EVALUATION BIOSYNTHESIZED SILVER NANOPARTICLES BY PHOMATROPICA AGAINST SOME MULTIDRUG RESISTANCE BACTERIAL ISOLATES

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ABSTRACT

Researchers describe the extracellular manufacture of silver nanoparticles (AgNPs) from Phomatropica and its effectiveness against certain multidrug-resistant pathogenic bacteria that were obtained from the Central Laboratory of Quran Hospital. These bacteria were pathogenic.

The AgNPs were synthesized and characterized by scan electrons microscopy, Fourier transform infrared spectroscopy, UV-visiblespectrophotometer that established the mostly spherical nanoparticles synthesis with size range between 55-99 nm. The potential antimicrobial activity was reported vs (Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia, and Escherichia coli)by well diffusion method.AgNPs showed different inhibitory areas at different concentrations, the 50µg/mlconcentration of AgNPsappeared inhibition zones varied from(0-21 mm), while at 100 µg/mlofAgNPs varied between (13-25mm) vs the tested pathogenic bacterial strains in this investigation. Nevertheless, the synergetic impact of AgNPs with antibiotics have beendetected in the increasing the inhibitory impact vs the pathogenic bacteria.

In conclusion, Extracellular biosynthesis appears to be a scalable and sustainable process. Because of their biogenic nature, these Ag-NPs might be a better medication candidate and have the potential to completely eliminate the issue of chemical agents.Antibiotic-resistant bacteria are proliferating at an alarming rate. To address this issue, the development of bactericidal agents is critical. AgNPs may provide a solution for drug-resistant bacteria.

KEYWORDS

Phomatropica; Extracellular; AgNPs ; Biosynthesized ;Multidrug resistant MDR

PAPER INDEX

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1. INTRODUCTION

Nanotechnology, which deals with particles on the microscopic scale ranging in size from 1 to 100 nanometers, is one of the most active study disciplines in current material research (Saif et al., 2016). AgNPs stand out among the many forms of metallic nanoparticles due to the broad-spectrum antibacterial effects they might produce (Prabhu and Poulose, 2012; Rai et al., 2014; Gupta et al., 2017; Loo et al., 2018). These nanoparticles are able to attach themselves to the membranes of bacteria as well as the cell walls, and they might even penetrate the cells themselves. They cause disruptions in the pathways that are used for signal transduction, they cause damage to cellular structures, and they create reactive oxygen species (Kim et al.,2011; Dakal et al., 2016) In the sectors of health and agriculture, AgNPs were utilized to suppress hazardous microbes (Kim et al., 2012; Mishra and Singh, 2015; Burdusel et al., 2018).

The vast majority of the currently available methods for producing nanoparticles have downsides, including the usage of potentially harmful chemicals and the production of waste that is detrimental to the environment (Iravani et al., 2014; Ahmed et al., 2016). Because of this, there was a significant increase in interest in methods of synthesis that are less harmful to the environment in the most recent few years. Microorganisms that are capable of degrading metal salts and producing nanoparticles of the required size and shape are used in the methods. These microbes include bacteria, fungus, and plants (Azmath et al., 2016)

An alternative that is safe, non-toxic, and beneficial to the environment is the biological reduction of metals, which results in the production of nanoparticles (Banu and Balasubramanian, 2014). Since they possess a high tolerance for metallic and are simple to handle, fungi are promising agents for the biogenic production of AgNPs (AgNPs). They also create a significant number of extracellular proteins, which contribute to the nanoparticles' already impressive level of stability (Balaji et al., 2009; Du et al., 2015; Netala et al., 2016). In comparison to bacterial cultures, fungal cultures provide a number of benefits, including a higher rate of biomass production and the elimination of the need for additional steps to obtain the filtrate (Gade et al., 2008).

2. MATERIALS AND METHODS

2.1. MATERIALS

Potato dextrose and potato dextrose agar (PDA) have been achieved from (Himedia, India) the antibiotics (Chloramphenicol30mg) was obtained from (Himedia, India), (Ampicillin 10mg) was obtained from (Rosteo, Italy). and silver nitrate (AgNO3) have beenbought fromSigma-Aldrich (Germany) and lactophenol cotton blue from Merke (India).

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2.2. ISOLATION AND IDENTIFICATION OF FUNGUS

Phomatropica was isolated from soil sample collected different location in Basrah (Southern Iraq) during the year 2022. Soil specimens was taken from approximately (2-5) cm depth.To isolate soil fungus, the serial dilution method was used. To obtain concentrations between one and four, a one-gram soil specimens has been consecutively diluted in sterilized purified water (10-1 to 10-4). Each dilution was transferred aseptically in 0.1 ml increments onto PDA plates. To distribute the sample evenly, a sterilized glass spreader was used. At pH 6.0 and 28 °C, the plates were cultured for 5-6 days. The fungal isolates are sub-cultured on (PDA) plates to produce pure culture. Pure-isolates are kept in a refrigerator at 4 ºC for future research. The isolated fungus was identified using visual characteristics, microscopic structure, and molecular identification.

2.3. COLONY CHARACTERIZATION

2.3.1. MORPHOLOGICAL AND MICROSCOPIC VIEW OF FUNGUS

Phomatropica which is used in the biosynthesis of AgNPs, was isolated from soil and kept alive by maintaining it on PDA medium at 28 degree centigrade and 4 degree centigrade. The colony morphology and micro morphology of the fungus, including the(color, shape, texture of the mycelia, spore formation pattern, etc), were used to identify it. The fungus was also cultured on a PDA medium at 28 degree centigrade for 10 days to analyze its colony morphology. Slide culture was used to investigate the fungus's micro morphological traits. Cultures were grown on PDA slides and cultured there for five days at 28 degree. The slides were then dyed with lactophenol cotton blue and investigated under a microscope light (Dongyanget al,2021). The taxonomic description led to the identification of the isolated fungus (R. Schneid. & Boerema,1975 ;Vaibhar, 2012).

