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RESEARCH ARTICLE

Biosynthesis and Characterization of Silver Nanoparticles from *Aspergillus niger* and its Antibacterial Activity

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ABSTRACT:

The fungal mediated biosynthesis of silver nanoparticles has been carried out in this study. The silver nanoparticles were synthesized from *Aspergillus niger* with an aqueous solution of $AgNO_3$. The biosynthesized silver nanoparticles were characterized through UV-visible spectrophotometer, Scanning Electron microscopy, Dynamic light scattering, and Fourier Transform Infrared Spectroscopy (FTIR). The maximum absorbance was noted between 400 nm to 450 nm in visible region corresponding to the Plasmon resonance of silver nanoparticles, confirms their presence. The size and shape of the silver nanoparticles was investigated using Scanning Electron Microscopy (SEM) and spherical shaped nanoparticles were observed under SEM. The solution with Silver nanoparticles were analysed by Dynamic light scattering to analyse the particle size, and the range of single nanoparticle size was about 3nm in diameter. The stabilizing agent that keeps the silver nanoparticles as stable and dispersed was identified from FTIR spectrum, FTIR spectrum of silver nanoparticles showed three distinct peaks, 548. 38, 1636. 17 and 3347. 85cm−1. The silver nanoparticles produced from *Aspergillus niger* showed potential antibacterial activity against *Staphylococcus aureus*, *Klebsiella pneumonia*, *Escherichia coli*, and *Salmonella typhi* where chloramphenicol was used as standard antibiotic.

KEYWORDS: *Aspergillus niger*, UV-vis spectrophotometer, Dynamic Light Scattering, Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM).

INTRODUCTION:

Synthesis of nanoparticles using microorganisms is inevitable, reliable, biocompatible, economic and eco friendly science for producing nanoparticles with inimitable physical and chemical properties[1,2,3]. Recent days, controlling of emerging multidrug resistant pathogens is a great challenge for pharmaceutical industries[4].

Due to these challenges, an alternative approach is needed to explore new bioactive compounds to meet the high demand of antibiotic resistance. Biosynthesis of nanoparticles by exploiting microorganisms has been creating a centre of attention due to their optical, chemical, photoelectron chemical and electronic properties.[5,6]. Silver nanoparticles have potential application in various fields such as, antibacterial effect, drug delivery, textile etc.[7]. The anti bacterial effects of silver are known since ages and silver is currently used to control bacterial growth in a variety of applications including dental work, catheters and burn wounds[8]. The various methods are employed to produce the broad spectrum of silver nanoparticles. However, for the environmental sustenance, there is essential to develop

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eco friendly procedures to avoid existence of toxic chemicals in the environment. Most of the bacterial and fungal species have antimicrobial activity through reducing the metal ions for production of metal nanoparticles. Recent studies showed that the potential bioactive compounds are produced by fungi than bacteria. This indicates that fungi are superlative candidates in the synthesis of metal nanoparticles, because of their ability to secrete large amount of enzymes[9]. Silver nanoparticles are synthesized using various fungi such as *Fusarium oxysporum*[10], *Fusarium semitectum*[11], *Apergillus fumigarus*[12], *Pleurotus sojar* caju[13], *Penicillium brevicompactum*[14], *Clostridium versicolor*[15].

The nanoparticle and its applications are extensively depending on their size. Hence in the present investigation is made to produce silver nanoparticles of small size using *Aspergillus niger.* Further the characterization of nanoparticles and its antibacterial activity is also studied in this study.

MATERIALS AND METHODS:

Isolation and identification of *Aspergillus niger*:

1. 0g of soil sample was collected from the medicinal garden and serial dilutions were made by suspending the soil sample in 10ml of sterile distilled water. Then 1ml of each dilution were added to sterile Petri dishes, containing sterile Sabouraud's Dextrose Agar medium with 1. 0% streptomycin solution for preventing bacterial growth, The plates were then incubated at 28ºC for 3-5 days. Dilutions of 10^{-3} and 10^{-4} were used to isolate fungi in order to avoid over-crowding of the fungal colonies. The isolated fungal culture was purified by sub-cultured using Sabouraud's Dextrose Agar and was maintained in slants for identification, on the basis of microscopic and macroscopic characteristics features as followed by[16].

