RESEARCH
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BIOSYNTHESIS OF SILVER NANOPARTICLES PRODUCED FROM *PLEUROTUS OSTREATUS* FUNGUS AGAINST *STAPHYLOCOCCUS AUREUS* ISOLATED FROM DIABETIC FOOT ULCERS

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Abstract:

Diabetic foot ulcers suffer from the risk of developing diabetic foot infections, as the development of bacterial resistance to antibiotics is a major problem that limits the use of antibiotics to treat diseases resulting from bacteria. From this perspective, our study aims to isolate and diagnose diabetic foot ulcers. A total of 105 specimens were collected, there were two different types of diabetes and type 1 diabetes. Their number reached 83 (79.04%), patients, and type 2 diabetes occurred in 22 (20.95%), out of 105 patients. The *Staphylococcus aureus* found naturally on the skin but can cause infection when it gets into wounds or ulcers it may be the cause of the infection diabetic foot ulcers .

In this study, we examined the effectiveness of silver nanoparticles extracted and manufactured from the fungus , *Pleurotus ostreatus* against *Staphylococcus aureus*, the (UV-Vis) spectroscopy was achieved to confirm AgNPs which The UV-vis spectrum revealed the formation of wavelength peak for AgNPs is at (415 nm) and absorption peaks at (1.2 nm).

synthesis using *P. ostreatus* fungus extract **.** All of *S.aureus* isolates were inhibited , decreased in number and desrtoyed in cell wall by AgNPs reduced by this fungus at different concentrations (50, 100, and 150) µg /ml . The largest inhibition zone of AgNPs was (4 cm) by a concentration of (150 µg /ml), whereas the lowest inhibition zone was (1.5 cm) at the concentration (50 µg/ ml).

Keywords: Diabetic foot ulcers,Silver nanoparticles, *Pleurotus ostreatus*, *Staphylococcus aureus*.

Introduction

The diabetic foot may be defined as a group of syndromes in which neuropathy, ischaemia and infection lead to tissue breakdown, resulting in morbidity and possible amputation[1]. foot complications account for more hospital admissions than any other diabetic complication. The major adverse outcomes of foot complications are foot ulcers and amputations. It is estimated that 15% of diabetics develop a foot ulcer within their lifetime [2,3].

The bacteria that can cause infections in the feet of a diabetic patient in the case of foot ulcers *S. aureus* is a gram-positive, commensal bacterium that colonizes 30% of healthy individuals from . The plays an important role in causing infections in both hospitals and the community ranging from minor to the damaged skin and wounds resulting from foot ulcers may be exposed to infection with bacteria. The cause of infections in the feet of a diabetic patient may be *S. aureus* it is considered one of the most important bacteria that causes infections in the skin,found naturally on the skin but can cause infection when it gets into wounds or ulcers it may be the cause of the infection the most frequently isolated and in several studies methicillin resistant *Staphylococci* are isolated in diabetic foot ulcer infections[4,5].

Among the important fungal species in the production of AgNPs is fungus *P. ostreatus*. Now today, many scientific reports, studies and researches have started for exploring the possibility of synthesis of metallic nanoparticles (NPs) using different genera of edible and medicinal fungi and mushrooms owing to the innumerable bioactive compounds with diverse biological activities present within them[6,7]. A wide variety of amino acids, proteins, and polysaccharides present in the mushrooms have been utilized in the synthesis of both extracellular and intracellular silver, gold, selenium, lead and iron nanoparticles[8]. In past few years of nanoscience, the primary focus on nanoparticle (NPs) synthesis has been on developing an environmentally benign method that produces NPs with the appropriate physicochemical qualities[9,10]. Silver nanoparticles (Ag-NPs) are the metal of choice for antibiotic resistant microbes. Synthesis of AgNPs through biological route is preferred over chemical route to promote green chemistry[11]. An eco-friendly process for the synthesis of nanomaterials, using a fungus *P.ostreatus*[12].

The aim of this study was to detect the biological activity of AgNps synthesis of the fungus *P. ostreatus* against *S. aureus* isolated from diabetic foot ulcers.