2.3.2. MOLECULAR IDENTIFICATION

2.3.2.1.DNA EXTRACTION

The extraction of DNA from fungi was performed using the method descrybed by (Alshehri and Palanisamy,2020) and following the protocol instructions included in the kit (Presto™ Mini gDNA Yeast Kit/ Genaid/ USA).

2.3.2.2.POLYMERASE CHAIN REACTION (PCR) USING UNIVERSAL PRIMERS

https://doi.org/10.17993/3ctecno.2023.v12n1e43.296-319

The fungus's internal transcribed spacer region was amplified using PCR. (Schochet al, 2012).the ITS region of 5.8S rDNA genewas amplified by PCR reaction, using universal forward and reverse

Primer ITS1-F :5'-TCCGTAGGTGAACCTGCGG-3'

and

Primer ITS4- R :5'- TCCTCCGCTTATTGATATGC-3'

(Raja et al, 2017).

2.3.2.3.NCBI BLAST

Basic Local Alignment search tool (BLAST) and National Center for Biotechnology Information (NCBI) both provided insurance for Phomatropica.

2.4. PREPARATION OF BIOMASS

For the manufacture of AgNPs, the Phomatropica was the organism of choice. 250 gm of potato and 20 gm of dextrose are to be added for every liter of purified water. To eliminate the medium component from the mycelia biomass, the mycelia were filtered out of the culture broth using Whatman filter paper No. 1 and washed three times in sterile Milli-Q deionized water. This process was done to remove the medium component. Ten days were spent incubating Erlenmeyer flasks on a rotary shaker at a temp of 25 degree centigrade and 120 revolutions per minute. At a temp of 25 degree centigrade, a 250 ml Erlenmeyer flask was agitated continuously for three days using the same method while it contained 10 g of biomass (wet weight) and 100 ml of deionized water. After incubation, the fungal cell filtrate (FCF) was collected by filtering the solution using Whatman filter paper and a Millipore filter with a pore size of 0.45 microns. Filtrate has beenclam and used in order to bring about the desired results of producing AgNPs.

2.5. FUNGAL MEDIATED SYNTHESIS OF AGNPS

For the creation of AgNPs, 100 ml of fungal cell filtrate (FCF) was mixed with 0.017 gm of AgNO3 (Sigma-Aldrich 99.9%, Germany) to achieve a final amount of 1 mM, and the mixture was then left to sit at 28°C in the dark for 72 hours. As a control, flaks with FCF but no AgNO3 were employed. The creation of AgNPswas verified by the color change response after a 72-hour incubation time in dark conditions. The colorless cell filtrate solution with AgNO3 solution turned into a brown color solution. An evaluation of the supernatant using a UV-visible spectrophotometer provided qualitative evidence of the decrease of Ag+.Using a UV-visible spectrophotometer, the absorbance of the sample supernatant was determined between 300 and 900 nm. The reaction mix was then centrifuged three times for 20 minutes at 6000 rpm with distilled water to concentrate the AgNPs. The pellet that was left behind was then dried in a hot air furnace at 40 degree centigrade. After drying, the sample was placed

https://doi.org/10.17993/3ctecno.2023.v12n1e43.296-319

in a glass vial together with nanoparticles that had been collected by scratching with a sterile spatula. AgNPs were then gathered for additional characterization.

2.6. SILVER NANOPARTICLE DETECTION AND CHARACTERIZATION

By transforming from colorless to light brown, the biosynthesized (AgNPs) in the fungal free-cell filtrate were visually evaluated. They were further validated by a UV-Vis spectrophotometer, scan electrons microscope (SEM), and Fourier transforms infrared spectroscopy (FT-IR).

2.6.1. SONICATION

After it has been cleansed and centrifuged, researchers employ it in our method, which involves using sound energy to agitate particle or discontinuity fibers that are suspended in liquid. In most cases, frequencies higher than 20 kilohertz (kHz) are used. Ultrasonication may be carried out with the assistance of either an ultrasonic bath or an ultrasonic probe (sonicator). For our experiment, we used an ultrasonic bath made by Binder in Germany (Deborah and Chung, 2017)

2.6.2. ASSAY USING UV-VISIBLE SPECTROPHOTOMETRY

The UV-Vis spectrophotometer (CECIL (CE,7200, England)) was used to measure the bio-decrease of Ag+ in aqueous solution after the fungal free-cell filtrate treated with AgNO3 had been incubated for 72 hours. 0.1 ml of the filtrate was obtained during the reduction process, diluted with deionized water, and then placed in a quartz UV-VIS cuvette. 300 to 900 nm was the range of the scanning. Free-cell filtrate that hadn't been altered served as a control. This was handled at the University of Basrah's Polymer Research Center.

2.6.3. FTIR (FOURIER TRANSFORM INFRARED SPECTROSCOPY) ANALYSIS

The free-cell filtrate underwent Fourier Transform Infrared (FTIR) (Bruker Tensor 27, Germany) examination after 72 hours of incubation. To identify the functional groups of the stabilizing and biomolecules capping the AgNPs, FT-IR measurements were conducted. After centrifuging the specimen solution having the nanoparticles at 5,000 rpm for 1200 second, it was filtered. The produced solid material was subsequently crushed with potassium bromide (KBr), and pellets were created. The pellet was examined using FTIR. This was handled at the University of Basrah's Polymer Research Center.

https://doi.org/10.17993/3ctecno.2023.v12n1e43.296-319

2.6.4. SCAN ELECTRONS MICROSCOPE (SEM)

This electron microscopy unit at Iran's University of Tehran employed a scan electrons microscope (SEM) (TESCAN MIRA3, French) to describe the size and shape of AgNPs(caroling et al., 2013).

