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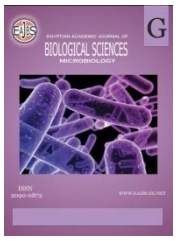

Silver Nanoparticles Synthesized from *Polyporus plorans*, A wild Mushroom with Detecting Its Physio-Chemical Characterization and Antimicrobial Activities.

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ABSTRACT

This study aimed to biosynthesize silver nanoparticles (AgNPs) using the aqueous extract of *Polyporus plorans*. The properties of the synthesized AgNPs were studied by UV-Vis, FTIR, AFM and SEM. The total antioxidant capacity was also detected for the first time in the world. The results showed that the AgNPs solution revealed an absorption maximum at 420 nm by ultraviolet-visible spectrum. Fourier transform infrared spectroscopy (FTIR) results showed that the AgNPs solution contained many functional groups belonging to the carbonyl group (C = O). The atomic force microscope (AFM) showed synthesized AgNPs were spherical in shape and the average size of the nanoparticles was 88.9 nm. The results of the scanning electron microscope (SEM) showed the prepared silver nanoparticles were in nano sizes 36.61-59.30 nm. The prepared AgNPs exhibited different antimicrobial activities against the diverse microorganisms at concentrations of 100%, 50%, and 25%. The AgNPs including pathogenic bacteria, *Escherichia coli* and *Staphylococcus aureus*, and some yeasts, *Candida galbrata*, *C. krusei*, *C. tropicalis*, *C. albicans*, and two dermatophyte species, *Trichophyton rubrum* and *T. mentagrophyte*. The results showed that the highest inhibition was for bacteria and dermatophytes at 100%, while for *Candida* it showed an inhibitory effect against *C. galbrata* and *C. krusei* only.The results of the antioxidant activity test showed an increase in its anti-oxidant effectiveness with an increase in the concentration solution. The results of current study indicated that the synthesized AgNPs from *P. plorans* may be developed as an effective agent against bacterial and fungal infections.

INTRODUCTION

Macrofungi include all fungi with sexual fruiting bodies observable by the unaided eye with about 14,000 species globally (Lu *et al.*,2020). These fruiting bodies usually grow either epigeous or hypogeous, constituting 1.2% of the supposed total number of fungi (Mueller *et al.*,2007) Macrofungi mostly belong to the basidiomycota and ascomycota, and few of them belong to the Zygomycota (Mueller *et al.*,2007).

Macrofungi are characterized by their nutritional value due to their high quality and content of carbohydrates, fibers, proteins, vitamins, minerals and secondary metabolites. Thus, a variety of macrofungi were used as edible sources such *Pleurotus* spp., *Agaricus* spp. and nutritional supplements such as vitamins and minerals (Shashkina *et al.*,2006).

Several studies have shown the effectiveness of its anti-inflammatory, antitumor, antiviral, antibacterial, antioxidant and liver diseases, in addition to its effectiveness in reducing hypotensive blood pressure and hypercholesterolemia (Bernardshaw *et al.*,2005).

In recent years, the manufacture of nanoparticles from edible and medicinal macrofungi, known as myconanotechnology, has received much attention from researchers. Sudheer *et al.* (2022) indicated that the high content of active compounds such as polysaccharides, enzymes and proteins nominated them as one of the most important reducing and coating agents in nanoparticle manufacturing processes. Macrofungi are environmentally friendly biofactories that have the ability to reduce metal ions to zero-valent or nano form (Kalia & Kaur, 2018), as well as the advantages of nanoparticles prepared from them, such as higher stability and longer shelf-life.

Studies indicate that most of the edible macrofungi that were used for the preparation of nanoparticles are basidiomycetes (Diego & Pardo-Gim´enez, 2017; Aygun *et al.*,2019) such as *Pleurotus* spp. and *Ganoderma* spp. (Martnez-Flores *et al.*,2020). Its preparation by biological methods or the so-called green technology is the most acceptable and eco-friendly method, which is less expensive, simple, repeatable, and releases less toxic environmental waste compared to its preparation by expensive and toxic physical and chemical methods (Khan *et al.*, 2018; Singh *et al.*, 2018). Based on what was mentioned, the study aimed to manufacture silver nanoparticles from the basidiomycetous *Polyporus plorans* for the first time in the world, as well as to detect their biological activity against human pathogenic microorganisms.

MATERIALS AND METHODS

Preparation of Aqueous Extracts from *P. plorans*:

The fresh basidiocarps of wild *Polyporus plorans* were collected and processed as in (Marie *et al.*, 2023) .Twenty grams of the *P. plorans* powder was soaked in 120 ml of deionized water and incubated at a temperature of 50-55 °C for 48 hours in a shaker water bath. The aqueous extract of the mushroom was filtered twice using Whatman No.1 filter paper and several layers of medical gauze. The collected filtrate was stored at a temperature of 4 °C until being used to prepare the nano solution (Narasimha *et al.*, 2011).

Biosynthesis of Silver Nanoparticles (AgNPs):

The synthesis of AgNPs was prepared by adding 50 ml of previous *P. plorans* extract into 950 ml of silver nitrate (1 mM, AgNO3) solution to reduce Ag^{+} to Ag^{o}. The mixture was agitated continuously under a temperature of 30 °C for 30 minutes. After that, the solution was filtered using plastic filters with a diameter of 0.22 microns several times. Then, it was transferred to the pelvic ultrasonic device under ultrasonic conditions, with an ultrasonic power of 100 watts and a frequency of 42 kHz for 20 minutes at 25 °C. The sonicated solution was incubated in a shaking incubator at 25 °C in the dark for 120 h (Alnuaimi et al.,2019). During the incubation period, the color changes that may occur in the color of the solution were monitored, which is evidence of the formation of silver nanoparticles as a result of the reduction process.

