



RESEARCH ARTICLE

Investigating the Inhibitory Effect of Silver Nanoparticles against Some Species of *Candida* and Pathogenic Bacteria

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ABSTRACT

Silver nanoparticles synthesized from aqueous extract of mushroom *Pleurotus ostreatus* exhibited inhibitory effect at the concentration of 12.5, 25, 50, and 100 mg/ml against some pathogenic bacteria and fungi such as *Candida albicans*, *Candida guilliermondii*, *Candida krusei*, *Candida zeylanoides*, *Geotrichum klebahnii*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. The maximum inhibition zone was observed against *C. zeylanoides* at the concentration of 100 mg/ml was 24.5 mm, while the minimum inhibition zone was observed against *Geotrichum* at the concentration of 25 mg/ml was 8 mm and the concentration of 12.5 mg/ml was not effective against some species.

Keywords: Pathogenic *Candida*, *Pleurotus ostreatus*, silver nanoparticles

INTRODUCTION

A new dimension of the metal microbial interaction has been explored for the synthesis of metal nanoparticles such as gold, silver, cadmium, zirconia, and silica titanium.^[1] Silver compounds have been used to treat burns, wounds, and infections as well as in preventing bacterial colonization of prostheses and catheters.^[2] Various salts of silver and their derivatives are used as antimicrobial agents. Nanosized silver particles exhibit antimicrobial properties. Nanoparticles of silver have been studied as a medium for antibiotic delivery, and to synthesize composites for use as disinfecting filters and coating materials.^[3] The nanoparticles were important due to their emission properties. These nanoparticles are used for wide range of application.^[4,5] Antibacterial activity is related to compounds that locally kill bacteria or slow down their growth, without being in general toxic to surrounding tissue.^[6] The high bactericidal activity is certainly due to the silver cations released from Ag nanoparticles (AgNPs) that act as reservoirs for the Ag + bactericidal agent.^[7] Severe fungal infections have significantly contributed to the increasing morbidity and mortality,^[8] immunocompromised patients who need intensive treatment including broad-spectrum antibiotic therapy,^[9,10] and *Candida* spp. represent one of the most common pathogens which are responsible for fungal infections often causing hospital-acquired sepsis with an associated mortality rate of up to 40%.^[11] Currently, the majority of yeast species are resistant to the available antifungal therapy^[12,13] such as on polyenes (amphotericin B), triazoles (fluconazole, itraconazole, voriconazole, and posaconazole) or echinocandins (caspofungin, micafungin, and anidulafungin) which exhibit their toxicity, adverse effects, and drug interaction.^[14,15]

The objective of this work is to evaluate the effect of a biosynthesized AgNPs product against some *Candida* spp. and bacteria species to determine how AgNPs interact with the growth of microbial cells.

MATERIALS AND METHODS

The mushroom *Pleurotus ostreatus* was obtained from fruit body grown on peach tree in Salah-Alddin Province.

Preparation of Hot Aqueous Extracts of Mushroom

The oven dried mushroom was blended; the obtained powder was soaked in distilled water at a ratio of 1:10 (w/v) and boiled with agitation at $60 \pm 2^\circ\text{C}$ for 30 min. The boiled mushroom powder was then left covered for 30 min. Residues were then removed by filtration through gauze and further centrifugation (10,000 rpm, 30 min, and

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4°C). Supernatants were then collected and filtered through Whatman No. 1 filter paper. After that, freeze dried extract powders were obtained using freeze dryer and stored at $4 \pm 2^\circ\text{C}$.^[16]

Biosynthesized AgNPs from *P. ostreatus*

Silver nitrate (1×10^{-3}) AgNO₃ stock solution was prepared in sterile deionised triple - distilled water and the subsequent dilutions were made from this stock solution. The bulk amount of (10) mg/ml of aqueous extract solution of is prepared with sterile distilled water and filtered through syringe filter (0.2) μm . Based on the result of a preliminary trial, 2–7 ml of 10 mg/ml of an aqueous extract of *P. ostreatus* (P2) were filled with sterile distilled water to a total of 10 ml.