2.7. ANTIBACTERIALACTIVITY OF AGNPS

Using the agar well diffusion assay method, the potential of AgNPs was evaluated for their antibacterial effectiveness (Perez et al., 1990). We evaluated four types of multidrug resistant pathogenic bacteria, including Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus and Pseudomonas aeruginosa. Each overnight-grown bacterial culture was streaked with swabs before being placed on sterile Muller-Hinton agar (MHA) plates. Utilizing a sterilized stainless steel Cork borer, wells in agar plates with a diameter of 5 mm were created (local,Iraq). Two concentrations of silver nanoparticle solutions (50 and 100 g/ml) have beenapplied to the wells. After incubation for 1 day at 37 degree centigrade, the wells have beeninvestigated for the existence of inhibitory zones, and the diameters of these placeshave been determined.

2.8. ASSAY FOR DETERMINING SYNERGISTIC EFFECTS.

To assess the bactericidal effectiveness of these nanoparticles both alone and in conjunction with antibiotics, the disk diffusion technique was utilized to measure the synergistic effect of extracellularly generated AgNPs with routinely utilized antibiotics (Ampicillin, Chloramphenicol) (Devi and Joshi, 2012). conventional antibiotic disks (Ampicillin, Chloramphenicol). Standard antibiotic discs (6 mm in diameter) were placed over the MHA medium that had been injected with test organisms after being impregnated with 20 l of freshly made AgNPs. Positive controls were standard antibiotic disks. Filtrate devoid of fungi served as the adverse control. For 24 to 48 hours, these plates were incubated at 37 degree centigrade. The inhibition places of the control and treatment plates have been assessed after incubation. The assays were all carried out in triplicate.

2.9. EVALUATION OF THE INCREASE IN FOLD AREA

The improvement in fold area was determined by comparing the mean contact area of the inhibitory place that was formed by an antibiotic by itself and by an antibiotic in combination with AgNPs. The fold increase area was determined utilizing the formula (B2- A2) / A2, that A represents the inhibition place diameter generated by the activity of antibiotics on their own, and B represents the inhibition place diameter induced by the activity of antibiotics in combination with AgNPs (Birla et al., 2009).

https://doi.org/10.17993/3ctecno.2023.v12n1e43.296-319

2.10.STATISTICAL ANALYSIS

We utilized the Statistical Package for the Social Sciences (SPSS), version 2020 (Copyright IBM Inc., USA). The least substantial difference (LSD) test was used in the statistical data analysis that was performed utilizing Guide. This test was used to compare the substantial differences that existed between the averages with a probability threshold of p less than 0.01.

3. RESULTS

3.1. FUNGAL IDENTIFICATION

The discipline of biological science is seeing fast advancements in the use of nanotechnology. In the course of this research, Phomatropica cell filtrate was used to effectively produce AgNPs. The fungus was extracted from the soil and cultivated at a temperature of 28 degrees Celsius on PDA medium. The fungus has been recognized as Phomatium due to the features of its colony morphology (the colonies appeared white, and with regular shape, mycelium pale white color (fig 1a), in the single colony there was a large number of conidia deposit in the middle of the colony) (Fig. 1b) and its micromorphology (Pycnidia, conidia subglobose, flaskshaped with conspicuous dark circumval.

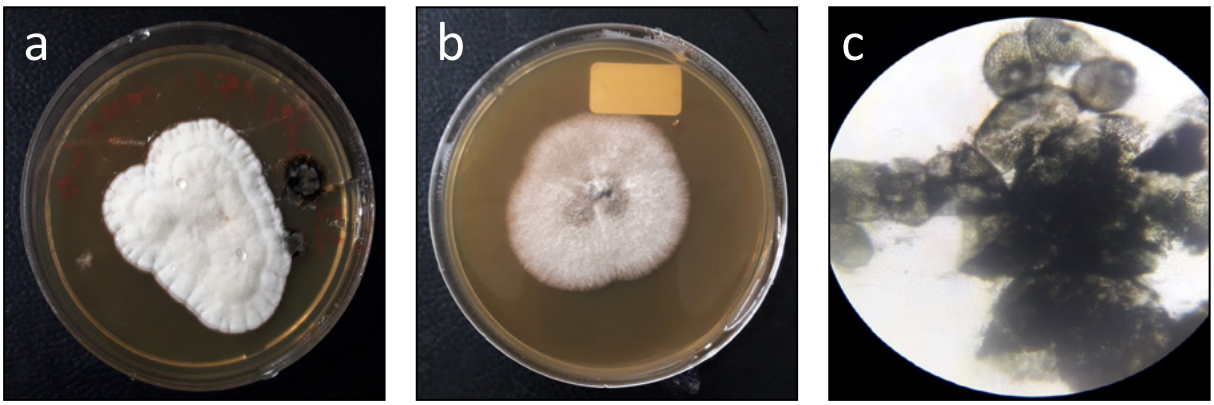

Figure 1. Morphology of Phomatropica

(a,b) Macroscopic morphology (7d and 28 degree centigrade); (c) microscopic morphology (40 x).

3.2. GENETIC IDENTIFICATION OF PHOMATROPICA

3.2.1. GENOMIC DNA EXTRACTION

The technique of electrophoresis for DNA extraction under UV transilluminator showed clear isolated DNA of Phomatropica (Figure2).

https://doi.org/10.17993/3ctecno.2023.v12n1e43.296-319

Figure 2. 0.8% of agarose gel Electrophoresis showed total DNA band of Phomatropica.

3.2.2. PCR AMPLIFICATION

The results of the molecular diagnosis of the isolate after electrolysis on agarose gel(1%) showed that the results of the DNA chain reaction using ITS1-ITS4 interfacial primers showed the existence of a

clear bundle resulting from the process of duplication of these genes, and the binding of the primer to its complement sequence in the DNA template ~550 bp (Figure 3).

