Biogenic silver nanoparticles by *Solanum torvum* and their promising antimicrobial activity

K. Govindaraju, S.Tamilselvan, V. Kiruthiga and G. Singaravelu*

ABSTRACT

Nanotechnology is gaining tremendous impetus in the present century due to its capability of modulating metals into their nanosize. Research in nanotechnology highlights the possibility of green chemistry pathways to produce technologically important nanomaterials. This report focuses on the biological synthesis of silver nanoparticles using *Solanum torvum* and its antimicrobial activity. Characterization of newly synthesized silver nanoparticles was made using UV-vis spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, X-ray diffraction (XRD) and High Resolution Transmission Electron Microscope (HR-TEM) studies. Resistance to antimicrobial agents by pathogen has emerged in recent years and is a major health problem. *Solanum torvum* mediated silver nanoparticles showed high antimicrobial activity against bacterial and fungal pathogens. Our results suggest that *S. torvun* mediated silver nanoparticles could act as an effective antimicrobial agent and prove as an alternative for the development of new antimicrobial agents to combat resistance problem.

Key words: *Solanum torvum,* silver nanoparticles, antimicrobial assay

INTRODUCTION

Nanotechnology refers broadly to a field of applied science and technology whose unifying theme is the control of matter on the atomic and molecular scale (Anima Nanda and Saravanan, 2009). Metal nanoparticles have received considerable attention in recent years because of their unique properties and potential applications in catalysis (Kamat, 2002), plasmonics (Maier *et al*., 2001), optoelectronics (Gracias *et al*., 2002), biological sensor (Mirkin *et al*., 1996; Han *et al.,* 2001) and pharmaceutical applications (Chan and Nie, 1998). Their performance depends critically on their size, shape and composition. Chemical synthesis methods are available for the synthesis of metal nanoparticles, many of the reactants and starting materials used in these methods are toxic and potentially hazardous in concern with biological applications (Ankamwar *et al*., 2005). Consequently, an array of biological synthesis protocols leading to the formation of nanostructures have been reported using bacteria (Kalimuthu *et al*., 2008; Anima Nandha and Saravanan, 2009), fungi (Bhainsa and Souza, 2006; Vigneshwaran *et al*., 2006; Basavaraja *et al.*, 2008; Kathirasen *et al*., 2009) and plants (Chandran *et al.*, 2006; Huang *et al*., 2007; Kasthuri *et al.,* 2009a and 2009b; Singaravelu *et al*., 2009). In this context it is noteworthy to mention that synthesis of inorganic nanoparticles by biological systems makes nanoparticles more biocompatible and environmentally benign. We have recently reported on the biological synthesis of gold nanoparticles using a phytochemcial (20β-acetoxy-2∞-3β-dihydroxyurs-12-en-28-oic acid) and their PTP 1B inhibitory activity and in another attempt biological synthesis of silver, gold and Ag shell-Au core nanoparticles using single cell protein *Spirulina platensis* and seaweed *Sargassum wightii* have been achieved (Singaravelu *et al*., 2007). Keeping the biological perspectives in mind, the results reported herein encompass the biological synthesis of silver nanoparticles and their antimicrobial activity.

MATERIALS AND METHODS

Biosynthesis of silver nanoparticles

Silver nitrate was purchased from Qualigens Fine Chemicals, Mumbai, India. *Solanum torvum* leaves were collected from Vellore zone, Tamilnadu, India. UV–visible spectra were recorded on Shimadzu UV-1601 spectrophotometer containing double beam in identical compartments each for reference and test solution fitted with 1-cm path length quartz cuvettes. The FT-IR spectra were recorded using Perkin-Elmer FT-IR spectrophotometer. The $AgNO_3$ reduced *Solanum torvum* solution was centrifuged at 9,000 rpm for 25 min individually. The deposited residue was dried and grinned with KBr to obtain pellet for the purpose of FT-IR analysis. X-ray diffraction (XRD) measurements of the bioreduced

silver solution drop-coated onto glass substrates were done on a Siefert X-diffractometer instrument operating at a voltage of 40 kV and a current of 30mA with Cu K∞ radiation. Transmission electron microscopic images were collected with a JEOL 3010 UHR TEM equipped with a Gatan Imaging Filter (Ankamwar *et al*., 2005).

