GTRP
Int. J. Biological Technology

Rapid biosynthesis of silver nanoparticles using fern leaflet extract and evaluation of their antibacterial activity

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Published: 15, August, 2013; Vol. No. 4(2):12-18; www.gbtrp.com;

Abstract

There are various conventional methods for the synthesis of silver nanoparticles such as physical, chemical and biological. Biological methods of nanoparticle synthesis are cost effective, easily scaled up and environmental friendly. A green synthesis of silver nanoparticles by the reduction of silver ions in the solution by leaflets extract of fern (*Cheilanthes forinosa* Forsk.) has been demonstrated. The biosynthesised silver nanoparticles were characterized by UV-Visible spectrophotometer, X-Ray Diffraction and Scanning Electron Microscopy analysis. Nanoparticles were crystalline in nature with diameter of 26.58 nm. Antibacterial activity was screened using synthesized silver nanoparticles. The silver nanoparticles have shown antibacterial activity against *Staphylococcus aureus* NCIM-2079 and *Proteus morgani* NCIM 2040.

Keywords: Silver nanoparticles, fern, antibacterial activity, *Cheilanthes forinosa* Forsk

Introduction

Silver nanoparticles have extensive application in the development of new technologies in the areas of electronics, material sciences and medicine at the nanoscale (Megudapathy *et al.*, 2001) therefore; silver nanometal has drawn attention of researchers (Safaepour *et al.,* 2009). Nanoparticles can be synthesized by various conventional methods. Some of these are chemical (Murry *et al.,* 1993) and physical (Ayyub *et al.,* 2001), but these methods for synthesis of nanoparticles require difficult and environmentally challenging techniques. Biological methods of nanoparticle synthesis using microorganisms, enzymes and plant extracts are possible and eco-friendly alternatives to chemical and physical methods (Kim and Song, 2009). Use of plants for nanoparticles synthesis can be advantageous over other biological processes by eliminating elaborative process of maintaining cell cultures. It can be easily scaled up for large scale synthesis. Now-a-days, the synthesis of nanoparticles has become very important due to their unique properties. Chemical and physical methods can produce pure, well defined nanoparticles but these are costly and harmful to the environment. Properties of these particles have diverse application including electronic devices (Wang *et al*., 2010), chemical and biological sensing (Sun *et al.,* 2005) and surface enhanced Raman spectroscopy (Dick *et al.,* 2002). Silver has an inhibitory effect on bacteria and fungi. The most important application of silver and silver nanoparticles is in medical industry to prevent infection against burn and open wounds (Ip *et al.,* 2006). The antibacterial activity of silver nanoparticles

strictly depends on the surface development of solid phase is in a nanoparticles form, the resulting antibacterial activity can be significantly increased and smaller silver nanoparticles may be several order of magnitude more active than the corresponding bulk solid. Therefore, silver nanoparticles adsorbed on to surfaces of various biomaterials are a potentially great choice when fabricating materials with antimicrobial properties (Sosa *et al.,* 2003). There are many reports on the synthesis of nanoparticles using extracts of plants belonging to gymnosperms and angiosperms. In the present investigation, we report the synthesis of silver nanoparticles by the reduction of aqueous silver ions with the leaflet extract of a fern *Cheilanthes forinosa* Forsk.

Materials and Methods

Plant material and preparation of extract

Fresh leaves of *C. forinosa* Forsk. were collected from the Bhimashankar forest of Western Ghats of Maharashtra, India. Leaflets were removed and washed with tap water, then with distilled water and dried with blotting paper and cut into small pieces. These were dispersed in 100 ml sterile distilled water and boiled for 30 min at 100 ^{0}C. It was filtered through Whatman No. 1 filter paper and volume of the filtrate was adjusted to 100 ml by adding sterile distilled water.

Synthesis of silver nanoparticles

1mM aqueous solution of silver nitrate was prepared and used for the synthesis of silver nanoparticles. 10 ml of *C. forinosa* Forsk. leaflet extract was added into 90 ml of 1 mM aqueous solution of silver nitrate. It was kept for 4 h. The colour change of reaction mixture from watery to dark brown was checked periodically. The colour change from colourless to brown indicated that silver nanoparticles were synthesized.

