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Extracellular Biosynthesis of Silver Nanoparticles Using *Aspergillus* sp. and Evaluation of their Antibacterial and Cytotoxicity

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Silver nanoparticles are extracellular synthesized by biomass filtrate of *Aspergillus* sp. Proteins in biomass filtrate of fungal isolate act as a reducing and capping agent to converting Ag^+ ion to Ag° metal and produce the silver nanoparticle s. During this study silver nanoparticles biologically synthesized by *Aspergillus* sp. and assessed their antibacterial and cytotoxicity effect on cancer cell represented by Caco-2 cell and normal cell represented by Vero cell.
Place and Duration of Study: The study was performed in Botany & Microbiology Department, Faculty of Science, Al-azhar University, Cairo, Egypt, from October 2015 until January 2017.
Materials and Methods: (1) Different fungal strains were isolated from soil sample collected from El-Wahat desert soil, Giza, Egypt. **(2)** Screening for extracellular biosynthesis of silver nanoparticles by fungal biomass filtrate. **(3)** The most potent fungal isolate was identified by cultural

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and morphological characters. **(4)** Silver nanoparticles were characterized by UV-vis spectroscopy, TEM, XRD, FTIR and Particle size analysis. **(5)** Study the antibacterial activity for biosynthesized AgNPs against human pathogenic bacteria by agar well diffusion methods and evaluate cytotoxic effect on two type of cell represented by Caco-2 cell and vero cell.

Results: During this study, the UV–Vis absorption spectra showed a maximum surface plasmon resonance peaks at 400 nm which confirmed the biosynthesis of silver nanoparticles by *Aspergillus* sp., TEM analysis revealed that, the silver nanoparticles were spherical shape with 5–30 nm in size. FTIR showed bands at 1639, 1383, 1115 and 516 cm^{-1} which corresponding to different functional groups possibly involved in the synthesis and stabilization of AgNPs while XRD revealed intense peaks corresponding to (111), (200), (220) and (311) which indicating the crystalline nature of the AgNPs synthesized by *Aspergillus* sp. The antibacterial activity of AgNPs against pathogenic bacteria showed, highest activity against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* respectively according to diameter of inhibition zone around the well filled by different concentration of AgNPs (100, 50, 25, 12.5, 6.25 and 3.125 µg/mL) in each wells. MTT assay method used to assessment cytotoxic effects of biosynthesized AgNPs against human colorectal adenocarcinoma cells (Caco-2) as cancer cell and kidney of African green monkey (normal Vero cells) when exposure to 1000, 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.90, 1.95, 0.97 and 0.48 µg/mL of AgNPs. According to results, IC_{50} for Caco-2 cell and Vero cell were 3.75 µg/ml and 280 µg/mL respectively. This study demonstrates the possible use of biologically synthesized silver nanoparticles in medical field.

Keywords: Silver nanoparticles; Aspergillus sp; TEM; antibacterial; anticancer; cytotoxicity; MTT assay.

1. INTRODUCTION

Nanotechnology has evolved as a favorable tool in the medicinal field by using nanoparticles for treatment of several diseases [1]. The biological synthesis of metal nanoparticles depended on the type and behavior of microorganism used. Since the beginning of this century, researchers have been working on intracellular and extracellular biosynthesis of metal nanoparticles by using bacteria, actinomycetes, yeast, fungi and other biological sources [2-4]. Metal nanoparticles have a high specific surface atoms and surface area, because of their outstanding physicochemical characteristics, including antibacterial properties, catalytic, optical, magnetic and electronic. Synthesis of metal nanoparticles is enormous due to their potential applicability in different areas such as medicine development, energy, electronics and chemistry [5]. The modern nanotechnology has facilitated the production of silver nanoparticles greater efficacy against bacteria with low toxicity to human. In general, silver ions can bind with a variety of negatively charged molecules like proteins, DNA and RNA. Silver nanoparticles synthesized biologically were found to be highly bactericidal activity against gram-positive and gram-negative bacteria, including highly multi-resistant strains such as methicillin-resistant *Staphylococcus aureus* [6]. The various silver-based materials and compounds containing metallic silver (Ag^0) [7] have been synthesized and shown to exhibit antimicrobial activity against various bacteria or ionic silver (Ag^+) [8-9]. The success of the introduction of the silver nanoparticles in different forms in bioscience, healthcare and consumer goods is well known [10-11]. Nanomaterials are expected hopefully to revolutionize cancer diagnosis and therapy. Human cancer cells are susceptible to the cytotoxic effects of AgNPs, but the biochemical pathways involved are often unclear. Because of the high heterogeneity of tumor cells, it is mandatory to dissect the molecular mechanisms responsible for AgNPs cytotoxicity using different cell lines. Indeed, tumor cell lines represent valuable preclinical models to decipher neoplastic circuitry and identify pharmacologically useful compounds because they are considered appropriate models for primary tumor cells [12]. Recently, the anticancerous efficacies of silver nanoparticles synthesized through different sources were evaluated against breast cancer line MCF-7 [13], Human colon adenocarcinoma cells [14,15], HT-29 cell lines [16], Vero cell line [17] and Hep2 cell line [18-20].

