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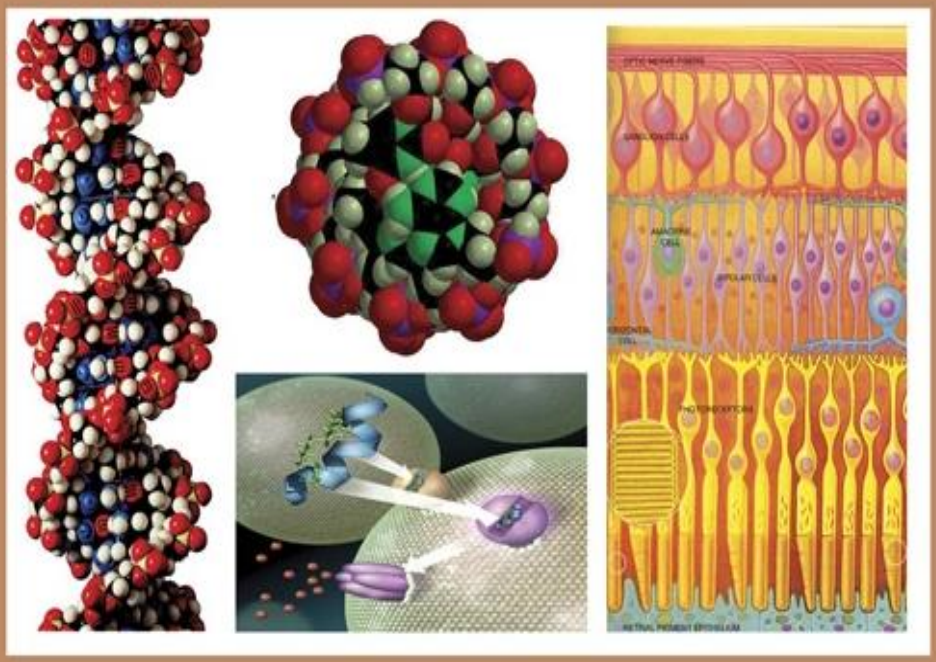

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Efficacy of Silver Nanoparticle-Coated Crude Extract of *Aspergillus fumigatus* Against Bacterial Infections from Surgical Sites Wound

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ABSTRACT

The chemical reduction was used to make nanoparticles of silver. Different methods, such as UV-Vis absorption spectroscopy, were used to observe the silver nanoparticles being formed. The UV-spectroscopy showed that Plasmon absorption was between 408- and 420 mm. silver nanoparticles are made of molecules and are about 410 nm in size. We used X-ray diffraction (XRD), transmission electron microscopy (TEM), and UV–Vis spectroscopy (UV–Vis) to look at the nanoparticles. The X-ray diffraction analysis of the nanoparticle dispersion provided evidence that elemental silver was present in the sample. There were no peaks found that belonged to any other impurity. Transmission electron microscopy (TEM) was used to determine the consistent dimensions and morphology of silver nanoparticles appropriately. TEM image shows restricted size distribution (40 and 50 nm). The XRD pattern shows crystalline and cubic metallic silver peaks. The Kirby-Bauer method determined nanoparticle dispersion antibacterial activity. It was observed that silver nanoparticles possess potent antibacterial and bactericidal capabilities against gram-negative bacteria and gram-positive bacteria such as Escherichia coli, *Pseudomonas aeruginosa*, and staphylococcus aureus. When assessed on *E. coli*, the greatest antibacterial activity revealed a zone of inhibition measuring 17 mm. In contrast, it was observed that the antibacterial activity of ethyl crude extract at a concentration of 50 l was very low when it was tested against S.typhi (13mm). The highest antibacterial activity against *E. coli* was seen at 100 ml, which indicated a zone of inhibition measuring 23 mm. At a concentration of 100 ml ethyl acetate crude extract and AgNPs, a zone of inhibition measuring 25 mm was seen against *S. typhi*. While a zone of inhibition measuring 29 mm was seen against *E. coli* when 100 microliters of ethyl acetate crude extract and AgNPs were used, respectively.

INTRODUCTION

Nanoparticles have obtained a lot more attention than other sizable materials because they have different properties (Bala & Arya, 2013; Farjana *et al*., 2014). Nanotechnology (NT) is a significant molecular technology that can operate on atoms, molecules, and supramolecular levels mostly in the range of 1-100 nm. Biological systems can be manufactured, enhanced, and utilized with the help of this technology; NT concepts and bio-assembled components are provided as examples by the scientific community.

