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Bioactivity of silver nanoparticles produced by the aqueous extract of local *Trichoderma longibrachiatum* isolates against some types of MDR bacteria

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The utilization of microorganisms in the biosynthesis of nanomaterials is one of the highlights of recent nanotechnology. This study stabilized and reduced biological silver nanoparticles outside of cells using an aqueous *Trichoderma longibrachiatum* extract. Three concentrations of aqueous silver salt ($AgNO_3$) were used: 0.5, 1.0, and 1.5 mM. The aqueous extract of *T. longibrachiatum* was combined with the aqueous salt in a 1:1 ratio at room temperature and pH 5.5. Initially, the formation of silver nanoparticles was indicated by a change in color. Surface plasmon resonance at 413 nm was employed for the detection of AgNPs, with their formation confirmed through UV-Vis spectroscopic analysis. Additional research conducted with Fourier transformation infrared (FTIR) showed bands at 1636, 2112, and 3322 cm^{-1}. The confirmation of spherical nanoparticles, exhibiting diameters ranging from 28 to 43 nm, was achieved via scanning electron microscopy. The specimen was identified based on morphological characteristics and molecular techniques. The sample's DNA was analyzed with PCR using universal primers ITS1 and ITS4. The PCR investigation indicated the presence of a 660 base pair band. Nucleotide sequences were compared with the BLAST program at NCBI. The new strain was accepted and added to NCBI with the scientific name *T. longibrachiatum* and accession number PP977534. The antimicrobial assays were conducted on the synthesized AgNPs against gram-positive bacteria such as *Staphylococcus aureus* and gram-negative bacteria such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli* using the well diffusion method. The present study illustrated that silver nanoparticles exhibited a wide range of inhibitory effects on all bacterial species under the current study, even at low concentrations. This efficacy was significantly greater than that of antibiotics and aqueous extracts of *T. longibrachiatum*. At low concentrations of 65, 98, and 130 ppm, silver nanoparticles were biosynthesized to be significantly more effective in inhibiting bacterial growth than the antibiotics used in this research or the aqueous extract of *T. longibrachiatum*. The inhibition diameter of *S. aureus* was 16 mm at the lowest concentration of silver nanoparticles, while the inhibition zone for the same type of bacterium was 10 mm at the highest concentration of *T. longibrachiatum* extract. Furthermore, all of the bacterial isolates employed in this investigation exhibited resistance to the ampicillin, sulfamethoxazole and trimethoprim antibiotics while simultaneously exhibiting sensitivity to levofloxacin. *P. aeruginosa* exhibited the maximum sensitivity to the levofloxacin antibiotic with a measurement of 15 mm. *In vitro* practical experiments have demonstrated that the synthesized AgNPs have the potential to assist in the management of these pathogens. The results indicated an enhancement in the inhibition zone at 65 ppm, with a considerable augmentation in the inhibition zone at 98 and 130 ppm. The current approach is a highly promising candidate for the industrial-scale manufacture of AgNPs, as our data clearly indicate, and it has the potential to control bacterial infections.

Keyword: AgNPs; $AgNO_3$; *Trichoderma longibrachiatum*; *Pseudomonas aeruginosa*; *Klebsiella pneumoniae*; *Escherichia coli*; *Staphylococcus aureus*.

Introduction

The excessive use of antibiotics by the population has led to genetic alterations in bacteria and the emergence of antimicrobial resistance, which is now challenging to address. To address this issue, it is essential to devise novel approaches for the management of antimicrobial active agents. Biocomposite systems, including silver nanoparticles, may serve as a viable medical alternative (Cadinoiu et al., 2022; Qassim et al., 2023). Antibiotics constitute the primary modality for treating bacterial infections. Nonetheless, microbes exhibiting resistance to many pharmaceuticals have emerged as a worldwide menace. Infections induced by these organisms are challenging to manage with presently available medications (Allawi & Al-Taee 2022; Chinemerem et al., 2022; Qassim et al., 2024). The creation of novel antibiotics is laborious and necessitates substantial financial inputs. Developing nations face significant challenges due to the growth and proliferation of infectious diseases caused by multidrug-resistant (MDR) bacteria (Dadgostar, 2019; Ejikeugwu et al., 2021). The utilization of microorganisms in the biosynthesis of nanomaterials is one of the highlights of recent nanotechnology (Sandhu & Goel, 2023).

Applications and the creation of nanomaterials make nanotechnology one of the most vital emerging domains in materials science (Pallavi et al., 2022; More et al., 2023). On the nanoscale realm, materials exist in a size range from 1 to 100 nm, exhibiting a variety of chemical, physical, and magnetic characteristics (Siddiqi et al., 2018a). These distinct attributes enable them to engage with a variety of microorganisms (Siddiqi et al., 2018b; Rodrigues et al., 2024). There are numerous prospective uses for metal nanoparticles in fields such as biomedicine, antimicrobial functions, optics, and catalysis (Sánchez et al., 2020). NPs have a higher surface-to-volume ratio than other particles of the same composition, making them more useful in biochemical and catalytic activities (Jyoti et al., 2016; Rafique et al., 2017). Their reactivity, durability, and other properties depend on their unique size, shape, and structure. Due to these qualities, they are suitable candidates for many uses, especially AgNPs, which have received considerable attention because they work well in medical settings as therapies and for delivering drugs (Kaur et al., 2019; Khan et al., 2019; Lee & Jun, 2019; Tian et al., 2020). Nanoparticles demonstrate antimicrobial effectiveness and provide a beneficial alternative in the treatment of most bacterial diseases, especially those involving organisms resistant to multiple drugs (Mba & Nweze, 2021). Microorganisms can be efficiently killed by nanoparticles because of their capacity to react to a wide range of exogenous and endogenous stimuli. Additionally, they facilitate improved medication delivery and release promising strategies (Qing et al., 2018; Ahmad et al., 2020; Yin et al., 2020). The metallic NPs appear to be more effective and promising. They demonstrate diverse actions against a range of