This study aimed to (1) biosynthesis and characterization of AgNPs produced through extracellular mechanism by *Aspergillus* sp. (2) Study the efficacy of produced silver nanoparticles by *Aspergillus* sp. against coded

test organisms represented by *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 29213 and *Bacillus subtilis* NCTC 10400*,* and (3) evaluate the cytotoxicity of silver nanoparticles to human epithelial colorectal adenocarcinoma (Caco-2) cells and Vero cell (kidney of African green monkey) *in vitro* by MTT assay methods.

2. MATERIALS AND METHODS

2.1 Isolation and Identification of Fungal Isolate

Aspergillus sp. was isolated from soil sample collected from El-Wahat desert soil, Giza, Egypt (GPS N: 2 21 38 E: 28 55 56.3). About 1.0 g of soil sample was diluted in sterile distilled water and plated onto malt extract agar (MEA) [21] and potato dextrose agar (PDA) [22] and incubated at 32± 2℃ for 3-4 days. Morphologically differed colonies were individually picked up and reinoculated on MEA or/ and PDA for purification, and then kept at 4℃ for further study [23]. Isolated colonies were sub-cultured and maintained in (MEA or PDA). The identification of the fungus was done due to cultural and morphological characteristics.

2.2 Extracellular Biosynthesis of Silver Nanoparticles

2.2.1 Biomass preparation

Two disk of freshly culture of *Aspergillus* sp. was inoculated into 100 mL of Czapex Dox Broth (CDB) medium (as a fermentative media) after adjusting pH 6.8-7.2 [24]. The flasks were incubated at 32±2℃ for 5 days in rotary orbital shaker at a speed of 120 rpm. The biomass was harvested after incubation periods by passing through four layers of wool cloth.

2.2.2 Preparation of biomass filtrate

The biomass filtrate was obtained regarding to the following procedures. In brief, the harvested fungus biomass is washed with sterilized distilled water to remove any medium components. Then, the washed biomass is suspended in 100 ml distilled water and agitated for 72 h at 32±2℃. Finally, the biomass filtrate is obtained by passing it through whatman filter paper N°.1. Filtrate is then collected and ready to use for further nanoparticles synthesis.

2.2.3 Preparation of silver nanoparticles using biomass filtrate.

The previously obtained biomass filtrate is being used for synthesis of silver nanoparticles as the following: 1.5 mM $AgNO_3$ was added to the biomass filtrate and kept for 48 h. at 35℃. After that, 4 mL sample was taken and the absorbance was measured by using a UV-vis spectrophotometer.

2.3 Characterization of Silver Nanoparticles

AgNPs synthesis by *Aspergillus* sp*.* was characterized by: **(A)** UV-Vis spectroscopy shows specific surface plasmon resonance peak (JENWAY 6305 Spectrophotometer). **(B)** The size and shape of AgNPs is often determined through Transmission Electron Microscopy (TEM - JEOL 1010 Japan). **(C)** The binding properties of silver nanoparticles using biomass filtrate of *Aspergillus* sp*.* were investigated by FTIR analysis. The characterization involved (FTIR) analysis of the dried powder of the biosynthesized AgNPs by conducting Fourier Transform Infrared Spectroscopy (FTIR) Agilent system Cary 630 FTIR model. **(D)** X-ray diffraction (XRD) studies of nanoparticles were carried out using Shimadzu Scientific Instruments (SSI), Kyoto, Japan. X-Ray Diffraction patterns for AgNPs were obtained with the XRD- 6000 series, including stress analysis, residual austenite quantitation, crystallite size/ lattice strain, crystallinity calculation, materials analysis via overlaid X-ray diffraction patterns Shimadzu apparatus using nickel-filter and Cu-Ka target, the estimation of the size of particles was performed by Scherrer's formula. **(E)** The particle size distribution of silver nanoparticles was evaluated using Dynamic Light Scattering (DLS) measurement conducted with a Malvern Zetazier Instrument. Measurements were taken in the range between 0.1 and 1000µm. Data obtained were analysed using Zetasizer software.

