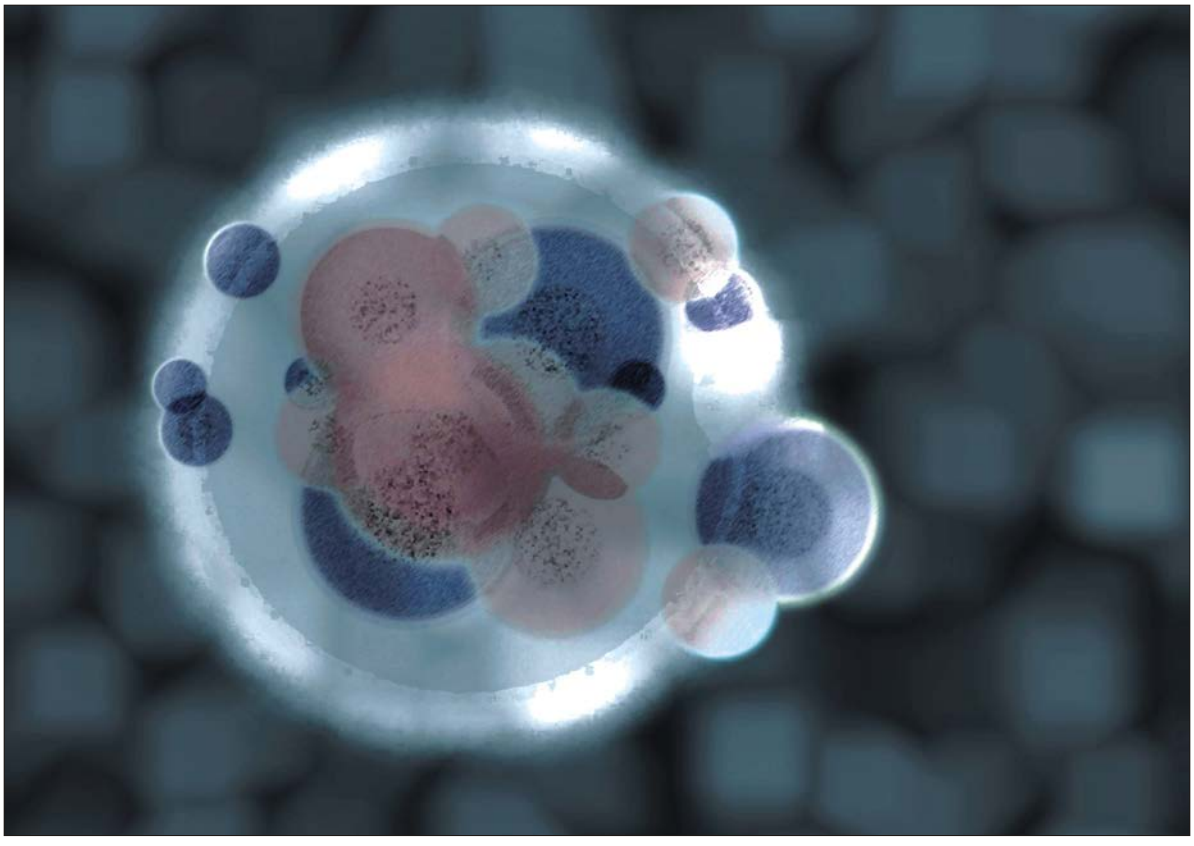

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Tuber Extract Mediated Biosynthesis of Silver Nanoparticles and its Antioxidant, Antibacterial Activity

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ABSTRACT
The biosynthesis of silver nanoparticles by the reduction of aqueous silver metal ions during revelation to both fresh, dry tuber extract and *in vitro* antioxidant, antibacterial activity of sweet potato were studied. Tuber extract of sweet potato was prepared for the synthesis of silver nanoparticles under different reaction time. The prepared materials were characterized by UV-visible spectroscopy, X-Ray Diffraction (XRD) and Fourier Transform Infrared Spectroscopy (FT-IR). During incubation period, the intensity of colour increased and its characteristic absorption was recorded at 428 and 439 nm. X-ray diffraction showed that the average particles size 4.77 and 5.6 nm. The FT-IR spectra of the tuber extract and synthesized silver nanoparticles revealed the reducing and capping actions of biomolecules such as amine, peptide, amide, lactone, polyphenol groups in protein linkages and it was found to be involved in the stabilization and biosynthesis of AgNPs. Scanning Electron Microscopy (SEM) showed silver nanoparticles was pure and the sizes were ranging from 1-10 µm. Green synthesized silver nanoparticles exhibited strong antioxidant and effective antibacterial activity against human pathogenic bacteria. Thus, green synthesis seems to be cost effective and alternate to conventional methods of silver nanoparticles synthesis. No synthetic reagent were used in this investigation and thus it is an environmentally safe method with potential for biomedical and agriculture application.

