



International Journal of Pharmacology

ISSN 1811-7775

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Antibacterial Activity of Biogenic Silver Nanoparticles Produced by *Aspergillus terreus*

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ARTICLE INFO

Article History:

Received: July 10, 2015

Accepted: August 18, 2015

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ABSTRACT

In the present study, *Aspergillus terreus* used for the biosynthesis of silver nanoparticle. The *A. terreus* cell free filtrate reacted with silver nitrate, resulting formation of silver nanoparticles (AgNPs). The AgNP was marked by visual analysis, UV-Vis absorption spectroscopy, Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM). The AgNP showed high absorbance at 450 nm in UV-Vis spectroscopy. The TEM micrograph exhibited poly disperse spherical and ellipsoid NPs in the size range from 20-50 nm. The fungus biogenic AgNPs established strong antibacterial activity against *Staphylococcus aureus* (MRSA), *Shigella boydii*, *Acinetobacter baumannii*, *Shigella sonnei* and *Salmonella typhimurium* compared with four antibiotics used. Biological way using the microorganisms is a new approach to the secure, less expensive and eco-friendly technique for the synthesis of silver nanoparticles is gaining importance in the field of nano-biotechnology.

Key words: *Salmonella typhimurium*, antibacterial activity, *Staphylococcus aureus*, silver nanoparticles

INTRODUCTION

In the recent years, traditional antibacterial agents have become progressively less significant and a large percentage which influential are extremely toxic making them worthless for using in the medicine and food (Goleva *et al.*, 2008). Silver salts from among certain metals that have antiseptic properties which are used in traditional medicine (Wber and Rutala, 2001; Besinis *et al.*, 2014). It is known from centuries silver in ionic form to treat venereal diseases, abscesses around the anus, burns pilgrimage, ophthalmic diseases and silver ion is also effective against several pathogenic human bacterial

species (Kurek *et al.*, 2011). Due to the unique physicochemical characteristics and antimicrobial activity of AgNPs, they are considered most functionalization and marketing of nanomaterials (Buzea *et al.*, 2007; Murphy *et al.*, 2015). The AgNPs has antibacterial, antifungal, antiviral, anti-inflammatory and anti-angiogenesis (Verma *et al.*, 2010; Kim *et al.*, 2012; Galdiero *et al.*, 2011; Wong *et al.*, 2009; Baharara *et al.*, 2014). The AgNPs prevent cell division, leading to the envelope of cell and cellular contents damage of the bacteria (Jung *et al.*, 2008). Regardless of its antibacterial nature, Ag⁺ has long been an effective recognized antioxidant agent as it show slow poisonousness in

living organisms and has several *in vitro* and *in vivo* applications (Buzea *et al.*, 2007). Different methods such as chemical, irradiative, physical, photochemical, electrochemical and biogenic procedures have been approached for the synthesis of nanoparticles (Pal *et al.*, 2007; Kim *et al.*, 2012; Li *et al.*, 2012). The present study aimed to (1) Green synthesis of AgNPs by the fungus *Aspergillus terreus*, (2) Characterize the biogenic AgNPs produced by *A. terreus* and (3) Antibacterial activity of AgNPs against the human pathogenic bacteria was studied.

MATERIALS AND METHODS

Aspergillus terreus was isolated from mangrove and served on potato dextrose agar at 25°C. The fungus was identified by morphological characteristics. Five human pathogenic bacteria were tested for their sensitiveness for AgNPs: *S. aureus* (MRSA), *Shigella boydii*, *Acinetobacter baumannii*, *Shigella sonnei* and *Salmonella typhimurium*. Two medium (PDA and Potato Dextrose Broth (PDB) and silver nitrate (AgNO₃) were purchased from Sigma-Aldrich ((St. Louis, MO, USA) and used as received.

