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Characterization of microbial synthesized silver and gold nanoparticles and evaluation of its antimicrobial and anticancer activities

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Abstract :In this study, *Aspergillus flavus var. columnaris* and *Penicillium goetzii* were used for bio-synthesizing of gold nanoparticles (AuNPs) and silver nanoparticles (AgNPs) respectively, using eco-friendly and cost-effective methods. After biosynthesizing of nanoparticles, it was characterized by UV–Visible Spectroscopy, Fourier-Transform Infrared (FTIR), Transmission Electron Microscopy (TEM), Selected Area Electron Diffraction (SAED) and Dynamic Light Scattering analysis (DLS). Bio-synthesized AgNPs and AuNPs were tested as antibacterial against gram positive and gram negative bacterial strains: *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella sp., Klebsiella sp.* and *Proteus mirabilis*, as antifungal against *Aspergillus niger* and *Candida albicans* and as antiviral against human adenovirus serotype 5 (HAdV-5). Furthermore Bio-synthesized AgNPs and AuNPs were tested as anticancer against HepG2 hepatocarcinoma cells. Maximum absorbance at 540 nm and 440 nm was obtained using UV–Visible absorption spectra for reaction mixtures of AuNPs and AgNPs respectively. DLS measurements results confirmed UV–Visible absorption results, that the highest intensity of the AgNPs and AuNPs were 84 nm and 73.9 nm, respectively. Some functional groups were identified using FTIR measurements (e.g. Alcohol, Alkane, cyclopentanone, carbon dioxide, Amine, conjugated alkene,…etc.) detected in *Penicillium goetzii* and *Aspergillus flavus var. columnaris* cultures filtrates that could be responsible for production of AgNPs and AuNPs. Particles size and morphologies of AgNPs and AuNPs were confirmed by the SAED pattern analysis and TEM. Green synthesized AuNPs and AgNPs showed good anticancer and antimicrobial activities.

keywords: AuNPs, AgNPs, antiviral, anticancer, antibiofilm

1.Introduction

The importance of microorganisms in our live, the importance of the secondary metabolites they produce, the danger of some of these microorganisms to the life of other organisms and to the life of each other cannot be denied. Infection with some of these microorganisms may lead to death, so it was necessary for researchers to find efficacious solutions through which they can killed or limit the spread of these harmful and pathogenic microorganisms and at the same time these solutions does not harm the environment such as using of silver and gold nanoparticles as antimicrobials.

A matter particle with a diameter between one and one hundred nanometers is referred to as a nanoparticle **[1]**. Because of their size, which are significantly smaller than the visible

light spectrum's (400–700 nm) wavelengths, nanoparticles cannot be observed with a regular optical microscope; instead, an electron microscope must be used. For the same reason, nanoparticles can be transparent when they are disseminated in transparent media **[2]**.

Due to their remarkable thermal, electrical, and optical capabilities, particularly in the medicinal field, AuNPs and AgNPs are among the most widely employed nanoparticles. Gold and silver nanoparticles have been produced by some physical and some chemical techniques; however, these techniques are all exceedingly expensive, complex, and produce toxic waste that pollutes and harms the environment **[3]**. Hence, there is a rising demand for more effective, economically viable, and ecologically friendly ways to synthesize nanoparticles. It has been shown that using biosynthetic techniques to produce nanoparticles is the best method for reducing harmful effects and is advised for maximizing the safety and sustainability of nanoparticle production **[4]**.

By substituting these relatively potent reducing agents, several biological techniques could enhance their environmental effects. The always used biological principles are using plant extract or fruit extract, bacterial and fungi extracts **[5]**.

2. Materials and methods

Isolation, cultivation and identification of the fungal isolates used for preparation of nanoparticles

The isolation of fungus that can produce AgNPs and AuNPs was done using an old gold ring. The gold ring was submerged for two hours in sterile double distilled water (ddw) while being shaken. For the synthesis of AgNPs and AuNPs, aliquots of this water were inoculated onto potato dextrose agar (PDA) plates altered with 0.25 mM $AgNO^3$ and 25 µg/ml chloramphenicol, respectively. These plates were incubated at 28^{o}C, and fungal colony growth was monitored. The distinct colonies were separated, moved to fresh plates, and kept on PDA slants **[6]**.

To find filamentous fungus, potato dextrose agar (PDA) (from Difco in the United States) was utilised. The media were prepared then autoclaved for 20 minutes at 121°C. 1 ml of the fungal suspension was then spread on the surface of agar plates and incubated for 5-7 days at 25^{o}C for filamentous fungus after the media had been distributed into Petri dishes and allowed to harden. According to the following studies, morphological characteristics such as pigmentation, colony diameter, extracellular exudates, the color of conidia and the color of reverse mycelium were used to identify the isolated fungi during this investigation. Microscopic features such as conidial heads, sporulation degree, fruiting bodies, and the homogeneity characteristics of conidiogenous cells were also examined by optical light microscope Olympus CH40 according to the following studies: **[7, 8, 9, 10, 11]**. The cultures were then kept in 4°C.

