



Research Article

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Influence of Physico-Chemical Parameters on the Fabrication of Silver Nanoparticles Using *Petrea volubilis* L. Stem Broth and Its Anti-Microbial Efficacy

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ABSTRACT

In this study we describe the phytofabrication of AgNPs through a green route as a cost-effective, instantaneous and an eco-friendly approach using *Petrea volubilis* L. stem broth. The influence of physico-chemical parameters - contact time, stem broth quantity, pH, temperature, and silver nitrate concentration were studied and optimised to engineer, nanoparticles of diverse sizes. Nanoparticles were characterized by UV-Vis spectroscopy, FTIR, XRD, Zeta potential, EDS, and HRTEM. The characterization using HRTEM showed that, the nanoparticles were spherical and with increase in contact time, stem broth quantity, pH, and temperature, the NPs size minimised whereas escalation in silver nitrate concentration, increased their size. Capping molecules were negatively charged and the NPs were passably stable according to zeta potential readings and they were crystalline as per XRD data. According to FTIR analysis, the bio reduction was attributed to alcohol, ethers, carboxylic acids, and esters. The highest anti-bacterial activity was observed against *S. aureus* and *S. typhi* whose ZOI diameter was 13 mm at 100µl in both bacteria. The highest anti-fungal activity of silver nanoparticles was observed against *A. flavus* whose ZOI diameter was 9 mm at 100µl compared to *P. chrysogenum* which is 3 mm at 100µl. The stem broth did not show any anti-microbial activity for the microbes. Anti-microbial activity of AgNPs is due to its small size and high surface area. Our findings clearly discloses that sizes of silver nanoparticles can be varied by varying the physico-chemical parameters and the small sized nanoparticles so formed are promising antimicrobial agents and has a great potential in various medical applications.

Keywords: *Petrea volubilis*, HRTEM, XRD, EDS, FTIR, Physico-chemical parameters, AgNPs.

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INTRODUCTION

Nanotechnology is the most promising areas of research in modern materials science. With increasing

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interest in minimization or total elimination of waste and implementation of sustainable processes through the adoption of the fundamental principles of green chemistry, the development of biological and biomimetic approaches for the fabrication of nanoparticles is a desirable attribute. [1-2] Nanotechnology sharply focuses on the synthesis, design, and altering the structure and size of the particles with dimensions lesser than 100 nm. Nanotechnology is a multidisciplinary field and is an

extension of the various existing sciences such as pharmaceutical sciences, applied physics, material sciences, colloidal science, device physics, molecular chemistry, mechanical and electrical engineering. Although UV irradiation, aerosol technologies, lithography, laser ablation, ultrasonic fields, and photochemical reduction techniques have been used successfully to produce nanoparticles, which are expensive and involve the use of hazardous chemicals. Green and eco-friendly nanoparticles are synthesized using bacteria, fungi, and plants. Biogenic synthesis is useful not only because of its reduced environmental impact when compared with some of the physical and chemical methods, but also because it can be used to produce large quantities of nanoparticles that are free of contamination and have a well-defined size and morphology. [3-6]

Highly stabilized silver nanoparticles (25–40 nm) were synthesized using a leaf extract *Ocimum tenuiflorum*. [7-8] The particles showed antibacterial activity towards Gram-negative and Gram-positive bacteria. Silver nanoparticles (20-30 nm) were also synthesized using a leaf extract of *Acalypha indica*. [9] The nanoparticles were shown to be antimicrobial against water borne pathogens such as *E. coli* and *Vibrio cholerae*. Silver nanoparticles were synthesized with cotton fibers loaded with silver ions. [10] The cotton fibers loaded with the silver nanoparticles were shown to be antibacterial towards *E. coli*. [10] Leaf extract of *Eucalyptus citriodora* (neelagiri) and *Ficus bengalensis* (marri) plants were used for the synthesis of nanoparticles. [10] The average size of the nanoparticles was ~20 nm.

In the present study we used *Petrea volubilis* L. stem broth to synthesize silver nanoparticles. Silver nanoparticles were synthesized by changing the physico-chemical parameters such as contact time, stem broth quantity, pH, temperature, and silver nitrate concentration. Thus synthesized AgNPs were subjected to characterization. a) The visual observation i.e. change in the colour of the reaction mixture from pale yellow to dark brown colour indicated the formation of AgNPs and this observation was confirmed by the SPR bands developed by UV-vis spectroscopy. b) FTIR spectroscopy analysis was performed to determine the phytochemical's present in the *Petrea volubilis* L. stem broth that brought about the reduction and capping of silver ions. c) Zeta potential measurements and XRD patterns illustrated that the capping molecules were negatively charged and the NPs were passably stable and crystalline. d) Energy-Dispersive X-ray Spectroscopy (EDS) indicated the presence of elemental silver in the reaction mixture. e) The morphology and sizes of the synthesized nanoparticles were analysed by HRTEM analysis. f) Antimicrobial activity of silver nanoparticles was tested using microbes such as *Staphylococcus aureus* MTCC3160 and *Salmonella typhi* MTCC3216 and fungus *Aspergillus*

flavus MTCC1883 and *P. chrysogenum* MTCC6879 which showed good results.

