

# Synthesis of plant-mediated silver nanoparticles using *Trianthema decandra* extract and evaluation of their anti microbial activities

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## Abstract

There is an increasing commercial demand for nanoparticles due to their wide applicability in various areas such as electronics, catalysis, chemistry, energy, and medicine. Metallic nanoparticles are traditionally synthesized by wet chemical techniques, where the chemicals used are quite often toxic and flammable. In this work, we describe a cost effective and environment friendly technique for green synthesis of silver nanoparticles from 1mM AgNO<sub>3</sub> solution through the extract of *Trianthema decandra* as reducing as well as capping agent. Nanoparticles were characterized using UV-Vis absorption spectroscopy, FTIR, XRD and SEM. X-ray diffraction and SEM analysis showed the average particle size of 15 nm as well as revealed their cubic structure. Further these biologically synthesized nanoparticles were found to be highly toxic against different multi drug resistant human pathogens. This is for the first time reporting that *Trianthema decandra* plant extract was used for the synthesis of nanoparticles.

**Keywords:** Silver nanoparticles, green synthesis, antibacterial activity, FTIR, XRD and SEM

## 1. Introduction

The field of nanotechnology is one of the most active areas of research in modern materials science. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. New applications of nanoparticles and nanomaterials are emerging rapidly (W. Jahn 1999; H. S. Naiwa 2000; C. J. Murphy 2008). Nanocrystalline silver particles have found tremendous applications in the field of high sensitivity biomolecular detection and diagnostics (S. Schultz *et al.* 2000), antimicrobials and therapeutics (M. Rai and A.Yadav 2009; Elechiguerra *et al.*2005) catalysis (Crooks 2007) and micro-electronics (Gittins *et al.*2000). However, there is still need for economic, commercially viable as well environmentally clean synthesis route to synthesize silver nanoparticles.

A number of approaches are available for the synthesis of silver nanoparticles for example, reduction in solutions (Goia and Matijevic 1998) , chemical and photochemical reactions in reverse micelles (Taleb 1997), thermal decomposition of silver compounds (Esumi *et al.*1990), radiation assisted (Henglein 2001), electrochemical (Rodriguez-Sanchez *et al.* 2000) , sonochemical (Zhu *et al.* 2000) , microwave assisted process (Pastoriza-Santos and Liz-Marzan 2002) and recently via green chemistry route (Begum *et al.* 2009; Bar *et al.* .2009; Song and Kim 2009) .

The use of environmentally benign materials like plant leaf extract (Parashar *et al.* 2009), bacteria (Saifuddin *et al.*2009) , fungi (Bhainsa and D'Souza 2006) and enzymes ( Willner *et al.*2007) for the synthesis of silver nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis protocol. Chemical synthesis methods lead to presence of some toxic chemical absorbed on the surface that may have adverse effect in the medical applications. Green synthesis provides advancement over chemical and physical method as it is cost effective, environment friendly, easily scaled up for large scale synthesis and in this method there is no need to use high pressure, energy, temperature and toxic chemicals.

Silver has long been recognized as having inhibitory effect on microbes present in medical and industrial process (Jose *et al.*2005; Lok *et al.*2007). The most important application of silver and silver nanoparticles is in medical industry such as topical ointments to prevent infection against burn and open wounds (Ip *et al.* 2006).

Further these biologically synthesized nanoparticles were found highly toxic against different multi drug resistant human pathogens.

## 2. Materials and Methods

### 2.1 Plant material and preparation of the Extract

*Trianthema decandra* were used to make the aqueous extract. *Trianthema decandra* roots weighing 25g were thoroughly washed in distilled water, dried, cut into fine pieces and were crushed into 100 ml sterile distilled water and filtered through Whatman No.1 filter paper (pore size 25  $\mu\text{m}$ ). The filtrate was further filtered through 0.6  $\mu\text{m}$  sized filters.

### 2.2 Synthesis of Silver Nanoparticles

1mM aqueous solution of Silver nitrate ( $\text{AgNO}_3$ ) was prepared and used for the synthesis of silver nanoparticles. 10 ml of *Trianthema decandra* root extract was added into 90 ml of aqueous solution of 1 mM Silver nitrate for reduction into  $\text{Ag}^+$  ions and kept at room temperature for 5 hours.

### 2.3 UV-Vis Spectra analysis

The reduction of pure  $\text{Ag}^+$  ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 5 hours after diluting a small aliquot of the sample into distilled water. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer UV-2450 (Shimadzu).