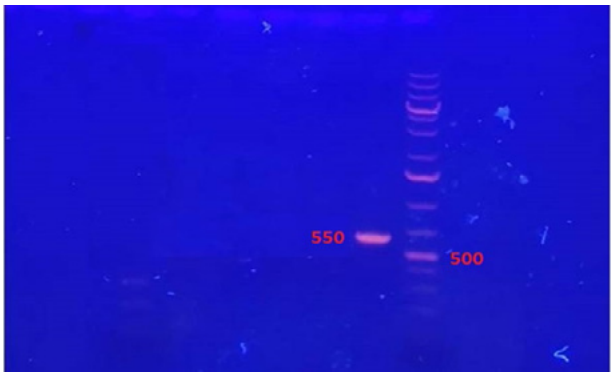

Figure 3. The electrophoresis of PCR.

3.2.3. SEQUENCING OF ITS GENE

The ITS nucleotidesequence is 100% homology toPhoma tropica (accession number JF 923821.1) as registered in the GenBankdatabase. Based onmolecular and morphological features, the fungal has been determined asPhomatropica.

3.3. SILVER NANOPARTICLE (AGNP) BIOSYNTHESIS

After 24 hours of incubation in the dark condition, P.tropica isolate's extracellular AgNPsbiosynthesis was visually detected to modify the color of the culture

https://doi.org/10.17993/3ctecno.2023.v12n1e43.296-319

supernatant in comparison to the control. Figures 4 and 5 displayed AgNPs harvest derived from isolation, which ranged in hue from colorless to brown.

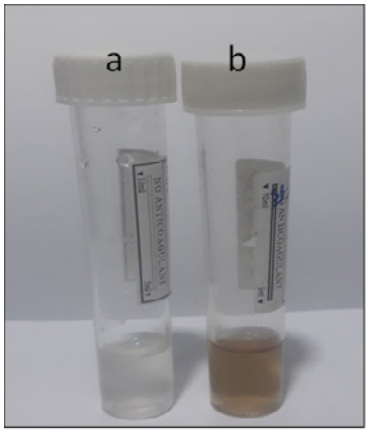

Figure 4. Color change observed in fungal cell filtrate (FCF) of P.tropica after exposure to AgNO3, a- without 1mM AgNO3 b- after 24h treated with AgNO3

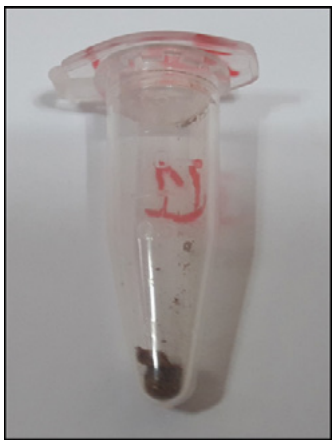

Figure 5. Nanoparticles synthesized byP. tropica

3.4. CHARACTERIZATION OFBIOSYNTHESISAGNPS

3.4.1. SONICATION OF AGNPS SOLUTION

Figure 6 shows how 0.001g of AgNPs were blended with 5 ml of purified water and sonicated for 15-20 minutes using an ultrasonic bath.

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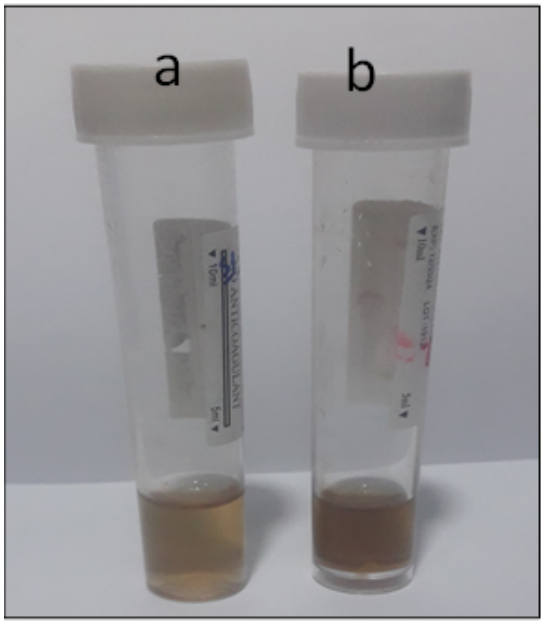

Figure 6. the AgNPs solution, the right(a) one before sonication and the left (b) one after sonication

3.4.2. UV-VISIBLE SPECTROPHOTOMETRY ANALYSIS

By employing a UV-visible spectrophotometer to conduct qualitative testing on the supernatant, the reduction of silver ions was verified. After 24 hours, 1 ml of the sample supernatant was removed, and the absorbance has been determined between 300 and 900 nm utilizing a UV-visible spectrophotometer (fig.7). At 423 nm, the absorbance peak was noted.

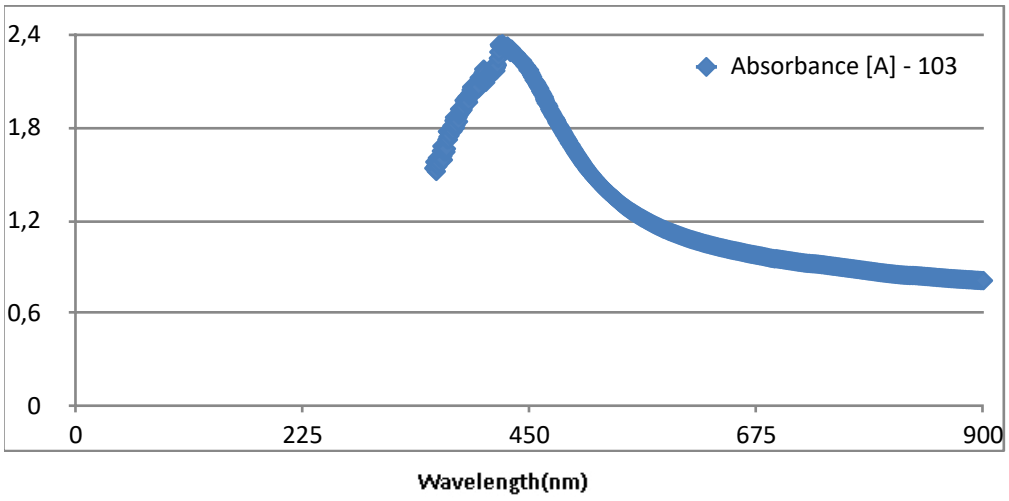

Figure 7. UV-Visible spectra of produced AgNPs by fungi.