MATERIALS AND METHODS

Materials

Silver nitrate (AgNO_3) used in the research work was purchased from Sigma-Aldrich chemicals. The glass wares were thoroughly cleaned with dilute nitric acid and milli-Q water, which were later dried in hot air oven. Silver nanoparticles were synthesized using *Petrea volubilis* stem broth. Healthy stem of *Petrea volubilis* were collected from the Botanical garden in Karnatak University campus, Dharwad, Karnataka, India. The stem was washed in the milli-Q water to remove any dust. About 5 g. of the cleaned stems were incised. They were boiled in 100 ml milli-Q water for 10 minutes on water bath at 50°C. The resulting broth was cooled to room temperature and filtered with whatman no. 1 filter paper to obtain the filtrate. The filtrate was collected in a 250 ml Erlenmeyer flask and stowed in the refrigerator at 4°C to be used within a week. This filtrate was later used for the synthesis of silver nanoparticles. 1×10^{-3} m AgNO_3 solution was prepared by dissolving 0.1699 grams silver nitrate (AR grade) in 1 liter milli Q water.

Characterization

UV-Vis spectral analysis was done on Jasco V-670 UV-Vis NIR spectrophotometer operated at a resolution of 1 nm at room temperature. The FTIR spectra of silver nanoparticles samples were recorded at room temperature with U-3010 spectrophotometer in the region of 500–4000 cm^{-1} at a resolution of 4 cm^{-1} . A small quantity of the synthesized silver nanoparticles sample (0.5–3 mg) was dried and mixed with 25 mg of KBr to form a pellet, which was used for FTIR measurements. For XRD measurements silver nanoparticles were dispersed into 10 ml of milli Q water and freeze dried. The dried mixture of silver nanoparticles was analysed by an X'Pert Pro x-ray diffractometer operated at a voltage of 40 kV and a current of 30 mA with $\text{Cu K}\alpha$ radiation in 2 θ configurations. Zeta potential of colloidal solution of nanoparticle was subjected to data acquisition using Zetasizer (Nano ZS) Instrument (Malvern instrument). The purity of the AgNPs were analysed in a range 2–4 keV by Energy-dispersive X-ray spectroscopy (F E I Quanta FEG 200). The surface morphology and sizes of the AgNPs were measured at different magnification at 100 keV using JEOL 3010 HRTEM.

Physico-chemical parameters

The different physico-chemical parameters were studied such as the contact time, stem broth quantity, pH, temperature, and silver nitrate concentration. The synthesis of nanoparticles was monitored at different contact time (1 h, 2 h, 4 h, 6 h, 24 h, 48 h and 72 h). The stem broth quantity was varied (5.0, 6.0, 7.0, and 8.0 ml). The effect of pH on nanoparticle synthesis was determined by altering the pH (pH 5, 6, and 7). The effect of different temperature (20°, 30°, 40° and 50°C)

on nanoparticle synthesis was investigated. The effect of various silver salt concentrations (1.0, 1.50, and 2.0 mM) was also determined.

ANTI-MICROBIAL ASSAY

Anti-bacterial Analysis

Peptone-10 g, NaCl-10 g, and Yeast extract 5g, Agar 20g in 1000 ml of distilled water. At first stock cultures of bacteria *Staphylococcus aureus* (gram positive bacteria), *Salmonella typhi* (gram negative bacteria), were inoculated in separate broth media and grown at 37°C for 18 hours. Later wells were made in the agar plates. Then 18 h old cultures (100µl, 10-4 cfu) were inoculated and spread evenly on the plate. The well diffusion method was used in the experiments. After 20 min the samples (20, 40, 60, 80, 100µl) were filled in the wells. All the plates were incubated at 37°C for 24 h. The diameter of inhibition zone was measured in mm.

Anti-fungal Analysis

Czapek-Dox Agar: Composition (g/l) Sucrose-30.0; Sodium nitrate-2.0; K₂HPO₄-1.0, MgSO₄. 7H₂O-0.5; KCl-0.5; FeSO₄-0.01; Agar-20; Initially, the stock culture of the fungi *Aspergillus flavus* and *P. chrysogenum* was inoculated in separate broth media and allowed to grow at 27°C for 48 hours. The 48 h old cultures (100µl 10⁴ CFU) were inoculated in each plate and spread evenly. Well diffusion method was used in the experiments. The wells were made in the agar plates. After 20 min the test compound (20, 40, 60, 80, 100µl) were filled in the wells at different concentrations. All the plates were incubated at 27°C for 96 h and the diameter of inhibition zone was measured in mm.

