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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

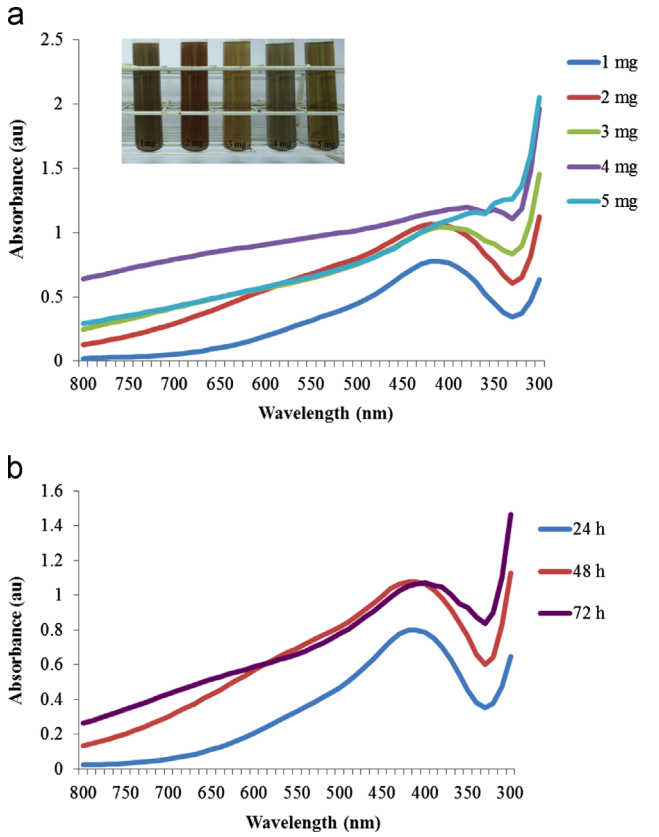

Fig. 1. Absorption spectra of AgNPs after bioreduction by *Pleurotus cornucopiae* var. *citrinopileatus* fresh mushroom aqueous extract. (a) Different concentrations used in bio-reduction, (b) Different time interval in bio-reduction.

