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Production and Characterization of Silver Nanoparticles from Culture Filtrate of Aspergillus Niger

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ABSTRACT

Silver nanoparticles (AgNPs) were greenly biosynthesized using the culture filtrate of *Aspergillus niger*. The reaction mixture turned deep brown after 24 hours of incubation indicating the formation of AgNPs. The synthesized AgNPs were characterized using UV-Visible Spectroscopy, X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Energy Dispersive X-ray Spectroscopy (EDX), Fourier Transform Infrared Spectroscopy (FTIR) and Gas Chromatography – Mass Spectrometry (GC – MS). Surface Resonance peak was observed at 400-500 nm. The XRD pattern showed the crystalline nature of the synthesized silver nanoparticles while SEM showed that the biosynthesized AgNPs from *A. niger* (AspAgNPs) have irregular shape and size ranging from 0.02 – 1.00 µm. EDX analysis showed strong peak of elemental silver while FTIR spectrum showed the different functional groups present in the AgNPs .GC –MS analysis showed that eleven (11) compounds were present in the biosynthesized AspAgNPs. AspAgNPs functionalized with Ciprofloxacin had the highest antagonistic activity against the indicator organisms. In conclusion, *A. niger* cell free filtrate bioreduced $AgNO_3$ for AgNPs biosynthesis and the AgNPs had antibacterial activity against the indicator organisms. Functionalization with Ciprofloxacin and Streptomycin enhanced the antibacterial potential of the AgNPs.

Keywords: Aspergillus niger; Culture filtrate; $AgNO_3$; AgNPs; Functionalization; Test pathogens

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1. BACKGROUND TO THE STUDY

Nanotechnology can be defined as the exploration, production, characterization and application of nano-sized (1-100nm) materials for the development of science. It has application in various fields such as pharmaceuticals, optics, textiles, cosmetics, food packaging, defense, transportation, electronics, medicine, healthcare, electronics, agriculture sports and aesthetics (Kathiresan *et al.,* 2009). A silver nanoparticle has good antimicrobial properties and hence has a wide application against multidrug resistant pathogens because they serve as an alternative to antibiotics (Xixi *et al.,* 2017). Green synthesis of AgNPs is mostly adopted due to their efficiency, ecofriendly, toleration and metal bioaccumulation ability (Sastry *et al*., 2003). Silver nanoparticles production are more suitable using fungi compared to other biological entities like bacteria, algae, plants among others. This is due to their ability of producing secondary metabolites which result in higher quantities of nanoparticles being produced. Secondary metabolites are low molecular weight products of secondary metabolism that are usually produced at the stationary phase of the organism (Lee *et al.,* 2016). *Aspergillus niger* belonging to the class Ascomycota, a black fungus that have the ability to produce different metabolites and industrial enzymes such as pectinases, α-amylases and cellulases for use in the food industries (Schuster *et al.,* 2002).

2. STATEMENT OF PROBLEM

Multidrug resistance is an important problem caused by the extensive use of chemical antimicrobial agents. The emergence of multidrug resistance microbes is a great concern as these pathogens are reported to be the leading cause of death all over the world.

3. OBJECTIVE

This research aimed at biosynthesis of silver nanoparticles using *Aspergillus niger* culture filtrate, characterization of the nanoparticles, Functionalization of the biosynthesized nanoparticles with antibiotics (Streptomycin and Ciprofloxacin) and determination of the antimicrobial potency of the nanoparticles against some MDR strains.

4. METHODOLOGY

4.1 Collection of culture and Production of Culture Filtrate

Culture of *Aspergillus niger* and the test pathogens (*Escherichia coli, Bacillus subtilis, Aeromonas* sp.*, Pseudomonas aeruginosa, Listeria* sp. and different species of *Staphylococcus aureus (*A_2, A_3, A_5, A_7 and A_8) were obtained from the culture collection of our previous work in the Department of Microbiology, University of Ibadan. The culture filtrate was produced using Potato Dextrose Broth (PDB).

