Green Synthesis and Antibacterial Effect of Silver Nanoparticles Using *Aspergillus flavus* in combination with Gemifloxacin

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Abstract- Nanotechnology is one of the finest fields of research and technology and has achieved the great progress in medical sciences. Green synthesis has importance in the study of nanoparticles' production instead of chemical and physical approaches, which has led to the development of bio mimetic approaches for the advanced nanomaterials. The present study focused on novel biological method for the extracellular synthesis of silver nanoparticles using the fungus *Aspergillus flavus*. The color of the solution changed upon addition of the $AgNo_3$ to the cell free extract in the conical flask indicated the formation of AgNPs. These nanoparticles were further characterized by FESEM and AFM which showed little monodispersity in shape and size within the range of 50-70nm and also the roughness of the silver nanoparticles. The AgNPs synthesized from *Aspergillus flavus* showed wider antimicrobial properties with the enhancement of bactericidal property against selected pathogens in combination with Gemifloxacin, which would be a novel substituent in the place of high dose antibiotics.

Index Terms - Green synthesis of AgNPs; UV-Spectrophotometer; AFM, FESEM; Antibacterial activity, Gemifloxacin

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1 INTRODUCTION

Nanoparticles are often referred to exhibit unique properties, which are quite different than those of larger particles, which show new 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 Nanoparticles formation 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, cost effective and don't involve in the use of any toxic chemicals for the synthesis of nanoparticles [6]-[8]

and it has been already reported that AgNPs size [9], [10], shape [11] and solubility [12], [13] affects AgNPs' toxicity. The studies have shown that biologically synthesised AgNPs have good antibacterial activity [14], which was proved by our previous work [15], [16]. The study on *Escherichia coli* has shown that AgNPs is showing cell lyses 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 also been demonstrated that bacteria become 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]. Previous studies show that silver ion, silver compounds and AgNPs have good antimicrobial and antiviral activity [18]-[21]. In this study, the AgNPs was synthesised from *Aspergillus flavus* by green method and were characterised. AgNPs were prepared using silver nitrate as silver precursor and *Aspergillus flavus* as reducing agent and stabilizer. The antibacterial effect of AgNPs was evaluated against five pathogenic bacteria, including *Escherichia coli* (Gram negative) and *Staphylococcus aureus* (Gram positive) using the disc diffusion method.

2. Materials and Methods

2.1 Isolation of airborne fungi

The air samplings by gravitation method were done in the vegetable market of Chennai by exposing Sabouraud's Dextrose agar media plates in order to isolate airborne fungi. The exposed media plates were brought to Biomedical and Microbiology Research Laboratory, Sathyabama University and were incubated at 25±3^{o}c for 3-7 days for the identification of different fungi.

2.2 Identification of *Aspergillus flavus*

The fungus, *Aspergillus flavus* was isolated and identified from the mixed culture by the author's expertise and available laboratory manuals. The pure culture of *A. flavus* was made and stored in 4^{o}c for further study.

2.3 Synthesis of silver nanoparticles

In the study seven day old culture of purified *Aspergillus flavus* was exploited 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 with alkaline PH-7. The flask was inoculated and incubated on

orbital shaker at 25^{o}C and agitated at 140rpm. After 72 hours, the biomass was filtered using What-man 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. The flask was incubated at 25^{0}C in a shaker at 140 rpm for 72 hours. The fresh and clean biomass was taken into the Erlenmeyer flasks containing 100ml of Milli-Q deionised water and further it was incubated at 25^{0}c in a shaker at 140 rpm for 72 hours. After the period of incubation the aqueous solution components were separated by filtration and the cell free extract was used with metal ion solution for the reduction of metals. 1mM $AgNO_3$ was prepared and 50ml was added to the cell- free–extract and kept in a shaking incubator at 25^{0}C and 140rpm for 24hours in dark condition for colour change.

2.4 **Characterization of Silver 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 Florescence Microscope (AFM), used to confirm particle size and agglomeration of nanoparticles through three dimensional images. The sample used in 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 AgNPs nanoparticles was studied via FESEM analysis. The synthesised nanoparticles were dried and being converted into powder form for FESEM analysis.

2.5 **Determination of antibacterial activity**

The efficacy of AgNPs was determined by performing antimicrobial susceptibility test using disk diffusion method against gram positive and gram negative organisms such as *Bacillus cereus, Escherichia coli, Proteus vulgaris, Staphylococcus aureus, and Vibrio chorea* in triplicates. The antibacterial assay of AgNPs was performed using Disk Diffusion method [22]. The Sterile disks impregnated with 25µg of solution of nanoparticles were placed on Nutrient Agar (Hi-Media). Standard antibacterial disk Gemifloxacin was used as positive

control and Gemifloxacin disks were impregnated with AgNPs were placed on nutrient agar medium inoculated with pathogens. Fungal filtrate used for biosynthesis of nanoparticles was used as Negative control and incubated at 37^{o}c for 18-24hrs. The zone of inhibition was measured and compared with the control. The experiments were done in triplicate.