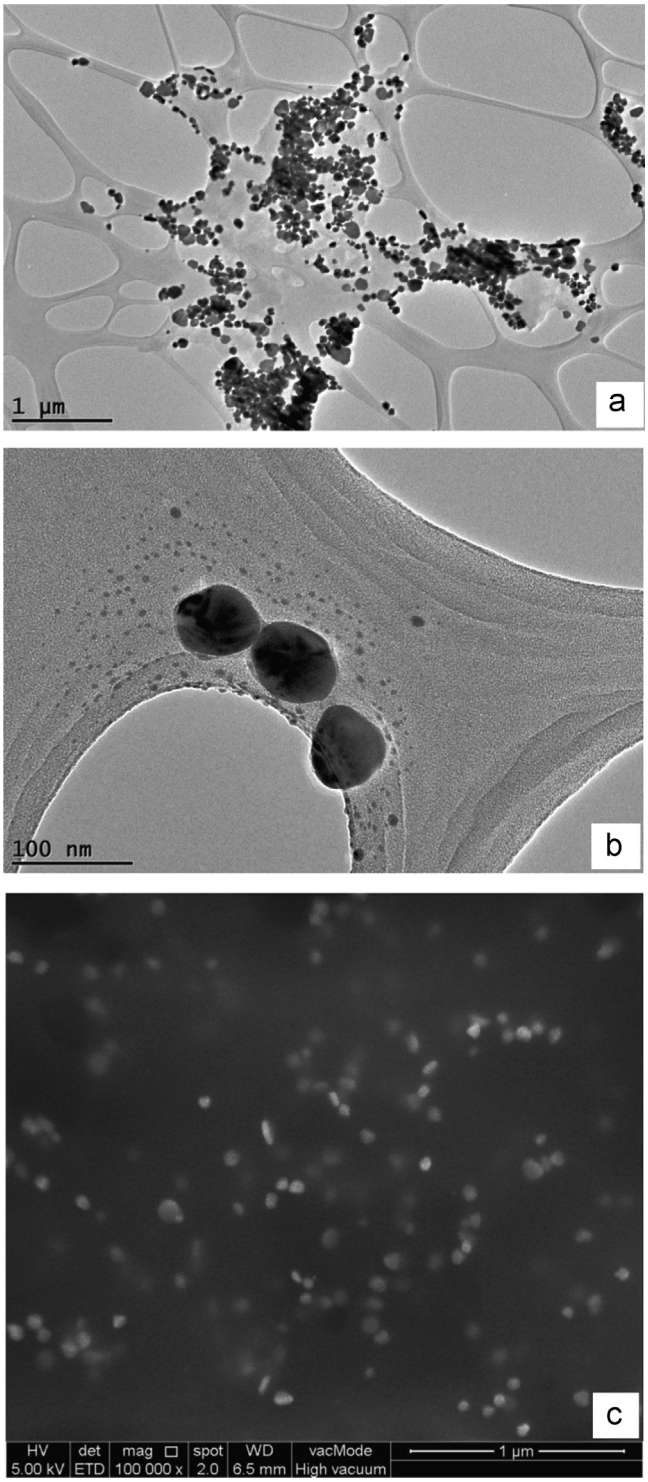

Fig. 2. Electron microscopic image of silver nanoparticles. (a). Silver solution formed the reaction of silver nitrate with mushrooms aqueous extracts for 24 h. (b) Individual nanoparticles through high resolution TEM with clear lattice fringes. (c) FESEM image of AgNPs.

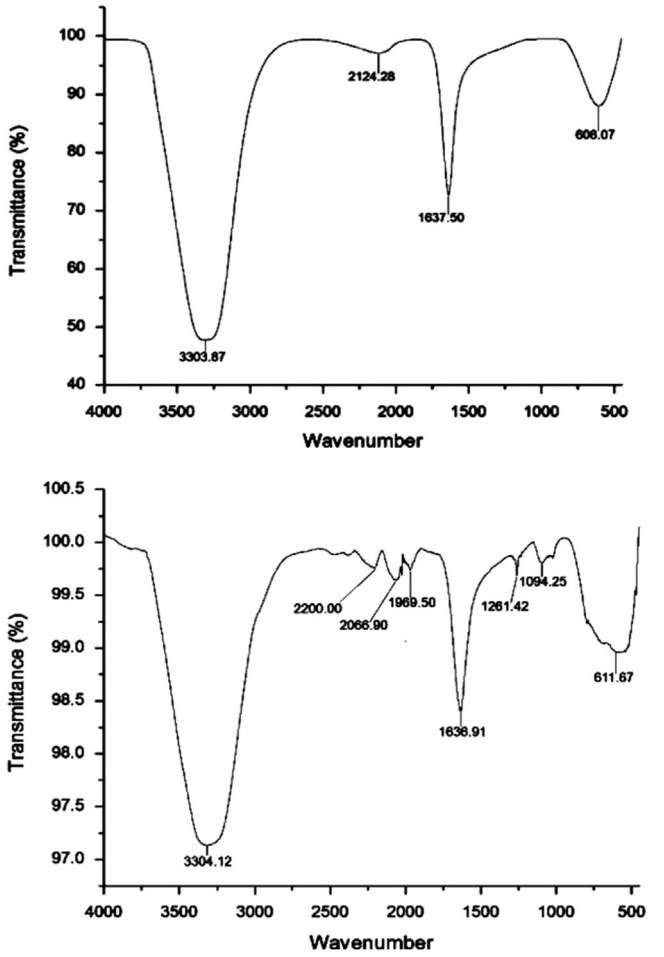

Fig. 3. FTIR image of AgNPs of *P. cornucopiae* var. *citrinopileatus* fresh basidiocarp aqueous extract. (a) *P. cornucopiae* aqueous extract, (b) *P. cornucopiae* var. *citrinopileatus* AgNPs.

submicron sizes. Similar absorption peaks were observed in AgNPs of *Coriolus versicolor* with a maximum absorption band at 440 nm [21].