Growth conditions of fungi, filtrates preparation and preparation of nanoparticles

The examined cultures were added to 50 ml of potato dextrose broth (PDB) in 500 ml Erlenmeyer flasks, where they were then cultured for five days at 28 °C at 150 rpm. The fungal culture was filtered via Whatman filter paper no. 1 to produce the exo-filtrate, which was then used to create mycelium-free filtrate. In contrast, to prepare endo-filtrate, the mycelium was washed several times in sterile double-distilled water before being suspended in 100 ml of the same water in an Erlenmeyer flask and shaken for 24 hours at 120 rpm and 30°C. The endo-filtrate was then filtered through Whatman filter paper no. 1 to create the final product. Centrifugation of these filtrates at 5000 rpm for 10 min was done. The resulting filtrates were used for the synthesis of AgNPs and AuNPs where 0.25 ml of filtrate was diluted by 0.25 ml of sterile distilled water then was added to 0.5 ml of reagent (2mM silver nitrate ($AgNO_3$) for silver nanoparticles preparation or 0.5 ml of Hydrogen tetrachloroaurate hydrate ($HAuCl_4$) for gold nanoparticles preparation). These mixtures were incubated at room temperature for appearing of color of formation of nanoparticles (yellow to brown for silver nanoparticles and purple to blue for gold nanoparticles) **[6]**.

Characterization of AgNPs and AuNPs

The biosynthesis of AgNPs and AuNPs were initially identified by the visual colour shift in

the reaction mixture from colourless to brown and from light yellow to purple or blue, respectively. The surface plasmon resonance peaks of the biosynthesized AgNPs and AuNPs were identified using a UV-visible spectral analysis (Carry 100 Ultraviolet-visible spectrophotometer, Agilent, USA) at a wavelength range between 300 and 700 nm. Using HRTEM (JEM 2100 HRT, Japan) and an operating voltage of 200 keV, the morphology, size, and SAED of the greenly synthesised AgNPs and AuNPs were determined. On a grid of copper that had been coated with carbon, a drop of colloidal AgNPs or AuNPs was applied, allowed to dry at room temperature, and then confirmed by HRTEM. The particle size distribution was determined using a nano-zeta sizer (Malvern Instrument ZS-Nano, UK) by monitoring the dynamic variations of light scattering intensity (DLS) brought on by the Brownian motion of the particles. The measurement provided the zeta potential, the polydispersity index (PdI), which describes the width of the particle size distribution, the peak values in the hydrodynamic diameter distribution, and the average hydrodynamic diameter of the sampled particles. On the PdI scale, 0 represents monodisperse and 1 represents polydisperse, respectively. With a temperature equilibration time of 1 min at 25°C and a scattering angle of 90, all experiments were done in triplicate. The presence of potential functional groups that could be responsible for the synthesis of AgNPs and AuNPs in the *Penicillium goetzii* and *Aspergillus flavus* var. columnaris culture filtrates was assessed using FTIR spectroscopy. Jasco 600 FTIR spectrophotometer (Japan) was used to monitor the FTIR spectra in three replicates with a resolution of 4 cm^{-1} over a 4000-400 cm^{-1} range.

Antibacterial and antifungal activities of silver and gold nanoparticles on selected bacterial and fungal strains

To reach the final concentration of 250 µg/ml; 180 µl of Luria-Bertani broth (LB broth) and 10 µl of a bacterial or fungal culture suspension (log phase) were placed to 96-well flat polystyrene plates. The nanoparticle suspension was then added (10µl). At 37°C, the plates were incubated overnight. After incubation, the tested nanoparticles' effective antibacterial actions were seen as clearance in the wells, however when the tested nanoparticles had no effect on the bacteria or fungi, the growth media in the wells seemed opaque. The absorbance was measured using a Spectrostar Nano Microplate Reader (BMG LABTECH GmbH, Allmendgrun, Germany) at optical density (OD) 600 after about 20 hours **[12]**.

Biofilm inhibition activity

To reach the final concentration of 250 µg/ml; 180 µl of Luria-Bertani broth (LB broth) and 10 µl of a bacterial or fungal culture suspension (log phase) were placed to 96-well flat polystyrene plates. The nanoparticle suspension was then added (10µl). The control (without the tested nanoparticles suspension) was also added. The plates were incubated for 24 hours at 37 °C. Following incubation, the wells' contents were taken out, they were cleaned three times with 200 µL of phosphate buffer saline (PBS) pH 7.2, to get rid of any free-floating bacteria, and they were then allowed to dry in sterile laminar flow for an hour. 200 L of crystal violet (0.1% w/v) per well were applied for staining, and after 1 hour, any surplus stain was removed, and the plates were stored for drying. A Spectrostar Nano Microplate Reader (BMG LABTECH GmbH, Allmendgrun, Germany) was used to measure optical density at 570 nm after washing dried plates with 95% ethanol **[12]**.

Antiviral activity

To assess the NPs' antiviral effects on the adapted species C human adenovirus serotype 5 (HAdV-C5). Different concentrations of the tested substances were incubated with MDBK cell monolayers maintained in Dulbecco's Modified Eagle Medium supplemented with 10% heat inactivated fetal bovine serum (FBS), 100 µg/ml streptomycin and 100 units/ml penicillin in a 5% CO_2 humidified environment at 37°C. As previously stated by [13], MTT (3-(4,5-dimethlethiazole-2-yl)-2,5-diphenyltetrazolium bromide) was used to calculate the maximum tolerated dilution (MTD) for antiviral experiments. Ten-fold dilutions of HAdV-C5 were made in FBS free growing media for the yield reduction experiment. After the compound was incubated at MTD for 1 hour (virucidal), after the cells

had received NP treatment (pre-treatment), and before the cells had received NP treatment (post-treatment), the cells were challenged with 100 µl of virus dilutions 10^{-4} to 10^{-9}. Microscopic testing for CPE was done 72 hours after infection. Using the Kärber method, the viral titer as 50% tissue culture infection dose (TCID50) was calculated [14]. The difference between the values of the virus with compound against the virus without compound was used to evaluate the reduction of the virus titer**.**

