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Amplification of antibacterial property of drugs in combination with silver nanoparticles synthesized from an airborne fungus

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Abstract: The synthesis of silver nanoparticles (AgNPs) has received considerable attention from last one decade with their potential biomedical applications. Green synthesis has shown tremendous importance in the study of nanoparticle synthesis, instead of chemical and physical approaches, which has led to the development of various biomimetic approaches for the growth of advanced nonmaterials. The present study was focused on novel biological method for the synthesis of silver nanoparticles using an airborne fungus, *Aspergillus* sp. The color of the solution altered being on addition of $AgNo_3$ to the cell free extract in the conical flask was the indication of the formation of silver nanoparticles which also showed absorbance peak at 411nm in the UV-Spectrophotometer. The synthesized AgNPs were further characterized by Atomic Force microscopy (AFM) and Field Emission Electron Microscopy (FESEM). The nanomaterials showed monodispersity in the range of 45-70nm. The antibacterial activity of the drugs viz., Gemifloxacin and Moxifloxacin was found to be more effective against selective clinical bacterial specimen when treated in combination with the synthesized nanoparticles. The biologically synthesized nanoparticles from *Aspergillus* sp. in combination with antibiotics would be the novel remedy substituent in the place of high dose antibiotics in near future.
Keywords: Amplification, AgNPs, UV-Spectrophotometer, Antibacterial property, Drugs.

Introduction

Nanoparticles are often referred to exhibit unique properties with relation to variation in specific characteristics like size, shape and distribution have been demonstrated[1]. In nanotechnology, the silver (Ag) is becoming a noble metal instead of other metals like (e.g., Ag, Pt, Au and Pd), because of having potential biomedical applications in the field of medicine[2]. Silver nanoparticles (AgNPs) have exclusive properties in various fields such as catalysts in chemical reactions[3], pharmaceutical components and in chemical sensing and biosensing[4,5]. The formation of these nanoparticles has been reported using chemical and physical methods but biological methods for the production of AgNPs are currently gaining importance in the field of medicine because they are environmentally friendly and cost effective. They don't involve in the formation of any toxic chemicals in the way of the synthesis [6-8]. It has been already reported different sizes of AgNPs [9-11], their shape[12] and solubility[13,14] which affect the AgNPs' toxicity. The studies have shown that biologically synthesised AgNPs are having good antibacterial activity[15]. As it was proved by the authors in our previous work, silver nanoparticles have inhibitory and bactericidal effect[15,16]. The study on *Escherichia coli* has shown that AgNPs is showing cell lysis and finally kills them, because it reacts with cell walls and cytoplasmic membranes, which results in pits in the cell wall of bacteria. It has been also demonstrated that bacteria becomes usually incapable

to develop resistance against AgNPs, because they can attack broad range of targets in microorganisms such as proteins with thiol groups, cell walls and cell membranes[17,18]. In previous studies it was being thoroughly investigated that Silver ion, silver compounds and AgNPs are having good antimicrobial and antiviral activity[19-22]. In this study, the AgNPs have been synthesised from an airborne fungus, *Aspergillus* sp. and were characterised using silver nitrate as silver precursor and the fungus as reducing agent and stabilizer. The antibacterial effect of AgNPs was evaluated in addition with available drugs against four pathogenic bacteria, including *Escherichia coli* (Gram negative) and *Staphylococcus aureus* (Gram positive) using the agar disc diffusion method[23].

Materials and Methods

Isolation of the airborne fungus

The *Aspergillus* sp. was isolated from the atmosphere of a vegetable market of Chennai by exposing Sabouraud's Dextrose agar media plates based on gravitation method and incubated for 3-7 days in the Microbiology Laboratory, Department of Biomedical University, Chennai-60011. After identification, the fungus was subcultured and stored in refrigerator at 4°C up to further studies.