Key words: Green synthesis, tuber extract, $AgNO_3$, XRD, antioxidant, antibacterial

INTRODUCTION

Nanotechnology is an efficient and cost effective method for the synthesis of nanoparticles. It is an emerging and promising area in the modern medical and agricultural science. Nanotechnology is one of the important fields with many applications in the revolutionary medicine (Song and Kim, 2009). The biological activity of nanoparticle can be enhanced with increase in surface area which in turn increases surface energy and catalytic reactivity (Singh *et al*., 2010). Many synthetic routes are available for production of nanoparticles. But it is promising to develop procedures that are eco-friendly, non toxic and cost effective (Kataoka *et al*., 2001). In this context, biological route for the synthesis of nanoparticles becomes effective and important method. Biomolecules are found suitable and reliable for the production of metal nanoparticles as it has highly controlled and hierarchical assembly properties (Parshar *et al*., 2009). Various research groups are involved in the synthesis of nanoparticle using plant extract. Antioxidants deactivate free radicals which cause various diseases before they attack cells and biological targets (Hermans *et al*., 2007). In this aspect inorganic nanoparticles are effective in scavenging oxygen based free radicals (Babu *et al*., 2007). In addition to antimicrobial and

antioxidant studies also have vital importance and numerous studies are in advancement to clarify these aspects (Kim and Song, 2010). It is first of its kind to report on the synthesis of silver nanoparticle using aqueous extract of sweet potato a tropical tuber crops belongs to family convolvulaceae.

In this study, biological route was adopted to synthesize silver nanoparticles using sweet potato tuber extract for reduction of Ag ions silver nanoparticles from silver nitrate solution within 8 days of reduction time at ambient temperature. Thus it was also shown that the average size of silver nanoparticles can be controlled to 4.77-5.6 nm by varying the concentration of silver nitrate and the volume of tuber extract. Hence, the present study was designed to biosynthesize and characterized silver nanoparticles and to investigate the antibacterial and antioxidant activities of the synthesized nanoparticles.

MATERIALS AND METHODS

Chemicals: Analytical grade silver nitrate ($AgNO_3$) was purchased from Hi-Media (Mumbai, India). All other reagents used in this investigation were of an analytical grade.

Collection and processing of plant samples: The disease free tubers of sweet potato (*Ipomoea batatas* L.) were collected during the month of Jan-2012 in and around the villages of Ezhusempon, in Villpuram District, located at Tamil Nadu, India. The collected tubers were immediately brought to the laboratory. In the laboratory, the tubers were washed thoroughly in tap water and finally were rinsed with distilled water to remove extraneous materials. A tuber tissues were cut into small size and dry in a closed room (24-28ºC) for approximately 7 days. The dried plant parts were pulverized with a sterile electrical blender (Preethi) to obtain a powdered form. The powdered samples were stored in an air tight container and protected from sunlight for further use.

Extract preparation: When need, the dried finely powdered sweet potato (20 g) was extracted in 200 mL Double Distilled Water (DDW) at 60ºC for 20 min. The extracts were filtered through Whatman No. 1 filter paper and filtrate was used for further experiments.

In addition, fresh sweet potato tuber was washed thoroughly in running tap water in the laboratory for 10 min in order to remove extraneous materials, cut into small pieces and the rinsed with sterile distilled water. The washed tuber (20 g) was chopped finely and boiled 200 mL sterile distilled water for 10 min. The extracts were filtered through Whatman No. 1 filter paper and the filtrate was used for further experiments. The fresh and dry extract was concentrated under reduced pressure with rotary evaporator, poured into a pre-weighed vial and further dried in a desiccating chamber until a constant dry weight was obtained. The ethanolic tuber extract was further used for studying the various antioxidant assays.

Bio-synthesis of silver nanoparticles from the aqueous tuber extract of sweet potato: Five grams of filtrate powder fresh and dried sample was mixed with 100 mL of double distilled water and then the mixture was boiled for 5 min, cooled and filtered through Whatman No. 1 filter paper. The extract was used fresh within 2-3 h (Song and Kim, 2009). Ten milliters of tuber broth was added to 90 mL of 1 mM aqueous $AgNO_3$ solution for the reduction of Ag+ions in a 250 mL Erlenmeyer flask (Singh *et al*., 2010). All 4 flasks were incubated for 7 days at room temperature in the dark. The bio-reduction of the silver ions in the solution was monitored periodically measuring the UV-vis spectroscopy (300-600 nm) of the solution (Raut *et al*., 2010). The formation of a white-golden yellow colored solution indicated the formation of the silver nanoparticles. The silver nanoparticles obtained from the solution were purified by repeated centrifugation at 12,000 rpm for 20 min followed by dispersion of the pellet in deionized water three times to remove the water soluble biomolecules such as proteins and secondary metabolites (Raut *et al*., 2010). The water suspended nanoparticles were frozen at -80ºC overnight and then kept under vacuum for 24 h to dry the nanoparticles. After freeze drying of the purified silver nanoparticles, their structure and composition were studied by Scanning Electron Microscopy (SEM) Fourier Transform Infra-Red (FTIR) spectroscopy XRD. After the synthesized nanoparticles were weighed, they were resuspended in deionized water and stored in a freezer for further study.

