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RESEARCH ARTICLE

BIOSYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL POTENCY OF SILVER NANOPARTICLES FABRICATED FROM *PHYMATODE SCOLOPENDRIA* (BURM. F.) CHING

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ABSTRACT

Plants that serve medicinal purposes contain phytoconstituents. These phytoconstituents are responsible for therapeutic uses of them. Nanomedicinal focus has majorly been on higher plants. Recently, nanomedicine have gained global attention in medicine. In this study, a cost effective and eco-friendly technique for biosynthesis of silver nanoparticles using 1mM, 2mM and 5mM $AgNO_3$ solutions with the extract of a fern *Phymatode scolopendria* as reducing and capping agent was described. The silver nanoparticles were characterized using UV–Vis absorption spectroscopy, Fourier Transform Infra-Red (FTIR), Energy Dispersive Spectrum (EDS), Scanning Electron Microscope (SEM), Transmission Electron Microscope (TEM) and XRD. The biosynthesized nanoparticles also were subjected to antimicrobial activities and compared with standard antibiotics. The result showed that the fabricated AgNPs as shown by the TEM image are spherical nano particles with an average size of 12.41nm. The XRD result shows that the AgNPs are considerably crystalline and polydispersed. The synthesized AgNPs exhibited good antibacterial activity against the bacterial pathogens *Salmonella typhi*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Escherichia coli* but showed least activity against the fungus *Candida albicans,* hence, *Phymatode* AgNPs are good antimicrobial agents.

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INTRODUCTION

Plant samples have recently been analysed and used to generate nanoparticles that can be incorporated into the world of nano medicine (Bhaskar *et al.,* 2012). The fabrication of nanoparticles from plants has shown to be an effective and alternate method for the production of nanoparticles that are novel. Obtaining silver nanoparticles from medicinal plants is an eco-friendly method and these nanoparticles could possess good antimicrobial efficiency against bacteria, viruses and other micro-organisms (Martinez-Castanon *et al.,* 2008). Martinez *et al.* (2008) proposed that investigations that supports the use of silver ion or metallic silver as well as silver nanoparticles should be exploited in medicine for many usefulness ranging, burn treatment, materials for dental purposes, materials for coating stainless steel, textile fabrics, treatment of water, sunscreen lotions etc and which possess low toxicity to the human cells, with high thermal stability, and low volatility. Bacteria and other pathogenic microbes are becoming consistently resistant to conventional drugs.

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However, the innovative scientific world and the need to determine an effective way to cope with this situation has led researchers to manage a new technology in this regard. Researchers reported that AgNPs can be effectively used against multi-drug-resistant bacteria (Ingle *et al.,* 2008; Rai *et al.,* 2012) because they possess large surface area and small size, making them easy to interact with various substances and increases their antibacterial activity. AgNPs (if well synthesized) can be the new generation of antimicrobial agents with the ability to inhibit the growth and life cycle of broad-spectrum microorganisms (Rai *et al.,* 2009 & 2014). Biosynthetic method of silver nanoparticle have some advantages over chemical and physical methods because the latter methods involve the use of chemicals that are toxic and also sometimes, certain reactions takes place at very high (Birla *et al.,* 2009; Rai *et al.,* 2008). However, biological methods for synthesizing AgNPs have shown to be cost effective and with less hazards. The biosynthesis of nanoparticles using plant sources is better than the usage of microorganisms due to the problem of maintenance and culturing of the latter sources. In this present study, we have used *Phymatode scolopendria* for the synthesis of AgNPs. This plant is a seedless vascular plant of the *Polypodiaceae* family.

It is a terrestrial or epiphytic fern with pinnately lobed leaves that are variable in shape. Fertile fronds have scattered, light brown, rather large sori. The leaf and rhizomes of this fern are used in the all-purpose combination medicines of some of the island's curers [3]. In Nigeria, the plant can be found only in the western part, hence, the choice of use.