### 2.4 XRD measurement

The silver nanoparticle solution thus obtained was purified by repeated centrifugation at 5000 rpm for 20 min followed by redispersion of the pellet of silver nanoparticles into 10 ml of deionized water. After freeze drying of the purified silver particles, the structure and composition were analyzed by XRD and SEM. The dried mixture of silver nanoparticles was collected for the determination of the formation of Ag nanoparticles by an X'Pert Pro x-ray diffractometer operated at a voltage of 40 kV and a current of 30 mA with  $\text{Cu K}\alpha$  radiation in a  $2\theta$  configurations. The crystallite domain size was calculated from the width of the XRD peaks, assuming that they are free from non-uniform strains, using the Scherrer formula.

$$D = 0.94 \lambda / \beta \cos \theta \rightarrow (1)$$

where D is the average crystallite domain size perpendicular to the reflecting planes,  $\lambda$  is the X-ray wavelength,  $\beta$  is the full width at half maximum (FWHM), and  $\theta$  is the diffraction angle. To eliminate additional instrumental broadening the FWHM was corrected, using the FWHM from a large grained Si sample.

$$\beta_{\text{corrected}} = (\text{FWHM}_{\text{sample}}^2 - \text{FWHM}_{\text{Si}}^2)^{1/2} \rightarrow (2)$$

This modified formula is valid only when the crystallite size is smaller than 100 nm (Boulch *et al* 2001).

### 2.5 SEM analysis of silver nanoparticles

Scanning Electron Microscopic (SEM) analysis was done using Hitachi S-4500 SEM machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

### 2.6 FTIR analysis of dried biomass after bioreduction

To remove any free biomass residue or compound that is not the capping ligand of the nanoparticles, the residual solution of 100 ml after reaction was centrifuged at 5000 rpm for 10 min and the resulting suspension was redispersed in 10 ml sterile distilled water. The centrifuging and redispersing process was repeated three times. Thereafter, the purified suspension was freeze dried to obtain dried powder. Finally, the dried nanoparticles were analyzed by FTIR.

## 2.7 Antibacterial assays

The antibacterial assays were done on human pathogenic *Escherichia coli* and *Pseudomonas aeruginosa* by standard disc diffusion method. Briefly Luria Bertani (LB) broth/agar medium was used to cultivate bacteria. Fresh overnight cultures of inoculum (100  $\mu$ l) of each culture were spread on to LB agar plates. Sterile paper discs of 6mm diameter (containing 50mg/litre silver nanoparticles) along with three standard antibiotic containing discs were placed in each plate.

## 3. Results and discussion

It is well known that silver nanoparticles exhibit yellowish brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles (Shankar *et al.* 2004). As the extract was mixed in the aqueous solution of the silver ion complex, it started to change the color from watery to yellowish brown due to reduction of silver ion which indicated formation of silver nanoparticles. It is generally recognized that UV-Vis spectroscopy could be used to examine size and shape controlled nanoparticles in aqueous suspensions (Wiley *et al.* 2006). Figure 1 shows the UV-Vis spectra recorded from the reaction medium after 4 hours. Absorption spectra of silver nanoparticles formed in the reaction media has absorbance peak at 450 nm, broadening of peak indicated that the particles are polydispersed. The biosynthesised silver nanostructure by employing *Trianthema decandra* root extract was further demonstrated and confirmed by the characteristic peaks observed in the XRD image (Figure 2) and the structural view under the scanning electron microscope. The XRD pattern showed three intense peaks in the whole spectrum of  $2\theta$  value ranging from 10 to 80. Average size of the particles synthesized was 15nm with size range 10 to 50nm with cubic and hexagonal shape. The typical XRD pattern (Fig. 2) revealed that the sample contains a mixed phase (cubic and hexagonal) structures of silver nanoparticles. The average estimated particle size of this sample was 15 nm derived from the FWHM of peak corresponding to 111 plane (figure 3). The SEM image showing the high density silver nanoparticles synthesized by the *Trianthema decandra* extract further confirmed the development of silver nanostructures.

Fig. 1. UV-Vis absorption spectrum of silver nanoparticles synthesized by treating 1mM aqueous  $\text{AgNO}_3$  solution with 10% *Trianthema decandra* root extract after 5 hrs.