Characterization of silver nanoparticles

UV-vis spectra analysis: The reduction of pure Ag+ions was monitored by measuring the UV-vis spectrum (Hitachi U-2910 spectrophotometer, japan) of the reaction medium at different time intervals by diluting a small aliquot (100 µL) of the sample 10 fold in deionized water. UV-vis spectroscopic analysis was performed by continuous scanning from 300-600 nm and 1 mM $AgNO_3$ solution was used for the baseline correction. The nanoparticles solution showed maximum absorbance at 428 and 439 nm.

FTIR analysis of $AgNO_3$ and aqueous tuber extract: To identify the biomolecules present in the fresh and dried tuber extract of sweet potato and the biomolecules within the AgNPs after the synthesis of the silver nanoparticles, FTIR spectra of the aqueous tuber extract and the purified AgNP powder were analysed by FTIR spectroscopy (FTIR Shimadzu 8400S, Japan). The FTIR analysis was performed with KBR pellets at Department of Chemistry, Annamalai University, Annamalai Nagar, Tamil Nadu, India. The FTIR was recorded in the range of 4000-500 cm^{-1}. The various modes of vibrations were identified and assigned to determine the different functional groups present in the extract and the AgNPs.

XRD-analysis: The XRD measurements of the silver nanoparticles solution drop-coated on glass were done on a

Shimadzu XRD-6000 model with 40 kV, 30 mA with CuK α-radiation at 2 θ angle. X-ray powder diffraction is a rapid analytical technique primarily used for phase identification of crystalline material and can provide information on unit cell dimensions. 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. $D = 0.94\lambda/\beta \cos\theta$.

SEM analysis: Purified AgNPs powder was characterized for nanoparticles shape and size with scanning electron microscope (JEOL-JSM6390, japan).

***In vitro* antioxidant assays**

Determination of Total Phenolic Content (TPC): The total phenolic content was determined by the Folin-Ciocalteau method (Ebrahimzadeh *et al*., 2008). Ethanolic tuber extract (0.5 mL, 1 mg mL^{-1}) and AgNPs (1 mg mL^{-1}) mixed with folin-ciocalteau reagent (5 mL, diluted 1:10 with distilled water) for 5 min, aqueous Na_2CO_3 (4 mL, 1 M) was then added to the mixture. The mixture was allowed to stand for 15 min and the phenolic content was determined by spectrophotometric method at 765 nm. The standard curve was prepared with 50, 100, 150, 200, 250 and 300 μg mL^{-1} solutions of Gallic acid in 50% methanol. The total phenolic content was expressed as Gallic Acid (GA) equivalents (mg GA g^{-1} dry weight).

DPPH free radical scavenging assay: The DPPH free radical scavenging assay was conducted based on the method of (Choi *et al*., 2002). One milliliter of 0.1 mM DPPH (in ethanol) was added to different concentration (50, 100, 150, 200, 250, 300 μg mL^{-1}) of ethanolic tuber extract and AgNPs. The reaction mixture was shaken and incubated in the dark for 30 min. The absorbance at 517 nm was measured against a blank (ethanol). Ascorbic acid was used as the standard. The lower absorbance of the reaction mixture indicated a higher percentage of scavenging activity. The percentage of inhibition or scavenging of free radicals was determined by the following formula.

$$\text{Inhibition (\%)} = \frac{\text{Control OD-sample OD}}{\text{Control OD}} \times 100$$

where, the control was prepared as described above without a sample.

Antibacterial screening

Bacterial pathogens and their growth conditions: Antibacterial pathogens *Staphylococcus aureus*, *Vibrio cholera*, *Proteus mirabilis*, *E. coli* and *Pseudomonas aeruginosa* were obtained from the Microbiology Laboratory of Raja Muthiah medical college, Annamalai University, Tamilnadu, India. The strains were maintained on nutrient agar slants at 4°C.

Preparation of inoculums: The active cultures for experiments were prepared by transferring a loop ful of cells from the stock cultures to Mueller Hinton Broth (MHB) tubes that were then incubated without agitation for 24 h at 37°C.

Agar well-diffusion method: Mueller-Hinton agar plates were swabbed on three axes with a sterile cotton-tipped swab that was first dipped in the freshly prepared diluted culture by standard method (Azoro, 2002). A 6 mm hole was bored aseptically with a sterile cork borer. The agar plugs were taken out carefully, without disturbing the surrounding medium. The holes were filled with 100 μL of different concentrations of AgNPs 10, 15, 20 μg mL^{-1} and allowed to stand for 1 h for the perfusion of the nanoparticles. The plates were kept for further incubation at 30°C for 24 h. After incubation, the petri dishes were evaluated for antibacterial activity, which was measured in terms of the diameter (millimeters) of the inhibition zone and ciprofloxacin was used as a control.

Statistical analysis: The group data were statistically evaluated using Student's t-test with SPSS/20 software. Values are presented as the Mean±SD. of three replicates of each experiment.