In microbiology, silver plays a crucial role as a bactericide. Nanotechnology (NT) enhances the chemical and physical properties of particles due to the larger number of surface atoms present in nanoparticles compared to microparticles. There are a few different chemical and physical methods that may be used to produce silver nanoparticles (also known as Ag-NPs), However, all of them have certain downsides due to the enormous amount of energy that is required to maintain high pressure and temperature. These drawbacks are caused by the necessity of maintaining high pressure and temperature. It is known that the process of synthesis makes use of several hazardous compounds, which may be harmful to human beings (Khalil *et al*., 2021; Khan *et al*., 2018). Some researchers, in response to the emergence and spread of several antibiotic-resistant microbes as well as the ongoing emphasis on the expenses of health care, have been working on developing new antibiotics. The development of antimicrobial drug resistance in bacteria and fungi has led to a rise in commercial costs and has become an issue on a global scale. Drug resistance is a major issue in all developing nations. It's potential that antiseptics based on silver, in contrast to antibiotics, have a broader range of potential applications and a lesser risk of developing resistant microbes (Li *et al*., 2011; Liu *et al*., 2015). Ag salt has been utilized as an antibacterial agent since the old period, and it is currently utilized to stop bacteria from growing in a variety of applications, including dental treatments, catheters, and inflammations, among many others (Mansori & Soelaiman, 2005). Aspergillus fumigatus is a type of fungus that is a member of the Aspergillus genus. Other families of the Aspergillus genus include Aspergillus oryzae and *Aspergillus nidulans*. Many species of the genus Aspergillus are capable of producing antibacterial compounds, such as aspergillic acid from *A. flavus*, penicillic acid from *A. ochraceus*, and fumagillin from *A. fumigatus* (Nierman *et al*., 2005; Pena *et al*., 2010). Keeping these facts in mind the present investigation was envisaged to test the effectiveness of a silver nanoparticle-coated crude extract of *Aspergillus fumigatus* against bacterial infections after surgery.

MATERIALS AND METHODS

Isolation of Fungus from Samples: Samples were taken from different locations.

Batch fermentation: An orbital incubator at 120 rpm grew the inoculation flask at 250°C for two weeks. Added, 500 mL potato dextrose broth medium (PDB) with 1000 mL Erlenmeyer flask. 15 minutes at 15 pressure heated sterile media to 121°C. Sterilize the PDB medium and add 10% inoculum. **Secondary Metabolite Extraction:** After fermentation, a 10% ethyl acetate solution was added to homogeneously mix the media containing the fungus and metabolites.

Biosynthesis of Silver Nanoparticles: At room temperature (25 ° C), mix around 10 g of the fungal crude extract with 10 mm of double-distilled water Leave sit for 48 hours. With the help of a Whatmann filter paper, we were able to purify get the extract. The Extract from silver nanoparticles was added to a solution of silver nitrate (99.9%) and 1 mM water**.** The pH was maintained at 8.0. 1 to 2 hours were devoted to shaking the conical flasks at 100 rpm in the dark at 25°C. A change in the solution's color was noted**.**

Characterization of Myco-Synthesized Silver Nanoparticles: UV-visible Spectroscopy was performed using a Perkin-Elmer Lambda 2 Spectrophotometer (UV-Vis). TEM and high energy electron diffraction (HEED) were used on a Phillips CM20-Ultra Dual microscope running at 200 kV to analyze the size, shape, and composition of the nanoparticles. The TEM images were used to create histograms of size distribution by measuring the diameters of at least 50

particles. Samples for TEM studies were prepared at the public health research lab (PHRL) of Khyber Medical University in Peshawar, Pakistan, by dropping silver nanoparticle solutions on carbon-coated TEM grids.

Evaluation of Surgical Wound Samples: Patients' surgical wound samples were collected using sterile cotton swabs at multiple hospitals, and research centre.

RESULTS

Characteristics of AgNPs Synthesized from the Extract of *A. fumigatus*:

UV and Visible Spectrometry: The use of UV-visible spectroscopy to characterize silver nanoparticles is one of the most common and widely employed structural characterization methods. Changes in color from pale yellow to brown were observed. At 410 nm, the existence of spherical particles as Ag nanoparticles were identified, confirming the synthesis of silver nanoparticles (Fig. 1**)**.

SEM Micrograph Showing the Synthesis of AgNPs: The structure of AgNPs was almost spherical, according to the SEM analysis. SEM analysis of AgNPs confirmed their spherical shape. The spherical silver nanoparticles in this scanning electron microscopy (SEM) picture are 20–100 nm in size. as shown in **Figure 2**.

TEM imaging confirmed: This image shows nanoparticle aggregations and microscopic particles. Silver particle size distributions reveal a diameter of 24 nm and a range of 8 - 50 nm **(**Fig. 3).

