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Research article

PHYTO-ASSISTED SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES FROM *AMARANTHUS DUBIUS*

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ABSTRACT: The aqueous extract of *Amaranthus dubius* was used for the green synthesis of silver nanoparticles from silver nitrate solution under various conditions. The silver nanoparticles were characterized by spectrophotometric, physical and theoretical methods. The size of silver nanoparticles ranged from 10-70nm. The present approach of biosynthesis of silver nanoparticles using aqueous extract of *A.dubius* appears to be cost efficient, eco-friendly and an easy alternative to conventional chemical method of synthesis.

Key words: *Amaranthus dubius*, XRD, SEM, FTIR, silver nanoparticles, Scherrer's formula.

INTRODUCTION

Nanotechnology is expected to be the basis of many main technological innovations in the 21st century. Research and development in this field is growing rapidly throughout the world (Mukunthan, et.al., 2011). An important aspect of nanotechnology, concerns the development of experimental processes for the synthesis of nanoparticles of different sizes, shapes 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 nanomaterials generate a large amount of hazardous byproducts. Thus, there is a need for 'green chemistry' that includes a clean, nontoxic and environment-friendly method of nanoparticle synthesis (Dubey, et.al., 2009; Mukherjee, et.al., 2001). Biological processes have recently been considered as possible methods for the synthesis of nanoparticles, especially the development of "green" synthetic approaches for nanoparticles. The biosynthesis of silver nanoparticles of different sizes and shapes has been reported using bacteria, fungi, plants, and plant extract (Lengke, et.al., 2007; Dubey, et.al., 2009; Mukherjee, et.al., 2001; Vijayaraghavan and Nalini, 2010). Plant extracts have been used for synthesis of silver nanoparticles, which has highlighted the possibility of rapid synthesis and may also reduce the steps involved in downstream processing, thereby making the process more economical and cost-efficient (Ghosh, et.al., 2012).

Surface Enhanced Raman Scattering (SERS) is a technique used to amplify inherently weak Raman signal and involves a catalyst, typically of the nanoscale. Nanoparticles of noble metals have been found particularly useful as they exhibit surface plasmon resonance (SPR). This SPR involves a collective oscillation of the conduction electrons in resonance with certain frequencies of incident light, where the plasmon resonance of the metallic substrate (colloids) provides the intense optical frequency fields responsible for the electromagnetic contribution to SERS method. Interest in the application of SERS as an effective analytical tool is increasing, with the potential of the development of highly selective and sensitive detection (Power, et.al., 2011). Silver nanoparticles have attracted intensive research interest because of their important applications as antimicrobial, catalytic and surface-enhanced Raman scattering effect. Silver has been used as an antimicrobial agent for centuries; the recent resurgence in interest for this element particularly focuses on the increasing threat of antibiotic resistance, caused by the abuse of antibiotics (Elumalai, et.al., 2010).

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Amaranth remains an active area of scientific research for both human nutritional needs and foraging applications. *Amaranthus dubius* or Spleen amaranth belongs to the economically important plant family Amaranthaceae. Properties of biological molecules present in *A. dubius,* cost and its availability in large quantities make them highly suitable for nanoparticle synthesis. Amaranthus grows well without much agricultural attention.

The green synthesis of Ag NP's involves three main steps, which must be evaluated based on green chemistry perspectives, including (1) selection of solvent medium, (2) selection of environmentally benign reducing agent, and (3) selection of nontoxic substances for the Ag NP's stability (Raveendran, et.al., 2003). In the present study, an easy and simple approach to the green synthesis of Ag nanoparticles has been reported by employing the aqueous extract of *A. dubius* to act as both a reducing as well as capping agent using environmentally benign solvents and nontoxic chemicals.

MATERIALS AND METHODS

Sample collection
Fresh leaves of *Amaranthus dubius* were collected from a retail shop in Coimbatore, Tamil Nadu, India.

Extraction of plant material
The fresh leaves of *A. dubius* (20 g) were washed thoroughly thrice in distilled water and cut into fine pieces, then transferred into a 500ml Erlenmeyer flask with 100ml of distilled water and boiled for 5 minutes. The extract was filtered through Whatman filter paper and stored at 4°C for further experiments.

Synthesis of silver nanoparticles
The plant extract (1ml) was mixed with different concentrations (6ml, 7ml, 8ml, 9ml and 10ml) of 3mM silver nitrate and left under room temperature (28 - 30°C) whereas in higher temperature the solution was maintained at constant temperature (75°C). The above solutions were also sonicated using ultrasonic bath (PCI Ultrasonics 1.5 L (H)). The formation of reddish brown colour was observed and λ max at different conditions were taken, using a spectrophotometer.