MATERIALS AND METHODS

Plant material and preparation of the Extract

The plant samples were collected within Adeyemi College of Education Campus (7^o 4.761' N 0^o 4.49.199' E 790ft and 7^o 6.109' N 4^o 48.417' E 833ft), Ondo, Ondo State, Nigeria. It was authenticated at the University of Ilorin Herbarium. They were subjected to air drying at room temperature to avoid the denaturing of some active principles under the high temperature of the oven. After drying, they were ground lightly to form granules. The prepared granules were soaked in methanol (plant material to solvent ratio was 1:10 w/v) and extracted for 72 h at room temperature with frequent agitation. The solution was subjected to filtration using Whatman filter paper. Filtrates of the extracts were dried in the laboratory oven at 40°C and the residue kept at -4^0C. The dried extracts were re-suspended in distilled water to prepare desired concentrations in mg ml^{-1} in further experiments.

Biosynthesis of AgNPs

Initial formation of AgNPs: The silver nitrate solution 1mM, 2mM and 5mM solutions were prepared in 100ml flask. 10ml of plant extract was added to 50ml of different molar concentrations of silver nitrate. The formation of reddish brown colour was observed after about 30minutes and maximum wavelength (λmax) at 15mins time intervals were taken for 2 hours, using a UV Visible spectroscopy to monitor the formation of silver nanoparticles. The AgNPs fabricated using different molar concentrations of $AgNO_3$ solutions were used for antimicrobial screening to identify which molar concentration will yield the AgNPs that will serve as the best antimicrobial agents. Then the solution was stored at room temperature for 24 hours for the complete settlement of nanoparticles.

UV-vis Spectra analysis: The bioreduction process of pure silver ions as well as the quantitative formation of silver nano-particles were observed using the plant extracts with 2mM concentration of $AgNO_3$ by observing the UV-Visible spectrum at different time intervals of 15minutes for 2 hours taking 1ml of the sample, compared with 1ml of distilled water used as blank. UV-Visible spectra analysis was done by using an Elico spectrophotometer at a resolution of 1nm from 340 to 650nm (Eman and Kevin, 2014) to monitor the progress of the silver nitrate reduction reaction and formation within metal ions and the plant extract. The synthesized silver nanoparticles were purified by centrifuging at 4000rpm for 15 mins. This process was repeated and the settled pellet was washed and dried in the oven at low temperature for further characterization study.

Characterization of the formed AgNPs

FTIR (Fourier-transform Infra-Red) Analysis: FTIR studies on the samples were carried out using Shimadzu 8400S FTIR spectroscopy to ensure the active principles in the plant extract involved in the formation of silver nanoparticles.

SEM and EDS analysis of AgNPs: A scanning electron microscope and Transmission Electron Microscope Hitachi S-4500 and Tecnai F20 were used respectively to record the micrograph images of synthesized AgNPs and elemental composition was identified using EDS attached to the SEM machine.

XRD (X-ray diffraction) Analysis: The XRD measurements of film of the biologically synthesized silver Nanoparticles cast onto glass slides were done and examined on a Phillips PW 1830 instrument.

Antimicrobial assay

The silver nanoparticles were studied for their antibacterial and antifungal activities. The Petri dishes were kept at 4 °C for 2 hrs, and 20ml of prepared agar solutions were poured in each of the petridish aseptically. Mueiller-Hinton Dextrose agar was used for both bacterial and fungal strains due to its universality for the growth of both. A loop was used to inoculate each of the microorganism strain on the entire surface of separate Petri-dishes containing the solidified agars in a sequential order for each of the microorganism and in respect of molar concentration of $AgNO_3$ solution to be introduced. The method that was used for the determination of the antimicrobial activity was disc diffusion method according to National Committee for Clinical Laboratory Standards (NCCLS, 2012). Care was taken to avoid mix up. The experiment was performed under strict aseptic conditions. The plates were inoculated with the different microorganisms strains accordingly. Discs of 6mm in size were incubated with 50ul each of the nanoparticle solutions and mounted unto the solidified agar surface. Already prepared discs of Streptomycin and Nystacin were used as positive control standards for bacterial and fungal strains respectively. The plates were incubated and zones of inhibitions were recorded.

RESULTS

Biosynthesis and intensity of AgNPs: In this study, the formation of silver nanoparticles by *Phymatode scolopendria* extract was investigated and this was confirmed by a colour change of light yellow to reddish brown colour in the reaction vessels. The surface plasmon resonance bands were investigated at the wavelength range of range of 340-650 nm (Eman and Kevin, 2014) using Elico U-Vis. Spectrophotometer to monitor the reduction reaction of silver nitrate (Fig. 1). It was shown that the reduction of silver nitrate to silver nanoparticles by methanolic extract of whole plant *Phymatode scolopendria* and surface plasmon absorption peak was at 460 nm. The rate of intensity of *Phymatode scolopendria* silver nanoparticles was measured at different contact time interval of 15 minutes for 1 hr 45 minutes (Fig. 2). Highest intensity was recorded at 90 minutes.