Synthesis of silver Nanoparticles

Seven day old culture of the fungus was used for the biosynthesis of silver nanoparticles. Fungal biomass was grown aerobically in a liquid medium containing KH_2PO_4 7.0, 2.0 k_2HPO_4 $MgSO_4$. $7H_2O$ 0.1, $(NH_4)2SO4$ 1.0, yeast extract 0.6, glucose 10.0 [22]. The flask was inoculated and incubated on orbital shaker at 25^oC and agitated at 140rpm. After 72 hours, the biomass was filtered using Whatman filter paper No.1 and extensively washed three times with Milli-Q water to remove the medium residues. The resulting fresh and clean biomass was taken into the Erlenmeyer flasks containing 100ml of Milli-Q demonized water under sterile conditions. The flask was incubated at 25^0C in a shaker at 140 rpm for 72 hours. After the period of incubation, the biomass was again filtered with Whatman filter paper No.1 to separate from biomass and the cell free extract was used in the following experiment. 1mM $AgNo_3$ was prepared and 50ml was added to the cell free extract and kept in a shaking incubator at 25^0C and 140rpm for 24hours in dark condition for colour change.

Characterization of nanoparticles

The characterization involves primarily qualitative analysis of silver ions (Ag+) through UV-spectrophotometer after change in colour. Periodically, small aliquots (1ml) of the reaction solution of supernatant was withdrawn and the absorbance was measured in between the ranges of 350-700nm against culture suspension without silver ions as control in the solution was observed after 24hrs. Observation peak was being measured continuously to check their stability.The synthesised nanoparticles were further characterised through Atomic Force Microscope (AFM) to confirm the particle size and agglomeration of nanoparticles through three dimensional images. The sample used in the study was sonicated for 7minutes and then centrifuged at 1000rmp for 5minutes and then a small volume of sample was spread on well cleaned glass cover slip and dried at room temperature for analysis. The surface morphology of silver nanoparticles was studied via FESEM analysis. The synthesised nanoparticles were dried and being converted into powder form for SEM analysis.

Determination of antibacterial activity

The efficacy of silver nanoparticles was determined by performing antimicrobial susceptibility test using disk diffusion method against gram positive and gram negative organisms viz., *Bacillus cereus, Escherichia coli, Proteus vulgaris* and *Staphylococcus aureus*. The bacteria were grown on nutrient agar medium (Qualigens, India). The antibacterial assay of AgNPs was performed using Disk Diffusion method. While performing the synergistic effect of nanoparticles with antibiotics, the antibiotics disks were impregnated on agar plates inoculated with pathogens with 25µL solution of AgNPs and incubated at 37^oc for 18-24hrs. The zone of inhibition was measured and compared with the control.

Results & Discussion

The interaction between fungal cultured filtrate containing extra cellular component and metal ion was observed by colour change from chalky white before the addition of 1mM silver nitrate solution into brownish colour on completion of reaction with Ag+ ions after 24hrs (Fig 1)[15]. The appearance of yellow brown colour in

the silver nitrate treated flask indicated the formation of silver nanoparticles due to the reduction of metal ions and Plasmon resonance.

a **b**

Fig 1: Synthesis of Silver Nanoparticles from *Aspergillus* sp.
(a) Before the additin of $AgNo_3$ (b) After the addition of $AgNo_3$

The analysis of synthesised silver nanoparticles was initially performed by Uv-Vis Spectroscopic analysis. The presence of absorption spectrum of silver nanoparticles prepared by biological reduction showed a surface Plasmon absorption band with a maximum of about 411nm (Fig 2) is characteristic of silver nanoparticle[16].