Fungal Biomass and AgNPs synthesis:

The identified fungal colonies of *Aspergillus niger* was cultured in Sabouraud's Dextrose broth and incubated at 28ºC and 100 rpm in an orbital shaker for 72 h. After incubation the fungal biomass was filter through Whatmann No. 1 filter paper. The biomass was washed thoroughly with sterile double distilled water to prevent the contamination of the medium. Approximately, 10g of fungal biomass was transferred into 250mL Erlenmeyer flask containing 100mL sterile double distilled water and incubated at 28ºC and 100 rpm in an orbital shaker for 72 h. The fungal filtrate was obtained by passing through Whatmann No. 1 filter paper and 100mL of fungal filtrate, $AgNO_3$ was added at a concentration of 1mM. The mixture was incubated at 28ºC and 100 rpm in an orbital shaker for 72 h. The fungal filtrate and $AgNO_3$ served as control.

Characterization of silver nanoparticles:

After 24 h of synthesis, the sample of AgNPs was centrifuged at 15, 000 rpm for 30 min at room temperature with repeated rinse were also performed to remove impurities. The residue of AgNPs was re-suspended in 1. 0ml sterile water. The silver nanoparticles formed in the fungal filtrate were monitored using:

i) **UV- Vis spectrophotometer:**

Change in colour of the cell free filtrate incubated with silver nitrate solution was visually observed over a period of time. Absorption measurements of the filtrate were carried out after 24h using UV – visible spectrophotometer[17.]

ii) **Scanning Electron Microscopy:**

The silver nanoparticles were evaluated for their surface and shape characteristics by scanning electron microscope (SEM).

iii) **Characterization using Dynamic Light Scattering:**

The Dynamic light scattering was performed to analyse the range of particle size. The sample was mixed well and scattering light with the angle of 173º and the temperature of the holder with 25. 0 °C.

iv) **FT – IR analysis:**

The sample mixture was kept in a hot air oven until its getting dried off. The dried sample was stored in a sterile eppendroff. Then it was used for the FT-IR analysis from region of 400 – 4000 cm-1.

Antibacterial activity:

Four different bacteria (*Staphylococcus aureus*, *Klebsiella pneumonia*, *Escherichia coli*, and *Salmonella typhi*) were taken from the stock culture and subcultured in 25 ml of nutrient broth kept for incubation 12 hrs. The synthesized silver nanoparticles using *Aspergillus niger* was tested for antibacterial activity by agar well-diffusion method against *Staphylococcus aureus*, *Klebsiella pneumonia*, *Escherichia coli*, and *Salmonella typhi*. The bacterial cultures were swabbed uniformly on the individual plates using sterile cotton swabs on the Muller Hinton Agar. The gel puncture was used to make a well (6mm in diameter) on Muller Hinton Agar. The synthesized silver nanoparticles (35 µl) A and Antibiotic B Chloromphenicol were added on the wells and the plates were incubated at 37°C for 24 h to observe the formation of zone of inhibition.

RESULTS AND DISCUSSION:

Fungal biomass and AgNPs synthesis:

The present work was carried out to synthesis silver nanoparticle from *Aspergillus niger* isolated from the soil (Fig. 1). Based on the microscopic and macroscopic characteristics, the fungal isolate was identified as *Aspergillus niger.*

The fungal free supernatant was collected and washed with demonized water followed by the washing of fungal biomass with sterile distilled water and it was filtered using the Whatman filter paper No. 1. Collected filtrate was colourless solution act as a reducing agent for the silver nanoparticle synthesis. The reaction bottle contain 100ml fungal filtrate with 1. 0 mM silver nitrate solution (positive) and another bottle contain only the fungal filtrate solution (control) were incubated in dark at 28ºC for 24 hr which results in the colour change of the solution from colourless to dark brown in the positive which indicates the formation of silver nanoparticle shown in (Fig. 2) Whereas no colour change in the control bottle confirms again that fungal filtrate is act as a reducing agent for AgNP synthesis, which correlate with the results attained by[18, 19, 20]. The possible cause of this colour change might be the presence of extra cellular protein reductase in the fungal filtrate reacts with silver nitrate solution results in silver ion reduction forming silver nanoparticle[21, 22]. However no colour change was observed in control flask indicates the absence of nitrate reductase does not result in silver ion reduction.