2.4 Antimicrobial Activity of Silver Nanoparticles Produced by *Aspergillus* sp.

Antimicrobial effect of biosynthesized AgNPs were assessed against coded test organisms represented by gram negative bacteria *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 8739*,* and gram positive

bacteria represented by *Staphylococcus aureus* ATCC 29213*, Bacillus subtilis* NCTC 10400 using agar well diffusion method. Mueller Hinton agar plates were seeded with overnight test organism. One hindered microliter (100 µL) of silver nanoparticles solution (100 µg/mL) was added to each well (0.8 mm) in plate. To determine minimum inhibitory concentration (MIC), different concentration of AgNPs solution (50, 25, 12.5, 6.25 and 3.125 µg/mL) were made. The plates were incubated at 37℃ for 24 h. After incubation, inhibition zone was observed. The diameter of inhibition zones was measured in mm and the results were recorded. The experiments were performed in three replicate and mean values were calculated.

2.5 *In vitro* Cytotoxicity of AgNPs Myco-synthesis from *Aspergillus* sp. against Cancer and Normal Cells

2.5.1 Cell culture methods

The human colorectal adenocarcinoma cells (Caco-2) and normal Vero cells (kidney of African green monkey) were procured from Holding Company for Biological Products & Vaccines (VACSERA), 51 Wezaret El-Zeraa st., Agouza, Giza, Egypt. Cell line incubated into culture bottle were checked using inverted microscope for its proper physical conditions, i.e. sheet, normal shape. The media overlaying cell monolayer was poured off, cells can be released from tissue culture flask by treatment with about 5 mL pre-warmed trypsin-EDTA solution. (Trypsin cleaves cell surface proteins that the cells used to adhere to the flask while EDTA chelates metal ions that are involved in cell adherence. The flask was rocked to trypsin completely cover the cell monolayer. The trypsin was aspirated with a pipette, then 2 ml of trypsin were dispensed, the bottle rocked and was incubated at 37℃. Cells were examined from time to time to avoid trypsin over action. The bottle was shaken with hand to completely dislodge the cells from the bottle surface. Cells were suspended in about 20 ml of growth media (1 X 10^5 cells/mL).

2.5.2 Cytotoxicity assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay was used to evaluate the cytotoxic effect of different concentrations of biosynthesized AgNPs (1000, 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.90, 1.95, 0.97 and 0.48 µg/mL) on human colorectal adenocarcinoma cells (Caco-2) and normal Vero cells (kidney of African green monkey). The selected doses were added to the cell monolayers in triplicate wells and the cytotoxicity of each individual dose was tested using a standard MTT assay for the rapid and sensitive quantification of cell proliferation and viability [25-28].

2.5.3 Determination of viability/cytotoxicity of cells (MTT protocol)

1- The 96 wells tissue culture plate was inoculated with 1 X 10^5 cells / ml (100 µL / well) and incubated at 37℃ for 24 hours to develop a complete monolayer sheet.
2- Growth medium was decanted from 96 well micro titer plates after confluent sheet of cells were formed, cell monolayer was washed twice with wash media.
3- Two-fold dilutions of tested sample were made in MEM medium with 2% serum (maintenance medium).
4- Each dilution contains 0.1ml was tested in different wells leaving 3 wells as control, receiving only maintenance medium.
5- Plate was incubated at 37°C and examined. Cells were checked for any physical signs of toxicity, e.g. partial or complete loss of the monolayer, rounding, shrinkage, or cell granulation.
6- MTT solution was prepared (5 mg/mL in phosphate buffered saline (PBS)
7- MTT solution (20 µL) was added to each well. Place on a shaking table, 150 rpm for 5 minutes, to thoroughly mix the MTT into the media.
8- Incubate (37C, 5% CO2) for 1-5 hours to allow the MTT to be metabolized.
9- Dump off the media. Dry plate on paper towels to remove residue if necessary.
10- Re-suspend formazan (MTT metabolic product) in 200 µL DMSO. Place on a shaking table, 150 rpm for 5 minutes, to thoroughly mix the formazan into the solvent.
11- Read optical density at 560 nm and subtract background at 620nm. Optical density should be directly correlated with cell quantity.

The percentage cell viability was then calculated with respect to control as follows:

Cell viability % = sample absorbance / control absorbance x100.

Cell toxicity % =100 – [(sample absorbance / control absorbance) x100].

2.6 Statistical Analysis

The means of three replications and standard error (SEr ±) were calculated for all the results obtained, and the data were subjected to analysis of variance means by sigma plot 12.5 program.

3. RESULTS

In the present study, the color change of biomass filtrate from colorless to brown color after adding $AgNO_3$ indicate the reduction of metal ions and formation of metal nanoparticles and confirmed this result by UV-Vis Spectroscopy which have characteristic absorption peak at 400 nm. The fungal isolate was subjected to identification based cultural and microscopic characters [29] and found that, the fungal isolate followed the *Aspergillus* species and named *Aspergillus* sp. (Fig. 1a).