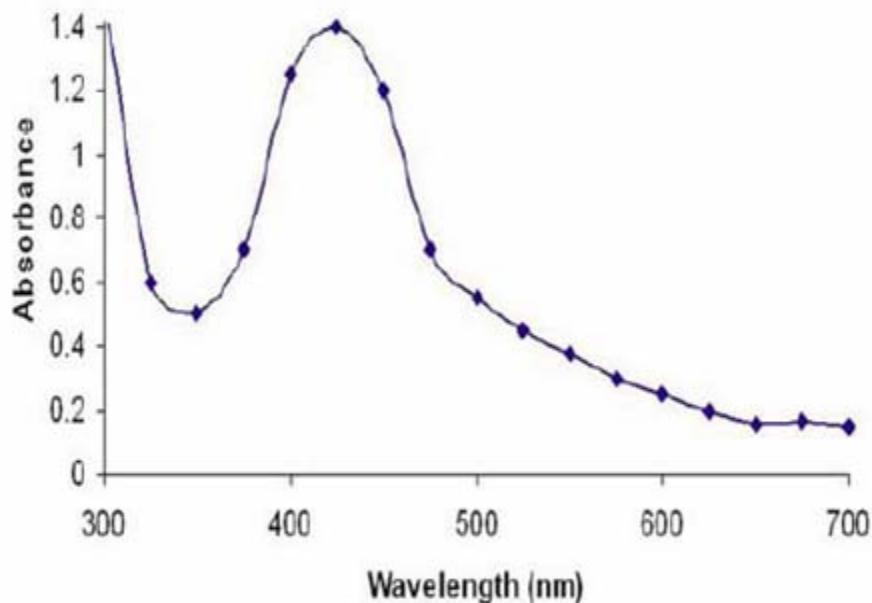
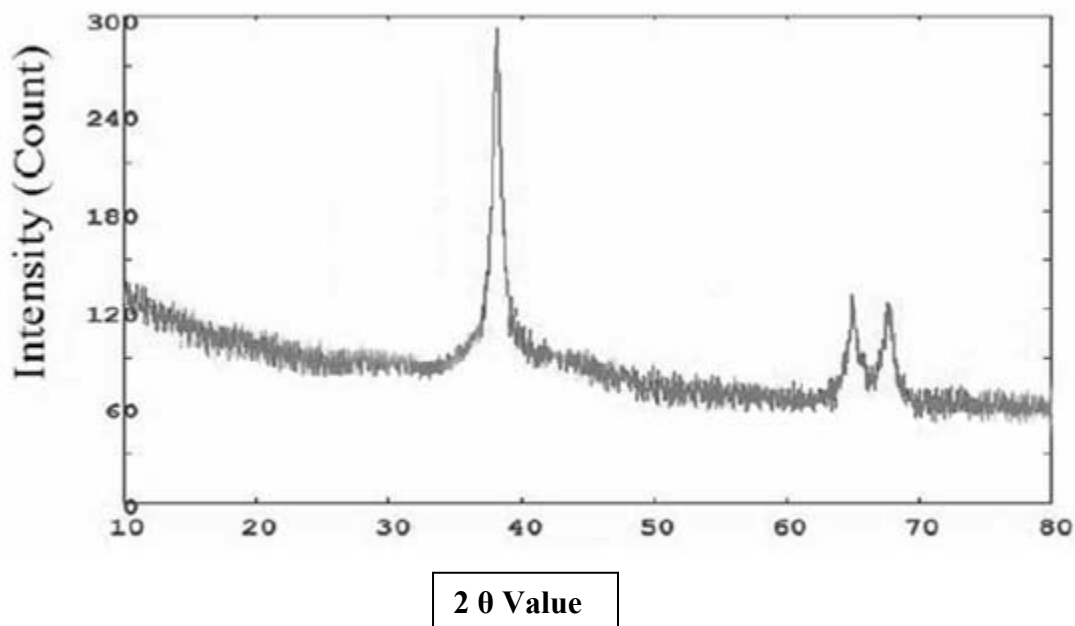


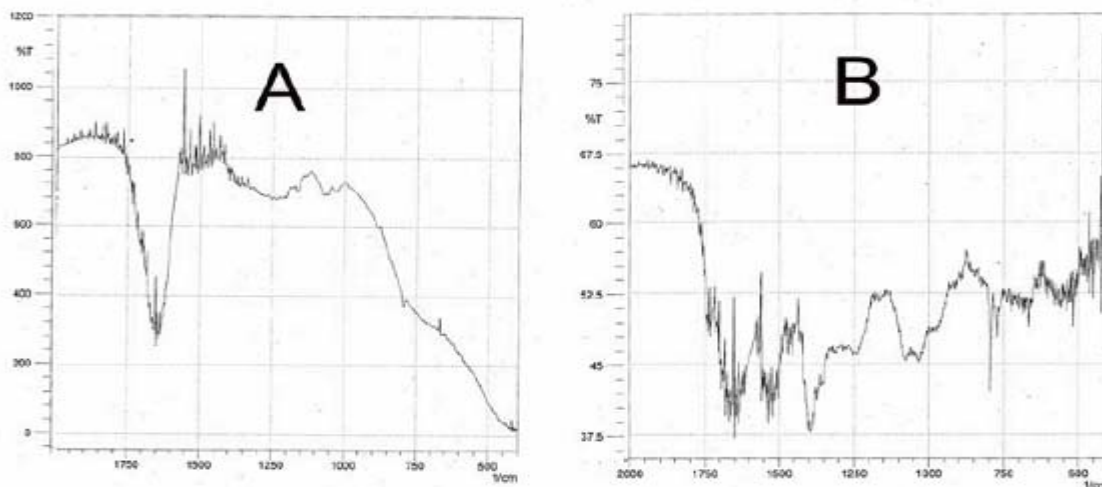
Fig. 2. XRD pattern of silver nanoparticles synthesized by treating 10% *Trianthema decandra* root extract with 1 mM aqueous AgNO<sub>3</sub> solution.



