Ch. Maruti Kesava Kumar et al /J. Pharm. Sci. & Res. Vol. 7(2), 2015, 76-82

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Synthesis, Characterization and Antimicrobial Studies of Stem Bark Mediated Synthesis of Silver Nanoparticles From *Adansonia digitata* (L.)

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Abstract: In the present study green synthesis of silver nanopartiles (SNPs) is prepared by using aqueous stem bark extract of *Adansonia digitata* (L.) as a reducing, stabilizing and capping agent. The synthesized SNPs are characterized by UV-VIS Spectroscopy, FTIR, XRD, AFM, SEM with EDAX and TEM. Validation of SNPs was performed on 07 bacterial species and 05 fungal species. For preliminary conformation of SNPs to observe the colour change from light brown to thick brown shows the formation silver nanoaprticles and the 450 nms of UV-VIS studies shows the further confirmation of synthesized nanoparticles are SNPs. FTIR studies shows that the Phenols, Alkynes and Primary amines of Proteins are mainly responsible for the reduction of nanopartilces and AFM, SEM and TEM studies shows that the particles are spherical in shape and having the size between 5 to 30 nms. The EDAX pattern shows the 2.69 wieght percentage of silver present in the synthesized sample solution and XRD studies shows that the particles are mostly crystalline in nature. Further these biologically synthesized nanoparticles were found to be highly toxic against different multi drug resistant bacterial and fungal pathogens. This is the first report on synthesis of SNPs from stem bark of *A. digitata* was used for synthesis of SNPs and its antimicrobial studies.

Keywords: *Adansonia digitata*, SNPs, UV-VIS, FTIR, XRD, AFM, SEM with EDAX, TEM and antimicrobial activity.

INTRODUCTION

Adansonia digitata L. (Baobab) belongs to the family Malvaceae and is a deciduous tree. In Europe and Ghana Baobab bark is used for the treatment of fever [1]. The gum obtained from stem bark is used for cleansing sores. In East Africa, the bark is used as an antidote to *Strophanthus* poisoning. In Congo Brazzaville, a bark decoction is used to bathe rickety children and in Tanzania as a mouthwash for toothache [2]. In Indian medicine, baobab bark is used internally as a refrigerant, antipyretic and antiperiodic. [3].

For several years, scientists have constantly explored different synthetic methods to synthesize nanoparticles. On the contrary, the green method of synthesis of nanoparticles is easy, efficient, and eco-friendly in comparison to chemical mediated or microbe mediated synthesis [4]. Recently different types of nanoparticles were bio-synthesized by using plant materials like Silver nanoparticles from *Shorea tumbuggaia* [5], Calcium nanoparticles from *Boswellia ovalifoliolata* [6], Zinc oxide nanoparticles from *Catharanthus roseus* [7], Gold nanoparticles from *Avena sativa* [8], Indium oxide nanoparticles from *Aloe vera* [9], Palladium nanoparticles from *Cinnamomum camphora* [10], Iron oxide nanoparticles from *Medicago sativa* [11], Copper nanoparticles from *Magnolia kobus* [12] and Cadmium Oxide Nanoparticles from *Achillea wilhelmsii* [13].

The use of environmentally benign materials like plant extract [14], bacteria [15], fungi [16] and algae [17] for the synthesis of silver nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis protocol. The rate of reduction of metal ions using plants has been found to be much faster as compared to micro-organisms and stable formation of metal nanoparticles has been reported [18]. In the present study, we synthesized stable silver nanoparticles with the bio-reduction method using aqueous stem bark extract of *A. digitata* and evaluated their antibacterial and antifungal activity against drug resistant bacterial and fungal isolates.

MATERIALS AND METHODS

Plant collection and extract preparation

The stem bark was collected from the campus of DKW Govt. Degree College for Women, Nellore and identified by the Flora of the Presidency of Madras [19]. The stem bark was washed several times with tap water to remove the dust particles and shade dried to evaporate the residual moisture. 25 gs of powdered stem bark were extracted with 100 ml of milli q water on boiling water bath for 30 min. The aqueous extract was separated by filtration with Whatman No. 1 filter paper and stored at room temperature for bio synthesis of silver nanoparticles.