3.1 Characterization of Biosynthesized AgNPs

3.1.1 UV-Vis spectroscopy

During the present study, the absorption spectra of AgNPs synthesized by *Aspergillus* sp. showed a maximum surface plasmon resonance peaks at 400 nm (Fig. 1B) has confirmed the reduction of silver ions to metallic silver. This result agreement with [30] while [31] found that, the absorbance band of the AgNPs synthesis by four *Aspergillus* species at 430 nm. The frequency and width of the surface plasmon absorption depends on the size and shape of the metal nanoparticles as well as on the dielectric constant of the metal itself and the surrounding medium [32].

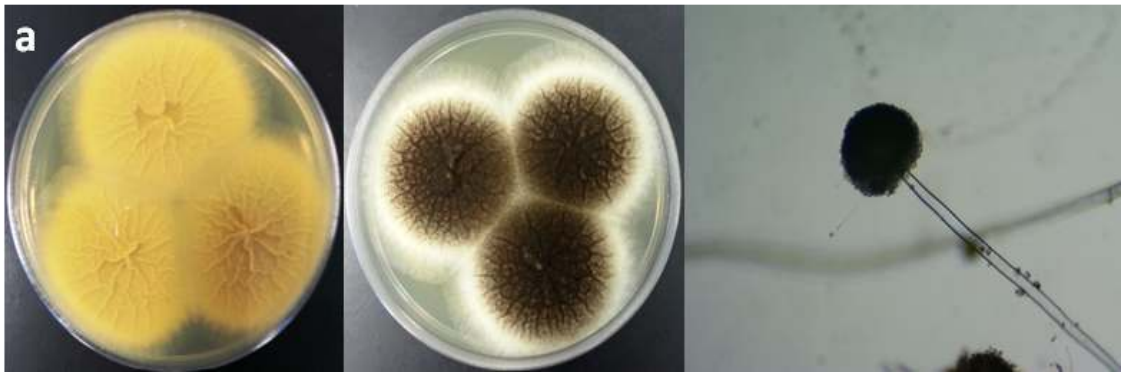

Fig. 1A. Culture and microscopic examination of *Aspergillus* sp. on MEA media

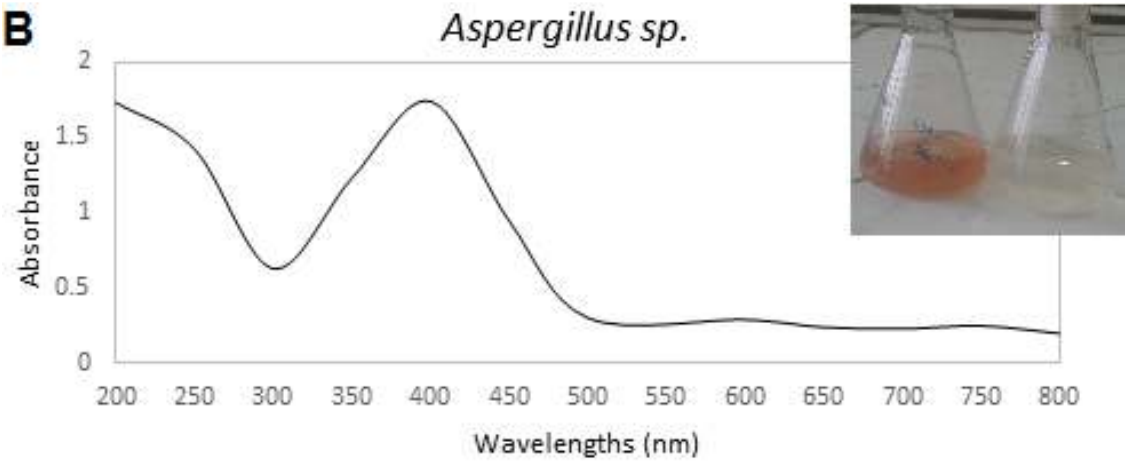

Fig. 1B. UV spectrum of reaction mixture of 1.5 mM silver nitrate with biomass filtrate of *Aspergillus* sp. shown Plasmon resonance at 400 nm

3.1.2 Transmission electron microscope (TEM)

The morphology and size of the AgNPs was viewed by TEM as shown in Fig 2. The synthesized nanoparticles from biomass filtrate of *Aspergillus* sp. was spherical in shape and dispersed without aggregation. The results showed that, the average diameters of AgNPs was around 5-30 nm. Reddy, et al., reported that, the *Aspergillus versicolor* strain ENT 7 had an average diameter of silver nanoparticles 3-40 nm [33].