Fig. 1. Aspergillus niger in sabouraud's Dextrose Agar

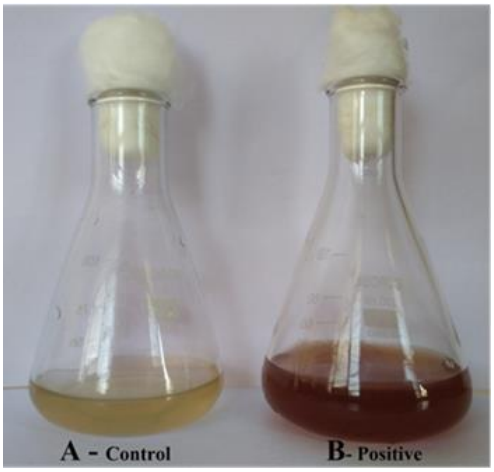

Fig. 2. A – Fungal filtrate, B – (positive) fungal filtrate with $AgNO_3$ after 24 hr incubation

Characterization using UV visible spectroscopy:

Within 48 hrs of incubation the colour change was observed due to excitation of surface Plasmon resonance[23,24,25]. The absorption spectra of synthesized silver nanoparticle were scanning the wavelength range from 250 - 750nm shows the absorption peak at 439 nm shown in (Fig. 3) which confirms the wide distribution of silver nanoparticle in the solution. The peak formed at 439 nm is the indication for the presence of the proteins and enzymes and the existence of these bioactive compounds are reason for the reduction of metal ions for synthesis of nanoparticle[26].

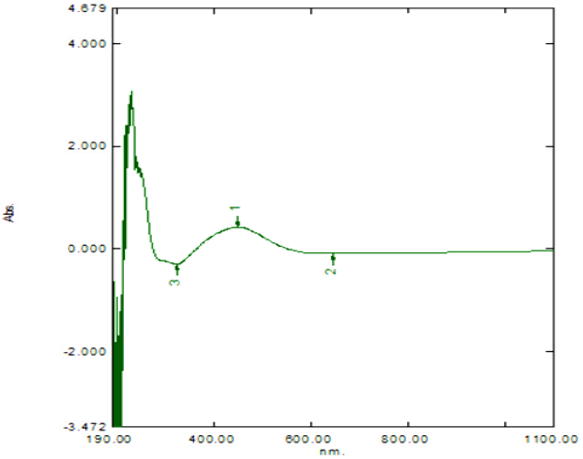

Fig. 3 UV – visible Spectra of Ag – NPs Synthesized by Aspergillus niger

Characterization using Scanning Electron Microscopy:

Scanning electron microscopy (SEM) images of AgNPs synthesized by *Aspergillus niger* were shown in (Fig. 4). The morphology of the nanoparticles was spherical in nature. The similar results were reported by [27, 28.]

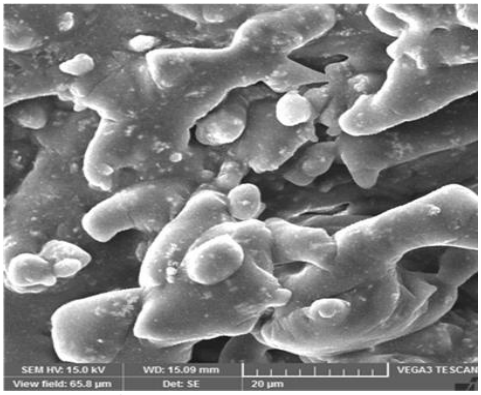

Fig. 4 SEM image of Ag – NPs synthesized by Aspergillus niger

Characterization using Dynamic Light Scattering:

The solution with Silver nanoparticles were analysed by Dynamic light scattering to analyse the range of particle

size. The sample were mixed well and Scattering light with the angle of 173º and the temperature of the holder with 25°C shows the range of particle size about the range of 3nm in diameter of a single nanoparticle was shown in (Fig. 5).