S.No	2 θ Value	Plane	Element	Phase
1	38.11	111	Ag	Cubic
2	63.51	110	Ag	Hexagonal
3	65.27	112	Ag	Hexagonal

FTIR analysis was used for the characterization of the extract and the resulting nanoparticles (Figure 3). FTIR absorption spectra of water soluble extract before and after reduction of Ag ions are shown in Fig. 3. Absorbance bands in Fig. 3A (before bioreduction) are observed in the region of 500–2000 cm<sup>-1</sup> are 1697, 1618, 1514, 1332, 1226 cm<sup>-1</sup>. These absorbance bands are known to be associated with the stretching vibrations for –C–C–O, –C–C– [(in-ring) aromatic], –C–C– [(in-ring) aromatic], C–O (esters, ethers) and C–O (polyols), respectively (Bar *et al.*2009). In particular, the 1226 cm<sup>-1</sup> band arises most probably from the C–O group of polyols such as hydroxyflavones and catechins. The total disappearance of this band after the bioreduction (Fig.3B) may be due to the fact that the polyols are mainly responsible for the reduction of Ag ions, whereby they themselves get oxidized to unsaturated carbonyl groups leading to a broad peak at 1650cm<sup>-1</sup> (for reduction of Ag). Further the nanoparticles syntheses by green route are found highly toxic against multi drug resistant human pathogenic bacteria at a concentration of 50 ppm. Silver nanoparticles exhibited antibacterial activity against *E. coli* and *Pseudomonas aeruginosa* as it showed a clear inhibition zone whereas the standard antibiotics like Ampicillin, Tetracycline and Rifampicin does not shown any inhibition zone. Antibacterial effects of Ag nanoparticles obeyed a dual action mechanism of antibacterial activity, *i.e.*, the bactericidal effect of Ag<sup>+</sup> and membrane-disrupting effect of the polymer subunits.

Fig. 3. FTIR spectra of vacuum dried powder of (a) *Trianthema decandra* root extract  
(b) nanoparticles synthesized by 10% *Trianthema decandra* root extract solution.



Reduction of silver ions present in the aqueous solution of silver complex during the reaction with the ingredients present in the *Trianthema decandra* root extract observed by the UV-Vis spectroscopy revealed the presence of silver nanoparticles may be correlated with the UV-Vis spectra. UV-Vis spectroscopy is well known to investigate shape and size controlled of nanoparticles. The XRD and SEM analysis showed the particle size between 25-50nm as well the cubic structure of the nanoparticles. FTIR analysis confirmed that the bioreduction of  $\text{Ag}^+$  ions to silver nanoparticles are due to the reduction by capping material of plant extract. The silver nanoparticles synthesized via green route are highly toxic to multidrug resistant bacteria hence has a great potential in biomedical applications. The present study showed a simple, rapid and economical route to synthesized silver nanoparticles.

#### 4. Conclusions

In conclusion, the bio-reduction of aqueous  $\text{Ag}^+$  ions by the root extract of the *Trianthema decandra* plant has been demonstrated. The reduction of the metal ions through leaf extracts leading to the formation of silver nanoparticles of fairly well-defined dimensions. But the capabilities of the other plant part such as root as a capping and reducing agent is not tested and not well defined. In the present study we found that roots can be also good source for synthesis of silver nanoparticles. This green chemistry approach toward the synthesis of silver nanoparticles has many advantages such as, ease with which the process can be scaled up, economic viability, etc. Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications, makes this method potentially exciting for the large-scale synthesis of other inorganic materials (nanomaterials). Toxicity studies of silver nanoparticles on human pathogen opens a door for a new range of antibacterial agents.

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