3 RESULTS AND DISCUSSION

The purified strain of *Aspergillus flavus* were exploited for the biosynthesis AgNPs. The interaction between fungal cultured filtrate containing extracellular component and metal ion was observed by change in colour from chalky white before the addition of 1mM silver nitrate solution into brownish colour on completion of reaction with Ag+ ions after 24hrs, figure1. 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 [23].

a **b**

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

3.1 Characterization of Silver nanoparticles

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 409nm, figure 2 is characteristic of silver nanoparticle.

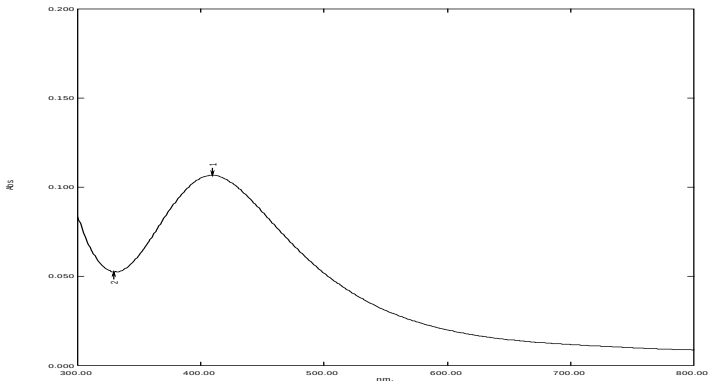

Fig. 2. Confirmation of AgNPs by UV-Spectrophotometry synthesised from *Aspergillus flavus*

This absorption band is called surface plasmon resonance, which would have been involved in the alteration of the solution color due the excitation of surface plasmon vibrations of the nanoparticles [24]. 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 50-70nm, figure 3. The nanoparticles were nano-sized and well dispersed. During analysis we found that silver nanoparticles formed were pre dominantly spherical and poly dispersed.

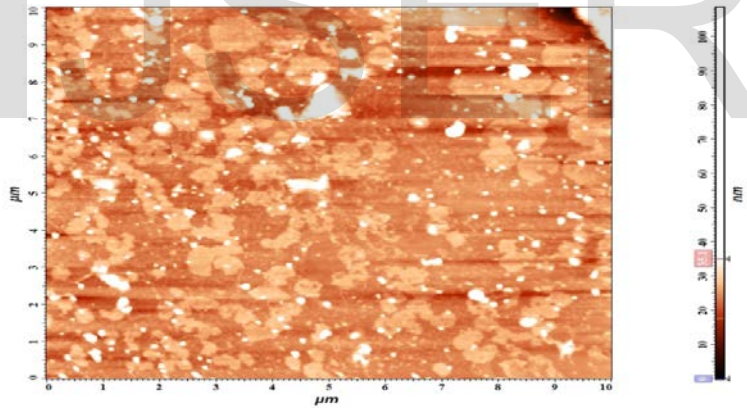

Fig. 3. Atomic Force Microscopy of silver Nanoparticles synthesized from *Aspergillus flavus* in the range of 50-70 nm.

The biologically synthesised nanoparticles were further analysed to determine the surface morphology by using FESEM and size of nanoparticles. The nanoparticles distributed uniformly showed that they were dispersed densely and having smooth surfaces and rough surfaces. The nanoparticles appearance showed that they were spherical to ovate in structure with having average dimensional size in the range of 50-70nm, figure 4.

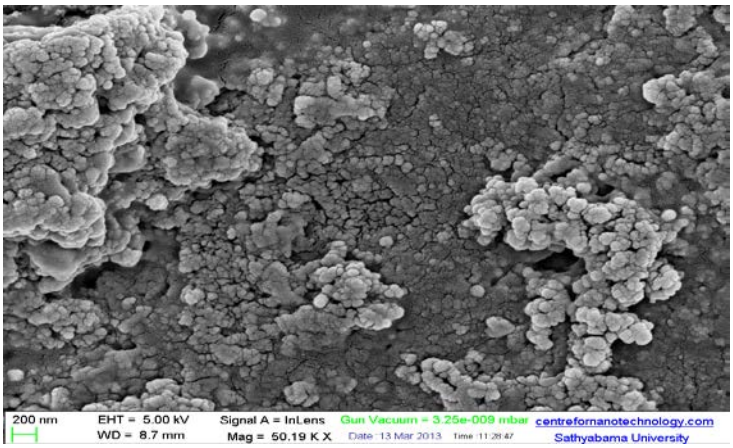

Fig.4 FESEM shows the silver nanoparticles are spherical in shape and size.