RESULTS

UV-vis Spectroscopic study: The change of colour intensity in fresh and dried tuber extract was noticed visually when the extract was incubated with the $AgNO_3$ solution. Absorption spectrum was recorded in incubated solution of fresh and dried sweet potato tuber extract at different wavelengths ranging from 300-600 nm revealed peak at 428 and 439 nm (Fig. 1). The intensity of absorption increased with duration of incubation and is shown in (Fig. 2a, b). The tuber extracts without $AgNO_3$ did not show any change in colour. The colour intensity is directly proportional to duration of incubation. The colour of the extract changed from white to golden yellow after one day of incubation and there was no significant change afterwards.

FTIR analysis: A precise evaluation of functional groups responsible for the stabilization of synthesized nanoparticles was carried out using FTIR measurements. The FTIR spectrum of fresh and dried sweet potato extracts. The fresh tuber extracts (Fig. 3a) showed resolved peaks at 3420, 2923, 2851, 1649, 1543, 1384, 1220, 1112, 825, 776 and 614 cm^{-1} unresolved peak at 1731, 1713, 1695, 1650, 1634, 1417 and 1453. The peaks obtained were due to amide I, II and III, aromatic rings, ether linkages, ketones, lactones, aldehyde, alcohol and saturated methyl groups found in the nanoparticles synthesized by sweet potato tuber extracts. The dried extracts (Fig. 3b) showed well resolved peak at 3427, 2923, 2847, 1630, 1384 unresolved peaks of 1154, 1114, 1077, 1022, 927, 854 and 757. The peaks obtained were due to amide I, II and

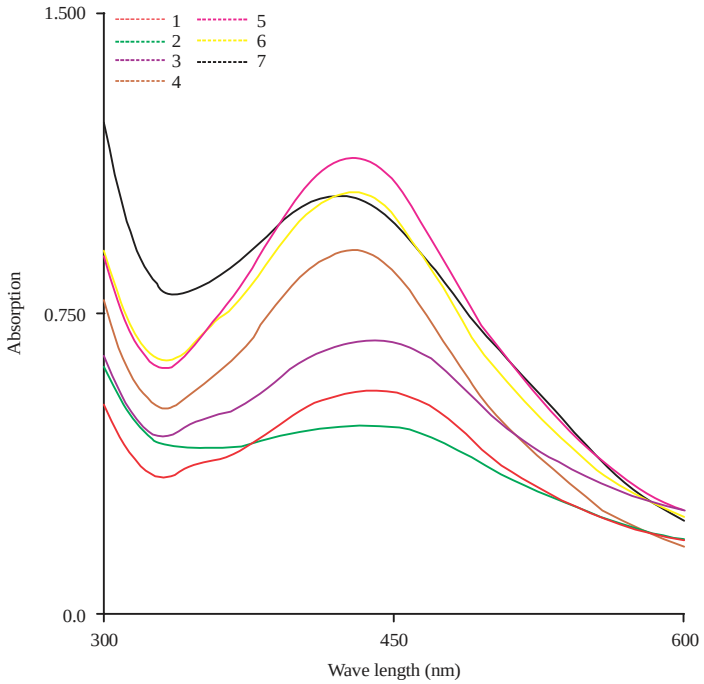

Fig. 1: UV-vis absorption spectrum of silver nanoparticle (1 mM) from fresh and dried tuber extract of the sweet potato at different time intervals (days), Dried sample: 1-3 (DSPTE: 439.00.0.770), Fresh sample: 4-7 (FSPTE: 428.50, 1.138)

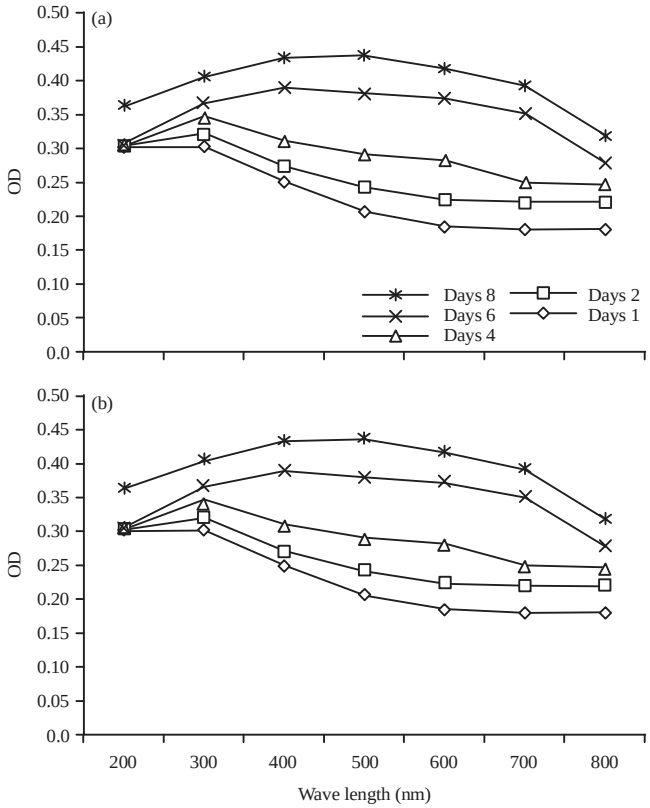

Fig. 2(a-b): Variation of UV-vis absorption spectrum of silver nanoparticle (1 mM) from fresh and dried tuber extract of the sweet potato at different time intervals (days)

