Green synthesis, characterization and antibacterial activity of silver nanoparticles (Agnps) from grass leaf extract *Paspalum conjugatum* P.J. Berguis

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The present study was conducted to evaluate the green synthesis of silver nanoparticles by using grass leaf extract *Paspalum conjugatum* as a bioreductant. Characterizations of silver nanoparticles were done by using ultraviolet-visible spectroscopy (UV-Vis), Atomic force microscopy (AFM) and Fourier transform infrared (FT-IR) spectroscopy. UV-visible studies indicated the surface Plasmon resonance at 442 nm confirmed the formation of silver nanoparticles. AFM revealed spherical nanoparticles ranging from 12–30 nm in size. FTIR analysis revealed that functional groups hydroxyl ("OH), amine (N-H) groups and the protein component in the form of enzyme nitrate reductase produced by grass leaf extract are mainly involved in reduction of Ag+ ions to Ag0 nanoparticles. Silver nanoparticle showed the antibacterial activity against *E. coli* (MTCC 40), *Pseudomonas aeruginosa* (MTCC 424) gram negative and *Bacillus subtilis* (MTCC 619), *Staphylococcus aureus* (MTCC 96) gram positive bacteria. Green synthesized silver nanoparticles may play a major role in the field of biocontrol of plant diseases.

Key words: *Paspalum conjugatum,* silver nanoparticles, UV-Vis spectra, atomic force microscopy, Fourier transform infrared spectroscopy and Anti bacterial activity

INTRODUCTION

In recent years, nanotechnology has been emerging as a rapidly growing ûeld with numerous applications in science and technology for the purpose of manufacturing new materials (Savithramma *et al*, 2011a,b). Nanoparticles are being viewed as fundamental building blocks of nanotechnology and deûned as particles having one or more dimensions in the order of 100 nm or less. The most important and distinct property of nanoparticles is that they exhibit larger surface area-to-volume ratio. Among the nanoparticles studied so far, extensive research has been done on silver nanoparticles (AgNPs) keeping in view of their potential bio-medical applications (Sasikala, 2014). Different types of metallic nanoparticles like copper, zinc, titanium, gold, and silver, among others, have been produced by biological methods. However, the interest in silver nanoparticles has been increasing due to their high antimicrobial activity against bacteria, viruses and eukaryotic microorganisms (Rai *et al,* 2009). Silver nanoparticles have wide application in biomedical science like treatment of burned patients, antimicrobial activity and used the targeted drug delivery, and so forth (Singh *et al*, 2010). The most important application of silver and silver nanoparticles is in medical industries, in such medicines as topical ointments to prevent infection against burns and open wounds (Ip *et al,* 2006). The development of new chemical or physical methods has resulted in environmental contaminations, since the chemical procedures involved in the synthesis of nano materials generate a large amount of hazardous by products (Zhang *et al,* 2008).

The synthesis of metallic nanoparticles using a green procedure is of great interest in terms of introducing environmentally friendly synthesis pro-

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cedures. The importance of these nanoparticles is testiûed by the publication of many reviews on the biosynthesis and properties of metallic nanoparticles in the last 4 years (Rai *et al*, 2008; Mohanpuria *et al*, 2008; Bhattacharya *et al*, 2008; Chen *et al*, 2008; Sharma *et al.* 2009; Singh *et al,* 2009; Sinha *et al*. 2009; Korbekandi *et al*, 2009; Krumov *et al.* 2009; Kumar *et al*, 2009; Dura ´n *et al,* 2011; Narayanan *and Santhivel*, 2010; Popescu *et al*, 2010; Gade *et al*. 2010; Thakkar *et al,* 2010; Arya, 2010; Blanco - Anduzaret *et al*, 2010; Zhang *et al*, 2011). In the present study we used the silver nanoparticles synthesized from the grass leaf extract of *Paspalum conjugatum* and its antibacterial effect on the bacteria, namely, *E. coli* (MTCC 40)*, Pseudomonas aeruginosa* (MTCC 424) gramnegative and *Bacillus subtilis* (MTCC 619), *Staphylococcus aureus* (MTCC96) grampositive bacteria.

MATERIALS AND METHODS

Sample collection

The Grass leaf sample *Paspalum conjugatum* was collected from Tripura university campus, Suryamaninagar and brought into the laboratory for further process.

Preparation of leaf extracts

A total of 5 g grass leaf sample of *P.conjugatum* was washed repeatedly with distilled water to remove any organic impurities. The cleaned leaf sample were then boiled with 100 ml double distilled water for 15 minutes. This solution was then filtered through whatman filter paper no. 41 and stored 4^0C for further experiment.

