Cihan University-Erbil Scientific Journal (CUESJ)

Research Article

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

Alaa M. Hasan, Sura M. Abdul Majeed, Rusol M. Al-Bahrani

Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq

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 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 ± 2°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

Corresponding Author:
Sura M. Abdul Majeed, Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.
E-mail: Suraalsadik80@gmail.com

Received: Apr 04, 2019
Accepted: Apr 24, 2019
Published: Jan 20, 2020

DOI: 10.24086/cuesj.v4n1y2020.pp32-36

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 ± 2°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."

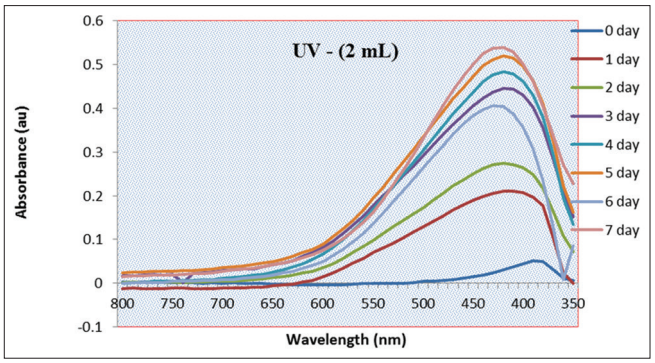

Figure 1: Ultraviolet-visible absorption spectra of silver nanoparticles after bioreduction by *Pleurotus ostreatus* mushroom aqueous extract

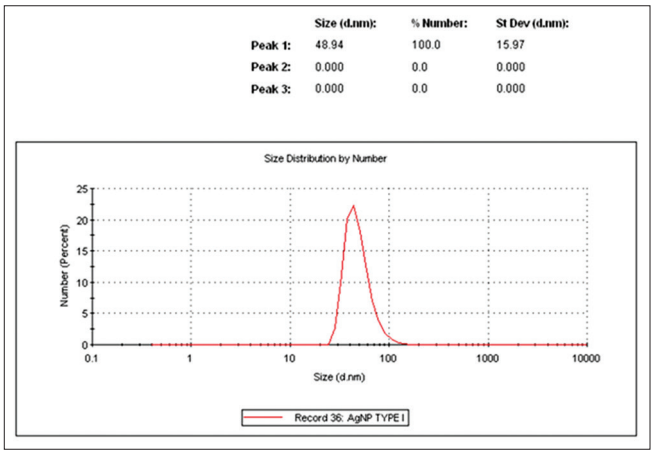

Figure 2: Size of silver nanoparticles

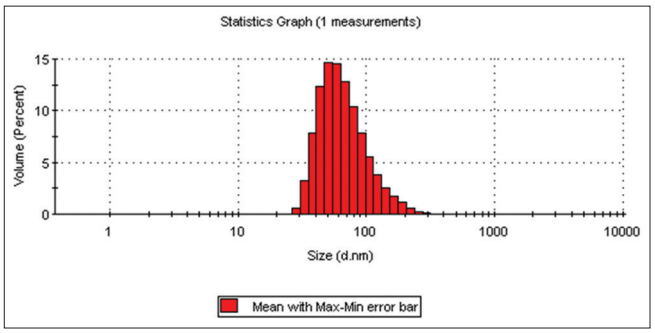

Figure 3: Intensity of particle size distribution of *Pleurotus ostreatus* dried mushroom aqueous extract