Anticancer activity

In the current investigation, HepG2 (Liver Hepatocellular Carcinoma) Cell Line was used. The cells were grown in 96-well plates with 5 x 10^4 cells and 100 µl of medium in each well. Following a 24 hour incubation at 5% CO^2 and 37 °C, different concentrations of nanoparticles (7.8, 15.6, 31.25, 62.5, 125, 250, 500, and 1000 µg/ml) were introduced to 200 µl each well. A further 24 hours were spent incubating the plates at 5% CO2 and 37°C. Then, 20 µl of MTT (5 mg/ml) were applied to each well. The culture medium was removed from the plates after 4 hours of incubation at 37 °C, and 150 µl of DMSO was then poured into each well. The absorbance (OD value) of each well (wavelengths 570 nm) was determined using a microplate reader after the crystals had been fully dissolved. Using the formula (A570 of treated cells/A570 of control cells X 100), the percentage of viable cells was estimated **[15]**.

3. Results and Discussion

Identification of fungal isolates used for preparation of nanoparticles

Penicillium goetzii growth on Czapek Yeast Agar (CYA) is characterized by fast rate, production of ascospores with size range of 3–4.5 × 2.5–4 µm and production of brown soluble pigments. If *Penicillium goetzii* cultivated on Dichloran Glycerol Agar (DG18), it produces larger colonies after incubation at 25 °C for 7 days (22–30 mm) (As shown in figure 1A)**.**

Aspergillus flavus var. columnaris colonies had a creamy green color and had fast growth rate when cultivated on PDA. Heads of conidia had diameter size ranged from 600.0 to 920.0 µm while conidiophore diameter size ranged from 5.50 to 12.50 µm. Vesicle diameter size ranged from 37.0 to 68.0 µm and ascospore diameter size ranged from 4.0 to 8.1 µm (As shown in figure 1B)**.**

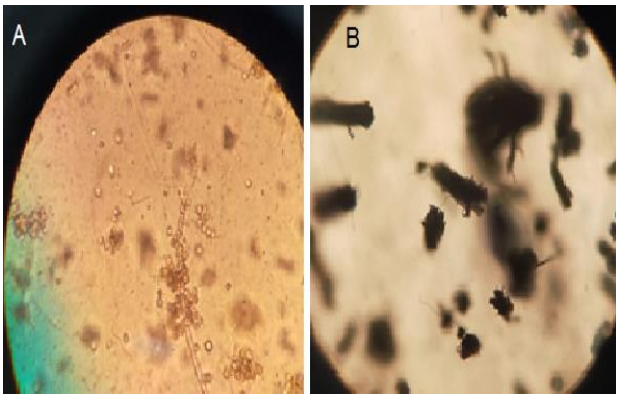

Figure 1. Fungal isolates were used for preparation of gold and silver nanoparticle; (A) Penicillium goetzii, (B) Aspergillus flavus var. columnaris.

Characterization of AgNPs and AuNPs

The reaction mixture color change

Conversion of colors from colorless to yellowish brown (Figure 2A) or from yellow to purple (Figure 2B) refers to the production of AgNPs and AuNPs, respectively. Changing of the solutions color is attributed to the presence of chemical compounds in cultures filtrates of *Penicillium goetzii* and *Aspergillus flavus var. columnaris*. In the absence of *Penicillium goetzii* and *Aspergillus flavus var. columnaris* cultures filtrates under similar conditions, there was no change in color was detected in $AgNO_3$ and $HAuCl_4$ solutions. The conversation of mixture color was considered as evidence on the reducing of Au^{3+} and Ag^{+} ions and thereby formation of AuNPs and AgNPs **[16]**.

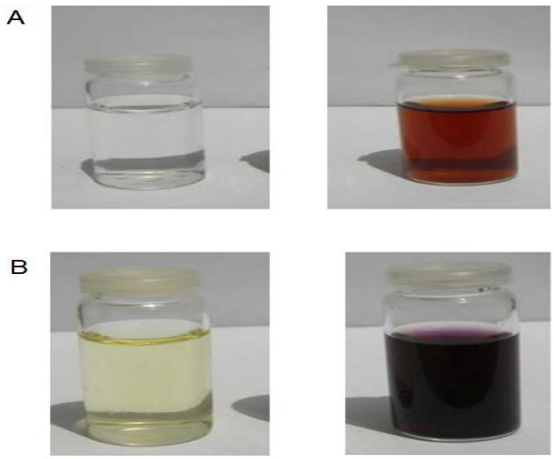

Figure 2. (A) Color change during biosynthesis of AgNPs, (B) Color change during biosynthesis of AuNPs.

UV-visible spectroscopy investigation

Figures 3A and 3B show the UV–visible absorption spectra in the range of 300-800 nm resulted from AgNPs and AuNPs biosynthesized from *Penicillium goetzii* and *Aspergillus flavus var. columnaris* which gave absorbance peak at 440 nm and 540 nm, respectively. In this study, maximum absorption wavelength (λ max) values confirm the production of AgNPs and AuNPs because it has been detected that the λ max of AgNPs was in the range 400-450 nm and λ max of AuNPs was in the range 50-550 nm, respectively **[16]**.