3.1.3 Fourier transform infrared spectroscopy (FTIR)

FT-IR measurement was used to find out the possible bio-molecules responsible for the reduction of Ag^+ ions and capping the biosynthesized AgNPs. Fig. 3 showed bands at 1639, 1383, 1115 and 516 cm^{-1}. Band at 1639 cm^{-1} correspond to the binding of amide I band of protein with N–H stretching [34]. The bands observed at 1383 and 1115 cm^{-1} can be assigned to C–N stretching vibrations of aliphatic and aromatic amines, while peak at 516 cm^{-1} corresponds to (=C-H bending) alkene. This amide I band designated that proteins found in biomass filtrate of *Aspergillus* sp can bind to Ag^+ ions across carboxylate ions or free amine groups to synthesis of AgNPs in the reduction process. These results are agreement with other reports found that, the proteins play key role in formation of silver nanoparticles act as capping and stability agents in synthesis of AgNPs [35-37].

3.1.4 X-ray diffraction (XRD) analysis

Data represented in Fig. 4 showed that, the AgNPs were essentially crystalline in nature due to intense peaks of XRD corresponding to (111), (200), (220) and (311) plane in the face centered cubic. Finally, found that the AgNPs formed in the extracellular filtrate of *Aspergillus* sp. are present in the form silver nanocrystals. This result agreement with [38-39] where found that, Silver nanoparticles synthesized using fungus *Phoma glomerata* (MTCC-2210) and *Mucor hiemalis* respectively were found to have diffraction signals (111, 200, 220 & 311).

3.1.5 Particle size analysis

The size measured in particle size analyzer technique is the hydrodynamic diameter of the theoretical area that diffuses with the similar speed as the measured nanoparticles, so that the size measured in DLS technique is bigger in comparison with microscopic techniques done in order to analyse the size distribution of synthesized nanoparticles with respect to the intensity [40]. Laser diffraction exposed that, the particles obtained were poly-dispersed mixture. The AgNPs synthesized by *Aspergillus* sp. showed the average diameter of the particles was found to be 76.45 nm (97.4%) (Fig. 5). While Aziz et al. found that, the mean average size of the silver nanoparticles was found to be around 150 nm [40].

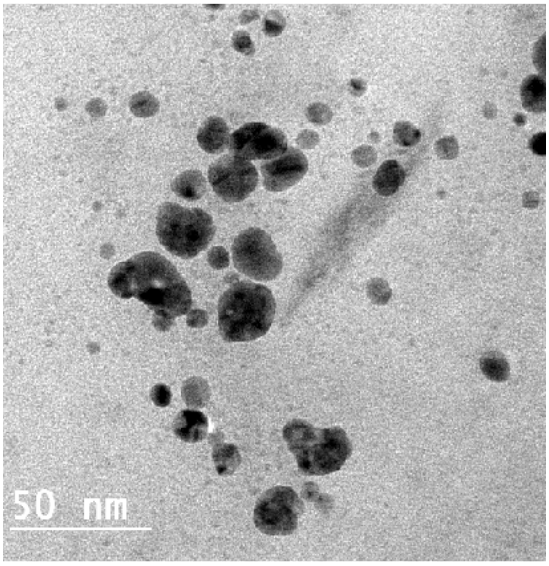

Fig. 2. TEM for AgNPs formed by *Aspergillus* sp showed spherical shape with diameter 5-30 nm

3.2 Screening for Antibacterial Activity

Data represented in Fig. 6 showed that, silver nanoparticles produced by *Aspergillus* sp. have antibacterial activity against both Gram-positive and Gram-negative bacteria. Due to appeared clear zone, AgNPs have very strong inhibitory action against *Bacillus subtilis* and *Staphylococcus aureus* (Gram-positive) than *Pseudomonas aeruginosa* and *Escherichia coli* (Gram-negative) with inhibition zones 18.9 -15.8 -15.4 and 12.8 mm respectively. The inhibitory action of the microbes may be attributed to the size of silver nanoparticles is suitable for penetration of bacterial cell wall for each Gram-positive and Gram-negative bacterium and hence loss of replication ability of DNA, besides the fact that expression of ribosomal sub-unit proteins as