The leaf extract (1mL) was added to 50mL of 10^{-3} M $AgNO_3$ aqueous solution and kept at room temperature. The time of addition of extract into the aqueous $AgNO_3$ solution was considered as the start of the reaction. Under continuous stirring conditions, after 10 min, the light yellow colour of $AgNO_3$ solution gradually changes to brownish yellow colour indicates the formation of silver nanoparticles. The bioreduction of $AgNO_3^-$ ions in solution was monitored by periodic sampling of aliquots (0.1mL) of aqueous component and measuring UV-vis spectra of the solution. The nanoparticles were characterized and confirmed by FT-IR, XRD and HR-TEM analysis (Chandran *et al*., 2006).

Antimicrobial assay

The silver nanoparticles synthesized using *S. torvum* was tested for antimicrobial activity by agar well-diffusion method against pathogenic bacteriae *Pseudomonas aeruginosa*, *Staphylococcus aureus*, pathogenic fungi *Aspergillus flavus* and *Aspergillus niger*. The pure cultures of bacterial and fungal pathogens were subcultured on nutrient agar and Potato Dextrose Agar (PDA) respectively. Wells of 10 mm diameter were made on nutrient agar and PDA plates using gel puncture. Each strain was swabbed uniformly onto the individual plates using sterile cotton swabs. Using a micropipette, different concentrations of the sample of nanoparticles solution (10 µl, 20 µl and 50 µl) was poured onto each well on all plates. After incubation at 37°C for 24 hours, the different levels of zone of inhibition of bacteriae were measured. The fungal plates were kept at room temperature for 48 hrs and the clear zones were measured.

RESULTS AND DISCUSSION

Synthesis and application of nanomaterials is in the limelight in modern nanotechnology. The present investigation demonstrates the formation of the silver nanoparticles by the reduct ion of the aqueous silver metal ions during exposure to the plant extract *S. torvum*. Formation of silver nanoparticles was monitored by UV-vis spectroscopy. Present results disclose that the reduction of the $AgNO_3$ ions and formation of silver nanoparticles was completed in 60 min of reaction. The colourless solution changed into brownish yellow colour which indicates the formation of silver nanoparticles. The UV-vis spectra shows no evidence of absorption in the range of 400-800 nm for the plant extract and the plant extract solution exposed to $AgNO_3$ ions shows a distinct absorption at around 434 nm which corresponds to SPR of silver nanoparticles established at 420 nm (Mulvaney, 1996) (Fig 1). It is observed that the silver surface plasmon resonance band occurs initially at 430 nm after completion of the reaction, the wavelength of the surface plasmon resonance band stabilizes at 434 nm. In order to assess the stability of the newly formed silver nanoparticles UV-vis spectral analysis was made which shows that the surface plasmon absorbance did not change even after six months indicating the stability of the silver nanoparticles.

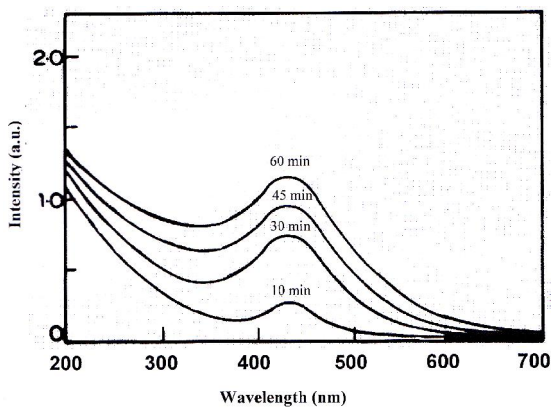

Figure 1. UV–vis spectra recorded as a function of the reaction time for the reaction of 1 mM $AgNO_3$ solution with *S. torvm* leaf extract.

FTIR measurements were carried out to identify the possible biomolecules responsible for the stabilization of the newly synthesized silver nanoparticles. Fig 2a represents the FTIR spectrum of the plain *S. torvum* leaf extract shows peaks at 1642, 1380, 1316, 1261 and 1020 cm^{-1}. The peaks observed for *S. torvum* stabilized silver nanoparticles at 1648, 1535, 1450 and 1019 cm^{-1}. The peak at 1450 cm^{-1} (-COO-) of carboxylate ions is responsible for stabilizing the silver nanoparticles (Fig 2b).