RESULTS AND DISCUSSION

Effect of contact time on the biosynthesis of AgNPs

Incubation time or contact time is the time duration required for the completion of all stages of the reaction in the reaction mixture. In the present investigation, visual observation shows the development of deep brown colour within 15 min on addition to *P. volubilis* stem broth. This change in the colour of reaction mixture indicates the formation of silver nanoparticles which is due to the excitation of surface plasmon vibrations. In the previous research with *Chenopodium album* leaf extract the nanoparticles were formed within 15 min of the reaction and increased up to 2 h, but only with a slight variation after 2 hours. [11] Formation of silver and gold nanoparticles was reported within 10 min of the reaction in Tansy fruit mediated synthesis. [12] According to UV-Vis spectroscopy observation a red shift in the peak wave length occurs at 457 nm which increases steadily in intensity and reaches stabilization at 416 nm in the blue shift region. The increase in incubation time periods viz. 1, 2, 4, 6, 24, 48 and 72 h has increased the respective intensities of the absorption peak (Fig. 1). The broadening of absorption spectra at lower contact time is due to the formation of large sized nanoparticles. As the incubation time accelerates further, intense peaks were formed indicating the synthesis of small sized nanoparticles. [13]

No change in absorbance was observed in incubation time after 72 hours, confirming stable and complete reduction of silver ions to silver nanoparticles. According to HRTEM microphotograph the silver nanoparticles thus formed are spherical in shape and their size ranged from 50-100 nm at 1, 2, 4, 6 and 24 h, 10-20 nm at 48 hrs and 5-10 nm at 72 h (Fig. 1). No agglomeration was observed. This can be explained by the fact that capping agents stabilizes the silver nanoparticles as soon as nucleation starts and so restricts the maximum size of the nanoparticles showing less aggregation. This data agrees with the results of the experiments conducted with *Chenopodium* leaf extract. [10] Zeta potential values of AgNPs synthesized using the stem broth of *P. volubilis* shows -20.7, -21.3, -21.9, -22.4, -22.5, -26.0, and -27.2, at 1, 2, 4, 6, 24, 48 and 72 hrs of contact time indicating the negatively charged capping biomolecules and moderate stability of the nanoparticle. Zeta potential results obtained from *P. volubilis* stem reaction mixture shows increase in the stability of the synthesized silver nanoparticles with a gradual rise in contact time.

Effect of Stem broth quantity on the biosynthesis of AgNPs

Quantity of stem broth plays an important role in the synthesis of silver nanoparticles. Samples were prepared separately by using 5, 6, 7, and 8 mL of the stem broth of *P. volubilis*. As the quantity of stem broth increased, respective colour changes were noted i.e. from reddish-yellow at 5.0 mL to deep brown colour at 8.0 mL. The UV-Vis spectra of AgNPs synthesized by using different stem broth quantity (5, 6, 7, and 8 mL) shows characteristic absorption peaks at 477, 443, 431, and 421 nm (Fig. 2). This decrease in absorbance indicates the reduction in the mean diameter of the nanoparticles. The HRTEM microphotograph reveals the sizes of AgNPs as 50-100 nm in 5, 6 and 7 ml and 10-20 nm in 8 ml sample (Fig. 2). These results reveal that the broad absorption peaks at 5 ml is due to large sized nanoparticles and narrow absorption peaks at 8 ml due to small sized nanoparticles whose images are clearly seen in the HRTEM microphotograph. Morphology of the nanoparticles according to HRTEM analysis shows that they are spherical in shape (Fig. 2). The observation of the images also clearly depict that there is no agglomeration. The above results show remarkable similarity with the results of the experiments conducted with bark extract of *Cinnamomum zeylanicum* and with the leaves of *Cinnamomum camphora*. [14-15] Moreover it is reported in *Pinus eldarica* bark extract that, the particle size of Ag nanoparticles decreases with an increase in the extract quantity. [16] The Zeta potential value of the AgNP reaction mixture synthesized using *P. volubilis* stem extract quantity (5, 6, 7 and 8 ml) shows -21.5, -22.7, -24.3 and -24.9 indicating that the nanoparticles are moderately stable and the capping molecules of silver nanoparticles are negatively charged groups. Increase in the broth quantity, results in more nucleation sites

being induced in the colloidal solution. It is evident that the phytochemicals present in the stem broth were responsible for the capping and stability of the nanoparticles.

Effect of pH on the biosynthesis of AgNPs

The experiments were conducted by varying the pH (pH 5.0, 6.0 and 7.0) of the reaction mixture. It was observed that with the gradual change in pH the color of the reaction mixture changed from light brown at pH 5.0 to deep brown at pH 7.0. The change in the colour of the reaction mixture indicates the synthesis of silver nanoparticles. The results of UV-vis spectra showed absorption bands at 443, 439 and 432 nm (Fig. 3). At pH 5, a characteristic peak was observed at 443 nm and more intense peaks were observed at pH 6 and 7 (Fig. 3). Due to the reduction in the size of the nanoparticles at elevated pH a blue shift in the wavelength of SPR band was observed in UV-Vis spectra. A much intense peak, signified the production of finely dispersed and spherical shaped AgNPs. HRTEM microphotograph of AgNPs synthesized show that the size of nanoparticles range in size between 20 and 50 nm in the reaction mixture with pH 5, 6 and 7 (Fig. 3). The zeta potential values of AgNPs synthesized are -20.6, -27.8 and -28.2 indicating that the capping molecules are negatively charged and are moderately stable. At higher pH due to reduction in the size, the absorption peaks became narrower and shift to shorter wavelength. This could be due to the presence of capping functional groups in the stem broth, and they were influenced by the variation in pH of the reaction mixture.

