

Enhancement of antidandruff activity of shampoo by biosynthesized silver nanoparticles from *Solanum trilobatum* plant leaf

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Abstract The present investigation describes simple and effective method for synthesis of silver nanoparticles via green route. *Solanum trilobatum* Linn extract were prepared by both conventional and homogenization method. We optimized the production of silver nanoparticles under sunlight, microwave and room temperature. The best results were obtained with sunlight irradiation, exhibiting 15–20 nm silver nanoparticles having cubic and hexagonal shape. Biosynthesized nanoparticles were highly toxic to various bacterial strains tested. In this study we report antibacterial activity against various Gram negative (*Klebsiella pneumoniae*, *Vibrio cholerae* and *Salmonella typhi*) and Gram positive (*Staphylococcus aureus*, *Bacillus cereus* and *Micrococcus luteus*) bacterial strains. Screening was also performed for any antifungal properties of the nanoparticles against human pathogenic fungal strains (*Candida albicans* and *Candida parapsilosis*). We also demonstrated that these nanoparticles when mixed with shampoo enhance the anti-dandruff effect against dandruff causing fungal pathogens (*Pityrosporum ovale* and *Pityrosporum folliculitis*). The present study showed a simple, rapid and economical route to synthesize silver nanoparticles and their applications hence has a great potential in biomedical field.

Keywords Antidandruff activity · Antimicrobial activity · Multidrug resistant pathogens (MDR) · Ag nanoparticle · *Solanum trilobatum* Linn

Introduction

Superbugs have become a serious hitch in public healthcare sector (Alanis 2005). Due to routine use of wide range antimicrobial drugs, various categories of multidrug resistant (MDR) pathogens have emerged (Davies and Davies 2010). These are the main source of nosocomial infections, blood stream infections, multidrug efflux pumps, histoplasmosis and paracoccidioidomycosis caused by *Enterococcus faecalis* (Saxena and Gomber 2010) *Acinetobacter baumannii* (Eiff et al. 2001) *Pseudomonas aerogenosa* (Nelson 2002) *Histoplasma capsulatum* (Hartmann et al. 2011) and *Paracoccidioides brasiliensis* (Kerr et al. 1988) respectively. These infections have a high mortality effect similar to MRSA bacteria (Chudasama et al. 2009). At this juncture the development of new techniques without involving antibiotics and hence the problem of resistance are required.

Nanoparticles and their applications are one of the recent approaches among them. Nanometer-size metallic particles show unique and considerably altered physical, chemical and biological properties compared to their macro scaled counterparts. The difference is mostly due to their high surface-to-volume ratio (Ansari et al. 2010). They exhibit transitional dimensions between bulk materials, atoms and molecules. The extensive applications they offer have warranted substantial research on them in recent years.

Silver nanoparticles exhibit size and shape-dependent properties (Rosarin and Mirunalini 2011; Sun and

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Changhua 2011) that are of interest for applications ranging from photonics (Maier et al. 2001), food packaging (Duncan 2011), catalysis (Astruc et al. 2005), sensing (Lopez-Acevedo et al. 2010), electronics (Nie and Emory 1997; Dick et al. 2001; Li et al. 2010a; Fendler 2001; Ozbay 2006), optoelectronics (Kamat 2002), and medicine (Giljohann et al. 2010; Brown et al. 2007). Biocidal or antimicrobial activity is one of the important properties of silver as it is highly toxic to most of the living cells including bacteria and fungi. These properties can also be influenced by alteration in structure and chemical–physical environment (Zhang et al. 2006). Therefore specific control on size and dimension during synthesis is important and is achieved through various methods including use of reducing agents and stabilizers (Lu et al. 2007).

Solanum trilobatum Linn (Solanaceae) plant is a thorny shrub, well known in Ayurveda and Siddha system as ‘Alarka’ and ‘Tuduvelai’, respectively (Zahir et al. 2010). The decoction of entire plant is has been administered to cases of acute and chronic bronchitis. Roots, berries and flowers are used for cough. Previous reports indicated that some chemical constituent, such as solasodine and β -solasamarine have been isolated from whole plant (Jahan et al. 2011). Pharmacological investigations have demonstrated that *Solanum trilobatum* Linn possess antioxidant, hepatoprotective, anti-inflammatory and analgesics activities. The present study has been designed to evaluate the Antidandruff activity of biosynthesized silver nanoparticles from *Solanum trilobatum* leaves.