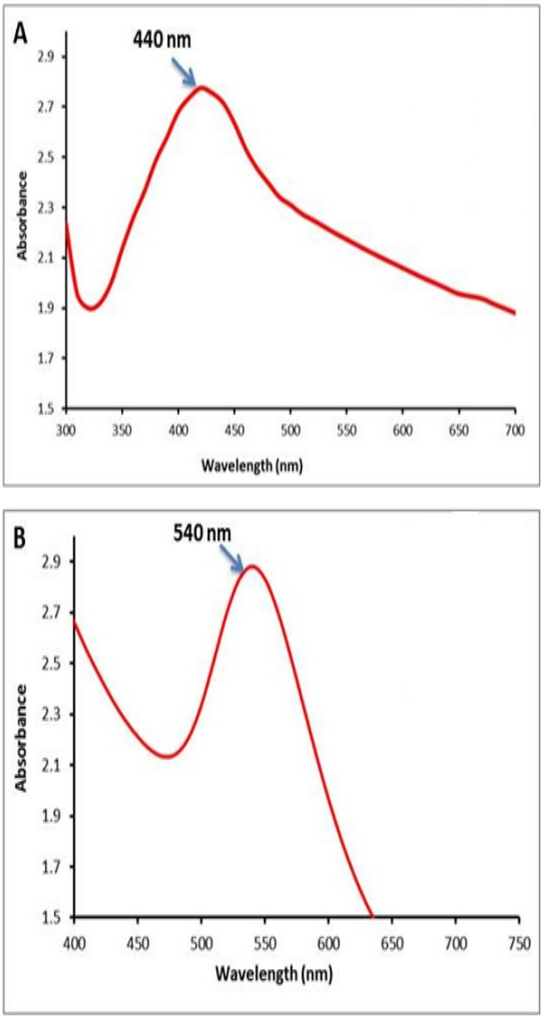

Figure 3. (A) Absorption peaks of the biosynthesized AgNPs by *Penicillium goetzii*, (B) Absorption peaks of the biosynthesized AuNPs by *Aspergillus flavus var. columnaris* using a UV–visible absorption spectrophotometer

Transmission electron microscopy (TEM)

Detecting the particle sizes of AgNPs and AuNPs and their morphologies was done using TEM micrograph. Spherical and irregular shapes appeared in TEM images with the size range of 16.7 – 21.8 mm for AgNPs and 10-23 nm for AuNPs (Figure 4 A and 4 B), other studies detected sizes smaller than those **[17, 18, 19]**. In addition, images of AgNPs and AuNPs using the selected area electron diffraction (SAED) assay showed very fine spots ring pattern, which indicated polycrystalline nature (Figure 4 A1 and B1). SAED analysis was almost compatible with previous results obtained from some biosynthesized AgNPs and AuNPs **[16, 20]**.

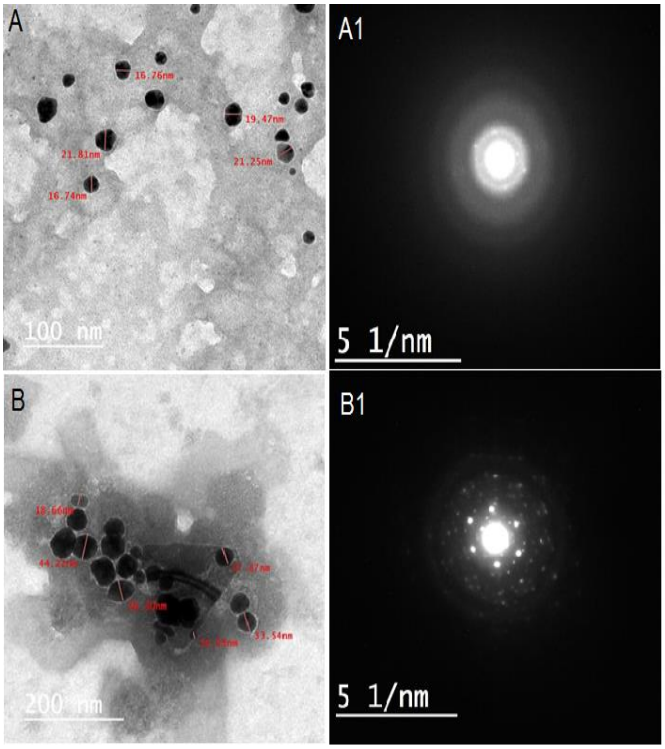

Figure 4. Transmission electron microscopy images of AgNPs (A and A1) and AuNPs (B and B1)

Fourier transform infrared spectroscopy (FTIR)

For identifying the probable active functional groups as capping and reducing agents exerted from *Penicillium goetzii* and *Aspergillus flavus var. columnaris* in cultures filtrates, FTIR spectra measurements were performed (Figure 5 A and B). As showed in Table 1, the FTIR spectrum of the green produced silver nanoparticles gave various absorption peaks at 2958, 2925, 2356, 2080, 2017, 1887, 1718, 1635, 1610, 1581, 813, 549 Cm^{-1} which are related to alcohol, carbon dioxide, isothiocyanate, cyclopentanone, aromatic compound, alkene, conjugated alkene, amine, cyclic alkene and halo compound

groups. FTIR spectra main peaks of AuNPs were showed in table 2 with absorption peaks at 3548, 2923, 1637, 1617, 1566, 1432, 1383, 1105, 796, 668 Cm^{-1} which are related to several functional groups such as primary amine, alkane, conjugated alkene, cyclic alkene, halo compound, aldehyde, alcohol, amine, 1,2,3,4-tetrasubstituted, 1,2,3-trisubstituted, carboxylic acid. In previous studies, Similar absorbance peaks with a wavenumber slightly variance had been observed **[21, 22, 23, 24, 25]**. This finding assures that *Penicillium goetzii* and *Aspergillus flavus var. columnaris* cultures filtrates contain phytochemical groups accountable for the reducing of $HAuCl_4$ and $AgNO_3$ and stabilizing of gold and silver nanoparticles **[22, 25]**.