FT-IR spectrum of AgNPs was used to identify the potential bioreductants of *P. cornucopiae* var. *citrinopileatus* aqueous extract involved in the reduction of AgNO3. Absorbance peaks at 3304, 2200, 2066, 1969, 1636, 1261, 1094 and 611 cm^{-1} representing the role of various functional groups in the bioreduction of $AgNO_3$ (Fig. 2). The absorbance peak at 3304 cm^{-1} corresponds to a C–H stretch alkene and O–H stretch carboxylic acids [22]. Peaks between 2200 and 1969 cm^{-1} correspond to a C=O vibration at the α- and β-unsaturated aldehydes; the peak at 1636 cm^{-1} indicates C=O stretch. The peaks at 1094 cm^{-1} and 611 cm^{-1} are the strong indications of heterocyclic compounds, such as alkaloids [23]. In the present study, the peaks at 1094 and 1261 cm^{-1} disappeared after nanoparticles synthesis, indicating the involvement of C–N group of amine, C–O group of alcohol, respectively in the bioreduction process.

The HRTEM and FESEM images showing the morphology of the synthesized AgNPs from aqueous extract of *P. cornucopiae* var. *citrinopileatus* is depicted in Fig. 3. It is evident from the micrograph that the particles are spherical in shape and have an average size ranging from 20 to 30 nm. The EDX spectrum showed peaks for the presence of silver, carbon, oxygen and potassium atoms in the range of 2.6–3.5 keV (Fig. 4). The existence of carbon, oxygen and potassium signals may have originated from the biomolecules bound to the surface of the nanoparticles [24]. However, Ag element is seen in greater percentage, which indicates that the major part of the product is AgNPs. The EDX pattern clearly showed that the Ag nanoparticles were formed by the reduction of silver ions using aqueous extracts *P. cornucopiae* var. *citrinopileatus*.

Antifungal assay: Possible anti-candida activity of the synthesized AgNPs was examined against four pathogenic *Candida* species (*C. albicans*, *C. glabrata*, *C. krusei* and *C. pseudotropicalis*) (Fig. 5) on SDA plates by well diffusion method. The aqueous extract of *P. cornucopiae* var. *citrinopileatus* at 20 and 40 μg/well showed no inhibitory activity against all *Candida* spp. However,

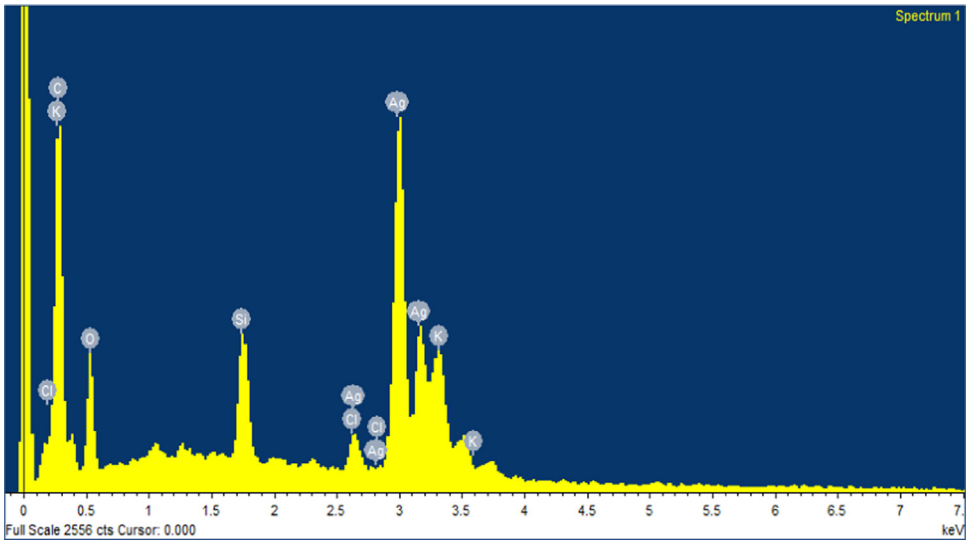

Fig. 4. EDX image of AgNPs solution formed the reaction of silver nitrate with aqueous extract of *P. cornucopiae* var. *citrinopileatus* for 24 h.