Materials and methods

Chemicals and collection of plant materials

Silver nitrate solution (99.8 %) purchased from Hi Media Laboratories Pvt. Limited, Mumbai, India. 1 mM AgNO_3 solution was prepared and stored in amber colour bottle. The leaves of *Solanum trilobatum* Linn. were collected from the coastal area of Kerala, India.

Preparation of extract by conventional and homogenization method

The fresh collected leaves of *Solanum trilobatum* Linn were washed several times with deionised water. For conventional method, 100 gm of finely cut *Solanum trilobatum* leaves were taken and boiled in 300 ml of deionised water for 3 min and filtered, followed by the step of centrifugation at 10,000 rpm for 15 min. Finally supernatant was collected and stored at 4 °C; while 100 g of washed leaves of *Solanum trilobatum* was homogenized in 300 ml water with the help of mortar and pestle and

filtered. Then the filtrate was centrifuged for 15 min at 10,000 rpm and supernatant was collected and stored at 4 °C.

Optimization and synthesis of silver nanoparticles under sunlight irradiation

Different concentration of leaf extracts (1, 3 and 5 ml) were taken in conical flask separately. 10 ml of 1 mM AgNO_3 solution was added with constant stirring and exposed under sunlight radiation and observed. The colour change of the solution was checked periodically, bioreduction of silver ions in the solution was monitored by measuring using UV–Vis spectra then the conical flask was incubated at room temperature for 48 h. The colour change of the leaf extract from yellow to dark brown indicated the silver nanoparticles were synthesized from the leaves. The content was centrifuged at 10,000 rpm for 15 min. The supernatant was used for the characterization of the silver nanoparticles. The same method was performed for both conventional and homogenized method of extracts.

Synthesis of silver nanoparticles under microwave irradiation

1, 3 and 5 ml of leaf extracts were mixed separately with 10 ml of 1 mM AgNO_3 solution with constant stirring, and the whole mixture was kept in a domestic microwave oven (National Model NN-GD 576 M). The sample was subjected to several short burst of microwave irradiation at frequency of 2.45 GHz, at power output of about 100 W in a cyclic mode (on 15 s, off 15 s) to prevent overheating as well as aggregation of metals. The irradiation process was conducted for a minimum of 5 up to maximum of 15 cycles. The reduction of Ag^+ ions was monitored by sampling an aliquot (2 ml) of the solution after 5, 7, 9, 12 and 15 cycles and measuring the UV–Vis spectra of the solution. Absorption measurements were carried out on a Genesys 10 UV–Vis spectrophotometer. The spectra were recorded at room temperature using a 1 cm quartz cuvette and characterized. The same was performed for both conventional and homogenized method of extracts.

Synthesis of silver nanoparticles under room temperature

10 ml of 1 mM AgNO_3 solution was added to different concentration of leaf extracts (1 ml, 3 ml and 5 ml) and incubated under room temperature for 2–3 days and observed the colour change. The colour change of the solution was checked at every 24 h. Bioreduction of silver ions in the solution was monitored by measuring using UV–Vis spectra. The colour change of the leaf extract from

yellow to dark brown indicated the silver nanoparticles were synthesized from the leaves. The content was centrifuged at 10,000 rpm for 15 min. The supernatant was used for the characterization of the silver nanoparticles. The same was performed for both conventional and homogenized method of extracts.

Production and recovery of silver nanoparticles by centrifugation

Among various concentration and methods used, sunlight irradiation method was very effective and 1 ml of homogenized leaf extract was shown maximum synthesis of nanoparticles. Further it was chosen for bulk production as 10 ml leaf extract in 100 ml of 1 mM AgNO₃. After bio-reduction, the solution consisting of hydrosols of silver nanoparticles was subjected to centrifugation (Remi, Cooling Centrifuge-C-24BL) at 10,000 rpm for 15 min, and the supernatant was discarded. The pellet formed was dissolved in 0.1 ml of toluene water and air dried.