Materials and Methods

In the study where 105 specimens were collected from diabetic foot ulcers patients suffering from diabetic foot ulcers (DFUs) from Najaf Governorate. Iraq, where the types of gram-positive bacteria and fungi causing diabetic foot ulcers were identified using a specimens collection method. Taking a swab of the foot from the wounds and openings on the soles of the feet and between the toes. A total of 105 specimens showed noticeable bacterial growth. All specimens were grown on MacConkey agar and mannitol salt agar.

Biosynthesis of Silver Nanoparticles from *Pleurotus ostreatus*

P.ostreatus is an oyster mushroom that uses reducing and stabilizing properties. *P.ostreatus*, grown on Sabouraud dextrose agar (SDA) medium, was transferred for (7) days, where the fungus grew, then it was grown on (SDA) medium for (21) days, after which it was filtered using filter paper to get rid of fungal remains. Next, the AgNPs were purified through a centrifugation process and using different characterization techniques to confirm their composition and determine their properties as shown in figure (1).

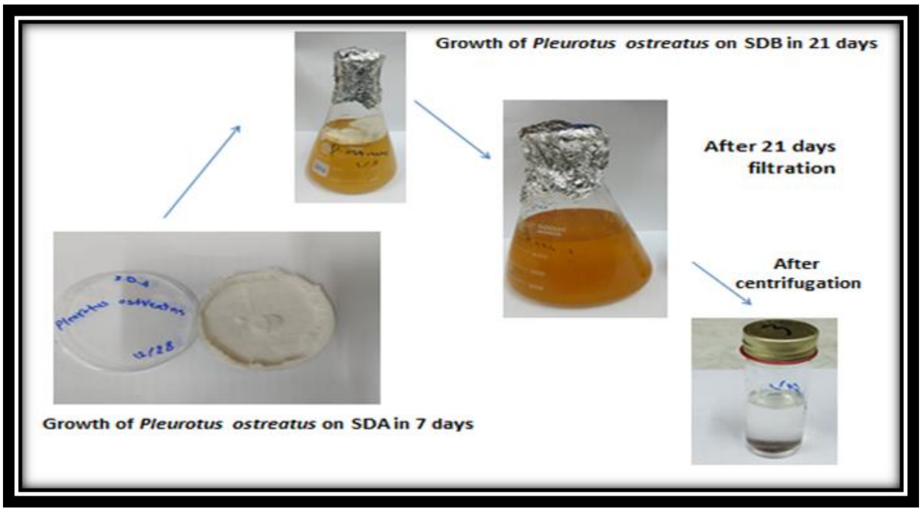


Figure (1): Steps for AgNPs synthesis by *P. ostreatus*

Results and Discussion

The results of this study appaered that DFUs appeared is of two types: type 1 and type 2 and as the highets percentage in diabets type 1 as compared with diabets type 2 results showed that out of 105 specimens collected, type 1 diabetes showed 83 (79.04%), the highest percentage being type 2 diabetes, which was 22 (20.95%) as shown in figure (2) there are many factors such as obesity and an unhealthy lifestyle which couces diabets type 1 and diabets type 2 this is consistent with [13].

