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Green synthesis of silver nanoparticles through reduction with *Euphorbia nivulia* Buch.-Ham., stem bark extract: Characterization and antimicrobial activity

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Nanoparticles, because of their diversified applications in the field of modern medicine, have gained a lot of importance thrust area. In the present investigation, synthesis and characterization of Silver nanoparticles (AgNPs) and their antimicrobial effect on certain pathogenic bacteria were studied. AgNPs were prepared by green synthesis process using stem extract of *Euphorbia nivulia*, from 1 mM $AgNO_3$ solution. The color change was observed after the addition of $AgNO_3$ due to the surface plasmon vibration. The detailed characterization of the nanoparticles was carried out using UV-Vis spectrometry at 400 to 700 nm; maximum absorption peak was observed at 432 nm. FTIR analysis showed the functional groups involved in the AgNPs formation. Scanning Electron Microscopy (SEM) revealed the structure and the size of nanoparticles spherical and 20-90 nm respectively. The antimicrobial activity screened for eight bacterial strains and one fungal strain. AgNPs showed highest inhibition (33.5±0.5) against *Escherichia coli,* followed by *Pseudomonas aeruginosa* (30.5 ±0.5), *Bacillus subtilis* (29 ±1) *Salmonella typhimurium* (28±1), *Bacillus cereus* (27±1) *Staphylococcus aureus* (24.5 ±1.5) and *Klebsiella pneumoniae* (23.5 ±0.5) and one fungal strain *Candida albicans* (26±1*).*

Key words: *Euphorbia nivulia*, stem bark, silver nanoparticles, characterization, antimicrobial activity.

INTRODUCTION

Silver nanoparticles have received enormous scientific, technological, and commercial attention due to their unique size and shape dependent properties (Nair and Laurencin, 2007). Noble-metal nanoparticles exhibit incredible physicochemical, optoelectronic and biochemical characteristics. They are being used for various purposes in industrial and pharmaceutical applications (Linic et al*.,* 2015; Thakkar et al*.,* 2010).

Despite the existence of numerous metals in nature, only a few of them such as gold, silver, palladium and platinum are synthesized extensively in nano -structured form (Saba et al., 2019) and extensive research has been

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devoted to explore the applications of silver nanoparticles in diversified fields including healthcare/biomedical (Kim et al., 2007; Jones and Hoek, 2010; Murphy et al., 2008). The synthesis processes of silver nanoparticles play a major role in the control of their size and shape, thus a wide range of physical, chemical, as well as biological methods has been established and reported (Abid et al., 2002) as chemicals, and plant extracts are extensively investigated due to their eco-friendly protocol and better morphological control (Narayanan and Sakthivel, 2010; Sastry et al*.,* 2003; Kaviya et al*.,* 2011). The establishment of resistance to antibiotics in bacteria, especially multidrug-resistant microorganisms, compelled scientists to explore novel compounds to halt them. Bactericidal activity of AgNPs without toxicity to human cells can make them a proper substitution for antibiotics (Leid et al., 2011; Modi et al., 2014).

Chemical reduction is the most frequently applied methods for the preparation of silver nanoparticles (AgNPs) as stable, in water or organic solvents. Initially, the reduction of various complexes with Ag^+ ions leads to the formation of silver atoms (Ag), which is followed by agglomeration into oligomeric clusters. These clusters eventually lead to the formation of colloidal Ag particles. When the colloidal particles are much smaller than the wavelength of visible light, the solution has yellow color with an intense band in the 380-400nm range and other less intense or smaller bands at longer wavelength in the absorption spectrum. This band is attributed to collective excitation of the electron gas in the particles, with a periodic change in electron density at the surface (surface plasmon absorption).