Synthesis of SNPs:

5 ml of aqueous stem bark extract were taken into the clean 250 ml conical flask and titrate with 50 ml of 1mM $Ag(NO_3)_2$ solution at the temperature range between 60-80^0 C for 60 min. and observed the colour change from light brown to thick brown which indicates the formation of SNPs (**Fig.1**). After 24 h. the contents was centrifuged at 10,000 rpm for 20 minutes to remove the presence of biological admixtures.

Fig. 1 Synthesis of SNPs from stem bark of *A. digitata* colour change pattern a. Plant extract b. Synthesized SNPs

Characterization of SNPs:

UV-VIS absorption spectra of SNPs was measured by using a Spectro UV 2080 Double beam 1200 L/mm spectrophotometer. Crystalline metallic silver nanoparticles were examined using an X-ray diffractometer (Shimadzu, XRD-6000) equipped with Cu Kα radiation source using Ni as filter at a setting of 30 kV/30 mA. Fourier transform infrared (FT-IR) spectra for extract of stem bark powder and silver nanoparticles was obtained in the range 4,000 to 500 cm^{-1} with an ALPHA interferometer (ECO-ATR), Bruker, Ettlingen, Karlsruhe, Germany, FT-IR spectrophotometer, by KBr pellet method. Atomic Force Microscopy (AFM) by NOVA NT-MDT SOLVER NEXT, RUSSIA. Scanning Electron Microscopy (SEM) and Content of silver ions of synthesized SNPs was done by using a FEI Quanta 200 FEG HR-SEM machine equipped with EDAX instrument. TEM analysis is performed by using HF-3300 advanced 300kV TEM/STEM from Hitachi.

Antimicrobial studies:

Biosynthesized silver nanoparticles were analyzed for their antimicrobial activity against two Gram positive bacteria's like *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* (ATCC 6538) and five Gram negative bacteria's like *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 43816), *Proteus vulgaris* (ATCC 13315), *Pseudomonas aeruginosa* (ATCC 15442) and *Salmonella typhimurium* (ATCC 14028). Antifungal studies were carried for five fungal species like *Alternaria solani* (ATCC 32904), *Aspergillus niger* (ATCC 16404), *Aspergillus flavus* (ATCC 9643), *Penicillium chrysogenum* (ATCC 11709) and *Trichoderma harzianum* (ATCC 20476) procured from Dept. of Microbiology, Sri Venkateswara University, Tirupati. Disc diffusion assay method was carried out by using standard protocol [20]. 20 µl of plnat extract, $Ag(NO_3)_2$, SNPs and Streptomycine/Flucanazole are used respectively and each extract was applied to separate filter paper discs (Whatman No. 1 filter paper with 6 mm diameter), and allowed to dry before being placed on the agar medium.

RESULTS AND DISCUSSION

UV-VIS analysis:

The reduction of pure silver ions was monitored by measuring the UV-Vis spectrum of the reaction medium from the wavelength of 190 nms-750 nms by using Spectro UV 2080 Double beam 1200 L/mm. The peak obtained from 450 nms indicates the presence of silver nanoparticles (Fig.2). The same type results were obtained in the case of extracellular synthesis of silver nanopartilces from *Bacillus* Sps. [15] and they are mostly spherical in shape were observed in *Piper nigrum* mediated SNPs [16].