3.4.3. SCAN ELECTRONS MICROSCOPE (SEM)

Images collected utilizing SEM with a magnification of 200Kx indicated that the AgNps have been collected and generally spherical in form, with diameters ranging from 55 to 99 nm.

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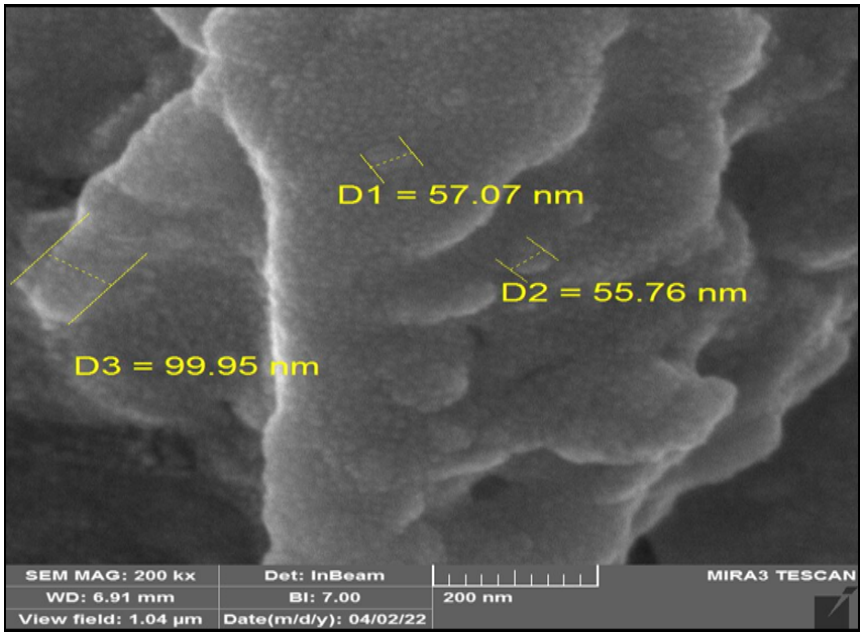

Figure 8. The biosynthesized AgNPs in the fungal free-cell filtrate were depicted in a SEM micrograph as spherical shapes aggregated with size ranges from 55 to 99 nm (magnification 200 K X).

3.4.4. FTIR ANALYSIS

It has been proven that FT-IR experiments have the potential to detect putative biomolecules essential in the bio removal of silver ions and the stability of AgNPs. The FTIR spectrum study indicates that the supernatant of Phomatropica includes biomolecules, which are responsible for the conversion of silver ions into AgNPs (Figure 9). The results of this research also demonstrated the existence of eight distinct stretch bands, and their values are as follows: 3358.43, 2925.48, 2845.13, 1745.26, 1645.62, 1539.88, 1455.99, and 1078.01. (cm-1).

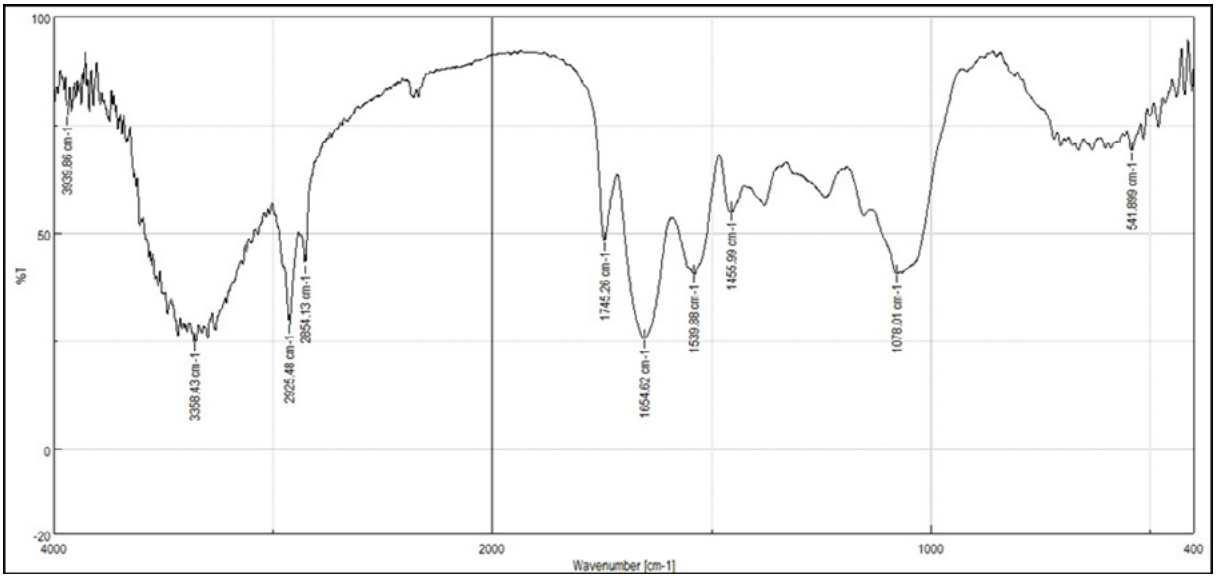

Figure 9. FTIR spectrum of AgNPs biosynthesized by P. tropica with distinct peaks.