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

***Candida* spp.**	**Inhibition zone (mm)**			
	12.5 mg/ml	**25 mg/ml**	**50 mg/ml**	**100 mg/ml**
Candida albicans	---	10	12	16
Candida guillermondii	---	10	15.5	20
Candida krusie	---	14.5	15.5	19
Geotrichum ktebahnii	---	8	14	20
Candida zeylanoides	15	18.5	21	24.5
Staphylococcus aureus	10	11	12	16
Pseudomonas aeroginosa	---	12	13	14
Escherichia coli	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**
Candida albicans	$<0.001^{d}$	0 ± 0.0^{d}	10 ± 0.57^{c}	12 ± 0.58^{b}	16 ± 0.58^{a}
Candida guillermondii	$<0.001^{d}$	0 ± 0.0^{d}	15 ± 0.58^{c}	15.6 ± 0.33^{b}	20 ± 0.57^{a}
Candida krusie	$<0.001^{d}$	0 ± 0.0^{d}	14.5 ± 0.28^{c}	15.6 ± 0.16^{b}	19 ± 0.57^{a}
Geotrichumktebahnii	$<0.001^{d}$	0 ± 0.0^{d}	8 ± 0.57^{c}	14 ± 0.58^{b}	20 ± 0.58^{a}
Candida zeylanoides	$<0.001^{e}$	15 ± 0.57^{d}	18.5 ± 0.28^{c}	21 ± 0.57^{b}	24.5 ± 0.28^{a}
Staphylococcus aureus	$<0.001^{d}$	10 ± 0.58^{c}	11 ± 0.57^{bc}	12 ± 0.57^{b}	16 ± 0.58^{a}
Pseudomonas aeroginosa	$<0.001^{c}$	0 ± 0.0^{c}	12 ± 0.58^{b}	13 ± 0.58^{ba}	14 ± 0.57^{a}
Escherichia coli	$<0.001^{d}$	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 ($P\leq0.05$), using (LSD test). AgNPs: Silver nanoparticles

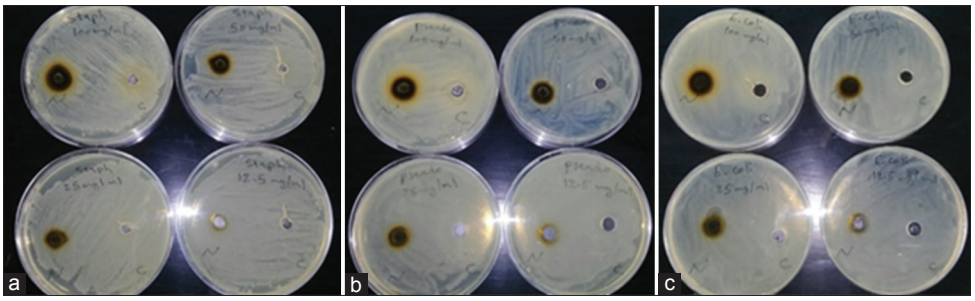

Figure 4: Inhibition zone of silver nanoparticles against several isolates of bacteria; (a) *Staphylococcus aureus*, (b) *Pseudomonas aeroginosa* (c), *Escherichia coli*

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 (*P. ostreatus*) 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].

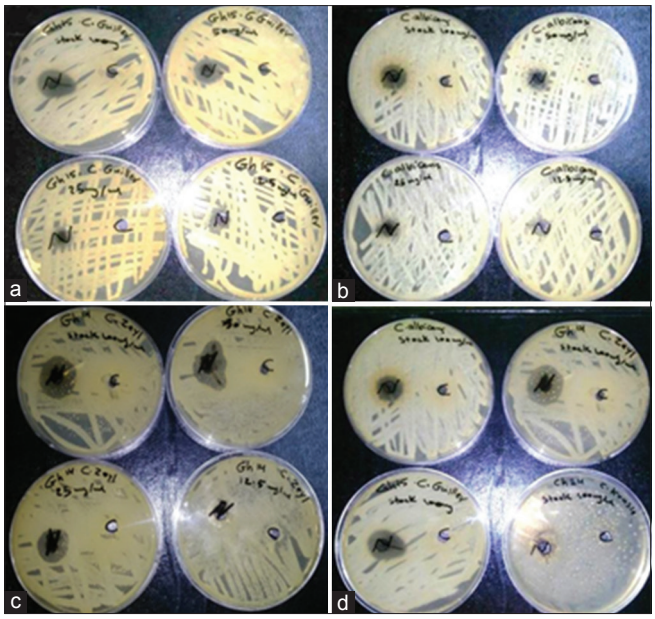

Figure 5: Inhibition zone of silver nanoparticles (AgNPs) (N) against several isolates of *Candida*; (a) *Candida guillermondii*; (b), *Candida albicans*; (c), *Candida zeylanoides*; (d), *Candida* spp. in concentration of 100 mg/ml of Ag NPs the way of measuring of diameter of inhibition zone. The aqueous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

