J. Appl. Environ. Biol. Sci., 8(3)142-147, 2018

ISSN: 2090-4274
Journal of Applied Environmental and Biological Sciences
www.textroad.com

Biosynthesis of Gold Nanoparticles using *Pleurotus ostreatus* (Jacq. ex. Fr.) Kummer Extract and their Antibacterial and Antifungal Activities

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Received: November 12, 2017
Accepted: February 25, 2018

ABSTRACT

The present work reports the green chemistry synthesis of gold nanoparticles (AuNPs) and the evaluation of their antibacterial and antifungal activities. AuNPs were synthesized by using an aqueous extract of *Pleurotus ostreatus*. AuNPs were characterized by UV–Vis-spectrophotometer, Infra-red (IR), spectroscopy, transmission electron microscopy (TEM). TEM studies showed the particles to be spherical in shape with an average size ~22.9 nm with a *SD* of ~ 6.6 nm ranging from 10.3 to 38.7 nm. In addition, the synthesized AuNPs were evaluated for their antibacterial and antifungal activities against *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Klebsiella pneumonia* ATCC 700603, *Pseudomonas aeruginosa* ATCC 254992 and *Staphylococcus aureus* ATCC 254996, while antifungal activity was investigated toward *Candida albicans* ATCC 10231. Obtained results showed that the functionalized AuNPs exhibited an important antimicrobial activity, respectively only toward *C. albicans*, *P. aeruginosa* and *S. aureus*. Results were discussed from the effectiveness point of view of gold nanoparticles, of *P. ostreatus* (Jacq. ex. Fr.) Kummer extract toward some microbial organisms.
KEY WORDS: AuNPs, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans*, *Pleurotus ostreatus*

INTRODUCTION

Recently, Metallic nanoparticles (NPs) are used in many research areas such as medical and environmental research [1, 2, 3] because they have unique biophysical properties, mainly because of the large accessible surface [4]. Nanoparticles can be synthesized by chemical routes such as the methods of [5, 6] but their main disadvantage is the production of toxic products for human health and the environment. For this reason it is necessary to develop environmentally friendly biosynthetic pathways. Green nanoparticles production using biological molecules have demonstrated advantages over chemical methods such as biomolecules produced by fungi include amino acids, polyphenols, carbohydrates and lipids [7, 8]. Having in fact a multiple role as they possess functional groups which can reduce Au^{3+} salt to Au^{0} and capped to the AuNPs resulting in very stable colloids. The biomolecule-based method can be used to overcome the side effects of the chemicals investigated for the manufacture of AuNPs. *Pleurotus ostreatus* is a commercially important edible fungus, reported to be anticancer, antiviral, anti-inflammatory and cholesterol lowering agents [9, 10, 11, 12]. In recent research, silver nanoparticles (AgNPs) syntheses using liquid mycelium culture of *P. ostreatus* have been reported as antibacterial, antifungal and anticancer factor [13, 14]. The aim of this study was to synthesize AuNPs using a simple, efficient and environmentally friendly method using the aqueous extract of *P. ostreatus* as a reducing agent. AuNPs have been tested against human pathogens microorganisms; antibacterial activity was achieved against *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Klebsiella pneumonia* ATCC 700603, *Pseudomonas aeruginosa* ATCC 254992 and *Staphylococcus aureus* ATCC 254996, while antifungal activity was investigated toward *Candida albicans* ATCC 10231.

MATERIAL AND METHODS

1. Obtaining of *Pleurotus ostreatus* basidiocarps

Technique of obtaining *P. ostreatus* basidiocarps was adopted from [15].

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2. Microorganisms used

The American Type Culture Collection (ATCC) strains used in this study were purchased from Becton Dickinson (BD). These strains were isolated on Cystine Lactose Electrolyte Deficient Deoxycholate Agar (CLED media). In total 5 bacterial strains (*Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC, 25922, *Klebsiella pneumonia* ATCC 700603, *Pseudomonas aeruginosa* ATCC 254992, *Staphylococcus aureus* ATCC 254996 and one fungal strain *Candida albicans* ATCC 10231) were tested.