infections that are resistant to multiple drugs (Rasheed et al., 2017; Mba & Nweze, 2020). The metal NPs are the most widely investigated; among the various nanoparticles, AgNPs have been the subject of the most extensive research and are the most commonly employed (Möhler et al., 2018; Bruna et al., 2021). Due to their potent inhibitory effect on microbial growth, they are currently considered the next generation of antibiotics. Currently, AgNPs, among the commercialized nanomaterials, are the most common nanoparticles. Research into their use as antibacterial agents has developed over time because they are less toxic than other nanoparticles. The green approach to nanoparticle manufacturing has low or no toxicity (Ahmed et al., 2024).

Researchers have reported various physical and chemical methods for the synthesis of nanomaterials, but these methods are often expensive and potentially harmful to the environment (Prabhu & Poulose, 2012; Nyabadza et al., 2023). Nanoparticles are of significant scientific interest due to their high potential in a wide range of industrial fields. Due to this, the synthesis method is essential for getting the best nanoparticle properties for a certain use (McNeil, 2011; Özçelik & Kara, 2023). Green synthesis using biological organisms such as plants and microbes is an environmentally friendly alternative to conventional chemical methods. This approach requires the use of non-toxic and environmentally friendly chemicals to manufacture nanoparticles (Majithia & Barretto, 2023). The use of microorganisms, including fungi, bacteria, and yeasts, for nanoparticle synthesis is a relatively recent development (Kim et al., 2018; Castillo et al., 2020; Singh et al., 2023). This study focuses on developing an environmentally friendly experimental protocol for the synthesis of silver nanoparticles with desired shape, size, and nature by the utilization of beneficial fungi, as they offer several advantages over other organisms, including the important role of their secreted enzymes. In the creation of silver nanoparticles (AgNPs), fungi reduce particle size and stabilize them. However, the precise mechanism by which they achieve this remains incompletely understood (Omran et al., 2019). Compared with other microorganisms, fungi are simple to isolate, process, and store, making them preferable for large-scale nanoparticle manufacturing (Moharrer et al., 2012; Adeleke et al., 2024). The study investigated the possibility of producing silver nanoparticles in an environmentally friendly manner by employing an extract from local *T. longibrachiatum* isolates and assessed how well it inhibited the growth of both Gram-positive and Gram-negative bacteria. Furthermore, we evaluate the quality of the resulting silver nanoparticles. This study suggests the possibility of producing silver nanoparticles on a large scale without involving any toxic chemicals or radiation. The study will also deepen the understanding of the molecular mechanisms of silver nanoparticle synthesis.

Material and methods

The samples were microscopically examined using morphological characteristics to isolate the samples belonging to *Trichoderma* spp. After initial identification, molecular diagnostic techniques were used. Then, DNA was carefully extracted from the isolated sample using the Mini Genomic DNA Kit (Taiwan).

PCR was conducted in a 20 µl volume reaction using GoTaq G2 Green Master Mix supplied by Promega (USA), which contains G2 DNA polymerase (a variant of Taq polymerase), dNTPs, MgCl2, and the polymerase buffer. Two dyes (blue and yellow) are pre-mixed in this master mix to assist in following the PCR bands (Ibrahim & Faisal, 2024). Ingredients for each 20 µL PCR reaction were as follows: 10 µL Master Mix, 2 µL of 10 µM forward primer ITS1 (5'TCCGTAGGTGAACCTGCGG'3), 2 µL of 10 µM reverse primer (5'TCCTCCGCTTATTGATATGC'3), a certain amount of DNA (up to 50 ng), and a variable amount of nuclease-free water. The elements were combined and put in a thermocycler device along with a 0.2 mL Eppendorf tube. According to the program, Table 1.

Subsequently, the samples' DNA and DNA ladders, produced by Biolaps, were introduced into the wells of agarose. The material was subjected to electrophoresis for 50 to 70 minutes at a voltage of 40 V/cm. A photograph of the gel was captured using the gel documentation system. Afterward, bands were removed from the gel and purified for DNA sequence analysis using a Geneaid DNA clean-up kit (Faisal & Younis, 2024).

Table 1
PCR program for amplification of the Internal Transcribed Spacer (ITS) region from *Trichoderma longibrachiatum*

Stage no.	Temperature, °C	Time, min.	Cycle number
Initial denaturation	95	3	1
Denaturation	95	0.5	
Annealing	55	1	35
Extension	72	1	
Final extension	72	5	1

According to Al-Rubaiey & Al-Juboory (2020), the polymerase chain reaction results obtained after electrophoresis on 2% agarose showed a band at 660 bp. The strip was visualized under UV light using a gel documenter and PCR product. The BLAST program on the National Center for Biotechnology Information (NCBI) website analyzed and compared the nucleotide sequences (Abdulrazzaq et al., 2024). They were then able to add it as a new strain to the NCBI, giving it the scientific name and accession number: *Trichoderma longibrachiatum* PP977534.

