ABSTRACT

Pleurotus ostreatus (P2) was the fungus isolated from Iraq and identified in this study, then mycosynthesis of silver nanoparticles from aqueous extract of Pleurotus ostreatus (P2) at (10) mg/ml. The antioxidant activity for synthesized silver nanoparticles from aqueous extract of fruiting body of Pleurotus ostreatus (P2) at concentration (10) mg/ml, in reducing power, total phenolic compounds and chelating ability were (5.33±0.11)nm, (13.80±0.10) mg/g and (85.36±0.55%) respectively. Reducing power for aqueous extract of fruiting body for Pleurotus ostreatus (P2) at concentration (10) mg/ml was (0.8±0.05) nm, total phenolic compounds (1.8+0.1) mg/g and chelating ability (26.10+0.10%), least significant differences (2.301).

KEYWORDS: Pleurotus ostreatus, fungus isolated.

INTRODUCTION

Many studies have found that some species of mushrooms especially from Basidiomycetes are having therapeutic properties such as antioxidant, antimiobial, anticancer, cholesterol lowering and immuno stimulatory effects. [1]

In the recent years, biosynthesis of nanoparticle has received a significant attention in the recent times owing to the use of mild experimental conditions such as temperature, pH, and pressure. 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. [2] Nanoparticles (NPs) like silver are known to induce reactive oxygen species (ROS) in various
cell types. In spite of this, the link between silver nanoparticles (AgNPs) and oxidative stress is not well established.\[3\]

**MATERIALS AND METHODS**

1-Mushrooms

*Pleurotus ostreatus* (P2) was the local fungus isolated and identified in this study. This fungus was obtained from the Mushrooms Production Unit in the College of Agriculture, University of Tikrit and routinely maintained on malt extract agar slants, then kept in refrigerated at (4) °C and sub culturing at least monthly.

2- Malt Extract Agar (MEA)

Dissolved (25) g of malt extract agar powder in (1) liter of distal water and sterilized by autoclave at (15) psi pressure and (121) °C for (15) minute.

3- Production fruit bodies of mushrooms

It was produced according to (4) and it including many steps

**Preparation of spawn**

Wheat grains (free from molds and insects) was boiled in water (1:5)W:V for (2) h, after removing the excess water, grains mixed well with (6%) w/w calcium carbonate (CaCo\(_3\)) and (2%) w/w calcium sulfate (CaSo\(_4\)), and then packed (150) g per flask with (1) liter capacity and then sterilized by autoclave for one hour, on the second day grains inoculated with a piece (1×2) cm of fungus colony for *P. ostreatus* (P2) and incubation at (25) °C for (14) days with shaking three times through incubation period to homogenize the mycelium growth and avoid adhesion and accumulation of grain. Spawn can be directly used or stored at (4) °C until use approximately one month.

**Preparation of substrate (production medium)**

Wheat straw was crushed, immersed over night in water to the next day, after removing the excess water, pasteurized with steam at (60)°C for (9) h then it cooled and become ready for spawning.

**Spawning**

Wheat straw substrate inoculated by through spawning method, the spawn was add at the rate (3%) w/w to the substrate, and packed in plastic bags have (30×50) cm dimensions. Each bag was filled with two kg of substrate, after that these bags were incubated in room incubation at...
(25) °C, these plastic bags raised from substrate after (2-3) weeks or until complete formation of mycelia for fruiting stage.

**Fruiting and harvesting**

To obtain better production, (80%) relative humidity must be provided by humidifier and spraying the walls, ground of incubation room and full growth substrates with tap water (2-3) times daily and exposing it to fluorescent light (6) h/day and reduced the temperature to (15)°C. After two weeks of these conditions fruitbodies were formed and harvested.

**4- Preparation of aqueous extract of Pleurotus streatus (P2)**

The oven dried mushroom was blended, the obtained powder was soaked in distal 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) this method according to.[5]

**5-Reducing Power**

The reducing power was determined according to the method of (6), (2.5) ml for aqueous extract for mushrooms were mixed with (2.5) ml of (200) mM sodium phosphate buffer pH (6.6) and (2.5) ml of (1%) potassium ferricyanide and the mixture was incubated at (50) °C for (20) min. After (2.5) ml of (10%) trichloroacetic acid was added, the mixture was centrifuged for (10) min, the upper layer (5) ml was mixed with (5) ml of distal water and (1) ml of (0.1%) ferric chloride and the absorbance was measured at (700) nm. A solution with all reagents without the extracts was used as a blank.

