Iraqi Journal of Science

ISSN: 0067-2904

Evaluation of inhibition activity of silver nanoparticles activity against pathogenic bacteria

Rusol, M. Al-Bahrani*, Jenan Atiyah Ghafil
Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq

Abstract
Silver nanoparticles synthesized from aqueoes extract for mushroom *pleurotus ostreatus*. Silver nanoparticles were showing good antibacterial activity. The antibacterial activity conducted against different pathogenic bacteria such as *Serratia marcescens, Pseudomonas fluorescens, Listeria, Methicillin-resistant Staphylococcus aureus (MARSA), Proteus mirabilis, Proteus vulgaris* and *Klebsiella pneumoniae*. The maximum inhibition zone was observed against *S. marcesance*. However, the lowest inhibition zone was found against *P. fluorescens*.

Keywords: Silver nanoparticles, *Pleurotus ostreatus, Pathogenic bacteria*

تقييم فعالية جزيئات الفضة النانوية ضد البكتريا

رسل محمد البحراني*، جنان عطية غافل
قسم علوم الحياة، كلية العلوم ، جامعة بغداد، بغداد، العراق

الخلاصة:
تم تخليق جزيئات الفضة النانوية من المستخلص المائي للفطر *pleurotus ostreatus* وان جزيئات الفضة النانوية لها فعالية عالية ضد البكتريا الممرضة مثل: *Serratia marcescens, Pseudomonas fluorescens, Listeria, Methicillin-resistant Staphylococcus aureusMARSA, Proteus mirabilis, Proteus vulgaris, Klebsiella pneumoniae* وان اعلى تثبيط كان ضد بكتريا *S. marcescens* وان اقل تثبيط كان ضد بكتريا *P. fluorescens*.

Introduction

Biosynthesis of nanomaterials has received a significant attention in the recent year. If harnessed to their full potential, biological synthesis could offer an extra advantage over the chemical methods by way of higher productivity and lower cost. In the recent years, 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]. Synthesis of metal nanoparticles has been reported from bacteria, yeasts, fungi and other biological sources and the same applied to diverse fields including drug delivery, biosensors, bioimaging, antimicrobial activity, food preservation etc. by exploiting their unique physical chemical and biological properties [2]. Antibacterial activity is related to compounds that locally kill bacteria or slow down their growth, without being in general toxic to surrounding tissue. Most current antibacterial agents are chemically modified natural compounds [3].The high bactericidal activity is certainly due to the silver cations released from Ag nanopaticles that act as reservoirs for the Ag+ bactericidal agent [4**].** The bactericidal activity of nanoparticles can be related to several mechanisms .The silver nanoparticles may also directly interact with the microbial cells. Silver ions can be studied to uncouple respiratory electron transport from oxidative phosphorylation, which inhibits respiratory chain enzymes or interferes through covering permeability to phosphate and protons e.g. interrupting transmembrane electron transfer, oxidizing cell components, disrupting,

*Email: nakm2004@yahoo.com

penetrating the cell covering or reactive oxygen species (ROS), or dissolving heavy metal ions that cause damage [5].

Material and methods

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 minutes. The boiled mushroom powder was then left covered for 30 minutes. Residues were then removed by filtration through gauze and further centrifuged (10,000 rpm, 30 min, and 4°C). Supernatants were then collected and filtered through Whatman No.1 filter paper. After that, freeze dried extract powders were obtained by using freeze dryer and stored at 4± 2°C.

Biosynthesis of silver nanoparticles

Biosynthesis of silver nanoparticles (AgNPs) Silver nitrate (1 x 10^{-3}) M, $AgNO_3$ 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 is prepared with sterile distilled water and filtered through syringe filter (0.2 µm). Based on the result of a preliminary trial, 2 ml to 7 ml of 10 mg/ml of aqueous extract of *P. ostreatus* respectively, were filled with sterile distilled water to a total 10 ml. After that the solution is added to 5 ml of 1 x 10^{-3} M aqueous $AgNO_3$ solution and exposed 7 days under UV-365 nm (Long UV). After 24 hours incubation, the light yellow colour of mixture solution turned to dark yellow indicating the formation of silver nanoparticles [6].

Bacteria isolates

All bacterial isolates *Serratia marcescens, Pseudomonas fluorescens, Listeria, Methicillin-resistant Staphylococcus aureus* (MARSA), *Proteus mirabilis, Proteus vulgaris, Klebsiella pneumoniae* were procured from AL-Yarmok teaching hospital. They were serially diluted and spread on nutrient agar plates. The plates were then incubated at 37°C for 24 h.

