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Mycosynthesis of silver nanoparticles by *Pleurotus cornucopiae* var. *citrinopileatus* and its inhibitory effects against *Candida* sp.

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ABSTRACT

The present study focuses on a simple, low-cost and rapid bio-reduction of silver nitrate to silver nanoparticles (AgNPs) using the hot water extract of fresh basidiocarps of *Pleurotus cornucopiae* var. *citrinopileatus*. *P. cornucopiae* is an edible mushroom with medicinal properties. The UV–visible (UV–vis) spectra showed intense absorption peaks at 400 to 500 nm, which are typical absorption bands of spherical AgNPs. Fourier transform infra-red (FT-IR) spectra confirmed the involvement of various functional groups present in the biomolecules for reduction and capping of AgNPs. The spherical shaped morphology and < 100 nm particle size of the sample AgNPs was confirmed by field emission scanning electron microscopy (FESEM) and high resolution transmission electron microscopy (HRTEM). Energy dispersive X-ray analysis (EDX) illustrated that the AgNPs were crystalline in nature. Mycosynthesized AgNPs significantly ($p < 0.05$) inhibited the growth of all *Candida* species tested.

1. Introduction

Metal nanoparticles have recently gained the attention of the scientific community because these nanoparticles have a plethora of biological applications in areas such as antimicrobial [1], anticancer [2,3] therapy and drug delivery [4]. Silver nanoparticles (AgNPs) are attracting considerable interest among the emerging nanoproducts in the field of nanotechnology due to their unique properties and application in treating a variety of diseases, including human breast cancer [5]. Green chemistry approaches for the synthesis of AgNPs via biological methods using bacteria, fungi, plant extracts or purified biomolecules have helped to offer reliable and environmentally friendly alternatives to conventional chemical and physical synthesis approaches. Several researchers have attempted to use fungi as a platform for synthesis of AgNPs and gold NPs (e.g., *Verticillium* sp., *Fusarium oxysporum*, *Aspergillus fumigatus*, *Volvariella volvacea*, *Pleurotus florida*, *Pleurotus djamor* var. *roseus*) [6–11]. Many biologically active compounds found in basidiomycota have raised interest in the phylum [12].

Candida is an opportunistic human pathogenic fungi that causes oral, vaginal, and systemic infections [13]. These infections are commonly associated with immune dysfunction, as they are frequently found in AIDS patients and bone marrow transplant patients. Research data carried out on *Candida* species so far have shown unequivocally that it develops resistance against conventional antifungal drugs and its infections are difficult to cure with conventional antifungal agents. Hence, there is a need to find newer materials for the treatment of *Candida* infections. A recent study reported that the growth of *Candida albicans* was markedly inhibited when the cells were incubated with quantum-sized AgNPs and the minimum inhibitory concentration was determined as 70 ng/mL [14]. Thus, the objectives of the present study were to produce AgNPs using the hot water extract of *Pleurotus cornucopiae* var. *citrinopileatus* and to evaluate the anti-candida activities of the synthesized AgNPs against four pathogenic *Candida* species.

2. Materials and method

Strains: *P. cornucopiae* var. *citrinopileatus* basidiocarps were obtained from Fungi and Plant Pathology Laboratory, College of

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Science, University of Anbar, Iraq. All the four pathogenic *Candida* spp.—*C. albicans* ATCC 90028, *Candida glabrata* ATCC 90300, *Candida krusei* ATCC 6258 and *Candida pseudotropicalis* were provided by courtesy of Prof. Dr. Ian Macreadie, RMIT, Australia and maintained in Mushroom Research Centre, University of Malaya, Malaysia.

Extraction: *P. cornucopiae* var. *citrinopileatus* basidiocarps were sliced and oven-dried (45 ± 2 °C for 48 h). The dried basidiocarps were milled to obtain fine powder. Ten grams of the basidiocarp powder was agitated and boiled in distilled water at a ratio of 1:10 (w/v) for 30 min at 60 ± 2 °C [11]. The boiled mushroom powder was left covered in room temperature for 30 min to cool and then filtered. Suspended residues were removed by centrifuging ($10{,}000 \times g$ for 30 min at 4 °C) and the supernatant 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 hot water extract unless otherwise stated.

Mycosynthesis of silver nanoparticles (AgNPs): The hot water extract of *P. cornucopiae* var. *citrinopileatus* was used for the reduction of Ag^+ ions to Ag°, wherein different concentrations (1–5 mg/mL) of hot aqueous extract was added to 5 mL of 1×10^{-3} M aqueous silver nitrate (AgNO3; Sigma Aldrich, St. Louis, MO, USA) solution and kept at $25 + 2$ °C [3]. The mixed solution was continuously stirred and incubated for 24, 48 and 72 h and the color change of $AgNO_3$ solution from light yellow to brownish yellow was monitored. After incubation, the solution was centrifuged at $20{,}000 \times g$ for 30 min. The supernatant was discarded and the residue was washed in sterile distilled water and dried. The samples were again centrifuged to wash off any substances that had been absorbed onto the surface of the AgNPs.

