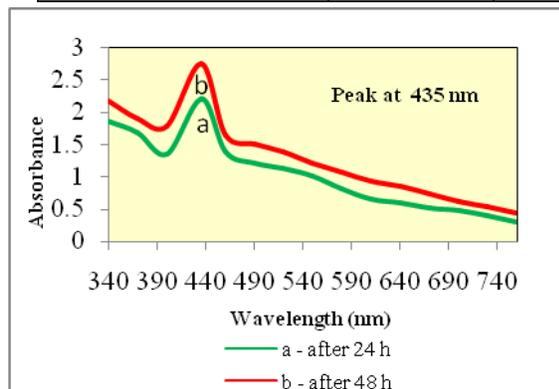
UV-Vis spectroscopy analysis showed that the SPR absorbance band of silver nanoparticles synthesized using *Coleus aromaticus* leaf extract centered at 435 nm and steadily increases in intensity as a function of time of reaction without any shift in the peak wavelength (fig 2.). The frequency and width 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 (Mukherjee *et al.*, 2002).

XRD pattern obtained for silver nanoparticles showed a characteristic peaks near the 2 $\theta$  value of 38.38<sup>0</sup> (fig.3). A Bragg reflection corresponding to the (111) sets of lattice planes are 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. 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). Crystallite size of

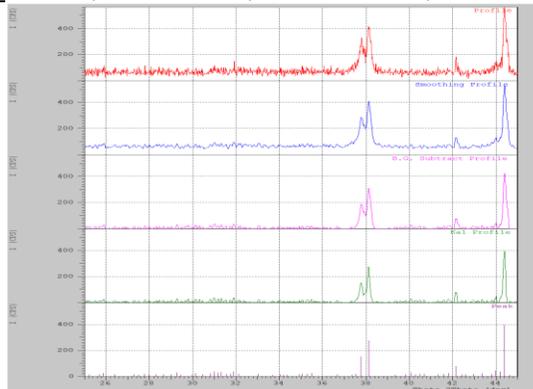
silver nanoparticles as estimated from the Full width at half maximum (FWHM) of the (111) peak using the Scherrer's formula exhibited average particles size 63 nm (Table.1).

**Table1. Crystalline size of silver nanoparticles synthesized using *Coleus aromaticus* leaf extract**

Plant extract	$\theta$ value (degree)	d- spacing (Å)	FWHM (degree)	Intensity (CPS)	Average Particle size (nm)
<i>Coleus aromaticus</i>	19.06	2.358	0.242	66.0	63.33

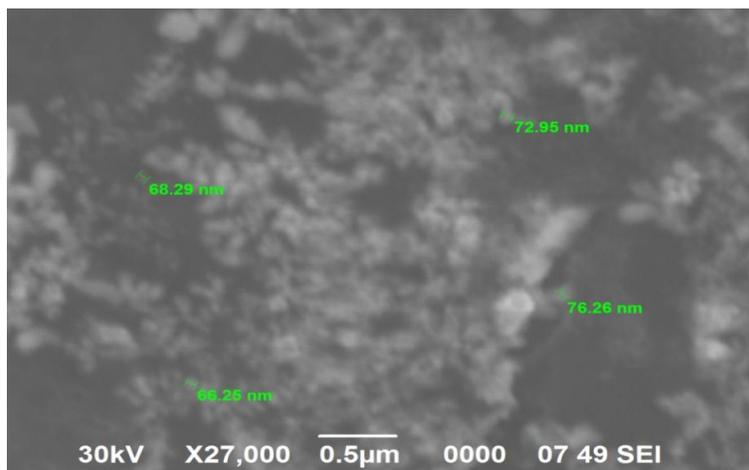


**Fig 2. UV-vis spectra of reduction of Ag ions to Ag nanoparticles**



**Fig 3. XRD pattern synthesized Ag nanoparticles**

SEM image has shown 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). The nanoparticles were not in direct contact even within the aggregates, indicating stabilization of the nanoparticles by a capping agent (proteins secreted by plant leaf extracts). The presence of secondary materials capping with the silver nanoparticles may be assigned to bio-organic compounds from leaf extracts (Rajesh *et al.*, 2009).



**Fig 4. SEM image of synthesized Ag nanoparticles**

## Conclusion

In conclusion, the bio-reduction of aqueous silver ions by the leaf extract of the *Coleus aromaticus* has been demonstrated. The reduction of the metal ions through leaf extract leading to the formation of silver nanoparticles extracellularly and the synthesized nanoparticles are quite stable in solution. The control of shape and size of silver nanoparticles seems to be easy with the use of plant leaf extracts. The synthetic methods based on naturally occurring biomaterials provide an alternative means for obtaining the nanoparticles. The approach of plant-mediated synthesis appears to be cost efficient, eco-friendly and easy alternative to conventional methods of nanoparticles synthesis. Use of plants in synthesis of nanoparticles is quite novel leading to truly 'green chemistry' route.

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