Investigation of silver nanostructures

UV–spectrophotometer (Model–U-2900) is used for characterization and monitoring the signature of silver nanoparticles. In particle, noble metal particles are ideal candidates for UV–Vis spectroscopy study, since they exhibit strong surface plasmon resonance absorption in the visible region and are highly sensitive to the surface modification (Mary and Inbathamizh 2012). XRD measurement was carried out by Philips X-Ray Diffractometer with Philips PW 1830 X-Ray Generator, at Department of Nuclear Physics, University of Madras, Chennai, India. The air dried nanoparticles were coated onto XRD grid and analysed for the formation of Ag nanoparticle at a voltage of 40 kV and a current of 30 mA with Cu K α radiation ($\lambda = 0.15406$). The diffracted intensities were recorded from 10' to 80' of 2θ angles at room temperature (300 K). Central workshop, Department of Mechanical Engineering, Anna University, Chennai, India, helps for the SEM analysis. The study was undertaken to know the size and shape of the silver nanoparticles biosynthesized by using *Solanum trilobatum*. After synthesis of nanoparticles, the sample was filtered through Millipore filters of 0.2 μ m pore size, to remove any contaminants interfering with the SEM images. Coating of the samples with platinum was done by using the coater (JEOL, Japan, Model No.JFC-1600), finally the images of nanoparticles were obtained in Scanning Electron Microscope at applied voltage of 15 kV, magnification at 10,000 \times and 5,000 \times . FTIR investigation was performed at Department of Chemistry, Anna University, Chennai, India. The dried Ag nanoparticles were subjected to FTIR analysis by KBr pellet (FTIR grade) method in 1:100 ratios and

spectrum was recorded in Nicolet Impact 400 FT-IR Spectrophotometer using diffuse reflectance mode operating at a resolution.

Antibacterial activity of silver nanoparticles against human pathogenic bacteria

Silver nanoparticles synthesized using *Solanum trilobatum* were tested for its potential antibacterial activity against few Gram positive and Gram negative human pathogens. *Staphylococcus aureus*, *Bacillus cereus*, *Micrococcus luteus*, *Klebsiella pneumoniae*, *Vibrio cholerae* and *Salmonella typhi* were used as the test organisms.

Agar diffusion assay was used to determine the antibacterial activity of silver nanoparticles from leaf extract. Sterile nutrient agar plates were prepared, latter swabbed with 24 h growing culture of test organisms. The wells (10 mm diameter) were made by using cork borer. Different concentrations (25, 50, 75, 100 μ g) of the silver nanoparticles were loaded in the wells. The plates were then incubated at 37 °C for 24 h. Antibacterial activity was measured by measuring zone inhibition.

Antifungal activity of silver nanoparticles against human pathogenic fungi-well diffusion assay method

Agar diffusion assay were used to determine the antifungal activity of silver nanoparticles from leaf extract. Potato Dextrose Agar (PDA) prepared was poured in the petri dish. 24 h growing culture *Candida albicans* and *Candida parapsilosis* were swabbed on it. The wells (10 mm diameter) were made by using cork borer. The different concentrations (25, 50, 75, 100 μ g) of the silver nanoparticles were loaded in the wells. The plates were then incubated at 37 °C for 24 h. The inhibition diameter was measured.

Antidandruff activity of silver nanoparticles from *Solanum trilobatum* leaf extract collection and maintenance of culture

Dandruff causing fungal pathogens, *Pityrosporum ovale* and *Pityrosporum folliculitis* were collected from Armats-biotek Training and Research Institute, Chennai, India and maintained on Leeming Notman and Potato Dextrose Agar, slant and broth.

Agar diffusion assay is used widely to determine the antidandruff activity of silver nanoparticles from leaf extract. The technique works well with defined inhibitors. Potato Dextrose Agar (PDA) prepared was poured in the petri dish. Twenty-four hours broth culture of *Pityrosporum ovale* and *Pityrosporum folliculitis* were swabbed over the surface of Potato Dextrose Agar. All the active nanoparticles and the

shampoos were dissolved in water at 10 mg/ml concentration. A circle of 10 mm diameter was cut at the surface of the agar and 100 μ l of the above prepared samples were loaded on the well. Plates were incubated at 37 °C for 3–5 days. After incubation the zone was measured using zone measuring scale and recorded.