***A. fumigatus* Extract-Synthesized XRD AgNPs:** The evaluation of the XRD spectrum showed that the sample depth was homogenized, and it showed that the materials were consistent throughout. In their natural state, the AgNPs are crystalline. Four distinct peaks at 2 values of 38.45, 46.35, 64.75, and 78.05 were indexed to the crystalline structure's (111), (200), (223), and (311) reflection planes as shown in Figure 4.

Fungal Metabolite and Nanoparticle Synthesis Using Fourier Transform Infrared Spectroscopy (FTIR): FTIR spectroscopy was utilized to explore the hypothesized interaction that occurs between silver and the bioactive molecules that are important for the formation and stability of silver nanoparticles. The FTIR analysis of the crude extract of *A fumigatus* and the produced AgNPs revealed distinct peaks in each of these two samples**.** Amines, carboxylic acids, and alkenes are all detectable in the FTIR spectrum, and each of these hydroxyl groups contributed to the synthesis, capping, and stabilization of AgNPs. Within the scope of the present investigation, functional groups are indicated by peaks that occur within the ranges of 510, 775, 822, 916, 1005, 1050, 1100, 1210, 1230, 1280, 1500, 1535, 1570, 1692, 2620, 2845, and 3630 cm-1. Examples of functional groups include alcohol, alkanes, carboxylic acid or ester, amide, alkanes, aliphatic amines or phenol, and amines. As a result of the reaction with AgNO3, new peaks formed. Crude extract from *A. fumigatus*, which was involved in the synthesis of AgNPs, contains Carboxylic, hydroxyl, and amide groups important uses alkenes, alkanes, alcohol phenol, and these vanish following AgNPs manufacture shown in Figure 5.

The Fungal Crude Extract Has Antimicrobial Activity: The agar well diffusion assay method was used to test the extract's antibacterial activity against a variety of pathogenic (clinical isolates) bacteria. These bacteria showed different zones of different bacterial counts, with the maximum zone of inhibition being reported. Ethyl acetate crude extract at 50 µL has the zone of inhibition concentrations of *E. coli* sp 17 ± 0.15 mm, *Pseudomonas sp* 18 ± 0.10 mm, *Klebsiella* sp, 19 ± 0.14 mm, Proteus 18 ± 0.15 mm, *S. aureus*, 17 ± 0.45 mm, *Salmonella* sp, 13 ± 0.9 mm, respectively. While in the 100 µL zone of inhibition, 23 ± 0.15 mm, 24 ± 0.10 mm, 22 ± 0.14 mm, 24± 0.26 mm, 26 ± 0.45 mm 18± 0.27 mm zone of inhibition against *E. coli* sp, *Pseudomonas aeruginosa Klebsiella sp, S. aureus*, and *Salmonella* sp,

respectively, as indicated in Table 1.

Antibacterial activity of synthesized AgNPs:The agar well diffusion assay method was used to test AgNPs' antibacterial activity against many pathogens. Each pathogen had different zones of inhibition. 50 µL and 100 µL wells were loaded. For different bacterial concentrations, the highest inhibitory zone. Synthetic AgNP activity. *E.coli, Pseudomonas, Klebsiella,* Proteus *S. aureus,* and *Salmonella* had inhibitory zones of 26 ± 0.36 mm, 28 ± 0.88 mm, 29 ± 0.47 mm, 26 ± 0.60 mm, 31 ± 0.56 mm, and 20 ± 0.98 mm. at 50 µL concentrations, respectively. *E.coli sp, Proteus sp, P.aeruginosa, S. aureus, Klebsiella sp, and Salmonella sp* were 29 ± 0.75 mm, 32 ± 0.75 mm, 29 ± 0.47 mm, 30 ± 0.98 mm, 33 ± 0.80 mm, and 25 ± 0.98 mm, respectively, in the 100 µL zone of inhibition, respectively, as shown in Table 2 and Figure 6.

