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Mycosynthesis and characterization of silver nanoparticles from *Pleurotus djamor* var. *roseus* and their *in vitro* cytotoxicity effect on PC3 cells

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ABSTRACT

The mycosynthesis approach for nanoparticle synthesis using edible mushrooms is attracting considerable interest over other conventional physical and chemical methods because this approach excludes the use of toxic chemicals. The present study reports the biological synthesis of silver nanoparticles (AgNPs) using an aqueous extract of *Pleurotus djamor* var. *roseus* and its cytotoxicity against human prostate carcinoma (PC3) cells. Nanoparticle formation was confirmed by UV–visible (UV–vis) spectroscopy, transmission electron microscopy (TEM), X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR) analysis. The UV–vis spectrum had an absorption peek at 440 nm, corresponding to the surface plasmon resonance of AgNPs. The TEM image confirmed the nanostructure and spherical shape of AgNPs, with sizes ranging from 5 to 50 nm. The FTIR spectroscopy results showed the binding properties of bio-constituents to identify the possible biomolecules responsible for capping and efficiently stabilizing the nanoparticles. The purity and crystalline structure were confirmed by XRD analysis. Furthermore, the cytotoxicity of biosynthesized AgNPs was evaluated using cell viability and nuclear fragmentation assays. It is evident from the results that the biosynthesized AgNPs inhibited proliferation of PC3 cells with an IC_{50} of 10 μg/ml during a 24-h incubation. These results suggest that AgNPs may exert their antiproliferative effect on the PC3 cell line by suppressing its growth, reducing DNA synthesis and inducing apoptosis. The morphological analysis of propidium iodide (PI) staining showed the presence of shrunken nuclei, DNA condensation and damage, which revealed cytotoxic effect on PC3 cells by AgNPs.

1. Introduction

Green chemistry approaches for the synthesis of silver nanoparticles (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. AgNPs have recently gained the attention of the scientific community because these nanoparticles have a plethora of biological applications in areas such as antimicrobial treatment [1], anticancer treatment [2,3], and drug delivery [4]. AgNPs are attracting considerable interest among the emerging nanoproducts in the field of nanotechnology due to their unique properties and applicability in treating a variety of diseases, including human breast cancer [5].

Several researchers have attempted to use fungi as a platform for synthesis of AgNPs and gold NPs (*e.g.*, *Verticillium*, *Fusarium oxysporum*, *Aspergillus fumigatus*, *Volvariella volvacea*, *Pleurotus florida*, *Ganoderma neo-japonicum*) [6–11]. Many biologically active compounds found in Basidiomycota have raised interest in the phylum [12]. Fungal mycelia, or the culture filtrate of the mushroom, including fruiting bodies, have been explored for their biological properties because they hold great promise as a source of novel antibiotics. Requiring a short growth and production period, the *Pleurotus* spp. are the easiest, fastest and cheapest species to

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cultivate. They belong to the oyster mushroom family (Pleurotaceae) and are known for being edible and nutritious mushrooms [13]. Fruiting bodies as well as active mycelia of *Pleurotus* species also possess a number of therapeutic properties such as anti-inflammatory, immunostimulatory and immunomodulatory [14], anticancer [15], ribonuclease [16], antiviral [17], anti HIV [18], anti-tumor [19] and antibacterial activities [20]. The photo-irradiated extracellular synthesis of AgNPs using an aqueous extract of the edible oyster mushroom *Pleurotus florida* as a reducing agent has been reported earlier [10]. However, the biosynthesis of AgNPs using *P. djamor var. roseus* has not been previously reported. In this present study, AgNP synthesis was achieved using a simple and rapid bioreduction approach. This is the first report on the mycosynthesis of AgNPs using *P. djamor* var. *roseus* as the source of the reducing agent.

Human prostate carcinoma (PC) is the most common cause of cancer in the male reproductive system. PC accounts for approximately 25% of newly diagnosed cancer patients. PC and colorectal cancer together represent the second leading cause of male deaths caused by cancer in the US after lung and bronchus cancer. Approximately 80% of prostate cancer occurs in the peripheral zone and 10–20% occurs in the transition zone. Cholesterol-lowering drugs, biopsy and conventional heating techniques involved in its treatment are expensive and known to induce side effects such as pain or discomfort *via* undesired reflection and scattering or absorption [21,22]. Accordingly, it is important to develop alternative therapies or drugs to overcome these drawbacks. The use of magnetic nanoparticles has been reported to be a feasible approach in treating patients with prostate cancer [23]. Toxicity due to the treatment can be considered moderate and quality of life is only temporarily impaired. Therefore, it is necessary to find novel therapeutic agents against cancer that are biocompatible and cost effective.

