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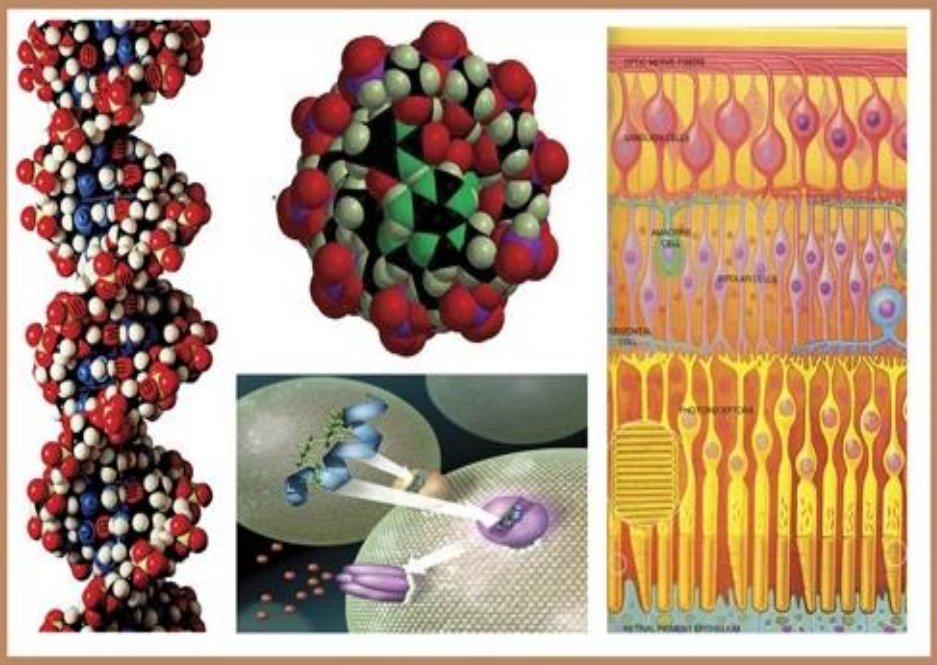

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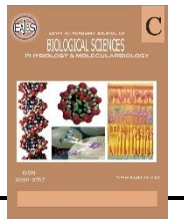

Aspergillus fumigatus-Mediated Biosynthesis of Silver Nanoparticles Efficiency, Characterization, and Antibacterial Activity Against Different Human Pathogens

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ABSTRACT

The misuse of antibiotics is one of the primary causes of the rapidly expanding problem of multidrug resistance. Fungi are responsible for the production of a variety of potent metabolites (Akhtar, *et al.,* 2019). Formation of nanoparticles of silver (AgNPs) is a simple non-toxic, and environmentally friendly method of the preparation and development of nanoparticles. Which considered a crucial step in nanotechnology. Producing AgNPs from *Aspergillus fumigatus* samples involved the use of X-ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), and Transmission Electron Microscopy (TEM) (Al-Abdullah, *et al.,* 2023). The effect of synthesized AgNPs and crude extract on several bacterial pathogens was observed. Both fungal crude extract and (AgNPs) showed the greatest antibacterial efficacy against bacterial isolates. The ethyl acetate crude extract showed the highest possible antibacterial activity, according to the reports against *E. coli* was seen at 16 mm at a 50µl concentration (12mg/1ml DMSO). Conversely ethyl crude extract has the least antibacterial action against *S.typhi* at 50µl concentration was (14mm) (Bala, M., *et al.,* 2013). The maximum activity of the ethyl acetate crude extract was observed against *E. coli* at 100 µl, which showed a zone of inhibition measuring 21 mm, while an inhibition zone of 18 mm was observed against *S.typhi*. Surface Plasmon Resonance (SPR) at 432 nm was found during UV-visible spectroscopy, confirming the production of AgNPs (Guilger, *et al.,* 2019). The spherical shape of AgNPs was seen in the SEM micrograph. The reduction of Ag+ ions into AgNPs was largely mediated by phenolic, carboxyl, and hydroxyl groups, according to the results of FTIR investigation (Farjana, *et al.,* 2014). The stabilization of AgNPs was accomplished through amino acid linkage. The produced peak of AgNPs' XRD revealed information about their nature, including their phase purity, size, and internal crystalline structure (Pena *et al.,* 2010). It is possible that the pharmaceutical and medical fields will find a great use for the AgNPs that are produced from the extract of *Aspergillus fumigatus.* Silver AgNPs and crude extract *Aspergillus fumigatus* enhance antibacterial activity, outlining their potential in future research.