Characterization of AgNPs: The FTIR analysis of P. scolopendria extract and AgNPs as presented in Fig. 3 & 4 revealed peaks mainly at 3317, 2945, 1774, 1635, 1448,1114, 1020 and 613 cm^{-1} conforming the presence of O-H stretch, C-H stretch, C=O stretch, N-H bend, C-C stretch (in-ring), C-N stretch, C-O stretch and C-H bend groups representing the hydroxyl group in alcohols or phenols, alkanes, carbonyls, primary amines, aromatics, aliphatic amines, alcohol carboxylic acids esters and ethers group and alkynes respectively (Fig. 3).



Plate 1. Colour change of solution showing synthesized AgNPs by *P. scolopendria*

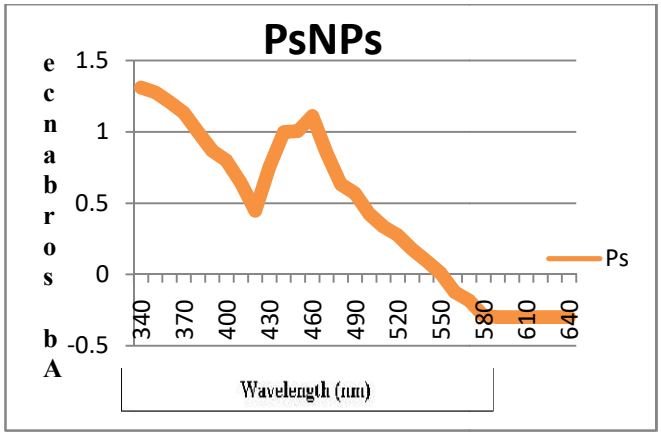


Fig. 1. UV-visible spectra of *P. scolopendria* extract containing (AgNPs)

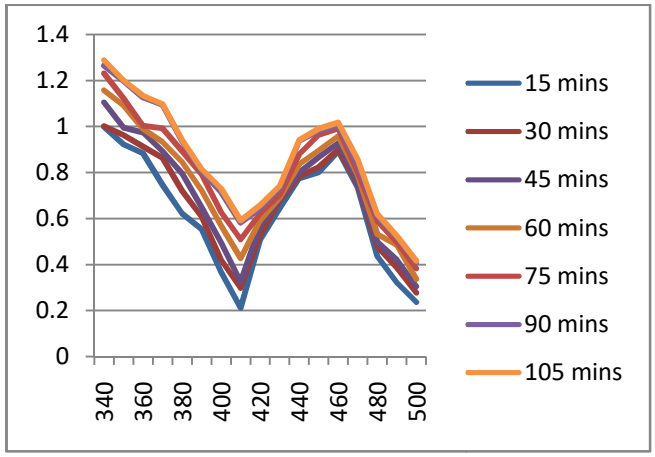


Fig. 2. UV-visible spectra of *P. scolopendria* extract containing (AgNPs) monitored at different contact time

The spectra in Fig. 4 above revealed the presence of prominent peaks at 3309, 2831, 1749, 1635, 1521, 1336 and 1141 cm^{-1} corresponding to different functional groups, namely, O-H stretch, C-H stretch, C=O stretch, N-H bend, N-O asymmetric stretch, N-O symmetric stretch and C-N stretch in synthesized nanoparticles. The peaks represents hydroxyl group in alcohols or phenols, alkanes, carbonyls, primary amines, nitro compounds and aliphatic amine group respectively. This shows that the carbonyls, hydroxyl (-OH) and Amine (-NH) groups of *Phymatode scolopendria* are mainly involved in the synthesis of silver nanoparticles due to their broad peaks. There are other minor peaks. The presence of elemental silver in the reaction mixture was confirmed by EDS analysis using the EDS attachment on the Hitachi S-4500 SEM machine (Fig. 5).

