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PHYTO-ASSISTED SYNTHESIS OF SILVER NANOPARTICLES BY LEAF EXTRACT OF *HIBISCUS ROSASINENSIS*

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Abstracts- Biosynthesis of nanoparticles by plant extracts is currently under exploitation. The present study deals with the synthesis of silver nanoparticles using the leaf extract of *Hibiscus rosasinensis*. The complete reduction of silver ions was observed after 48 h of reaction at 30⁰ 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 formation of silver nanoparticles was characterized through UV-Visible spectroscopy, X-Ray Diffraction (XRD) and Scanning Electron Microscopy (SEM). The synthesized silver nanoparticles were predominately spherical in shape, polydispersed and ranged in size from 30-60 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, *Hibiscus rosasinensis*, Silvers nanoparticles, Ecofriendly

I. INTRODUCTION

An important aspect of nanotechnology concerns the development of experimental processes for the synthesis of nanoparticles of different sizes, shape and controlled disparity. 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. As a result, researchers in the field of nanoparticles synthesis and assembly have turned to biological system of inspiration. Among the biological system plants have found application particularly in metal nanoparticles synthesis. 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 and in a controlled manner according to their size, shape and dispersity [3]. Biosynthesis of nanoparticles by plant extracts is currently under exploitation. 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) [8]. Silver nanoparticles were synthesized by treating silver ions with *Capsicum annum* 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 ± 2 nm) [4]. Hence, the present study was carried out to synthesis of silver nanoparticles through biological method using the *Hibiscus rosasinensis* leaf extract.

II. MATERIALS AND METHODS

Preparation of leaf extract

The fresh and young leaf samples of *Hibiscus rosasinensis* was collected and washed thoroughly with sterile double distilled water (DDW) and finally surface sterilized with 0.1 % HgCl₂ 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.

Synthesis of silver nanoparticles

Silver nitrate was used as precursor of synthesizing the silver nanoparticles. Five ml of leaf extract was added to 100 ml of 1 mM AgNO₃ (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 °C and reaction was carried out for a period of 48 h.

UV-visible spectroscopy analysis

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

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 on plate 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 α radiation with a wavelength of 1.5418 Å.

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; λ - X-ray Wavelength; β - Angular FWHM of the XRD peak at the diffraction angle; θ - Diffraction angle.

SEM analysis

The thin film of the samples were 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 for 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.

FT-IR measurement

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

III. RESULT AND DISCUSSION

The extracellular synthesis of silver nanoparticles occurred during the exposure of *Hibiscus rosasinensis* leaf extract to 1 mM aqueous silver nitrate solution. The complete reduction of silver ions was observed after 48 h of reaction at 30 °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 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 and is known as Surface Plasmon Resonance-SPR [1].



Fig 1. Optical photograph

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

UV-vis spectroscopy analysis showed that the SPR absorbance band of silver nanoparticles synthesized using *Hibiscus rosasinensis* leaf extract centered at 430 nm (fig 2.) and steadily increases in intensity as a

function of time of reaction without any shift in the peak wavelength. 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 [5].

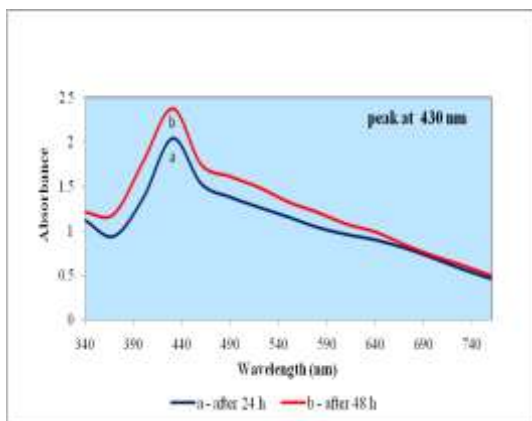


Fig 2. UV-vis spectra of reduction of Ag ions to Ag nanoparticles

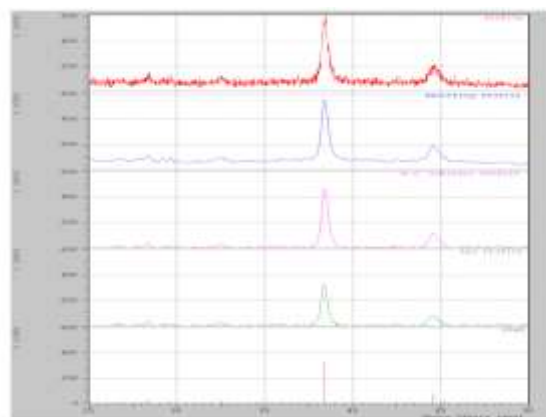


Fig 3. XRD pattern of Ag nanoparticles

XRD pattern obtained for silver nanoparticles showed characteristic peaks near the 2θ value of 38.38° (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 [2]. 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 [7].