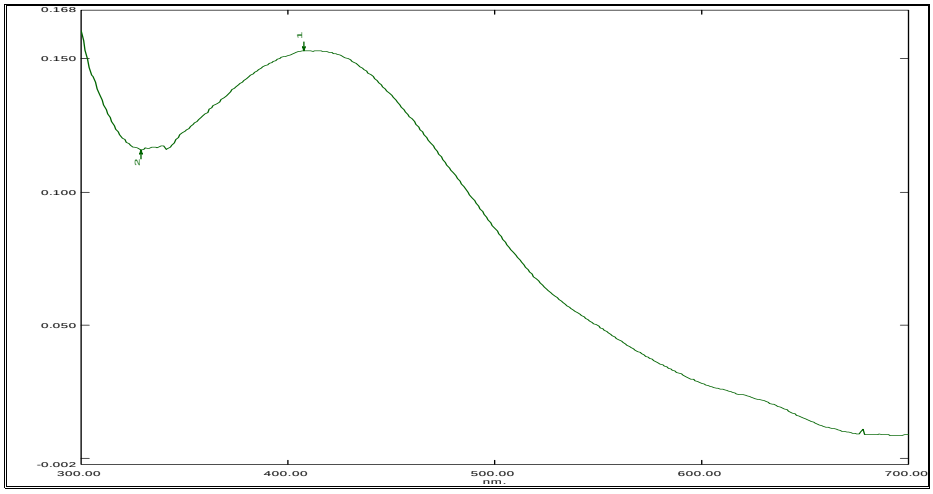

Fig 2: Confirmation of AgNPs by UV-Spectrophotometry synthesised from *Aspergillus* sp.

Porosity, roughness and fractal dimensions of synthesised silver nanoparticles were evaluated by analysing the AFM images to determine the average particle size which was in the range of 50-75nm (Fig 3)[12].

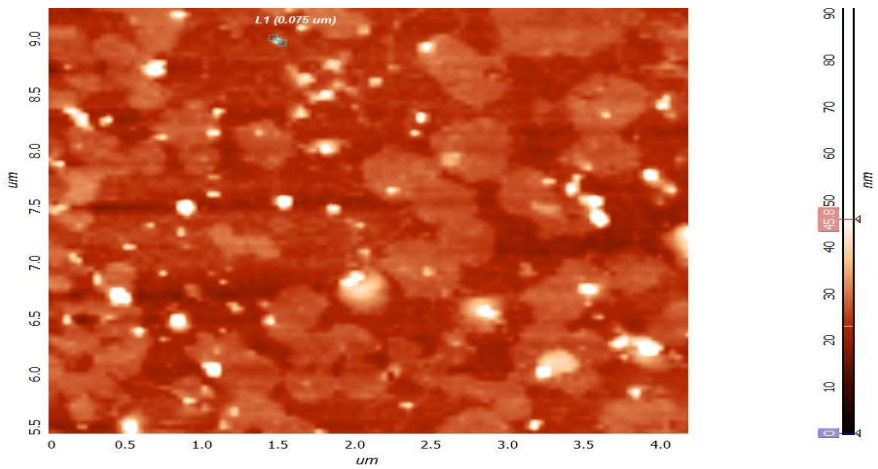

Fig 3: Atomic Force Microscopy micrograph of silver nanoparticles synthesized from the *Aspergillus* sp. in the range of 50-75 nm.

Based on the FESEM analysis, it was found that the nanoparticles distributed uniformly and were dispersed densely and have smooth & rough surfaces. The nanoparticles appearance showed that they were spherical to ovate in structure with having average dimensional size in the range of 45-70nm (Fig 4). The biologically synthesised nanoparticles were characterised by qualitative as well as Quantities techniques to confirm the Absorption peaks (UV-Spectrophotometry), topography and average roughness (AFM) and morphology and size of nanoparticles (FESEM)[2,3]. The absorbance maxima of synthesised nanoparticles was at 411nm, representing surface Plasmon resonance of the silver, while topography and average roughness was in the range of 45-70nm.