The X- ray diffraction patterns obtained for the silver nanoparticles synthesized using *S. torvum* leaf extract is shown in Figure 3. The presence of intense peaks of silver nanoparticles corresponding to the 1 1 1, 2 0 0 and 2 2 0, which are indexed as crystalline silver face-centered cubic (fcc) phase (Leff *et al*., 1996). According to Scherer's formula (Jeffrey, 1971), $t = 0.9l/ B\cos\theta$, an average crystal size (t) of the silver nanoparticles can be estimated from the X- ray wavelength of the Cu Kα radiation (l=1.54A°), the Bragg angle (θ), and the width of the peak at half height (maximum) (B) in radians. The average size of the silver

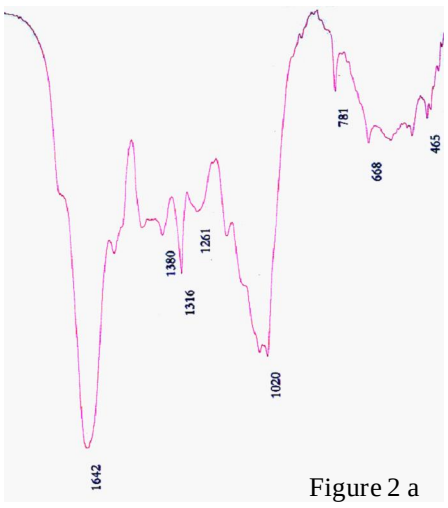

Figure 2 a

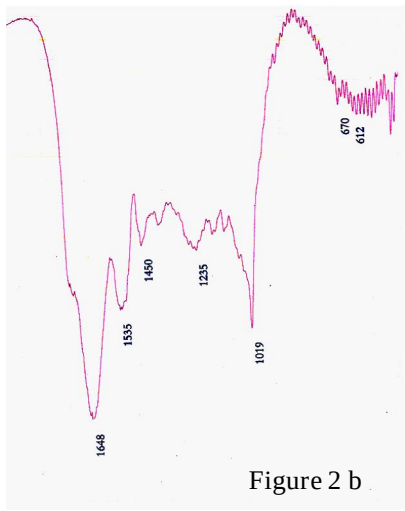

Figure 2 b

Figure 2 a and b FTIR spectrum of (a) Plain *S.torvum* leaf extract and (b) silver nanoparticles synthesized using *S.torvum* leaf extract

nanoparticles as calculated using the peak at 38° (which is the characteristic (111) peak of silver) is 14 nm. This result is quite comparable with what is observed from the TEM image of the reduction of $AgNO_3$ by *S .torvum* extract.

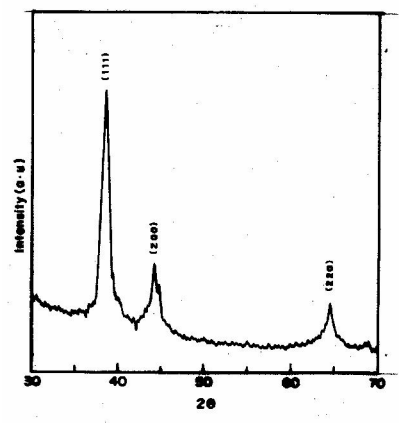

Figure 3. X-ray diffraction pattern of the silver nanoparticles. Silver nanoparticles were synthesized from 1mM silver nitrate-treated *S. torvm*

High Resolution Transmission Electron Microscopy (HR-TEM) has provided further insight in the morphology and size details of the silver nanoparticles.A representative HR-TEM image recorded from the silver nanoparticles is shown in Fig 4 a. The silver nanoparticles are spherical in structure. All the nanoparticles are well separated and no agglomeration was noticed. From the HR-TEM images we obtained the average size of silver nanoparticles of 14 nm. The histogram of the silver nanoparticles size distribution (Fig.4b) was obtained by measuring the size of about 125 silver nanoparticles.