REFERENCES

1. A. Mohammed, M. Girilal, S. A. Mahdy, S. S. Somsundar, R. Venkatesan and P. T. Kalaichelvan. "Vancomycin bound iogenic gold nanoparticles: A different perspective for development of anti RSA agents". *Process Biochemistry*, vol. 46, pp. 636-641, 2011.
2. J. Hardes, H. Ahrens, C. Gebert, A. Streitbuerger, H. Buerger and M Erren. "Lack of toxicological side-effects in silver-coated megaprostheses in humans". *Biomaterials*, vol. 28, pp. 2869-2875, 2007.
3. J. R. Morones, J. L. Elechiguerra, A. Camacho, K. Holt, J. B. Kouri and M. J. Yacaman. "The bactericidal effect of silver nanoparticles". *Nanotechnology*, vol. 16, pp. 2346-2353, 2005.
4. B. Y. Geng, L. D. Zhang, G. Z. Wang, T. Y. Xie and G. W. Zhang. "Synthesis and photoluminescence properties of ZnMnS nanobelts". *Applied Physics Letters*, vol. 84, pp. 2157-2159, 2004.
5. K. Dunn and V. Edwards-Jones. "The role of acticoat with nanocrystalline silver in the management of burns". *Burns*, vol. 30, pp. 1-9, 2004.
6. F. Von Nussbaum "Antibacterial natural products in medicinal chemistry - exodus or revival". *Angewandte Chemie International Edition*, vol. 45, pp. 5072-5129, 2006.
7. S. Sarkar, A. D. Jana, S. K. Samanta and G. Mostafa. "Facile synthesis of silver nanoparticles with highly efficient anti-microbial property". *Polyhedron*, vol. 26, pp. 4419-4426, 2007.
8. M. A. Pfaller and D. J. Diekema. "Epidemiology of invasive candidiasis: A persistent public health problem". *Clinical Microbiology Reviews*, vol. 20, pp. 133-163, 2007.
9. G. S. Martin, D. M. Mannino, S. Eaton and M. Moss. "The epidemiology of sepsis in the United States from 1979 through 2000". *New England Journal of Medicine*, vol. 348, pp. 1546-1554, 2003.
10. P. G. Pappas, J. H. Rex, J. Lee, R. J. Hamill, R. A. Larsen and W. Powderly. "A prospective observational study of candidemia: Epidemiology, therapy, and influences on mortality in hospitalized adult and pediatric patients". *Clinical Infectious Diseases*, vol. 37, pp. 634-643, 2003.
11. T. F. Patterson. Focus on candidemia. In: *"Treatment and Prevention of Fungal Infections"*. New York: Applied Clinical Education, pp. 7-8, 2007.
12. T. C. White, S. Holleman, F. Dy, L. F. Mirels and D. A. Stevens. "Resistance mechanisms inclinical isolates of *Candida albicans*". *Antimicrobial Agents and Chemotherapy*, vol. 46, pp. 1704-1713, 2002.
13. S. Perea, J. L. Lopez-Ribot, W. R. Kirkpatrick, R. K. McAtee, R. A. Santillan, M. Martinez. "Prevalence of molecular mechanisms of resistance to azole antifungalagents in *Candida albicans* strains displaying high-level fluconazole resistance isolated from human immunodeficiency virus-infected patients". *Antimicrobial Agents and Chemotherapy*, vol. 45, pp. 2676-2684, 2001.
14. M. D. Levin, J. G. den Hollander, B. van der Holt, B. J. Rijnders, M. Van Vliet, P. Sonneveld and R. H. N. van Schaik. "Hepatotoxicity of oral and intravenous voriconazole inrelation to cytochrome P450 polymorphisms". *Journal of Antimicrobial Chemotherapy*, vol. 60, pp. 1104-1107, 2007.
15. L. J. Worth, C. C. Blyth, D. L. Booth, D. C. M. Kong, D. Marriott, M Cassumbhoy, J. Ray, M. A. Slavin and J. R. Wilkes. "Optimizing antifungal drug dosing and monitoring to avoid toxicity andimprove outcomes in patients with haematological disorders". *Internal Medicine Journal*, vol. 38, pp. 521-537, 2008.
16. H. Sher, M. Al-Yemeni and K. Khan. "Cultivation of the oyster mushroom (*Pleurotus ostreatus* (Jacq.) P. Kumm.) in two different agroecological zones of Pakistan". *African Journal of Biotechnology*. vol. 10, no. 2, pp. 183-188, 2011.
17. S. Gurunathan, J. Raman, S. N. Abd Malek, P. A. John and S. Vikineswary. "Green synthesis of silver nanoparticles using *Ganoderma* neo-japonicum Imazeki: A potential cytotoxic agent against breast cancer cells". *International Journal of Nanomedicine*, vol. 8, pp. 4399-4413, 2013.
18. S. Berardinelli and D. J. Opheim. "New germ tube induction medium for the identification of *Candida albicans*". *Journal of Clinical Microbiology*, vol. 22, pp. 861-862, 1985.
19. S. Sujatha, S. Tamilselvi, K. Subha and A. Panneerselvam. "Studies on biosynthesis of silver nanoparticles using mushroom and its antibacterial activities," *International Journal of Current Microbiology and Applied Sciences*, vol. 2, no. 12, pp. 605-614, 2013.
20. A. J. Huh and Y. J. Kwon. "Nanoantibiotics: A new paradigm for treating infectious diseases using nanomaterials in the antibiotics resistant era". *Journal of Controlled Release*, vol. 156, pp. 128-145, 2011.
21. A. Panáček, M. Kolář, R. Večeřová, R. Prucek, J. Soukupová, V. Krystof, P. Hamal, R. Zboril and L. Kvítek. "Antifungal activity of silver nanoparticles against *Candida* spp". *Biomaterials*, vol. 30, pp. 6333-6340, 2010.
22. K. J. Kim, W. S. Sung, B. K. Suh, S. K. Moon and J. S. Choi. "Antifungal activity and mode of action of silver nano-particles on *Candida albicans*". *Biometals*, vol. 22, pp. 235-242, 2009.
23. K. J. Kim, W. S. Sung, S. K. Moon, J. S. Choi, J. G. Kim and D. G. Lee. "Antifungal effect of silver nanoparticles on dermatophytes". *Journal of Microbiology and Biotechnology*, vol. 18, pp. 1482-1484, 2008.
24. M. Stevanović, S. Škapin, I. Bračko, M. Milenković, J. Petković, M. Filipičd and D. P. Uskokovića. "Poly (lactide-co-glycolide)/silver