3. Preparation of Extract

20 g of *P. ostreatus* basidiocarps were powdered and placed in 250 mL of deionized water in a 500 mL conical flask and boiled for 15 min with continuous stirring using a magnetic stirrer. The aqueous *P. ostreatus* extract was then cooled to room temperature, filtered through Whatman No. 1 filter paper and stored in a sterilized bottle at 4°C until further use.

4. UV-Vis spectroscopy analysis: UV-Vis spectral analysis was done by using UV-Vis spectrophotometer (Jasco V-670) covering a wavelength range from 190 to 800 nm and equipped with 1 cm wide quartz cells. The reduction of pure Au^{3+} ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 24 hrs, after diluting a small aliquot of the sample into distilled water.

FTIR analysis of Au-NPs. FTIR analysis was done using Thermo Scientific Nicolet iS5 Infrared Spectrometer using the KBr pellet method over the range 400–4000 cm^{-1} with a resolution of 4 cm^{-1}.

TEM analysis of Au-NPs. Sample for TEM analysis was prepared, as mentioned in IR sample preparations. The sample was first sonicated (Vibronics VS 80) for 5 minutes. Au-NPs were loaded on carbon-coated copper grids, and solvent was allowed to evaporate under Infra light for 30 minutes. TEM measurements were performed on Phillips model CM 20 instrument, operated at an accelerating voltage at 200 kV.

5. Synthesis of Au nanoparticles using *P. ostreatus* Extract.

Gold nanoparticles were synthesized by the reduction of $HAuCl_4\,3H_2O$ solution by *P. ostreatus*. Briefly, 5, 10 and 15 mL of this extract was slowly added to 50 mL of 1 mM $HAuCl_4$ in a 100 mL conical flask with continuous stirring at room temperature. The reduction of Au^{3+} to Au^0 nanoparticles by *P. ostreatus* extract was monitored visually by the change in color of the solution from brown to reddish purple (Fig. 1). The synthesized nanoparticles were collected by centrifugation (16,000 rpm) for about 20 min., washed with Milli-Q water, air dried and stored in a clean dry small bottle at 4 °C until further use and used for further analysis.

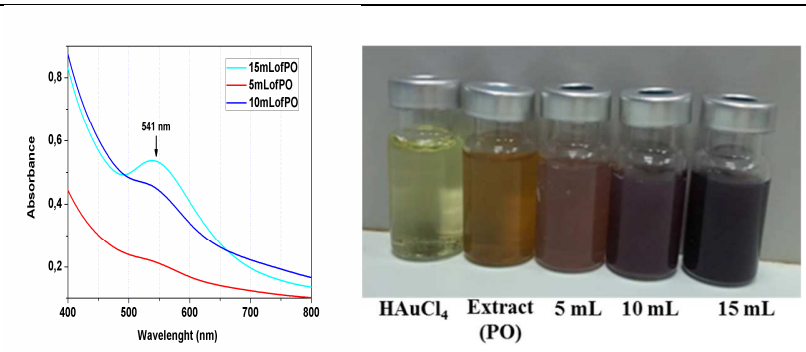

Fig. 1: The UV–vis spectra of gold nanoparticles obtained with different volume of *P. ostreatus* extract (PO), and photos of corresponding colloids taken after an incubation time of 30 min.

6. Screening for antibacterial and antifungal activities

Agar well Diffusion:

The ATCC microorganisms were activated on CLED media. The 0.5 Macfarland standards were used to adjust the turbidity to prepare inoculum from overnight grown bacteria. Muller Hinton Agar (MHA) and Sabouraud Dextrose agar (SDA) media were prepared according to standard aseptic technique; the first media used to test antibacterial activity, while the second one used to test antifungal activity. Four Wells of 6 mm were made aseptically, in these two media, by using sterile cork-borer and the sterility test was performed before inoculating. Bottoms of wells were