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3.5. AGNPS HAVE ANTIBACTERIAL ACTIVITIES

The studied strains of Gram negative and Gram positive bacteria were resistant to the biosynthesized AgNPs' antibacterial action. According to the findings, bacterial growth was slightly less inhibited by AgNPs at a 50 g/ml concentration (0-21 mm inhibition zones) than it was by a 100 g/ml concentration (13-25 mm inhibition zones) (Fig.10). AgNPs had the lowest growth inhibitory activity vsK. pneumonia and the highest vsP. aeruginosa. Without AgNPs, no inhibitory zones could be seen in the fungal free cell filtrate (FCF). Similar findings were reported using AgNPs produced by Papulaspora pallidula by Tawfik and Ahmad (2015).

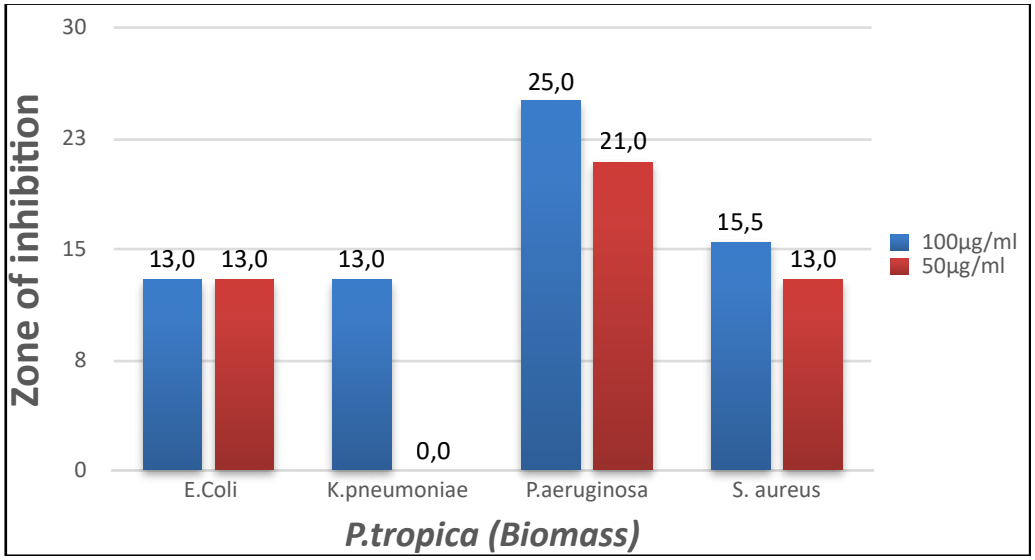

Figure 10. The growth inhibition zones that four strains of human pathogenic bacteria displayed in response to two nanoparticles of silver doses (AgNPs) produced by the fungus P. tropica.

3.6. COMBINATION EFFICACY OF AGNPS WITH AMPICILLINAND CHLORAMPHENICOL

This particular research utilizing the disk diffusion technique, we tested the effectiveness of these AgNPs in conjunction with antibiotics at concentrations of 50 and 100 g/ml versus gram-negative and gram-positive bacteria. The width of the inhibitory zone, measured in millimeters, surrounding antibiotic disks with and without resistance vs test bacteria is illustrated in Fig. 11 and 12. In all of the instances, the inhibition places diameter for antibiotics alone and in conjunction with AgNPs demonstrated a substantial increase in fold area. This was the case with ampicillin and chloramphenicol at concentrations of 50 and 100 µg/ml of AgNPs (Table 1). The synergistic activity of AgNPs at 50 µg/ml concentration with antibiotics were found to be higher vsE. Coli and P. aeruginosa as compared to S.aureusand. K. pneumonia The synergistic activity of AgNPs at 100µg/ml concentration with antibiotics have been detected to be greatervsP. aeruginosa and as E. coli compared to K. pneumonia andS. aureus.

https://doi.org/10.17993/3ctecno.2023.v12n1e43.296-319

Table 1. Mean inhibition place (mm) brought about by various antibiotics with/withoutAgNPs created utilizing the fungus p tropicavs the test organisms

Inhibition Zone (mm)												
Bacterial strains	AgNPs 50µg/ml						AgNPs100µg/ml					
	Ampicillin	Am + AgNPs	Increase in fold area	chloramphenicol	C+ AgNPs	Increase in fold area	Ampicillin	Am + AgNPs	Increase in fold area	chloramphenicol	C+ AgNPs	Increase in fold area
E. coli	-	12	3	24	25	0.085	-	12	3	24.5	25.5	0.083
K. pneumonia	-	7.5	0.563	22.5	22.5	0	-	8.5	1.007	23	24.5	0.134
P. aeruginosa	-	10.5	2.063	16	20.5	0.641	-	13.5	4.063	18	25	0.929
S.aureus	15.5	18	0.349	24.5	26	0.126	15.5	18.5	0.425	23	26.5	0.327

The increase in fold area of places of inhibition has been determined by comparing the inhibition place created by antibiotic only with the places of inhibition obtained for antibiotics paired with AgNPs manufactured utilizing the fungal isolate.

* The values represent the averages of three replicates.

differences that are significant at P 0.01.**

-The diameter of the disc, which was measured to be 6 millimeters, was utilized in the lack of the growth of bacteria inhibition places in order to compute the fold increase in columns 1, and 7.