After that, the solution is added to 5 ml of (1×10^{-3}) M aqueous AgNO₃ solution and kept at room temperature and exposed under ultraviolet (UV) (365) nm (long UV). After 24 h incubation, the light yellow color of the mixture solution turned to dark yellow indicating the formation of AgNPs.^[17]

Candida Isolates

All *Candida* isolates *Candida albicans*, *Candida guilliermondii*, *Candida krusei*, *Candida zeylanoides*, and *Geotrichum klebahnii* were procured from AL-Yarmouk Teaching Hospital. Mycological identification was carried out for all samples by commercial carbohydrate assimilation systems such as the API 20 C test kit.^[18] All yeast isolates were inoculated in a primary isolation medium such as Sabouraud Dextrose Agar (SDA) medium for 2–3 days at 37°C.

Antimicrobial Activity of AgNPs (Well Diffusion Method)

The synthesized AgNP was tested for antimicrobial activity by agar well diffusion method against pathogenic microbes for all the previously mentioned bacteria and yeast species. The pure cultures of bacteria and yeast were subcultured on nutrient and SDA subsequently. Each strain was swapped homogeneously onto the individual plates using sterile cotton swabs. Wells of 10 mm diameter were done. The concentration of AgNPs was poured on each well. After 24 h of incubation the diameter of inhibition zone was measured. Three replicates of experiments were carried out.^[19]

RESULTS

The formation of AgNPs was confirmed by the UV-visible spectrophotometry, which showed a strong peak within the range of 200–500 nm, “Figure 1.”

Intensity of Particle Size Distribution Analysis

To know the size of synthesized AgNPs, size distribution analysis was performed using light scattering in aqueous solution. The results showed that the size of the particles ranged from 16 to 104 nm. The average size of AgNPs was determined to be 49 ± 16 nm as in “Figures 2 and 3.”

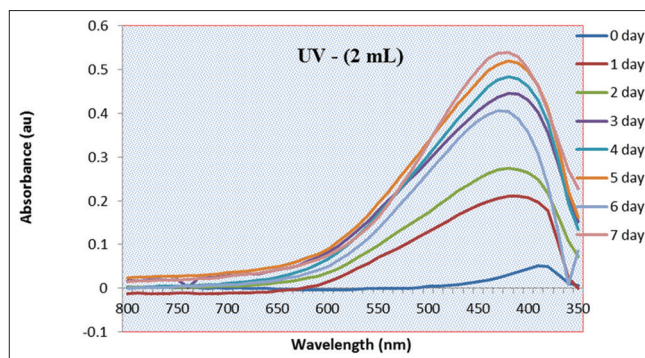


Figure 1: Ultraviolet-visible absorption spectra of silver nanoparticles after bioreduction by *Pleurotus ostreatus* mushroom aqueous extract

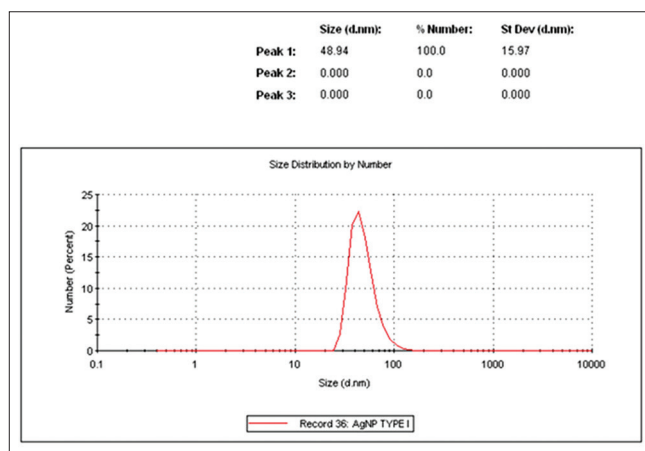


Figure 2: Size of silver nanoparticles

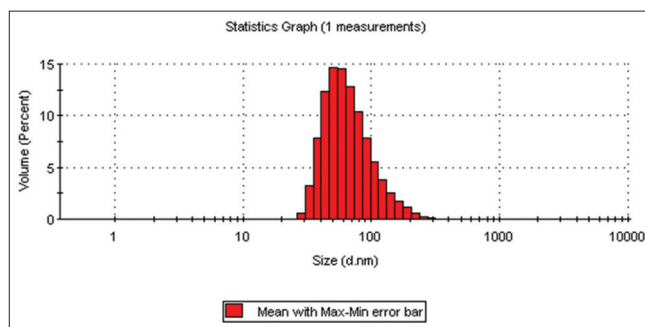


Figure 3: Intensity of particle size distribution of *Pleurotus ostreatus* dried mushroom aqueous extract

