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SHORT COMMUNICATION

Study on UV-visible for detection of biosynthesis of silver nanoparticles by oyster mushroom's extracts

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ABSTRACT
Twelve extracts belong to four species of edible oyster mushrooms were screened for their chemical value (viz. proteins, carbohydrates and phenols were assessed) and their capability to biosynthesize silver nanoparticles (AgNPs). In limitative conditions, dark incubation and temperature 25 °C, three modes of extracts preparation were developed. Properties of silver nanoparticles creation from extracts solution with 1 mM $AgNO_3$ were investigated by color and UV-Visible spectroscopy to confirm silver nanoparticles formation. The bright yellow oyster mushroom (*Pleurotus cornucopiae* var. *citrinopileatus*) was finding as a potential candidate for the synthesis of AgNPs. Brown color of aqueous solution was given indication for AgNPs formation. Results showed that AgNPs absorption band was located at a peak of 440 nm for *P. cornucopiae* var. *citrinopileatus*. Although others *P. ostreatus* (grey & white) and *P. salmoneostramineus* (pink) were not form AgNPs due to no change in color of extracts with Ag ions when incubated under the same conditions, which indicative for no silver nanoparticles synthesis. Thus, AgNPs formation depended on species of oyster mushroom, method of extracting, concentration of extract and the conditions of incubation (of light and temp.), but no on content of proteins, carbohydrates and phenol in crude extract leastwise in this study.

Keywords: *Pleurotus* spp., Chemical value, Extraction method, Silver nanoparticles, Nanotechnology.

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INTRODUCTION

Nanotechnology is the concept that research and development on new materials in size between 1 to 100 nm. It was used in medicinal, industrial and agricultural applications [1]. Recently, mushrooms are efficient in metal nanoparticles synthesis [2]. Using AgNPs increased in medicinal applications and health care in the world to know the role of their antiviral, anticancer, antibacterial and antifungal activities [3, 4]. Nowadays, numerous studies have be discussing the producing and using various nanoparticles in many directions [5].

Generally, study of nanomaterials synthesis from fungi is recently and it's important because a large amount of enzymes is producing [6]. Also, using of their extracts viz. proteins, amino acids, polysaccharides and vitamins involved as the reducing, stabilizing and capping agents in process of the nanoparticles [7, 8]. Eco-friendly nanoparticles were produced from silver and gold salts with fungal extracts [9]. Importance of nanoparticles was belonging to their goals, cancer cells and viruses, lead to use it in the producing of nano-drugs [10, 11]. Size and shape of AgNPs depends on the preparation temperature and type of mushroom's extract [9]. Chemical and biological experiments

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appeared inhibition of pathogenic bacteria because the interaction between silver ions in nanoparticles and group of thiol in the surface of microbes [12].

Because of the used material in synthesis of NPs must be non-toxic for producing drugs [13], new biomaterials were used to biosynthesize of newly metallic NPs; edible mushrooms were used as NPs bio resources: *Pleurotus sajor caju* [14, 15], *Coriolus versicolor* [16], *Agaricus bisporus* [17], *Pleurotus* spp. [18], *Pleurotus florida* [19], *Pleurotus ostreatus* [20, 21], *Pleurotus sanguineus*, *Schizophyllum commune*, *Lentinus sajor caju*, *Trametes feei*, *Trametes pocas* [22], *Pleurotus sapidus* [23], *Lentinus edodes* [24], *Inonotus obliquus* [25], *Ganoderma lucidum* [26] and *Pleurotus cornucopiae* [27]. Though, Numerous articles were given methods of synthesis newly metal nanoparticles in shape and size depending on specific requirements such as salts of silver, gold and cadmium which were used with mushrooms to biosynthesize AgNPs and AuNPs and CdNPs respectively[28], also, ferrous sulphate ($FeSO_4$) with *Pleurotus* spp. was used to form FeNPs [18] while zinc sulfide was used with *P. ostreatus* to synthesize ZnNPs [29].