INTRODUCTION

Nanoscience is a well-known branch of modern science that evaluates the fundamental precepts of nano-sized objects (Ijaz, *et al.,* 2022). The suffix nano makes reference to a nanometer of a unit, or 109 units. The origin of term "nano" is a Greek word that means "extremely small." Nanotechnology is one of the modern scientific research fields. Inorganic and organic nanoparticles are the two major categories of nanoparticles (Khan, S., *et al.,* 2022). At the turn of the 20th century, nanoparticles were prepared using a variety of conventional techniques, including chemical and physical methods (Mansoori, *et al.,* 2005). Approximately 1 to 100 nanometers is the size range of nanoparticles (Nps). Silver nanoparticles are widely considered to be the most significant and reliable due to their many potential uses (Noshad, *et al.,* 2019). Thus, scientists and researchers are increasingly interested in using plants and biological organisms like bacteria, algae, and fungi to prepare metal oxide and metal nanoparticles because they are environmentally, clinically adaptable, stable, and cost-effective. In addition to their potential as a drug carrier with low cytotoxicity. Silver AgNPs have been shown to be effective against a wide range of bacterial and fungal infections (Khan, et al., 2021). The antibacterial properties of silver nanoparticles have been reported to be effective against multidrug-resistant human pathogens. NPs are used in an anti-cancer treatment as well as drug delivery (Khan. S *et al.,* 2022). In particular, AgNPs made from fungi could be the best choice because they have good antimicrobial properties and can be used in most biologically, medically, and industrial applications. This study has importance to use beneficial fungi because they have many advantages over other microbes, (Mahmoud, et al., 2023), such as their ability to act as reducing and stabilizing agents in silver nanoparticle synthesis. Multiple disciplinary make use of products derived from fungi, including usage in agriculture, medicine, and even environmental (Pena *et al.,* 2010). Despite the fact that fungal species produce a large number of antimicrobial agents, more consideration and research is required in order to develop novel antimicrobial agents from these species due to the prevalence of resistant bacteria (Mahmoud, *et al.,* 2023). The ability of fungi to produce secondary metabolites such as antibiotic chemicals is their most important characteristic. Interestingly, soil fungi are capable of producing numerous unique secondary metabolites. Such metabolites have shown different attractive capabilities, such as antibacterial, antiviral, anticancer, and antioxidant chemicals that have medicinal, agricultural, and industrial significance. These fungus spp produces various secondary metabolites with important medicinal properties (Nierman, *et al.,* 2005). *Aspergillus fumigatus* is a fungus under the genus *Aspergillus*, Fungi are classified under the polyketide family, where 40 different species were capable to producing secondary metabolites (Pena *et al.,* 2010). Secondary metabolites, such as gliotoxin, helvoic acid, and fumagillin, can be produced by the fungus *Aspergillus fumigatus* (Akhtar, *et al.,* 2021). The large biomolecule known as fumagillin possesses the ability to combat a variety of infectious diseases. Several *Aspergillus species* can produce antibacterial compounds, such as aspergillic acid (*A. flavus*), penicillic acid (*A. ochraceus*), and fumagillin (*A. fumigatus*) (khan. S *et al.,* 2022). Fungi are liable for the production of 20 of the world's most popular and successful drugs. As a result, the current work was predicated on the utilization of secondary metabolites produced by *Aspergillus fumigatus* (Li, W.-*et al.,* 2021) as well as a silver nanoparticle-coated (Fig. 1) crude extract in order to combat pathogenic bacteria isolated from human site infection.