Uses of plant

All parts of the test species *Euphorbia nivulia* possess medicinal properties. The juice of the leaf is used as a purgative, diuretic etc (Raghunath and Badguja, 2011). The paste of the leaf, made with neem oil is applied externally in rheumatism. Plant latex is used for treating jaundice, dropsy, enlargement of liver and spleen, and applied to hemorrhoids. Coagulated latex is used for bronchitis (Khare, 2004). The latex of this plant possesses vesicant, wormicidal and purgative properties (Pullaiah, 2006). Milky latex of this plant is reported for its bronchodilating activity (Savithramma et al., 2007). Kumar and Chaturvedi (2010) mentioned the application of ethnomedicine derived from stem of this plant in curing the bone fractures and antiseptic utility of latex. Although there is a wide range of potential useful medicinal phytoconstituents of the plant, the research in this area is infantile. The synthesis of nanoparticales has been reported in *Euphorbia hirta* (Durga Devi et al., 2014) and *Euphorbia wallichii* (Abdul Rehaman et al*.,* 2020). The focus of this study was on the synthesis of silver nanoparticles mediated by the stem extract of *E. nivulia*. In addition, we have also demonstrated the antimicrobial activity of the prepared nanoparticals on Gram negative, Gram positive bacterial and fungal strain to finding out the potential properties of the generated nanoparticles for various environmental and biomedical applications.

MATERIALS AND METHODS

Preparation of extract

The extract was made using stem bark, which was collected from Nallamalla forests of Kurnool District, Andhra Pradesh State. A photograph of habit and stem bark is shown in Figure 1. Prior to extracting crude drug, stem bark was cleaned thoroughly using deionized water. Washed stem bark was cut into small pieces and used for extraction. 100 ml boiling deionized water was added to 2 g of stem pieces and left for 5 min to boil, and then solution was removed from the heat source and allowed to cool at room temperature. Following this step, the extract was then filtered through a coarse sieve to remove remnants and the filtrate was refrigerated for long run use. The filtrate was used for green synthesis of AgNps.

Synthesis of silver nanoparticles

The silver nitrate $AgNO_3$ (Sigma Aldrich) was used in this experiment. Stem bark extract of 500 µl was added to 4.5 ml of 1 mM $AgNO_3$ and the reaction was left for 30 min to take place under ambient conditions. The observed change in colour from colorless to transparent yellow and finally dark brown with time indicates the formation of silver nanoparticles. As the reaction mixture reached a dark brown color, it was centrifuged at 15,000 rpm for 45 min. The pellet containing silver nanoparticles was washed 3-4 times with deionized water to remove silver ions and extract residue; it was then centrifuged for 60 min. Reduction of Ag^+ ions was monitored using UV−Visible spectral analysis.

UV-visible spectra analysis

The reduction of pure Ag+ ions was monitored by measuring the UV-Vis spectrum after 30 min of reaction. A small aliquot of the sample was taken for UV-Vis spectrum analysis and peak was observed from 400-700 nm.

Fourier transform infrared spectroscopy (FTIR)

To remove any free biomass residue or compound that is not the capping ligand of the nanoparticles, the residual solution of 100 ml after reaction was centrifuged at 15000 rpm for 10 min and the resulting suspension was washed with sterile distilled water. Thereafter, the purified suspension was dried to obtain stable powder. Finally, the dried nanoparticles were analyzed by FTIR, 600-4000 cm^{-1} range.

Energy dispersive x-ray analysis (EDAX)

In order to carryout EDAX analysis, the plant extracts that reduced silver nanoparticles were dried and drop coated onto carbon film; it

Figure 1. (A) Habit of *Euphorbia nivulia, (*B) Stem bark.

was performed with an Hitachi S-3400 NSEM instrument equipped with a thermo EDAX attachments .

Scanning electron microscopic (SEM)

SEM analysis was carried out by using thin films of the sample prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid. Extra solution was removed using a blotting paper and then the film on the SEM grid was allowed to dry under a mercury lamp for 5 min.

Antimicrobial assay

Antibacterial activity using disc diffusion method

The antimicrobial activity of synthesized silver nanoparticles was determined using disc diffusion assay method. The test microorganisms were obtained from the microbial type culture collection centre, Institute of Microbial Technology (IMTECH), Chandigarh, India. Gram-positive strain: *Bacillus cereus* (MTCC-4079), *Micrococcus luteus* (MTCC-7256) *Bacillus subtilis* (MTCC-1133). Gram-negative strains *Staphylococcus aureus* (MTCC-7443), *Escherichia coli* (MTCC-1668), *Klebsiella pneumoniae* (MTCC-7028), *Pseudomonas aeruginosa* (MTC-7296), *Salmonella typhimurium* (MTCC-98) and one fungal strain *Candida albicans* (MTCC-7315). Bacterial strains were spread on the Petri dishes which contained autoclaved Luria–Bertani (LB) medium containing agar. Then, the disks (6 mm diameter) soaked in Ampicillin 100 mg/ml used as a control for bacteria, Tetracyclin 100mg/ml for fungi, 1 ml of plant extract, $AgNO_3$ and biosynthesized AgNPs were separately placed on Petri dishes containing LB media. Petri dishes were incubated at 37ºC for 24 h. Inhibition zone of each disk was measured by ruler.