Silver and Agnanoparticles have antimicrobial properties and were used in antimicrobial applications [3, 8]. In addition, the biological synthesis of nanoparticles is highly eco-friendly compared as the more traditional physical and chemical methods which often involve the use of hazardous chemicals. In this study, in limitative conditions, the use of four varieties of oyster mushroom [*Pleurotus ostreatus* (grey), *P. ostreatus* (white), *P. cornucopiae* var. *citrinopileatus* (bright yellow) and *P. salmoneostramineus* (pink)] in form fresh and dried extracts in the biosynthesis of silver nanoparticles is a relatively recent addition to the list of mushrooms in field of nanotechnology.

MATERIALS AND METHODS

Strains

Four fruing bodies of oyster mushrooms species: *Pleurotus ostreatus* (grey), *Pleurotus ostreatus* (white), *Pleurotus cornucopiae* var. *citrinopileatus* (bright yellow) and *Pleurotus salmoneostramineus* (pink) were obtained from Fungi and Plant Pathology Lab., College of Science, University of Anbar, Iraq.

Preparation of Extracts of Mushroom

Hot extraction of the dried oyster mushroom

Ten grams of the fresh mushroom of each species were dried at 45 °C and milled to obtained fine powdered materials, separately. Then extracted in 200 ml of hot distilled water (60 °C), shacked using a magnetic starrier for 60 min, centrifuged using the Ultracentrifuge (Beckman J2-M1) at 10000 rpm and 4 °C for 30 min to remove the insoluble matters and collected the aqueous supernatant. The residue was extracted again in the same method. The clear aqueous supernatant was freeze dried by the Lyophilizer (Chaist, Germany), collected the powder namely **(A)**, weighted and stored at 4 °C for this study.

Hot extraction of fresh oyster mushroom

Separately, one hundred grams of each species of fresh mushrooms were extracted in 800 ml of hot distilled water (60 °C), crushed by the Blander, shacked using a magnetic starrier for 60 min, centrifuged at 10000 rpm and 4 °C for 30 min and collected the clear aqueous supernatant. The residue was extracted again by the same method. The aqueous supernatant was freeze dried by the Lyophilizer, collected the powder that namesake (B), weighted and stored at 4 °C until use.

Cold extraction of fresh oyster mushroom

Separately, one hundred grams of each species of fresh mushrooms were extracted in 800 ml of distilled water (25 °C), crushed by Blander, frizzed at -20 °C for 48 h, dissolved at 25 °C, centrifuged at 10000 rpm and 4 °C for 30 min and collected the aqueous supernatant. The residue was extracted using the same method, too. The aqueous supernatant was freeze dried by Lyophilizer, collected the powder that namesake **(C)**, weighted and stored at 4 °C for this study.

Chemical compositions of extracts of mushroom

Total protein quantitative determinations were performed using the Bradford Dye Binding method. The reading of absorbance at 595 nm was achieved; total protein was calculated from a protein standard curve of bovine serum albumin between (10-100 µg/ml) [30]. While, total carbohydrates quantitative determinations were performed using the Phenol-Sulphuric Acid method. The reading of absorbance at 490 nm was achieved [31]. From another side, the total phenol content was performed using Arnow's method. The reading of absorbance at 515 nm was achieved; total phenol was calculated from a pure phenol standard curve eight concentrations between (1.88-16.91 g/L) [31].

Characterization of silver nanoparticles

Optical detection

One gram of each extract kind was dissolved in 100 ml of sterilized distilled water, separately. Serial dilutions of each extract were made (1, 2, 3, 4, 5, 6, 7, 8, 9 and 10) mg/ml. Ten milliliters of each concentration mixed with 5 ml of 1 mM of $AgNO_3$ (Sigma-Aldrich, USA) in glass test tube separately, incubated in dark condition at 25 °C. Then, change of solution color to brown was checked daily for seven days.

UV-Vis spectroscopy analysis

The reduction of Ag^+ was monitored by measuring the UV-Vis spectrum of the reaction medium daily at 24 h time interval until seventh day by drawing 2 ml of the sample and the absorbance was recorded at 350-800 nm using UV-Vis Spectrophotometer [32].

Statistical Analysis

Experimental values are given as means. Statistical significance was determined using ANOVA (two ways analysis) by GenStat program version 7 DE3 (VSN International Ltd., UK). Differences at level less than 0.05 were considered to be significant.