Antibacterial Activity of Silver Nanoparticles

Well Diffusion Method

The silver nanoparticle synthesized was tested for antimicrobial activity by agar well diffusion method against pathogenic microbes for *Serratia marcescens, Pseudomonas fluorescens, Listeria, Methicillin-resistant Staphylococcus aureusMARSA, Proteus mirabilis, Proteus vulgaris, Klebsiella pneumoniae.* The pure cultures of bacteria were subcultured on nutrient broth. Each strain was swapped homogeneously onto the individual plates using sterile cotton swabs. Wells of 10 mm diameter were on Muller Hinton agar using gel puncture. Concentration of silver nanoparticle 25µ/ml was poured on each well. After 24 hours incubation the various levels of zone of inhibition was measured. Three replicates of experiments were carried out [7].

Result and Discussion

Characterization of AgNPs using UV-Visible Spectroscopy AgNPs were characterized by UV-Visible spectroscopy. The UV-Visible absorption spectra of the AgNPs were measured in the range of 200-800 nm using a UV-Visible spectrophotometer. UV-Visible spectroscopy is an important and valuable technique for the characterization of nanoparticles. A strong and broad, surface plasmon peak located at 460 nm was observed for the AgNPs prepared using dried basidiocarp extracts of *P. ostreatus* Figure-1. The strong surface plasmon resonance centered at 460 nm clearly indicates the formation of AgNPs, which is extremely stable, with no evidence of flocculation of the particles even after onemonth. AgNPs synthesized from the aqueous extract of *P. ostreatus* exposure to UV (365 nm). The formation of AgNPs was confirmed by the UV-Vis spectrophotometry.

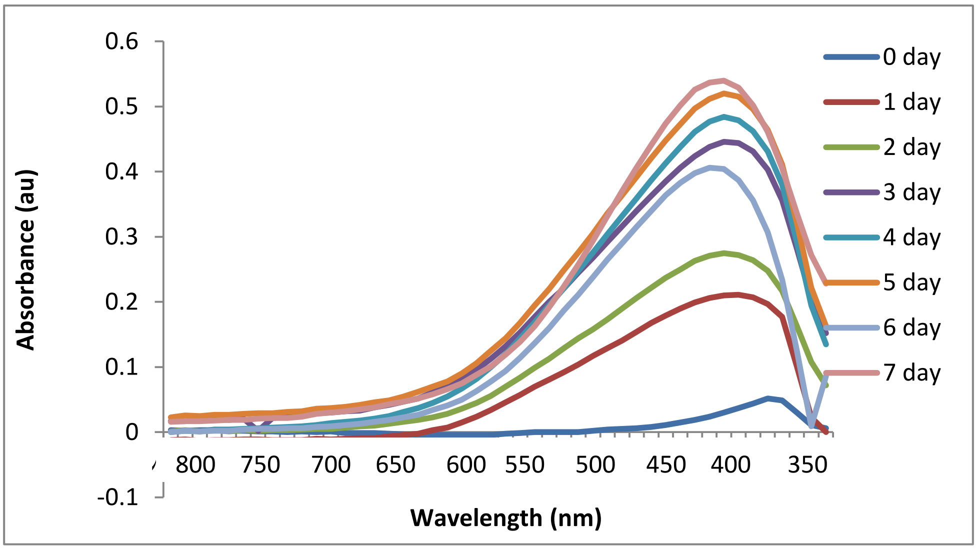

Figure 1- Strong beak within the range of 200 to 500 nm

Silver nanoparticles have a broad spectrum antimicrobial activity against several pathogens they are increasingly incorporated into various matrices to extend their utility in materials and biomedical applications. They are used as additives in health related products such as bandages, catheters, and other materials to prevent infection, particularly during the healing of wounds and burns [8].

The optical properties of silver nanoparticles are related to the excitation of plasma resonance or inter-band transition, particularly on the size effect.

Intensity of Particle Size Distribution Analysis (PSD)

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 range from range from 16 to 100 nm.