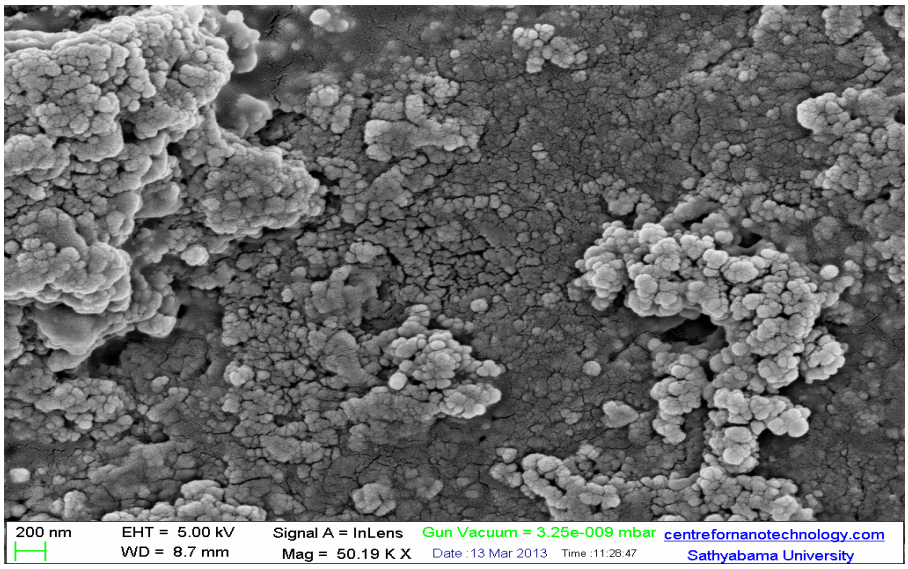

Fig 4: FESEM analysis of silver nanoparticles synthesised from the *Aspergillus* sp.

The synergistic effect of the drugs and the synthesized AgNPs was found significant with regards to the zone of inhibitions, the diameter of inhibitions zones in (mm) and enhanced effect (Table 1). Gemifloxacin showed good activity against *Staphylococcus aureus (20)* followed by *Escherichia coli* (19), *Proteus vulgaris* (18), *and Bacillus cereus* (18) while Moxifloxacin showed good activity against *Bacillus cereus* (27), and *Staphylococcus aureus* (26) followed by *Proteus vulgaris* (24) and *Escherichia coli* (21). Moxifloxacin along with silver nanoparticles showed enhanced effect on gram positive bacteria instead of gram negative bacteria while as the Gemifloxacin shows equally effect on both the bacteria[23]. Antibacterial results had shown that AgNPs synthesised to posses' discrete antibacterial activity against clinically isolated pathogens along with Moxifloxacin and Gemifloxacin[16,23].

Table 1: Synergistic activity of the drugs, Gemifloxacin (5mcg) and Moxifloxacin (5mcg) in combination with AgNPs against pathogenic bacteria.

Clinical bacterial pathogens	AgNPs 25µl/disk (zone in mm)	Gem. 5mcg/disk (zone in mm)	Gem. + AgNPs (zone in mm)	Mox. 5mcg/disk (zone in mm)	Mox.+ AgNPs (zone in mm)
Bacillus cereus	16	12	18	21	27
Escherichia coli	12	14	19	15	21
Proteus vulgaris	15	13	18	20	24
Staphylococcus aureus	14	14	20	21	26

AgNPs: Silver nanoparticles, Gem.: Gemifloxacin, Mox.: Moxifloxacin

Conclusion

The impregnated form of AgNPs and antibiotics showed enhanced bactericidal activity against gram positive and gram negative bacteria studied here in the present study. It is concluded that the silver nanoparticles amplify the antibacterial activity of Moxifloxacin and Gemifloxacin drugs in order to prevent the

growth as well as to lyse the bacteria. Thus it would be said that the nanomaterials are the leading requirements in the field biomedical research in the direction of finding new drugs with potentiality, but further studies are also required to understand the cellular and mechanism behind the biosynthesis of nanoparticles and their mode of action on pathogens.

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