Separation of silver nanoparticles
The reddish brown silver solution was centrifuged at 13000 rpm for 15 minutes in a centrifuge (Spectrofuge 7M), followed by re dispersion of the particles in distilled water by sonication and again centrifuged for 15 minutes, to remove the plant cell debris. The dispersed solutions were refrigerated at 4°C.

Characterization techniques
UV-Visible absorption spectra were measured using Double beam spectrophotometer 2202- (SYSTRONICS). The size of synthesized silver nanoparticles was examined by X-ray diffraction analysis (SHIMADZU Lab X XRD-6000) with a Cu Kα radiation monochromatic filter in the range 10–80°. Morphology and size of silver nanoparticles were investigated by Scanning Electron Microscope using TESCAN instrument provided with Vega TC software. The FTIR spectrum (BRUKER FTIR Tensor-27) was also recorded for the synthesized nanoparticles.

RESULTS AND DISCUSSION

The present study deals with the biosynthesis of silver nanoparticles from silver nitrate solution using the aqueous extract of *Amaranthus dubius*. Comparative experiments were carried out to study the effect of variation of concentration of aqueous extract of *Amaranthus dubius* and different concentrations of silver nitrate on the rate of bioreduction of silver ions. The reduction of silver ions occurs rapidly as the concentration of silver nitrate solution increases with (1 ml) of the plant extract under various conditions to be given in table (1). On treating the aqueous extract and silver nitrate, the colour changed gradually from yellow to reddish brown within 30 mins shown in fig (1) which may be due to excitation of surface plasmon vibrations in the silver metal nanoparticles (Mulvaney, 1996).

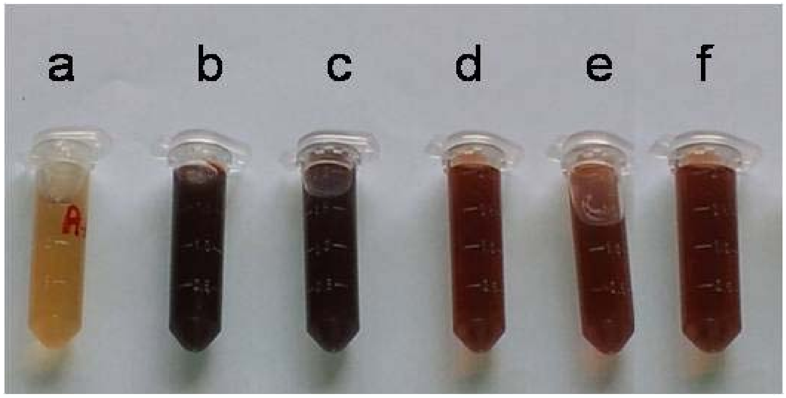

Figure 1: (a) Aqueous extract of *A.dubius* (b,c,d,e & f) silver nanoparticles synthesized from silver nitrate solution at different concentrations with 1ml of plant extract.

Figure 2 shows the UV visible spectra recorded from the aqueous extract of *Amaranthus dubius* and silver nitrate solution. It is observed that the surface plasmon resonance band occurs at 420 nm and steadily increases in intensity as a function of concentration without any shift in the peak wavelength.

Table 1: Comparative study on the synthesis of silver nanoparticles and its variation with time

Plant Extract + 3mM $AgNO_3$ (ml)	Time (minutes)		
	Room temperature	Higher temperature (75°C)	Sonication
1 + 6	30	10	24
1 + 7	25	10	22
1 + 8	20	8	15
1 + 9	18	5	12
1 + 10	15	5	11

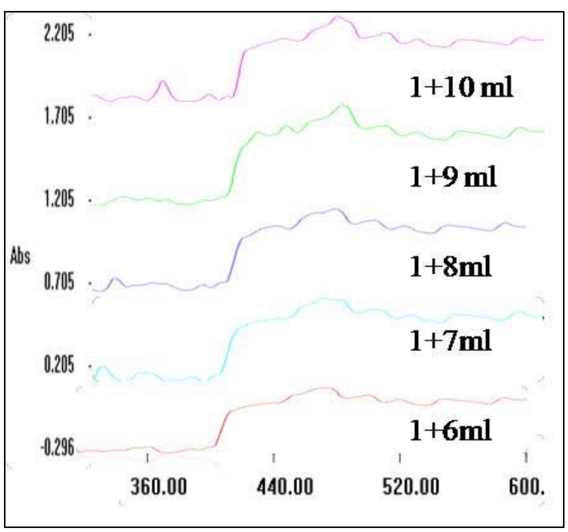

Figure 2: UV Visible absorption spectra of silver nanoparticles from extract of *Amaranthus dubius* and silver nitrate solution at different concentrations

