Green synthesis of silver nanoparticles using tree oyster mushroom 

**Pleurotus ostreatus** and its inhibitory activity against pathogenic bacteria

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**ABSTRACT**

The present study reports the biological synthesis of silver nanoparticles (AgNPs) using the aqueous extract of fresh basidiocarps of the tree oyster mushroom, *Pleurotus ostreatus*. The AgNPs solution exhibited an absorption maximum at 440 nm, corresponding to surface plasmon resonance of AgNPs. The field emission scanning electron microscopy (FESEM) and high resolution transmission electron microscopy (HRTEM) analyses revealed that the synthesised AgNPs were spherical in shape and the particle size was less than 40 nm. The energy dispersive x-ray analysis (EDX) spectrum showed peaks for the presence of silver, carbon and oxygen atoms in the range of 2.8–3.2 keV. Fourier transform infra-red spectroscopy (FTIR) results showed the binding properties of bio-constituents responsible for capping and stabilizing the nanoparticles. The synthesised AgNPs significantly (p < 0.05) inhibited the growth of all bacterial species tested. The MIC of the tested bacterial spp. were in the range 13–27 µg/mL. The results of this study indicated that the synthesised AgNPs may be developed as an effective agent against bacterial infections.

**Keywords:** Nanoparticles, *Pleurotus ostreatus*, Mushroom, Anti-bacterial, Biomaterials

1. Introduction

In recent years, silver nanoparticles (AgNPs) have attracted the scientific community in the field of nanotechnology due to their unique properties and biological applications. Green synthesis of nanoparticles is considered as a clean, nontoxic and environmental-friendly method compared to other physical and chemical methods [1]. The green synthesised AgNPs have been widely used in many biological applications such as antimicrobial [2], anticancer treatment [3] and in drug delivery [4]. Several researches have successfully used fungi as reducing agents in AgNPs synthesis [5,6]. The therapeutic properties of the genus *Pleurotus* have been well documented. The photo-irradiated extracellular synthesis of AgNPs using the aqueous extract of the edible oyster mushroom *Pleurotus florida* as a reducing agent has been reported earlier [7]. The AgNPs synthesised from *Pleurotus djamor* var. *roseus* showed effective against human prostate carcinoma [8]. The mycosynthesised AgNPs using *P. cornucopiae* var. *citrinopileatus* inhibited the growth pathogenic *Candida* species tested [9]. *Pleurotus ostreatus* is a commercially important edible mushroom, which is also known as the oyster mushroom. The various medicinal effects of *P. ostreatus*, such as anticancer, immunomodulatory, antiviral, antibiotic anti-inflammatory and cholesterol-lowering activities are known worldwide [10–12]. The antifungal activity of AgNPs synthesised using the culture supernatant of *P. ostreatus* have been reported [13]. However, there are no reports on the green synthesis of AgNPs using the basidiocarps of *P. ostreatus*. The present study was thus focused to synthesise AgNPs by a simple, efficient, environmentally benign method using the aqueous extract of basidiocarps of *P. ostreatus* as the reducing agent. The AgNPs were tested against human pathogenic bacteria species viz., *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

2. Materials and method

2.1. Mushroom sample and strains

*Pleurotus ostreatus* fresh basidiocarps were collected from Tikrit, Iraq. The basidiocarps were sliced, oven-dried at 50 ± 2 °C for 24 h and ground to fine powder. Ten grams of the *P. ostreatus* powder was soaked in distilled water in a ratio of 1:10 (w/v) and boiled for 30 min.
at 60 ± 2 °C. The boiled mushroom powder was left covered in room temperature for 30 min and filtered. Suspended residues were removed by centrifuging the filtrate (10,000×g for 30 min at 4 °C) and the supernatant collected was filtered through Whatman No.1 filter paper.

The filtrate was freeze-dried (Christ, model Alpha 2-4 lyophilizer, The Netherlands) at −53 ± 2 °C for 48 h. The freeze-dried powder was used as the aqueous extract and stored at 4 °C prior to use.

2.2. Biosynthesis of silver nanoparticles (AgNPs)

Different concentrations (1–6 mg/mL) of the aqueous extract of *P. ostreatus* was added to 5 mL of 1 mM aqueous silver nitrate (AgNO₃; Sigma Aldrich, St. Louis, MO, USA) solution and kept at 28 ± 2 °C in dark incubation for the bioreduction of Ag⁺ ions to Ag°. The mixed solution was continuously stirred and incubated for 6, 12, 18, 24, 30, 36 and 40 h. The color change of AgNO₃ solution was monitored. The fully reduced solution was centrifuged at 20,000×g for 30 min. The residue was retained after discarding the supernatant. The residue was washed in sterile distilled water and dried.