Dynamic light scattering analysis (DLS)

For analysis of poly dispersity index (PDI) and biosynthesized AuNPs and AgNPs particle size, DLS measurements were used. The highest intensity of the AgNPs were 45.4 nm with PDI equal to 0.371 (Figure 6 A), while the highest intensity of the AuNPs were 9.06 nm with PDI= 0.522 (Figure 6 B). The mean size distribution of biosynthesized AgNPs in previous studies ranged from 30-90 nm **[26, 27, 28, 29]** whereas biosynthesized AuNPs ranged from ranged from 17.4-95 nm **[27, 30, 31, 32]**.

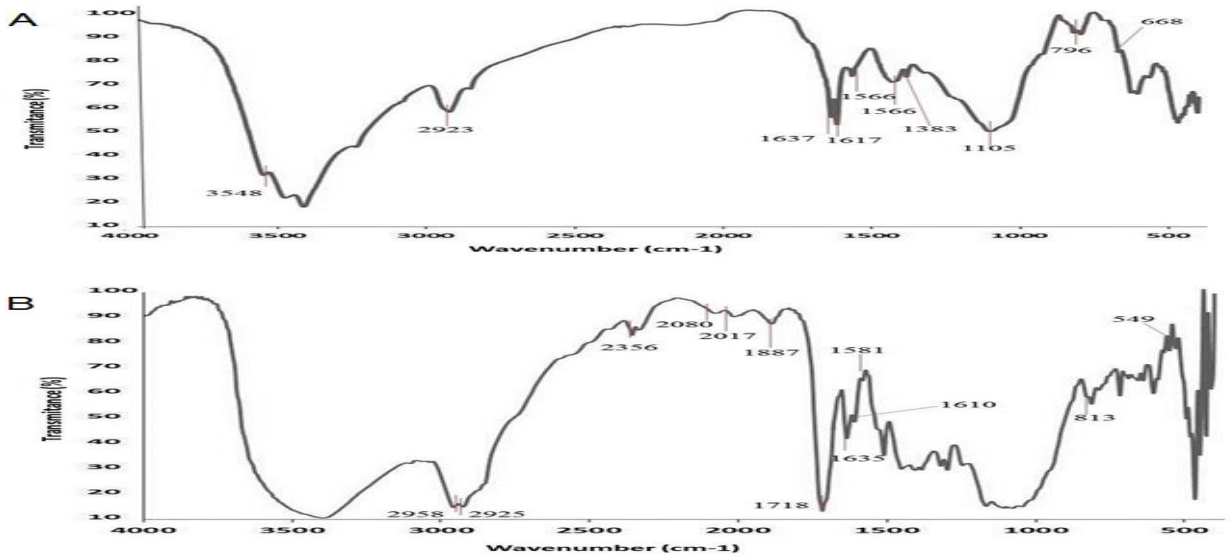

Figure 5. FTIR spectra of silver (A) and gold (B) nanoparticles

Table 1. FTIR analysis: Functional groups of *Penicillium goetzii* mediated AgNPs synthesis

Wave Frequency numbers (cm^{-1})	**FTIR Functional Group**		
	Group	**Class**	**Peak Details**
2958	O-H stretching	alcohol	weak, broad
2925	O-H stretching	alcohol	weak, broad
2356	O=C=O stretching	carbon dioxide	strong
2080	N=C=S stretching	isothiocyanate	strong
2017	N=C=S stretching	isothiocyanate	strong
1887	C=O stretching	cyclopentanone	strong
1718	C-H bending	aromatic compound	weak
1635	C=C stretching	alkene	medium
1610	C=C stretching	conjugated alkene	medium
	N-H bending	amine	medium
	C=C stretching	cyclic alkene	medium
1581	N-H bending	amine	medium
	C=C stretching	cyclic alkene	medium
813	C=C bending	alkene	medium
549	C-Br stretching	halo compound	strong

Table 2. Functional groups of *Aspergillus flavus var. columnaris* mediated AuNPs synthesis: FTIR analysis.

Wave Frequency numbers (cm^{-1})	**FTIR Functional Group**		
	Group	**Class**	**Peak Details**
3548	N-H stretching	primary amine	medium
2923	C-H stretching	alkane	medium
1637	C=C stretching	alkene	medium
1617	C=C stretching	conjugated alkene	medium
1566	C=C stretching	cyclic alkene	medium
1432	O-H bending	carboxylic acid	medium
1383	C-H bending	aldehyde	medium
	O-H bending	alcohol	medium
1105	C-N stretching	amine	medium
796	C-H bending	1,2,3,4-tetrasubstituted	strong
	C-H bending	1,2,3-trisubstituted	strong
668	C-Cl stretching	halo compound	strong

Table 3. Antibacterial activities of biosynthesized AgNPs and AuNPs against some bacterial strains

Nanoparticles	Escherichia coli		Proteus mirabilis			Klebsiella sp.	Salmonella sp.		Pseudomonas aeruginosa		Staphylococcus aureus	
	Growth Optical Density	Biofilm inhibition ratio (%)	Growth Optical Density	Biofilm inhibition ratio (%)	Growth Optical Density	Biofilm inhibition ratio (%)	Growth Optical Density	Biofilm inhibition ratio (%)	Growth Optical Density	Biofilm inhibition ratio (%)	Growth Optical Density	Biofilm inhibition ratio (%)
AgNPs	0.355	49.4	0.505	41.0	0.064	91.7	0.322	45.1	0.431	48.7	0.080	89.3
AuNPs	0.466	33.6	0.246	71.2	0.275	64.6	0.421	28.2	0.841	0.00	0.361	51.8