RESULTS

UV-Vis spectra analysis

The UV-Vis spectroscopy was the preliminary technique for the characterization of the silver nanoparticles. The UV-Vis absorption was analyzed after centrifuging and redispensing the particles in deionized water. The maximum broad absorption peak was observed at 432 nm which was confirmed that poly dispersed nanoparticles were formed (Figure 2).

FTIR analysis of silver nanoparticles

FTIR analysis is the technique used for the identification of change in functional groups. A broad band at 2361 cm^{-1} due to the presence of –OH stretching was observed; a sharp absorption band located at 2100 cm^{-1} can be attributed to CH group stretching and a band at 1646 cm^{-1} (due to the ring stretching) was also observed. Other important peaks observed from 1396 $^{-1}$ 1125 and 1252 were due to the C– O –C stretching from the glycosidic linkages and O– H bending from alcohols. A considerable modification can be noticed in the well-defined spectrum of aqueous solution of stem bark and aqueous poly-AgNPs (Figure 3).

EDAX analysis

EDAX confirmed the presence of the signal characteristic

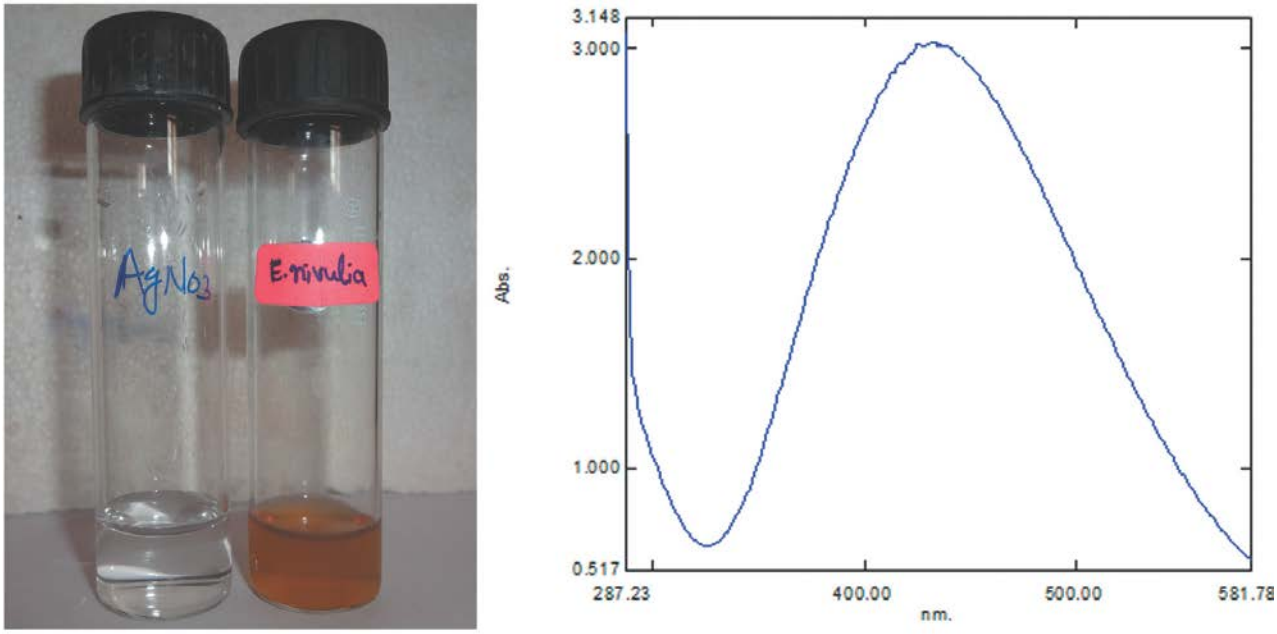

Figure 2. UV-Vis spectra analysis of silver nanoparticles.