Biomass preparation: The Erlenmeyer flasks (250 mL) containing 100 mL PDB were inoculated with spores suspension of *Aspergillus terreus* (1×10⁶ conidia mL⁻¹). All flasks were incubated at 25°C on a rotary shaker (200 rpm) for 7 days. The biomass was harvested under filter paper (Whatman No. 1) and then washed with distilled sterilized water to eliminate any components of the medium. Twenty five grams biomass was added to flasks having 150 mL sterile distilled water and incubated under the conditions previously mentioned for 24 h. The biomass was filtered with Whatman paper No. 1 and the crude cell filtrate was collected to experiment subsequent.

Biogenic of silver nanoparticles (AgNPs): The AgNPs were synthesized by 200 mL filtrate mixed with 20 mL AgNO₃ solution (10 mmol L⁻¹) in a 500 mL flask and incubated at 25°C in dark for 24 h. An Erlenmeyer flask without AgNO₃ solution was served as control. Silver nanoparticles were concentrated at 10000 rpm for 14 min, then collected for further description (Li *et al.*, 2012).

Characterization of silver nanoparticles

UV-visible spectroscopy analysis: After incubation of AgNO₃, the color of filtrate was changed and optically observed over various the incubation periods. Silver ion bioreduction was detected by specimen of 1 mL aliquot and absorption measuring was done on UV-visible spectroscopy (name of spectra). Absorbance was measured through 300-800 nm.

Transmission Electron Microscope (TEM): The images of AgNPs were obtained by transmission electron microscope (JOEL, Japan, Model-6360, in Department of Geology, UOM, Guindy). A drop of synthesized silver nanoparticles was put on the carbon covered copper grids and reserved for curt. After drought of specimen grid loaded on to a sample holder and figure get captured within 2 min (Shelar and Chavan, 2014).

Antibacterial activity assay: The antimicrobial activity of AgNPs against MRSA, *S. boydii*, *A. baumannii*, *S. sonnei* and *S. typhimurium* was evaluated by the agar diffusion technique (CLSI., 2009). Muller-Hinton agar medium was poured in petri plates to a uniform depth of 5 mm. After solidification of the medium, 0.9% saline solution bacteria were speared on Muller-Hinton agar plates with cotton swab. Wells were introduced in the agar plates with a sterilized 3 mm cork borer. The 50-75 µL from AgNPs solution were added in the wells and four discs were added to petri plates. All plates were incubated at 35°C for 24 h and three replicates were used per treatment. The inhibition zone was measured and compared with antibiotics treatments.

RESULTS AND DISCUSSION

Visual analysis and UV spectrophotometer: In the present study, silver nanoparticles were synthesized by a reduction of Ag ion using the culture supernatants of *A. terreus* at 28°C. It was mostly known that silver nanoparticles generated yellow solution in water (Fig. 1). This change in color is due to the Surface Plasmon Resonances (SPR) impact and reduction of silver nitrate (Wiley *et al.*, 2006). The fungus free extract was changed to brown from yellow within 3 h, conversely no change in color in the culture without AgNO₃ (Fig. 1). Therefore, color change of the extract obviously showed the formation of silver nanoparticles. The color density of cell



Fig. 1: Color of the fungal cell free *Aspergillus terreus* without AgNO₃ and with AgNO₃

extract continued with AgNO₃ even after 24 h of incubation, which noted that the nanoparticles were fully scattered in the solution and there was no clear assembly. The UV spectrophotometer of cell filtrates of the fungus with the silver nitrate presented properties SPR adsorption band at 450 nm (Fig. 2).

EDX: The existence of Ag was assured through Fig. Energy dispersive analysis X-ray (EDX) spectrum recorded in the spot-profile form from one of the heavy populated silver nanoparticle region on the film surface. Powerful signals from Ag atoms in the nanoparticle were noticed (Fig. 3 and 4). Whereas, weaker signals from O, Al, Si, C and Cl atoms were also observed. Vertical axis show the number of X-ray count, while the horizontal axis presents energy in KeV.