Antibacterial activity of biosynthesized silver and gold nanoparticles

Six bacterial strains were utilized to investigate the effectiveness of silver and gold nanoparticles as antibacterial agents, five of which were gram-negative strains: *Klebsiella sp.*, *Escherichia coli, Pseudomonas aeruginosa*, *Proteus mirabilis* and *Salmonella sp.* and one gram positive strain: *Staphylococcus aureus*. Silver nanoparticles biosynthesized using *Penicillium goetzii* acted as a good antibacterial agent where the inhibition ratio of different used bacterial strains ranged from 41.0% to 91.7%. The best antibacterial activity was against *Klebsiella sp*.by inhibition ratio of 91.7%. Gold nanoparticles biosynthesized using *Aspergillus flavus var. columnaris* had variable antibacterial activity; it had no antibacterial effect on *Pseudomonas aeruginosa* while its inhibition ratio for other bacterial strains was ranged from 28.2% to 71.2%, the best antibacterial activity was against *Proteus mirabilis* with inhibition ratio of 71.2%., as shown in table (3) and figure (7).

In this study, antibacterial effect of biosynthesized AgNPs and AuNPs was reported against *Escherichia coli* with inhibition ratios of 49.4 and 33.6 respectively. Kaushik et al [33], Hashemi et al [34], Vimala et al [35], Li et al [36] and Elbehairy [37] found that biosynthesized AgNPs acted as good antibacterial agent against *Escherichia coli*. Vimala et a [35], Zhu et al., [38], Punnoose & Mathew [39], Purbowati et al [40] and Gnanamoorthy et al [41] found that the antibacterial efficacy of AuNPs against *Escherichia coli* was remarkable. In contrast Dang et al [42] and Rajathi et al [43] found that gold nanoparticles had no antibacterial effect on *Escherichia coli.*

In this study, antibacterial effect of biosynthesized AgNPs and AuNPs was

reported against *Proteus mirabilis* with inhibition ratios of 41.0 and 71.2 respectively. Hashemi et al [34], Vimala et al [35], DJ et al [44] and Shirzadi et al [45] found that silver nanoparticles had antibacterial effect on *Proteus mirabilis*. Vimala et al [35] and Fanoro et al [46] found that gold nanoparticles had antibacterial effect on *Proteus mirabilis*.

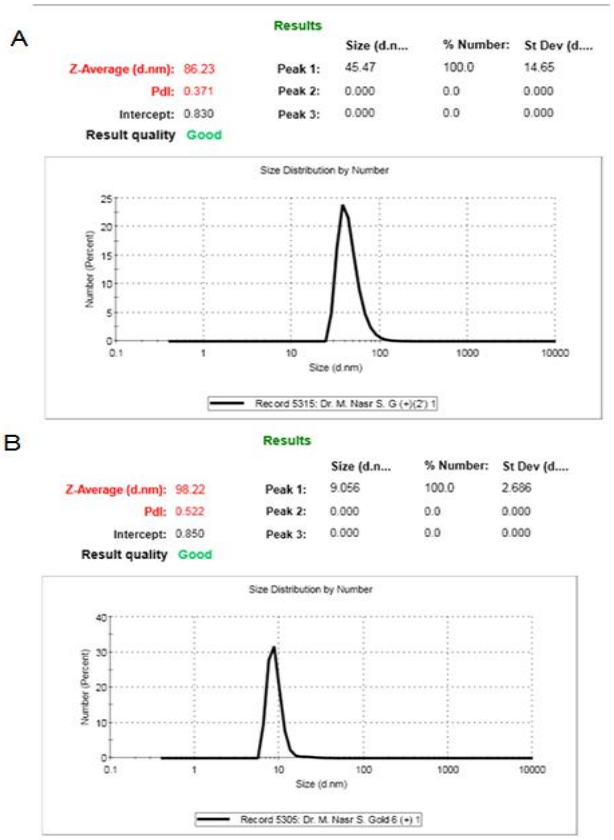

Figure 6 (A) DLS of AgNPs synthesized by *Penicillium goetzii* shows the size distribution by number, (B) DLS of AuNPs synthesized by *Aspergillus flavus var. columnaris* shows the size distribution by number.

In this study, antibacterial effect of biosynthesized AgNPs and AuNPs was reported against *klebsiella sp*.with inhibition ratios of 91.7 and 64.6 respectively. Hashemi et al [34], Vimala et al [35], DJ et al [44] and Shirzadi et al [45] found that silver nanoparticles had antibacterial effect on *klebsiella sp*. Vimala et al [35], Fanoro et al [46] and Mahmood et al [47] found that gold nanoparticles had antibacterial effect on *klebsiella sp*.

In this study, antibacterial effect of biosynthesized AgNPs and AuNPs was reported against *Salmonella sp*. with inhibition ratios of 45.1 and 28.2 respectively. Sosani et al [48], Ahmad et al [49] and Neupane et al [50] found that silver nanoparticles had antibacterial effect on *Salmonella typhi.* Dhas et al [51] found that AuNPs had antibacterial effect on *Salmonella typhi.* Abdalhamed et al [52] found that AuNPs had antibacterial effect on multi-drug resistant *Salmonella sp.*

In this study, biosynthesized AgNPs had antibacterial activity against *Pseudomonas aeruginosa* with inhibition ratios of 48.7, while AuNPs had no antibacterial effect. Hashemi et al [34], DJ et al [44] and Shirzadi et al [45] found that silver nanoparticles had antibacterial effect on *Pseudomonas aeruginosa.* Punnoose & Mathew [39] and Abdulazeem et al [53] found that AuNPs exhibited remarkable antibacterial activity against *Pseudomonas aeruginosa*.