nanoparticles: Synthesis, characterization, antimicrobial activity, cytotoxicity assessment and ROS-inducing potential". *Polymer*, vol. 53, pp. 2818-2828, 2012.
25. M. A. El-Sayed. "Some interesting properties of metals confined in time and nanometer space of different shapes". *Accounts of Chemical Research*, vol. 34, pp. 257-264, 2001.
26. I. Sondi and B. Salopek-Sondi. "Silver nanoparticles as antimicrobial agent: A case study on *E. coli* as a model for gram-negative bacteria". *Journal of Colloid and Interface Science*, vol. 275, pp. 177-182, 2004.
27. A. T. Le, T. T. Le, H. H. Tran, D. A. Dang, Q. H. Tran and D. M. Vu. "Powerful colloidal silver nanoparticles for the prevention of gastrointestinal bacterial infections". *Advances in Natural Sciences: Nanoscience and Nanotechnology*, vol. 3, p. 045007, 2012.
28. A. de. Sousa, D. Mehta and R. W. Leavitt. "Bactericidal activity of combinations of silver-water dispersion™ with 19 antibiotics against seven microbial strains". *Current Science*, vol. 91, pp. 926-929, 2006.
29. R. Vazquez-Muñoz, M. Avalos-Borja and E. Castro-Longoria. "Ultrastructural analysis of *Candida albicans* when exposed to silver nanoparticles". *PLoS One*, vol. 9, no. 10, p. e108876, 2014.