UV-Vis Spectra analysis

UV-Vis spectroscopy is commonly used to examine size and shape controlled nanoparticles in aqueous suspensions (Wiley *et al.,* 2006). The reduction of pure Ag^+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium after 4 h after diluting 100 µl of the sample with 1 ml sterile distilled water. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer UV-2450 (Simatzu).

XRD analysis

The silver nanoparticle solution thus obtained was purified by repeated centrifugation at 10000 rpm for 20 min followed by re-dispersion of the pellet of silver nanoparticles into 10 ml sterile distilled water. After freeze drying of purified silver nanoparticles, the structure and composition were analysed by XRD using RIGAKUD machine. The crystalline domain size was calculated from the width of XRD peaks using Scherrer's formula.

Debye-Scherrer's equation
$D = K\lambda / \beta \cos\Theta$

Where, $\beta = \pi / 180*FWHM$ (FWHM = Full Width half Maximum)
$\lambda = 1.540598\ A^o$

$K\lambda = 0.94*1.540598\ A^o$
$= 1.4482$

SEM analysis of silver nanoparticles

Scanning Electron Microscopic (SEM) analysis was done using PHILIPS-XL-30SEM machine. Thin films of samples 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 films on the SEM grid were allowed to dry by putting under a mercury lamp for 5 min.

Antibacterial assays

The antibacterial assays were done on pathogenic bacteria *Staphylococcus aureus* NCIM-2079 and *Proteus morgani* NCIM 2040. Nutrient agar medium was used to cultivate bacteria. 20 ml molten and cooled medium (Nutrient agar) was poured in sterilized petriplates. The plates were left overnight at room temperature to check for any contamination to appear. *Staphylococcus aureus* NCIM-2079 and *Proteus morgani* NCIM 2040 were grown in nutrient broth for 24 h. A 100 ml nutrient broth culture of bacterial organism (1 x 10^5cfu/ml) was used to prepare bacterial lawn. Sterile paper discs of 6 mm diameter were prepared and placed in each plate. 30 µl diluted compound was loaded on the paper discs with the help of micropipette and plates were incubated at 37 ^{0}C for 24-48 h for observing

inhibition rate and others with 30 µl of positive control drugs. The plates were examined for evidence of zones of inhibition, which appear as a clear area around the disc. The diameter of each zone of inhibition was measured.

Results

As the *C. forinosa* Forsk. leaflet extract was mixed in the aqueous solution of silver nitrate, it started to change colour from watery to yellowish brown due to reduction of silver ion which indicated formation of silver nanoparticles. Silver nanoparticles exhibit yellowish brown colour in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles (Shankar *et al.,* 2004).

UV-Vis spectra recorded from the reaction medium after 4 h is shown in Fig.1. Absorption spectra of silver nanoparticles formed in the reaction media has absorption peak at 435 nm, broadening of peak indicated that the particles are polydispersed.

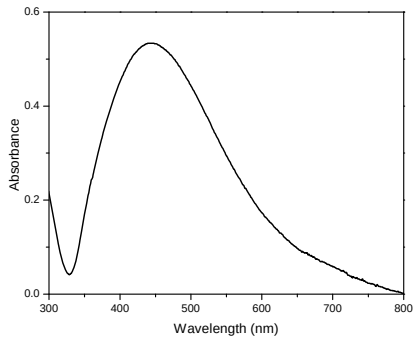


Fig.1 UV-Visible spectra of Ag nanoparticles

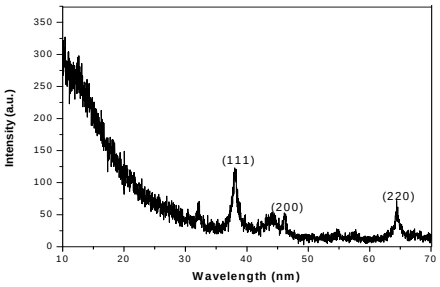


Fig. 2 XRD pattern recorded for the silver nanoparticles

The dry powder of the silver nanoparticles was used for XRD analysis. The diffraction intensities were recorded from 10^o to 70^o at 2Θ angles. Fig. 2 reveals three intense peaks in the whole spectrum of 2Θ value ranging from 10^o to 70^o, corresponding to three diffraction facets of silver. A number of Bragg reflections corresponding to the (111), (200), (220) sets of lattice planes were observed. An average size of silver nanoparticles synthesized was 26.58 nm and these were spherical in shape.