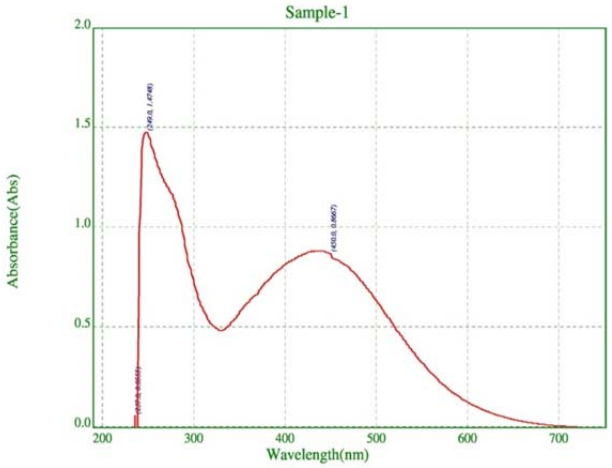


Fig. 2 UV-VIS analysis of synthesized SNPs from stem bark of *A. digitata* shows broad peak at 450 nms

FTIR analysis

The functional groups of stem bark extract and synthesized nanoparticles were identified by using FT-IR spectroscopy between the scan range of 500-4000 cm^{-1}. The FT-IR spectrum obtained for stem bark extract **(Fig. 3a)** displays a number of absorption peaks like 3281 cm^{-1} assigned for O-H bond of Alcohols/Phenols, 2917 cm^{-1} for C-H Stretch of Alkanes, 2308 cm^{-1} for N-H bond of Ammonium ions, 1603 cm^{-1} for N-H bend of amines, 1419 cm^{-1} for O-H bend of Carboxylic acids, 1316 cm^{-1} for C-H bend of alkenes, 1021 cm^{-1} for C-N bond of aliphatic amines, 774 cm^{-1} for C-H Bond of Aromatic Benzene and 654 cm^{-1} for C-H deformation of Alkynes reflecting its complex nature. The FT-IR spectrum of synthesized SNPs **(Fig. 3b)** shows the 3323 cm^{-1} assigned for O-H bond of Alcohols/Phenols, 2113 cm^{-1} for C-C bond of terminal alkynes and 1636 cm^{-1} for N-H bond of primary amines proteins. Most of the peaks appeared in the stem bark extract was disappeared after the synthesis of SNPs. Based on the FT-IR analysis it is confirmed that the broad peaks of Phenols and Proteins acts as a reducing **(Fig. 3a)**, stabilizing and capping agents **(Fig. 3b)** and for silver nanoparticles from the state of Ag^0 to Ag^+.

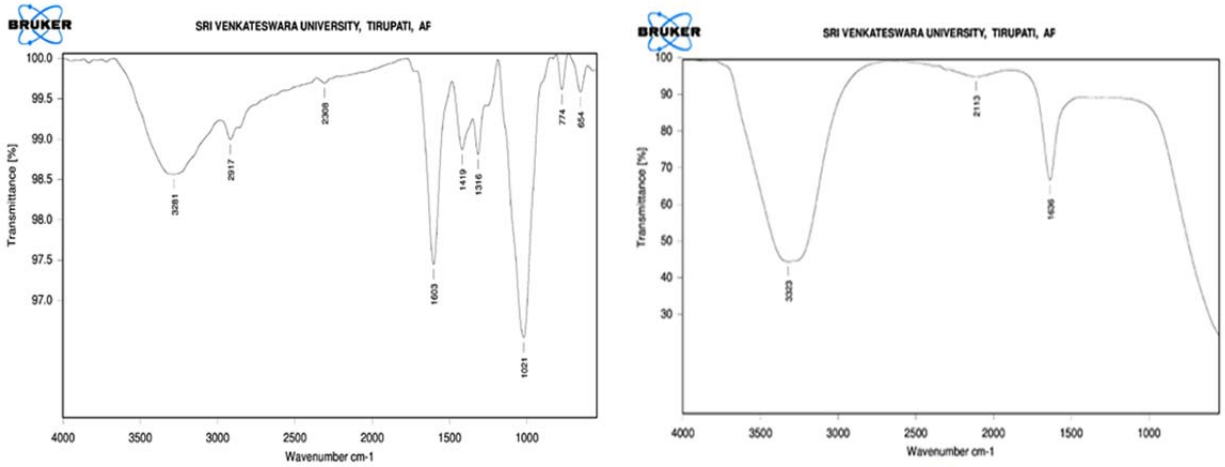


Fig. 3a. FTIR spectra of aqueous stem bark extract 3b. FTIR spectra of synthesized SNPs

XRD Analysis:

X-ray diffraction analysis was carried out to confirm the nature of the nanoparticles. The peaks at 2θ values of X axis shows 38.09^0, 44.27°, 64.58°, and 77.43° corresponding to 420, 135, 112 and 100 Bragg reflections of Y axis respectively, which may be indexed based on the face centered cubic structure of silver. X-ray diffraction results clearly shows that the silver nanoparticles formed by the reduction of Ag+ ions by the stem bark extract are crystalline in nature and they are mostly spherical in shape **(Fig. 4)**.