Results and discussion

Biosynthesis of silver nanoparticles

Plant materials were collected and plant leaf extraction was prepared both by conventional (Govindarajan et al. 2008) and homogenized method (Fig. 1). Biosynthesis of silver nanoparticles by the filtrate of *Solanum trilobatum* was confirmed by change in the color of the filtrate to brown after addition of silver nitrate (Fig. 2). This arises due to excitation of surface plasmon vibrations in silver nanoparticles (Govindaraju et al. 2008). As the *Solanum trilobatum* plant leaf extract was mixed in the aqueous solution of the silver ion complex, it started to change the



Fig. 1 Plant leaf extracts **a** conventional method. **b** Homogenization method

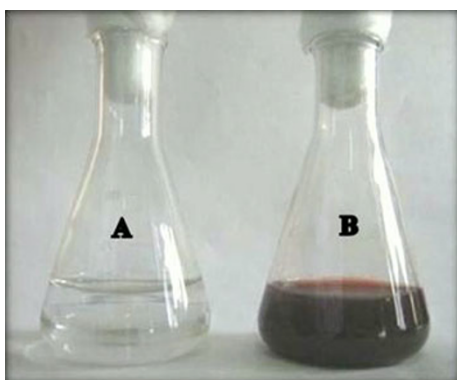


Fig. 2 Synthesis of silver nanoparticle **a** before, **b** after reaction

color from watery to yellowish brown due to reduction of silver ion, which indicated formation of silver nanoparticles (Kanchana et al. 2010, 2012).

Investigation of *Solanum trilobatum* synthesized silver nanoparticle

The bioreduction of Ag^+ with examination of size and shape of nanoparticles in aqueous suspensions was monitored by periodic sampling of the reaction mixture at regular intervals by using UV–Vis spectroscopy (Wu et al. 2010; Raghunandan et al. 2011). Control flasks maintained with silver nitrate solution (without culture filtrates) did not show any change of color and its absorbance maximum was found to be at 340 nm, which is specific for silver nitrate solution. Whereas sunlight irradiated reaction mixture consisted of culture filtrate with silver nitrate (Fig. 3) showed a strong characteristic absorbance peak at around 430 nm, broadening of peak indicated that the particles are