The bacterial isolates *S. aureus*, *P. aeruginosa*, *K. pneumoniae*, and *E. coli* used in this study were procured from the Department of Biology, College of Science, University of Mosul, Iraq. These isolates were mostly identified by their morphological features. They were then put through the Vitek 2 system to confirm the identification and diagnosis and find out which antibiotics worked best on them.

The disk diffusion method, which is also called the Kirby-Bauer method (Baur et al., 1966). We used ampicillin 25 µg, sulfamethoxazole, trimethoprim 25 µg, and levofloxacin 5 µg to test how well Gram-positive *S. aureus* and Gram-negative *E. coli*, *P. aeruginosa*, and *K. pneumoniae* bacteria responded to antibiotics. The protocol followed the guidelines set out by the Clinical Laboratory Standard Institute (Bakthavatchalam et al., 2024).

An Erlenmeyer flask containing 200 mL of sterile potato dextrose broth (PDB) was used to culture four disks (6 mm in diameter) of *Trichoderma longibrachiatum* harvested after 7 days from the edges of actively growing PDA cultures. The broth culture (pH 5.5) was incubated for seven days at 28 °C and a rotation speed of 130 rpm. The yellowish-white fungus formed a circular mat (Fig. 2b). The biomass was collected by filter paper, Whatman No. 1. To ensure that no medium particles remained attached to the biomass, it was washed thoroughly with deionized water. To prepare the 100 mg/mL stock solution of aqueous extract of *T. longibrachiatum*, 10 g of the biomass was mixed in 100 mL of distilled deionized water and incubated at 28 °C for three days. The aqueous extract of *T. longibrachiatum* was obtained by filtering the mixture through Whatman No. 1. Three concentrations of 5, 10, and 15 mg/mL were prepared from stock solutions of aqueous extracts of *T. longibrachiatum* (Elamawi et al., 2018).

It is possible to make a solution with 1 mM of $AgNO_3$ by mixing 0.1699 g of silver nitrate with 1000 mL of deionized water (DW) (Tailor et al., 2024).

Three doses of aqueous silver nitrate ($AgNO_3$) at 0.5, 1.0, and 1.5 mM were combined in equal proportions (1:1) with *T. longibrachiatum* extract (Devi et al., 2013; Noshad et al., 2019). 50 mL of silver nitrate, prepared at a concentration of 0.5 mM, were mixed dropwise with 50 mL of distilled *T. longibrachiatum* aqueous extract at a concentration of 5 mg/mL. Before being mixed with a magnetic stirrer at 1000 rpm at 30 °C for 20 minutes, the solution was ultrasonically treated with a flow rate of 0.2 mL/min, ultrasonic power of 100 W, frequency of 42 kHz, and time of 15 minutes. After that, it was stored in opaque bottles for 24 hours. The combination was centrifuged for 15 minutes at 10,000 rpm and 4 °C to extract a clear liquid, which was then stored in opaque vials as a colloid preparation. For a period of five days, the solution's color change was carefully observed. The experiment was repeated for the remaining concentrations of silver nitrate (Al-Hayanni et al., 2022; Alnuaimi et al., 2023).

Following the attainment of a steady color change, the solution was centrifuged for 10 minutes at 5000 rpm to eliminate any precipi-

tate. The supernatant was subsequently centrifuged for 30 minutes at 12,000 rpm. To remove any remaining culture extract, the pellet was rinsed with deionized water. The final particles were resuspended in distilled water before analysis (Srećković et al., 2023).

A UV-vis spectrophotometer (Shimadzu, Japan) was used to observe the creation of the reduced silver nanoparticles in the colloidal solution. The supernatant's absorption spectra were recorded using a UV-vis spectrophotometer within the 200–800 nm range. As a blank, we utilized deionized water (Fahim et al., 2024).

An FT-IR spectrometer (Shimadzu, Japan) was employed to assess the solution of AgNPs and *T. longibrachiatum* extract within the 500–4000 cm^{-1} range, with a resolution of 8 cm^{-1}. Peaks were detected for the functional groups in the produced particles (Al-Hayanni et al., 2022).

The microstructure was examined using a scanning electron microscope (EVO ZEISS-Germany) at a voltage range of 10 to 25 kV at the College of Pharmacy, Nineveh University, Mosul. It is worth noting that the samples were uniformly spread across the conductive paste surface and subsequently coated with a thin layer of gold using a sputter coater (Ahmed et al., 2018).

Silver nanoparticles and an aqueous extract were tested for antibacterial efficacy against pathogenic bacteria using the agar well diffusion method (Crisan et al., 2024; Hossain, 2024). The bacteria under test were grown in nutrient broth medium. Mueller-Hinton agar was placed on sanitized Petri dishes and hardened at laboratory temperatures. To test each bacterial isolate, 0.1 mL of a standard inoculum (1.5 x 10^8 CFU/mL, 0.5 McFarland's standard) was added to a separate plate using clean cotton swabs. A cork borer was used to create 5 mm diameter wells in the Mueller-Hinton agar. Each well contained 100 µL of AgNPs and 100 µL of *T. longibrachiatum* extract. A negative control, distilled deionized water, was used (Khac et al., 2023).

The disk diffusion method used standard antibiotics as positive controls, such as ampicillin (25 µg), sulfamethoxazole and trimethoprim (25 µg), and levofloxacin (5 µg) discs. The plates were incubated at 37 °C for 24 hours. Three replicate trials were performed.