3.2 Antibacterial Activity

The antibacterial activity of silver nanoparticles, its comparative analysis in a synergistic mode along with Gemifloxacin was studied against gram positive and gram negative bacteria, which was found satisfactory in the present study. While analysing the synergistic effect of AgNPs with Gemifloxacin, there was significant increase in inhibitions zones. The diameter of inhibitions zones in (mm) and enhanced effect was measured to confirm the synergistic role of nanoparticles along with antibiotics against selected pathogens (Table-1). The AgNPs were evaluated singly and in combination with standard antibiotic Gemifloxacin (5mcg/disk) at a concentration of 25µg/disk. The results showed that the antibacterial activity of Gemifloxacin in presence of nanoparticles was increased in combined formulation of against *Vibrio cholerae* (21±1), *Staphylococcus aureus* (20±1) *followed by Escherichia coli* (19±1), *Proteus vulgaris* (18±0.79) *Bacillus cereus* (18±0.96) tested bacteria. From the above investigations it was clear that biologically synthesised AgNPs enhances the antibacterial potency of standard drugs against clinical pathogens Fig-5, but further studies are required to understand the cellular mechanism behind the biosynthesis of nanoparticles and their mode of action on pathogens.

Table1. Synergistic activity of AgNPs+Antibiotics (Gemifloxacin-5mcg/disk +25µg/disk

AgNPs) against pathogenic bacteria.

Pathogens	Filtrate	AgNPs	Gem	Gem+AgNPs
	zone of inhibition (mm)			
Staphylococcus aureus	07±0.71	14±0.78	14±1.00	20±1.0
Bacillus cereus	06±0.89	12±0.60	14±1.01	19±0.96
Proteus vulgaris	07±0.99	15±1.01	13±0.39	18±0.79
Escherichia coli	08±0.91	16±0.75	12±0.79	18±1.0
Vibrio cholerae	06±0.67	15±0.77	15±0.99	21±1.01

*AgNPs (Silver nanoparticles), *Gem (Gemifloxacin)

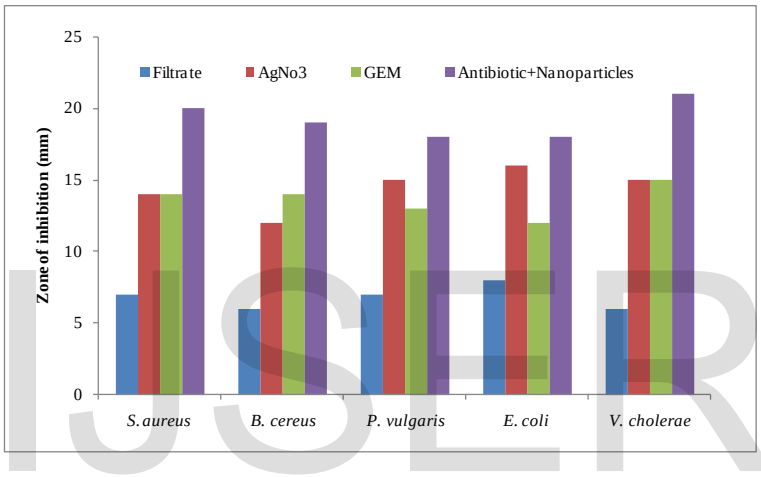

Fig.5. Graphical representation of synergistic effect of Gemifloxacin and AgNPs against test pathogens

4 CONLUSION

Aspergillus flavus was isolated from air (vegetable market) Chennai, India and were subjected to synthesis of silver nanoparticles. The bioreduction of silver ions was monitored by observing the colour change of the solution from chalky white to brownish yellow. The biologically synthesised nanoparticles were characterised by qualitative as well as quantitative techniques to confirm the absorption peaks (UV-Spectrophotometry), topography and average roughness (AFM) and morphology and size of nanoparticles (FESEM), were found within a range between 50-70nm. Bactericidal results showed that AgNPs synthesised posse's discrete antibacterial activity against clinically isolated pathogens. Enhanced bactericidal activity was found when the AgNPs were impregnated with antibiotic, Gemifloxacin in combined formulations. Thus it would be concluded that combined formulations of standard drugs with silver nanoparticles would be an alternative remedy to

control multi drug resistant pathogens, but further studies are required to confirm its mode of action and toxicity on human beings.

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