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Research Article

Silver Nanoparticles Synthesis, Stabilization and Characterization by Different Concentrations of *Acacia senegal (l.)* Willd.Extract and Evaluation of their Antibacterial Activity

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ABSTRACT
Metallic nanoparticles are conventionally synthesized using chemical techniques, which use toxic and flammable techniques. The present work reports a cost effective and eco friendly technique for green synthesis of silver nanoparticles using the leaf extract of different concentration species of *Acacia senegal*. Leaf extract. The leaf extract such as triterpenes, eugenol and flavonoids are responsible for the formation of nanoparticles. Absorption spectroscopy was used to monitor the formation of silver nanoparticles. The particle size was analyzed using Transmission electron microscope. The Infrared Spectroscopy was performed to identify types of chemical bonds, i.e. functional groups in a molecule. The antimicrobial activities of the synthesized silver nanoparticles were tested against Gram positive bacteria Staphylococcus aureus and Bacillus subtilis bacteria and Gram negative (Pseudomonas putida and Klebsiella pneumonia, E.coli by disc diffusion method technique.

Key words: *Acacia senegal*, silver nanoparticles, antimicrobial activity

INTRODUCTION

Nanotechnology consists of applied biology, chemistry, material science, physics, and engineering working at the nano level creating materials on the atomic level and alterations at molecular level. The silver particles that range from 1 to 100 nm are silver nanoparticles and are one of the most commonly utilized nanomaterials as they have unique optical, electrical, thermal and anti-microbial (Parameswari et al., 2010) properties and are being incorporated into products that range from photovoltaic to biological and chemical sensors. A number of approaches are available for the synthesis of silver nanoparticles by chemical and photochemical reactions, thermal decomposition of silver compounds, microwave assisted process and recently via green chemistry route. The use of environmentally benign materials like plant leaf extract (Parashar et al., 2009), bacteria (Govindraju et al., 2009), fungi (David et al., 2010) (Roy et al.,2010) (Saifuddin et al.,2009) and enzymes for the synthesis of silver nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications (Shrivastava et al., 2007, Verma et al,, 2010, Rai et al., 2009) . In continuation of earlier work with silver nano particles synthesis using Cuminum cyminum, *Stigmaphyllon littorale* , *Boswellia serrata* , *Moringa oleifera lam*, *Sterculia foetida* fruit (Kudle *et al.* 2012, and 2013) and *Securinega leucopyrus* (Donda *et al.*, 2013), we here in report the synthesis of nano particles using *Acacia Senegal* leaf extracts at different concentrations.. *Acacia farnesiana* (Yallappa *et al.*, 2013) was the only species which was used earlier for the silver nanoparticles.

Experimentation: Fresh *Acacia senegal* leaf was washed 3 times with de-ionized water. 10g of the leaf was finely cut and were crushed and stirred with 100 ml de-ionized water and filtered by Whatman No.1 filter paper to get the extract. The filtrates are used as reducing agent and stabilizer. UV-Visible absorption spectra, FTIR, TEM was used for the characterization studies. Antibacterial Activity of AgNPs was analyzed by methods similar to those followed by Kudle *et al*. (2012, and 2013).

RESULTS AND DISCUSSION

The reduction of pure silver ions was monitored by measuring the UV-Vis spectrum of the reaction medium by diluting a small aliquot of the sample in distilled water. From UV results obtained, it is evident that the silver nanoparticles were formed and this was confirmed by the surface plasmon resonance exhibited in the range of 300-750nm. For *Acacia senegal*, silver nanoparticles exhibited maximum absorption peak at 439 nm. The flat curves of the graph represent the poly dispersed particles in the solution. (Fig2-b) Fourier-transform infrared spectroscopy was used for the analysis of the reduced silver. The