A similar pH effect was reported at elevated pH in addition to the complete and rapid reduction of the AgNPs. [17] The experiments with respect to the effect of pH during synthesis of silver nanoparticles using *Cinnamomum zeylanicum* powder and bark extract over a wider pH range (1–11) was conducted and concluded that the pH of the reaction mixture decreased in most of the cases after the AgNPs synthesis. [11] It is observed that acidic condition suppresses while the basic condition accelerates the formation of silver nanoparticles. This is because extremely acidic conditions render the biomolecules present in the plant extracts to be inactive. The aggregation of silver nanoparticles to form large nanoparticles at low pH (pH 5), is favored over the nucleation. It was speculated that at acidic pH, the availability of a large number of functional groups facilitates a higher number of Ag (I) to bind and form a large number of smaller nanoparticles. [14]

Effect of temperature on the biosynthesis of AgNPs

As per the visual observation with increase in temperature (20°C, 30°C, 40°C and 50°C) the color of the reaction mixture turns deep brown. The AgNP reaction mixture synthesized by using stem broth of *P. volubilis* showed the absorption peaks at 456, 451, 448 and 422 nm at the different temperature's (20°C, 30°C, 40°C and 50°C) (Fig. 4). Results of the UV-Vis

spectroscopy showed that, broader peaks are observed at 20°C whereas at 50°C peak is sharp (Fig. 4). In a similar study it was observed that using Tansy (*Tanacetum vulgare*) fruit extract, increase in temperature from 25 to 150°C there was increase in the sharpness of absorption peaks in both silver and gold nanoparticles. [12] The AgNPs synthesized using *P. volubilis* stem broth were analysed by HRTEM and the microphotographs show that the sizes range between 20 and 50 nm at 20 – 50°C temperature (Fig. 4). Zeta potential values of the AgNP reaction mixture show -22.6, -23.0, 24.1, and -24.6 by varying the temperature, indicating that the capping molecules are mainly composed of negatively charged groups and the nanoparticles formed are moderately stable.

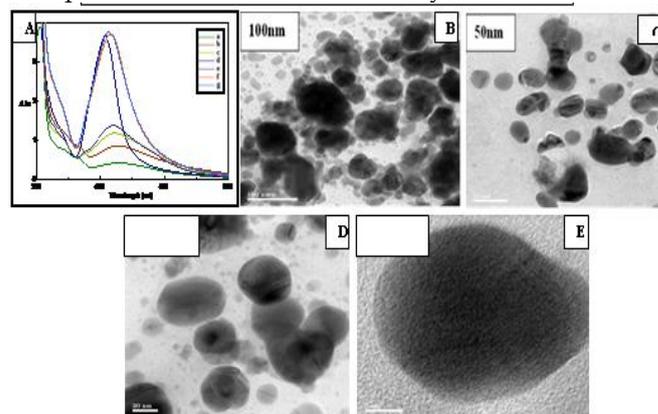


Fig. 1: A) UV-vis absorption spectra of AgNPs at different contact time. [a- 1 h, b- 2 h, c- 4 h, d- 6 h, e- 24 h, f- 48 h & g- 72 h]. HRTEM images of AgNPs at different contact time B) & C) 1, 2, 4, 6 & 24 h-100nm & 50nm D) 48 h-20nm & E) 72 h-5nm.

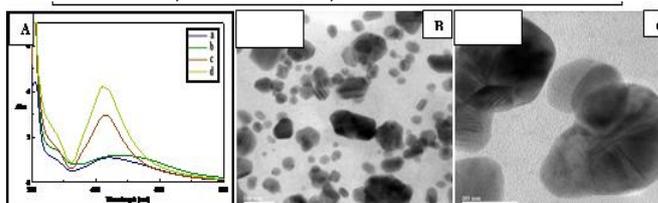


Fig. 2: A) UV-Vvis absorption spectra of AgNPs at different stem broth quantity. [a- 5 ml, b- 6 ml, c- 7 ml & d- 8 ml]. HRTEM images of AgNPs at different stem broth quantity. B) 5 ml, 6 ml & 7 ml - 50nm C) 8 ml - 20nm.

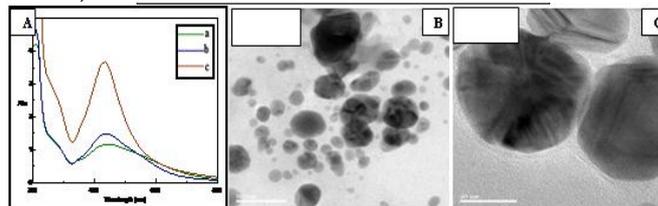


Fig. 3: A) UV-Vis absorption spectra of AgNPs at different pH. [a- pH 5, b- pH 6 & c- pH 7]. HRTEM images of AgNPs at different pH B) pH 5 & pH 6 - 50nm C) pH 7- 20nm.

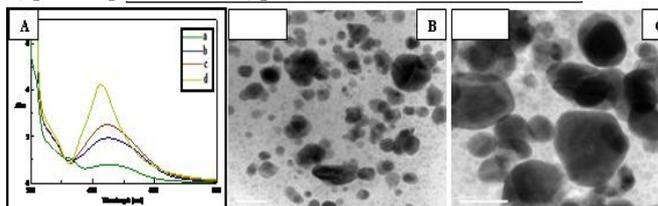


Fig. 4: A) UV-Vis absorption spectra of AgNPs at different incubation temperature. [a- 20°C, b- 30°C, c- 40°C & d- 50°C]. HRTEM images of AgNPs at different incubation temperature. B) 20°C- 50nm C) 50°C -20nm.