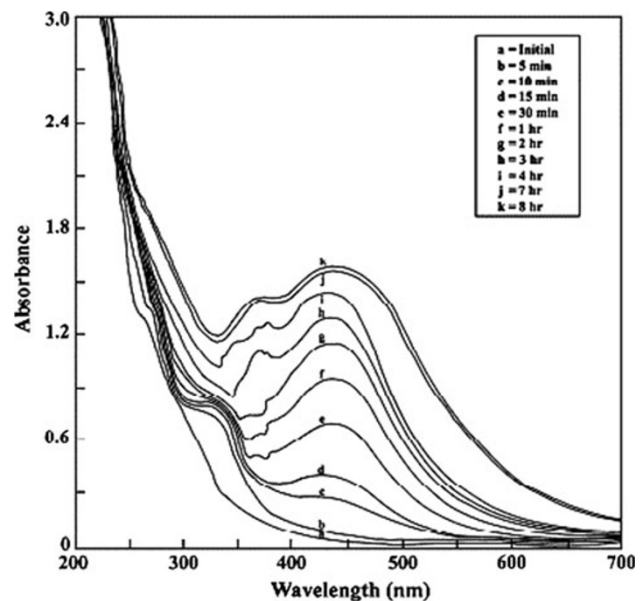


Fig. 3 UV–Vis spectra of silver nanoparticle

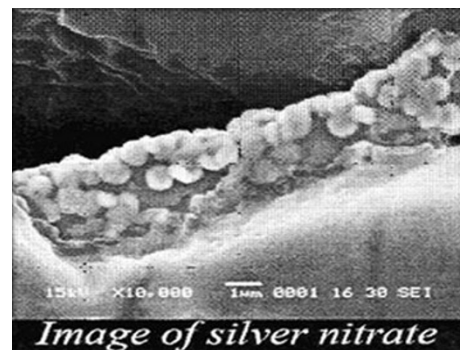


Fig. 4 SEM analysis of silver nitrate

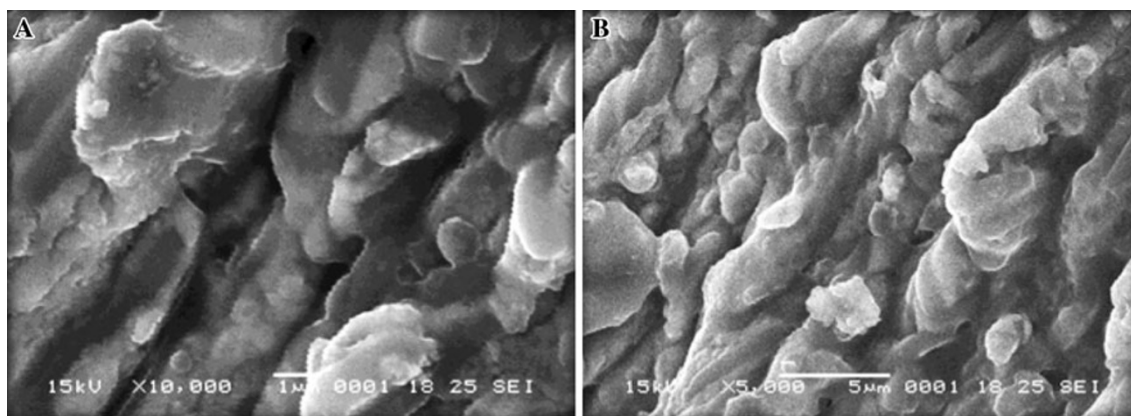


Fig. 5 SEM analyses of **a** silver nanoparticle in 1 μm and **b** silver nanoparticle 5 μm

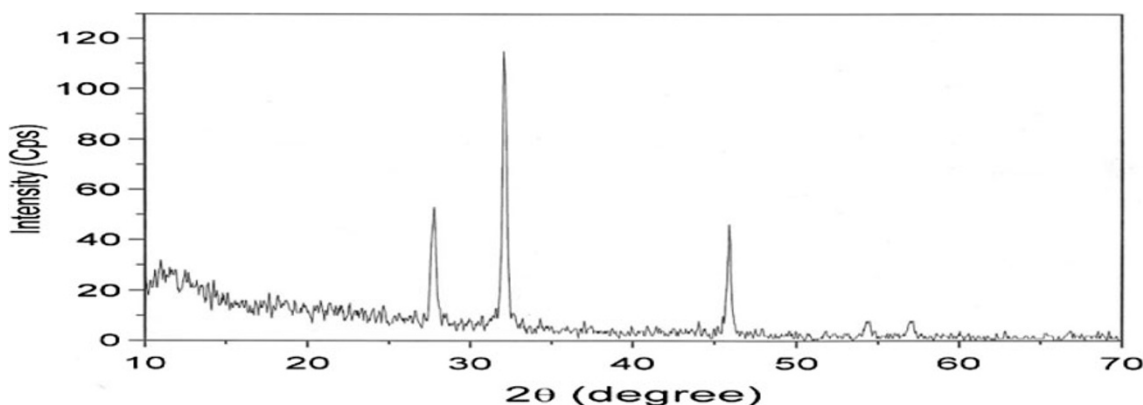


Fig. 6 The XRD pattern of the silver nanoparticles

polydispersed. Analysis by spectrophotometer was made up to 8 h with all the combinations, in which homogenized method followed by sunlight irradiation optimize the best results therefore used for further studies.

Scanning electron microscopic analysis of the silver nitrate solution (Control) and reduced form of silver nitrate solution, clearly distinguishable owing to their size difference. It is clear from the SEM pictures that control silver nitrate particles are more than 1,000 nm size, whereas silver particles in the bioreduced colloidal suspensions measured 15–20 nm in size. Figure 4 is the SEM of silver nitrate and Fig. 5 is the SEM of bioreduced silver nitrate. The SEM image showing the high density silver nanoparticles synthesized by the *Solanum trilobatum* plant leaf and confirmed the development of silver nanostructures.

The biosynthesized silver nanostructure employing *Solanum trilobatum* plant leaf extract was further demonstrated and confirmed by the characteristic peaks observed in the XRD image. The XRD pattern showed intense peaks at 28.18°, 38.18°, 46.18°, 64.1° and can be indexed the angle values of (111), (200), (220), (240) crystalline planes of cubic Ag in the whole spectrum of 2θ value ranging from 10 to 80 (Marimuthu et al. 2011; Bee et al. 2002; Cihlar

et al. 1999). This analysis revealed that average size of the particles synthesized was 15 nm derived from the FWHM of peak corresponding to 111 plane with size range 10 to 50 nm with cubic and orthorhombic crystals. The high peaks in the analysis indicate the active silver composition with the indexing (Fig. 6). The typical XRD pattern revealed that the sample contains a mixed phase (cubic and hexagonal) structures of silver nanoparticles.