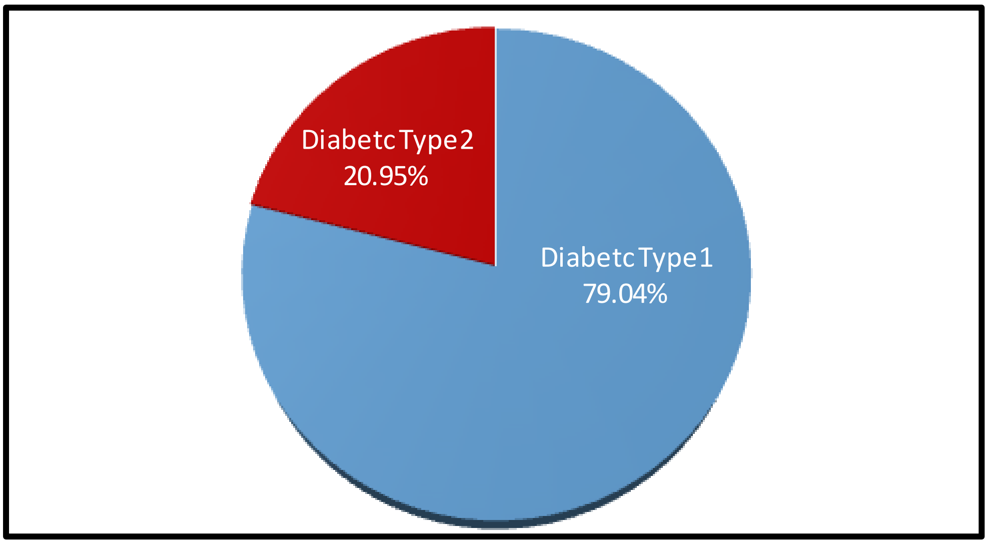


Figure (2) The percentage types of DFUs

The results showed that the total number of Gram-positive bacteria was 42 out of 80 isolates. The highest incidence is *S.aureus* (40%), while *S.hemolyticus* has the lowest incidence (2.5%). as shown in the table (1).

Table No. (1) The Gram-positive bacteria isolates from DFUs

NO	Gram-positive bacteria	Number	Percentage %
1	***Staphylococcus aureus***	**32**	**40**
2	***Staphylococcus sciuri***	**8**	**10**
3	***Staphylocuccus haemolyticus***	**2**	**2.5**
	Total	**42**	**41.16**

Infection with "Gram-positive" the cause of infections in the feet of a diabetic patient may be *S. aureus* it is considered one of the most important bacteria that causes infections in the skin,found naturally on the skin but can cause infection when it gets into wounds or ulcers it may be the cause of the infection [14,15].

Visual confirmation of silver nanoparticles produced by *Pleurotus ostreatus*

The results showed a color change from silver nitrate (AgNO3) , which was clear in color to a very dark brown color as shown in figure (3). The color of silver nanoparticles depends on their size and shape, and when the particle size is in the nanometer range, they show different colors based on the surface plasmon effect. The color of AgNPs is usually at the point in the optical spectrum of visible light, which means that they appear yellow or brown. When AgNPs are deposited on the surface of the material, the concentration of the particles and change in their distribution causes achange in the color of the solution. This is observed in the color change from clear in color to dark brown color when the AgNPs start depositing on the surface and aggregate significantly which is a positive sign of AgNPs formation.

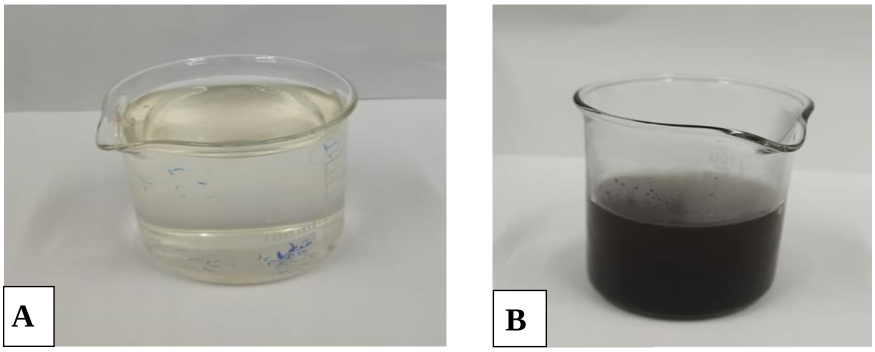


Figure (3) Color change after reduction of Ag+ to silver nanoparticles by *P.ostreatus* fungus extracts. (A): Before adding the extract to the silver nitrate (B): Formation of silver nanoparticles after adding fungus *P.ostreatus* extract.

Ultraviolet and visible spectroscopy (UV-Vis)

(UV-Vis) spectroscopy was achieved to confirm AgNPs synthesis using *P.ostreatus* extract. The UV-vis spectrum revealed the wavelength of AgNPs (415 nm). formation of absorption peaks at (1.2 nm), as shown in figure (4). Our results were close to at absorbans [16]. who demonstrated that the maximum absorption peak for AgNPs synthesis by *P.ostreatus* is at (400 nm).