Results and discussion

Following sample collection, morphological traits and molecular approaches were employed to identify the samples that exhibited a band at 660 bp (Fig. 1a). Figure 1b *T. longibrachiatum* was subsequently incorporated as a new strain into the NCBI, assigned the scientific name and accession number: *Trichoderma longibrachiatum* PP977534.

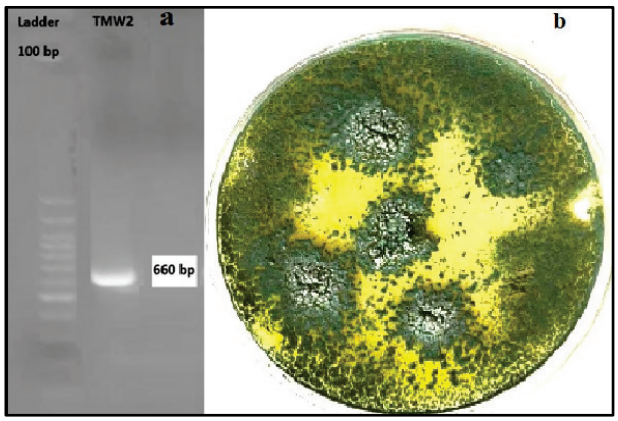

Fig. 1. PCR reaction product of *T. longibrachiatum* studied for the ITS region and a reaction product of 660bp, carried over by a 2% agarose gel (*a*), and *T. longibrachiatum* voucher PDA N189-2 (*b*)

For the synthesis of silver nanoparticles, mycelial extract of *T. longibrachiatum* was used as a reducing and capping agent. Visual observation revealed a color change in the reaction mixture. After adding the *T. longibrachiatum* extract to the previously prepared concentrations of silver nitrate solution at a ratio of 1:1, the solution initially showed a yellowish color. The bioreduction of silver ions to metallic silver (Ag^+ to Ag^0) was used to create the silver nanoparticles during this process, and the bioactive molecules found in the mycelial cells subsequently stabilized them (Herrera et al., 2024). The color intensity showed a gradual increase to dark brown with increasing incubation time, as shown in (Fig. 2a).

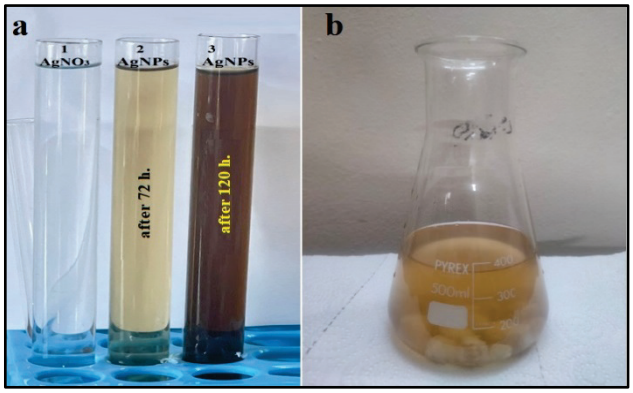

Fig. 2. Color changes During the bio-reduction of $AgNO_3$ into AgNPs using *T. longibrachiatum* aqueous extract (*a*), and circular mat of mycelium from an aqueous extract of *T. longibrachiatum* (*b*)

The analysis of these spectra is a powerful tool for understanding the properties of silver nanoparticles, such as their size, shape, and size distribution. A distinct absorption peak is evident. All spectra show a strong absorption peak in the UV and visible region (around 400–450 nm). The surface plasmon resonance (SPR) phenomenon, a characteristic of metallic nanoparticles, is responsible for this peak. This peak indicates the presence of silver nanoparticles, which are nanosized and have a relatively narrow size distribution. We observe that the intensity of absorption at the 413 nm wavelength of the SPR peak increases as the concentration of silver nanoparticles increases. The maximum intensity of AgNPs increased as the concentration of $AgNO_3$ increased from 0.5 to 1.5 mM (Fig. 3). This indicated an increase in the production of AgNPs over time (Jayaprakash & Kannappan, 2022). This means that increasing the concentration leads to an increase in the number of silver nanoparticles in the solution, which increases the total absorption. Increasing the concentration may also change the peak's position and shape, reflecting the size and shape of the nanoparticles. The width of the spectral peak is usually an indicator of the size distribution of the nanoparticles. A narrow peak indicates a narrow particle size distribution, while a broad peak indicates a broad size distribution. In this figure, the peak width appears to change very little as the concentration changes, indicating that the size distribution of the nanoparticles remains relatively constant. This suggests the exclusion of aggregation within the system of reactions and the consistency of the nanoparticles' dimensions and morphology (Elamawi et al., 2018; Thepbandit et al., 2024).