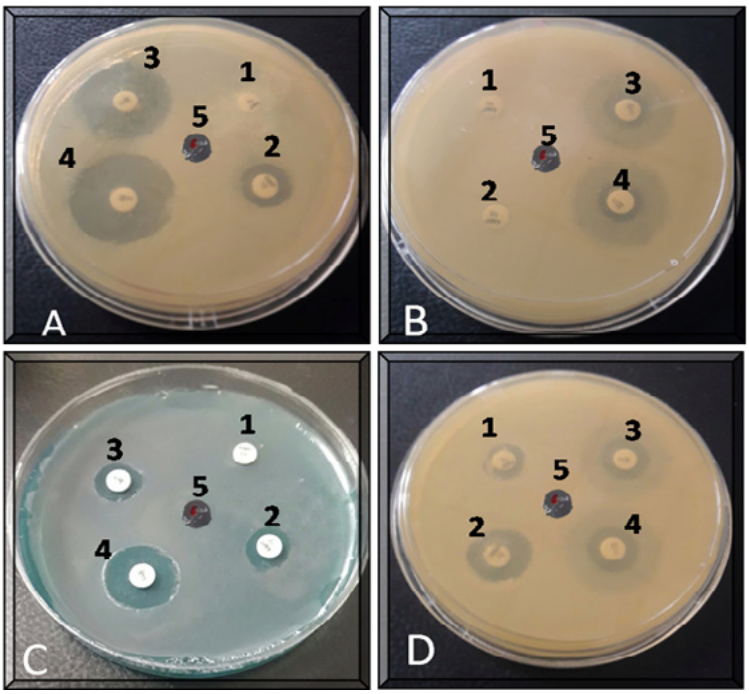

Figure 11. Antibacterial activities of biosynthesized nanoparticles of silver at amount (50 g/ml) vsE. coli (A), K. pneumonia (B), P. aeruginosa (C), S. aureus (D) commercial antibiotic Ampicillin (1) and a combination of AgNPs with Ampicillin (2) commercial antibiotic chloramphenicol (3) and a combination of AgNPs with chloramphenicol (4) Fungal cell-free filtrate (5)

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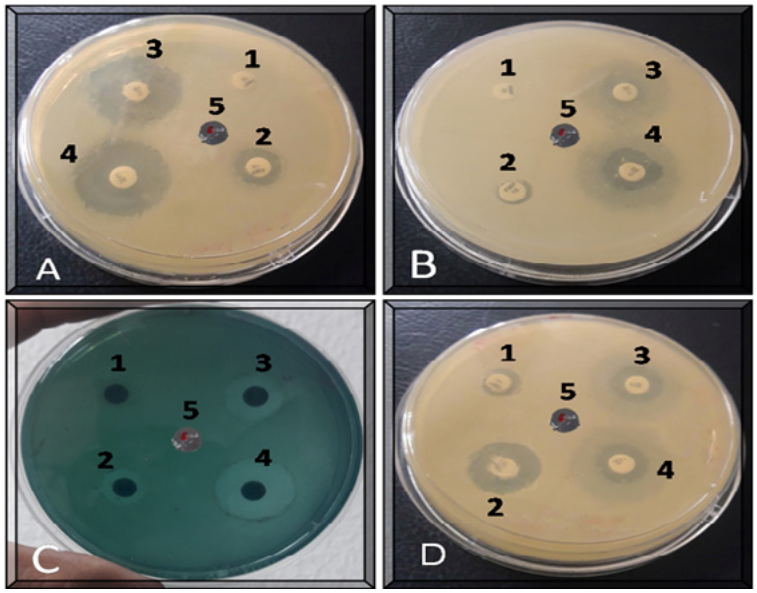

Figure 12. The inhibition places (mm) displayed by biosynthesized nanoparticles of silver at concentrations (100g/ml) vsE. coli (A), Klebsiella pneumonia (B), P. aeruginosa (C), and S. aureus (D) commercial antibiotic Ampicillin (1) and an AgNPs/Ampicillin combination (2) commercial antibiotic chloramphenicol (3) and an AgNPs/Chloramphenicol combination (4) Fungal cell-free filtrate (5).

4. DISCUSSION

4.1. ISOLATION AND IDENTIFICATION OFPHOMATROPICAFROM SOIL

Phomatropica has been chosen for the production of nanoparticles because it is simple to extract from soil, it is straightforward to cultivate on straightforward media such as PDA, and most importantly, it has consistent biochemical properties (Rai et al, 2009). In addition, there is no research done so far on selecting Phomatropica for the production of AgNPs. This is something that has to be done.

(Boerema et al., 2004) conducted research on the Phoma species based on the physical and cultural aspects of each species. The recognition of Phoma depending only on morphological characteristics was relatively inconsistent, which contributed to confusion over its identity. Because of this, molecular-based approaches were used, which turned out to be a superior choice for the detection and investigation of genetic differences amongst fungi (deGruyter et al., 2009; Aveskamp et al., 2010).

Several of the most frequent options for phylogenetic inference at the genus level or lower is bi-parental, nuclear ITS regions. This is because these areas have a greater rate of base replacement than the genes found in most organelles. The phylogenetic connections between Phoma and the groups to which it is closely related were examined in great detail utilizing ITS sequence data (Iryini et al,2009 :Aveskamp et al, 2009).

https://doi.org/10.17993/3ctecno.2023.v12n1e43.296-319

4.2. BIOSYNTHESIS AND CHARACTERIZATIONOF AGNPS

Since dangerous bacteria have been showing signs of antibiotic resistance during the past ten years, researchers are concentrating on creating new antibacterial substances. Ag-NPs as antibacterial agents have emerged as viable candidates in the current medical landscape (Duran et al. 2007). AgNPs can be produced by microorganisms like fungus, which has significant promise for numerous applications (Alghuthaymiet al., 2015). Due to their capacity to create AgNPs, many Phoma species were shown to be able to synthesis nanoparticles of silver (Aniketet al, 2013 ; Sudhiret al,2016 ; Aniketet al, 2011).This is the first account of the environmentally friendly extracellular production of AgNPs by P. tropica. As evidenced by the color shift from colorless to brownish after 3 days of incubation after being exposed to a solution of 1 mM AgNO3, the current investigation demonstrated that the chosen fungus, P. tropica, displayed a great potential for the AgNPs synthesis in culture medium. These results are consistent with earlier research utilizing several fungi species (Asemet al. 2017;Tejal Barkhade.. 2018 ;Bahimbaet al., 2011: Mohamed et al, 2021).