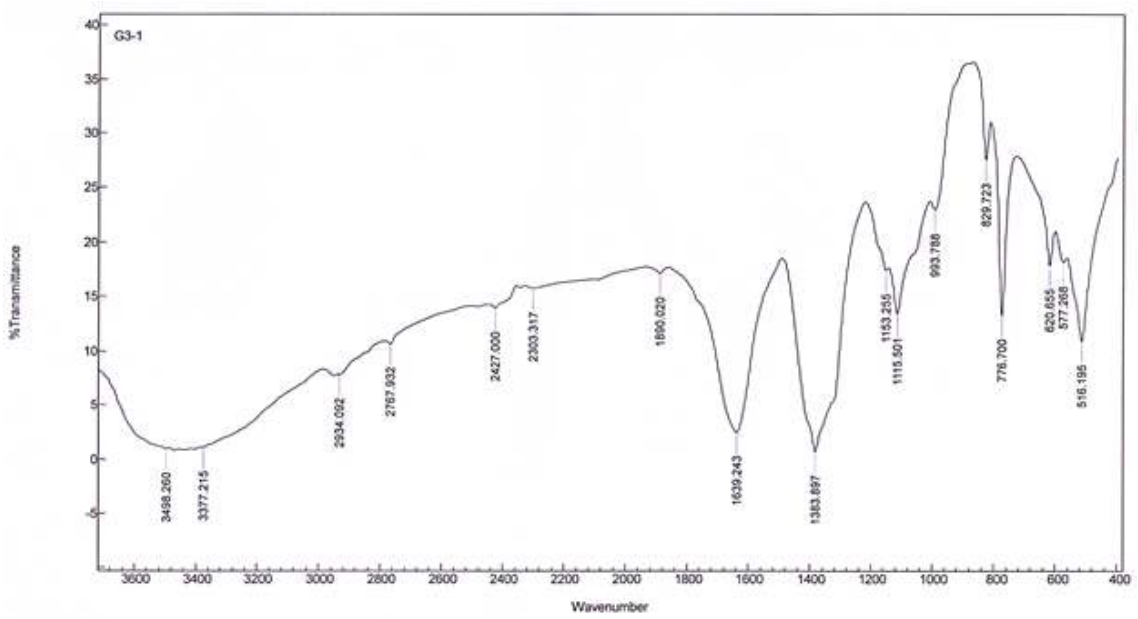

Fig. 3. FTIR spectra showing the presence of proteins as capping agents for AgNPs synthesized by *Aspergillus* sp.

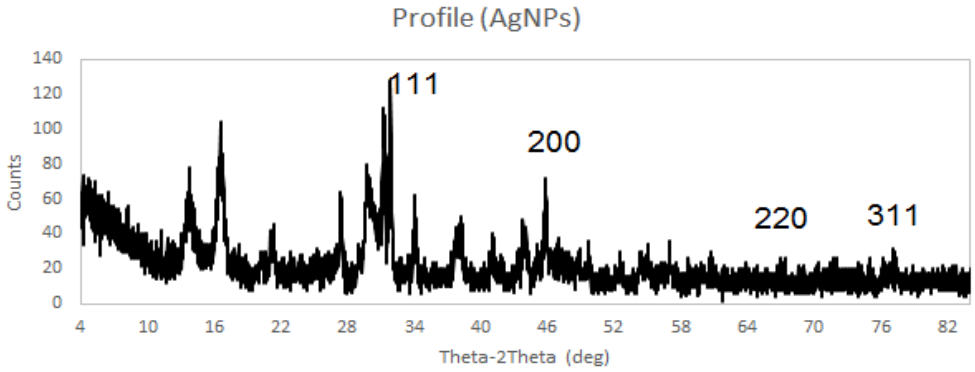

Fig. 4. XRD pattern of AgNPs synthesized by *Aspergillus* sp.

well as some other cellular proteins and enzymes essential to ATP production becomes inactivated. Awal et al., reported that, the mechanism of inhibition of AgNPs on microorganisms is not well known but AgNPs binds with cytoplasmic membrane and killed the bacterial cell [41]. This is because the electrostatic interaction between positively charged AgNPs and negatively charged cell membrane of microorganisms. Other explanation for the antimicrobial activity of AgNPs was AgNPs react with thiol (-SH) groups of vital enzymes / proteins and inactivates them [42] and causing disruption of bacterial membranes [43].

3.3 Minimum Inhibitory Concentration (MIC) for AgNPs Biosynthesized

When concentration of AgNPs decreased, the diameter of inhibition zone also was decreased. The results indicated that antibacterial effect was dose dependent so that it must be determine the MIC to AgNPs for each bacterial strains. To achieve this goal, different concentrations of AgNPs were be used (50, 25, 12.5, 6.25 and 3.125 µg/mL). According to data represented in Table 1, the MIC for *Bacillus subtilis* and *Staphylococcus aureus* were 6.25 and 12.5 µg/mL with clear zone 9.9 and 10.4 respectively. While MIC for gram negative bacteria was