Purification of Biosynthesized AgNPs:

After obtaining the heterochromia, the solution containing prepared silver nanoparticles was placed in aerosolized test tubes and then in a refrigerated centrifuge at a speed of 10000 rpm for 10 minutes to obtain a clear liquid (Agrawal *et al.*, 2014).

Characterizations of Biosynthetic Silver Nanoparticles:

Spectrophotometer UV-Vis:

The biosynthetic silver particles were confirmed, and their optical properties were determined using UV-visible spectroscopy. This process included taking 2 ml of the reaction mixture and diluting it ten times with deionized water to reduce false readings. The absorbance values of the biosynthetic silver

particles were detected and recorded by calibrating the absorption spectra within 190–850 nm (Al-Shammari& Al-Zubaidi,2016).

Atomic Force Microscopy (AFM):

Atomic force microscopy works by fixing nano sizes and shapes. A thin film of silver nanoparticles sample is placed on a glass slide by adding 100 µl of sample solution on the slide, leaving it to dry for 5 minutes. The slides are then scanned using AFM (Kámán *et al.,* 2019).

Scanning Electron Microscopy (SEM):

The physical properties such as the surface topography and the crystal size of silver nanoparticles obtained from filtrated mushrooms were studied using a scanning electron microscope. The sample was examined under different magnification powers and constant voltage and then photographed after being coated with gold using a Sputter coater device (Dimitrijevic *et al.,* 2013). electron microscope (SEM) was used to determine the structural, shape and size properties of the nanoparticles

Bioactivity Study of Biosynthetic Silver Nanoparticles:

Preparation of Some Bacterial and Fungal Microorganisms:

Two isolates of Gram-negative and Gram-positive bacteria *E. coli* and *Staphylococcus aureus*, and two Dermatophytes *T. mentagrophyte* and *T. rubrum* were obtained from external laboratories in Salah al-Din Governorate - Tikrit. Also, isolates of some *Candida* including *C. albicans*, *C. glabrata*, *C. krusei*, and *C. tropicalis* were obtained from external laboratories of the College of Sciences-Biology Department, University of Tikrit. All isolates were activated after being cultured on nutrient agar media (Mast) and Sabouraud dextrose agar (Bangalore) for detecting antimicrobial activity. The yeast and bacterial suspensions were prepared by the method of Clayton *et al.(*2004).

Bioactivity Evaluation of AgNPs for Antibacterial and Anticandidal Activities:

The biological activity of the nano solution was estimated according to the method of Daragon *et al.* (2021). The method included preparation of nutritional media, SDA and NAM, media sterilization, and pouring afterward. The two media were separately distributed in sterile plastic containers (50 ml). The yeast suspension or bacterial suspension was separately added to the plastic container containing the SDA or NAM respectively. These flasks were shaken in a circular motion to homogenize the mixture. Then, both media were individually poured into sterilized petri dishes and allowed to cool. After plate solidification, sterile 6 mm paper discs saturated with different concentrations of the silver nanoparticle solution 25%, 50% and 100% (Korcan *et al.*,2021), were placed on the surface of the inoculated plates. The dishes were incubated at a temperature of 30-35°C for 24 hours. After incubation, the diameters (mm) of the inhibition zones around the discs were measured using a ruler. The method above was adopted with the replacement of the nanoparticle solution with other treatments, including deionized water, aqueous extract of macrofungi, and the antibiotics, Nystatin as anti-candida and Amoxicillin as anti-bacteria, as controls. Other treatments were also applied to separately evaluate the synergistic effect of the nano-solution (at the concentration that achieved the highest inhibition rates) with the antibiotics. All the above experiments were carried out with three replications.

Evaluation of the Effectiveness of Nano-Solutions against Dermatophytes:

The biological activity of different concentrations of silver nanoparticle solutions on human pathogenic fungi was evaluated using the Poisoned Food Technique (Grover and Moore, 1962). The SDA media was placed in a sterile plastic container (50 ml) and cooled at room temperature. Then, the nano solutions with concentrations of 100%,50% and 25%. were separately added to SDA flasks. The flasks were well shaken to mix the solutions, then poured into sterilized petri dishes, and labeled according to their concentrations. Fungal discs (5 mm diameter) were taken from the edges of the 7-day-old fungal colonies of *T. mentagrophytes* and *T.*

rubrum and put on the solidified plates. Additionally, the control treatments included adding deionized water, an aqueous extract of macrofungi, and the antibiotic, Nystatin, and the synergistic effect of the nano-solution with the Nystatin. The dishes were incubated at a temperature of 25-27 °C. After the incubation, the rates of colony diameters (mm) for the treatments were calculated by measuring the orthogonal diameters using a ruler. The experiment was carried out with three replicates for each treatment and then taking average values for all replicates.

Estimation of Antioxidant Activity *in vitro*: Prepare and Test the Total Antioxidant Capacity:

The test solution of the total antioxidant capacity was prepared according to the method of Prieto *et al.* (1999). Three cm^3 of the prepared solution was taken. The tubes were covered with aluminium foil and incubated in a boiling water bath at 95ºC for 90 min. After the sample tubes were cooled at the laboratory temperature, the absorbance of the solutions was measured at the wavelength of 695 nm. After filtering on the parameter representing the solvent (water) used, a solution of silver nanoparticles was prepared at concentrations of 5%, 10%, 20%, 40% and 80% to measure the total antioxidant content.

Statistical Analysis.

Statistical analysis of the studied traits was carried out using the SAS (2002) statistical program.

The Completely Randomized Designee (CRD) was performed for the investigated treatments and the Duncan multilevel test was applied to d ct the significant differences between treatments (Duncan, 1955). The P-values < 0.05 were considered significant.