4.2. Biosynthesis of Silver Nanoparticles

Biosynthesis was done according to the modified method of Basavaraja *et al*., (2008) using 50 mL of 1mM $AgNO_3$ solution and 50mL of cell free filtrate of *A. niger.*

4.3. Characterization of the biosynthesized Silver Nanoparticles (AspAgNPs)

Visual observation, UV-Visible Spectroscopy, X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Energy Dispersive X-ray Spectroscopy (EDX), Fourier Transform Infrared Spectroscopy (FTIR) and GC-MS analysis were done using standard methods (Ingle *et al*., 2008; Pavani *et al.,* 2013;Aguilar-Méndez *et al.,* 2011; Raut *et al.,* 2010; Devi *et al.*, 2012; Ruthiran *et al.,* 2017)

4.4. Functionalization and Antibacterial potential of the nanoparticles

The nanoparticle was functionalized using Ciprofloxacin and Streptomycin and the antibacterial potential against some pathogenswas determined

5. RESULTS

The metabolites of *Aspergillus niger* was able to bioreduce $AgNO_3$ for the biosynthesis of AgNPs and Colour changes from light yellow to brown indicate AgNPs biosynthesis. An absorption peak ranging from 400 - 500 nm was observed and the Surface Plasmon Resonance peak was 400 nm (Figure 1). SEM micrograph shows that the AgNPs is irregular in shape with size ranging from 0.02 – 1.00 µm (Figure 2). Fourteen (14) absorption peaks were present in the range of 3434.68cm^{-1} - 452.11cm^{-1} from the FTIR spectrum (Figure 3). Alcohol, amides Halo compound were among the functional groups present.

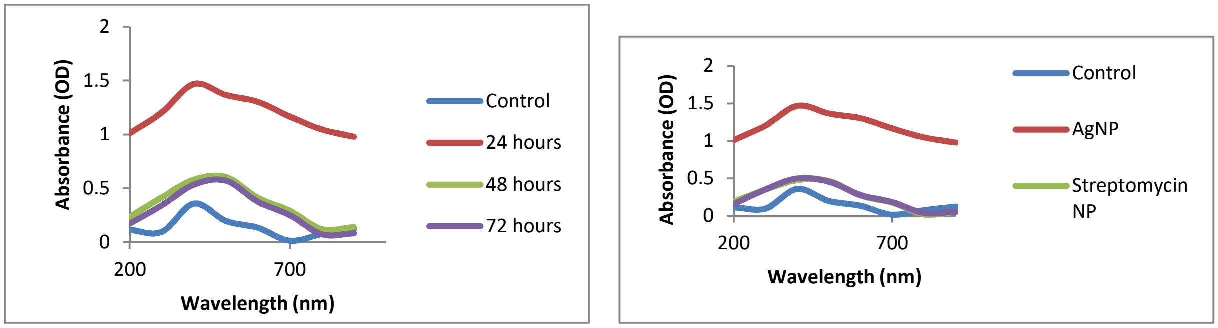

Figure 1a -b: UV-Visible Spectrophotometer of AspAgNPs and functionalized AnAgNPs
KEY: Control – $AgNO_3$; AgNP – Silver nanoparticles synthesized from *A. niger* metabolites (AspAgNPs);
Streptomycin NP – AspAgNPs functionalized with Streptomycin;Ciprofloxacin NP - AspAgNPs functionalized with Ciprofloxacin.