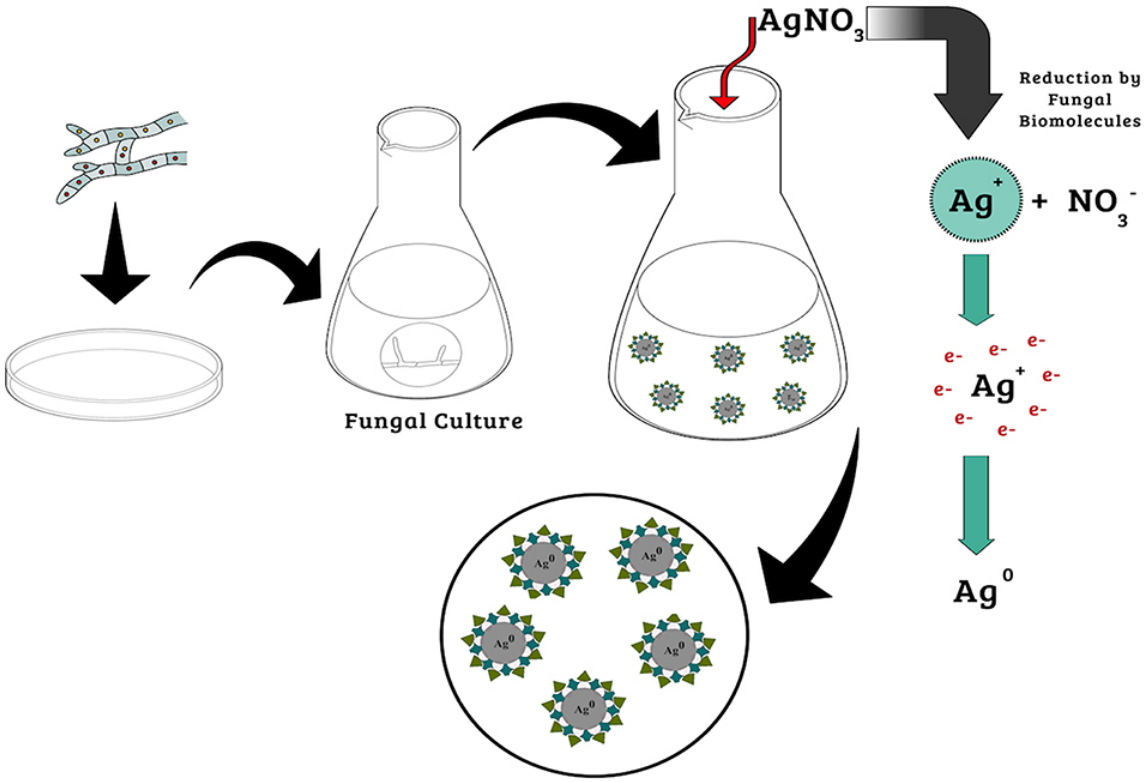

Fig. 1: Mechanisms of biogenic synthesis of silver nanoparticles.

MATERIALS AND METHODS

Fungi Isolation: Sterilized polyethylene bags collected soil samples from several locations. Serial dilutions were performed using Potato Dextrose Agar (PDA) medium in 10 test tubes and one conical flask that had been sterilized at 121°C for 15 minutes at 15 psi with 9 ml of distilled water per sample (Khan, M *et al.*, 2021).

Batch Fermentation: At the main research labs where activity perform, 500 ml of Potato dextrose broth (PDB) was added to a 1000 ml Erlenmeyer flask. The sterile media were sterilized for 15 minutes at 121°C. After sterilization, 10% inoculum was added to the PDB medium that had already been made. Inoculated flasks were put in an orbital shaker incubator and kept at 25°C for two weeks at 120 rpm (Liu, *et al.*, 2015).

Secondary Metabolite Extraction: When the process of fermentation was done. 10% ethyl acetate solution, the medium with the fungus and metabolites was mixed well (Pena *et al.*, 2010).

Solvent Extraction Technique: Organic solvents like methanol and ethyl-acetate were utilized to separate the secondary metabolites from the rest of the mixture. After 10 min of mixing, the mixture was left at a steady boil for 5 minutes (Al-Daamy *et al.*, 2018). After 5 minutes, the container contained two separate, immiscible layers. As the pellet of fungal crude extract included our target substance, we isolated and utilized the organic solvent layer above it. The organic solvent was evaporated with the use of a rotary evaporator. After that leftover extract and the evaporated organic solvent were both employed to create the secondary metabolite that was needed (Pena *et al.*, 2010).

Biosynthesis of Silver Nanoparticles: A total of 10 grams of the crude extract of the fungus was mixed with 100 ml of double-distilled water (ddH2O) and incubated at 25°C with continuous agitation at 120 rpm

in a 250 ml flask for 48 hours. During a 48-hour incubation period, the cell filtrates were collected by passing them through Whatman filter paper. The flask containing the filtrates had 1 mM of silver nitrate solution (AgNO3) added to it, and then the flask was placed in a dark room at 28°C temperature for 24 hours (Prabhu, *et al.*, 2012).

Mycosynthesized Silver Nanoparticles Characterization by X-ray Diffraction: Ultra-violet visible spectroscopy, TEM, FTIR, and XRD spectroscopy were used to characterize mycosynthesized AgNPs in accordance with standard operating procedures. In the UV-visible spectroscopy using a spectrophotometer was used to confirm the synthesis of AgNPs. SEM), XRD, and FTIR were used to characterize AgNPs (El-Rafie, *et al.*, 2010).

Sample Evaluation: sterile cotton swabs were used to collect human pathogenic samples from patients at different hospitals, labs, and healthcare centers. Pathogenic bacteria were grown and identified in the bacteriology section of the microbiology lab.

RESULTS

Characterization of *A. fumigatus* Extract-Synthesized AgNPs:

1. Spectrophotometry of Ultraviolet and Visible Light: When the fungal extract was added to the solution containing the silver nitrate, the color changed from a light yellow to a greyish color. As can be seen in Figure 2**,** there was a significant peak at 432 nm that was distinct from the AgNP synthesis process (Gajbhiye, *et al.*, 2009).