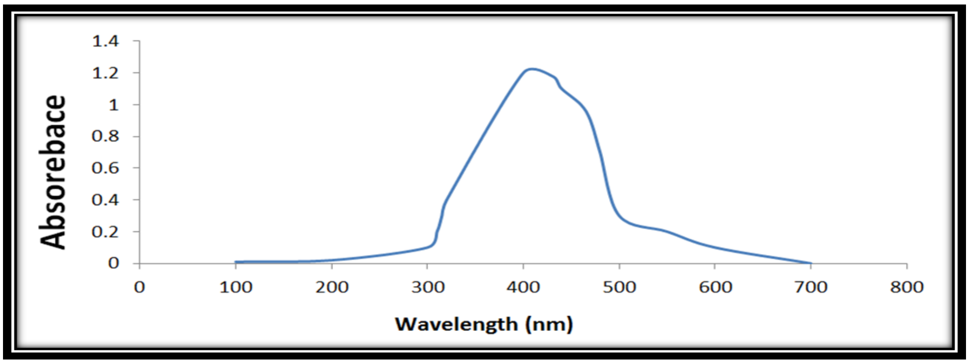


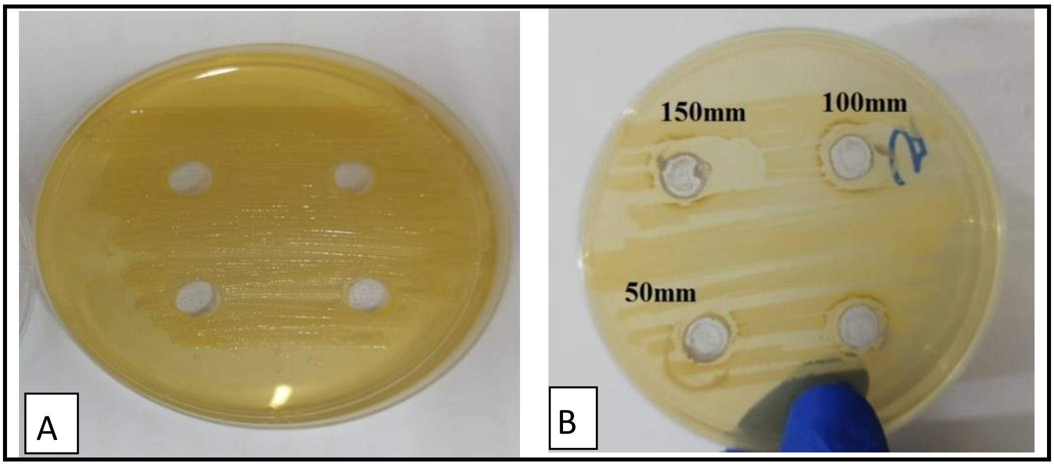
Figure (4): UV-vis spectrum of AgNPs synthesis by *Pleurotus ostreatus*

Antibacterial activity of Silver Nanoparticles

The antibacterial activity of the NPs was tested against 20 isolates that were multidrug resistant (MDR). The bacteria were grown on Mueller-Hinton Agar medium to test drug sensitivity figure(5).

The bactericidal activity of green synthesis AgNPs was determined using the agar well diffusion technique. All of *S.aureus* isolates were inhibited by Silver NPs at different doses (50, 100, and 150 µg /ml). The largest inhibition zone of AgNPs was (4 cm) by a concentration of (150 µg /ml), whereas the lowest inhibition zone was (1.5 cm) at the concentration (50µg/ ml).

Kirby Bauer method some AgNPs are placed on a plate on which bacteria grow. If the bacteria are sensitive to antibiotics, a clear ring or inhibited zone will appear around the foil to indicate poor growth test immune susceptibility to antibiotics as shown in figure (5).



Figure(5) The effect of silver nanoparticles on the growth of *S.aureus* in Muller Hinton medium.: (150, 100, 50)µg/ml. (A): Control with out treated AgNPs (B):After treated with AgNPs.