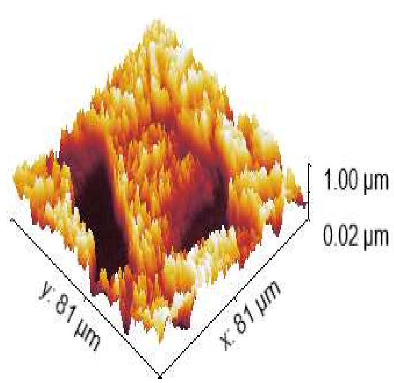

Figure 2: SEM micrograph of AspAgNPs

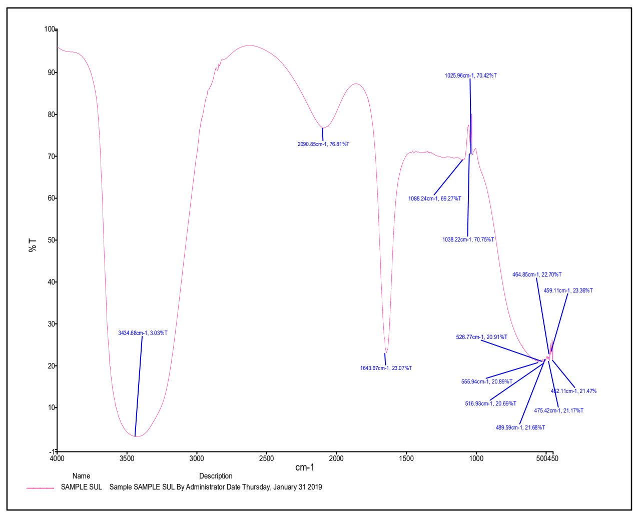

Figure 3: FTIR spectrum of AspAgNPs

The diffracted intensities were recorded from 0 to 80 (2θ) and the XRD peaks were observed at 36.02°, 45.91° and 62.34°(Figure 4). Strong signals of silver in the nanoparticles was confirmed from the spectrum peak (Figure 5).
The GC-MS of the synthesized AspAgNPsshows that 11 compounds were present (2,4-Dodecadienal; 2,4-Nonadienal (RT: 8.133); 1-(2,4-Di-methyl phenyl) ethanol (RT: 8.367); Tetradecanoic acid (RT: 17.992); 9,12 Octadecadienoic acid 11,14,17-Eicosatrienoic acid; 9-Hexadecenoic acid; Eicosanoic acid; Decane, 5-methyl- 5-Methyldecane; 9,12-Octadecadienoyl chloride, (Z,Z)- Linoleoyl chloride and 12-Methyl-E,E-2,13-octadecadien-1-ol (Table 1).

The AspAgNPs and FAspAgNPsS and FAspAgNPsC had antibacterial activity against the test pathogens (Figure 5). *Staphylococcus* aureus (A_8) had the highest susceptibility while *Bacillus subtilis* and *Listeria sp.* had the lowest. The functionalized particles had a better antibacterial potential in which *Aeromonas* sp. had the highest zone of inhibition (42 mm).