From (figure 3), the particles were confirmed as elemental Ag (o) using XRD. In fig (3), a couple of Bragg's reflections are distinctly exhibited, which may be indexed on the basis of the face centered cubic structure of silver. It exhibits a sharp and intense peak at 38 and 32 corresponding to diffraction from the (111) and (101) planes of silver with fcc lattice (JCPDS no. 04-0783) (Varshney, et.al., 2009).

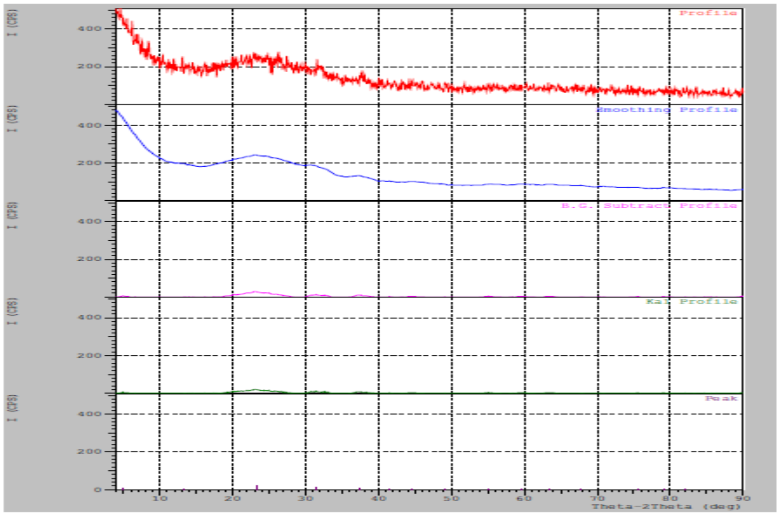

Figure 3: XRD patterns of silver nanoparticles synthesized using *Amaranthus dubius*

The crystalline size of synthesized nanoparticles was calculated from the width of the XRD peaks, assuming that they are free from non-uniform strains, using the Scherrer formula.

Debye-Scherrer's equation

The Debye-Scherrer's equation is commonly used to determine the crystalline size of nanoparticles.

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

where,

D – Average crystalline size (nm)

k – Dimensionless shape factor (0.9)

λ – X ray wavelength (0.1541 nm)

β – Angular / line broadening at FWHM of the XRD peak at the diffraction angle

θ – Diffraction angle

Table 2: Determination of crystalline size of AgNP's by using Debye-Scherrer's equation

S.No	2θ	FWHM	β = π*FWHM/180	θ	Cosθ	D = k λ / β. Cosθ
1.	31.4500	0.5000	0.008722	15.72	0.9625	16.51
2.	37.3500	0.9000	0.0157	18.67	0.9473	9.32

Average size of the nanoparticle = 16.51 + 9.32 /2 = 12.9 nm

The average size of the silver nanoparticles was estimated from the FWHM of the (111) peak using the Scherrer formula is 12.9 nm. Smaller particle size tends to enhance antibacterial properties (Jones and Hoek, 2010).

The SEM images recorded from drop coated films of AgNP's synthesized from aqueous extract of *Amaranthus dubius* and silver nitrate solution is shown in (figure 4 a). It quite obvious from the figure, that there is high density distribution of particles, having diameter in the range 60-70 nm and possessing spherical shapes. The SEM images recorded from vacuum dried pellet (figure 4 b) shows that the compounds in aqueous extract probably secondary metabolites help to stabilize AgNP's with bigger sizes. Understanding the nature of capping agent conferring stability to the nanoparticles obtained by this method is quite important. Higher stability produces higher antibacterial properties (Shrivastava et.al., 2007).