The presence of some functional group as revealed by IR spectral is shown in Fig. 7. The FTIR spectral analyses of silver nanoparticles show certain common absorption band at 3,398 cm^{-1} is a characteristic of hydroxyl ν (O–H) and ν (N–H) vibrational frequency which are interchangeable. A common 3 vibrational peak between 2,899 and 2,977 cm^{-1} are characteristic of a ν (C–H) symmetrical vibration of saturated hydrocarbon. The vibrational frequency ν (C–O) was observed in the spectra of the extracts at 1,047 and 1,087 cm^{-1} . Deviation from this region to a higher wave number was observed which is indicative of a secondary amide. These peaks were sharper than the ν (O–H) peaks due to reduction in hydrogen bonds which increases with electronegativity. Vibrational peaks at 1,654 cm^{-1} in extract signify the possibility of an aromatic compound

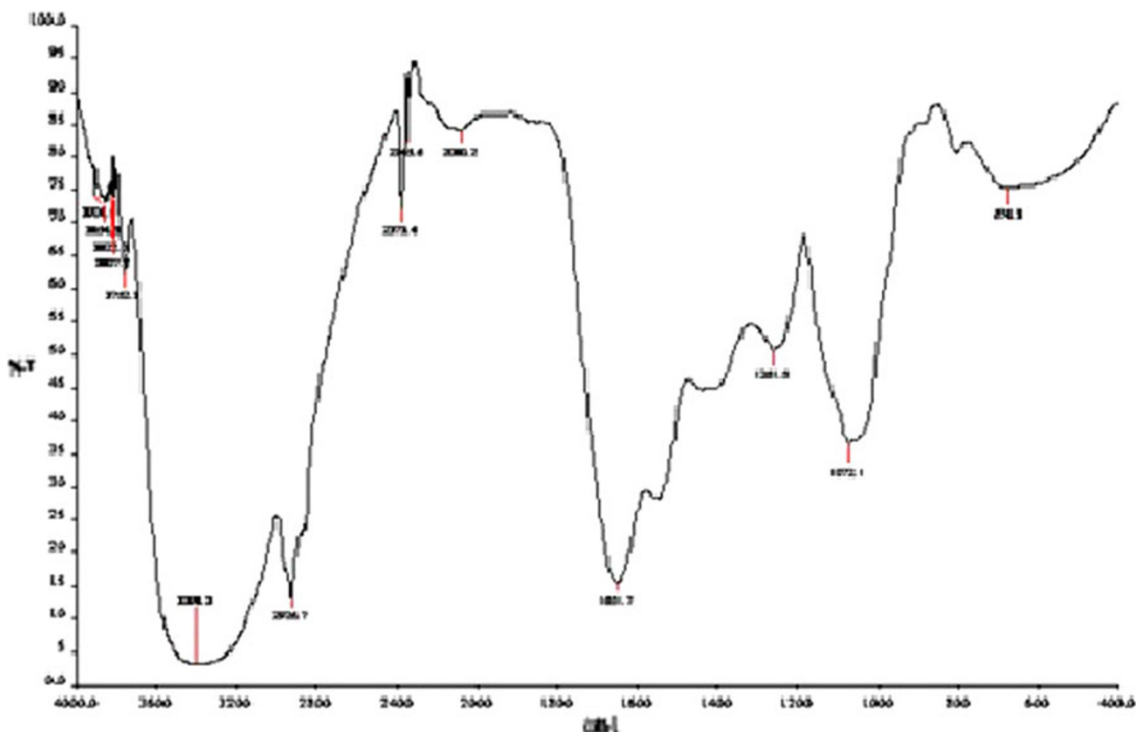


Fig. 7 The FTIR spectrum of the silver nanoparticles

(Bahgat and Ragheb 2007). Based on the physical state (oily) of the extracts and the characteristic features of the infrared vibrational peaks in the spectra, terpenoids, long chain fatty acids and secondary amine derivatives are possible compounds in the particles.