Crystallite size of silver nanoparticles as estimated from the Full width at half maximum (FWHM) of the (111) peak of silver using the Scherrer's formula exhibited average particles size of 35 nm.

Crystalline size of synthesized silver nanoparticles

| Plant extract | θ value [degree] | d- spacing [Å] | FWHM [degree] | Intensity [CPS] | Average Particle size [nm] |
|------------------------------|-------------------------|----------------|---------------|-----------------|----------------------------|
| <i>Hibiscus rosasinensis</i> | 19.19 | 2.343 | 0.428 | 78.0 | 35.83 |

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 and this capping may be assigned to bio-organic compounds from leaf extracts [6].

Fourier Transform Infra-Red (FT-IR) spectroscopy analysis showed that the synthesized silver nanoparticles are capped with biomolecule compounds which are responsible for reduction of

silver ions. 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 *Hibiscus rosasinensis* 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 3400 to 3200 cm^{-1} and 3000 to 2850 cm^{-1} were assigned to O-H stretching of alcohol and phenol compounds and aldehydic -C-H- stretching of alkanes, respectively. The peaks in the region of 1640 to 1550 cm^{-1} and 1450 to 1375 cm^{-1} correspond to N-H (bend) of primary and secondary amides and C-H (-CH₃ - bend) of alkanes, respectively. The peaks at the region of 1350 to 1000 cm^{-1} correspond to -C-N- stretching vibration of the amine or -C-O- stretching

of alcohols, ethers, carboxylic acids, esters and anhydrides. FT-IR analysis reveals that the carbonyl group from amino acid residues and proteins has the stronger 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 suggests that the biological molecules could possibly perform dual functions of formation and stabilization of silver nanoparticles in the aqueous medium (Sathyavathi *et al.*, 2010).

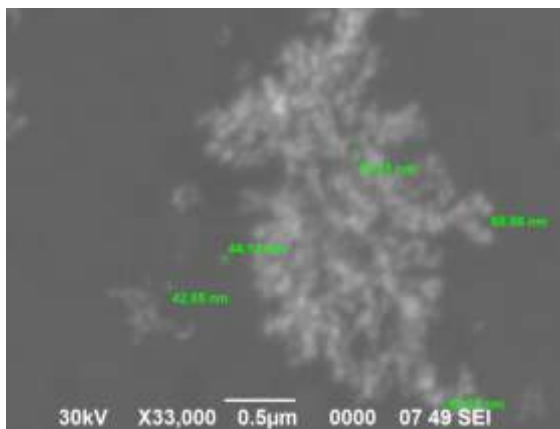


Fig 4. SEM image of Ag nanoparticles

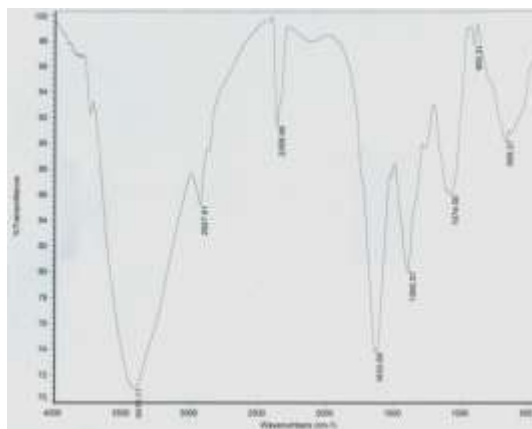


Fig 5. FT-IR spectrum of Ag nanoparticles

IV. CONCLUSION

The reduction of the metal ions through leaf extract of *Hibiscus rosasinensis* 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 synthesis methods based on naturally occurring biomaterials provide an alternative means for obtaining the nanoparticles. Use of plants in synthesis of nanoparticles is quite novel leading to truly 'green chemistry' route. The approach of plant-mediated synthesis appears to be cost efficient, eco-friendly and easy alternative to conventional methods of nanoparticles synthesis.

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