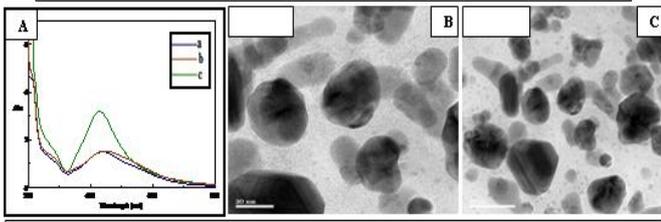


Fig. 5: A) UV-vis absorption spectra of AgNPs at different silver nitrate concentration. [a- 1.0mM, b- 1.50mM & c- 2.0mM]. HRTEM images of AgNPs at different silver nitrate concentration. B) 1.0mM -20nm, & C) 1.50 & 2.0mM -50nm.

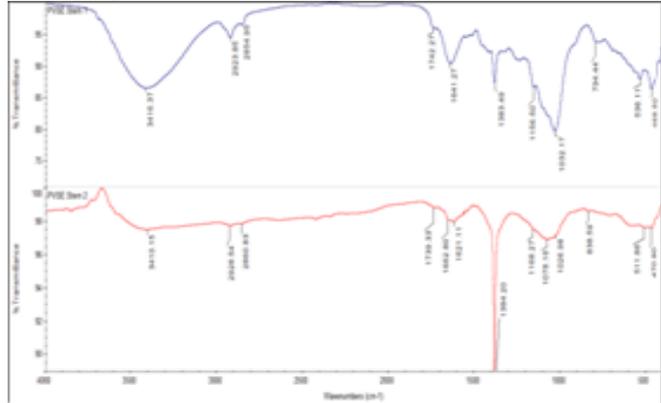


Fig. 6: FTIR spectra of *Petrea volubilis* stem broth and Silver nanoparticles soln.

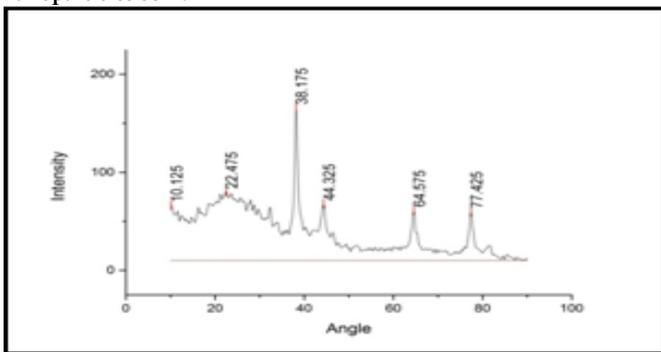


Fig. 7: XRD pattern of the biosynthesized silver nanoparticles using *Petrea volubilis* stem broth

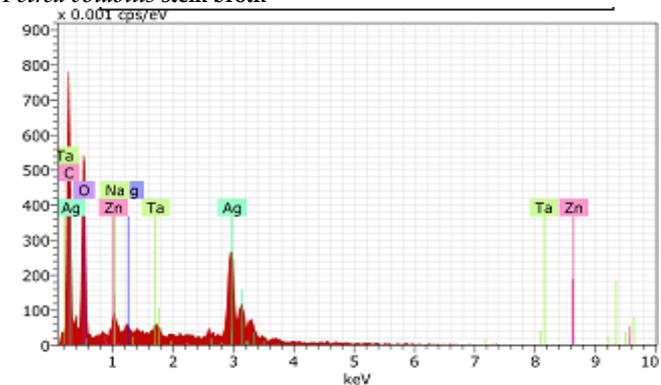


Fig. 8: EDX of Silver nanoparticles synthesized using *Petrea volubilis* stem broth.

Research on the synthesis of silver nanoparticles in lemon verbena extracts revealed that rise the reaction temperature increases the reduction of AgNPs. [18] Moreover, reports show that spherical nanoparticles were synthesized more frequently at high temperatures when compared with the nanoparticles formed at low temperature. With an increase in temperature the yield of silver nanoparticles shows a positive correlation. Increase in the rate of reduction of the metal ion to nanoparticles was reported at higher temperature. [19] A

rapid synthesis rate of silver nanoparticles at higher temperatures was reported. [17]