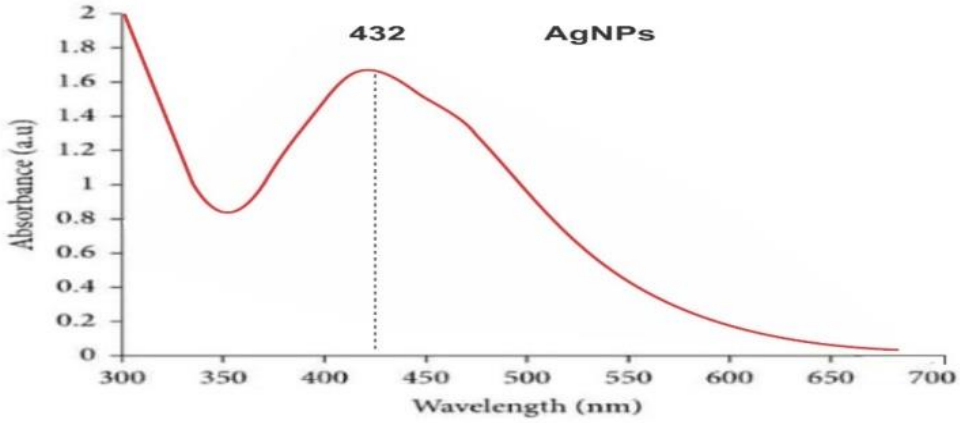

Fig. 2: The UV-visible spectra of AgNPs synthesized from *A. fumigatus* crude extract show a peak at 432 nm.

2. Synthesized AgNPs in SEM Micrograph: According to the SEM analysis, the structure of the silver nanoparticles AgNPs was almost spherical and round (Pena *et al.*, 2010). The formation of nanoparticle aggregates suggested that the produced AgNPs had become more stable. As can be seen in the picture, scanning electron micrographs demonstrate that the silver nanoparticles have a spherical form, and the observed sizes range from 15 to 90 nm. **(**Fig. 3**)**.

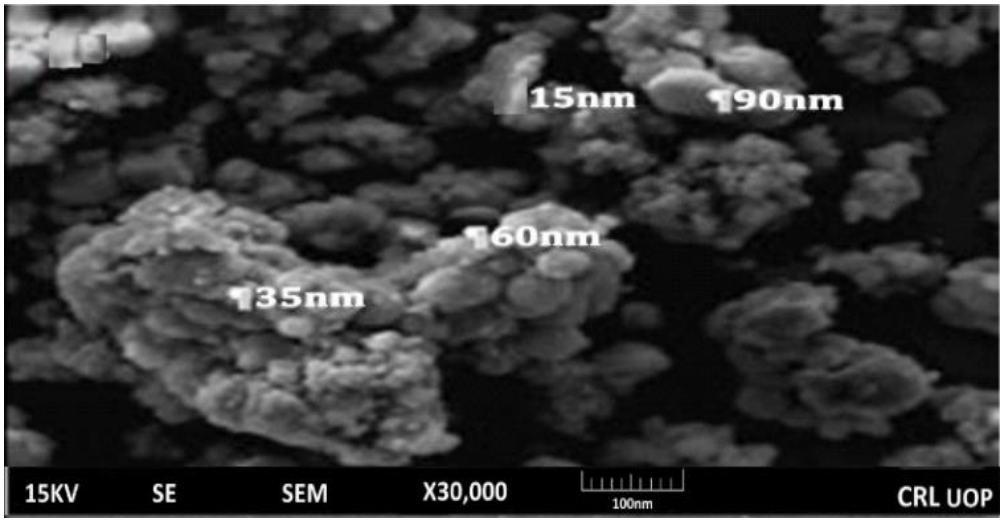

Fig. 3: SEM micrograph of synthesized AgNPs.

3. TEM (Transmission Electron Microscopy) analysis: TEM analysis was used to record the exact dimensions and morphology of the silver nanoparticles that were synthesized. TEM micrographs indicate clearly that the structure of pentagonal bio prisms is divided and sphere-shaped, and their size is greater than 100 nanometers (Gajbhiye, *et al.*, 2009). The result of our TEM analysis is 25 nanometers, as shown in Figure 4.

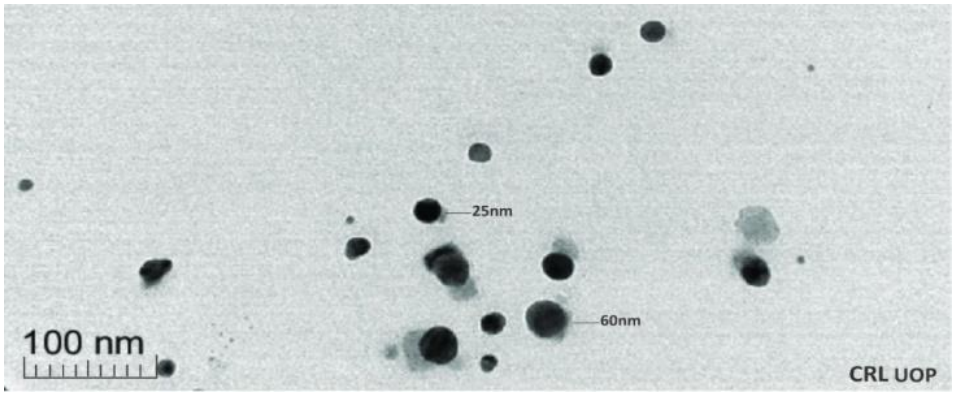

Fig. 4: TEM shows a scale 25 to 100 nm in size.