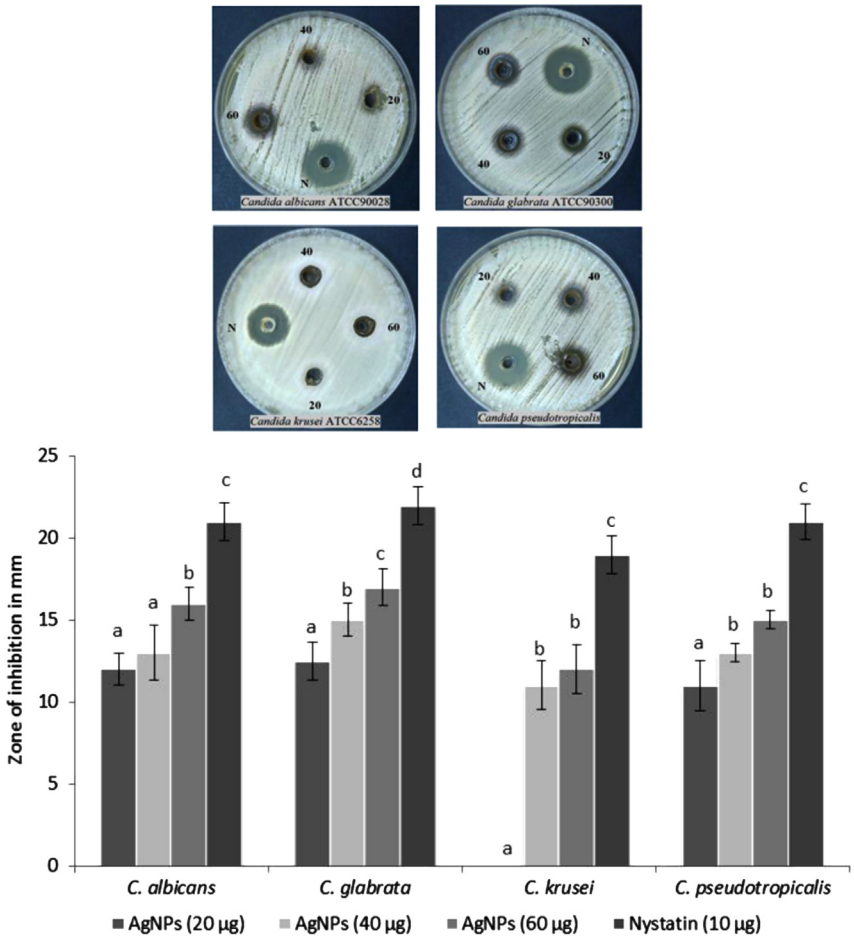

Fig. 5. Anti-candida activity of AgNPs synthesised with aqueous extracts of *P. cornucopiae* var. *citrinopileatus*N. positive control (Nystatin); 20µg, 40µg and 60µg of synthesized AgNPs by P. cornucopiae var. citrinopileatus. The differences among different concentrations in each sample were evaluated using Duncan multiple range test, where $p < 0.05$ was considered an indication of significance. .