RESULTS AND DISCUSSION

Characterizations of biosynthetic silver nanoparticles:

Visual Detection:

The optical properties of the prepared silver nanoparticles were studied by observing the color changes of the solution after adding the aqueous extract of the *P. plorans* to the silver nitrate solution. The color of the solution was changed from light yellow to yellowish brown indicating the formation of AgNPs, as shown in Figure (1). That is because of the reduction of silver metal ions +Ag to silver nanoparticles Ag^o as a result of the surface plasmon resonance phenomenon excitation of surface plasmon vibration in the NPs.

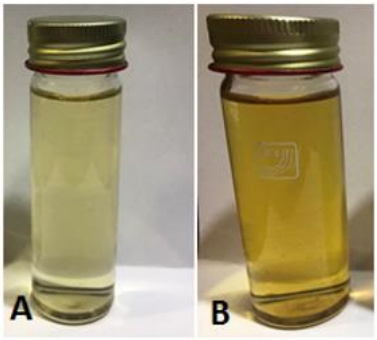

Fig. 1. Color changes appeared when preparing silver nanoparticles using the aqueous extract of *P. plorans,* (A) Before silver nanoparticle formation and (B) After silver nanoparticle formation.

This phenomenon happened on the surfaces of some metals such as gold, silver and copper and is responsible for their different colors when the elements reached the nanosize (Xia *et al.*, 2005).

The current results are consistent with several studies, such as the study of Gudikandula *et al.* (2017), which specified clear color changes from light yellow to brown in nano solutions prepared from the macrofungi *Trametes ljubarskyi* and

Ganoderma enigmatic, which indicates the reduction of the silver ion and the production of silver nanoparticles. Similarly, Birla *et al.* (2020) showed a different color of the nano solution prepared from the aqueous extract of the macrofungi *Inonotus hispidus* from light yellow to yellow-brown indicating the nanoparticle formation.

UV-VIS Spectra Analysis:

The surface plasmon excitability of bio-nanoparticles in the prepared solution of *P. plorans* was measured. The surface Plasmon resonance (SPR) peak was observed at 420 nm (Fig. 2).

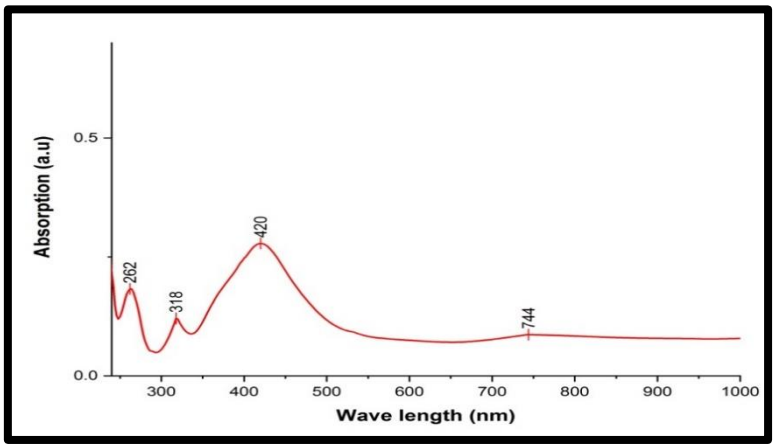

Fig. 2. The absorption spectrum of the prepared silver nanoparticles *P. plorans* using a Spectrophotometer UV-VIS

The current results were identical to several studies in which absorption peaks of 420 nm were recorded for silver nano solutions prepared from the *G. lucidium* and *Agaricus bisporus* (Ul-Haq *et al.,* 2014). Also, AL-Ansari *et al.* (2020) specified that the formation of these peaks confirms the formation of AgNPs from aqueous AgNO3 solution and their spherical nature. Macrofungi are characterized by their high ability to produce different types of enzymes, in particular the reductase enzyme that works to reduce and stabilize the nanoparticles (Kitching *et al.*, 2015), and due to this, fungi have a great ability to form large quantities of nanoparticles compared to bacteria (AL-Ansari *et al.*, 2020).

Fourier-Transform Infrared Spectroscopy:

The types of chemical bonds or functional groups were determined in the prepared AgNP solution of *P. plorans* using the FTIR device by producing an infrared absorption spectrum. FT-IR spectrum revealed strong absorption peaks from 3436 cm^{-1} to 667 cm^{-1}. The absorbance peaks at the frequency 3436 cm^{-1}, returning to a stretching bond group O-H. The appearance of two absorption bands at frequencies 2921 and 2851 cm^{-1} is due to the stretching of the aliphatic C-H bond. The emergence of an absorption band at frequency 2357 cm-1 belonging to the CO_2 group, with the appearance of a clear decrease in the frequency of the carbonyl group C=O to appear at frequency 1635 cm^{-1}. This is due to the succession between the carbonyl group and the double bond, which leads to a decrease in the value of the strength constant of the double bond, which reduces its frequency. It was also observed that two absorption bands appeared at 1557 and 1435 cm^{-1} corresponding to the stretching of the C=C bond and the appearance of an absorption beam at the frequency 667 cm^{-1}. The C-Br bond is stretchy, and the appearance of a few bands indicates that the prepared samples are nanoscale, as in Figure (3).