4. Characterization of XRD AgNPs Synthesized from the Extract of *A. fumigatus*: The XRD analysis showed that the sample was homogeneous and powder in form and crystalline AgNPs were produced during the synthesis process (Mahmoud, *et al.*, 2023). Crystalline reflection planes (110), (111), (200), and (211), (220), (311) were indexed to the four separate diffraction peaks at 2 values. The XRD analysis showed that the sample was homogeneous and finely ground. Crystalline AgNPs were produced during the synthesis process (Pena *et al.*, 2010). Crystalline reflection planes (110), (111), (200), and (211), (220), (311) were indexed to the four separate diffraction peaks at second values as shown in Figure 5.

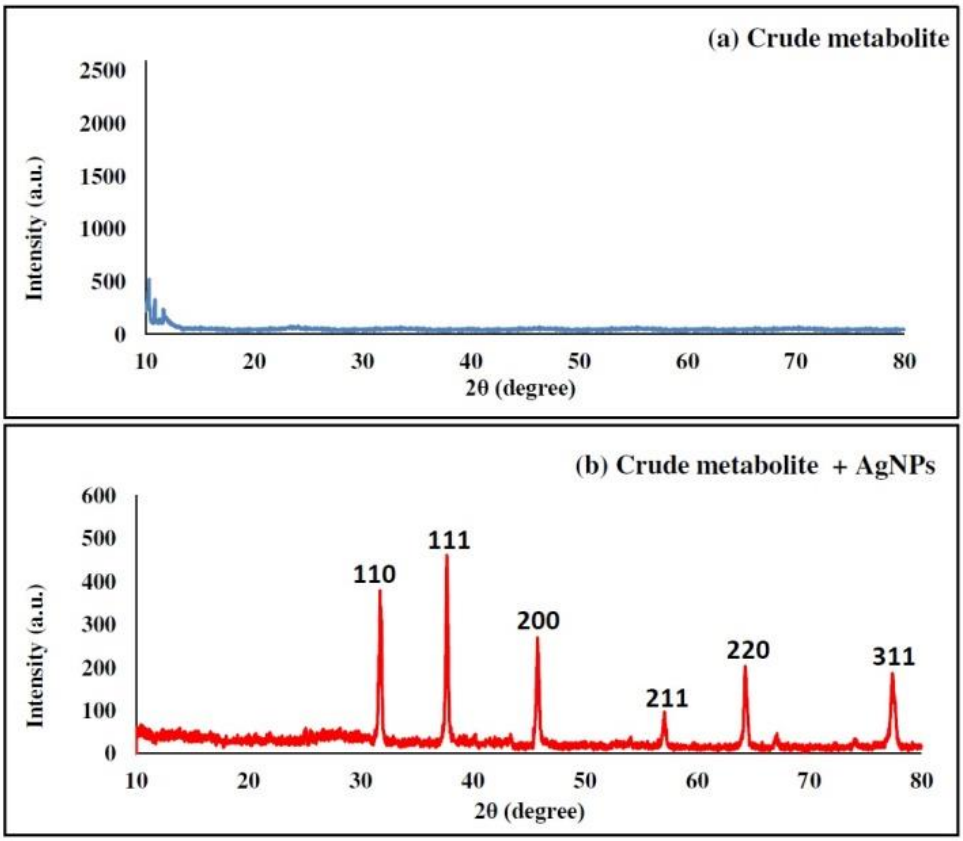

Fig. 5: XRD spectra displaying the crystalline-like nature of synthesized AgNPs.

5. Fourier Transform Infrared Spectroscopy (FTIR) of Fungal Metabolite and Synthesize Nanoparticles:

Silver's interaction with bioactive chemicals that create and stabilize silver nanoparticles were examined using FTIR spectroscopy. During the FTIR analysis of crude, numerous peaks of interest were discovered. The extract of *A. fumigatus* and artificially produced AgNPs. The FTIR spectrum contains peaks for amines, carboxylic acids, and alkenes, among many other functional groups. This assisted in the capping of AgNP as well as its stability and synthesis. The frequency band at which the peaks were seen in the present investigation was as follows: 1040, 1245, 1545, 1640, 1720, 1900, 2200, 3000, 2900, and 3300 cm-1 (Pena *et al.*, 2010). Among the functional groups that were shown as alcohol, alkanes, carboxyl groups or ethers, amine groups, alkanes, aromatic amines or phenol, and amines. After the reaction with AgNO3, new peaks were discovered, exhibiting carboxylic, OH, and amide groups whereas alkenes, alkanes, alcohol, and phenol disappeared following the synthesis of AgNPs inside the crude extract of *A. fumigatus*, which played a vital role in the synthesis of silver nanoparticles AgNPs as shown in Figure 6.