Although some studies reported in the literature are related to the anticancer activity of AgNPs, to the authors' knowledge, there are no studies concerning the effect of these synthesized AgNPs from aqueous extract of *P. djamor* var. *roseus* (AEPDR) on PC3 cells. Thus, the present study also evaluated the effect of AgNPs on cell viability and their potential to promote cell death.

2. Materials and methods

2.1. Chemicals

The PC3 cell line was obtained from the National Centre for Cell Science (NCCS), Pune, India. Silver nitrate ($AgNO_3$), Dulbecco's modified Eagle's medium (DMEM), trypsin–EDTA, fetal bovine serum (FBS), 3-(4,5-dimethyl thiazol-2yl)-2,5-dimethyltetrazolium bromide (MTT), propidium iodide (PI), dimethyl sulfoxide (DMSO), sodium bicarbonate and antibiotic solution were purchased from Hi-Media Laboratories Ltd., Mumbai, India and Sigma Chemicals (St. Louis, USA).

2.2. Mushroom sampling and identification

Fresh *P. djamor* var. *roseus* basidiomata were harvested from decomposed wood (*e.g.*, *Ficus* tree) in the forests of the Indian Institute of Technology campus, Chennai, India, and their macroscopic and microscopic characters were recorded. The molecular and classical taxonomy results supported the identification of the isolated strain as *P. djamor* var. *roseus*. The sequence data were deposited in the GenBank database (Maryland, USA) under accession number GU350628. The basidiocarp specimen was deposited in the Fungal Biotechnology, Natural Product and Tissue Culture Lab (FBNPTL) of the Centre for Advanced Studies in Botany, University of Madras.

2.3. Biomass production

A well-established spawn (fungal seed) of *P. djamor* var. *roseus* was used for cultivation. The spawn was introduced into a pathogen free 5 cm chopped golden yellow color paddy straw substrate (previously soaked in purified water for a period of 12 h). The spawn was inoculated using a layer-by-layer method. The inoculated bags were incubated in the dark for a period of 12–14 days at approximately 80–90% humidity to promote mycelial formation. When mycelial growth covered the whole substrate of the bag, perforations were made in the polythene bags. The bags were then transferred to the glass house and exposed to a light intensity of approximately 2000–3000 lx units to promote basidiomata development. The humidity (80–90%) was maintained by regularly spraying water twice a day. Basidiocarps appeared after 20–27 days from the day of inoculation. The basidiocarps were harvested from the mushroom bags by twisting the fruiting bodies and used as biomass (Fig. 1).

2.4. Preparation of aqueous extracts from P. djamor var. roseus (AEPDR)

The fresh basidiocarps were boiled in distilled water at a ratio 1:10 (w/v) for 30 min under agitation at $60 \pm 2\,^{\circ}C$. Soaked mushrooms were then left covered for 30 min and filtered after cooling. Residues were removed by filtration using gauze and then centrifuged (10,000 g, 30 min, $4\,^{\circ}C$). The collected supernatant was filtered using Whatman No.1 filter paper. The filtrate was freeze-dried (Christ, model Alpha 2–4 lyophilizer, The Netherlands) at $-53 \pm 2\,^{\circ}C$ for 48 h. One hundred milligrams of the extract (with a concentration assumed to be 100%) was dissolved in 100 ml of sterile distilled water and filter sterilized through 0.2 μm pores.

2.5. Instruments

UV–vis spectroscopy measurements were carried out using a JASCO V 550 spectrophotometer operated at a resolution of 1 nm. Fourier transform-infrared spectra (FTIR) were recorded using Perkin-Elmer FTIR spectrophotometer at a resolution of $4\,cm^{-1}$. The AgNP solution was dried and ground with KBr to obtain a pellet for the purpose of FTIR analysis. XRD measurements were carried out using a Philips PW 1830 instrument operating at a voltage of 40 KV and a current of 30 mA with Cu Kα radiation. A drop of nanoparticle solution was placed on formvar film coated copper-grid and the solvent was evaporated under vacuum. The grids were observed by transmission electron microscopy (TEM).

2.6. Synthesis of silver nanoparticles

Silver nitrate (1×10^{-3} M, $AgNO_3$) stock solutions were prepared using sterile deionized triple distilled water, and the subsequent dilutions were made from this stock solution. One milligram per ml of AEPDR was added to 5 ml of 1×10^{-3} M aqueous $AgNO_3$ solution and kept at room temperature. The time of addition of aqueous extracts into the metal ion solution was considered as the start of the reaction. After 12 h, the light yellow color of the $AgNO_3$ solution became a dark yellow color indicating the formation of AgNPs [24]. The fully reduced solution was centrifuged at 8000 rpm for 30 min. The supernatant liquid was discarded and the residue was dispersed in Millipore water. The samples were again centrifuged to wash off any substances had been absorbed onto the surface of the AgNPs.