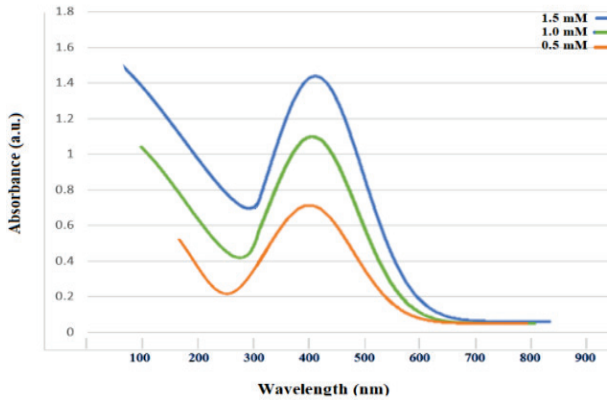

Fig. 3. AgNPs Uv/vis absorption

The diagnosis of nanoparticles utilizing the FTIR approach (Fig. 4) revealed bands corresponding to the functional groups, as

previously recorded in the research by Elamawi et al. (2018). Using Fourier transform infrared spectra is an important way to look at the functional groups that help keep synthesized nanoparticles stable (Gong et al., 2024). The FTIR spectrum shows the bands that represent the vibration frequencies of the different chemical bonds in the molecule. By analyzing these bands, we can identify the functional groups present in the extract that play a crucial role in the nanosynthesis process. The identification of functional groups aids in comprehending the mechanism that reduces silver ions and forms nanoparticles. For example, electron-donating groups such as hydroxyl groups (OH) in phenols can donate electrons to silver ions, leading to their reduction and nanoparticle formation. Similarly, complex-forming groups, such as carbonyl groups (C=O), can form complexes with silver ions, facilitating the reduction process. To fully understand how proteins interact with AgNPs, FTIR spectroscopy is a must. It is possible to measure the secondary structure of the metal nanoparticle-protein interaction. AgNPs, mixed with AgNO3 for 120 hours, underwent FTIR spectroscopy (Fig. 4). The FTIR spectrum included bands at 1636, 2112, and 3322 cm^{-1}. An FTIR spectrum showed bands at 1636, 2112, and 3322 cm^{-1}. Protein amide I and II bands exhibit bending vibrations at 1636 cm^{-1}. This frequency range is the same as the stretching vibration of the carbonyl (C=O) group in peptide bonds (Gupta et al., 2022). This band is a characteristic feature of proteins and peptides. When you look at an FTIR spectrum, the band at 2112 cm^{-1} usually means that phenylacetylene has a carbon-nitrogen triple bond (C≡N) or a carbon-carbon triple bond (C≡C) that is stretching. When *T. longibrachiatum* extract was used to make silver nanoparticles (AgNPs), the broad peak at 3322 cm^{-1} was due to the primary amines changing shape. These are connected to the OH group that is bonded to hydrogen within the molecule (Dobrucka et al., 2019). This shows that the (OH) functional group in *T. longibrachiatum* extract can contribute to the conversion of Ag^{+} to Ag^{0}. Previous research has shown that reducing hydroxyl groups in polysaccharides can lead to the creation of silver and gold nanoparticles (Dhaka et al., 2023).

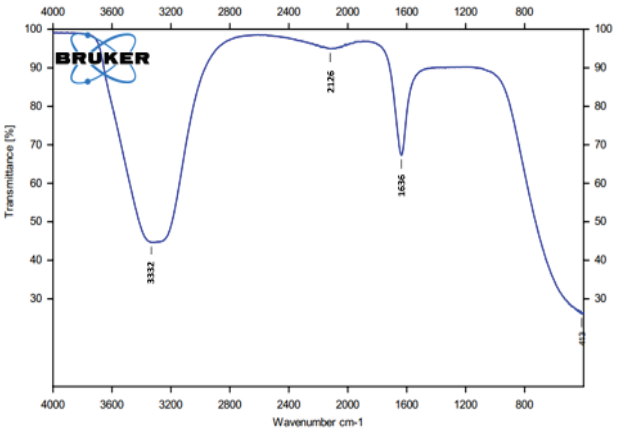

Fig. 4. FTIR spectra of AgNPs synthesized using aqueous extract of *T. longibrachiatum*

Characterization by SEM is the most efficient diagnostic of nanoparticle production, whereas the production of nanoparticles depends mainly on the change in the size, shape, and nature of the surface of the particles (Kazemi et al., 2023). The spherical shape and aggregated particles are seen in the SEM pictures of AgNPs; the findings definitely revealed the AgNPs' very porous surface is caused by agglomeration characteristics with a range of particle size between 28 and 43 nm (Fig. 5).

Different standard concentrations of silver nitrate ($AgNO_3$) ranging from 0.25 to 1.25 mmol were prepared in distilled deionized water, and a calibration curve was constructed using these standard solutions (Fig. 6). After performing the experiment using the calibration curve, the unknown concentrations of silver nanoparticles were determined in terms of their absorbance, which were 65, 98, and 130 ppm (Al-Hayanni et al., 2022; Alnuaimi et al., 2023).

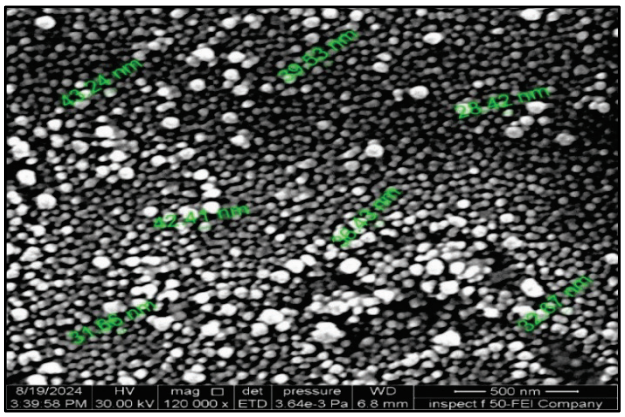

Fig. 5. Electron microscope images of AgNPs produced from *T. longibrachiatum*

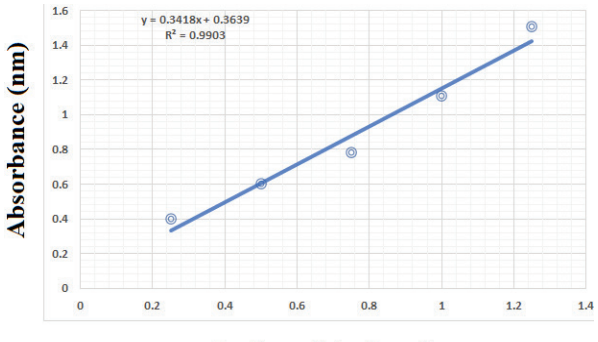

Fig. 6. The standard curve made of $AgNO_3$ dilutions for the determination of silver ion concentration