In this study, antibacterial effect of biosynthesized AgNPs and AuNPs was reported against *Staphylococcus aureus* with inhibition ratios of 89.3 and 51.8 respectively. Hashemi et al [34], DJ et al [44] and Shirzadi et al [45] and Mohamedin et al [54] found that silver nanoparticles had antibacterial effect on *Staphylococcus aureus.* Punnoose & Mathew [39], Gnanamoorthy et al [41] and Fanoro et al [46] found that AuNPs exhibited remarkable antibacterial activity against *Staphylococcus aureus*.

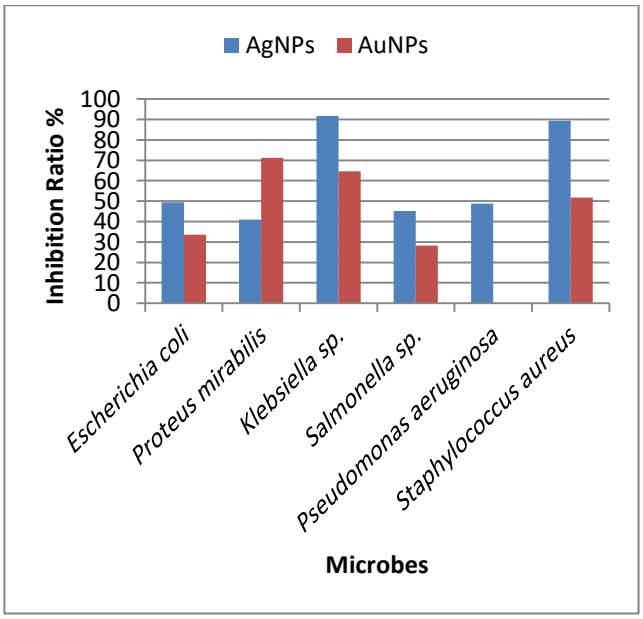

Figure 7. Antibacterial activities of biosynthesized AgNPs and AuNPs using microbes against some bacterial strains

Antifungal activity of biosynthesized silver and gold nanoparticles

Two genera were used for evaluation of antifungal activity of silver and gold nanoparticles: *Candida albicans* and *Aspergillus niger*. Silver nanoparticles had weak antifungal activity on *Candida albicans* while had no effect on *Aspergillus niger*. Gold nanoparticles had moderate antifungal activity on *Aspergillus niger* while had no effect on *Candida albicans* as shown in table (4). Ahmad et al [49] found that silver nanoparticles had moderate antifungal activities against *Candida albicans*. Mohamedin et al [54], Rajamohamed et al [55], Jabber & Al-Khafaji [56] and Abdallah & Ali [57] and found that silver nanoparticles had antifungal effect on *Candida albicans*. Kareem & Samaka [58], Tian et al [59] and Nidhin et al [60] found that gold nanoparticles had antifungal effect on *Candida albicans*. Owaid et al [61], Mittal et al [62] and Anshiba et al [63] found that silver nanoparticles acted as antifungal against *Aspergillus niger*. Tian et al [59] and khan et al [64] found that gold nanoparticles had antifungal effect on *Aspergillus niger*.

Table 4. Antifungal activities of biosynthesized AgNPs and AuNPs against *Candida albicans* and Aspergillus niger

Nanoparticles	***Candida albicans***	***Aspergillus niger***
	Growth Optical Density (O. D.)	Growth Optical Density (O. D.)
Control (Without NPs)	0.666	3.070
AgNPs	0.424	3.070
AuNPs	0.666	1.968

Antiviral activity of biosynthesized silver and gold nanoparticles

The antiviral properties of AgNPs or AuNPs against HAdV-5 infection in vitro were assessed in the current study using TCID50, and the results were compared with ribavirin (Table 6 and figure 9). Additionally, it was studied how antivirals work and whether nanoparticles directly or indirectly affect viruses. AgNPs reduced the viral titer by 2.25, 2.5, and 2.0 log10 TCID50 in the virucidal, pre-treatment, and post-treatment experiments, respectively. In the virucidal, pre-treatment, and post-treatment experiments, respectively, AuNPs reduced the virus titer by 2.25, 3.0, and 2.25 log10 TCID50, indicating excellent antiviral activity against HAd-5. AgNPs and AuNPs have been shown to have antiviral properties against a variety of human adenovirus strains, including serotype 5 [65, 66, 67, 68. 69, 70, 71], also against other viruses such as SARS-CoV-2, murine norovirus, HIV, Herps Simplex, Measles virus, African swine fever virus and influenza A virus [67, 72, 73, 74, 75, 76]. In this study, the best antiviral activity of AgNPs and AuNPs was shown in pretreatment assay, suggesting that these nanoparticles directly blocked the virus receptors in the host cells surface and prevent the attachment of virus with it. Previous studies [77, 78, 79] demonstrated that AgNPs and AuNPs might suppress virus multiplication by their attachment to virus coat, preventing viral adhesion to host cell. According to Luceri et al [80], treatment of cells with silver and gold nanoparticles prior to viral entry reduced virus infections by 2.5 and 1.25 log10 TCID50, respectively. They speculated that this might be because the nanoparticles interacted with host receptors to stop the virus from penetrating the host cell. Low antiviral action was shown when NPs were introduced to cells after virus entry, indicating that NPs may also be able to stop the spread of viruses by attaching to their DNA [81].

Table 5. The antiviral activities of AgNPs and AuNPs on HAdV-5 by Karber methods.