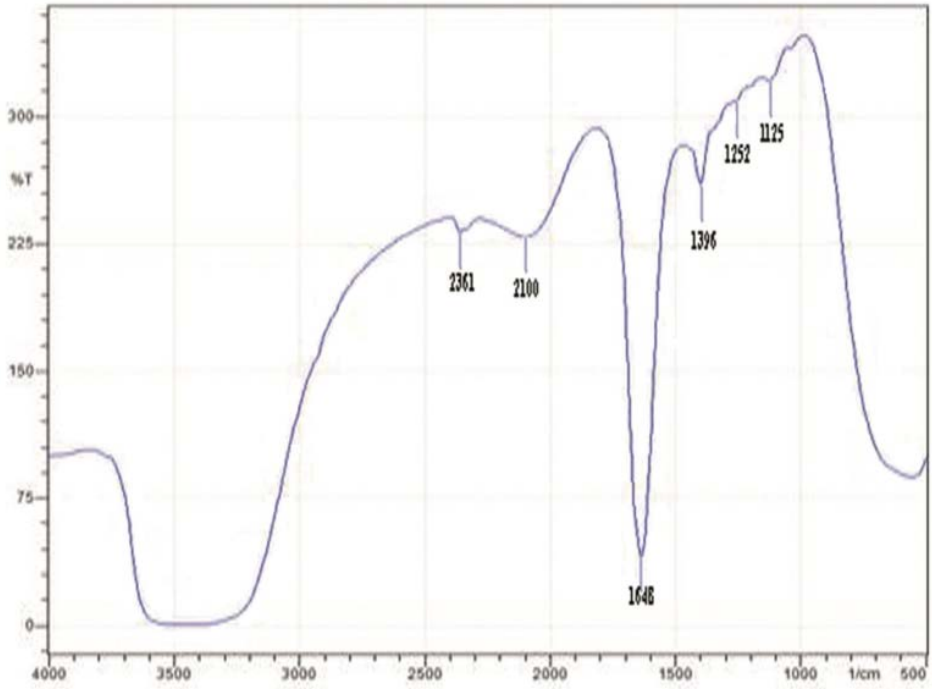

Figure 3. FTIR analysis of Silver nanoparticles.

of elemental silver. The peaks of Ag observed Peak for Ca and C are from the grid used and the peaks for S, P and N correspond to the protein capping over the AgNPs. Silver nanocrystallites display an optical absorption band peak approximately 3 keV, which is typical of the absorption of metallic silver nanocrystallites due to

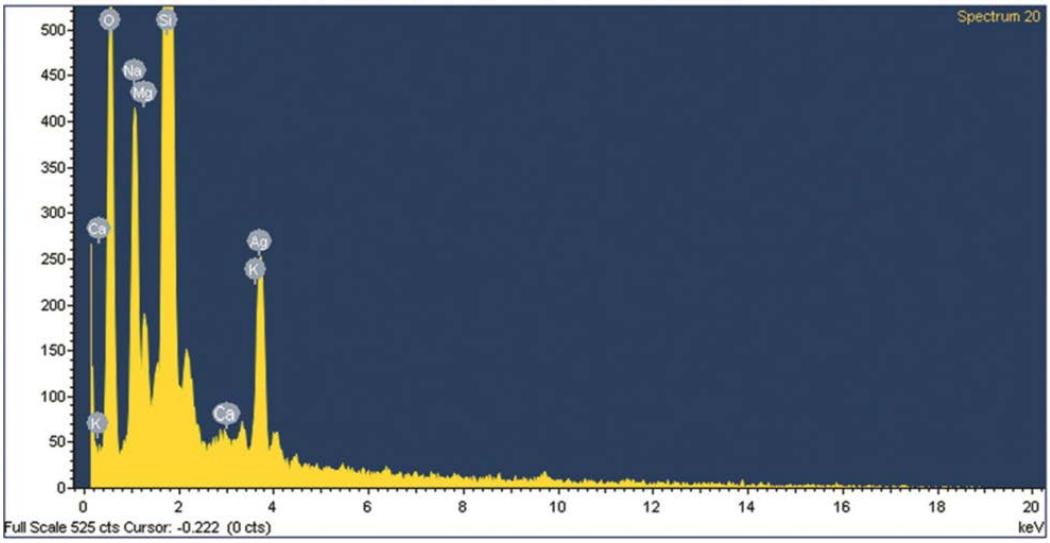

Figure 4. Energy dispersive X-ray Analysis of silver nanoparticles.