Biological synthesis of silver nanoparticles

An aqueous solution of 1mM of silver nitrate (99.99% Sigma Aldrich) was prepared and used for the synthesis of AgNPs. Five ml of leaf extract was added into 95ml of 1mM silver nitrate to reduce Ag^+ to Ag^0 and incubated in Shaker incubator at 150 rpm at 37°C for 3 days which resulted a change in color.

Characterization of silver nanoparticles

UV- Vis spectra analysis

Synthesis of silver nanoparticles by reducing the silver ions solutions with leaf extract may be easily absorbed by UV-visible spectroscopy. The absorption spectra of the reaction mixture were measured using 200-700 nm range on “Perkin Elmer Lamda 25” spectrophotometer with scanning speed of 300 nm/minutes. For UV-Vis spectral analysis absorbance data were collected from spectrophotometer and plotted the data on software origin7.0.

Atomic Force Microscopy (AFM) analysis

The silver nanoparticles (AgNPs) extracted by the above protocol were visualized with an Atomic Force Microscopy (AFM). A thin film of the sample was prepared on a silicon wafer glass chip and was allowed to dry for 5min, the slides were then scanned with the AFM (Bruker).

Fourier Transforms Infra-Red Spectroscopy (FTIR) analysis

The powder sample of AgNPs was prepared by centrifuging the synthesized AgNPs solution at 10,000 rpm for 20 min. The solid residue formed is then washed with de-ionized water to remove any unattached biological moieties to the surface of the nanoparticles, which are not responsible for biofunctionalization and capping. The resultant residue was then dried completely and the powder obtained was used for FTIR measurements. FTIR spectra in solid phase were recorded as KBr pellets with a Perkin- Elmer FTIR –100 Spectrophotometer to identify the possible biomolecules in the leaf extract responsible for the reduction of ions and also the capping agents responsible for the stability of the biogenic nanoparticle solution.

Antibacterial Assays by Disc Diffusion method

Biosynthesized silver nanoparticles produced by the grass leaf extractof *Paspalum conjugatum* were tested for antibacterial activity against Gram-positive and Gram-negative bacteria by the disc diffusion method. Test bacterial strains were procured from IMTECH Chandigarh, India. The selected bacterial strains gramnegative *E. coli* (MTCC 40*), Pseudomonas aeruginosa* (MTCC 424) and grampositive *Bacillus subtilis (*MTCC 619), *Staphylococcus aureus (*MTCC 96) were cultured. Disc diffusion was carried out for the bacterial suspension containing10^6 cells. About 20 ml of sterilized nutrient agar media was poured into each sterile petriplates and allowed to solidify. 100 µl of the test

bacterial strains were evenly spread over the prepared plates by using a sterile glass spreader. Each Paper disc pouring with 10µl of distilled water (Negative control), grass leaf extract, silver nitrate solution (1mM), silver nanoparticle solution and antibiotic streptomycin (1mg/ml positive control) were then placed on prepared plates. These plates were incubated at 37°c for 24-48 hours. After incubation period, the results were recorded and the inhibition zone was expressed in mm.

RESULTS AND DISCUSSION

Five ml leaf extract of *Paspalum conjugatum* was added to 95ml aqueous silver nitrate solution (1mM), resulting in a rapid change in dark orange colour within 80 min due to excitation of surface plasmon vibration in metal nanoparticles (Fig. 1)

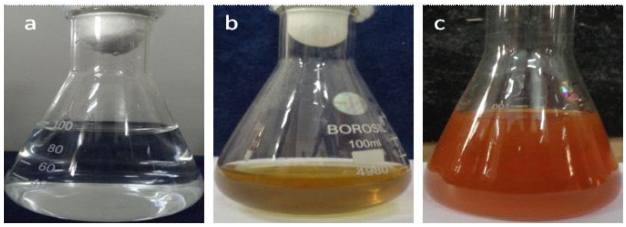

Fig.1: a) Silver nitrate (AgNO3) solution, b) Leaf extract and C) Silver nanoparticles (AgNPs) solution

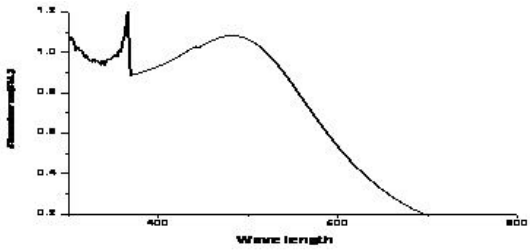

Fig. 2 : UV-Vis spectrum of bio functionalized AgNPs showing surface plasmon peak at 442nm

The absorbance scan taken by UV-Vis spectrophotometer (Perkin Elmer Lamda 25) showed a sharp Plasmon peak at 442 nm confirming the presence of silver nanoparticles (Fig: 2).