Antiviral	**Virucidal**			**Pre-treatment**			**Post-treatment**		
	A	B	R	A	B	R	A	B	R
Ribavirin	6.5	2.25	4.25	6.25	4.5	1.75	6.25	1.25	5.0
AgNPs	6.25	4.0	2.25	6.25	3.75	2.5	6.5	4.5	2.0
AuNPs	6.25	4.0	2.25	6.25	3.25	3.0	6.25	4.0	2.25

A: the virus titers before treatment; B: virus titers after treatment; R: Reduction in virus titer calculated as the difference between treated and untreated.

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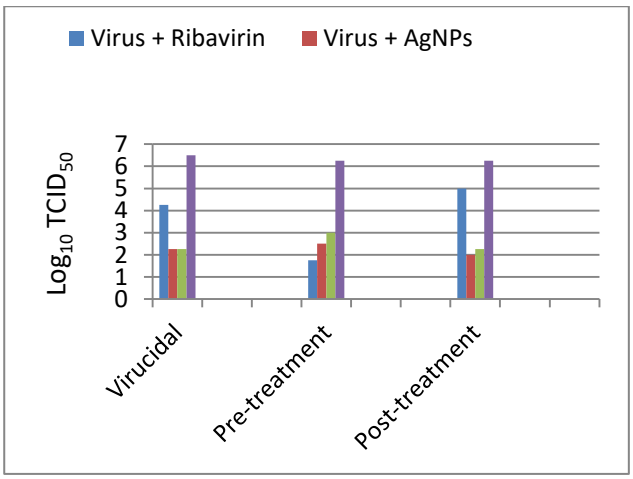

Fig.8. Antiviral activity of the biosynthesized AgNPs and AuNPs.

Anticancer of of biosynthesized silver and gold nanoparticles

To evaluate the antiproliferative activities of the *Penicillium goetzii* and *Aspergillus flavus var. columnaris* cultures filtrates extracts mediated biosynthesized AgNPs and AuNPs, various dilutions of the AgNPs or AuNPs were added to HepG2 liver cell lines and incubated for 24h. A significant increase in the call viability (%) was detected with decreasing of AgNPs and AuNPs concentrations (increasing dilution). By increasing nanoparticles dilution from 1/2 µg/ml to 1/256 µg/ml, the cell viability of the HepG2 cells was increased from

17.4% to 98.5% when incubated with AgNPs. While when HepG2 liver cell lines were incubated with AuNPs dilutions from 1/2 µg/ml to 1/256 µg/ml, the cell viability increased from 14.4% to 98.5%, as shown in table 5 and figure 8.

Table 6. Cell viability percent after adding different concentrations of silver and gold nanoparticles

Nanoparticles		**Nanoparticles Concentration (µg/ml)**								
		1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	Control
AgNPs	Viability (%)	17.4	28.0	36.4	90.2	93.2	97.0	97.7	98.5	100
AuNPs		14.4	14.4	26.5	36.4	47.0	91.7	97.7	98.5	100

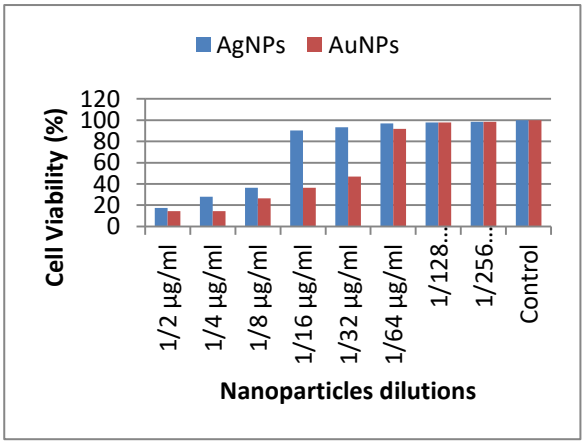

Fig 9. Biosynthesized AgNPs and AuNPs anticancer activity against HepG2 liver cell lines

Previous investigations [82, 83, 84, 85] revealed the anticancer effects of AgNPs and AuNPs against HEPG-2. According to research by Wang et al [86], silver nanoparticles may prevent the growth of tumors by producing reactive oxygen species (ROS), which can destroy cellular DNA. According to Pei et al [87], DNA damage may change how a protein is expressed, leading to apoptosis. Additionally, according to Costa et al [88], AgNPs might damage the mitochondrial chain, causing superoxide anion leakage. According to Blagoi et al [89], AuNPs can have cytotoxic effects when gold atoms interact with the functional groups of intracellular proteins, or the phosphate groups and nitrogen bases found in DNA. It's interesting to note that the size of nanoparticles affects ROS production, the smaller nanoparticles leading to an overproduction of ROS [90]. This may explain the higher anti-HepG2 liver cell activity of AgNPs than AuNPs in this study.

Conclusion

In this study, *Penicillium goetzii* and *Aspergillus flavus var. columnaris* synthesized AgNPs and AuNPs. Biosynthesized silver and gold nanoparticles were characterized using UV–visible spectrum, DLS, TEM, and FTIR which showed the spherical and irregular shapes of NPs with size ranging from 16.7 – 21.8 mm for AgNPs and 10-23 nm for AuNPs. Various phytochemical groups were present in Penicillium goetzii and Aspergillus flavus var. columnaris culture extracts showed by FTIR, these groupes may be responsible for production of NPs by reduction of $AgNO_3$ and $HAuCl_4$. AgNPs and AuNPs showed antibacterial and antifungal activities.

Additionally, they showed strong antiviral effects against HAdV serotype 5 and anticancer effect against Liver Hepatocellular Carcinoma cells. Consequently, these nanoparticles may become suitable alternatives for application in various medical and industrial uses

4. References

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