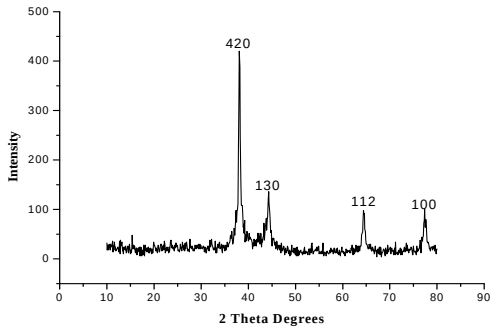


Fig. 4 XRD analysis of synthesized SNPs from stem bark of *A. digitata*

AFM Analysis:

Surface topology of the synthesized SNPs was studied by 1µm×1µm atomic force microscopy (AFM) analysis (**Fig.5a**). AFM was used as the primary method to monitor SNPs dissolution and agglomeration pattern. The topography of micrographs clearly indicate that the formulated SNPs possess spherical shape and have the calculated sizes in the range of 20 to 50 nm and no agglomerations are observed**.** A statistical treatment of AFM images was performed using specially designed image processing software (NOVA-TX). To further exploit these measures should be extracted from these images and explore the 3D nanostructures to know height of the nanoparticles **(Fig.5b)** and average size of nanoparticles in a particular area is (24 nms) is calculated **(Fig.5c)**.

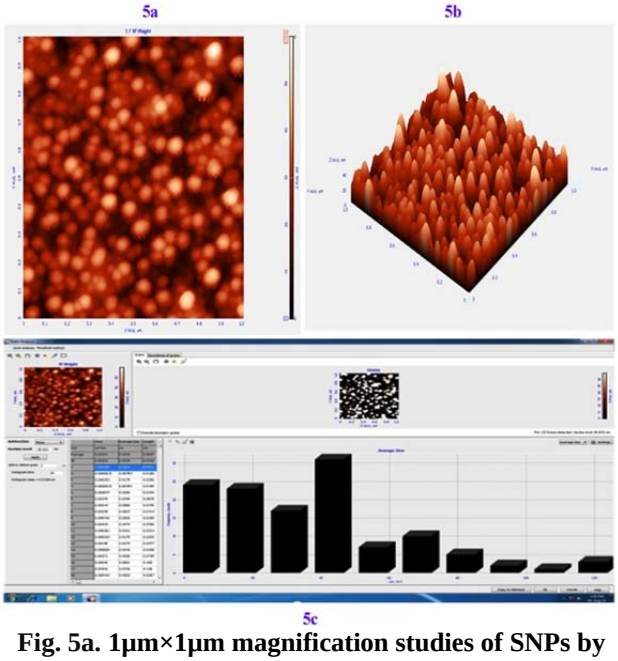
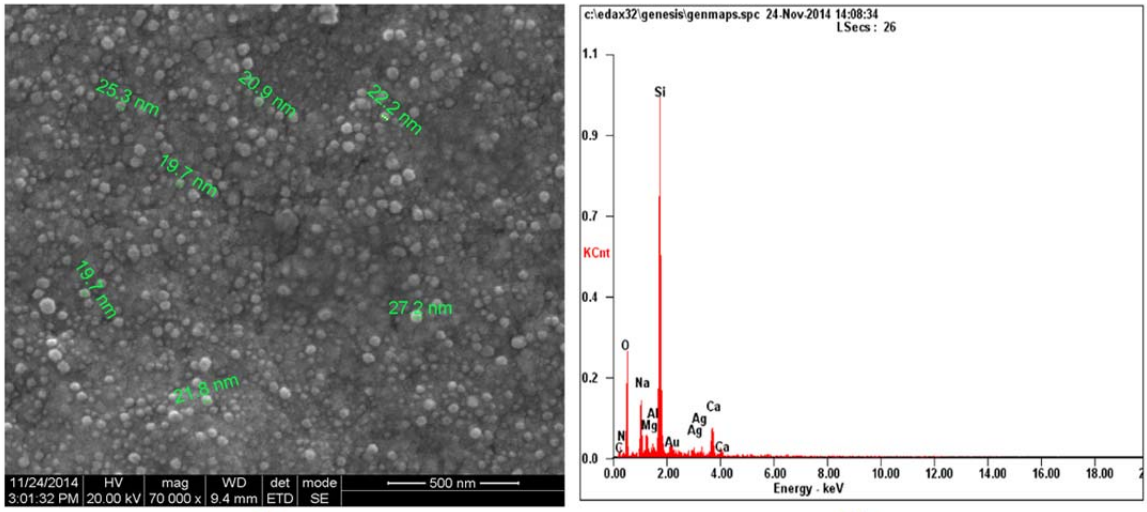