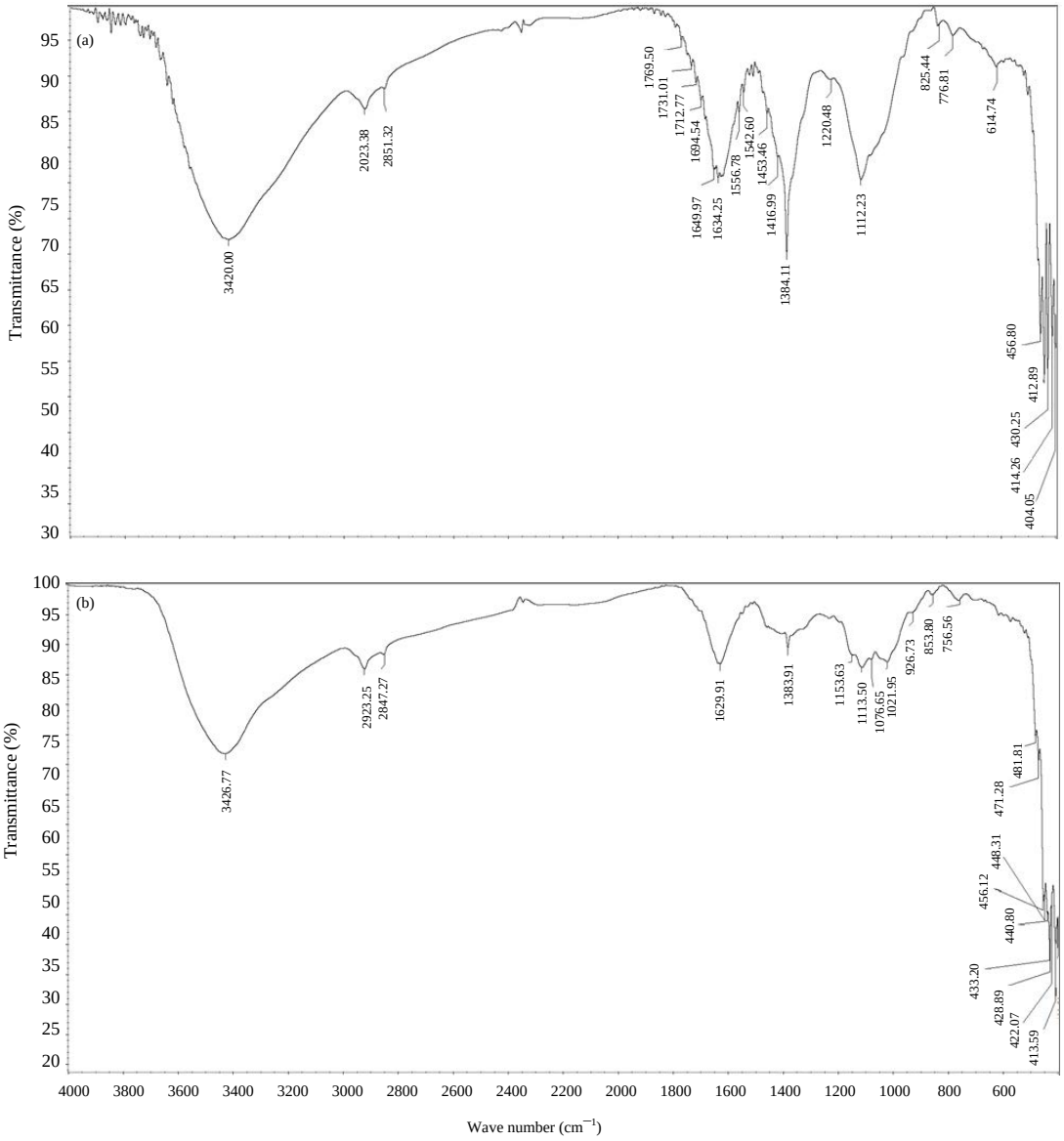

Fig. 3(a-b): FTIR spectrum of fresh sweet potato tuber extract

III, aromatic rings, ether linkages, alcohol, aldehyde and saturated methyl groups were found in the nanoparticles synthesized by sweet potato tuber extracts.

XRD analysis: The X-ray diffraction pattern of the biosynthesized silver nanostructure produced by the fresh and dry tuber extracts was demonstrated and confirmed by the characteristic peaks observed in the XRD image (Fig. 4a, b). The XRD pattern showed three strong peaks 32.12º, 36.22º, 40.24º and four intense peaks 38.18º, 44.33º, 64.53º, 77.43º in the whole spectrum of 2 θ values ranging from 10-80 and indicated that the structure of silver nanoparticles. The average grain size of the silver nanoparticles formed in the bioreduction process was determined using Scherrers formula and was estimated as 4.77-5.6 nm (Table 1).