DISCUSSION

Antimicrobial mechanisms of nanomaterials are not fully understood, but it is proposed that when they come into contact with cells, they provoke the production of reactive oxygen specie, cell membrane disruption, mitochondrial damage, and DNA damage.^[20]

Antifungal activity by AgNPs has been proved against different *Candida* species, including *C. albicans*, since microbial cells were inhibited using AgNPs.^[21] Furthermore, it was reported that AgNPs damage the structure of the cell membrane in *C. albicans* producing “holes” on the surface of the cells and thus inhibiting the budding process.^[22]

Table 1: The inhibitory effect of AgNPs (mm) against *Candida* and bacterial species

<i>Candida</i> spp.	Inhibition zone (mm)			
	12.5 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml
<i>Candida albicans</i>	---	10	12	16
<i>Candida guilliermondii</i>	---	10	15.5	20
<i>Candida krusie</i>	---	14.5	15.5	19
<i>Geotrichum ktebahnii</i>	---	8	14	20
<i>Candida zeylanoides</i>	15	18.5	21	24.5
<i>Staphylococcus aureus</i>	10	11	12	16
<i>Pseudomonas aeruginosa</i>	---	12	13	14
<i>Escherichia coli</i>	10	12	13	15

AgNPs: Silver nanoparticles

Table 2: Statistical analysis of the inhibitory effect of AgNPs (mm) on *Candida* and bacterial isolates

Microbial species	Control	AgNPs (mg/ml) s			
		12.5	25	50	100
<i>Candida albicans</i>	<0.001 ^d	0±0.0 ^d	10±0.57 ^c	12±0.58 ^b	16±0.58 ^a
<i>Candida guilliermondii</i>	<0.001 ^d	0±0.0 ^d	15±0.58 ^c	15.6±0.33 ^b	20±0.57 ^a
<i>Candida krusie</i>	<0.001 ^d	0±0.0 ^d	14.5±0.28 ^c	15.6±0.16 ^b	19±0.57 ^a
<i>Geotrichumktebahnii</i>	<0.001 ^d	0±0.0 ^d	8±0.57 ^c	14±0.58 ^b	20±0.58 ^a
<i>Candida zeylanoides</i>	<0.001 ^e	15±0.57 ^d	18.5±0.28 ^c	21±0.57 ^b	24.5±0.28 ^a
<i>Staphylococcus aureus</i>	<0.001 ^d	10±0.58 ^c	11±0.57 ^{bc}	12±0.57 ^b	16±0.58 ^a
<i>Pseudomonas aeruginosa</i>	<0.001 ^c	0±0.0 ^c	12±0.58 ^b	13±0.58 ^{ba}	14±0.57 ^a
<i>Escherichia coli</i>	<0.001 ^d	10±0.57 ^c	12±0.58 ^b	13±0.57 ^b	15±0.58 ^a

The findings were described in the table represent the average of three replicates±standard error. Small letters (a, b, c, d, e, ba, and ac) indicate to comparison between means in column, similar letters are non-significantly differences between means at (P≤0.05), using (LSD test). AgNPs: Silver nanoparticles