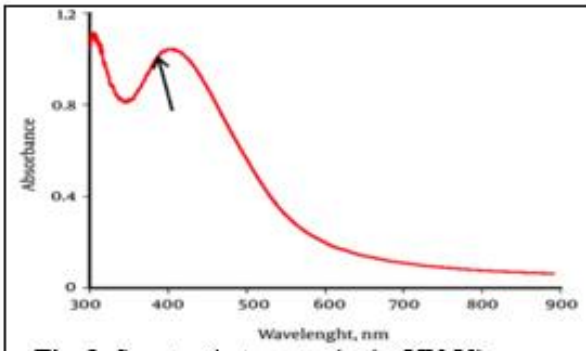

Fig. 1. Spectrophotometry in the UV-Vis range

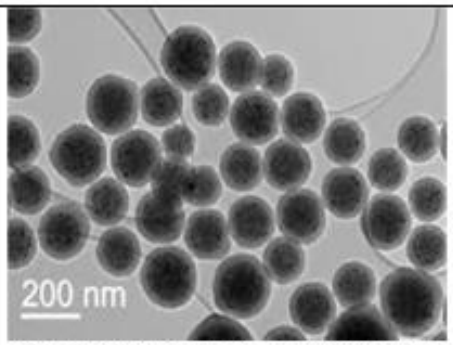

Fig 2. SEM micrograph of synthesize AgNPs

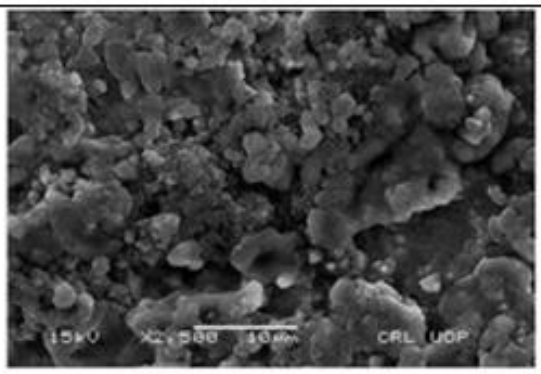

Fig 3. TEM imaging

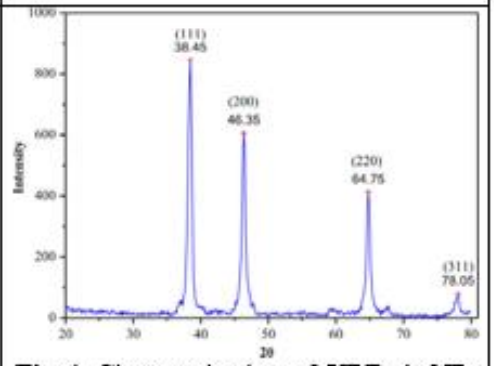

Fig 4. Characterization of XRD AgNPs synthesized from the extract of *A. fumigatus*

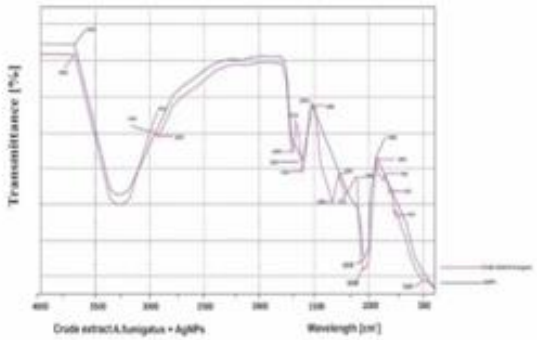

Fig 5. Fungal metabolite and manufacture nanoparticles using Fourier Transform Infrared Spectroscopy (FTIR)

Table 1. Antibacterial activity of Fungal crude extract of *Aspergillus fumigatus*

Bacterial Strains	Fungal Metabolites		Standard Deviation 50 µl	Standard Deviation 100 µl	Average 50 µl	Average 100 µl	Ciproflox acin'
	50 µl	100 µl					
E. coli	17	23	0.15mm	0.17 mm	19.6	17.3	25
P. aeruginosa	18	24	0.10mm	0.41 mm	18.2	21.63	25
K. pneumoniae	19	22	0.14mm	0.36 mm	16.8	19.66	23
P. mirabilis	18	24	0.16mm	0.27 mm	19.2	20.6	24
S. aureus	17	26	0.18mm	0.44 mm	17.6	22.4	31
S. typhi	13	18	0.9mm	0.24 mm	15.7	18.33	22

Table 2. Antibacterial activity of AgNPs and Fungal crude extract of *Aspergillus fumigatus*

Bacterial Strains	Synthesized silver nanoparticles		Standard Deviation 50 µl	Standard Deviation 100 µl	Average 50 µl	Average 100 µl	Ciproflo satin
	50 µl	100 µl					
E. coli	26	29	0.36mm	0.76 mm	23.7	23.8	25
P. aeruginosa	28	32	0.87mm	0.77mm	21.6	23.4	29
K. pneumoniae	29	31	0.46mm	0.46 mm	24.3	24.4	28
P. mirabilis	26	30	0.62mm	0.97 mm	22.4	26.2	29
S. aureus	31	33	0.58mm	0.81 mm	21.5	25.6	30
S. typhi	20	25	0.53mm	0.96 mm	22.5	23.3	24