Antimicrobial resistance is a big global concern that varies by area. As a result, nanotechnology and silver particles provide alternate solutions to the problem of antimicrobial resistance (Hetta et al., 2023). Figure 7 and Table 2 show the antibiotic susceptibility test results obtained using the Kirby-Bauer method. All of the examined bacteria strains were entirely unaffected by ampicillin 25 µg or sulfamethoxazole-trimethoprim 25 µg (Khalifa et al., 2021; Ramatla et al., 2022; Sethuvel et al., 2023). This indicates that these antibiotics are not effective against many germs. Although certain bacteria are sensitive to levofloxacin (5 µg), partial resistance renders the treatment useless. The findings suggest that the restricted treatment choices for infections caused by these strains make treatment more challenging and increase the risk of adverse consequences. Furthermore, excessive, inappropriate, or unneeded usage of antibiotics contributes to the formation of resistant strains. The transfer of resistance genes, which give resistance across strains, aids in the establishment of resistant strains. This raises the likelihood of resistant infections, which can lead to serious problems. Based on these findings, we urge the sensible use of antibiotics, using them only when absolutely essential and after sensitivity testing. We want to enhance community awareness by educating the public about the importance of using antibiotics responsibly.

The bioactivity of AgNPs was tested against both Gram-positive and Gram-negative strains. Research has confirmed that silver nanoparticles have an excellent ability to kill *P. aeruginosa*, *E. coli*, and *K. pneumoniae* due to their being characterized by having a thin peptidoglycan layer and outer lipid membrane (Tripathi & Goshisht, 2022). while Gram-positive bacteria possess a thick peptidoglycan layer, which may prevent nanoparticles from entering their cells (Slavin et al., 2017; Meikle et al., 2020). The research showed that the small NPs give off more silver cations than the large NPs. The small particles were also better at killing bacteria than the large ones (Slavin et al., 2017). As observed in the results of the experiment, the silver nanoparticles (AgNPs) outperformed both the *T. longibrachiatum* extract and the control in their ability to inhibit bacterial growth. The antibacterial activity of silver nanoparticles (AgNPs) was assessed

against tested bacteria. According to the previous studies, AgNPs have the ability to inhibit bacterial growth (Pineda et al., 2024).

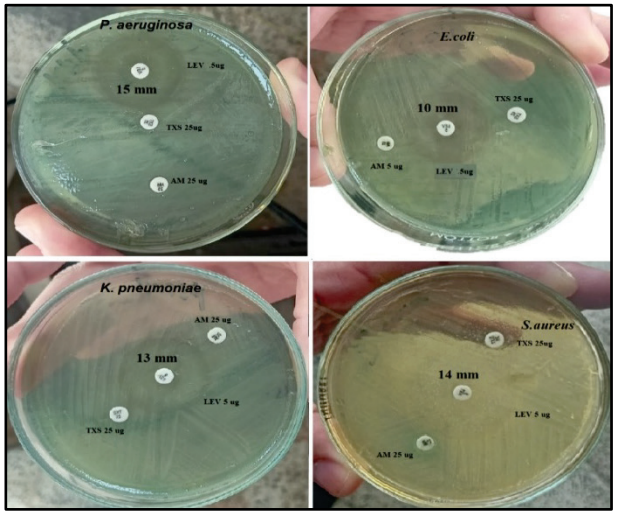

Fig. 7. Disk susceptibility testing results for antibiotics Kirby Bauer methodology

Table 2
Antibiotic susceptibility test (mm) results using the Kirby-Bauer method

Bacteria types	Antibiotics			
	control	levofloxacin 5 µg	ampicillin 25 µg	sulfamethoxazole and trimethoprim 25 µg
P. aeruginosa	0	15	R	R
K. pneumoniae	0	13	R	R
E. coli	0	10	R	R
S. aureus	0	14	R	R

The lower concentration of silver nanoparticles caused an inhibition zone that was 16 mm wide. This was wider than the highest concentration of *T. longibrachiatum* extract, which caused an inhibition zone that was 13 mm wide, as shown in Tables 3 and 4. Also, the inhibition zone increased significantly when the concentration increased. All tested bacteria show that the zone of inhibition grows as the concentration of silver nanoparticles goes up. This means that silver nanoparticles are antimicrobial and can kill all tested bacteria. This appears since *P. aeruginosa* and *E. coli* demonstrate more sensitivity to silver nanoparticles than *K. pneumoniae* and *S. aureus*, as evidenced by wider zones of inhibition at the same concentrations (Fig. 8). Various bacteria exhibit varying degrees of sensitivity to AgNPs and the aqueous extract (Fig. 9); this variation may be due to differences in cell wall composition, defense mechanisms, or the ability of the bacteria to expel nanoparticles. The sensitivity of different bacterial species to silver nanoparticles can vary. Nanoparticles can either saturate bacterial surfaces or limit the surface area of bacterial contact by generating nanoparticle clusters. This response is consistent with previous research demonstrating the potent antibacterial properties of silver nanoparticles against gram-negative bacteria (Girma et al., 2024). This may occur because gram-negative bacteria possess a negative charge in their lipid polysaccharides, which facilitates the attachment of nanoparticles to their cell walls, or because they contain specific proteins in their cell walls, which enable direct interaction with silver nanoparticles and the release of silver ions (Ag^{+}) (Dakal et al., 2016; Tripathi et al., 2022). This indicates the vulnerability of antibiotic-resistant bacteria to silver nanoparticles (Hochvaldová et al., 2024). Many antibacterial properties of silver nanoparticles have been studied by researchers. These include breaking down cell walls, creating reactive oxygen species, and taking in and releasing Ag^{+} ions. Based on our results, this approach is promising for manufacturing silver nanoparticles on an industrial scale, which could be useful in combating bacterial infections.