RESULTS AND DISCUSSION

Chemical Value of extracts

Significantly, at level less than 0.05, the total carbohydrates content of C extract of *Pleurotus ostreatus* (white) is 50.46% followed of 49.93% and 49.06% by B extracts of *P. ostreatus* (white) and *P. ostreatus* (grey) respectively. Whereas B extract of *P. cornucopiae* var. *citrinopileatus* shows lower concentrations of carbohydrates of 31.67% (table 1). The best total protein is 2.20% by C extract of *P. salmoneostramineus*, followed of 2.15% and 2.03% by C and B extracts of *P. ostreatus* (white) respectively, while the lower content is 0.64% and 0.94% by A extracts of *P. salmoneostramineus* and *P. cornucopiae* var. *citrinopileatus* successively. The total proteins content increased in extracts of cold water (C), followed B then A extracts in all mushroom species according to the method of extraction that due to the cold water which no destroyed more of proteins, while the heat treatment lead to destroyed it.

The total phenols increase to 153 mg/100g significantly ($p< 0.05$) with A extract of yellow oyster mushroom *P. cornucopiae* var. *citrinopileatus*, followed by 149, 80 and 79 mg/100g for A extracts of pink (*P. salmoneostramineus*), white and grey (*P. ostreatus*) oyster mushroom successively. The lower value is 17.5 mg/100g followed 20, 22 and 35 mg/100g for C extracts of yellow, white, grey and pink oyster mushroom, respectively. Generally, the total phenols increased with extract of dried oyster mushrooms by hot water extraction method followed by extracts of fresh oyster mushroom by same extraction method, then declined with extracts which prepared using cold water method. The reason of the high content of total phenols in the produced extract by hot water method compared as cold water method may be return to that hot water lead to unbound phenol groups thus appeared in the measure. However, the cold water keeps the phenols in the jointed chemical groups thus never showed in the apparatus during measurement.

The differences among contents of total proteins, carbohydrates and phenols may be belong to differing genetic information of species of oyster mushrooms [33]. Also, chemical value of edible mushroom depends on agro-wastes type as substrates for the cultivation, which lead to differ biodegradation ability according to species of mushroom. Weights of the produced powder are variable (Table 1). The C extract of *Pleurotus ostreatus* (white) had been higher weight 8.54 g than others, followed of 6.27 g and 5.50 g for B and C extracts of white and grey strains successively. The less weights of powder are 2.25, 2.40 and 2.44 grams for extracts; C of *P. cornucopiae* var. *citrinopileatus*, A of *P. salmoneostramineus* and B of *P. cornucopiae* var. *citrinopileatus*, respectively.

Formation of silver nanoparticles

Optical detection and UV-Visible spectral analysis

Fig. 1 shows the optical photograph of color change of extracts of oyster mushrooms in dark condition and temperature 25 °C when challenged with 1 mM silver nitrate ($AgNO_3$), which changed color from yellow to bright or dark brown color just with extract of *P. cornucopiae* var. *citrinopileatus* according to its concentrations after seven days. That is attained maximum peak at 440 nm indicative of synthesis of silver nanoparticles in small monodisperse [15, 32]. The light brown color gives best absorbance than dark brown at same wave length as seen in Fig. 1. The AgNPs absorbance band is increasing in positive correlation with long period of incubation. The extracts of white, grey and pink oyster mushrooms show no change in color and stay yellow when incubated under same conditions, which indicative to no nanoparticles formation. Thus NPs formation is depends on the kind of oyster mushroom, extracting method, concentration of extract and the

Table 1: Chemical composition of oyster mushrooms' extract (for each 100 g dry matter)

Oyster Mushrooms	Extraction method	Total carbohydrates (g $100g^{-1}$) (%)	Total proteins (g $100g^{-1}$) (%)	Total phenols (mg $100g^{-1}$)	Weight of the produced powder (gram)
P. ostreatus (grey)	A	44.26	1.640	79.50	2.96
	B	49.06	1.927	26.00	5.14
	C	48.60	2.077	22.00	5.50
P. ostreatus (white)	A	46.03	1.157	80.50	2.88
	B	49.93	2.030	23.00	6.37
	C	50.46	2.150	20.00	8.54
P. cornucopiae var. *citrinopileatus*	A	43.46	0.937	153.00	2.94
	B	31.67	1.660	27.50	2.44
	C	42.03	1.737	17.50	2.25
P. salmoneostramineus	A	45.69	0.640	149.00	2.40
	B	43.94	1.467	36.50	2.55
	C	48.28	2.200	35.00	5.16
LSD ($p< 0.05$)		1.738	0.1221	2.242	-