The addition of AgNO3 to the fungal free-cell filtrate caused a color shift as a result of the excitation of silver's surface plasmon resonance vibration that verified the decrease of silver ions as reported by (Chitra and Annadurai,2013).

4.3. UV-VISIBLE SPECTROPHOTOMETRY ANALYSIS

The current research demonstrated that UV-Vis spectrophotometry examination revealed a maximum with great absorbance at 423 nm, which stated that the investigated fungus had been replicating AgNPs, indicating that the production of AgNPs has been complete after 3 days of incubation with free-cell filtrate. This is in line with a few other pieces of art that have been done (Mohamed et al 2021; Aniket et al, 2013).

When compared to previous studies, it appears that there have been some variances in the features of the AgNPs generated by distinct species of fungus. These variations have been seen in the AgNPs (Birla et al. 2009; Chitra and Annadurai, 2013; Maliszewska et al., 2009; Raheman et al., 2011). These variations might be attributable to the origin of the fungal isolates or strains as well as the environment under which they grew (Marambio-Jones and Hoek, 2012). Based on the findings of Neethu et al. (2018), the amount of fungal mycelium was directly correlated to the amount of AgNP that was synthesized.

4.4. BIOSYNTHESIZED DESCRIPTION AGNPS BY SCAN ELECTRONS MICROSCOPY (SEM)

Different reaction parameters affect the form and size of biosynthesized nanoparticles of silver in solution.

The AgNPsbiosynthesized morphology by the chosen fungus was scattered with size of 55-90 nm, as shown by SEM ostly spherical and pictures.

https://doi.org/10.17993/3ctecno.2023.v12n1e43.296-319

Numerous studies have noted that different fungus species, pH levels, and temperatures affect the AgNPs shape and size that are generated (Martinez-Castanonet al., 2008; Marambio-Jones and Hoek, 2010; Muhsin and Hachim, 2015 ;Aniketet al, 2013).

4.5. FT-IR ANALYSISA OF THE BIOSYNTHESIZED AGNPS

P. tropica has been shown to contain biomolecules that turn silver ions into AgNPs. These biomolecules occur in 8 different stretching bands, which are: 3358.43, 2925.48, 2845.13, 1745.26, 1645.62, 1539.88, 1455.99, 1078.01, and others (cm-1).

The distinctive hydrogen-linked OH set that could be the result of the formation of nanoparticles in an aqueous phase, is connected to the bending vibrations of the OH alcohol bonds, phenols, and the N-H stretching vibration of main protein amides, all of which make a contribution to the peak at 3358.43cm-1. These vibrations are responsible for the existence of the peak.

The C-H stretching associated with the methylene protein set and the N-H stretching associated with the amine salt are both candidates for the causes of the maxima at 2925.48 and 2845.13 cm-1, respectively. This finding is undeniably linked to the modification of the electric surroundings of the methylene and methane sets that was brought about by the close proximity of the AgNPs and carbonyl. The stretching vibrations of C=O was the source of the experimental group that could be seen at 1745.26 cm-1. In the FTIR spectrum, there are two bands that are visible. These bands have been connected with the stretching vibration of the amide I band and the amide II band of the protein, respectively. The bands have a wavelength of 1645.62 cm-1 and 1539.88 cm-1. (Joshi,2012) suggests that the absorption peak at 1455.99 cm-1 might be related to geometric bending vibration of amino acid residue sets with free protein carboxylate sets -COO- (carboxylate ion), 1078.01 (ethers, esters, and C-O alcohol stretched oxalic acids), and C-N stretched of aliphatic amines.

4.6. AGNPSANTIBACTERIAL ACTIVITY ON PATHOGENIC BACTERIA

At two different doses (100 g/ml and 50 g/ml), the biosynthesized AgNPs were shown to limit the development of human pathogenic bacterial strains, which were examined. This was another finding from the present investigation. In contrast, the effectiveness of AgNPs as an antibacterial agent was inconsistent. Even though the mechanism by which AgNPs prevent bacteria from growing is not extensively established, it is possible that it is linked to the impact of Ag+ by leading to damage of bacterial cell membranes, the damage of enzymes, or the conformational changes of DNA. This is what has been proposed by other study results (Kim et al. 2007; Marambio-Jones and Hoek, 2010).

https://doi.org/10.17993/3ctecno.2023.v12n1e43.296-319

4.7. COMBINATION EFFICACY OF AGNPS WITH AMPICILLIN AND CHLORAMPHENICOL

According to increased fold area, the biosynthesized AgNPs and the medicines ampicillin and chloramphenicol significantly boosted efficiency vs the chosen human pathogenic bacteria (Birla et al. 2009). These results are consistent with those of earlier research that investigated the synergic impact of AgNPs produced from a variety of fungal species when used in conjunction with a selection of various commercialized antibiotics and put to the test versus Gram-negative and Gram-positive bacteria (Fayaz et al., 2010; Devi and Josh, 2011; Gudikandula et al., 2015; Shareef et al., 2017).

(Fayaz et al. 2010) also showed an increase in the antibacterial activity of chloramphenicol, erythromycin, kanamycin, and ampicillin when used in conjunction with AgNPsvs Salmonella typhi, Escherichia coli, Staphylococcus aureus, and Micrococcus luteus.

(Devi and Joshi, 2011) showed an improvement in the antibacterial activity of ciprofloxacin, chloramphenicol, erythromycin, and methicillin when combined with biosynthesized AgNPs versus Enterococcus faecalis, Salmonella enterica, Streptococcus pyogenes, and Staphylococcus aureus.

5. CONCLUSION

The production of AgNPs using P. tropicawas investigated in the current study this finding is the first for this fungus species in Iraq. The generated AgNPs demonstrated activity versus Gram-positive and Gram-negative human pathogenic bacteria throughout a wide range. This fungus shows potential as a natural source for the synthesis of AgNPs, which have applied in the medical product and pharmaceutical manufacturing industries.

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