Characterization of AgNPs: The bioreduction of silver ions was monitored by measuring the absorbance of the sample at 4 h time intervals using UV–vis Spectrophotometer (JASCO V 550 spectrophotometer) in the range of 350 to 800 nm. For Fourier transform infra-red spectroscopy (FT-IR) analysis, the AgNP solution was dried and ground with KBr to obtain a pellet. FT-IR was performed using Perkin-Elmer FT-IR spectrophotometer at a resolution of 4 cm^{-1}. 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).

Anti-candida activity: Anti-candida activity of the AgNPs was measured by well diffusion test performed on Sabouraud dextrose agar (SDA) lawned with selected *Candida* spp. (*C. albicans*, *C. glabrata*; *C. krusei* and *C. pseudotropicalis*). The density of the inoculum was adjusted to 10^5 cfu/mL. A sterile cotton swab was dipped into the standardized suspensions and was used to lawn on the surface of the SDA medium to ensure an even distribution of the inoculum. The plates were left undisturbed for 3 to 5 min to allow absorption of excess fluid. Selected antifungal agent (Nystatin 10 μg/well) and about 20, 40 and 60 μg/well of the AgNPs were introduced into the bore wells on the agar using sterile dropping pipette. The plates were then incubated at 37 ± 2 °C for 24 h and examined for measuring any inhibition zone.

3. Results and discussion

Characterisation of AgNPs: Production of AgNPs through fungi has several advantages over other approaches. They include tolerance towards high metal nanoparticle concentration in the medium, easy management in large scale production of nanoparticles, good dispersion of nanoparticle and higher amounts of protein expression. As a result for large scale production of nanoparticles fungi is preferred over other method [15]. The fungal system is the better alternative for the biological synthesis of metal nanoparticles.

The yellow exotic oysters mushroom *P. cornucopiae var. citrinopileatus* is easy to culture in bulk due to high lignolytic activity [16]. Interest in these species has increased considerably in the last decade because of their gastronomic value [17], numerous multifunctional biological activities, such as melanin biosynthesis inhibitory activity, antioxidant [18]. A previous study has reported the presence of lectin, peptide and proteins in water extract of *Pleurotus citrinopileatus* [19]. Due to these dissimilatory properties of *P. citrinopileatus* it could be widely used for the rapid and eco-friendly biosynthesis of metal nanoparticles.

In the present study, the color change of $AgNO_3$ solution from pale yellow to dark brownish yellow containing various concentrations of *P. cornucopiae* var. *citrinopileatus* aqueous extract under different incubation periods indicated the formation of AgNPs. The color change is due to the excitation of surface plasmon vibration in the NPs [20]. The results of color change indicated that the active molecules like polysaccharides and proteins present in the hot water extract of *P. cornucopiae* var. *citrinopileatus* reduced the silver metal ions to form AgNPs. The formation of AgNPs was confirmed by UV–vis absorption spectra at 400 to 500 nm, where intense absorption peaks at wavelengths of 450 and 420 nm are the typical absorption bands of spherical AgNPs due to their surface plasmon resonance (Fig. 1). A broad absorbance peak was obtained using 2 mg/mL of the aqueous extract compared to other concentrations. The optimum reaction kinetics was observed in 24 h incubation. Hence, 2 mg/mL concentration of *P. cornucopiae* var. *citrinopileatus* aqueous extract at 24 h dark incubation is essential for optimized bioreduction of $AgNO_3$ solution. The width of the peak is indicative of polydispersed nanoparticles ranging in

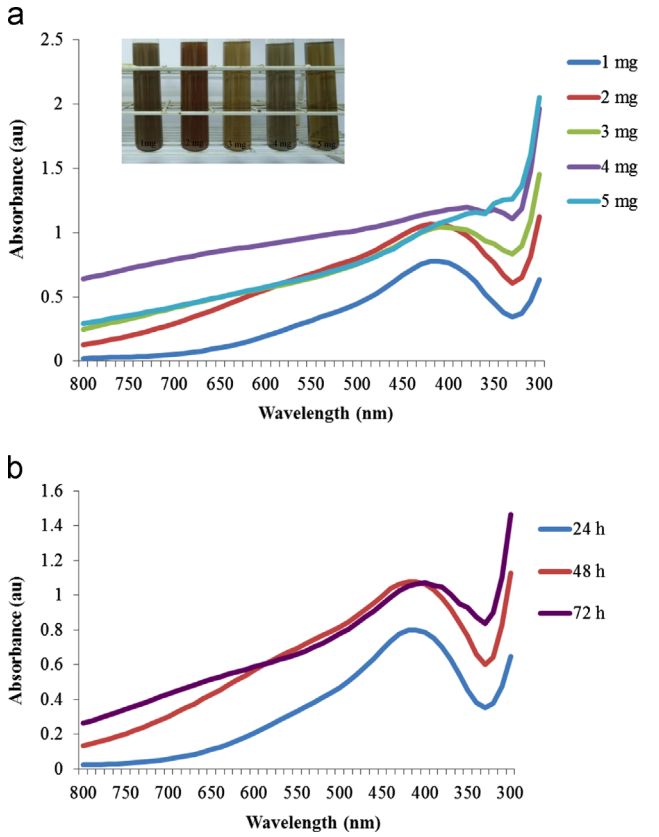

Fig. 1. Absorption spectra of AgNPs after bioreduction by *Pleurotus cornucopiae* var. *citrinopileatus* fresh mushroom aqueous extract. (a) Different concentrations used in bio-reduction, (b) Different time interval in bio-reduction.