Effect of silver nitrate concentration on the biosynthesis of AgNPs

The visual observation of the reaction mixture shows brown color at 1.0 mM of the metal salt concentration. In the reaction mixture containing 2 mM of the metal salt the color of the reaction mixture turns slightly lesser brown when compared to the solution containing 1 mM of the metal salt concentration. This data agrees with the UV-Vis spectra which show a sharp peak at 1 mM and a broad peak at 2 mM of the metal salt concentration (Fig. 5). As the silver nitrate concentration increases from 1.0 to 2.0 mM, the peak absorbance increases and shifts to higher wavelengths. In case of silver nanoparticles, the absorbance peak broadens with increase in metal ion concentration indicating synthesis of large sized nanoparticles. [11] HRTEM microphotograph of AgNPs shows that they are spherical in shape. HRTEM shows the sizes of AgNPs range between 20 – 50 nm at 1, 1.5, and 2 mM of silver salt concentration (Fig. 5). This could be due to many silver ions adsorbed on the surface of preformed nuclei, where the secondary reduction process takes place leading to the formation of larger nanoparticles. [20] Similar results were obtained using the seed extract of *Jatropha curcas* with different concentrations of silver nitrate. [21] The zeta potential values of AgNP reaction mixture shows -25.3, -24.9 and -23.9 at 1, 1.5 and 2 mM of silver salt concentration. The results indicate that the capping molecules are negatively charged and are moderately stable. Zeta potential reports reveal that the stability of silver nanoparticles decrease with an increase in metal salt concentration.

Table 1: FTIR measurements of the *Petrea volubilis* stem broth and Silver nanoparticles synthesized.

S. No	Absorption peak cm ⁻¹ of stem broth	Absorption peak cm ⁻¹ of Silver nanoparticles	Functional groups
1.	3416.37	3410.15	O-H stretching
2.	1742.27	1739.33	C=O stretching
3.	1641.27	1621.11	(C=O)NH ₂ group
4.	1032.17	1026.98	-C-O
5.	536.11	511.86	C-Br stretching mode

Fourier transform infrared spectroscopy (FTIR)

The major shift in the absorbance bands present in the spectrum are 3416.37 - 3410.15cm⁻¹, 1742.27 - 1739.33cm⁻¹, 1641.27 - 1621.11cm⁻¹, 1032.17 - 1026.98 cm⁻¹, 536.11 - 511.86 cm⁻¹ (Fig. 6 and Table 1). The shift in the bands from 3416.37 to 3410.15cm⁻¹ can be assigned to O-H stretching due to carboxylic acids. C=O stretching mode of the carbonyl functional groups in alcohol, ethers, carboxylic acids and esters can be assigned to a prominent shift in 1742.27 - 1739.33cm⁻¹ (Table 1). The band position at 1641.27 - 1621.11cm⁻¹ are due to (C=O) NH₂ group of aromatic group as expected for this plant. The band positions at 1032.17-1026.98cm⁻¹ corresponds to -C-O group. A weak

absorption band at 536.11 - 511.86 cm^{-1} is due to C-Br stretching mode respectively (Table 1).

X-ray diffraction (XRD)

XRD analysis of the biosynthesized silver nanoparticles using *P. volubilis* stem is as follows. XRD pattern obtained for synthesized silver nanoparticles shows a characteristic high intensity peak at $2\theta = 77.43^\circ$ (Fig. 7). This corresponds to pure silver showing Bragg reflections of (311) set of lattice plane, which is well coordinated with the existing JCPDS File No. 04-0783. According to the FWHM data of the peak it is estimated that the size of silver Nano crystallites was in accordance with the above characterizations. There are three peaks with a low intensity at around 22.48° , 38.18° , and 44.33° (Fig. 7). The peaks at 2θ values of 38.18° , 44.33° , 64.58° , and 77.43° can be indexed as (111), (200), (220), and (311) planes of fcc silver (Fig. 7). An XRD spectrum compared with the standards confirmed that the silver nanoparticles formed in this experiment were crystalline in nature. According to the results of the synthesized silver nanoparticles the lattice plane (111) is known for its high antibacterial activity.

Energy Dispersive X-ray Analysis (EDX)

In *P. volubilis* stem strong signal energy peaks in the range of 2–4 keV are reported for silver atoms with weaker signals for tantalum, sodium, zinc, and oxygen which may be due to the other biomolecules capping the nanoparticles (Fig. 8). Among the different signals, the C signal may probably be due to X-ray emission from proteins, enzymes; carbohydrates in *P. volubilis* stem broth. The major emission energy at 3 keV reveals that the silver is accurately identified.

Antimicrobial assay

Antimicrobial activity studies on pathogen provide an insight for nanotechnology applications in medicine. Since time immemorial silver has been employed in antimicrobial activity. Antibacterial activity is related to compounds that kill the microbes or slow down their growth, without being harmful to host organisms. The antimicrobial activity of the synthesized silver nanoparticles was tested against two bacterial (gram positive and gram negative) and two fungal species using well diffusion method. Resistance to drugs causes high-dose administration of antibiotics, resulting in intolerable toxicity.

P. volubilis stem synthesized AgNPs were tested against bacteria *S. aureus* which showed the ZOI of 10 mm, 12 mm, and 13 mm at 60 μl , 80 μl and 100 μl . No ZOI was observed at 20 μl and 40 μl (Fig. 9). AgNPs against *S. typhi* showed a ZOI of 8 mm, 11 mm, 12 mm and 13 mm at 40 μl , 60 μl , 80 μl and 100 μl . No ZOI was observed at 20 μl (Fig. 9). Both *S. Aureus* and *S. typhi* a gram +ve and -ve bacteria show a ZOI diameter of 13 mm at 100 μl which indicates that the nanoparticles are active against both the bacteria. The fungus *A. flavus* showed the ZOI of 5 mm and 9 mm at 80 μl and 100 μl . No ZOI was observed at 20, 40, and 60 μl (Fig. 9). *P. chrysogenum* showed ZOI of 3 mm at 100 μl . No ZOI was observed at 20, 40, 60, and 80 μl (Fig. 9). The highest anti-fungal

activity by *P. volubilis* stem was observed against *A. flavus* whose ZOI diameter is 9 mm at 100 μl compared to *P. chrysogenum* which is 3 mm at 100 μl .