Table 3
The inhibitory effect (mm) of three concentrations of AgNPs

Bacteria types	Silver nanoparticles			
	control	65 ppm	98 ppm	130 ppm
P. aeruginosa	0	19	21	25
K. pneumoniae	0	17	19	22
E. coli	0	19	20	24
S. aureus	0	16	18	20

Table 4
The inhibitory effect (mm) of *T. longibrachiatum* extract concentrations

Bacteria types	*T. longibrachiatum* aqueous extract			
	control	5 mg/mL	10 mg/mL	15 mg/mL
P. aeruginosa	0	12	13	13
K. pneumoniae	0	11	13	12
E. coli	0	12	14	13
S. aureus	0	8	9	10

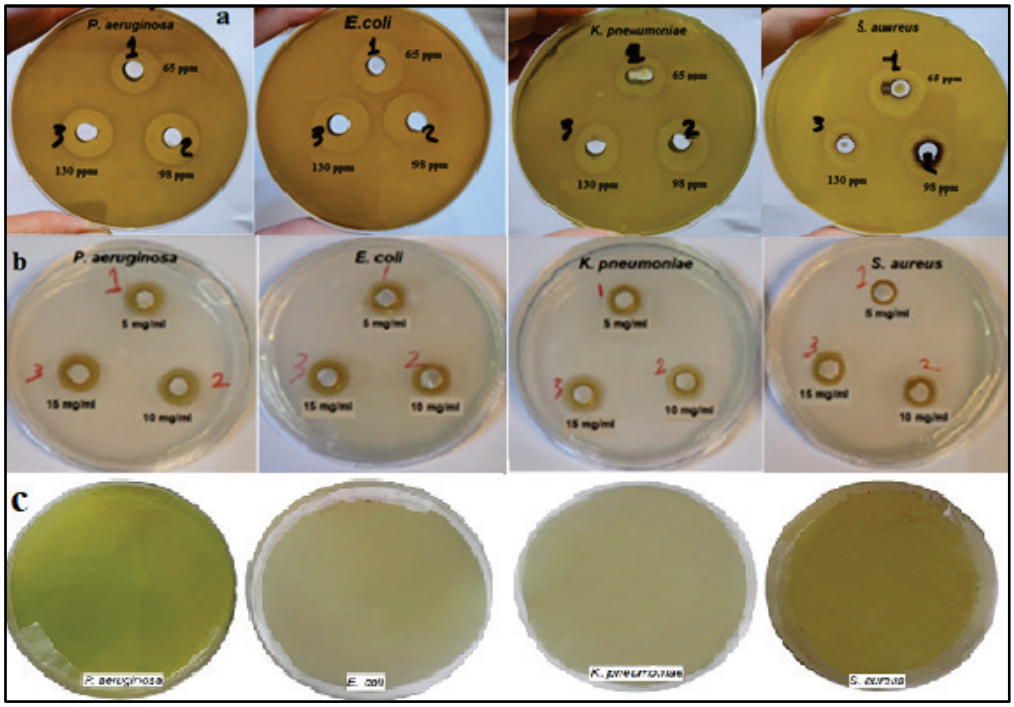

Fig. 8. Effect of AgNPs on tested bacteria (*a*), effect of aqueous extract of *T. longibrachiatum* on tested bacteria (*b*), and control (*c*)

Silver nanoparticles showed outstanding effectiveness in inhibiting the growth of all types of bacteria tested. We notice that the inhibition zone increased significantly with increasing concentrations of silver nanoparticles, indicating a direct relationship between concentration and antimicrobial activity. *P. aeruginosa* was the most sensitive bacteria to silver nanoparticles, while *S. aureus* was the least sensitive to them. As for the effectiveness of the *T. longibrachiatum* extract, it also showed antimicrobial activity against all types of bacteria. The increase in the inhibition zone with increasing concentrations of the extract was less pronounced compared to silver nanoparticles. In general, the inhibition zone resulting from the extract was smaller compared to the inhibition zone resulting from the same concentration of silver nanoparticles.