SEM analysis: To characterize the topology and the size the nanoparticles synthesized SEM analysis of fresh and dried sweet potato tuber extract was carried out. The particles synthesized exhibits polydispersity of various sizes ranges from 1-10 µm (Fig. 5a, b). The SEM analyses showed most of the nanoparticles are aggregated and few of them are scattered.

***In vitro* antioxidant activity:** It has been found from the literature review that the antioxidant activity of the biosynthesized silver nanoparticles was not reported earlier.

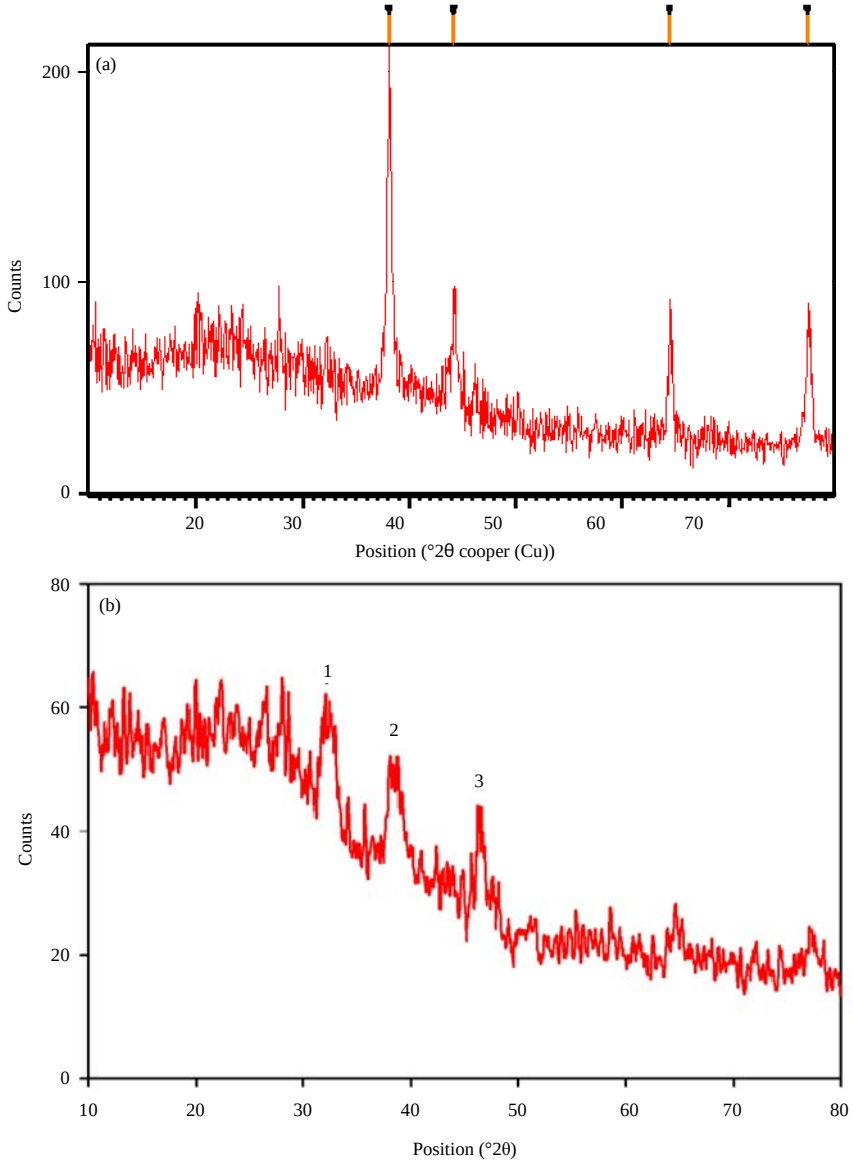

Fig. 4(a-b): XRD analysis of fresh and dried sweet potato tuber extract

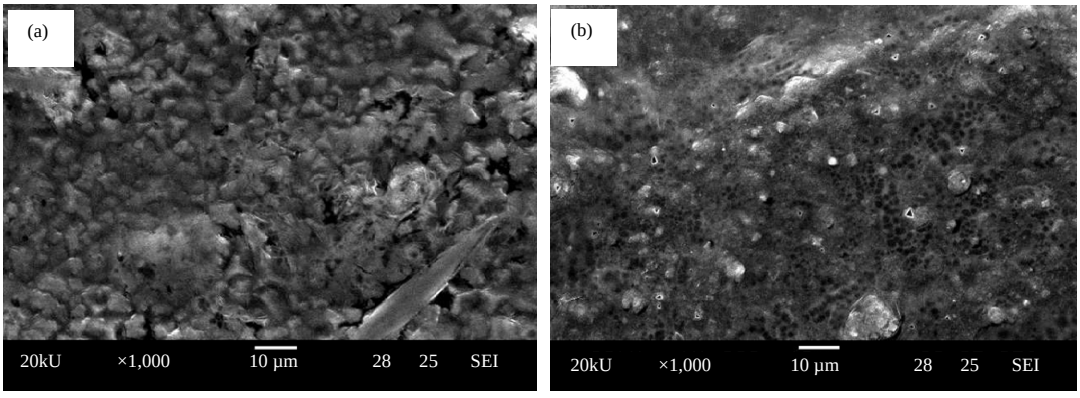

Fig. 5(a-b): SEM image of silver nanoparticles synthesized from fresh and dried sweet potato tuber extract