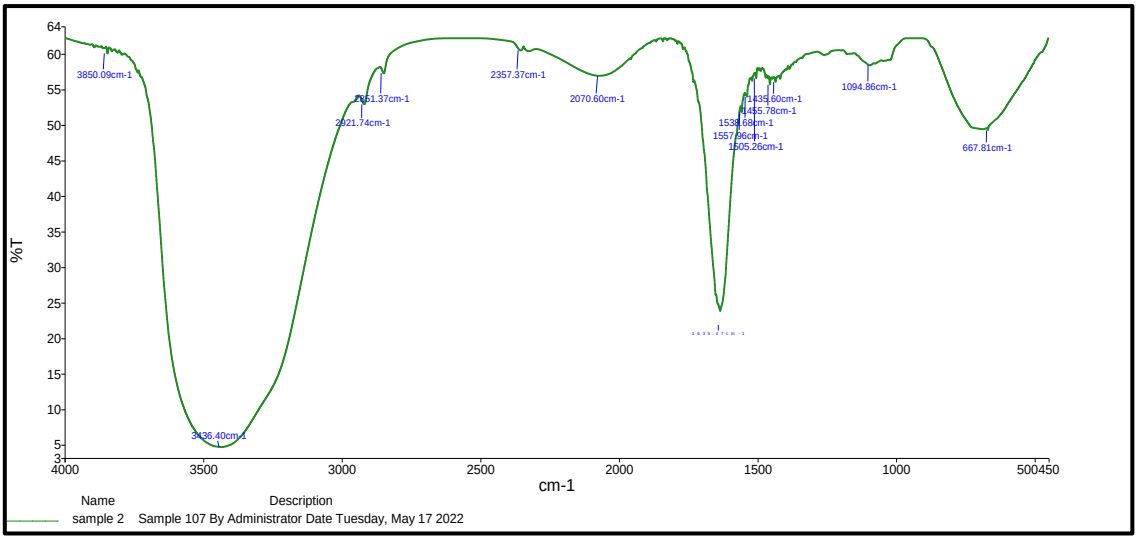

Fig. 3: Infrared spectroscopy of silver nanoparticles prepared by aqueous extract of *P.ploran*.

The obtained results were in agreement with the findings of Narasimha *et al.* (2011) and Eskandari-Nojedehi *et al.* (2018) for gold NPs of *Agaricus bisporus*. They found the absorption peaks of FT-IR spectrum between 500 and 4000 cm^{-1}, confirming the presence of proteins, carbonyl groups, and carboxylic acids. In this study, the presence of the functional groups is an indication of their important role in the process of biological reduction of silver nitrate and the production of nanoparticles. It is assumed that groups might have roles as stabilizer and enveloper and prevents the nanoparticles from aggregating (Niraimathi *et al.*, 2013).

Atomic Force Microscopy (AFM)

The current results of detecting the nature of the surface of the nanoparticles prepared from the *P. plorans* using the atomic absorption microscope showed that the nanoparticle shape was spherical. The average size distribution of that silver nanoparticles was 88.9 nm. (Fig. 4).

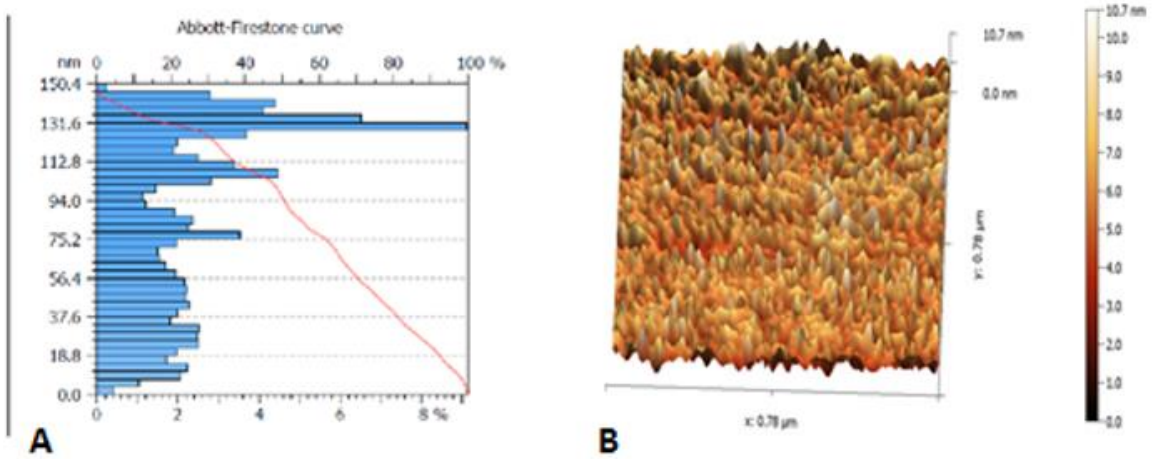

Fig. 4. (A) Diagram of the size range of silver nanoparticles prepared from the fungus *P. plorans*. (B) 3D image of silver nanoparticles prepared from the fungus *P. plorans*.

The shape, size, and distribution of nanoparticles depend on physical and chemical properties such as temperature, incubation period, and pH. The importance of the physical, chemical and biological properties of nanoparticles depends on their size and shape of nanoparticles (Kredy, 2018).

Scanning Electron Microscopy (SEM):

The shape and size of silver nanoparticles prepared from the macrofungus under study were determined. Figure **(**5**)** shows that the prepared silver nanoparticles have a spherical shape and particle sizes ranging from 36.61-59.30 nm.