LSD: Least Significant Difference (Probability less than 0.05). A: extract of the dried mushroom that extracting by hot water, B: extract of fresh mushroom that extracting by hot water, C: extract of fresh mushroom that extracting by cold water.

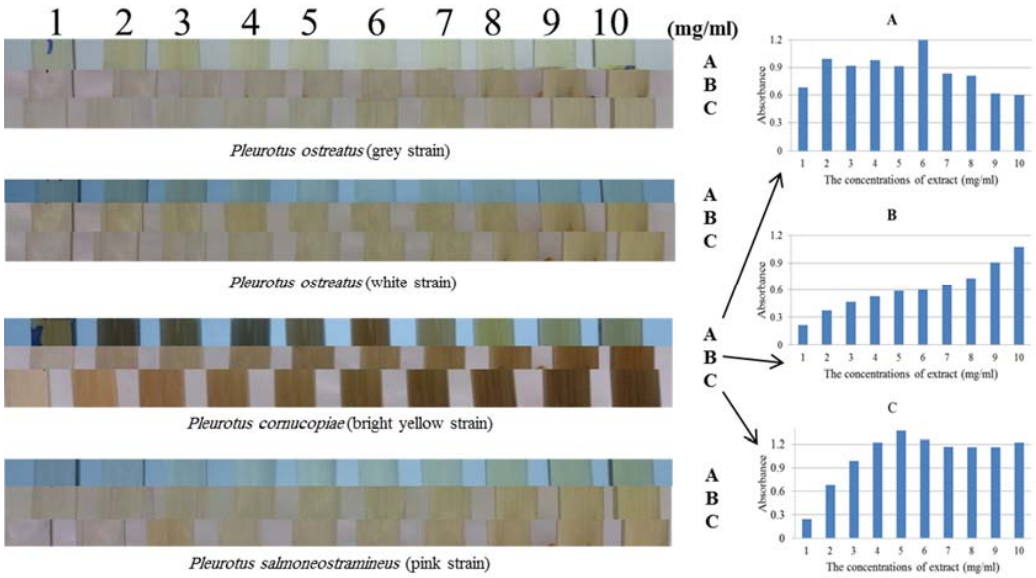

Fig 1. Colors with UV-Vis spectroscopy at 440 nm of AgNPs of *P. cornucopiae* var. *citrinopileatus* after seven days.

A: extract of the dried mushroom that extracting by hot water was exposed to 5 ml of 1 mM of AgNO3 solution, B: extract of fresh mushroom that extracting by hot water was exposed to 5 ml of 1 mM of AgNO3 solution, C: extract of fresh mushroom that extracting by cold water was exposed to 5 ml of 1 mM of AgNO3 solution, mg/ml: concentrations of oyster mushroom extracts in 10 ml.

conditions of incubation (of light and temp. place), but no on chemical value of crude extract as showed in this study.

These results agreed with Owaid et al. [27] who reported mycobiosynthesis of spherical silver nanoparticles from *P. cornucopiae* var. *citrinopileatus* mushroom with size ranged from 10 to 20 nm. Other studies recorded biosynthesis of silver nanoparticles from oyster mushroom such *Pleurotus* spp. [18], *Pleurotus florida* [19], *Pleurotus ostreatus* [20], *Pleurotus sajor caju* [15], *Pleurotus sanguineus* [22] and *Pleurotus sapidus* [23]. Finally, *Pleurotus* spp. had highest use (40%) in mushroom nanoparticles synthesis [34].

CONCLUSION

As resultant, formation of silver nanoparticles may be depended on species of mushroom, method of extracting, concentration of extract and the conditions of incubation (light and temp.), but no on proteins, carbohydrates and phenol content in crude extract leastwise in this study.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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