Fig. 5a. 1µm×1µm magnification studies of SNPs by AFM; 5b. 3d structure; 5c. Calculation of average number of SNPs in 1µm×1µm magnification respective to fig 5a

SEM analysis:

Thin films of the sample were prepared on clean glass slide by just dropping a very small amount of the sample and extra solution was removed by using a blotting paper and then the film was allowed to dry for 10 min. in Hot air oven. For conventional imaging in the SEM Analysis biological or insulating samples require thin conductive coating. The surface of the thin films are coated by gold acts as a electrically conductive agent. SEM analysis shows uniform distribution of SNPs on the surfaces with spherical shape with particle size range from 19.7 nms to 27.3 nms **(Fig 6a)**.

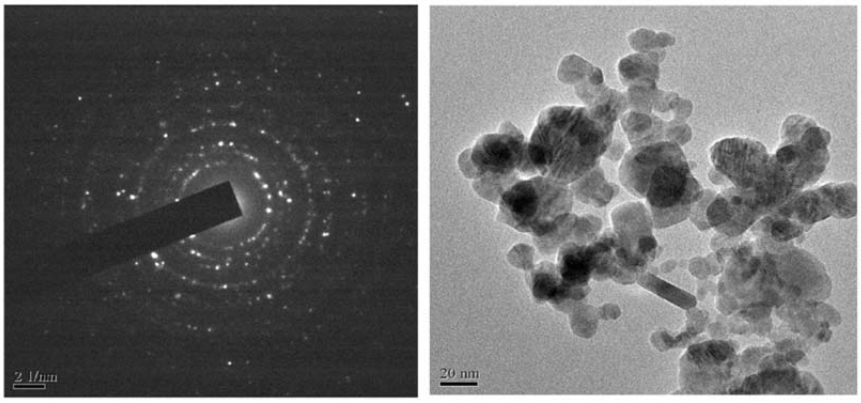


6a 6b

Element	CK	NK	OK	NaK	MgK	AlK	SiK	AuM	AgL	CaK	Matrix
Wt%	09.97	03.64	29.45	07.91	02.35	00.89	32.75	04.95	02.69	05.39	Correction
At%	17.46	05.47	38.71	07.23	02.03	00.69	24.52	00.53	00.52	02.83	ZAF

6c

Fig. 6a. 500 nms magnification studies of SNPs by HR-SEM; 6b. EDAX measurement of content of Ag metal in the sample; 6c. EDAX data of different metals in the sample



7a 7b

Fig. 7a Selected Area Electron diffraction pattern of SNPs from stem bark of *A. digitata*; 7b. 20 nms magnification studies of SNPs by TEM

EDAX analysis:

EDAX analysis was performed to knowing percentage of SNPs in the sample. For this the synthesized SNPs was characterized by using FEI Quanta 200 FEG HR-SEM equipped with EDAX instrument. The EDAX spectra shows the different types of elements with their weight percentage like Carbon (09.97%), Nitrogen (03.64%), Oxygen (29.45%), Sodium (07.91%), Magnesium (02.35%), Aluminum (00.89%), Silicon (32.75), Aurum (04.95%) Silver (02.69%) and Calcium (05.39%) in the sample. **(Fig.6b & 6c).**

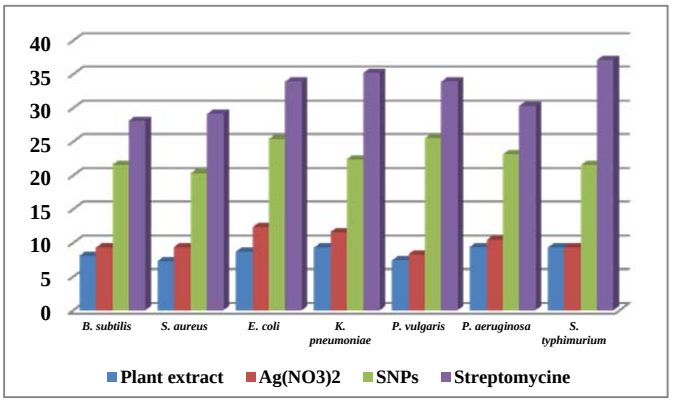
TEM analysis:

Morphological structure and distribution of synthesized silver nanoparticle were characterized at high magnifications was done by TEM. For TEM analysis the SNPs are coated on copper grids and analyzed by Hitachi HF-3300 advanced with 300kV. The TEM images of SNPs signify that the synthesized nanoparticles are poly dispersed and predominantly spherical in shape **(Fig. 7)**. The overall morphology of the silver nanoparticles produced by reduction of Ag+ ions with 1mM $Ag(NO_3)_2$ is composed of almost in uniform distribution.

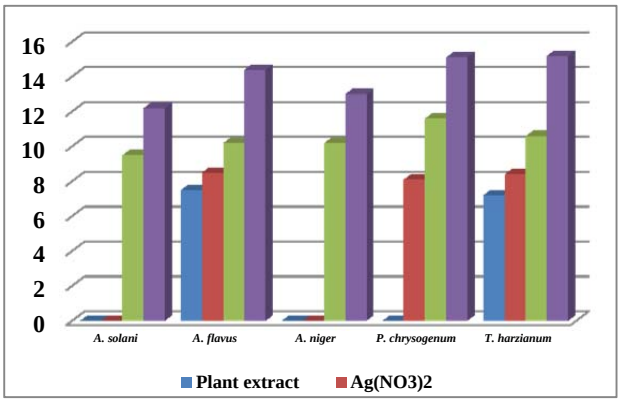
Antimicrobial studies

Biosynthesized SNPs were analyzed for their antimicrobial activity against two gram +Ve bacteria's like *B. subtilis*, *S. aureus* and five gram negative bacteria's like *E. coli*, *K. pneumoniae*, *P. vulgaris*, *P. aeruginosa* and *S. typhimurium*. Antifungal studies are taken out from five fungal species like *P. chrysogenum*, *A. niger*, *A. flavus*, *T. harzianum* and *A. solani*. The obtained results are indicative of the diameters of zone of inhibition due to microbial susceptibility. The synthesized SNPs shows

highest inhibiting effect on *P. vulgaris* followed by *E.coli*, *P.aeruginosa*, *K. pneumoniae*, *S. typhimurium*, *B. subtilis* and *S.aureus* **(Graph 1; Fig. 8a)**. Whereas in the case of fungi the highest inhibition zones were observed in *P.chrysogenum followed by T. harzianum*, *A. flavus*, *A. niger* and *A. solani* **(Graph 2; Fig. 8b)**.