The silver nanoparticles were characterized by AFM for its detail size, morphology and agglomeration of silver. A 2 µm × 2 µm, AFM image is shown on Figure 3. A statistical treatment of AFM images was performed using specially designed image processing software (WS X M 4.0 Beta 7.0). From the AFM study it is very clear and can be predicted the shape of nanoparticles are nearly spherical with some ir-

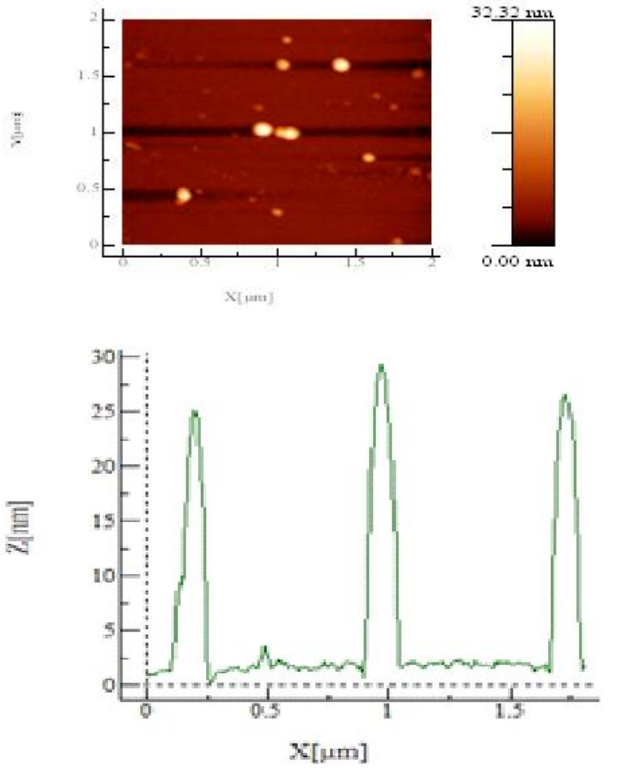

Fig 3 : Atomic force microscopy topographical image shows formation of spherical silver nanoparticles by leaf extract of *Paspalum conjugatum*

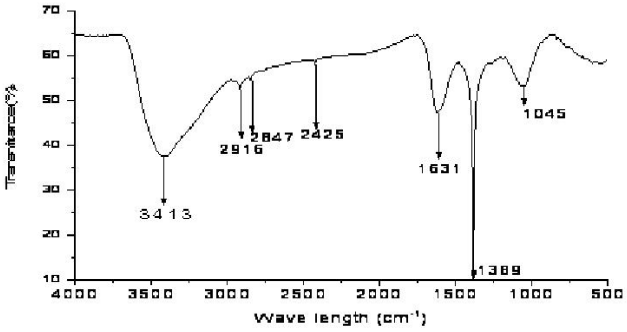

Fig. 4: FT-IR spectra representing the functional groups associated with the reduction and stabilization of leaf extract mediated silver nanoparticles

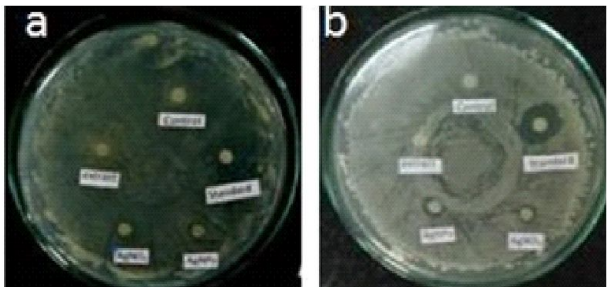

Fig. 5 : a) *Staphylococcus aureus* and b) *E. coli* shows antibacterial activity of silver nanoparticles (AgNPs)

regular shaped particles and are randomly distributed. The silver nanoparticles have average size 12-30 nm.

Fourier Transforms Infra-Red Spectroscopy (FTIR) analysis

FTIR measurement was carried out to identify the possible bio molecules in *Paspalum conjugatum* leaf extract responsible for capping leading to efficient stabilization of the AgNPs. The FTIR analysis was done for AgNPs. Fig. 4 showed the spectral data revealed two types of vibrations (stretching and bending) in the wavelength range of 4000 – 500 cm^{-1}. The representative spectra of nanoparticles obtained manifests absorption peaks located at about 3413 cm^{-1} corresponds to strong broad O-H stretching and H - bonded of alcohols and phenols groups; 2916 cm^{-1} represents to medium C-H Stretching of alkanes group; 2847 cm^{-1} corresponds to medium O-H stretching of carboxylic acids group; 1631 cm-1 corresponds to medium N-H bend of 1^0 amines group; 1389 cm^{-1} corresponding to medium and assignment of C-H deformation.1045 cm^{-1} corresponds to C-N stretching bend of alphatic amines group;

As compared to standard antibiotic streptomycin at a concentration of 1mg/ml among AgNPs, AgNO3 and grass leaf extract, the AgNPs showed the highest antibacterial activity against *E. coli (*11mm), *Staphylococcus aureus (*10mm) *Pseudomonas aeruginosa* (9 mm), and *Bacillus subtilis (*9 mm) in diameter. There was no inhibition zone of control (distilled water) and leaf extract against test bacterial strain (Fig.5).