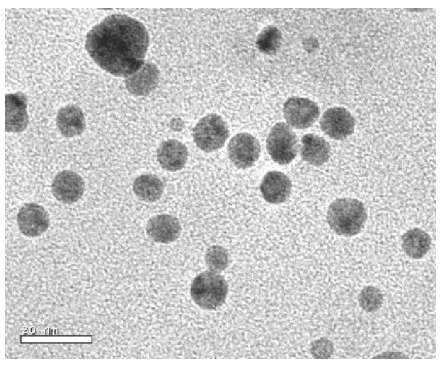

Figure 4a. TEM image of silver nanoparticles by *S. torvum*

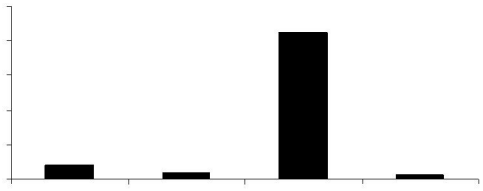

Figure 4b. Percentage distribution of *S. torvum* mediated silver nanoparticles

Synthesis and characterization of nanomaterials have become an area of intense research over the last few years. Several material scientists have reported the preparation

Table 1. Zone of inhibition (mm) of *S. torvum* mediated silver nanoparticles (µl)

Test organism	nanoparticles in µl		
	10 µl	50 µl	100 µl
Pseudomonas aureginosa	4.7	12.5	16.9
Staphylococcus aureus	5.2	11.9	17.6
Aspergillus flavus	4.3	10.7	15.2
Aspergillus niger	4.9	11.5	14.8

of nanomaterials of metals such as Au, Ag, CdS and CdSe using chemical and physical methods (Rockenberger *et al*., 1999; Murray *et al*., 1993; Sarathy *et al*., 1997; Duff *et al*., 1993). To the best of our knowledge, this is the first report on the synthesis of silver nanoparticles using the plant extract of *S. torvum*. Presently, silver nanoparticles are finding a variety of applications starting from biological tagging to electronic devices (Rao *et al*., 2003). A key challenge in the application of these materials is prevention of agglomeration of the nanomaterials, which was overcome in the present study and it may be due to the surface function utilization / stabilization of the *S. torvum* extract.

The antimicrobial activity of *S. torvum* mediated silver nanoparticles was performed against pathogenic bacteriae and fungi of silkworm *Bombyx mori*. Pathogens subjected in the present study were *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Aspergillus flavus* and *Aspergillus niger* using agar well diffusion method. The mean of three replicates of zone of inhibition (mm) around well with *S. torvum* mediated silver nanoparticles is presented in the Table 1.

The number of bacterial colonies grown on agar plates as a function of the different concentration of silver nanoparticles when gradually declined when the concentration of nanoparticles increased. Results clearly demonstrate that newly synthesized silver nanoparticles are promising antimicrobial agent against the pathogens employed.

The mechanism of the bactericidal effect of silver colloid particles against bacteriae is not very well-known (Ales Panacek *et al*., 2008). Silver nanoparticles may attach to the surface of the cell membrane and disturb its power function such as permeability and respiration. It is reasonable to state that the binding of the particles to the bacteria depends on the surface area available for interaction. Smaller particles having the larger surface area available for interaction will give more bactericidal effect than the larger particles (Ales Panacek *et al*., 2008). Morones *et al*. (2005) demonstrated using the Scanning Tunneling Electron Microscopy (STEM) and the X-ray Energy Dispersive Spectrometer (EDS), showed silver nanoparticles not only at the surface of cell membrane, but also inside the bacteria. This then suggests the possibility that the silver nanoparticles may also penetrate inside the bacteria and fungi, causing damage by interacting with phosphorus- and sulphur-containing compounds such as DNA. Silver tends to have a high affinity to react with such compounds. One more possibility would be the release of silver ions from nanoparticles, which will have an additional contribution to the antimicrobial properties of silver nanoparticles. Currently, the increase of bacterial resistance to antimicrobial agents poses a serious problem in the treatment of infectious diseases as well as in epidemiological practice. Increasingly, new bacterial strains have emerged with dangerous levels of resistance, including both of Gram-positive and Gram-negative bacteria. Dealing with bacterial resistance will require precautions that lead to prevention of the emergence and spreading of multiresistant bacterial strains, and the development of new antimicrobial substances (Ales Panacek *et al*., 2008).

Our results demonstrate the ability of the *S. torvum* on synthesizing silver nanoparticles and their antimicrobial activity represent a significant advancement in the nanomaterial with realistic implications. The green chemistry approach addressed in the present work on the synthesis of silver nanoparticles is simple, cost effective and the resultant nanoparticles are highly stable and reproducible.

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