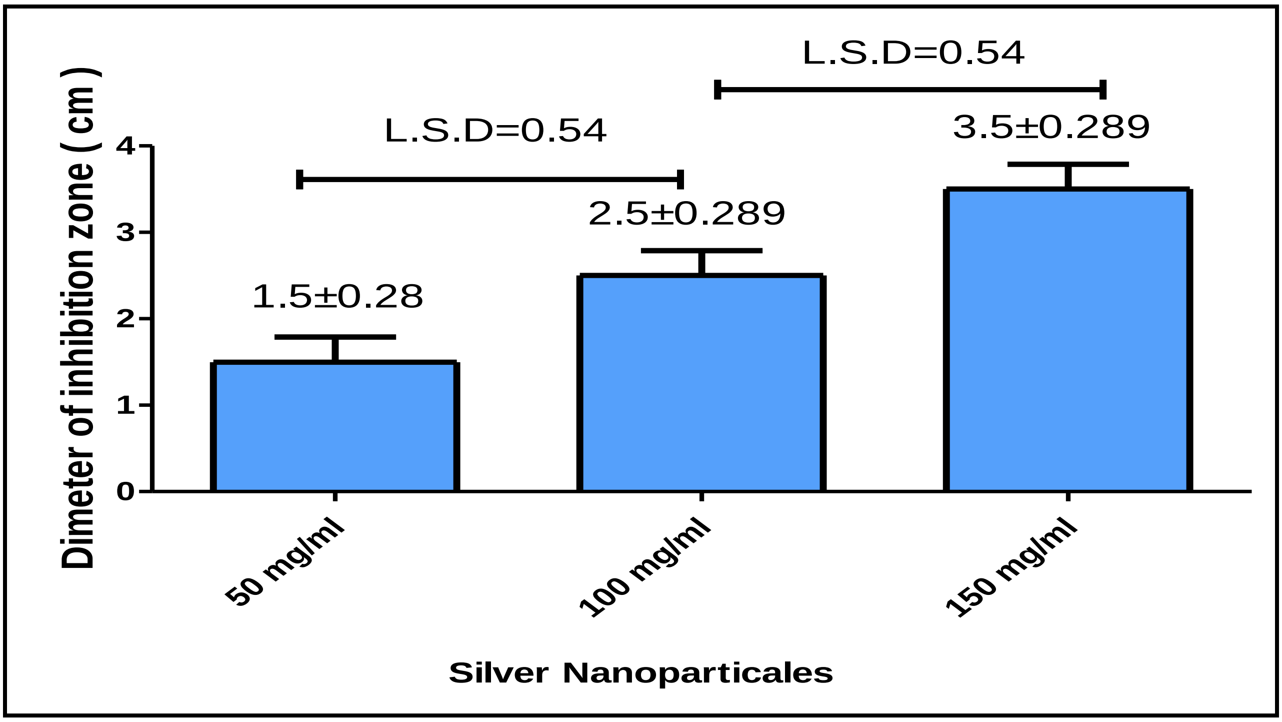
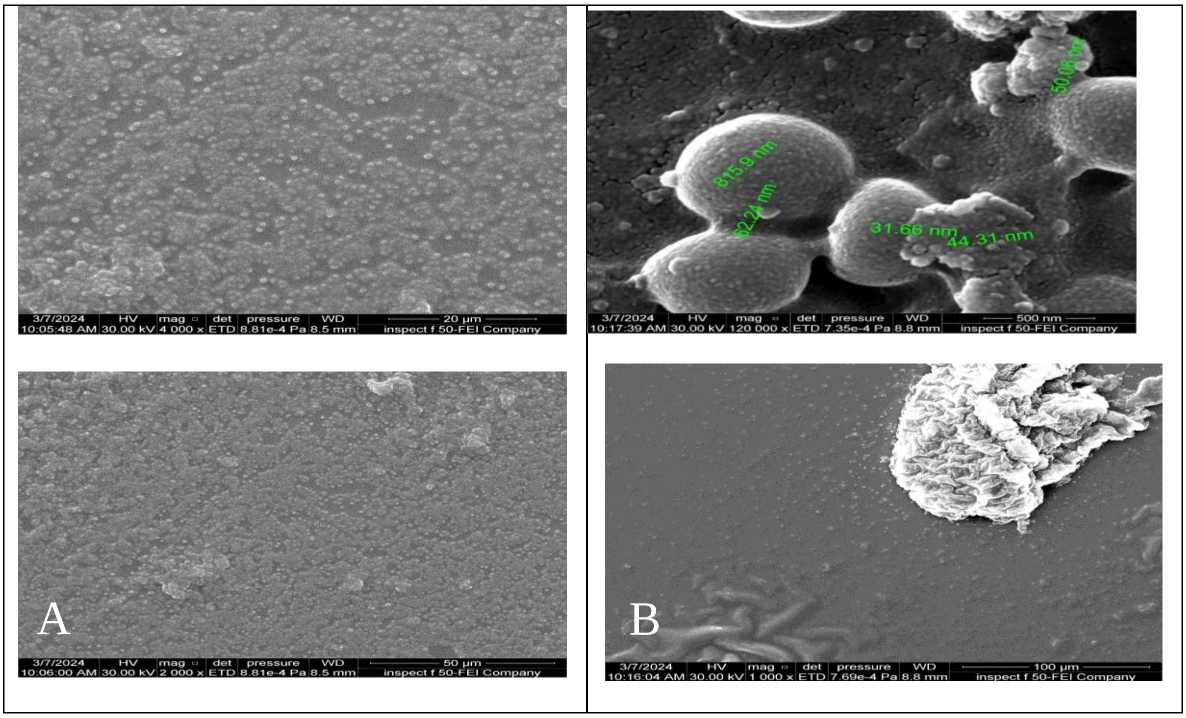