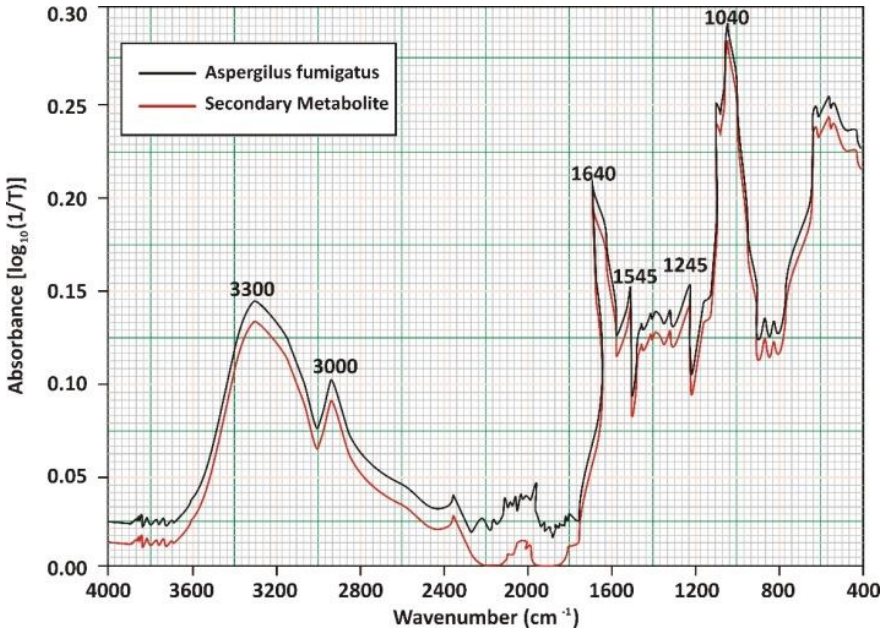

Fig. 6: The FTIR spectrum of synthetic AgNPs and crude *A. fumigatus* extracts**.**

Antimicrobial Activity of Fungal Crude Extract:

The extract's antibacterial activity was examined using the agar well diffusion assay method against a wide range of bacteria (clinical isolates), and the results revealed varied zones of inhibition for each different pathogen. The wells were loaded with varied concentrations of 50 µL and 100 µL (Mahmoud, *et al.,* 2023). The greatest zone of inhibition was reported at varied bacterial concentrations. Maximum bacterial concentration-inhibited zone sizes were reported (Tasleem, *et al.,* 2022). The zone of inhibition produced by a 50 µL concentration of crude ethyl acetate extract was 16±0.14 mm against *Escherichia coli spp,* 19±0.3 mm against *Pseudomonas aeruginosa spp,* 18± 0.4 mm against *Staphylococcus aureus,* and 14 ± 0.34 mm against *Salmonella spp* (Momenah, *et al.,* 2023). While the zone of inhibition was measured in 100 µL, it was found to be 21 ± 0.15 mm for *E. coli spp,* 22 ± 0.16 mm for *Pseudomonas spp,* 24 ± 0.14 mm, *S.aureus spp,* 18 ± 0.14 mm and *Salmonella spp* 18 ± 0.16 as shown in Table 1.

Table 1: Antibacterial activity of fungal crude extract.

Bacterial Strains	Fungal Metabolites		Standard Deviation 50 µl	Standard Deviation 100 µl	Average 50 µl	Average 100 µl	Ciprofloxacin
	50 µl	100 µl					
E. coli spp	16	21	0.14mm	0.16mm	16.4	18.40	25
Pseudomonas spp	19	22	0.3mm	0.14mm	18.3	21.53	28
S. aureus spp	18	24	0.4mm	0.18mm	17.7	22.3	29
Salmonella spp	14	18	0.34mm	0.16 mm	15.9	18.34	23

Synthesized Silver Nanoparticles AgNPs Antibacterial Activity:

An agar well diffusion experiment was utilized to examine the antimicrobial activity of AgNPs against a variety of bacteria (**Fig. 7**). The wells in the assay were loaded with varying doses of AgNPs in two different volumes (50 µL and 100 µL) (Khan, S *et al.,* 2022). This research found that the different zone of inhibition occurred at varying concentrations depending on the bacteria strain. The

maximum activity of silver nanoparticles AgNPs zone of inhibition by a 50 µL concentration against *E. coli spp,* 23 ± 0.35 mm, *Pseudomonas spp,* 23 ± 0.36 mm, *S,aureus spp,* 22 ± 0.35 mm,, and *Salmonella spp,* 18 ± 0.32 mm, respectively, on the other hand, the activity of 100 µL zone inhibition was observed 27 ± 0.75 mm, 27 ± 0.75 mm, 28 ±. 0.43 mm, 23 ± 0.91 mm, zone of inhibition against *E. coli spp, P,aeruginosa spp, S,aureus spp,* and *S, thyphi spp,* respectively as shown in Table 2 and Figure 8 (Dablool, *et al* 2023).