AgNPs at 60 µg/well showed a significant ($p < 0.05$) increase in the inhibition of *Candida* spp. The inhibition zones for all the *Candida* spp. tested range from 11 ± 2 to 17 ± 1 mm (*C. albicans* 12 ± 3 to 15 ± 2 mm, *C. glabrata* 12 ± 2 to 17 ± 4 mm, *C. krusei* 11 ± 3 to 12 ± 2 mm and *C. pseudotropicalis* 11 ± 2 to 15 ± 2 mm). Nystatin (10 µg/well) was used as the comparative standard. The mechanism of the inhibitory effects of Ag ions on fungi is partially known. Bioactivity of AgNPs against the *Candida* spp has been reported by several authors and it is necessary to elucidate how AgNPs interact with fungal cells in order to generate information that could be

useful to develop new clinical AgNPs applications. The previous studies have reported that the positive charge on the Ag ion is crucial for its antifungal activity through the electrostatic attraction between negative charged cell membrane of microorganism and positive charged nanoparticles [25,26], cell permeability and progressive release of membrane constituents [27], free radical generation [28]. Thus, the in vitro anti-candida results obtained in the present study demonstrate that AgNPs have moderate inhibitory activity against *Candida* infections.

4. Conclusion

The present study, for the first time, showed that *P. cornucopiae* var. *citrinopileatus* aqueous extract can effectively serve as a reducing as well as a stabilizing agent in mycosynthesis of AgNPs in an environmentally friendly manner. UV–vis spectra, FT-IR, FESEM, HRTEM and EDX analysis confirmed the formation, binding constituents, size and shape, and silver ion signal of AgNPs. Silver nanoparticles prepared by the reduction method described here have great promise as antifungal agents and the results obtained in this study complement existing research on the potential use of nanomaterials in biomedicine. Anti-candida activity will provide a safe and efficient way to disrupt cutaneous to subcutaneous and even for controlled systemic infection management.