FTIR absorption spectra of silver nanoparticles shows absorbance bands (before bioreduction) are observed in the region of $500\text{--}2,000\text{ cm}^{-1}$ are $1,697$, $1,618$, $1,514$, $1,332$, $1,226\text{ cm}^{-1}$. These absorbance bands are known to be associated with the stretching vibrations for --CC--CO , --CC-- [(in-ring) aromatic], --C--C-- [(in-ring) aromatic], C--O (esters, ethers) and C--O (polyols), respectively (Edwards et al. 2003). In particular, the $1,226\text{ cm}^{-1}$ band arises most probably from the C--O group of polyols such as hydroxyflavones and catechins (Begum et al. 2009). The total disappearance of this band after the bioreduction 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 $1,650\text{ cm}^{-1}$ (for reduction of Ag).

Antibacterial activity of silver nanoparticles against human pathogenic bacteria

Zone of inhibition in the plate showed that silver nanoparticles synthesized using *Solanum trilobatum* have the antibacterial activity against all test pathogens namely *Staphylococcus aureus*, *Bacillus cereus*, *Micrococcus*

luteus, *Klebsiella pneumoniae*, *Vibrio cholerae* and *Salmonella typhi* (Citarasu et al. 2003; Galina et al. 2009). On comparison with the silver nitrate and filtrate silver nanoparticles outperformed in the bactericidal effect (Fig. 8).

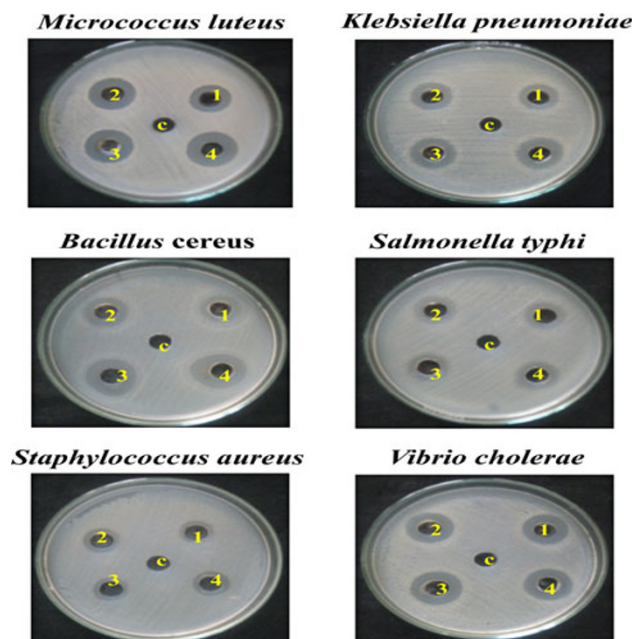
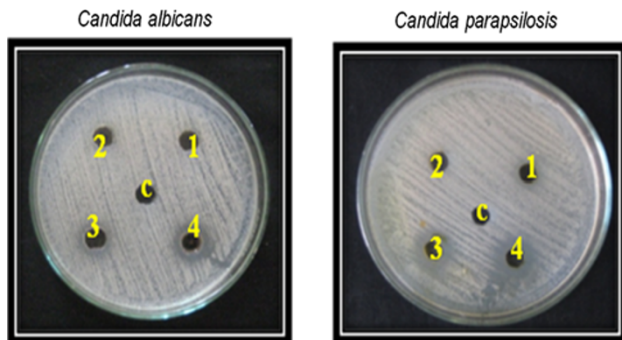


Fig. 8 Antibacterial activity of silver nanoparticles against human pathogenic bacteria, C Control, 1 25 μg , 2 50 μg , 3 75 μg , 4 100 μg

Table 1 Antibacterial activity (Zone of inhibition in mm) of silver nanoparticles against human pathogens

S. no	Concentration of silver nanoparticles ($\mu\text{g/ml}$)	Microorganism					
		Gram positive			Gram negative		
		<i>M. luteus</i>	<i>B. cereus</i>	<i>S. aureus</i>	<i>K. pneumoniae</i>	<i>S. typhi</i>	<i>V. cholerae</i>
1	25	22	20	18	19	17	18
2	50	24	21	20	21	18	20
3	75	25	23	21	22	21	20
4	100	26	24	22	23	22	21

**Fig. 9** Fungistatic and fungicidal assay of silver nanoparticles against human pathogenic fungi. C Control, 1 25 μg , 2 50 μg , 3 75 μg , 4 100 μg