2.3. Characterisation of AgNPs

Biosynthesised AgNPs were confirmed by sampling the reaction mixture at regular intervals and the absorption maxima was scanned at 350–800 nm using JASCO V 550 UV–vis spectrophotometer. The size and shape of the AgNPs were measured using field emission scanning electron microscopy (FESEM) and high resolution transmission electron microscopy (HRTEM) images. The crystalline structure of the particles was determined by recording their elemental spectra by an energy dispersive x-ray spectroscopy (EDX; FEG Quanta 450, EDX-OXFORD). Fourier transform infra-red spectroscopy (FT-IR) analysis was performed using Perkin-Elmer FT-IR spectrophotometer at a resolution of 4 cm⁻¹. The sample for FT-IR was prepared by grinding dried AgNPs with potassium bromide (KBr) to obtain a pellet.

2.4. Antibacterial activity of synthesised silver nanoparticles

2.4.1. Disc diffusion assay

The disc diffusion test was performed on Muller Hinton agar (MHA) lawn with selected bacterial spp. The density of the inoculum was adjusted to 10⁶ CFU mL⁻¹, separate sterile cotton swabs were dipped into the standardised *Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa* suspensions. Sterile swabs were used to lawn the suspension on the surface of the MHA medium and to ensure an even distribution of the inoculum. The plates were left undisturbed for 3–5 min to allow absorption of excess fluid. Selected antibacterial agent (10 µg), *P. ostreatus* aqueous extract (200 µg) and AgNPs coated discs (25, 50, 75, 100, 125 and 150 µg/disc) were placed on the inoculated plates and pressed firmly into the agar with sterile forceps. The plates were then incubated at 37 ± 2 °C for 24 h.
2.4.2. Minimal inhibitory concentration (MIC) of silver nanoparticles

MIC was determined according to a modified CLSI method using 96-well microtiter plates [14]. The bacterial spp. (concentration of $6 \times 10^6$ CFU/mL) were exposed to two-fold dilution series of the AgNPs ranging from 0.2 to 50 $\mu$g/mL. All the dilutions and controls were prepared in triplicates. Sterile Mueller Hinton broth was used as the negative control and inoculated broth was used as the positive control. The plates were incubated in aerobic conditions for 16 h at 37±2 °C. The MIC was then determined as the concentration (0.2–0.02 $\mu$g/mL) when there was no increase in optical density at 575 nm wavelength.

3. Results and discussion

3.1. Characterisation of AgNPs

The color change of AgNO$_3$ solution from pale yellow to dark brownish yellow indicated the formation of AgNPs. The color change is due to the excitation of surface plasmon vibration in the NPs [15]. The active molecules present in the *P. ostreatus* basidiocarp extract reduced the silver metal ions into AgNPs. The formation of AgNPs was confirmed by intense absorption peaks at wavelengths in the range of 400–470 nm, which are typical absorption bands of spherical AgNPs due to their surface plasmon resonance (Fig. 1). The AgNPs synthesised using low concentrations (10 and 20 mg/mL) of *P. ostreatus* aqueous extract showed broad absorbance, however the peak turned narrower with further increase in the concentration. At concentrations above 30 mg/mL, the absorbance was not recorded in the range 400–470 nm, this might be because of the high quantity of different types of bioconstituents present in the solution, which results in coupling and clumping of AgNPs. The UV–vis spectrum taken on different time intervals showed the optimum reaction kinetics at 24 h incubation. Hence, 20 mg/mL concentration of *P. ostreatus* aqueous extract at 24 h dark incubation was determined as the optimum conditions required for effective bioreduction of AgNO$_3$ solution. The width of the peak is indicative of polydisperse nanoparticles ranging in submicron sizes. Similar absorption peaks were observed in AgNPs of
P. cornucopiae var. citrinopileatus with a maximum absorption band at 420–450 nm [9].

The HRTEM and FESEM images provide further insight into the structure and morphology of the synthesised AgNPs (Fig. 2a–c). The images depict that the AgNPs are spherical in shape and well dispersed without any aggregation, with an average size ranging from 10 to 40 nm. The size distribution analysis showed the average size of the AgNPs as 28 nm (Fig. 2d). The presence of different size distribution of AgNPs as evidenced in size distribution study, which is due to the involvement of various biomolecules in capping and bioreduction of AgNO₃ solution. In general, metallic AgNPs exhibit typical absorption peak at 3 keV due to surface plasmon resonance [16]. The EDX analysis confirmed the presence of elemental silver. At 3 keV, 13% of Ag besides C and O, were found to be present in AgNPs (Fig. 2e). The existence of C and O atoms might be due to the presence of other active principles in the aqueous extract of P. ostreatus. FT-IR spectrum revealed the functional groups involved in the reduction of AgNO₃ (Fig. 2f). The spectrum showed strong absorption peaks from 3318 cm⁻¹ to 534 cm⁻¹. The absorbance peak at 3318 cm⁻¹ corresponds to N–H stretching vibrations [17], band at 2944 cm⁻¹ corresponds to C–H stretch alkenes and O–H stretch carboxylic acid [18]. Peak at 1612 cm⁻¹ is characteristic of a C=O vibration at the α- and β-unsaturated aldehydes. The presence of a carbohydrate group is evident from the peak at 1411 cm⁻¹ [19]. Hence, it is assumed that proteins and carbohydrates of the aqueous extract might be attached to the AgNPs and involved in the bioreduction.