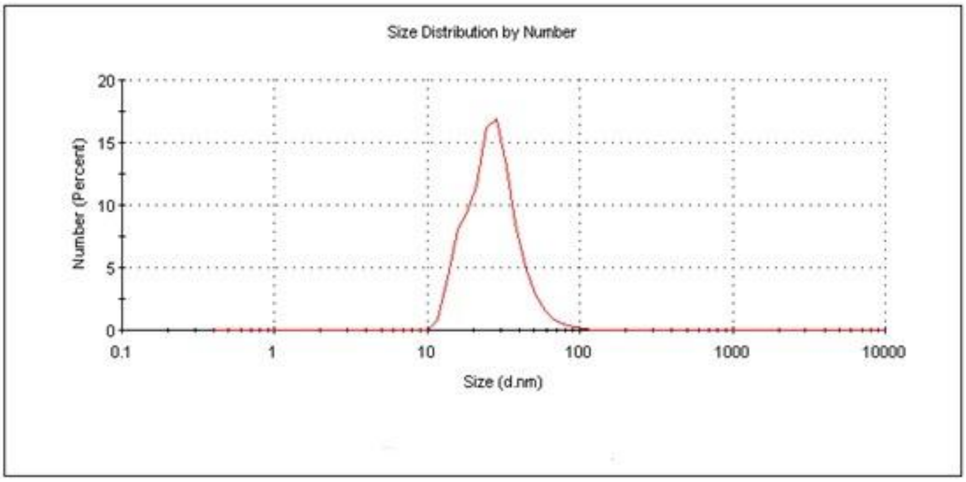

Figure 2- Size of silver nanoparticles

Antimicrobial activity of AgNPs

Antibacterial Activity by Well Diffusion Method

Silver nanoparticles having good zone of inhibitions were shown in Table-1. The antibacterial activity conducted against the pathogenic bacteria such as *Serratia marcescens*, *Pseudomonas fluorescens*, *Listeria*, *Methicillin-resistant Staphylococcus aureusMARSA*, *Proteus mirabilis*, *Proteus vulgaris*, *Klebsiella pneumoniae*. In that bacterial synthesized AgNPs were energetically involved in the antibacterial activity against *P. fluorescens* had the minimum zone of inhibition because of the maximum resistant capacity of the bacterial isolates. The maximum zone against Serratia. Silver nanoparticles, as an antibacterial agent, have been effective in a range of equipment, including glass, polymers, and titanium [9].

Table 1- Diameter of inhibition zone of AgNPs against different clinical isolates of bacteria

	Bacterial isolates	Diameter of inhibition zone (mm).
1	*Pseudomonas fluorescens*	20
2	*Listeria*	25
3	*MRSA*	22
4	*Proteus vulgaris*	28
5	*Klebsiella pneumonia*	28
6	*Serratia marcescens*	30
7	*P. mirabilis*	25
8	Control (Distilled water)	0.0

The bactericidal activity of nanoparticles can be related to different ways by direct react with microbial cells or effect on respiratory electron transport from oxidative phosphorylation that inhibits respiratory chain enzymes or interferes through covering permeability to phosphate and protons. That can be yielded different strategies to reduce food borne outbreaks from bacterial contaminations [10].