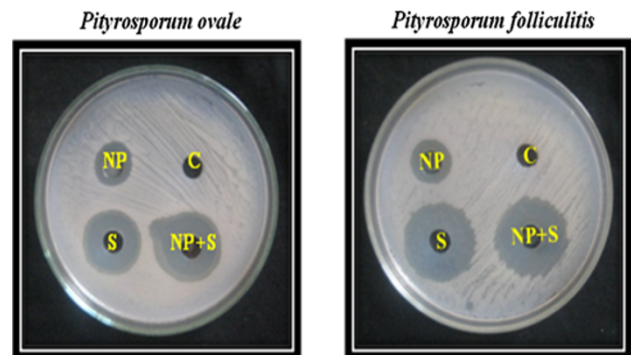
Mechanism of Ag nanoparticles was similar as described by Li et al. 2010b and Singhal et al. 2011 (Table 1).

Fungistatic and fungicidal assay of silver nanoparticles against human pathogenic fungi

Zone of inhibition in the plate showed that silver nanoparticles synthesized using *Solanum trilobatum* has the antifungal activity against test pathogens namely *Candida albicans* and *Candida parapsilosis* (Shahjahan et al. 2005; Bhutani et al. 2010). On comparison with the silver nitrate and filtrate silver nanoparticles outperformed in the fungicidal effect (Fig. 9; Table 2).

Table 2 Fungistatic and fungicidal assay (Zone of inhibition in mm) of silver nanoparticles against human pathogenic fungi

S. no	Concentration ($\mu\text{g/ml}$)	Microorganism	
		<i>Candida albicans</i>	<i>Candida parapsilosis</i>
1	25	13	11
2	50	16	11
3	75	19	14
4	100	20	19

**Fig. 10** Anti-dandruff activity of silver nanoparticles against human pathogenic dermatophytes. C control, NP nanoparticle and S shampoo

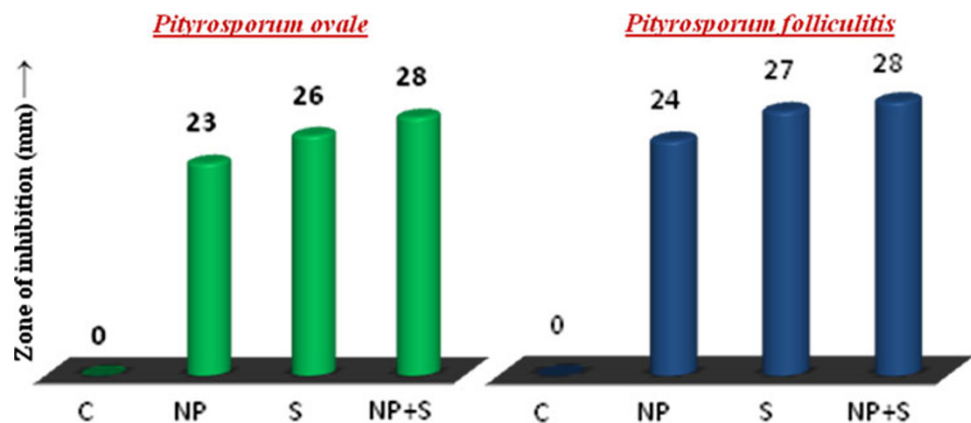
Antidandruff activity

Among two different dandruff fungal species, nanoparticles showed maximum inhibitory activity (Ayyanar and Ignacimuthu 2011). 24 and 22 mm zone of inhibition were obtained by *P. ovale* and *P. folliculitis*, respectively (Fig. 10). The nanoparticles effectively inhibit all the test pathogen. The silver nanoparticles from *Solanum trilobatum* was chosen as best treatment for dandruff and chosen for further studies because of its high inhibitory activity (Fig. 11).

Conclusion

The bio-reduction of aqueous Ag^+ ions by the leaf extract of the *Solanum trilobatum* 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. In the present study we found that leaves can be 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 applications, makes this method potentially exciting for the

Fig. 11 Antifungal activity (Zone of inhibition in mm) of silver nanoparticles against human pathogenic fungi causing dandruff. C control, NP nanoparticle and S shampoo



large-scale synthesis of other inorganic materials (nanomaterials). Antimicrobial studies of silver nanoparticles on human pathogen open a door for a new range of Antidandruff agents.

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