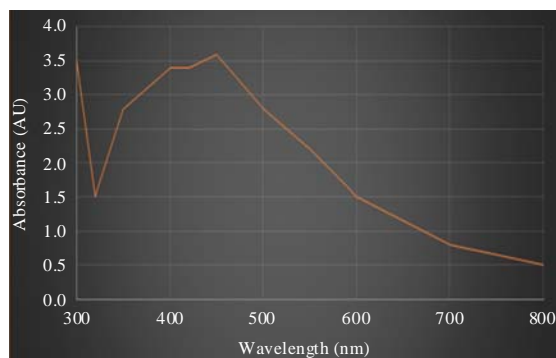


Fig. 2: UV-Vis spectrum recorded for AgNPs synthesized by *Aspergillus terreus*

Visual absorption band peak in 3 KeV is perfect for the absorption of Ag nano-crystallites (Magudapathy *et al.*, 2001; Jegadeeswaran *et al.*, 2012).

SEM: The SEM micrograph of nanoparticles exhibited that Ag nanoparticles were mostly cubical well dispersed with sizes ranging from 20-50 nm (Fig. 5). It was recognized the form of metal nanoparticles a significant change their visual and electronic characteristics (Selvi and Sivakumar, 2012; Gopinath *et al.*, 2013).

Transmission electron microscopy: The TEM images extended specifics morphological structure of AgNPs. Special form and size of poly disperse AgNPs were obtained from TEM images (Fig. 6). The AgNPs were globular and oval.

Antimicrobial activity: Inhibition zone (mm) was obtained for AgNPs solutions tested against MRSA, *S. boydii*, *A. baumannii*, *S. sonnei* and *S. typhimurium*. Data presented in Table 1 showed that silver nanoparticles gave high antibacterial activity against one Gram-positive bacteria and four Gram-negative bacteria (Table 1). The AgNPs significantly inhibited the growth of all pathogenic bacterial compared with antibiotics. Inhibition zone diameter around the disk containing AgNPs in MRSA was 21 mm. This showed that AgNPs were nearly 100 and 190.9% effective when compared to both tetracycline and gentamicin, respectively. It was observed that AgNPs inhibited *A. baumannii* growth giving 20 mm when compared to ampicillin neomycin, gentamicin and tetracycline that produced 0, 0, 22 and 25 mm inhibition zone, respectively.