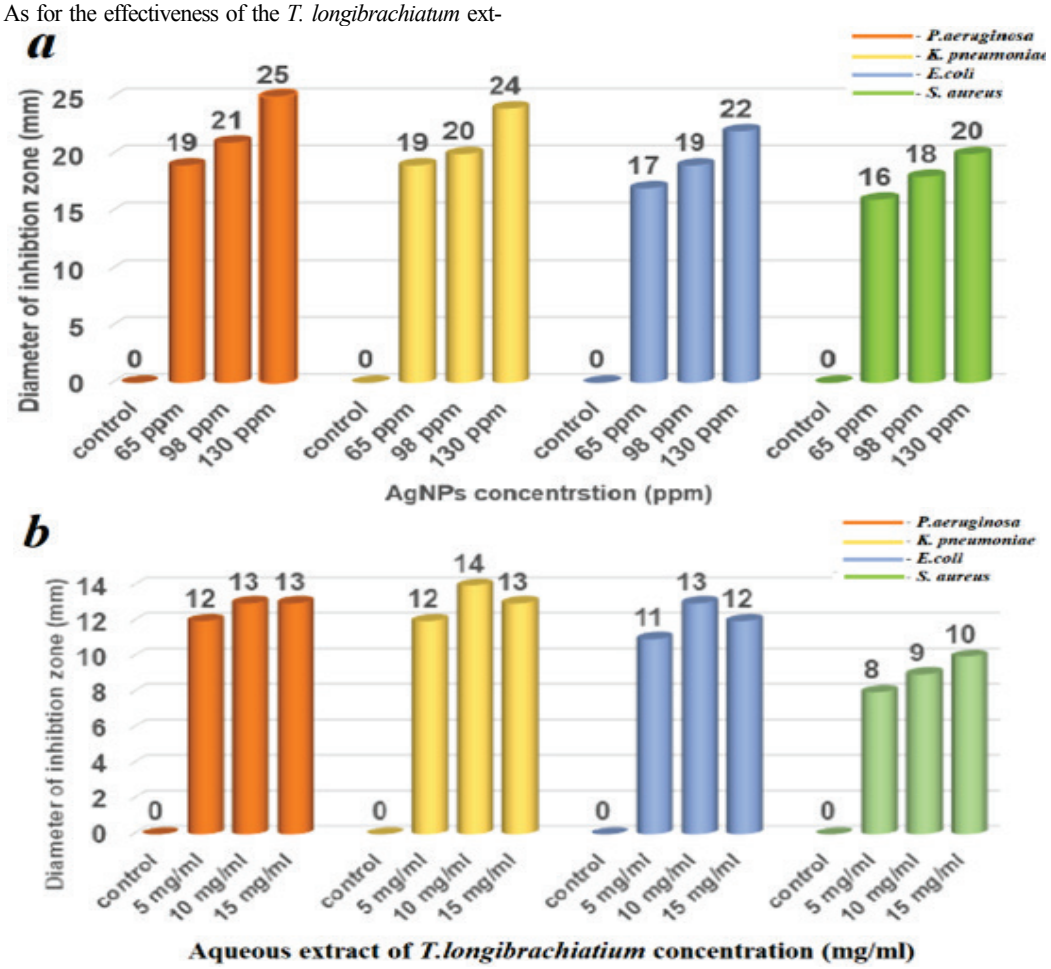

Fig. 9. Impact of varying concentrations of the AgNPs on the tested bacteria (*a*), and impact of varying concentrations of the aqueous extract of *T. longibrachiatum* on the tested bacteria (*b*)

Conclusion

Silver nanoparticles showed greater efficacy in inhibiting bacterial growth than the mycelium extract, especially at higher concentrations. The relationship between the silver nanoparticle concentration and the inhibition zone was more pronounced than that between the extract concentration and the inhibition zone. Both silver nanoparticles and *T. longibrachiatum* fungal extract influence bacterial cells through distinct mechanisms. The mechanisms of action of silver nanoparticles may involve damaging the cell membrane and disrupting metabolic processes. In addition, this result may be attributed to the shape and smallest-sized spherical particles of AgNPs due to the high surface-to volume ratio. The size and shape of silver nanoparticles may affect their ability to penetrate the bacterial cell wall and interact with cellular components. Fungal extract components may act by inhibiting specific enzymes or forming complexes with bacterial cell components. The chemical composition of silver nanoparticles is different from that of fungal extract, leading to differences in their physicochemical properties and biological efficacy. Silver nanoparticles and *T. longibrachiatum* extract both possess antimicrobial properties. Silver nanoparticles were more effective in inhibiting bacterial growth than the fungal extract at the concentrations tested. These results were based on experimental conditions and may vary with different experiments. Further studies are needed to determine the exact mechanisms of action of silver nanoparticles and *T. longibrachiatum* extract and to evaluate their safety and efficacy in complex biological systems. The results indicate that the limited options for treating infections caused by these strains increase the difficulty of treatment and the risk of complications. Furthermore, the overuse, incorrect use, or unnecessary use of antibiotics contributes to the emergence of resistant strains. Furthermore, the spread of resistance genes, which confer resistance between different strains, contributes to the emergence of resistant strains. This increases the incidence of resistant infections, potentially leading to serious complications. Based on these results, we advocate for the rational use of antibiotics, ensuring their use only when necessary and following sensitivity testing. as We aim to raise community awareness by educating the public on the significance of using antibiotics correctly. Based on these results, silver nanoparticles are promising options for developing novel therapeutics to combat bacterial infections. The data indicates high resistance among the gram-negative bacteria to antibiotics. *K. pneumoniae*, *E. coli*, and *P. aeruginosa* are causes of concern at the current stage, which is a critical stage. Thus, there is a need to stop misuse of antibiotics with immediate effect and to implement a strong antimicrobial stewardship program. The present research has demonstrated an efficient and inexpensive biological approach to producing metal nanoparticles, which could be useful in developing a novel antibacterial agent to combat harmful bacteria and other microbes.

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