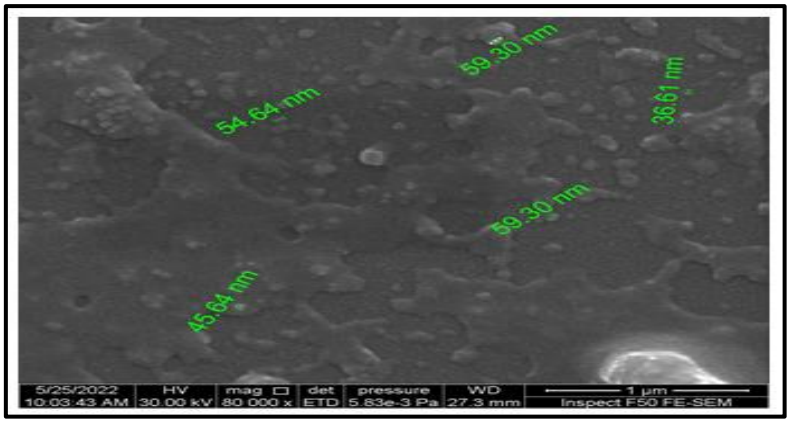

Fig. 5. SEM image of silver nanoparticles synthesized by the fungus *P. plorans*. 80000X magnification**.**

The results of the current study came close to the results of Jameel et al. (2020), as the sizes of silver nanoparticles prepared from *A. bisporus* reached 50.44 nm. At the same time, the sizes of silver nanoparticles prepared from *Schizophyllum commune* ranged between -54 and 99 nm (Arun et al., 2014). The results of the current study were identical to the results of Mohanta et al. (2018) and Alnuaimi (2019), in which they showed the spherical shape of the nanoparticles using electron microscopy.

Antibacterial and Antifungal activity of the synthesized silver nanoparticles

Silver nanoparticles prepared from the aqueous extract of *P. plorans* showed inhibition activity against the gram-positive bacteria, *S. aureus* and Gram-negative bacteria, *E. coli* (Table (1) and Figure (6)). The highest diameter of zone inhibition was with *E. coli*, without significant differences between the concentrations of 100% and 50%, which amounted to 0.88 ± 18.66 mm and 0.14 ± 21.25 mm, respectively compared with the control treatment.

Table 1. Inhibition diameters (in mm) for some pathogenic bacteria treated with silver nanoparticles prepared from fungi *P. plorans* and control treatments.

Microorganisms	Transactions (damping diameter/mm)						
	Concentrations of biosynthetic silver nanoparticles			control transactions			The combined effect
	100%	50%	25%	deionized	The aqueous extract of the mushroom	Nystatine	nanoparticles with the antibiotic nystatin)
E. coli	0.88±18.66 c	0.76±15.50 c	1.16±13.83 d	0.0±0.00 e	0.0±0.00 e	40.00 a±0.0	0.57±24.50 b
S. aureus	0.14±21.2b	0.60±17.16c	0.28±15.00 d	0.0±0.00 e	0.0±0.00 e	25.00 a±0.0	0.57±24.50 b

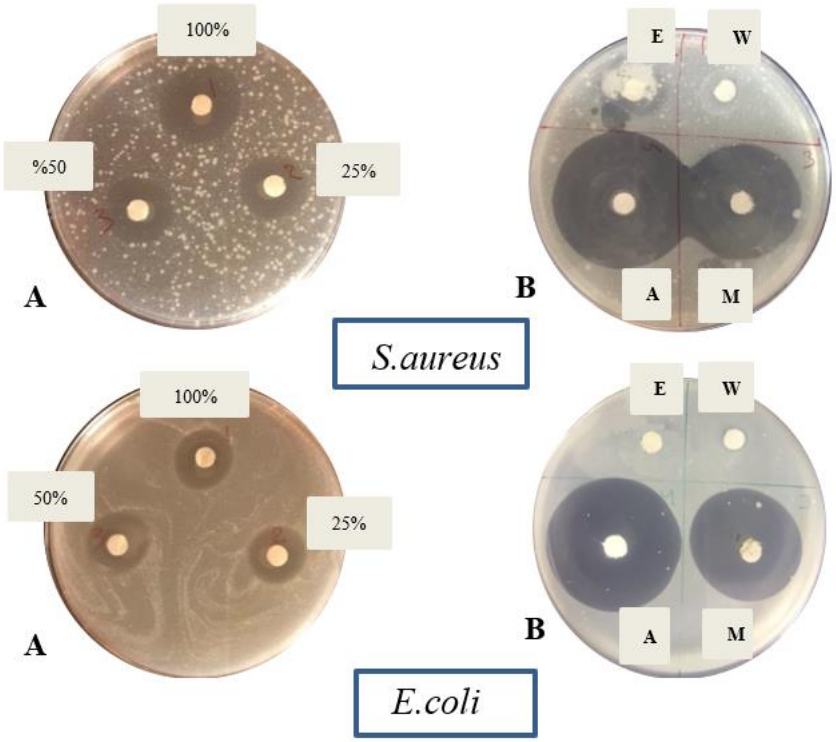

Fig. 6. Inhibitory effect of nano-solution prepared from fungus *P. plorans* against *E. coli* and *S. aureus* bacteria. A: (100%, 50%, and 25%) represent the effectiveness of the concentrations prepared from the nanoparticles; B: (W, E, M, and A) represent the control parameters. W: deionized water; E: aqueous extract of *P. plorans* M; mix; A: Amoxicillin antibiotic.

However, the effectiveness of silver nanoparticles was less efficient or did not show any inhibitory activity with yeasts. The *P. plorans* nanoparticles showed the highest inhibition activity at 100% and 50% concentration with *C. glabrata* and *C. krusei* species, which were 0.66 ± 11.33 mm and 0.0 ± 10. 0 mm at the concentration of 100% and 10.16 mm, and 8.83 mm at the concentration of 50%, respectively without any significant differences between both concentrations. Also, it was noted that the nano concentrations did not give any inhibitory activity with the *C. tropicalis and C. albicans* compared with the control treatment, as shown in Table (2) and Figure (7).

Table 2. Inhibition diameters (in mm) for some *Candida* treated with silver nanoparticles prepared from fungus *P. plorans* and control treatments.