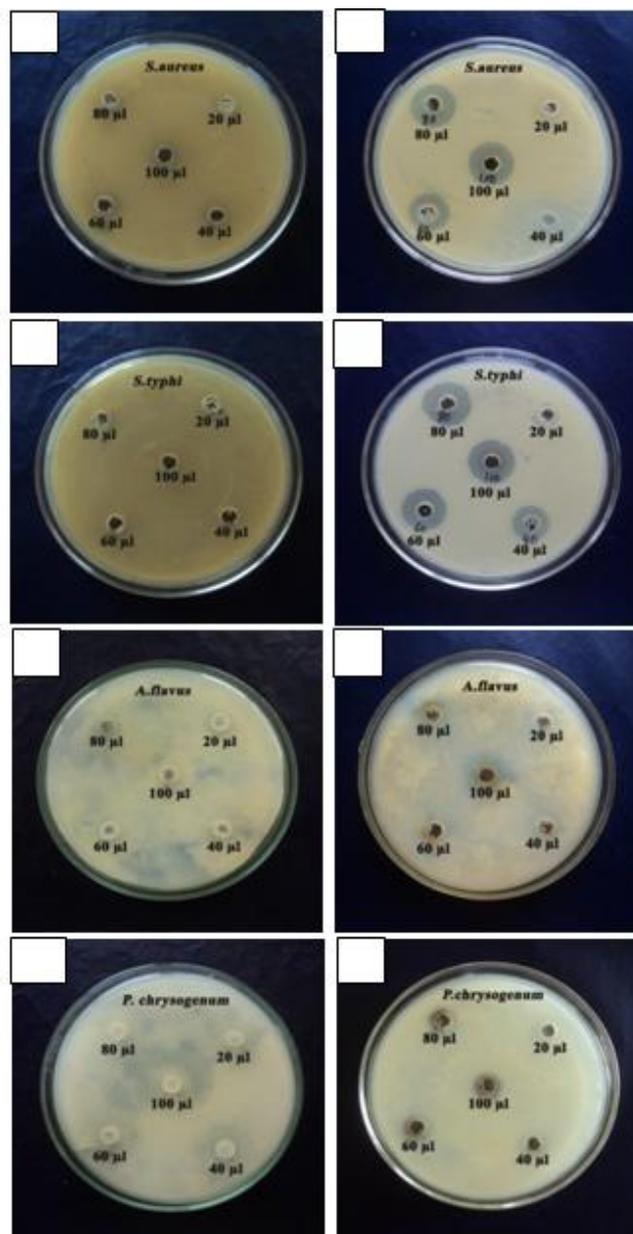


Fig. 9: A), C), E) & G) *S. aureus*, *S. typhi*, *A. flavus* & *P. chrysogenum* with stem broth No zone of inhibition, B) *S. aureus* with AgNPs, ZOI - 10 mm, 12 mm and 13 mm at 60 μl , 80 μl and 100 μl D) *S. typhi* with AgNPs, ZOI - 8 mm, 11 mm, 12 mm and 13 mm at 40 μl , 60 μl , 80 μl and 100 μl F) *A. flavus* with AgNPs, ZOI - 5 mm and 9 mm at 80 μl and 100 μl . H) *P. chrysogenum* with AgNPs, ZOI - 3 mm at 100 μl .

Meagre reports on the mechanism of the bactericidal effect of silver nanoparticles are not very well understood. However three types of antimicrobial mechanisms were observed i.e. (i) Plasmolysis, cytoplasm of bacteria separated from bacterial cell wall, was observed in Gram positive bacteria and Gram negative bacteria, (ii) inhibited cell wall synthesis and (iii) induces metabolic disturbances to pathogenic bacteria. [22]

The exact mechanism of anti-fungal effects of silver nanoparticles has not been well understood. Reports on

the mechanism of anti-fungal action of silver ions on fungi have shown that, DNA loses its potential to replicate resulting in the inactivation of ribosomal subunit proteins. Certain other cellular proteins and enzymes essential for ATP production also become inactive. It has also been stated that the functioning of membrane-bound enzymes, such as those in the respiratory chain have primarily been affected by the silver ions.