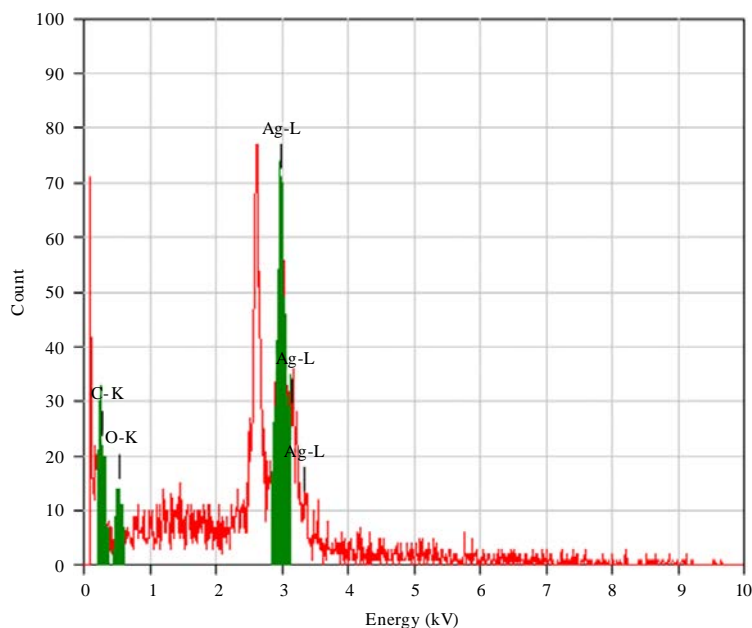


Fig. 3: EDX spectrum recorded of AgNPs synthesized by *Aspergillus terreus*

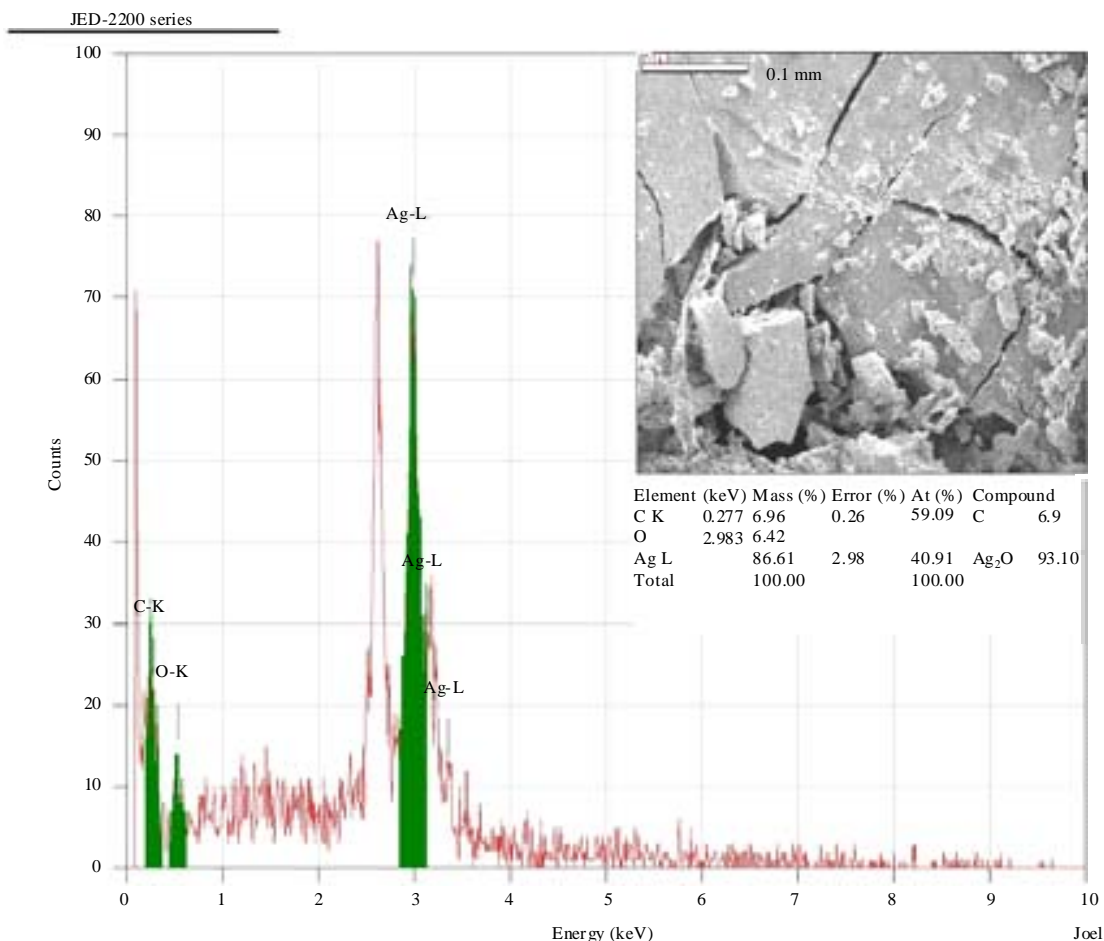


Fig. 4: EDX spectrum and SEM of AgNPs synthesized by *Aspergillus terreus*

Table 1: Antibacterial activity of biogenic silver nanoparticles against pathogenic bacteria compared to four antibiotics

Organisms	Inhibition zone (mm)				
	Gentamicin	Ampicillin	Tetracycline	Neomycin	<i>Aspergillus terreus</i>
<i>Shigella boydii</i>	16	20	15	10	19
<i>Acinetobacter baumannii</i>	22	0	25	0	20
MRSA	11	0	25	0	21
<i>Shigella sonnei</i>	18	0	16	19	18
<i>Salmonella typhimurium</i>	24	10	16	24	19