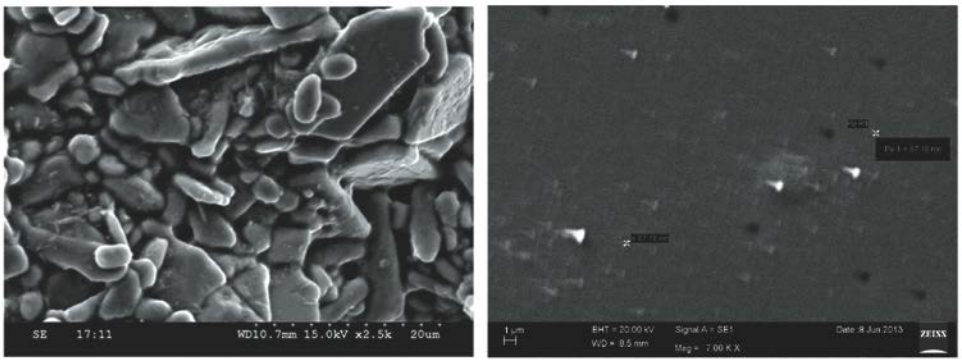

Figure 5. SEM image of Silver nanoparticles formed by *Euphorbia nivulia*

surface (Figure 4).

SEM analysis

The SEM analysis of the sample, AgNPs in the solution has an average size of about 20- 90 nm. The nanoparticles were oval, spherical in shape. Most of the nanoparticles were aggregated, and few individual particles were also observed. The image shows agglomerates of small grains and some dispersed nanoparticles, confirming the results obtained by SEM (Figure 5).

Antimicrobial activity

Antibacterial activity of green synthesized silver nanoparticles against the test isolates at different concentrations showed that they revealed a strong dose-dependent antibacterial activity. It was seen that, as the concentration of green synthesized nanoparticles was increased, bacterial growth decreases in all cases. The zones of inhibition of silver nanoparticles against Gram positive bacteria and Gram negative bacteria are shown in Figure 6 and Table 1. The results indicated that silver nanoparticles synthesized from *E. nivulia* stem bark extract have effective antibacterial activity in Gram positive, Gram negative bacterial and fungal strain. AgNps showed highest inhibition (33.5±0.5) against *Escherichia coli,* and *Pseudomonas aeruginosa* (30.5 ±0.5). The fungal strain *Candida albicans* shows 26±1.