sealed by pouring molten Agar in sterile conditions. The plate containing MHA were swabbed with 24 hour culture of standard ATCC bacterial strains. Dispense 50 µl, 100 µl, 150 µl and 200 µl of the obtained AuNPs in the wells, the fifth well is the positive control which received 15 µg of tetracycline (CAS Number: 60-54-8) dissolved in distilled sterile water. The plates were incubated aerobically at 37° C for 24 hours according to Clinical and Laboratory Standards Institute (CLSI) guidelines. The results obtained were recorded when the zone of inhibition was greater than 6mm and compared with the positive control Tetracycline. Antimicrobial activities were measured by taking the diameter of clear inhibition zone around each well and calculated mean and standard deviation for the three reading. Concerning antifungal activity, same procedures have been done with the second medium (SDA), except positive control which was Fluconazole (CAS Number: 86386-73-4) dissolved in DMSO and the control well was received 25 µg of Fluconazole.

RESULTS AND DISCUSSION

The biosynthesis of gold nanoparticles (AuNPs) was realized out by adding hydrosol extract of *P. ostreatus* into $HAuCl_4$ solution with 1mM. The phytochemicals in the *P. ostreatus* extract acted as both reducing and stabilizing agents. The reduction of ionic gold Au^{3+} to gold metal Au^0 lead to a change in the color of the solution from yellow to pink then violet, color within 30 min of reaction [16, 17, 18]. Previous studies [19, 20, 21, 22, 23] show that this *P. ostreatus* contains considerable amounts of amino acids, polysaccharide glycoproteins, eritadenine, L-ergothioneine, phenolic substances, alkaloids, lactones, terpenes and ceramides. The OH of the polyphenol group reduced the Au^{3+} ions to Au^0 while the polysaccharide and amide group in amino acids stabilized the AuNPs. The formation of AuNPs was studied by UV–visible spectroscopy by measuring the surface plasmon resonance (SPR) of the wavelength range from 400 to 700 nm. More study shows that AuNPs synthesize by natural extract display a SPR band at around 500 - 550 nm, according to its size and shape [16, 17]. The band characteristic of surface plasmon resonance (SPR) of AuNPs was observed at 541 nm (Fig. 1). The TEM images provide size and morphology of the synthesized AuNPs (Fig. 2). The images depict that the AuNPs are spherical in shape with an average size ~22.9 nm with a *SD* of ~ 6.6 nm ranging from 10.3 to 38.7 nm (Fig 3).

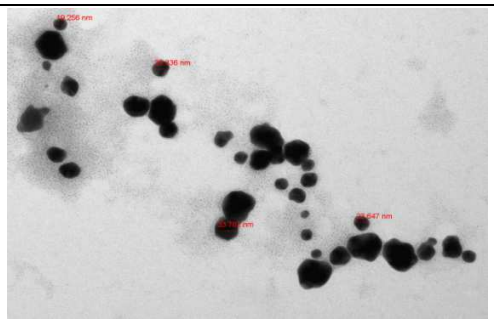

Fig. 2: A selected TEM image of *AuNPs*

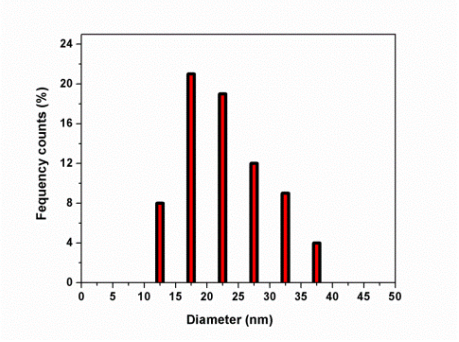

Fig. 3: Particle size distribution histogram

In order to study the synthesis of AuNPs, FTIR measurements were performed. Figure 4 shows the FTIR absorbance spectra of biosynthesized AuNPs with *P. ostreatus* extract. The spectrum confirms the presence of functional groups of some biomolecules such as carbohydrate, proteins, polyphenol, capping the AuNPs.