Synthesized silver nanostructure was further demonstrated and confirmed by the structural view under the scanning electron microscopy (Fig. 3). The SEM image showing the high density spherical shape silver nanoparticles synthesized by the *C. forinosa* Forsk. leaflet extract.

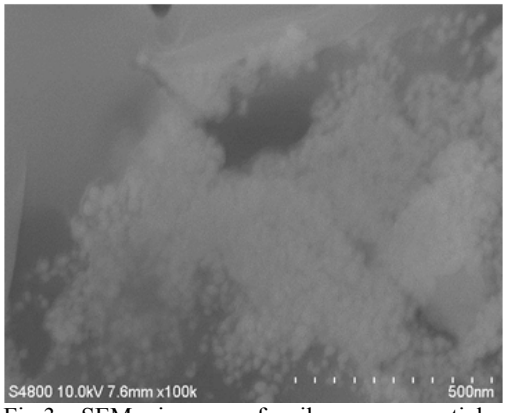
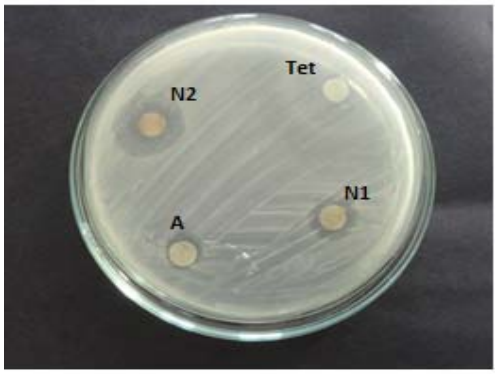


Fig.3 SEM image of silver nanoparticles synthesized using leaflets extract of *Cheilanthes forinosa* Forsk.

Fig. 4 a) *Staphylococcus aureus* NCIM-2079



b) *Proteus morgani* NCIM 2040

The inhibitory activity in culture media of silver nanoparticles is reported in Fig. 4 comparable with the standard antibacterial. The inhibitory zone of silver nanoparticles was 15 mm for *Staphylococcus aureus* NCIM-2079, whereas tetracycline also showed the similar inhibitory activity with 15 mm size inhibitory zone. In *Proteus morgani* NCIM 2040, 20 mm size inhibitory zone was observed which is comparable to the zone of inhibition (22 mm) of tetracycline.

Discussion

Plant extracts are effective against various plant pathogens (Misra and Dixit, 1970). The use of plant extracts has opened awareness for the control of diseases. When the leaflet extract of *C. forinosa* Forsk. was added in the aqueous solution of $AgNO_3$, colour change was observed from water colour to yellowish brown due to reduction of Ag^+, which indicated the synthesis of silver nanoparticles. Colour change was due to excitation of surface plasmon vibrations (Mulvaney 1996). UV-Vis spectrograph of the colloidal solution of silver nanoparticles formed has been recorded as a function of time. Absorption spectra of silver nanoparticles formed in the reaction media after 4 h has absorbance peak at 435 nm, broadening of peak indicated that the particles are polydispersed (Fig. 1). UV-Vis absorption spectra show peaks characteristic of surface plasmon resonance of nanosized particles (Huang *et al.,* 2007).

The diffraction intensities were recorded from 10^o to 70^o at 2Θ angles. Fig. 3 revealed three intense peaks in the whole spectrum of 2Θ value ranging from 10^o to 70^o, corresponding to three diffraction facets of silver. A number of Bragg reflections corresponding to the (111), (200), (220) sets of lattice planes were obtained, which may be index based on the face centered cubic structures of silver nanoparticles. The average size of silver nanoparticles calculated using Scherrer's formula was 26.58 nm. The silver nanoparticles were spherical in shape. Hence from the XRD pattern it is clear that silver nanoparticles formed using leaflets of *C. forinosa* Forsk. were essentially crystalline in nature.

SEM image (Fig.2) showed aggregates of spherical nanoparticles formed. Similar results were reported using *Aloe vera L.* leaf extract (Chandran et al. 2006); *Cinnamomum camphora* plant extract (Huang *et al.,* 2007); leaf extract of *Citrullus colocynthis* (Satyavani *et al.,* 2011) and *Andrographis paniculata* (Sulochana *et al.,* 2012).