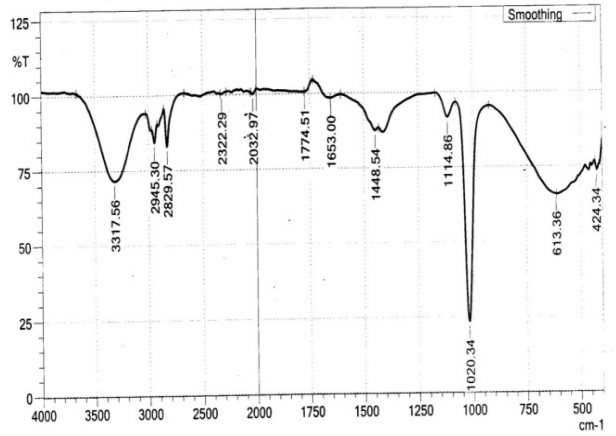


Fig. 3. FTIR Spectra of *P. scolopendria* extract

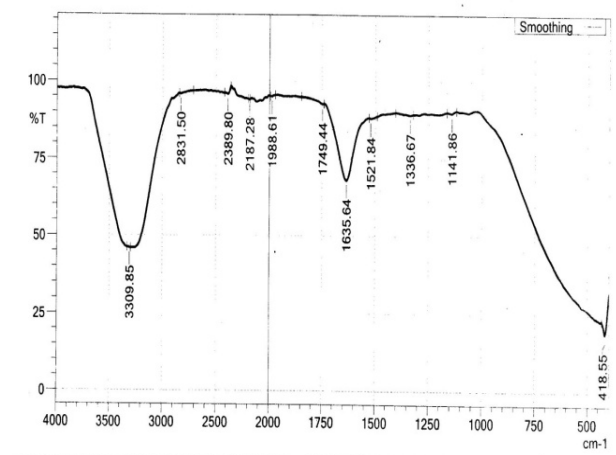


Fig. 4. FTIR Spectra of *P. scolopendria* AgNPs

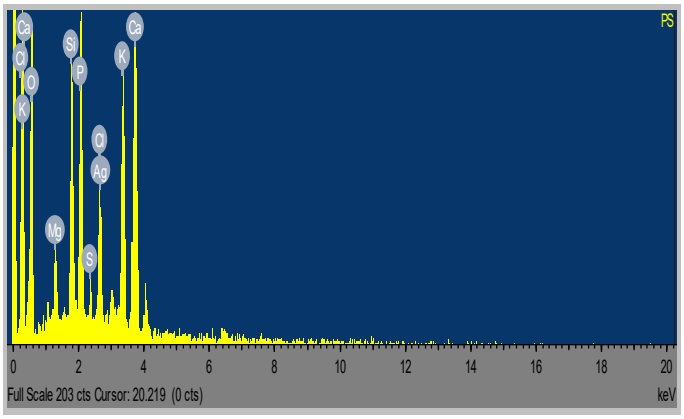


Fig. 5. EDS spectra of *P. scolopendria* AgNPs and elemental composition

The silver nanoparticles exhibited an optical absorption band peak at 3 keV which is characteristic of the absorption power of metallic silver nanoparticles. The SEM-EDS analysis displayed spectra peak for silver and thus showed its presence as well as other elements. The percentage yield of silver was shown to be 30%. The morphology of synthesized nanoparticles of *P. scolopendria* characterized by Scanning and Transmission Electron Microscopes, these micrographs were taken using Tecnai F20 TEM (FEI, Eindhoven, The Netherlands) operating at an acceleration voltage of 200kV and Hitachi S-4500 SEM machines. Images were collected with a Gatan US 4000 digital camera using the Digital Micrograph platform. The AgNP formed were found to be uniformly spherical in shape and polydispersed.