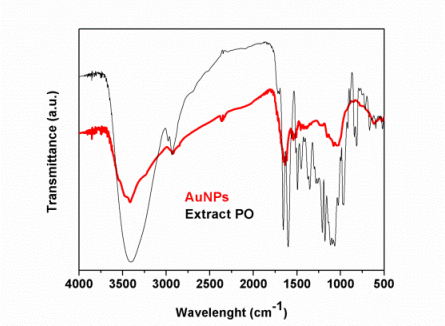

Fig. 4: IR spectrum of the dried AuNPs. IR spectrum of *P. ostreatus* (PO) extract is also given for comparison.

The hydroxyl (OH) of polysaccharide and phenols, carbonyl of the polyphenols and amide group of proteins, C–N of the amines and alkaloids were identified. In the synthesized AuNPs, the broadness of the bond assigned to the hydroxyl (OH) stretching vibration shifted from 3405 to 3414 cm^{-1}. In addition, the C=O of the amide which observed at 1654.8 cm^{-1} in the (*P. ostreatus*) was shifted to 1636 cm^{-1}. Furthermore, the bands at 1600, 1492 cm^{-1} assigned to the C=C of aromatics ring, C–N of amides, C–N of amines and C–O of ester stretching vibrations in the *P. ostreatus* shifted to 1440.06, 1396.95, 1278.89, 1192.51, respectively. This further confirmed the reduction and capping of the AuNPs by the *P. ostreatus* extract. Thus, the reduction and stabilization of the AuNPs could be attributed to the presence of polyphenols carbohydrates and amino acids in this extract. Many scientists reported that *Pleurotus ostreatus* have significant antibacterial effect towards Gram-negative and gram-positive bacteria [24, 25]. Our obtained results showed that the functionalized AuNPs exhibited varied antimicrobial activity on the tested bacteria and fungi. These activities have been manifested, respectively in importance point of view, only on *C. albicans*, *P. aeruginosa* and *S. aureus*. While no effects observed of these AuNPs toward *E. faecalis*, *E. coli* and *K. pneumonia*. Antimicrobial activity may be demonstrated by the diameter of zone of inhibition; this zone becomes more important with augmentation of the quantity of AuNPs from 50 to 200 µl, table 1.

Table 1. Zone of Inhibition induced by Gold nanoparticles of *P. ostreatus* extract.

Organisms	Method	Quantity of AuNPs			
		Zone of inhibition (mm) ± S.D.*			
		50 µl	100 µl	150 µl	200 µl
Candida albicans ATCC 10231	Well diffusion	21.66±1.527	23±1	26.33±1.527	32.33±2.081
	control	40.66±0.577			
Pseudomonas aeruginosa ATCC 254992	Well diffusion	19.33±1.527	23.66±0.577	26.33±1.040	29.33±1.258
	control	23.33±0.577			
Staphylococcus aureus ATCC 254996	Well diffusion	0	8.77±0.608	10.88±0.692	12.44±0.346
	control	29.33±0.577			

* $n_=$ 3 (0) = no inhibition zone

Using 50 µl of AuNPs gives zone of inhibition for *C. albicans* and *P. aeruginosa* but not for *S. aureus*. If we compare this zone with a previous study for the same author [15] in which he used hydro crude extract of the same species of fungi, and same strains of bacteria, we can notice that AuNPs have positive effect on *C. albicans* and *P. aeruginosa* for the mentioned quantity of extract. While *S. aureus* was affected by 100, 150 and 200 µl for prepared AuNPs and hydro crude extract obtained previously [15]. Inversely to this behaviour, it's very important to demonstrate that for the three tested bacteria (table 1), AuNPs in this study have less effect as antimicrobial agent than hydro crude extract of the same species in our previous study for 100, 150 and 200 µl of extract [15].

CONCLUSION

Saprophytic edible fungi *P. ostreatus* may constitute a source of bioactive compound. It is possible to synthesize AuNPs of *P. ostreatus* extract and is confirmed by UV, IR and TEM analysis. AuNPs can be used as an inhibitory agent against human pathogenic fungi and resistant human pathogenic bacteria.

ACKNOWLEDGMENTS

The authors wish to acknowledge the approval and the support of this research study by the grant No. 6945-SCI-2017-1-7-F from the Deanship of the Scientific Research at Northern Border University, Arar, KSA.

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