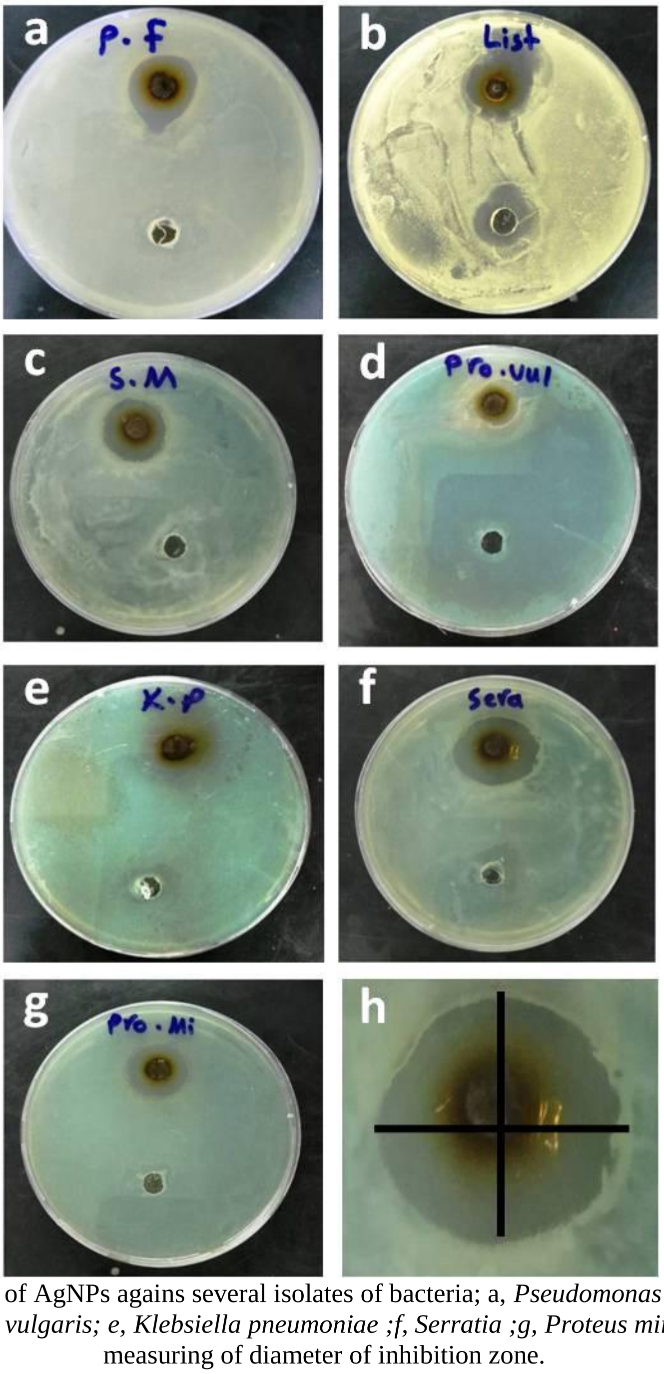

Figure 3- Inhibition zone of AgNPs agains several isolates of bacteria; a, *Pseudomonas fluorescens: b,Listeria,; c,MARSA ;d, proteus vulgaris; e, Klebsiella pneumoniae ;f, Serratia ;g, Proteus mirabilis,*h, the way of measuring of diameter of inhibition zone.

An interesting evolution of using nanoparticles against bacterial biofilms is represented by silver-coated magnetic nanoparticles, in fact, engineered multimodal nanoparticles comprising a magnetic core and a silver ring showed promising results [11].

References

1. Mohammed, A., Girilal, M., Mahdy, S.A., Somsundar, S.S., Venkatesan, R. and Kalaichelvan, P.T. **2011**. Vancomycin bound iogenic gold nanoparticles: A different perspective fordevelopment of anti RSA agents. *Process Biochem*, 46, pp: 636–641.
2. Vipul, B., Debabrata, R. and Absar, A. **2004**. Biosynthesis of zirconia nanoparticles using the fungus Fusarium oxysporum. *J Mater Chem*, 14, pp:3303–3305.
3. Von Nussbaum, F. **2006**. Antibacterial natural products in medicinal chemistry – exodus or revival? Angew. *Chem. Int. Ed*. 45, pp:5072–5129.
4. Sarkar, S., Jana, A.D., Samanta, S.K. and Mostafa, G.**2007**. Facile synthesis of silver nanoparticles with highly efficient anti-microbial property. *Polyhedron*., 26, pp: 4419–4426.
5. Tiwary, C.S., Sarkar, P., and Kumbhakar, A.K. **2008**. Synthesis and optical characterization of monodispersed Mn 2+ doped CdS nanoparticles. *Phys Lett A* , 372 (36), pp:5825-5830.
6. Gurunathan, S., Raman, J., AbdMalek, S.N., John, P.A. and Ikineswary, S. **2013**. Green synthesis of silver nanoparticles using Ganoderma neo-japonicumImazeki: a potential cytotoxic agent against breast cancer cells. *Int J Nanomedicine*, 8, pp:4399-4413.
7. Sujatha1, S. Tamilselvi, K. Subha and Panneerselvam1 A. **2013**. Studies on biosynthesis of silver nanoparticles using mushroomand its antibacterial activities. *Int. J. Curr. Microbiol. App. Sci* 2(12), pp: 605-614.
8. Strathmann M. and Wingender, J. **2004**. Use of an oxonol dye in combination with confocal laser scanning microscopy to monitor damage to Staphylococcus aureus cells during colonisation of silver-coated vascular grafts. *Int. J. Antimicrob. Agents*, 24 (3), pp: 234–240.
9. Jorgensen, M. and Ferraro, J. **2009**. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. *Clin. Infect. Dis*. 49, pp: 1749–1755.
10. Parikh, D. V., Fink, T. and Rajasekharan, K. **2005**. Antimicrobial silver/sodium carboxymethyl cotton dressings for burn wounds, *Textile Res. J.* 75 (2), pp: 134–138.
11. Melaiye, A. and Youngs, W. J. **2005**. Silver and its application as an antimicrobial agent, *Expert Opinion on Therapeutic Patents*. 15 (2), pp: 125–130.