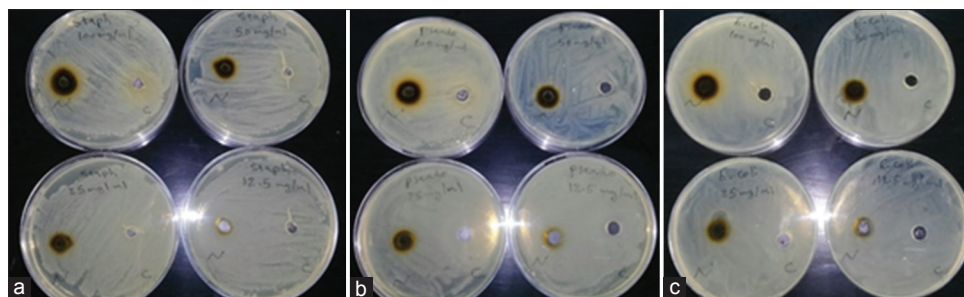


Figure 4: Inhibition zone of silver nanoparticles against several isolates of bacteria; (a) *Staphylococcus aureus*, (b) *Pseudomonas aeruginosa* (c), *Escherichia coli*

The antifungal properties of AgNPs against *C. albicans* have been demonstrated in some other studies, although reported minimum inhibitory concentration values are different from the ones we found in this work.^[21-24] Such differences could be due to the nature of the particles used, the difference in size being particularly important. It is known that size and shape of metallic nanoparticles influence their chemical, optical, and thermal properties.^[25]

The effectiveness of AgNPs against bacteria is clearly demonstrated, and in fact, AgNPs were shown to be effective against *E. coli*, with cells showing formation of “pits” in the cell wall. The AgNPs were found to accumulate in the bacterial membrane and some of them were reported to successfully penetrate into the

cells.^[26] Similar results were found in *E. coli* and *Vibrio cholera*; it was established that AgNPs provoked changes mainly in the cell membrane morphology, producing a significant increase in their permeability, thus affecting the proper transport through the plasma membrane, and resulting eventually in cell death. They also reported that silver NPs with small diameters penetrated into the cells.^[27] In our study, Ag NPs were found surrounding *C. albicans* cells, similar to the results found in bacteria.^[27-29]

In this study, the aqueous extracts of mushroom (*Postreatus*) gave showed (ve-) results against all microbial species when used as a control [Figures 4 and 5], while the biosynthesized AgNPs gave showed (ve+) result as an inhibition zones for different microbial species [Figures 4 and 5, Tables 1,2].

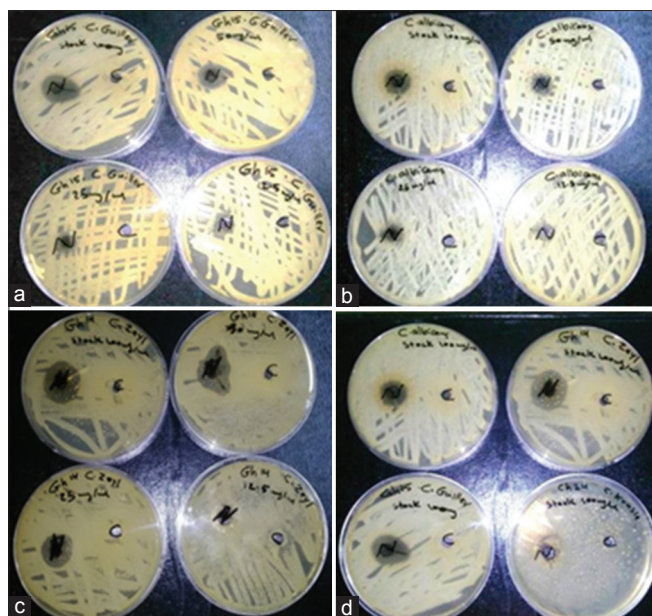


Figure 5: Inhibition zone of silver nanoparticles (AgNPs) (N) against several isolates of *Candida*; (a) *Candida guilliermondii*; (b), *Candida albicans*; (c), *Candida zeylanoides*; (d), *Candida* spp. in concentration of 100 mg/ml of Ag NPs the way of measuring of diameter of inhibition zone. The aqueous solution of *Pleurotu zostreatus* (C)

However, despite the clear antimicrobial properties of AgNPs, their potential use in the clinic should be carefully evaluated since there is a lack of basic knowledge on the potentially different antimicrobial properties of AgNPs which may vary depending on many factors, including the method of synthesis, size, shape, functionalizing agent, and application method and also their interaction in more complex systems such as plants, animals, and humans.

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