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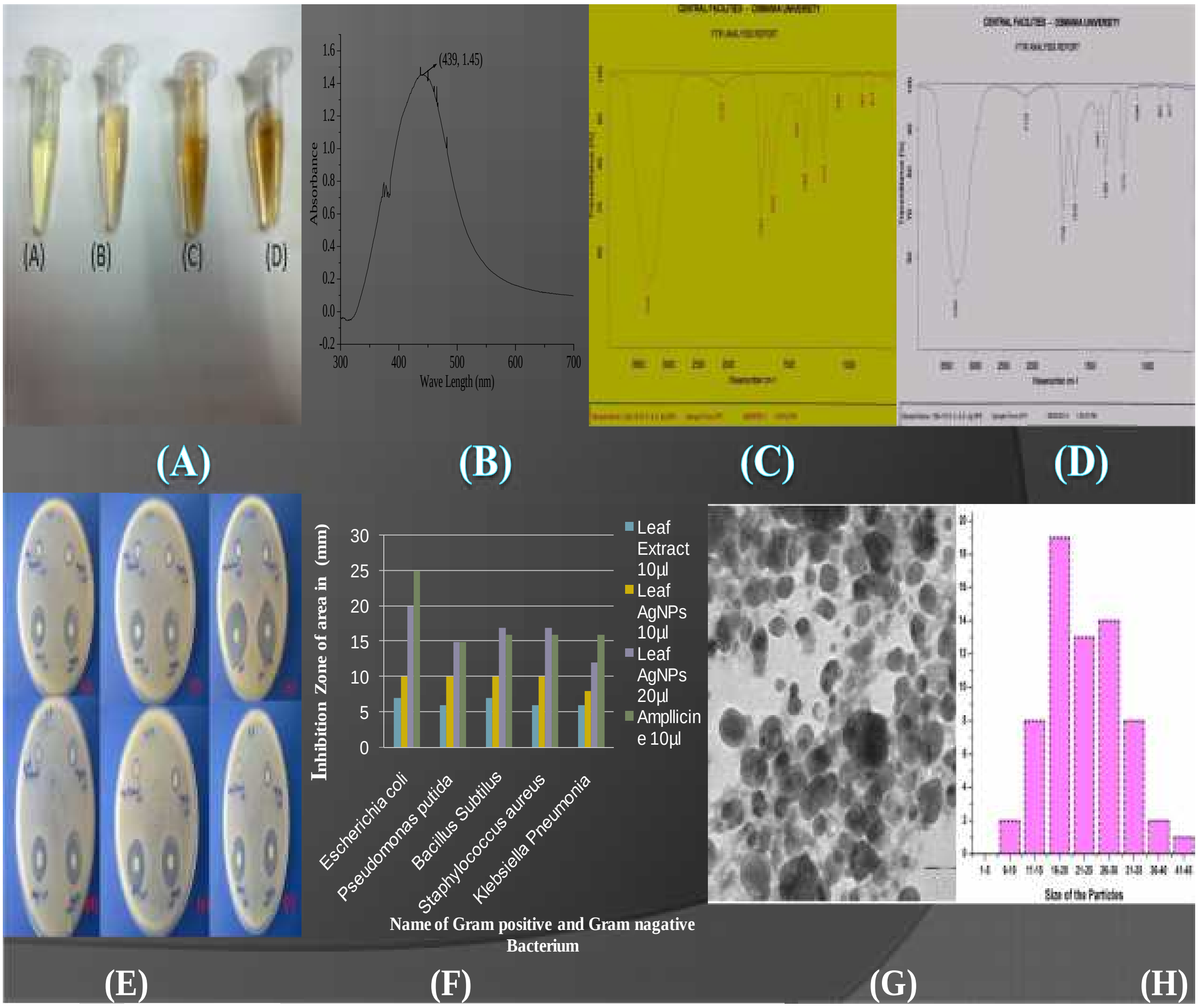

Fig. 1: The Acacia Senegal leaf extracts different concentrations and silver nano particle (A) Uv-Visble Spectroscopy (B) leaf extract (C) AgNPs FTIR (D) antimicrobial activity zones (E) Inhibition zone area in (mm) (F) AgNPs Image of TEM (G) and Size of the particles (H).

spectrum was taken in the mid-IR region of 500–4000 cm-1. The spectrum was recorded using attenuated total reflectance (ATR) technique. The FTIR spectrum of leaf extract and silver nanoparticles is shown in Figure (2c-d). The bands obtained for different AgNPs produced from *Acacia senegal* are almost same with little variations at absorbed wavelengths and percentage transmittance. The leaf extract band near 3349 cm^{-1} corresponds to AgNPs, 3350 cm^{-1} O-H stretching H-bonded alcohols and phenols. The peak leaf extract and AgNPs around 1638 cm-1 corresponds to N-H bond primary amines. The extract and AgNPs peak around 1369 cm-1 corresponds to C-N stretching of aromatic amine group.

Zone of Inhibition is the area on an agar plate where growth of a control organism is prevented by an antibiotic (Ampicillin) and leaf extract also usually placed on the agar surface. The activity of the sample was observed by the formation of zone of inhibition after 12 hours incubation The zone of inhibitions of different bacteria is given in the Figure (2-e). The control plates show the growth of bacteria in the absence of antibacterial agents. The clear zone surrounding the sample in the remaining plates shows the activity of the sample. The zone surrounding the sample is clear that shows complete zone of inhibition. The space surrounding the complete zone of inhibition is partial zone of inhibition where the activity decreases than complete zone of inhibition. Comparing the four compounds concentration (fig. 2-f), inhibitory activity of silver nanoparticles on two of the organisms is almost same. Transmission electron microscopy (TEM) analysis of the synthesized silver nanoparticles was performed. A drop of the silver nanoparticle suspension was placed on carbon coated copper grids and allowing the solvent to evaporate prior to analysis and show morphology, size of nanoparticles (fig2-G and H).

The reduction of aqueous Ag+ ions by the leaf extract at different concentrations of *Acacia senegal* plants is clearly seen. Reduction of the metal ions took place by the leaf extracts leading to the formation of silver nanoparticles. UV-Visible spectroscopy clearly shows that the size of particles differs as concentration of leaf extract changes. According to TEM images, *Acacia senegal* produced a mixture of rods and spherical nanoparticles, *Acacia senegal* sample is found to be the purest containing highest

% of silver with very less impurities and the other species with less amount impurities. From the analysis of FTIR peaks we can conclude that the AgNPs were surrounded by terpenoids, flavanoids and eugenol having functional groups of alcohols, phenols, amines, carboxylic acids, ethers and esters. the spherical shape of the particles confirms and particles to aggregate. Particle size analysis was performed for the silver nanoparticles synthesized using different concentration of curry leaf extract and the obtained results**.** The result indicates that the average particle size of the synthesized silver nanoparticles is highly influenced by the concentration of leaf broth. The similar trend was observed in TEM analysis. The smallest particle size was found to be 6- 45nm, which was obtained using the leaf broth to silver nitrate solution ratio of 1:1. The antibacterial activity of silver nanoparticles was confirmed by Zone of inhibition. As the diameter of the zone of inhibition is high, we can conclude that silver is a very effective antibacterial agent. The activity of silver is effective against both the bacteria which gives a conclusion that it is effective against gram +ve and gram –ve bacteria. The further activity of silver on other micro organisms like fungi, virus, etc should be studied. Basing on these results silver can be used to cure the diseases caused by bacteria. Toxicity studies of silver nanoparticles on human pathogen opens a door for a new range of antibacterial agents.

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