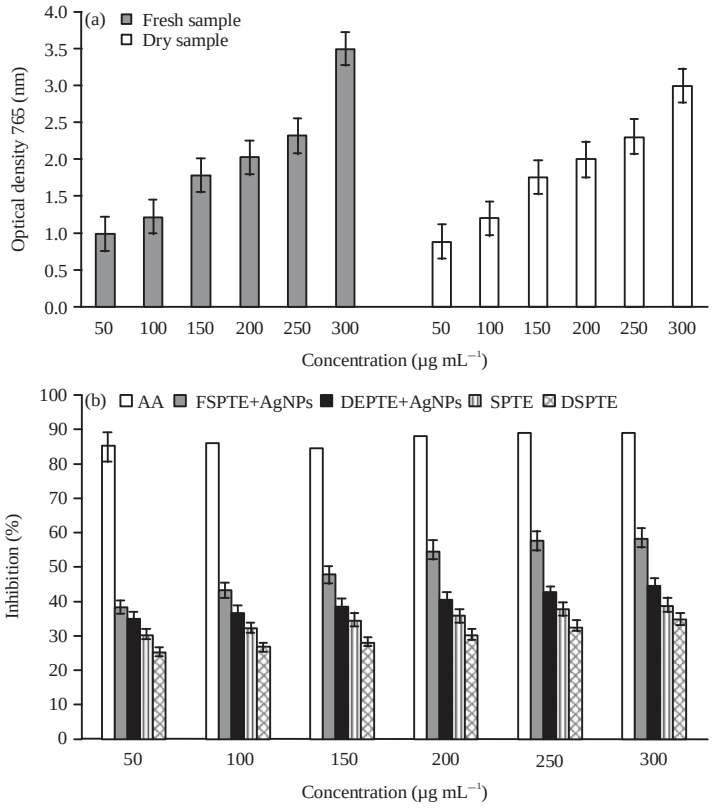

Fig. 6(a-b): Total phenolic and DPPH assay of the AgNPs from fresh and dried sweet potato tuber extract

Table 1: XRD analysis confirmed the size of nanoparticles

Position (°2θ)	FWHM, β (°θ)	Particle size, D (nm)
32.12	2	4.01
36.22	2	4.12
40.24	1	6.20
		Mean = 4.77
38.18	0.39	3.58
44.33	0.44	4.21
64.53	0.40	6.53
77.43	0.60	8.30
		Mean = 5.6 nm

Folin-Ciocalteau method was adopted to measure the phenolic content of nanoparticle (Fig. 6a). The phenolic content fresh and dried sample was recorded at 8th day and the values are 3.508±0.105 and 3.002±0.0900.

Positive DPPH tests showed that (8th FSPTE, DSPTE) and (8th DAgNPs) are free radical scavengers. The DPPH scavenging assay depicts effective inhibition activity of fresh and dried extract when compared with the standard ascorbic acid (Fig. 6b). The DPPH activity of nanoparticles was increased with increase in dose concentration. The (8th DAgNPs) showed more inhibition with (75%) scavenging activity of DPPH than SPTE.

Antibacterial studies: In the present investigation, the antibacterial effect of AgNPs at different concentrations (10, 15, 20 µg mL^{-1}) was quantitatively assessed on the basis of the zone of inhibition (Table 2). The AgNPs exhibited a maximum effect against *Staphylococcus aureus* with a zone of inhibition of 10.67±0.7 mm. When these results were compared with those for standard ciprofloxacin.

Table 2: Antibacterial activities of biosynthesized AgNPs at different concentration against human pathogenic bacteria

	Zone inhibition (mm)			
	AgNPs			Control
Concentration (µg mL^{-1})	10	15	20	15
Antibacterial agent				
Pseudomonad aeruginosa	7.0±0.4	9.0±0.7	12.0±0.6	7.0±0.6
Staphylococcus aureus	9.0±0.6	12.0±0.8	11.0±0.5	7.0±0.6
Vibrio cholera	8.0±0.5	8.0±0.6	10.0±0.8	6.0±0.5
Proteus mirabilis	6.0±0.4	7.0±0.4	9.0±0.7	0.5±0.5
Escherichia coli	7.5±0.5	8.0±0.5	6.0±1.0	0.5±0.4

Zone inhibitation values are expressed as Mean±SD

DISCUSSION

The development and biosynthesis of nanoparticles by reliable eco-friendly and easy methods stimulated the interest for its synthesis and applicability for the mankind (Bhattacharya and Gupta, 2005). When added to plant extract the reduction of silver ion is followed by its change of colour in our study the silver nanoparticles maintain golden yellow

colour (Yang *et al*., 2008; MubarakAli *et al*., 2011). This yellow coloration found is due to Plasmon absorption band in the range of 300-428 nm (Kong and Jang, 2006; Philip, 2011). The characterization of synthesized silver nanoparticles from sweet potato tuber extract was done using UV-vis spectroscopy at wave length range of 300-600 nm. The absorption of maxima at 428-439 nm for fresh and dried sweet potato confirmed the formation silver nanoparticles. This characteristic peak is similar to surface Plasmon vibration prepared by chemical reductions (Ahmad *et al*., 2003).