Microorganisms	Transactions (damping diameter/mm)						
	Concentrations of biosynthetic silver nanoparticles			control transactions			The combined effect
	100%	50%	25%	deionized	The aqueous extract of the mushroom	Nystatine	nanoparticles with the antibiotic nystatin)
C.galbrata	0.66±11.33 c	0.16±10.16 c	0.16±5.83 d	0.0±0.00 e	0.0±0.00 e	0.86±30.00 a	0.83±29.16 a
C.krusei	0.0±10. 00 c	0.72±8.83 c	0.72±5.16 d	0.0±0.00 e	0.0±0.00 e	0.33±25.33 a	0.0±20.00 b
C.tropicalis	0.0±0.00 c	0.0±0.00 c	0.0±0.00 c	0.0±0.00 e	0.0±0.00 e	0.83±31.66 a	0.72±27.16 b
C.albicans	0.0±0.00 c	0.0±0.00 c	0.0±0.00 c	0.0±0.00 e	0.0±0.00 e	0.83±29.16 a	0.0±20.00 b

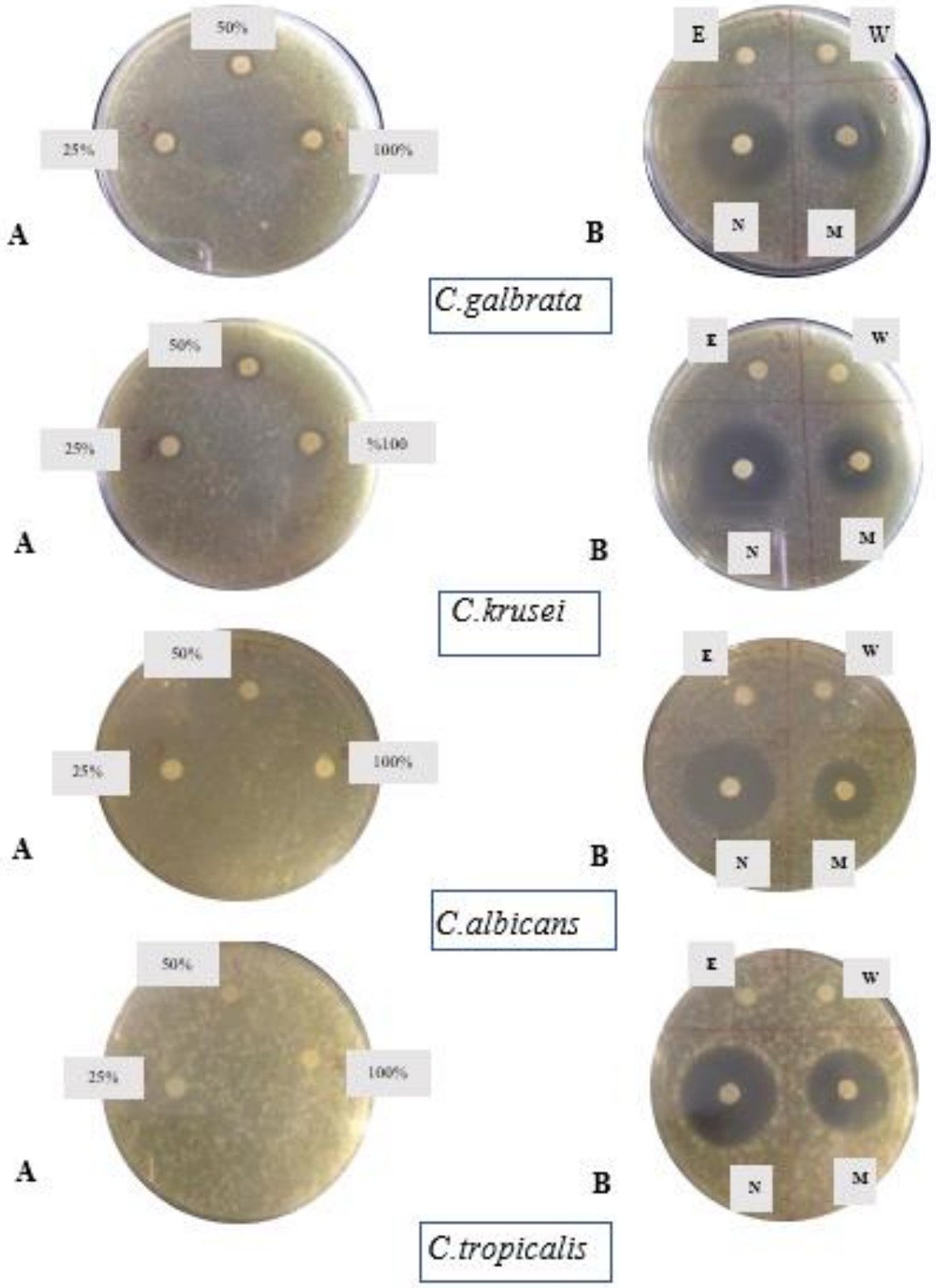

Fig. 7. Inhibitory effect of nanoparticles prepared from *P.plorans* fungus against *Candida* A_ (100%, 50%, and 25%) represents the effectiveness of the concentrations prepared from the nanoparticles. B_ (W, E, M, and N) represents the control parameters. W: deionized water; E: aqueous extract of *P.plorans* M; mix; N: the antibiotic Nystatin.

Similar to bacteria, the effectiveness of the prepared nano-solution was greater with Dermatophytes *T. mentagrophytes* and *T. rubrum* (Table (3) and Figure (8).). The last colony diameters of the *T. mentagrophyte* and *T. rubrum* were 0.76 ± 28.50 mm and 0.0 ± 30.00 mm respectively at the concentration of 100% compared to the control treatments. There were no significant differences between the concentrations of 50% and 25% for both species.

Table 3. The values of colony diameters (in mm) for selected dermatophyte fungi growing on SDA medium treated with silver nanoparticles prepared from *P.plorans* and control treatments.