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References

[1] Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, et al. Antimicrobial effects of silver nanoparticles. Nanomed Nanotechnol 2007;3:95–101.
[2] Byrne JD, Betancourt T, Brannon-Peppas L. Active targeting schemes for nanoparticle systems in cancer therapeutics. Adv Drug Delivery Rev 2008;60:1615–26.
[3] Gurunathan S, Raman J, Abd Malek SN, John PA, Vikineswary S. Green synthesis of silver nanoparticles using *Ganoderma neo-japonicum* Imazeki: a potential cytotoxic agent against breast cancer cells. Int J Nanomed 2013;8:4399–413.
[4] Emerich DF, Thanos CG. The pinpoint promise of nanoparticle-based drug delivery and molecular diagnosis. Biomol Eng 2006;23:171–84.
[5] Franco-Molina MA, Mendoza-Gamboa E, Sierra-Rivera CA, Gómez-Flores RA, Zapata-Benavides P, Castillo-Tello P, et al. Antitumor activity of colloidal silver on MCF-7 human breast cancer cells. J Exp Clin Cancer Res 2010;29:148.
[6] Mukherjee P, Ahmad A, Mandal D, Senapati S, Sainkar SR, Khan MI, et al. Bioreduction of AuCl(4)(-) ions by the fungus, *Verticillium* sp. and surface trapping of the gold nanoparticles formed. Angew Chem Int Ed Engl 2001;40:3585–8.
[7] Ahmad A, Mukherjee P, Senapati S, Mandal D, Khan MI, Kumar R, et al. Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. Colloids Surf., B: Biointerfaces 2003;28:313–8.
[8] Bhainsa KC, D'Souza SF. Extracellular biosynthesis of silver nanoparticles using the fungus *Aspergillus fumigatus*. Colloids Surf., B: Biointerfaces 2006;47:160–4.
[9] Philip D. Biosynthesis of Au, Ag and Au-Ag nanoparticles using edible mushroom extract. Spectrochim Acta A: Mol Biomol Spectrosc 2009;73:374–81.
[10] Bhat R, Deshpande R, Ganachari SV, Huh do S, Venkataraman A. Photo-irradiated biosynthesis of silver nanoparticles using edible mushroom *Pleurotus florida* and their antibacterial activity studies. Bioinorg Chem Appl 2011;2011:650979. http://dx.doi.org/10.1155/2011/650979.Epub2011Dec10.
[11] Jegadeesh R, Rajasekhar Reddy G, Hariprasath L, Veerapandian S, Babu G, Raman N, et al. Mycosynthesis and characterization of silver nanoparticles from *Pleurotus djamor* var. *roseus* and their in vitro cytotoxicity effect on PC3 cells. Process Biochem 2015;50:140–7.
[12] Yashvant P, Ram N, Singh VK. Medicinal properties of *Pleurotus* species (*Oyster mushroom*): a review. World J Fungal Plant Biol 2012;3:1–12.
[13] Odds FC. Candida and candidosis. 2nd ed. London: Bailliere Tindall; 1988.
[14] Selvaraj M, Pandurangan P, Ramasami N, Rajendran SB, Sangilimuthu SN, Perumal P. Highly potential antifungal activity of quantum-sized silver nanoparticles against *Candida albicans*. Appl Biochem Biotechnol 2014;173:55–66.
[15] Takakura Y, Oka N, Kajiwara H, Tsunashima M, Usami S, Tsukamoto H, et al. A versatile affinity tag for protein purification and immobilization. J Biotechnol 2010;145:317–22.
[16] Kaviyarasan V, Natarajan K. Changes in extracellular enzyme activities during growth and fruiting of *Pleurotus cornucopiae* var. *citrinopileatus*. In: Rai RD, Dhar BL, Verma RN, editors. Indian mushroom conf advances mushroom biology and production, India; 1997. p. 310–28.
[17] Bao YY, Zhang Y, Tolgor LY. Chemical components of *Pleurotus citrinopileatus* Singer. Mycosystema 2004;23:262–9.
[18] Meng TX, Ishikawa H, Shimizu K, Ohga S, Kondo R. Evaluation of biological activities of extracts from the fruiting body of *Pleurotus citrinopileatus* for skin cosmetics. J Wood Sci 2011;57:452–8.
[19] Janga JH, Jeonga SC, Kimb JH, Leeb YH, Jub YC, Leea JS. Characterization of a new antihypertensive angiotensin i-converting enzyme inhibitory peptide from *Pleurotus cornucopiae*. Food Chem 2011;127:412–8.
[20] Sastry M, Mayya KS, Bandyopadhyay K. pH Dependent changes in the optical properties of carboxylic acid derivatized silver colloidal particles. Colloids Surf A 1997;127:221–8.
[21] Sanghi R, Verma P. A facile green extracellular biosynthesis of CdS nanoparticles by immobilized fungus. Chem Eng J 2009;155:886–91.
[22] Rajeshkumar S, Kannan C, Annadurai G. Synthesis and characterization of antimicrobial silver nanoparticles using marine brown seaweed *Padina tetrastromatica*. Drug Invention Today 2012;4:511–3.
[23] Meghwal M, Goswami TK. Chemical composition, nutritional, medicinal and functional properties of black pepper: a review. Sci Rep 2012;1:1–5.
[24] Punuri JB, Sharma P, Sibyala S, Tamuli R, Bora U. Piper betle-mediated green synthesis of biocompatible gold nanoparticles. Int Nano Lett 2012;2:18–32.
[25] Hamouda T, Myc A, Donovan B, Shih A, Reuter JD, Baker Jr JR. A novel surfactant nanoemulsion with a unique non-irritant topical antimicrobial activity against bacteria, enveloped viruses and fungi. Microbiol Res 2000;156:1–7.
[26] Dibrov P, Dzioba J, Gosink KK, Häse CC. Chemiosmotic mechanism of antimicrobial activity of Ag(+) in *Vibrio cholerae*. Antimicrob Agents Chemother 2002;46:2668–70.
[27] Amro NA, Kotra LP, Wadu-Mesthrige K, Bulychev A, Mobashery S, Liu G. High-resolution atomic force microscopy studies of the Escherichia coli outer membrane: structural basis for permeability. Langmuir 2000;16:2789–96.
[28] Danilczuk M, Lund A, Saldo J, Yamada H, Michalik J. Conduction electron spin resonance of small silver particles. Spectrochim Acta Part A 2006;63:189–91.