Concentration of silver nanoparticles was determined using SEM. At 3 keV the silver nanoparticles showed absorption peak due to the surface plasma resonances (Prasad *et al*., 2013; Jain *et al*., 2009; Veerasamy *et al*., 2011). The concentrations of silver nanoparticles in the sweet potato tuber extract like fresh 1-10 µm and dried 1-10 µm after eight day of incubation (Philip *et al*., 2011; Jaidev and Narasimha, 2010).

The identification of functional group responsible for stabilization of synthesized nanoparticles was done using FTIR measurement. The FTIR spectra of silver nanoparticles showed a strong peak at 1543 cm^{-1} which corresponds to the bending vibration of secondary amine of proteins. Another band at 1634 cm^{-1} was due to stretching vibration of (NH) C = O group. The decrease in intensity at 1543 cm^{-1} after the reduction of $AgNO_3$ indicates the involvement of the secondary amine in the reduction process (Guidelli *et al*., 2011). The shift of band 1634 cm^{-1} was due to the binding of (NH) C=O group with nanoparticles. These changes may also be due to the poly phenol which is oxidized to unsaturated carbonyl group (Sivaraman *et al*., 2009). The metal nanoparticles adsorbs on the surface is characteristic of flavonones (Carlson *et al*., 2008). The proteins/enzymes show the characteristic peaks in the amide I, II regions and are responsible for synthesis and stabilization of metal nanoparticles (Ahmad *et al*., 2003; Mukherjee *et al*., 2001). The free amine group or cysteine residues in the protein can bind silver nanoparticles (Gole *et al*., 2001) and bound proteins on the surface stabilize the silver nanoparticles during synthesis. Poly phenols are efficient reducing agent for nanoparticles synthesis (Krishnaraj *et al*., 2012; Yang *et al*., 2008). Thus the polyphenols and amide are responsible for the reduction of silver nanoparticles.

Silver is well known as one of the most important antimicrobial substances. The silver ion and silver-based compounds are highly toxic to microorganisms, which show a strong biocidal effect against the microbial species. The silver ion or salts impose limited usefulness as anti microbial agents due to its discontinuous release of inadequate concentration of silver ion from the metal ion and interfering effects of salts. However, these effects can be overcome by silver nanoparticle, which are more reactive due to large surface area (AshaRani *et al*., 2009; Jha and Prasad, 2010). Biosynthesized silver nano particle, displayed excellent antibacterial activity against all human pathogens. As the antibacterial activity was dose dependent, it increased linearly with increased concentration of test sample. In our study, the nanoparticle concentration was fixed at 250 g mL^{-1} (moderate dose) as higher dose may be toxic towards the host of pathogen. It has been reported by AshaRani *et al*. (2009), that silver nano particle exhibit a metabolic arrest of fibroblast cells (IMR-90) at higher concentration (200-400 g mL^{-1}) and toxicity is dependent on size of nano particle.

Superoxide anions are reactive species produced by transfer of one electron and involves in the formation of other reactive oxygen species such as hydrogen peroxide, hydroxyl radical or singlet oxygen in living system (Stief, 2003). Because of scavenging power, antioxidants turn to be effective in managing with disease like ulcer, stomach problems, cancer and AIDS. Antioxidant when reacts with nitric oxide forms peroxynitrite which can produce toxic radicals such as hydroxyl radicals (Halliwell, 1997; Sivakumar and Gajalakshmi, 2013). When compared the antioxidant activity of SNPs with standard ascorbic acid, it was found that antioxidant activity of nanoparticles was lower than standard in the DPPH free radicals scavenging assay. But in terms of reducing power assay, SNPs was found stronger than that of standard ascorbic acid. However, 8th DAgNps exhibited high free radical scavenging activity than ethanolic tuber extract. The tuber extract contains higher phenolic content which act as capping agent and influence the growth process of nanoparticles and in particular close packing sequences without any agglomeration.

CONCLUSION

The overall result emphasize that the synthesized nanoparticle (AgNPs) was characterized by UV, XRD, FTIR SEM. The antioxidant and antibacterial activities were more pronounced in the AgNPs. The higher level of phenolic content in the fresh and dried sweet potato tuber extract indicate that it can act as capping agents, for the stable growth of nanoparticle in particular closed packing sequences without agglomeration.

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