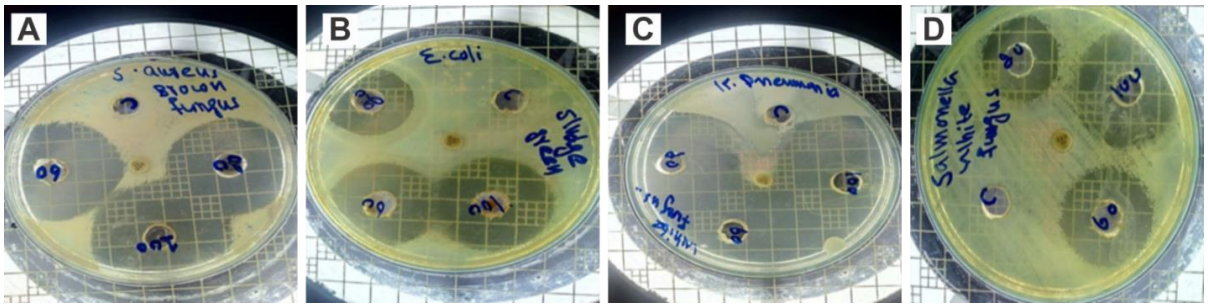

Fig. 7. Synthesized silver AgNPs and fungal metabolites activity.

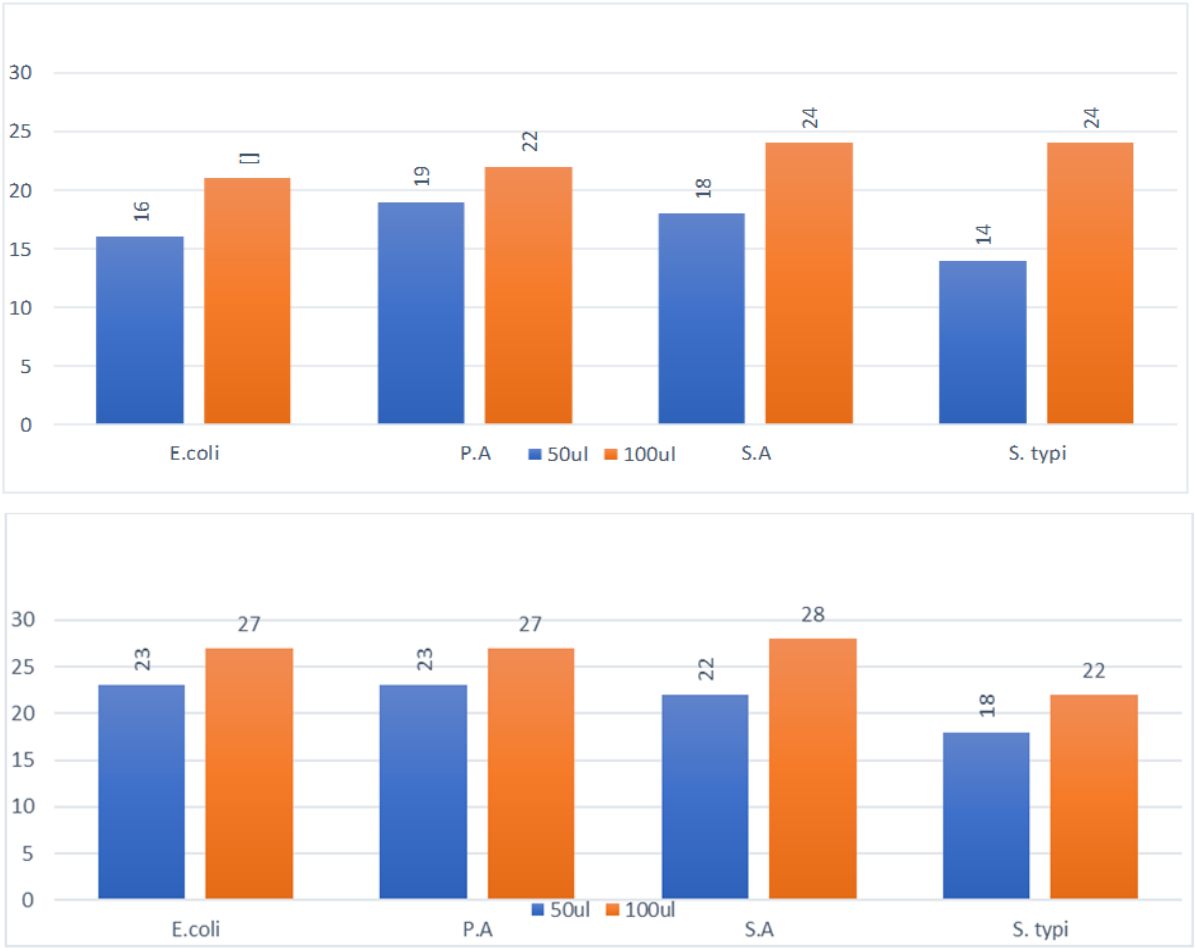

Fig 8. Key: *E. coli spp (Escherichia coli spp), P. A spp (Pseudomonas aruginosa spp), S.A spp (Staphylococcus aureus spp), S.typi spp,(Salmonella typhi spp).*

Table 2: Synthesized silver nanoparticles (AgNPs) having antibacterial activity.