**6- Determination of total phenolic compounds**

Total amount of phenolic compounds was measured according to (7). A folin ciocalteure reagent was diluted with distal water (1:10) and added (4) ml to (1) ml of ethanolic extract for mushroom and water extract, the color was developed by adding (5) ml of (7.5%) sodium carbonate solution in distal water. The absorbance was read at (765) nm after (30) min on an UV-VIS spectrophotometer. Gallic acid was used as a standard substance for calibration.
Standard curve of Gallic acid
Prepared stock from dissolved (250) mg from gallic acid in (10) ml from distal water then get stock in concentration (25) mg/ml and from this stock prepared serial dilution (0,5,10,15,20,25) mg/ml ,then the total phenolic compounds was determined according to the method of (7).

7- Chelating ability on ferrous ions
Chelating ability was determined according to the method of (8) included (1) ml from mushrooms extract were completed by ethanol in alcohol extract or water in aqueous extract to (3.7) ml then added (0.1) ml of (2) mM ferrous chloride. The reaction was initiated by the addition of (0.2) ml of (5) mM ferrozine. After (10) min at room temperature, the absorbance of the mixture was determined at (562) nm against a blank. Citric acid and Ethylene Diamino Tetraacetic Acid (EDTA) were used for comparison.

Citric acid
Prepared stock from dissolved (500) mg from Citric acid in (10) ml from distal water then get stock in concentration (50) mg/ml and from this stock prepared serial dilution (0,5,10,15,20,25) mg/ml, then the chelating ability was determined according to the method of (8).

EDTA
Prepared stock from dissolved (500) mg of EDTA in (10) ml from distal water to get stock solution in concentration (50) mg/ml and from this stock prepared serial dilution (0,5,10,15,20,25) mg/ml ,then the chelating ability was determined according to the method of (8).

8- Biosynthesis of silver nanoparticles (AgNPs)
Silver nitrate (1x10⁻³) M AgNO₃ stock solution was prepared in sterile deionised triple - distilled water and the subsequent dilutions were made from this stock solution. AgNPs were made according to the method described by (9). 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, ( 2-7) ml of (10) mg/ml of aqueous extract of P. ostreatus (P2) respectively, were filled with sterile distilled water to a total (10) ml. After that the solution is added to (5) ml of (1X 10⁻³) M aqueous AgNO₃ solution and kept at room temperature and exposed under UV (365) nm (Long UV). After (24) h incubation, the light
yellow colour of mixture solution turned to dark yellow indicating the formation of silver nanoparticles. The AgNPs remained stable at room temperature for more than two months as no changes in the absorption spectrum were observed.

9- Evaluate antioxidant activity of synthesized silver nanoparticles
The silver nanoparticles synthesized from *Pleurotus ostreatus* (P2) at concentration (10) mg/ml were evaluated for antioxidant activity as methods (5), (6) and (7). Then compared the antioxidant activity with untreated aqueous extract of *P. ostreatus* (P2).

10- Statistical analysis
All analyses were performed in triplicate. Analysis of variance (ANOVA) was performed using Duncan’s multiple range test to compare treatment means at (P < 0.05) using SPSS software version (16) (SPSS Inc., USA).

RESULTS
1- Reducing power
Reducing power (p < 0.05) showed in aqueous extract for fruiting body of *P. ostreatus* (P2) was (5.4±0.1) nm. In the present study, assay of reducing activity was based on the reduction of Fe 3+/ferricyanide complex to the ferrous form in presence of reductants (antioxidants) in the tested samples (10).

2- Total phenolic components
Total phenolic component (p < 0.05) showed in aqueous extract for fruiting body of *P. ostreatus* (P2) at (10) mg/ml was (1.8±0.1) mg/g. (11) reported that phenolic compounds were associated with antioxidant activity and play an important role in stabilizing lipid peroxidation. (12) Suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when up to (1.0) g was ingested daily from a diet rich in fruits and vegetables. (13) Suggested that highest content of phenols in mushrooms might be the key components accounting for the better results found in antioxidant activity, reducing power, scavenging abilities as compared to other mushrooms. Numerous studies have conclusively showed that consumption of foods high in phenolic content can reduce the risk of heart disease by slowing the progression of atherosclerosis, because they act as antioxidants, therefore edible mushrooms may have potential as natural antioxidants in food. This result indicates that phenol may be the main antioxidant compounds found in mushrooms, in agreement with several authors. Phenolic compounds as a scavenger of free radicals is widely accepted which stabilizes membranes and control oxidative reaction (14).
3-Chelating ability

