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Antibacterial Activities of Silver Nanoparticles Synthesized from plants and Fungi

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ABSTRACT

The present study aimed to synthesize silver nanoparticles (AgNps) having antibacterial properties. Two fungal strains, viz *Aspergillus niger* and *A. terreus* were procured from NCIM, Pune. And were used to synthesize silver nanoparticles by reduction and precipitation of $AgNO_3$. The aqueous plant extract of Neem (*Azadiracta indica*) leaves was prepared for the synthesis of AgNps. All the AgNps derived from fungi and plant sources were evaluated for antibacterial properties against known laboratory pathogens. The obtained nanoparticles were characterized using a UV-visible spectrophotometer and SEM. Silver nanoparticles were produced significantly from *A. niger* at 400nm and *A. Terreus* at 400 nm. The aqueous extract of the neem plant showed maximum absorption at 500 nm. Also, the extract nanoparticles showed the highest antibacterial activity against *E. coli*, *Proteus vulgaris, Pseudomonas* spp, *Salmonella paratyphi-A* and *S. aureus.*

Key words : *Antibacterial, Silver nanoparticles, Aspergillus niger, A. terreus, Azadiracta indica.*

Introduction

Using the dissimilatory properties of fungi, the biosynthesis of inorganic nanomaterials using eukaryotic organisms such as fungi may be used to grow nanoparticles of gold. The study of the biosynthesis of nanomaterials offers valuable contributions to materials chemistry. The ability of some microorganisms such as bacteria and fungi to control the synthesis of metallic nanoparticles should be employed in the synthesis of new materials. Silver nanoparticles are widely used for its unique properties in catalysis, chemical sensing, biosensing, photonics, electronics, and pharmaceuticals (Garima *et al.*, 2011). Silver nanoparticles have a great potential for use in biological including antimicrobial activity (Ingle *et al.*, 2009). Fungi have a high metal tolerance ability and so canbe used for synthesis of silver nanoparticles. Silver nanoparticles are reported to possess antifungal, anti-inflammatory, antiviral, anti angiogenesis, and anti platelet activity (Jain *et al.*, 2009). Thus the present study was aimed to synthesise silver nanoparticles from the fungi , Aspergillus and neem *Azadiracta indica*) leaf extracts.

Materials and Methods

Fungal cultures

The fungal cultures were procured from NCIM, Pune

Production and separation of fungal Biomass

The spore suspension (10^8 spores/ml) of *Aspergillus niger* and *A. terreus* were inoculated in 100 ml Sabouraud's broth in 500 ml capacity flasks. for mycelial growth. These flasks were incubated at 28

°C for 48 h. The biomass was separated by using the filtration technique. The broth was filtered through Whatman's filter paper No 1. The filtrate was discarded. The mycelia were washed with sterile distilled water to remove the traces of the medium by centrifugation. The mycelial growth was resuspended into 250 ml of sterile distilled water and incubated at 28 ^{0}C for 48-h to extract cell material.

Preparation of plant extract

Crude extracts were prepared by using the fresh leaves of Neem. These leaves were thoroughly washed under running tap water. 20 g of leaves were crushed in 50 ml of distilled water. It was then filtered using Whatman's filter paper No. 1, yielding the crude extract.

Silver nitratereduction and production of silver nanoparticles (Garima *et al.*, 2011; Kumar *et al.*, 2012 and Sarkar *et al.*, 2010)

The biomass in the distilled water was filtered through the Whatman's filter paper No.1. The filtrates were collected and the cell mass is discarded. These filtrates as well as crude extract of leaves was treated with equal volumes of $AgNO_3$ (1x10'M) solution and contents of flasks were incubated for 24-48 h at 4 ^{0}C. After incubation dark brown colour developed which was taken as indication of the formation of nanoparticles.

Characterisation of silver nanoparticles

UV-Visible spectrophotometer (Shankar *et al.*, 2005)

The absorbance of the reaction solution was measured at various wavelengths (200-1000 nm) and particular periods (0, 24, 48 h).

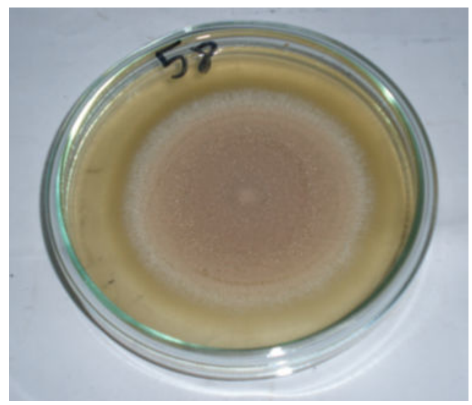
Antimicrobial activity (4, 5, 6, 8 and 9)

The antimicrobial activity of the reaction mixture was tested against pathogens *Staphylococcus aureus*, *Salmonella paratyphi-A*, *Proteus vulgaris*, *Pseudomonas* spp, and *E. coli* by the agar well diffusion method using Sabouraud's agar and 100 µl each of samples were used for inoculation in the wells.

Results

Aspergillus niger and Aspergillus terreus were cultured on Sabouraud's agar as shown in photoplates 1 & 2 Results of silver reduction properties by fungi and plant extract are shown in Figs. 1, 2, and 3. In the spectrophotometric analysis, the reaction mixture of *Aspergillus niger*, *Aspergillus terreus* & Neem exhibited a strong absorption between 400, 400 and 500 nm, respectively.

Photo plate 1. Colonial growth of *Aspergillus niger*



Photoplate 2. Colonial growth of Aspergillus terrus

SEM micrograph

Scanning Electron Microscopy (SEM) shows silver nanoparticle aggregates (photoplate-1). In this micrograph, it was observed the size of spherical nanoparticles was (20-50 nm).