Graph 1



Graph 2

Graph 1& 2: Graphical representation of zone of inhibition for bacterial and fungal species respectively of various extracts of *A. digitata*

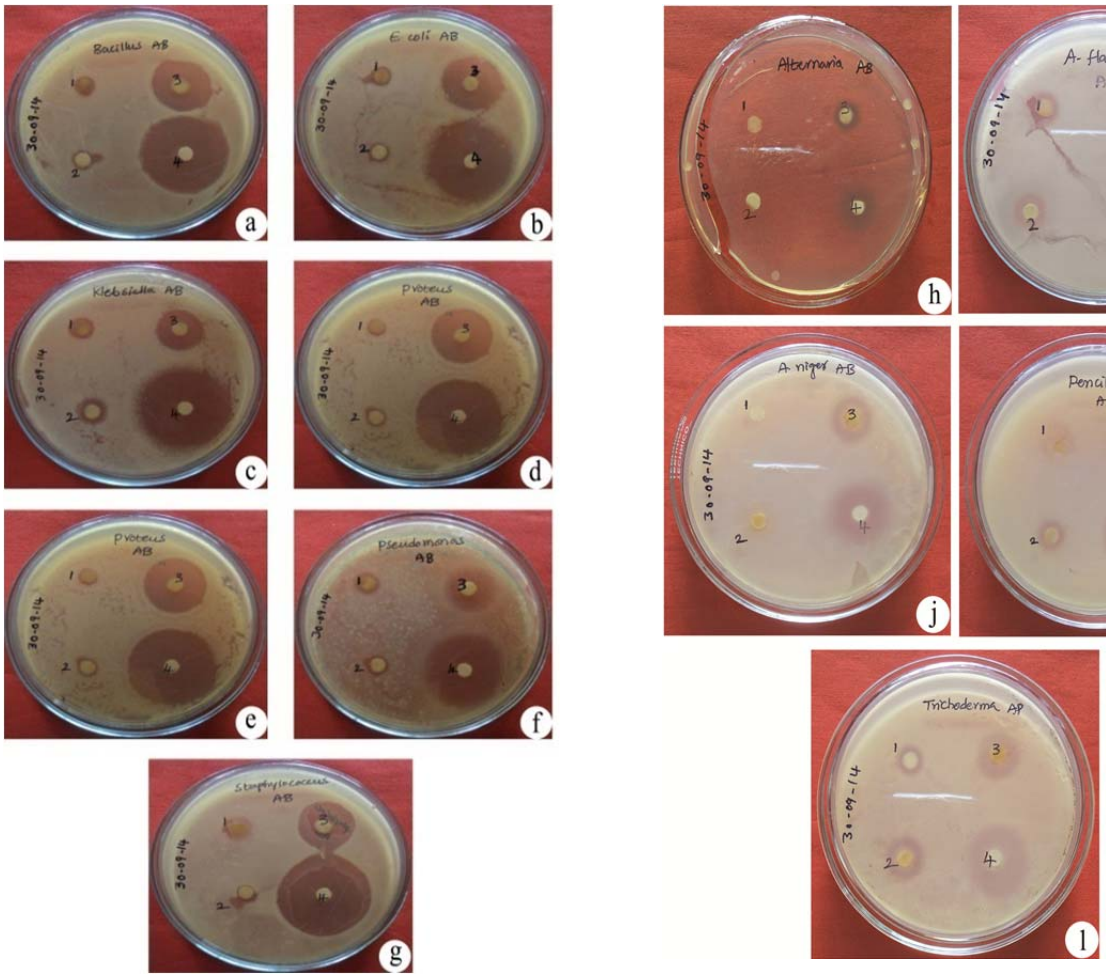


Fig. 8a & 8b Photographic images of zone of inhibition for Bacteria and Fungi

a. *B. subtilis*, **b.** *S. aureus*, **c.** *E. coli*, **d.** *K. pneumoniae*, **e.** *P. vulgaris*, **f.** *P. aeruginosa*, **g.** *S. typhimurium*, **h.** *A. solani*, **i.** *A. flavus*, **j.** *A. niger*, **k.** *P. chrysogenum* and **l.** *T. harzianum*