Chelating ability in aqueous extract for fruiting body of *P. ostreatus* (P2) was (70.26±0.15) at (10) mg/ml. The chelating ability of a compound is defined as the formation of bonds between two or more separate binding sites within the same molecule and a single central atom (15). This result agree with(16) that found chelating ability (88.2%) at (10) mg/ml. Chelating agents may serve as secondary antioxidants, because they reduce the redox potential thereby stabilizing the oxidized form of the metal ions. As ferrous ions are the most effective pro-oxidants in food systems, the high ferrous ion chelating ability of the various extracts from the fruiting bodies of *P. ostreatus* could be beneficial (17). (18) Believed that transition metals are serving as the catalysts for the initial formation of radicals, chelating agents, on the other hand may stabilize transition metals in living systems and inhibit generation of free radicals, consequently reducing free radical mediated damage.

4-Mycosynthesis of AgNPs

Mycosynthesis of AgNPs was achieved by using the aqueous extract of *P. ostreatus* (P2). The stacked UV-Visible spectra of silver nanoparticles formed from the reaction of aqueous AgNO₃ with dried basidiocarp aqueous extracts were shown in figure (1). Addition of extracts to the aqueous AgNO₃ resulted in a change in color from pale yellow to brown after (3) days under long UV (365) nm exposure, which indicated the formation of AgNPs in the reaction mixture as figure (1). Results of color change indicated *P. ostreatus* (P2) dried basidiocarp extracts could be used as a reducing and stabilizing agent for the synthesis of AgNPs. The formation of the colour change is due to the excitation of surface plasmon vibration in metal nanoparticles and the formation of AgNPs was confirmed by UV-Vis spectroscopy.

Figure (1): Synthesis of silver nanoparticles (AgNPs) using *P. ostreatus* (p2) extract .The photo shows containers with samples of AgNO (A), AgNO₃ with the *P. ostreatus* (p2) dried basidiocarp extract (B) after exposure UV (365) nm
5- 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* (p2) as figure (2). 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 one month.

AgNPs synthesized from the aqueous extract of *P. ostreatus* (p2) after (24) hours exposure to UV (365) nm. The formation of AgNPs was confirmed by the UV-Vis spectrophotometry, which showed a strong peak within the range of (200-500) nm.

![UV (2 mL)](image)

**Figure (2):** UV-Vis absorption spectra of silver nanoparticles after bio-reduction by *P. ostreatus* (p2) mushroom aqueous extract at (10) mg/ml

6- 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 (16 -104) nm.
7- Compared antioxidant activity between synthesized AgNPs and aqueous extract of *P. ostreatus* (P2) at (10) mg/ml

In the table (1) showed reducing power, total phenolic compounds and chelating ability (p < 0.05) have significant different in antioxidant activity between synthesized AgNPs from aqueous extract of *P. ostreatus* (P2) at concentration (10) mg/ml and aqueous extract from *P. ostreatus* (P2) at concentration (10) mg/ml. These result agree with (19) reported that nanotechnology is concerned with the development of experimental processes for the synthesis of nanoparticles of different sizes, shapes and controlled disparity. This provides an efficient control over many of the physical and chemical properties and their possible application. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size distribution and morphology.

**Table (1): Reducing power, total phenolic component and chelating ability between synthesized AgNPs from *P. ostreatus* (p2) (10) mg/ml and aqueous extract of *P. ostreatus* (p2) (10) mg/ml**

<table>
<thead>
<tr>
<th>Type</th>
<th>Reducing power (nm)</th>
<th>Total phenolic component (mg/g)</th>
<th>Chelating ability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>synthesized AgNPs from <em>P. ostreatus</em> (P2) (10) mg/ml</td>
<td>5.33±0.11</td>
<td>13.80±0.10</td>
<td>85.36±0.55</td>
</tr>
<tr>
<td><em>P. ostreatus</em> (P2) (10) mg/ml</td>
<td>0.8±0.05</td>
<td>1.8±0.1</td>
<td>26.10±0.10</td>
</tr>
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REFERENCES