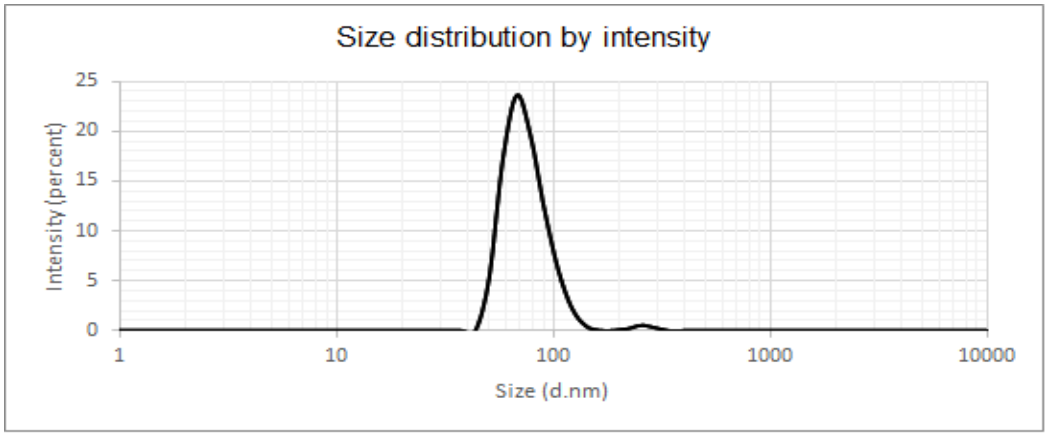

Fig. 5. Particle size distribution for AgNPs synthesized by *Aspergillus* sp.

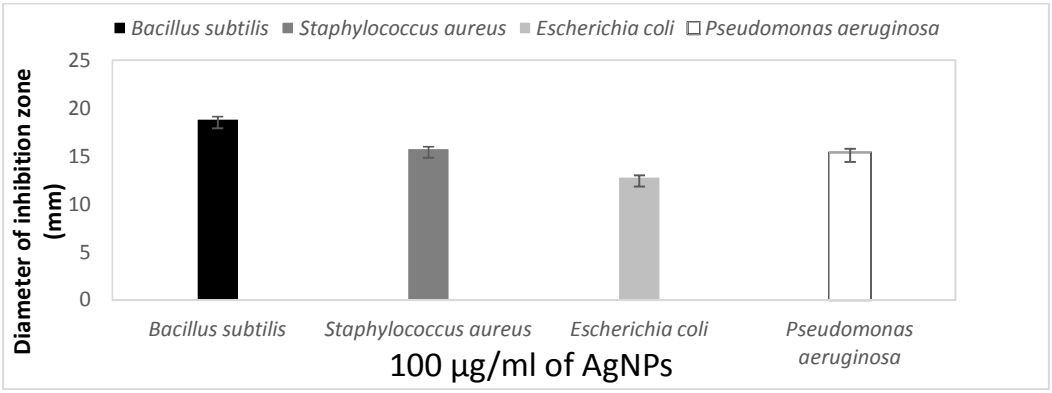

Fig. 6. Antibacterial activity of biosynthesized AgNPs (100 µg/mL) by *Aspergillus* sp. against different coded test organisms

Table 1. Determination of MIC (µg/ mL) with inhibition zone (mm) for nano-silver against coded test organisms

Coded test organisms	MIC (µg/mL)	Inhibition zone (mm)
Bacillus subtilis	6.25	9.9 ± 0.2
Staphylococcus aureus	12.5	10.4 ± 0.3
Escherichia coli	25	9.7 ± 0.16
Pseudomonas aeruginosa	25	10.8 ± 0.16

25 µg/mL with clear zone 9.7 and 10.8 for *Escherichia coli* and *Pseudomonas aeruginosa* respectively. Buszewski, et al., reported that, bacteriostatic activity of bio (AgNPs) toward *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus* was (6.25 µg/ml), while, *Pseudomonas aeruginosa* was 25 µg/mL [44].

3.4 MTT Assay

The *in-vitro* cytotoxic effects of biosynthesized AgNPs were screened against human colorectal adenocarcinoma cells (Caco-2) as cancer cell and kidney of African green monkey (normal Vero cells) and the percentage of cell inhibition was confirmed by MTT assay. At the end of incubation period, the AgNPs at concentration 0.48 µg/mL shown 100% viability, 1.95 µg/mL shown 89.6% viability, 3.9 µg/mL shown 49.6% viability with toxicity percent 0.0%, 10.3%, 53.3% and 92.3% respectively for Caco-2 cell (Table 2). While normal Vero cell showed 100% viability at 15.62 µg/mL and viability decreased by increasing AgNPs concentration (Table 2). The AgNPs at concentration 3.75 µg/mL and 280 µg/mL decreased the viability of Caco-2 cell and Vero cell to 50% of the initial level respectively, so that, these values were chosen as IC_{50}.