### Table 1

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Streptomycin (10 µg)</th>
<th>P. ostreatus hot aqueous extract (200 µg)</th>
<th>AgNPs in different concentrations</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Zone of inhibition in mm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. subtilis</td>
<td>25.00 ± 0.45</td>
<td>-</td>
<td>25 µg</td>
</tr>
<tr>
<td>B. cereus</td>
<td>18.60 ± 0.52</td>
<td>-</td>
<td>50 µg</td>
</tr>
<tr>
<td>S. aureus</td>
<td>23.60 ± 0.52</td>
<td>-</td>
<td>75 µg</td>
</tr>
<tr>
<td>E. coli</td>
<td>24.00 ± 0.18</td>
<td>-</td>
<td>100 µg</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>27.00 ± 0.36</td>
<td>-</td>
<td>125 µg</td>
</tr>
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<td></td>
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<td>150 µg</td>
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3.2. Antibacterial assay

Dose-dependent growth inhibition of bacterial strains was used to assess the anti-bacterial activity of AgNPs. Fig. 3 demonstrates the growth inhibition of five different bacterial strains (Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa) treated with various concentrations of mycosynthesised AgNPs. The aqueous extract of P. ostreatus at 25, 50, 75 µg/disc showed no inhibitory activity against all bacterial spp. However, AgNPs at 100, 125 and 150 µg/disc showed a significant (p < 0.05) increase in the inhibition of bacterial spp. (Fig. 3). The inhibition zones for the bacterial spp. tested ranged from 8 to 14 mm (B. subtilis 8.80 ± 0.18–14.00 ± 0.1 mm, B. cereus 9.00 ± 0.45–12.00 ± 0.10 mm, S. aureus 8.00 ± 0.18–11.00 ± 0.36 mm, E. coli 9.00 ± 0.1–12.00 ± 0.8 mm and P. aeruginosa 9.00 ± 0.0–13.00 ± 0.55). Streptomycin
(10 µg disc−1) was used as the comparative standard (Table 1). AgNPs exhibited broad-spectrum anti-bacterial activity towards five different bacterial strains and this antibacterial effect was found to be size as well as dose-dependent.

The AgNPs were bactericidal against all the bacterial spp. tested at the minimum concentrations. Among the bacterial spp. tested, B. cereus showed the lowest MIC (13.21 ± 1.00 µg/mL) followed by E. coli and P. aeruginosa at MIC of 16.13 ± 2.00 and 18.96 ± 2.00 µg/mL, respectively. The bactericidal efficacy of the bacterial spp. tested in the present study is moderate compared to the efficacy of antibiotics such as streptomycin, ampicillin, gentamycin. However, these commercial antibiotics may cause toxic adverse events such as nephrotoxicity, neuropathy, hepatitis and so on [20]. The AgNPs would be an alternative in the treatment of bacterial pathogens.

The mechanism of the inhibitory effects of Ag+ ions on microorganisms is only partially known. Some studies have reported that the positive charge on the Ag+ ion is crucial for its antimicrobial activity through the electrostatic attractions between the negatively charged cell membrane of microorganisms and the positively charged nanoparticles [21,22]. Another tactic could be alteration in structural integrity or physicochemical changes in the bacterial cell wall. Amro et al. suggested that metal depletion may cause the formation of irregularly shaped pits in the outer membrane and change membrane permeability, which is caused by the progressive release of lipopoly-saccharide molecules and membrane proteins [23]. Although their inference may involve some sort of binding mechanism, the mechanism of the interaction between AgNPs and components of the outer membrane is still unclear. Further studies are needed to explore the mechanism.

4. Conclusion

The biosynthesis of AgNPs using P. ostreatus aqueous extract, as a reducing as well as stabilizing agent, was shown to be an efficient and eco-friendly system. Hence, the biological approach appears to be cost efficient alternative to conventional physical and chemical methods of AgNPs synthesis and would be suitable for developing a biological process for large-scale production. The synthesised AgNPs showed moderate antibacterial activity compared to the efficacy of antibiotics such as streptomycin, ampicillin, gentamycin. Hence, these AgNPs may be explored to treat bacterial infections and related applications in health sectors. These AgNPs may be utilized as nano-coatings for surgical devices, instruments, in wound healing creams/gels and sanitary bandages.

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References