Many reports well documented on the biosynthesis of silver nanoparticles using several plant extracts. Silver nanoparticles were synthesized using latex of *Jatropha curcas* (Bar *et al.,* 2009); extract of *Acalypha indica* (Krishnaraj *et al*., 2010); *Rosa rugosa* (Dubey *et al.,* (2010); *Murraya koinigi* (Philip *et al.,* 2011); mangosteen (Veerasamy *et al.,* 2011); *Mangifera indica* (Philip, 2011); *Polyalthia longifolia* (Kaviya *et al.,* 2011); *Andrographis paniculata* (Sulochana *et al.,* 2012); *Sesbania grandiflora* (Das *et al.,* 2013) and *Ocimum sanctum* (Ramteke *et al.,* 2013).

Molecular basis for the biosynthesis of silver nanoparticles is not known, but it is speculated that reducing sugars are responsible for the reduction of silver nitrate to silver nanoparticles (Shankar *et al.,* 2005). The organic matrix contains silver binding proteins that provide amino acid moieties that serve as the nucleation sites. Proteins / enzymes that have been found to be responsible for the reduction of metal ions when plant extracts are used for the synthesis of silver nanoparticles (Sawale *et al.,* 2008). In another study, it has been suggested that different compounds such as caffeine and theophylline bring out the reduction process and thus nanoparticles synthesis (Krishnaraj *et al.,* 2010). According to Zhou *et al.,* (2010), natural antioxidants have strong reducing ability. Polyols are mainly responsible for the reduction of Ag^+ (Geethalakshami and Sarada, 2010). Polyol compounds and the water-soluble heterocyclic compounds are mainly responsible for the

reduction of silver ions and the stabilization of the nanoparticles, respectively (Elumalai *et al.,* 2010).

Silver nanoparticles exhibited antibacterial activity against *Staphylococcus aureus* NCIM-2079 and *Proteus morgani* NCIM 2040 as it showed clear inhibition zones of 15 mm diameter and 20 mm respectively. Similar antibacterial activity of silver nanoparticles was reported against *E. coli* and *Pseudomonas aeruginosa* (Jain *et al.,* 2009); *Bacillus cereus* and *Pseudomonas aeruginosa* (Elumalai *et al.,* 2010); *Salmonella enterica* and *Staphylococcus aureus* (Das *et al.,* 2013); *Staphylococcus aureus, Escherichia coli, Salmonella typhi and Candida albicans* (Dar *et al.,* 2013). There are many views regarding the mechanism of action of silver nanoparticles on bacteria. Cell wall ruptures due to silver ions and silver nanoparticles (Lok *et al.,* 2007). The attachments of both silver ions and nanoparticles to the cell wall caused accumulation of envelop protein precursors which resulted in dissipation of the proton motive force. Silver nanoparticles also exhibited destabilization of the outer membrane and rupture of the plasma membrane there by causing depletion of intracellular ATP. The mode of action of both silver nanoparticles and silver ions were reported to be similar, although the nanoparticles were reported to be effective at significantly lower concentration than that of the ions. However it was proposed that the bactericidal mechanism of silver nanoparticles and silver ions are distinctly different. For treatment with silver nitrate, a low molecular weight central region was formed within the cell as a defence mechanism, whereas for treatment with nanoparticles, no such phenomenon was observed, although the nanoparticles were found to penetrate the cell wall (Morones *et al.,* 2005). It was suggested the possibility that the silver nanoparticles may also penetrate the bacteria and cause damage by interacting with phosphorus and sulphur containing compounds such as DNA (Hatchet and White, 1996). The study concluded that leaflet extracts of the fern *C. forinosa* Forsk. is capable of synthesizing silver nanoparticles in aqueous solution. These silver nanoparticles revealed to possess an antibacterial activity against *Staphylococcus aureus* NCIM-2079 and *Proteus morgani* NCIM 2040. The mechanism of synthesis of silver nanoparticles by plants can be used in the drug production for diseases which are caused by multidrug resistant microorganisms.

Acknowledgements

Authors are grateful to Rayat Shikshan Sanstha, Satara and Principal, Annasaheb Awate College, Manchar (Affiliated to University of Pune) for granting permission of executing the work and providing laboratory facilities at Plant Tissue Culture Research Laboratory.

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Manuscript Progress Date
Received : 04.06.2013
Revised : 06.07.2013
Accepted : 24.07.2013