Microorganisms	Transactions (damping diameter/mm)						
	Concentrations of biosynthetic silver nanoparticles			control transactions			The combined effect
	100%	50%	25%	deionized	The aqueous extract of the mushroom	Nystatine	nanoparticles with the antibiotic nystatin)
T. rubrum	0.0±30.00 b	0.41±37.91 a	1.08±42.06 a	1.01±46.75 a	1.87±39.25 a	0.0±0.00 d	0.0±10. 00 c
T. mentagrophyte	0.76±28.50 c	0.83±36.66 b	0.16±37.33b	0.72±46.25 a	0.72±38.73 a	0.0±0.00 d	0.0±10. 00 c

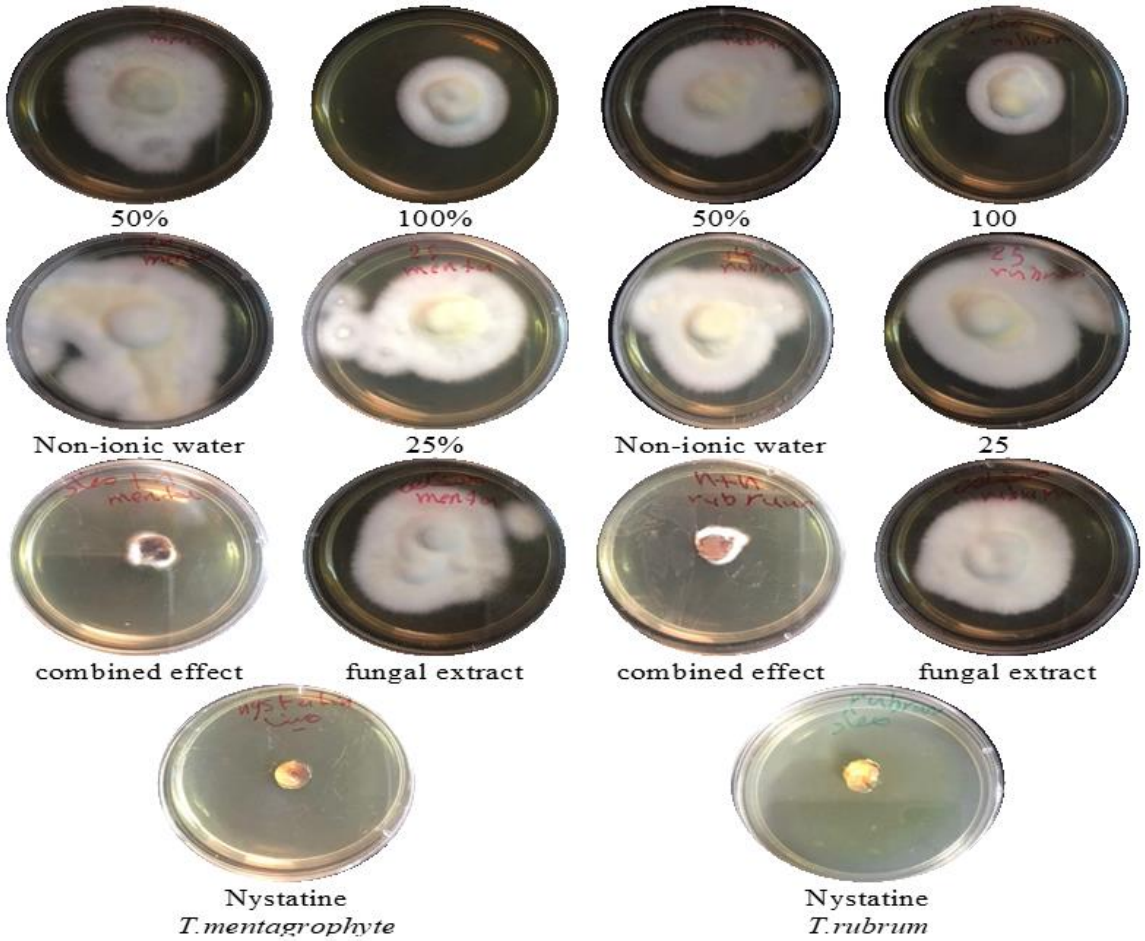

Fig. 8. The effect of the concentrations of the nano solution prepared from the fungus *P. plorans* with the rest of the studied treatments against the two dermatophytes *T. rubrum* and *T. mentagrophyte.*

The results of this study were consistent with the results of several studies in which the effectiveness of silver nano solutions prepared from macrofungi against pathogenic bacteria and fungi was confirmed. The study of Bhat *et al.* (2011) showed the anti-activity of silver nanoparticles prepared from *Pleurotus florida* against *Staphylococcus aureus*, *Salmonella typhi*, *Providencia alcalifaciens*, and *Proteus mirabilis*. Arun *et al.* (2014) demonstrated that silver nanoparticles prepared from *S. commune* had antibacterial activity against *E. coli* and dermatophytes, which included *T. simii* and *T. mentagrophytes* and *T. rubrum*. Additionally, the study of Mallmann *et al.* (2015) showed that silver nanoparticles prepared from the fungus *G. lucidum* had antifungal activity against the yeast *C. tropicalis*, while other studies demonstrated the effectiveness of silver nanoparticles prepared from *G. lucidum* against the bacteria *E. coli* and *S. aureus* (Mohanta *et al.*,2016), and the activity of silver nanoparticles prepared from the fungus *Pleurotus sajor-caju* against the yeast *C. albicans* (Musa *et al.*, 2017).