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REFERENCES

1. Raveendran P, Fu J, Wallen SL. Completely "Green" Synthesis and Stabilization of metal nanoparticles. *J Am Chem Soc.* 2003; 46: 13940-1.
2. Rao CNR, Kulkarni GU, John TP, Edwards PP. Size-dependent Chemistry: Properties of Nanocrystals. *Chemistry A European Journal.* 2002; 8: 28-35.
3. Anastas PT, Zimmerman JB. Green nanotechnology. Why we need a green nano award and how to make it happen. Washington DC: Woodrow Wilson International Center for Scholars; 2007.
4. Dahl JA, Maddux BLS, Hutchison JE. Toward Greener Nanosynthesis. *Chem Rev.* 2007; 107:2228-69.
5. Shankar SS, Rai A, Ahmad A, Sastry M. Rapid Synthesis of Au, Ag, and Bimetallic Au core Ag Shell Nanoparticles Using Neem (*Azadirachta indica*) Leaf Broth. *J Colloid Interface Sci.* 2004; 275:496-502.
6. Hutchison JE. Greener Nanoscience: A Proactive Approach to Advancing Applications and Reducing Implications of Nanotechnology. *ACS Nano.* 2008; 2: 395-402.
7. Patil SV, Borase HP, Patil CD, Salunke BK. Biosynthesis of Silver Nanoparticles Using Latex from Few Euphorbian Plants and Their Antimicrobial Potential. *Appl Biochem Biotechnol.* 2012a; 167: 776-90.
8. Patil R, Kokate M, Kolekar S. Bioinspired Synthesis of Highly Stabilized Silver Nanoparticles Using *Ocimum tenuiflorum* Leaf Extract and Their Antibacterial Activity. *Spectrochim Acta A Mol Biomol Spectrosc.* 2012b; 91: 234-8.
9. Krishnaraj C, Jagan EG, Rajasekar S, Selvakumar P, Kalaichelvan PT, Mohan N. Synthesis of Silver Nanoparticles Using *Acalypha indica* Leaf Extracts and its Antibacterial Activity against water borne Pathogens. *Colloids Surf B.* 2010; 76: 50-56.
10. Ravindra S, Mohan YM, Reddy NN, Raju KM. Fabrication of Antibacterial Cotton Fibres Loaded With Silver Nanoparticles via "green approach". *Colloids Surf A.* 2010; 367:31-40.
11. Dwivedi, A. D. and Krishna G. (2010). Biosynthesis of silver and gold nanoparticles using *Chenopodium album* leaf extract. *Colloids and Surfaces A: Physicochem. Eng. Aspects.* 369: 27-33.
12. Dubey SP, Lahtinen M, Sillanpa M. Tansy Fruit Mediated Greener Synthesis of Silver and Gold Nanoparticles. *Process. Biochem.* 2010; 45:1065-1071.
13. Zhang WZ, Qiao XL, Chen JG. Synthesis and Characterization of Silver Nanoparticles in AOT Microemulsion System. *Chemical Physics.* 2006; 300:495-500.
14. Sathish kM, Sneha K, Won SW, Cho CW, Kim S, Yun YS. "Cinnamon zeylanicum Bark Extract and Powder Mediated Green Synthesis of Nano-crystalline Silver Particles and its Bactericidal Activity". *Colloids and Surfaces B.* 2009; 73: 332-338.
15. Huang J, Li Q, Sun D, Lu Y, Su Y, Yang X, Wang H, Wang Y, Shao W, He N, Hong J, Chen C. Biosynthesis of Silver and Gold nanoparticles by Novel Sundried *Cinnamomum camphora* Leaf. *Nanotechnology.* 2007; 18:105104-105114.
16. Iravani S, Zolfaghari B. Green Synthesis of Silver Nanoparticles Using *Pinus eldarica* Bark Extract. *BioMed Research International.* 2013; 639725, dx.doi.org/10.1155/2013/639725.
17. Andreescu D, Eastman C, Balantrapu K, Goia DV. A Simple Route for Manufacturing Highly Dispersed Silver Nanoparticles. *J. Mater. Res.* 2007; 22: 2488-2496.
18. Cruz D, Pedro LF, Ana M, Pedro DV, Serralheiro ML, Ana R, Lino L. Preparation and Physicochemical Characterization of Ag Nanoparticles Biosynthesized by *Lippia citriodora* (Lemon Verbena). *Colloids and Surfaces B: Biointerfaces.* 2010; 81: 67-73.
19. Huang J, Lin L, Li Q, Sun D, Wang Y, Lu Y, He N, Yang K, Yang X, Wang H, Wang W, Lin W. Continuous-flow Biosynthesis of Silver Nanoparticles by Lixivium of Sundried *Cinnamomum camphora* leaf in Tubular Microreactors. *Ind. Eng. Chem. Res.* 2008; 47: 6081-6090.
20. Yang N, Wei-Hong Li. Mango Peel Extract Mediated Novel Route for Synthesis of Silver Nanoparticles and Antibacterial Application of Silver Nanoparticles Loaded onto Non-woven Fabrics. *Industrial Crops and Products.* 2013; 48: 81-88.
21. Bar H, Bhui DK, Sahoo G P, Sarkar P, De SP, Misra A. Green Synthesis of Silver Nanoparticles Using Latex of *Jatropha curcas*. *Colloids and Surfaces A: Physicochem. Engin. Aspects.* 2009; 339:134-139.
22. Song HY, Ko KK, Oh LH, Lee BT. Fabrication of Silver Nanoparticles and Their Antimicrobial Mechanisms. *Eur Cells Mater.* 2006; 11(Suppl 1):58.

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