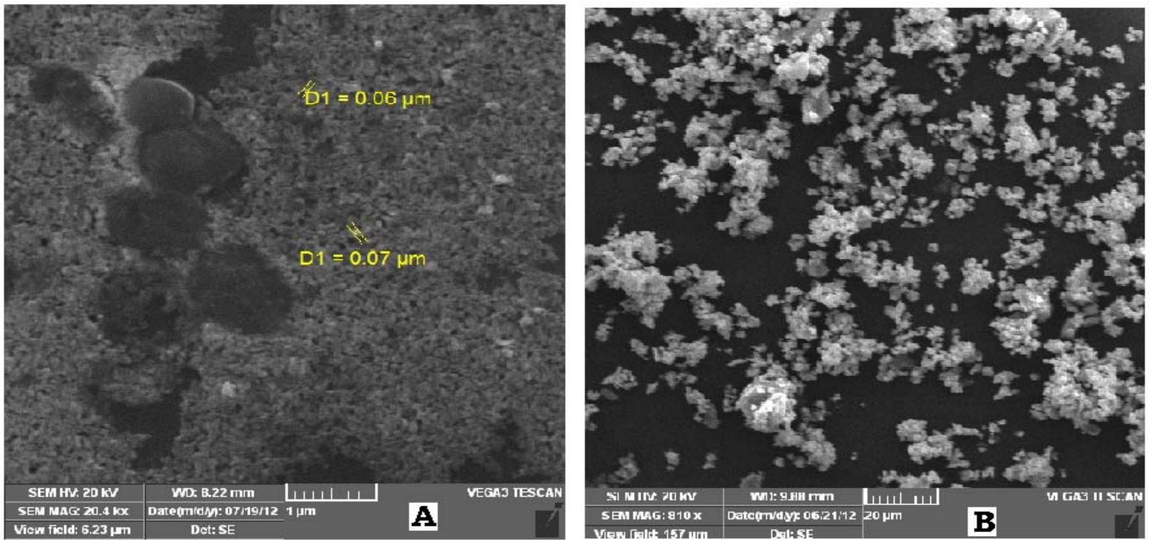

Figure 4 (a & b): SEM images of silver nanoparticles synthesized using *Amaranthus dubius*

FTIR spectra of aqueous silver nanoparticles prepared from the aqueous extract of *A. dubius* shown in fig.5. The peak located at 3285 cm^{-1} may be due to the presence of functional groups like –NH or –OH. The peak at 1634 cm^{-1} revealed the presence of carbonyl stretching in proteins. The peaks near 2471 cm^{-1}, 2411 cm^{-1} and 2115 cm^{-1}, 2172 cm^{-1} assigned to C-H and CC or CN triple bond stretching respectively. In fig 5, the peaks from 455-607 cm^{-1} shows significant changes upon reduction can be observed. IR spectroscopic study confirmed 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.

It can be concluded that the present green synthesis shows that the environmentally benign aqueous extract of *A. dubius* can be used as an effective capping and reducing agent for the synthesis of silver nanoparticles. The particle size of silver nanoparticles is ranged from 10 to 70 nm which was confirmed by XRD and SEM analysis.

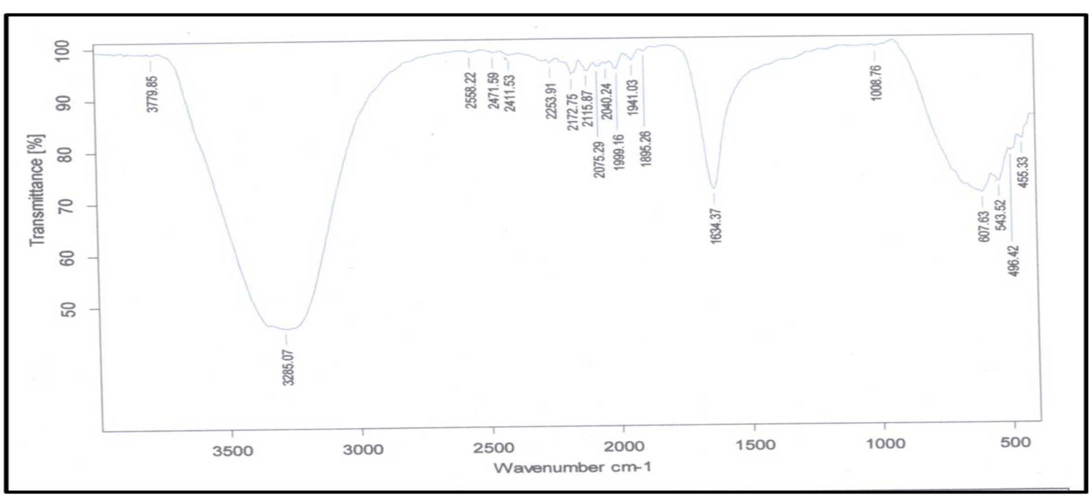

Figure 5: FTIR spectra of silver nanoparticles synthesized using *Amaranthus dubius*

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