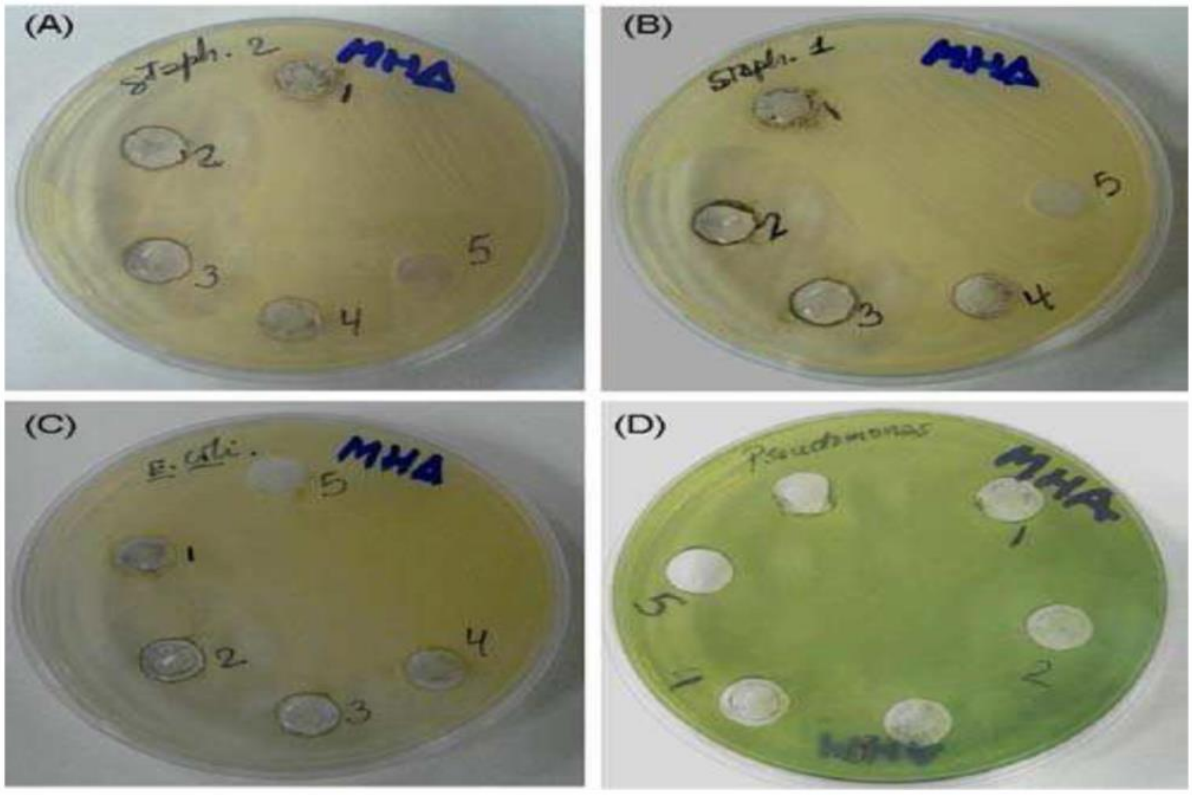

Fig 6. Antimicrobial Activity of Fungal crude extract and AgPs.

DISCUSSION

A particle's diameter must be 100 nm or smaller for it to be considered a nanoparticle according to this definition (Pinruan *et al.*, 2007). Nanoparticles operate as both a medium and a carrier for antibiotics and other naturally occurring substances that are antibacterial (Prabhu & Poulose, 2012; Rashmi *et al*., 2005). This allows for the production of nanoparticles

that are non-toxic and safe for the environment. When it comes to the production of nanoparticles, microorganisms play a crucial role. The use of microbes to synthesize nanoparticles expands their biological uses. Silver nanoparticles are the most significant and dependable metal nanoparticles.

In addition to serving as a drug carrier and having a wide range of therapeutic applications, AgNPs are also among the least cytotoxic. The SEM technique was used to determine the AgNPs' overall topology. *A. fumigatus* extract was used to synthesize AgNPs, and The AgNPs' monodispersed and spheroidal topology was confirmed by 30,000X SEM micrographs of 15-100 nm particles (Sethi *et al*., 2013).

Conclusion

It is concluded from the investigation that all the examined pathogens had responded favorably to the $AgNO_3$ nanoparticle which resulted in enhanced bacteriostatic effectiveness. The increase in the quantity and size of $AgNO_3$ nanoparticles also responded to increased antibacterial activity. The ZnO nanoparticles have the potential to be effective antibacterial agents based on the fact that their zones of inhibition are effective against Gram-positive and Gram-negative bacteria.

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