The efficacy of AgNPs showed better activity to controlling the gram-negative than against the gram-positive bacteria. This was probably related to the density of the peptidoglycan layer that may restrain the action of Ag⁺ through the cell of bacteria. These results are in agreement with other studies (Morones *et al.*, 2005; Singh *et al.*, 2008; Kim *et al.*, 2011; Pettegrew *et al.*, 2014). Feng *et al.* (2000) reported that AgNPs showed antibacterial activity by obstruction proteins and inhibiting replication. AgNPs immediately conjugate with protein and are steady by thiol-bearing cysteine remains (Elechiguerra *et al.*, 2005). Xiu *et al.* (2012) presumed that silver nanoparticles will

interact slowly with naked peptides on the well of in gram-negative bacteria but rapidly with well of gram-positive bacteria because the well in gram-negative covered with extra layers. Also, silver nanoparticles aggregation on the cell membrane and uptake inside the cell of other bacteria such as *S. typhus* and *P. aeruginosa* (Xiu *et al.*, 2012). Lok *et al.* (2006) reported that silver nanoparticles were destabilized the cell and plasma membrane of bacteria, resulting in exhaustion of intracellular adenosine triphosphate. Silver and silver ion had exposed strong antimicrobial activity as these have large surface area with strong reaction places (Morones *et al.*, 2005).

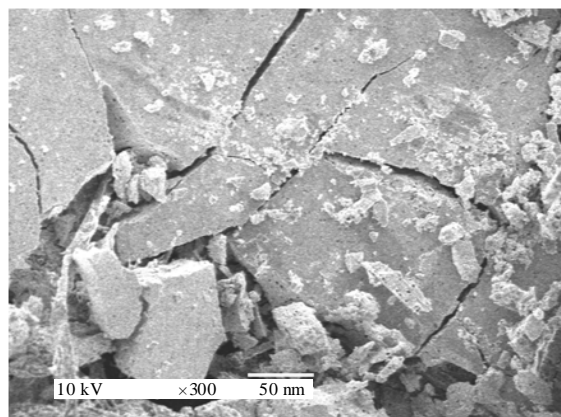


Fig. 5: SEM micrograph of AgNPs synthesized by *Aspergillus terreus*

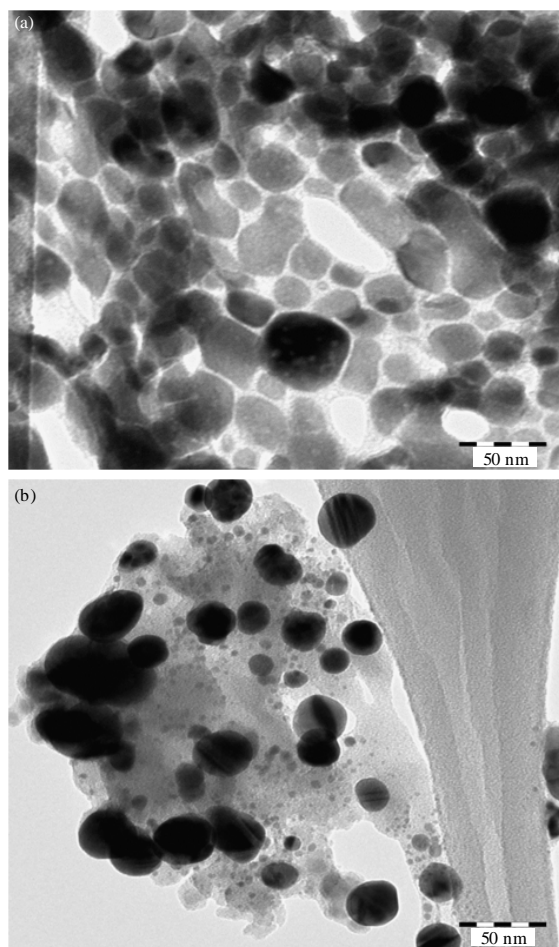


Fig. 6(a-b): TEM images of AgNPs synthesized by *Aspergillus terreus*

ACKNOWLEDGMENT

This project was supported by King Saud University, Deanship of Scientific Research, College of Science Research Center.

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