In the present study leaf extract of *Paspalum conjugatum* was used for green synthesis of silver nanoparticles. Leaf extract 5 ml added to an aqueous 95 ml silver nitrate solution (1Mm), resulting in a rapid change in dark orange color within 80 min due to excitation of surface Plasmon vibrations in metal nanoparticles. It was reported that silver nanoparticles exhibit dark orange color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles(Mulvaney, 1996; Nagajyothi *et al*, 2014). Silver nanoparticles are known to exhibit a UV–Visible absorption maximum in the range of 400–500 nm . In this report the formation of AgNPs was initially confirmed using UV– Visible spectroscopy due to Surface Plasmon Resonance phenomenon-SPR (Chudasama *et al,* 2009). According to Krasovskii and Karavanshii (2008) the formation and stability of silver nanoparticles can be monitored by UV- vis spectral analysis. In the present study UV–Vis spectra of the solution of leaf extract with silver nitrate (1 Mm) showed a strong broad peak at 442 nm, which indicated the presence of AgNPs.. The FTIR spectrum of biosynthesized AgNPs showed peaks around at 3413,2916, 2847, 1631,1389 and1045 cm^{-1}. According to Manivasagan *et al*, 2013 the FTIR analysis revealed that the protein component in the form of enzyme nitrate reductase produced in grass leaf extract may be responsible for reduction and act as capping agents. In the present study overall observation confirms hydroxyl (-OH) and amine (N-H) groups in leaf extract are mainly involved in reduction of Ag+ ions to Ag0 nanoparticles. FT-IR spectra supports the presence of a protein type of compound on the surface of biosynthesized nanoparticles, conûrming that metabolically produced proteins acted as capping agents during production and prevented the reduced silver particles agglomeration. In the present study AFM analysis showed the topographical image of silver nanoparticles indicated that they are agglomerated, distinct spherical nanoparticles and synthesized AgNPs have an average size 12- 30 nm. This report also correlates with the work of

Table 1: Antibacterial activity of silver nanoparticles

Bacterial strain	Inhibition zone in diameter (mm)				
	Standard (positive control)	Silver nitrate (AgNO3)	Silver nanoparticles (AgNps)	Leaf extract	Distilled water (Nagetive control).
Staphylococcus aureus	16	7	10	0	O
Pseudomonas aeruginosa	14	6	9	0	0
Bacillus subtilis	22	7	9	0	0
E. coli	11	6	11	0	0

Ingle *et al,* (2008) and Saware and Vernataraman (2014), Alahmad *et al,* (2013) and Nagajyothi *et al,* (2014).

According to Gong *et al*, (2007) silver nanoparticles has made a remarkable comeback as a potential antimicrobial agent and proved to be most effective as it has good antimicrobial efficacy against bacteria, viruses and other eukaryotic micro-organisms. The use of silver nanoparticles is also important, as several pathogenic bacteria have developed resistance against various antibiotics. In the present study the AgNPs synthesized by green route were found to be very destructive against pathogenic Gram-positive and Gram-negative bacteria, like *Escherichia coli, Pseudomonas aeroginosa, Staphylococcus aureus, and Bacillus subtilus* using disc diffusion method on nutrient agar (NA) media. In the study synthesized AgNPs exhibited very good antibacterial activity against both Gram-positive (*Staphylococcus aureus, Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*) than silver nitrate ($AgNO_3$). Several workers have also investigated the antibacterial activity of biosynthesized silver nanoparticles against *Staphylococcus aureus, Escherichia coli, P. aeruginosa* and *K. pneumonia. (* Kora and Arunachalam, 2011; Amin *et al*, 2012; Jose *et al*, 2005; Lok *et al*, 2007; Kotakadi *et al*, 2013; Ruparelia *et al*, 2009; Gaddam *et al*, 2014). From the present study it is revealed that the silver nanoparticles can be biologically synthesized from grass leaf extract of *Paspalum conjugatum* collected from Tripura. Synthesized Silver nanoparticles can be used as effective growth inhibitors in antimicrobial control systems. The antibacterial property of the synthesized nanoparticles can be used in generation of drugs in various pharmaceuticals.

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