The antimicrobial activity of macrofungi may be related to their high content of bioactive compounds including secondary metabolites such as alkaloids, phenols, proteins, amino acids, terpenes, steroids, anthraquinones, benzoic acid derivatives, and quinolones. Other primary metabolites such as oxalic acid also have important roles. Others suggested that acids, peptides, and proteins may play great importance in the antimicrobial activity of nanoparticle solutions ((Alves *et al.*, 2012). Bhat *et al.* (2011) indicated that the flavins (flavoproteins) present in the oyster mushroom extract are responsible for reducing the silver ion in silver nanoparticles. Cor *et al.* (2018) attributed the effectiveness of the aqueous extract of *G. lucidium* to its D-glucose content. Klaus *et al.* (2020) also reported the effectiveness of crude polysaccharides isolated from the fruiting bodies of *A. bisporus, A. brasiliensis*, and *Tropicoporus linteus* against a group of clinically isolated pathogens such as *P. aeruginosa, C. albicans*, and *E. coli*.

The results of the current study, as well as the results of previous studies, show that biologically prepared nano-solutions can become an alternative to industrial treatments, in addition to their few side effects, as the free electrons produced by the surfaces of silver nanoparticles have a fatal effect on the surface membrane of the pathogenic organism (Bhat *et al.*, 2011).

Estimation of Antioxidant Activity *in vivo*:

The results show that silver nanoparticle solutions prepared from the *P. plorans* have antioxidant activities (Fig. 9). These activities increase with the concentration of the nano-solution. The highest effectiveness of 0.098 was achieved at a concentration of 80%. The total antioxidant activity (TAC) assay using phosphomolybdenum is the most popular method used for assessing the effectiveness of antioxidants in a biological sample (Rubio *et al.*, 2016). The technique depends on the reduction of Phosphate-Molybdenum (Mo (VI)) to Phosphate-Molybdenum (Mo (V)) by the compounds of the sample analyte resulting in green phosphate / (Mo (V) complex (Chen and group, 1956). In the current results, the TAC assay was successful in characterization of silver nanoparticle solutions. Additionally, this method is inexpensive because it does not measure all independently composed, fast, simple and can be performed using automated, semi-automatic or manual methods (Bartosz, 2010; Marques *et al.*, 2014).

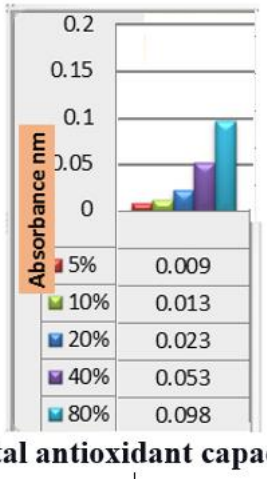

Fig. 9. Antioxidant activity of silver nano solutions prepared from *P. plorans*.

The results of the current study came in agreement with the results of the study of Aygun et al. (2019), which showed that silver nanoparticles prepared from *G. lucidium* have antioxidant activity. Other studies also confirmed the antioxidant effectiveness of silver nano solutions prepared from *Inonotus obliquus* and *I. hispidus*, which increased with increasing concentrations of the prepared nano solutions (Nagajyothi *et al.*, 2013; Jaloot *et al.*, 2020). In addition, several other studies indicated the effectiveness of antioxidant silver nano solutions prepared from Basidiomycetuos fungi such as *Auricularia polytricha*, *Pleurotus* spp. and *Termitomyces* spp (Puttaraju and co., 2006) and *Lentinus squarrosulus* (Lau and Abdullah, 2016).

The results of the FTIR examination of the current study shown in Figure (3) indicate the presence of hydroxyl groups and their role in the manufacture and fixation of nanoparticles. Thus, the presence of these functional groups (Bhakya *et al.*, 2015) and other secondary metabolites as antioxidants, such as terpenoids, polyketides, steroids, phenolic compounds, flavonoids, polysaccharides, glycosides, and tocopherols, as well as organic acids (Kozarski *et al.*, 2015; Mwangi *et al.*, 2022). Studies revealed that the presence of these compounds in *G. sessiliforme* mushroom extract, for example, has a significant role in stabilizing silver nanoparticles and increasing their antioxidant effectiveness (Karwa *et al.*, 2011; Mohanta *et al.*, 2018). The efficiency of the antioxidant *Leccinum scabrum* is due to its phenols and its total flavonoid content. Similarly, the efficacy of the *Lenzites betulina* is related to its possession of free radical scavenging proteins (Sytu & Camacho, 2018). Elias *et al.* (2011) indicated the effectiveness of mushroom extracts *Agaricus Brasilicenses* alcoholic and aqueous antioxidants because of their content of phenolic compounds such as gallic acid, syringic acid, and pyrogallol. The phenols are among the secondary metabolites of mushrooms, and they are antioxidants with redox properties that allow them to act as reducing agents, Hydrogen donors and free radical scavengers (Dimitrios, 2006). The researchers, Eze and Nwabor (2020), showed that the resistance to free radicals by biologically prepared silver nano solutions is based on electron donation known as the single electron transfer (SET), or may depend on donating a hydrogen atom known as hydrogen atoms transfer (HAT) originates from the biomolecules covering the prepared silver nanoparticles.

According to the available references, the current study is the first in the world to prove the effectiveness of the antioxidant nano-silver solution prepared from the *P. plorans*.

In conclusion, the green synthesis of silver nanoparticles from the macrofungal extracts of *the P. plorans* and their

characterization using UV spectral analyses, AFM, SEM and FTIR was successfully done in the present study. Also, the silver nanoparticles from *P. plorans* have been proven to have antimicrobial effects on bacteria, yeast and microfungi. These antimicrobial activities were different depending on species and concentrations. The study proved for the first time in the world the prepared nano-silver solution has strong antioxidant activity. Both antimicrobial and antioxidant activities may be related to the presence of bioactive molecules on the surface of silver nanoparticles of the *P. plorans* extracts.

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