Table 1. Antimicrobial Activity of Phymatode scolopendria AgNPs

P. scolopendria SNP at different concentrations of $AgNO_3$ solution				
	Zones of Inhibition measured in mm			
Microorganisms	Streptomycin/ Nystacin	5mM	2mM	1mM
Pseudomonas aeruginosa	12^a	12^a	10^b	-
Salmonella typhi	12^c	20^a	14^b	10^c
Escherichia coli	12^a	11^a	10^b	-
Klebsiella pneumonia	12^a	10^b	5^c	5^c
Candida albicans	14^a	-	-	-

Mean values with different letters are significantly different at $P \leq 0.05$

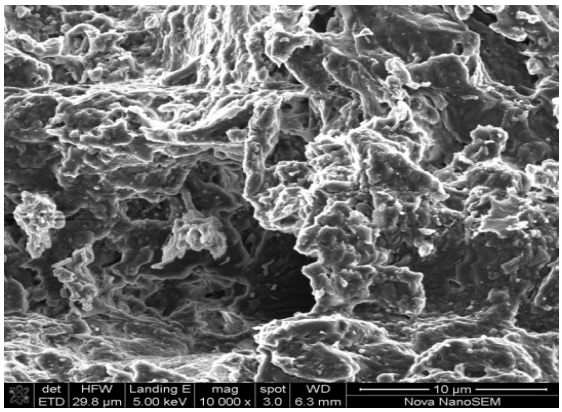
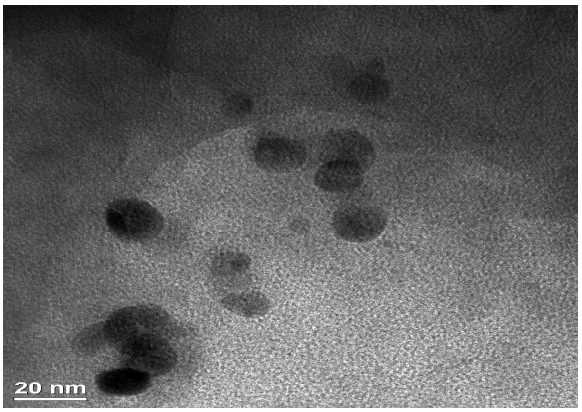


Plate 2 (a). SEM Image of *P. scolopendria* extract



(b) TEM Image of AgNPs synthesized by *P. scolopendria* extract

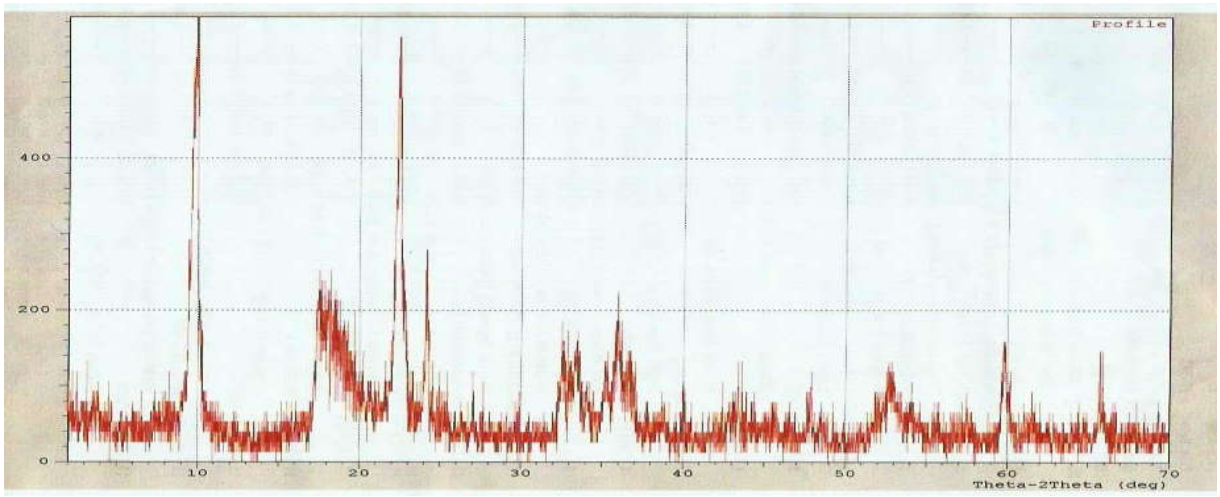


Figure 7. The XRD spectra of *P. scolopendria* AgNPs

The TEM image exposed that the AgNPs have an average size of 12.41 nm. This was measured using Image J statistical software package (Plate 2 a and b). The XRD analysis result showed obtained diffraction nano peaks at 32.58°, 37.84° and 64.87° and which are respectively assigned to (101), (111), (200) and 220 planes (Fig. 6). This corresponds to the JCPDS-International Center for Diffraction Data No. 65-2871.This indicated that the synthesized Ag nanoparticles are crystallised in face centered cubic (fcc). The XRD pattern thus clearly shows that the silver nanoparticles are crystalline in nature according to JCPDS International Center for Diffraction Data, PCPDFWIN v. 1.30, 31-1238.