Figure (4-15) Comparison between (50,100 and 150) µg/ml using the spss v25 computer software by l.s.d test; p- value lees than 0.05.

Effect of silver nanoparticles on the *S .aureus* using field emission Scanning Electron Microscopy (SEM)

The effect of AgNps extracted from the fungus *P.ostreatus* the microscope SEM result showed that the number, inhibited, desroyed and reduced by the concentrations (50, 100, and 150) µg /ml.

on the *S.aureus* and examining it using shown with a microscope (SEM) as in figure No. (7)



Figure(7): SEM Micrograph the effect of AgNps extracted from the fungus *P.ostreatus* in inhibiting *S.aureus*.A:(Control with out treated AgNPs) B: (After treated with AgNPs).

The size of the nanoparticles plays an important role in antibacterial activity. Silver ions are continuously released from AgNPs, which may consider a mechanism for killing microbes. Silver ions can easily adhere to the cell wall and cytoplasmic membrane as they are more closely related to sulfur proteins and also due to electrostatic attraction[17]. the bacterial envelope is disrupted because when silver ions attach to the cell wall or cytoplasmic membrane, it enhances the permeability of the cell and ultimately leads to cell disruption. When free silver ions are uptake by cells, they deactivate respiratory enzymes, generating reactive oxygen species interrupting adenosine triphosphate production. ROS is the principal species that provokes the activity of DNA modification and cell membrane disruption. In DNA, sulfur and phosphate are essential components. Still, the interaction of AgNPs with sulfur and phosphorus in DNA can cause difficulties in DNA replication and cell reproduction or even result in the termination of bacteria. Sometimes, the ribosome's denaturation in the cytoplasm occurs as the silver ions can inhibit protein synthesis[18].

Conclusion

In this research, type 1 diabetic had the highest rate of 82 and type 2 diabetes had the highest rate of 22 and was observed. In the age groups from (51 to 60) years are the most susceptible to developing diabetic foot ulcers, with a number of 33 ,compared to the age groups from (81 to 90) years, which had the lowest incidence of infection 1 ,the proportion of gram-positive bacteria isolated from diabetic foot ulcers was highest and the common bacteria is *s. Aureus*. All of *s.aureus* isolates were inhibited , degresd in number and desrtoyed in cell wall by silver nps reduced by this fungus at different concentration (150 µg/ml), and the smallest zone of inhibition for biosynthesized agnps against *s. Aureus* was at a concentration of (50 µg/ml).

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