1. Plant extract, 2. Ag $(NO_3)_2$, 3. SNPs, 4. Streptomycine/Flucanazole

In this biological synthesis shows that the environmentally benign and renewable source of *A. digitata* stem bark used as a effective reducing and capping agent for the synthesis of SNPs. The colour change pattern shows the preliminary conformation of synthesized nanoparticles are SNPs. Same type results were obtained in *Svensonia hyderabadensis* [22] and 450 nms of peak obtained in UV-VIS spectroscopy further confirms the formation of nanoparticles are SNPs and FT-IR studies indicates phenols and proteins are most responsible for reducing, stabilizing and capping agents towards the formation of SNPs. The same type of results are found in *Myristica fragrans* seed extract synthesized SNPs [23]. AFM studies indicates the nanoparticles are spherical in shape with diameter range from 10 nms to 50 nms and the overall average size is 24 nms and 3D image shows the height of the nanoparticles and no further agglomeration were confirmed by AFM. The SEM results indicate that the particles are mainly spherical in shape having diameter between19.7 nms to 27.2 nms and uniform distribution of particles was seen. The EDAX analysis shows the 2.69 weight percentage of Ag present in the synthesized biological sample. The TEM analysis indicates the nanoparticles are poly dispersed and spherical in shape. Further the above SNPs prove their antimicrobial activity. Among them gram negative bacteria's are more susceptible when compare to the gram positive bacteria. The gram +Ve bacteria having thick layers of peptidoglycons when compare to the gram –Ve bacteria and the penetration of SNPs through cell membrane is easy. The exact mechanism of antibacterial activity is not known but some of the scientists state that SNPs may attach to the surface of the cell membrane and disturb its permeability and cause structural changes on cell membrane leads to cell death [24]. Few studies have showed that silver nanoparticles may kill fungal spores by destructing the membrane integrity [25]. Morones [26] and Baker [27] suggested that the possibility of SNPs may also penetrate inside the bacteria and fungi causing damage by interacting with electron phosphorous and sulphur containing compounds such as DNA and proteins resulting in cell death. In the present study the synthesized SNPs from *A. digitata* shows Spherical shaped with diameter ranging between 5 nms to 30 nms confirmed by XRD, AFM, SEM and TEM shows potential and effective against different resistant micro organisms. Smaller particles have larger surface area available for interaction and will give more bactericidal effect than the larger particles [16]. The same type of results were found in *Euphorbia hirta* leaf mediated synthesis of SNPs having the size between 40-50 nms shows highest zone of inhibition on *Bacillus* and *Staphylococcous. Acalypha indica* leaf mediated synthesis of SNPs having diameter range between 20-30 nms had excellent antimicrobial activity against water borne pathogens *E. coli*, *V. cholera* and *Cochlospermum religiosum* leaf synthesized SNPs with 40 nms act as a potential antimicrobial agent [28].

CONCLUSION:

In this study we have developed a eco friendly and environment safe green method for the synthesis of silver nanoparticles from *A. digitata* stem bark extract with rapid speed. The stem bark extract very much suitable for the synthesis of small sized silver nanoparticles. The colour change from light brown to thick brown indicates the presence of different phytochemicals responsible for reduction, stabilization and capping of silver nanopartilces, which is confirmed by UV-VIS spectroscopy and FTIR. FTIR reveals that the Phenols and Primary amines of Proteins are mainly responsible for reduction and capping to this nanoparticles to prevent agglomeration and provide stability to the medium. The nanoparticles are very small in range between 5-30 nms confirmed by XRD, AFM, SEM, TEM and analysis of total content silver nanoparticles by EDAX instrument. Further the antimicrobial studies indicated that the nanoparticles are toxic to different types of drug resistant microorganisms. Finally we conclude that the stem bark of *A. digitata* is ideal material for the rapid synthesis of SNPs and act as a potential antimicrobial agents.

ACKNOWLEDGEMENT:

The authors are very grateful to DST-PURSE and Dept. of Chemistry in S.V. University. JNTU, Hyderabad. SAIF, IIT Madras for analysis of SNPs.

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