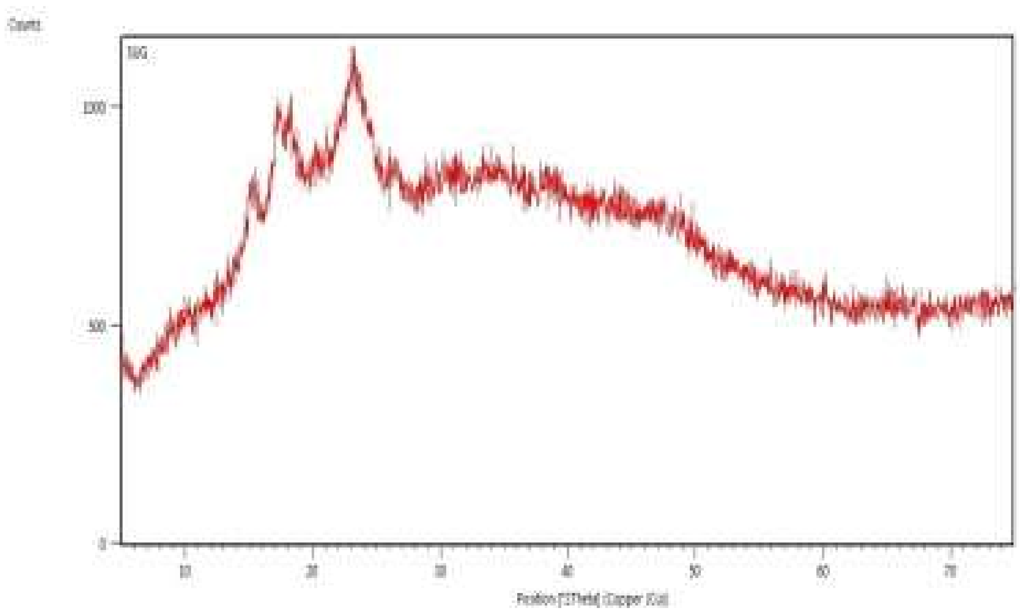

Figure 4: XRD Analysis of AspAgNPs

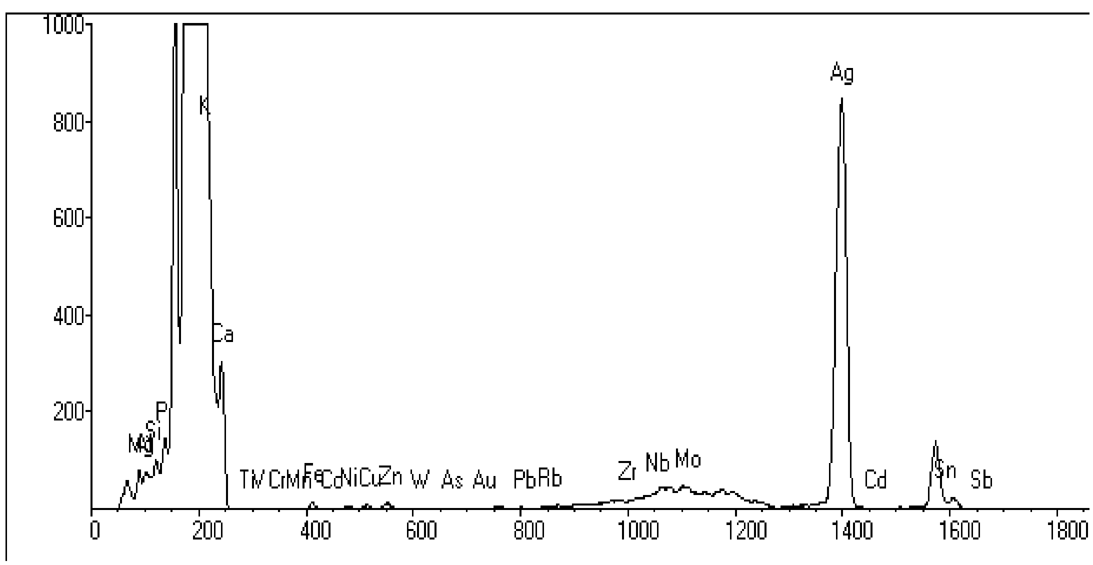

Figure 5: EDX Analysis of AspAgNPs

Table 1: GC - MS Analysis of different components of AgNPs from *Aspergillus niger*

S/N	Retention Time	Peak Area (%)	Compound Name	Molecular Weight	Molecular Formula	Structure
1	7.725	0.43	2,4-Dode- cadienal	180	$C_{12}H_{20}O$	
2	8.133	0.86	2,4-Nonadienal	138	$C_9H_{14}O$	
3	8.367	0.49	1-(2,4-Di-methyl phenyl) ethanol	150	$C_{10}H_{14}O$	
4	17.992	15.55	Tetradecanoic acid	228	$C_{14}H_{28}O_2$	
5	19.925	1.05	9,12-Octadecadie- noic acid	294	$C_{19}H_{34}O_2$	
6	20.033	3.72	11,14,17-Eicosatrienoic acid	320	$C_{21}H_{36}O_2$	
7	20.975	62.43	9-Hexade- cenoic acid	254	$C_{16}H_{30}O_2$	
8	21.150	9.98	Eicosanoic acid	312	$C_{20}H_{40}O_2$	
9	22.483	1.02	Decane, 5-methyl- 5-Methyldecane	156	$C_{11}H_{24}$	
10	24.292	2.77	9,12-Octadecadienoyl chloride, (Z,Z)- Linoleoyl chloride	298	$C_{18}H_{31}ClO$	
11	26.358	1.71	12-Methyl-E,E-2,13-octadeca- dien-1-ol	280	$C_{19}H_{36}O$	