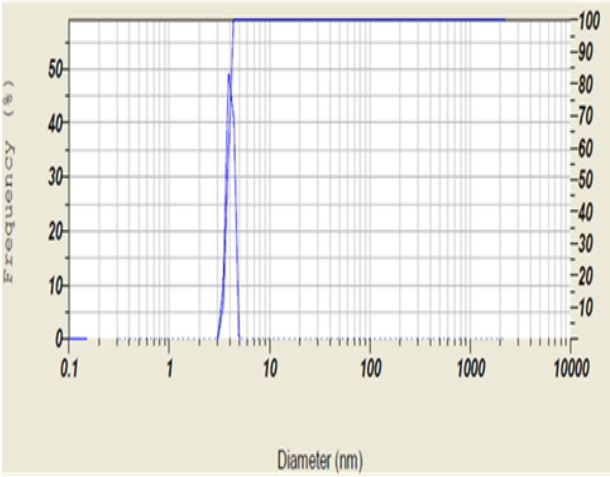

Fig. 5 Particle size analysis of AgNPs in Dynamic light scattering

Characterization using Fourier Transformation Infrared Spectroscopy (FTIR):

The silver nanoparticles are stable and well dispersed. The stabilizing agent that keeps the silver nanoparticles as stable and dispersed was identified from FTIR spectrum, FTIR spectrum of silver nanoparticles showed three distinct peaks, 548. 38, 1636. 17 and 3347. 85cm−1 were shown in (Fig. 6). The peak at 3347. 85cm−1 refers to the stretching vibrations of primary amines while the peak at 1636. 17cm−1 is due to the carbonyl stretch vibrations in the amide linkages of proteins and 548. 38cm−1 is the fingerprint. The carbonyl groups of amino acid residues and peptides have strong ability to bind to silver. Thus the result indicates the amines; carboxylic acid and amide groups of *Aspergillus niger* are involved in the fabrication of silver nanoparticles showed the similarities with [29].

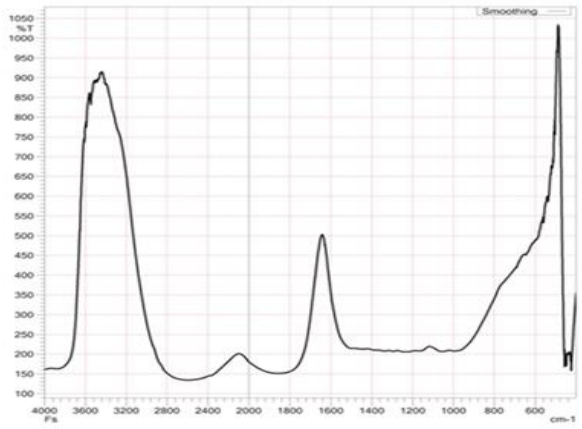

Fig. 6 FTIR spectrum of Ag - NPs synthesized by *Aspergillus niger*

Antibacterial activity of synthesized AgNPs:

The silver nanoparticles produced from *Aspergillus niger* were assayed for their antibacterial activity with chloramphenicol used as standard antibiotic against *Staphylococcus aureus*, *Klebsiella pneumonia*, *Escherichia coli*, and *Salmonella typhi.* The maximum zone of inhibition was observed with *Staphylococcus aureus* (22mm), the zone of inhibition was formed by *E. coli* (17mm), and followed by *Salmonella typhi* (14mm), *Klebsiella pneumonia* (12mm) showed this similarities with [30].

CONCLUSION:

The fungal mediated biosynthesis of silver nanoparticle can be a promising method for the preparation of nanoparticles and it can be precious in the biotechnological applications. The fungal mediated biosynthesis of nanoparticles provides an environmental friendly, simple and efficient method of nanoparticle synthesis. In conclusion, the fungus *Aspergillus niger* has shown potential for synthesis of Ag-NPs and its showed remarkable antibacterial activity against *Staphylococcus aureus*, *Klebsiella pneumonia*, *Escherichia coli*, and *Salmonella typhi.*

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