DISCUSSION

In the present study we have demonstrated the potential

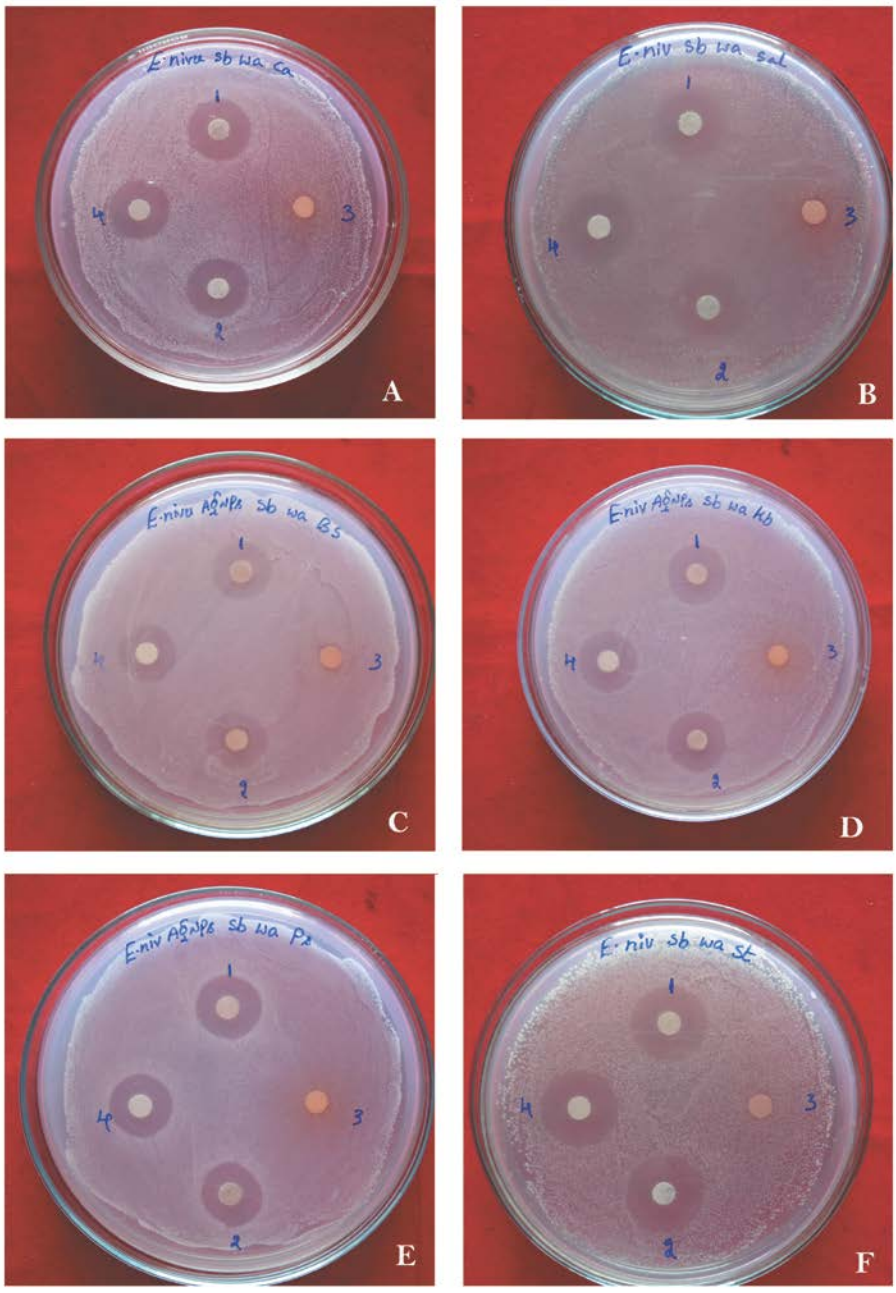

Figure 6. Antimicrobial activity of AgNPs of *Euphorbia nivulia* A. *Candida albicans*, B. *Salmonella typhimurium* C. *Bacillus subtilis*, D. *Klebsiella pneumoniae*, E. *Pseudomonas aeruginosa*; F. *Staphylococcus aureus*. Inhibition Zones: 1. AgNps; 2. Control; (Antibiotics) 3. Plant extract, 4. $AgNO_3$.

of stem bark extract of the *E. nivulia* in reducing aqueous Ag^+ to Ag^o ions and the formation of eco-friendly silver nanoparticles with fairly well-defined dimensions. The present study provides evidence that the stem bark is good source for synthesizing stable silver nanoparticles in lesser time. 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, shelf life and viability, etc. These eco-friendly nanoparticles could be used as an excellent source against multi drug resistant bacteria, enhancing wound healing process, and act as anticancer, anti-stress agent.

Table 1. Antimicrobial activity of *E. nivulia* (Sb).

Microorganisms	*E. nivulia* (Sb)			
	Inhibition zone (mm-^{1})			
Bacterial strains	**Ampicillin100 mg/ml**	**AgNPs**	**$AgNO_3$**	**Plant extract**
Bacillus cereus	10	27±1	10	6
Staphylococcus aureus	9±1	24.5±1.5	13±1	8.5±0.5
Micrococcus luteus	15.5±0.5	30±1	13.5±1.5	11±1
Bacillus subtilis	20.5±0.5	29±1	19±1	13.5±0.5
Escherichia coli	20.5±0.5	33.5±0.5	18.5±0.5	10.5±0.5
Klebsiella pneumoniae	10.5±0.5	23.5±0.5	13±2	12±1
Pseudomonas aeruginosa	13.5±0.5	30.5±0.5	14.5±0.5	6±1
Salmonella typhimurium	13	27±1	15	7±1
Fungal strain	**Tetracyclin100 mg/ml**	**AgNPs**	**$AgNO_3$**	**Plant extract**
Candida albicans	12	26±1	13.5±0.5	6

The green synthesis of nanoparticles can also be used in large-scale for synthesizing nanoparticles from other inorganic materials. The results reported in this study open the possibility for further investigations of biologically synthesized AgNPs. Purification of different compounds from extracts and detailed characterization of active bio- organic compound of stem bark extract catalyzing AgNPs synthesis and stabilization are further parts of the study.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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