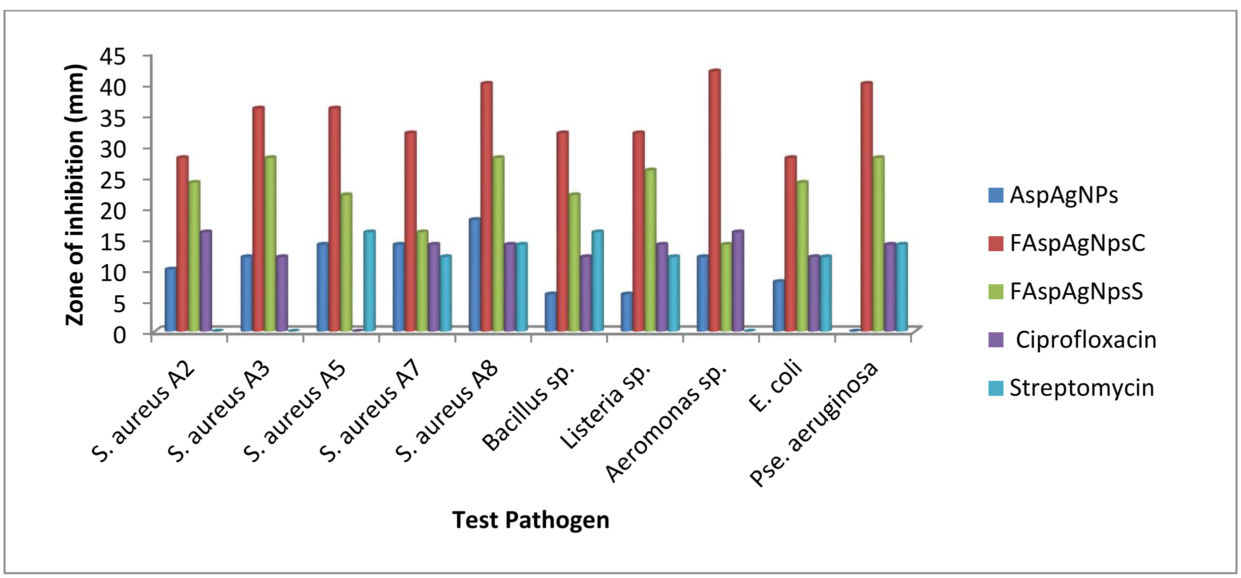

Figure 6: Antibacterial activity of AnAgNPs, FAnAgNPsC and FAnAgNPsS

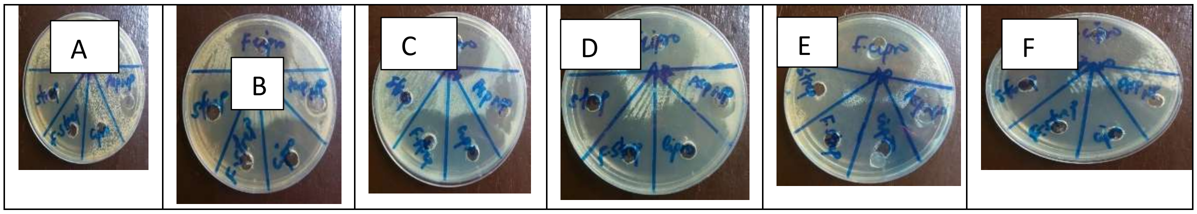

Figure 7: Antibacterial potential of the AspAgNPs, FAspAgNPsC FAspAgNPsS against: (a) *Listeria* sp.; (b) *S. aureus* A_3; (c) *S. aureus* A_5; (d) *S. aureus* A_7; (e) *S. aureus* A_8; and (f) *Aeromonas* sp.

Key:Asp NP – Silver nanoparticle biosynthesized from *Aspergillus niger;*F Cipro – AnAgNPs functionalized with Ciprofloxacin;Strep – Streptomycin;F Strep - AnAgNPs functionalized with Streptomycin Cipro - Ciprofloxacin

6. DISCUSSION OF FINDINGS

Aspergillus niger culture filtrate bio-reduced $AgNO_3$ for AgNPs biosynthesis which was confirmed by changes in colour. This may be due to the excitation of Surface Plasmon Resonance of AgNPs (Soheyla *et al.,* 2013). The observation of dark brown colouration indicates the reduction of silver nitrate ions by the proteins present in the fungal cell free filtrate resulting in the synthesis of AgNPs which was in accordance with the work of Kathiresan *et al*., (2010).

SEM micrograph of the biosynthesized AgNPs from *A. niger* shows that the particles are irregular and spherical shaped and ranged from 0.02 – 1.00 μm in size**.**

FTIR analysis carried out on the synthesized AgNPs shows the presence of fourteen (14) absorption peaks in the range of 3434.68cm^{-1} - 452.11cm^{-1}. Dattu *et al.,* (2014) reported that different functional groups absorb characteristic frequencies of FTIR radiation.

For the crystalline nature of the AgNPs, intense XRD peaks were observed corresponding to the (111), (200) and (220) planes at 2θ angles of 36.02°, 45.91° and 62.34°. This result is in agreement with the common standard JCPDS file no. 04-0783. Prema and Rincy (2009) reported similar result

EDX spectrum showed strong silver signal. Varshney *et al.,* (2009) reported strong signals from the silver atoms in the nanoparticles synthesized using fungi. GC –MS of the biosynthesized AgNPs shows the presence of eleven (11) compounds. Nielsen *et al.,* 2009, reported Tetradecanoic acid and 9-octadecenoic acid from *Aspergillus niger* AspAgNPs, FAspAgNPsC and FAspAgNPsS had antibacterial potential against the pathogens. Manju and Vedpriya (2013); Mudasir *et al*., (2013) had similar report.

7. CONCLUDING REMARKS

The culture filtrate of *A. niger* was a good biomolecules for biosynthesis of nanoparticles, the particles were irregular in shape and 0.02 -1.00 μm. the SRP was at 400 nm, Silver had the strongest peak from EDX analysis, different functional groups and 11 chemical compounds were detected. The AspAgNPs, FAspAgNPsC and FAspAgNPsS had antibacterial activity against the test pathogen. Functionalization enhanced the antibacterial potential of the nanoparticles.

8. CONTRIBUTION TO KNOWLEDGE

The research has been able to establish that metabolites from *A. niger* had some components that can bioreduced $AgNO_3$ for AgNPs biosynthesis. 11 chemical compounds were present in the AspAgNPs and the AspAgNPs exhibited antibacterial activity. Functionalization improved the antibacterial potential of the nanoparticles

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