Bacterial Strains	Synthesized silver nanoparticles (AgNPs)		Standard Deviation	Standard Deviation	Average	Average	Ciprofloxacin
	50 µl	100 µl	50 µl	100 µl	50 µl	100 µl	
E. coli spp	23	27	0.38mm	0.70 mm	22.7	23.9	22
Pseudomonas spp	23	27	0.81mm	0.70mm	22.6	23.1	24
S. aureus spp	22	28	0.58mm	0.82 mm	21.5	25.5	25
Salmonella spp	18	23	0.56mm	0.97 mm	21.5	23.5	24

DISCUSSION

A particle size that is 100 nm or less might be considered to be a nanoparticle and developing of silver nanoparticles AgNPs, which are environmentally friendly, and acceptable and non-toxic in nature. Nanoparticles, work as a substrate and as well as a transporter for antibiotics (Uzair, *et al.,* 2018). The production of nanoparticles relies heavily on microbes and their natural antibacterial compounds, despite their biological uses of nanoparticles can be attributed to their manufacturing through microbes. Most credible nanoparticles are silver nanoparticles and it's non-cytotoxic in nature. AgNPs can treat bacterial and fungal infections as well as a transporter of drugs (Mahmoud, *et al.,* 2010). In this research, different fungal species were isolated from the plant, soil, and *Aspergillus spp* was chosen to produce secondary metabolites. *Aspergillus spp* was identified morphologically and microscopically with the help of lactophenol cotton blue techniques. To create mycochemicals, isolated *Aspergillus spp* were allowed to undergo fermentation, (Khan *et al.,* 2022), afterwards metabolites were extracted using ethyl acetate. Ethyl-acetate extracts contained steroids, terpenoids, alkaloids, flavonoids, and tannins. In this study, silver nanoparticles were produced from *A. fumigatus* extract and evaluated by UV-visible spectroscopy, FTIR, SEM, TEM, and XRD. The sample absorbed energy at 432 nm, AgNPs' max value, according to UV-visible spectroscopy. *A. fumigatus* extract's Ag+ NP content affects AgNP absorption. The configuration of AgNP was analyzed using an SEM technique and the shape was a spheroidal and uniform distribution of the silver nanoparticles (Hariri, *et al.,* 2023). AgNPs synthesized with *A. fumigatus* extract were confirmed by 30,000X SEM micrographs. These micrographs showed that the particle sizes ranged from 15-100 nm. The TEM results showed that *A. fumigatus* extract was a potent reducing agent, creating spherical. AgNPs and TEM studied nanoparticles were between 25 to 60 nm. Moreover, XRD was a fast analytical method used to determine a crystalline material's phase (Pena *et al.,* 2010). There were many peaks in the FTIR spectrum that corresponded to amines, carboxylic acids, and alkanes that produced and stabilized AgNPs. In this study, the antibacterial effectiveness of mycosynthesized silver nanoparticles and fungal crude metabolites was tested in two different concentrations 50 µL and 100 µL were evaluated against all known human pathogens (Jalal, *et al.,* 2023). The crude metabolite ethyl acetate extract demonstrated significant inhibitory zones between 15 mm and 31 mm against all of tested isolates. The inhibitory zones significantly expanded when we utilized more crude metabolites against these isolated bacteria.

CONCLUSIONS

These results led to the following inferences about the crude ethyl acetate extract. It contained a variety of phytochemicals, such as tannins, terpenoids, and flavonoids (Khan. S, *et al.,* 2010). The UV-visible spectroscopy was able to confirm the synthesis of AgNPs at a wavelength of 432 nm, suggesting that *A. fumigatus* extract may be able to decrease

silver during room-temperature AgNPs synthesis (Al-Abdullah *et al.*, 2023). The size of the produced AgNPs was reported to be on the nanoscale by the SEM micrograph. According to the results of an FTIR examination, the phenolic, carboxyl, and hydroxyl groups of *A. fumigatus* extract were responsible for the reduction of silver, whereas the amide linkage amino acid was responsible for the stabilization of AgNPs (Pena *et al.*, 2010). According to the results of the XRD analysis, the naturally occurring structure of the AgNPs was crystalline in nature. TEM images exhibited a spherical shape for the AgNPs**.**

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