Further the microscopic observation of Caco-2 cells and Vero cell treated with IC_{50} concentration AgNPs revealed unhealthy cells with lost their typical shape morphology, partial or complete loss of monolayer, rounding, shrinking or cell granulation when compared to untreated control cells showed a well-organized cytoskeleton (Fig. 7). Other reported found that, the 10 µg/mL of AgNPs reducing the activity of mitochondrial for colon carcinoma cells to 53% and smaller particles easily penetrated the cells [14]. Previous study showed the different concentration 10, 25, 50, 100 and 200 µg/mL of AgNPs on Caco-2 cell and found relative cell activity is 79.17%, 63.44%, 66.41%, 68.69%, and63.25% respectively [45].

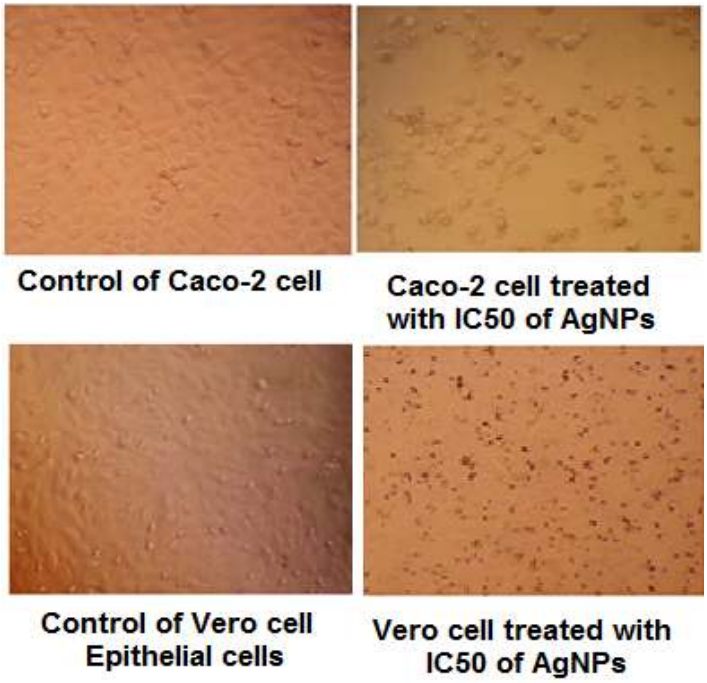

Fig. 7. Morphological observation of AgNPs for untreated Caco-2 cells, Caco-2 cells treated with IC_{50} concentration of AgNPs produced from *Aspergillus* sp., (with lost their typical shape morphology, partial or complete loss of monolayer, rounding, shrinking or cell granulation) and untreated Vero cell lines, Vero cell lines treated with IC_{50} concentration of AgNPs produced from *Aspergillus* sp., (with altered morphology and complete loss of shape cells)

Table 2. Viability and toxicity percent for Caco-2 cell and Vero cell treated with different concentration of AgNPs

AgNPs concentration	Caco-2 cell		Vero cell	
(µg/ml)	Viability %	Toxicity %	Viability %	Toxicity %
1000	7.65	92.34	39.49	60.50
500	7.40	92.59	46.70	53.29
250	8.39	91.60	55.41	44.58
125	7.65	92.34	69.42	30.57
62.5	8.39	91.60	82.16	17.83
31.25	8.39	91.60	93.63	6.36
15.62	10.37	89.62	100.36	0.00
7.81	19.50	80.49	0.00	0.00
3.9	46.66	53.33	0.00	0.00
1.95	89.62	10.37	0.00	0.00
0.97	93.82	6.17	0.00	0.00
0.48	100.67	0.0	0.00	0.00

4. CONCLUSION

In the present study, *Aspergillus* sp*.* has shown high potential for extracellular biosynthesis of silver nanoparticles, as confirmed by UV-Vis spectroscopy which showed a maximum surface plasmon resonance peaks at 400 nm. TEM image showed spherical shape with diameter range between 5-30 nm. The AgNPs showed greater antimicrobial activity against common human pathogenic bacteria such as*, Bacillus subtilis* NCTC 10400*, Staphylococcus aureus* ATCC 29213*, Pseudomonas aeruginosa* ATCC 9027 and *Escherichia coli* ATCC 8739*.* The present study showed significant cytotoxic effects for biologically synthesized silver nanoparticles against Caco-2 cancer cells which inhibit the half-cell viability at low concentration ~3.75 µg/ml with loss shape of cell morphology, but at low concentration the normal Vero cell not effected. The toxicity of silver nanoparticles against cancer and normal cells was depended on particle size and dose of AgNPs.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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