The size of rod-shaped nanoparticles ranges from 5-10 nm

Antimicrobial activity

Antibacterial activity of Ag nanoparticles against *E. coli and S. aureus*: Bacterial inhibition tests against *E.coli, Proteus vulgaris, Pseudomonas* spp *Salmonella paratyphi-A and S. aureus* are studied, the representative results are shown in shown in Photoplates: 2 & 3, clear zone diameters of the bacterial inhibition was correlated to the antibacterial activity of silver particles.

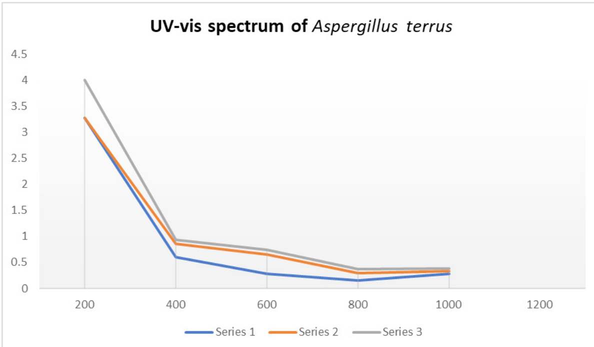


Fig. 1. UV visible spectrum of *Aspergillus terrus*

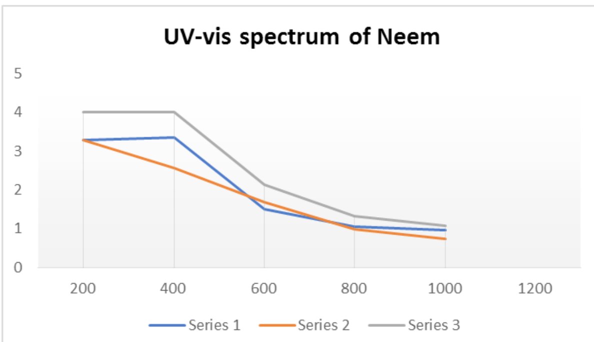


Fig. 2. Reduction of silver nanoparticles by *neem extract*

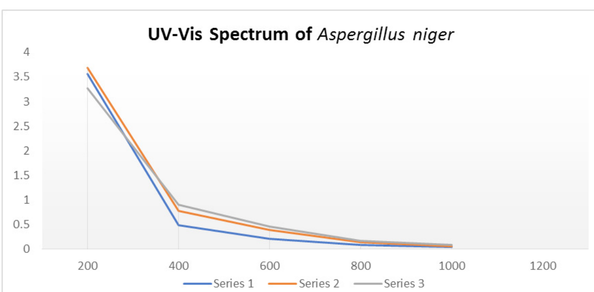
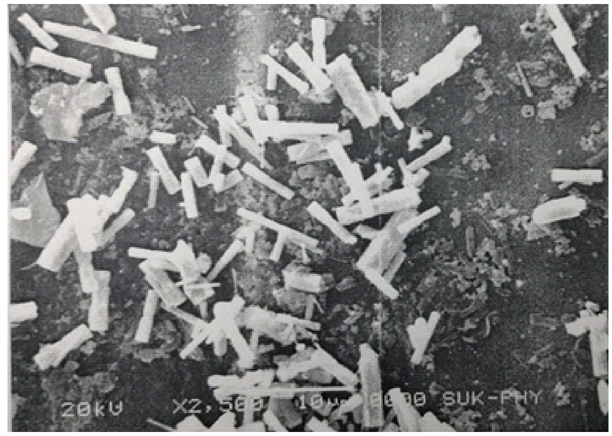
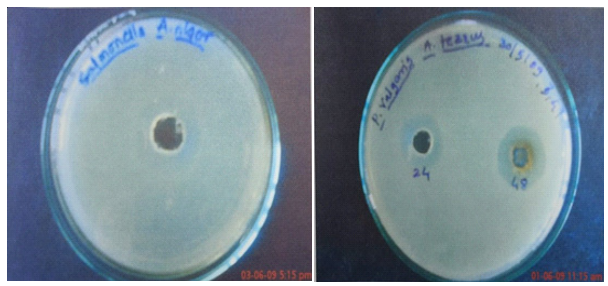


Fig. 3. Reduction of silver nanoparticles by *Aspergillus niger*

Fungal cell filtrates treated with silver nitrate solution is known to show peak around 420nm with high absorbance (Kumar *et al.*, 2012). The similar results of absorbance peaks were seen in the above studies. These data are consistent with previously reported studies in which silver ions had effective antimicrobial properties at concentrations of 1x10 M. It has been reported that AgNPs exhibit a dark brown color in aqueous solution as a result of surface Plasmon resonance (Kumar *et al.*, 2012). Silver nanoparticles were fairly toxic to *Pseudomonas aeruginosa* while they showed a moderate toxicity



Photoplate 3. Scanning electron micrograph of silver nanoparticles from *Aspergillus niger*



Photoplates 4 & 5. Showing antimicrobial properties of silver nanoparticles against known bacterial pathogens.

against *P. vulgaris*, *E. coli*, *B. subtilis*, and *P. putida* studied (Sap-Lam *et al.*, 2010).

Conclusion

The silver nanoparticles produced using *Aspergillus niger* and *A. terreus* and *Azadiracta indica* extract possess antibacterial properties against pathogens generally known multiple drug resistant organisms which indicates that these nanoparticles have potential in controlling such pathogens.

Conflict of Interests

There are no conflicts of interests amongst authors.

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