DISCUSSION

One of the most widely used methods for the synthesis of silver nanoparticles is biological material synthesis. This study investigates the use of *P. scolopendria* for the synthesis of silver nanoparticles with active antimicrobial effect.

Studies have indicated that many biomolecules are responsible for reducing the ions to the nanoparticle size and that they also serve as stabilizing agents (Anuradha *et al.,* 2014; Vedpriya, 2010). The nanoparticles fabricated from *P. scolopendria* were preliminarily characterized by UV-Visible Spectroscopy, which is proved to be a very useful technique for nanoparticles analysis. The plant extracts were mixed with $AgNO_3$ solution it was changed from light yellow to reddish brown colour due to excitation of the surface plasma vibrations which indicates the formation of the Silver nanoparticles (Anuradha *et al.,* 2014; Surekha *et al.,* 2016). The UV spectrum absorption obtained from our result was recorded at 340-600nm and maximum absorption peak was recorded at 460nm. This peak falls within the range of specification for nanoparticles (Gnana Dhas *et al.,* 2012; Mousa and Mina, 2012). It was also observed that the peaks were broad which is an indication for the polydispersity of the AgNPs (Wang *et al.,* 2010). Our result also shows the UV-Visible Spectra which are recorded after the completion of the reaction at different time intervals of 15 minutes for 1hr 45 minutes at room temperature, it was observed that no obvious

synthesis occured after 90 minutes . Though the colour of the silver nanoparticles changed from light yellow to reddish brown within 30 minutes. This is characteristic of the nanoparticles due to the excitation of surface plasmon vibrations in the silver nanoparticles synthesized (Donda *et al.,* 2013; Gnana Dhas *et al.,* 2012). The intensity increased with increase in the reaction time while the highest intensity was recorded at 90 minutes. The FTIR spectrum of silver nanoparticles are shown in Fig. 4. The major and broad bands includes; the band at 3317 cm-1 is assigned to the O-H stretching of H-bonded alcohols and phenols, the band at 1635 cm-1 corresponds to the N-H bending of primary amine, the band at 1118 cm-1 are related to the C-N streching of aromatic amine group. Whereas in the region 1020 cm-1which corresponds to the C-C streching of alcohols, carboxylic acids, ethers and esters are the binding metal with to form a silver nanoparticle is confirmed (Collera-Z´u˜niga *et al.,* 2005). Other functional groups inclusive of carbonyls, hydroxyl (-OH) and Amine (-NH) groups of *P. scolopendria* fern are also involved in synthesis of silver nanoparticles since they represents the broad peaks. Other minor peaks indicated that the formed silver nanoparticles were surrounded by proteins, terpenoids and other secondary metabolites (Donda *et al.,* 2013) as also represented in the raw extract. The Carboxylic acids, esters and ether as well as the alkanes and the aliphatic amines are responsible as capping/stabilizing agents. Studies have shown that biomolecules like proteins, phenols, and flavonoids not only play a role in the reduction of silver ion to nanoparticles, but also serves as capping agents (Vedpriya, 2010). The EDX spectra obtained from our result revealed the purity of the material and the complete chemical composition of synthesized silver nanoparticles. The AgNPs were found to be 16% which was considered to be averagely high in percentage and in occurrence with some elemental components found in the extract (Anuradha *et al.,* 2014). The SEM and TEM images showing the silver nanoparticles synthesized by the fern extract, further confirmed the development of silver nano structure. The TEM image shows the formation of spherical nano particles. The average size was confirmed to be 12.41nm. The synthesized silver nanoparticles exhibited good antibacterial activity against the bacterial pathogens *Salmonella typhi*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli.* It showed least activity against the fungus *Candida albicans*. Though the antibacterial effect depended on size & dose was more pronounced against gram-negative bacteria than gram-positive bacteria. This supports earlier reports in previous studies (Abd, 2011; Baker *et al.,* 2005). The antimicrobial efficacy of AgNPs has also been reportedly found to depend on the dimension of the particles, the smaller the particles, the greater antimicrobial effects (Singh and Prasad, 2007).

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