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Biosynthesis and characterization of silver nanoparticles from fungal species and its antibacterial and anticancer effect

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Abstract

Silver nanoparticles have gained considerable importance in recent years due to their diverse medicinal activities. In the present study, we have explored filamentous fungi *Penicillium italicum* for the extracellular biosynthesis of silver nanoparticles (AgNPs) and evaluated its antibacterial and anticancer effects. The nanoparticles were characterized by using UV−Visible and Fourier transform infra-red (FTIR) spectroscopy and transmission electron microscopy (TEM) analysis. UV−Visible spectra showed specific absorption peak at 422.67 nm which confirmed the presence of nanoparticles. FTIR spectroscopy analysis revealed the presence of alcohols, phenols, alkenes, and amines that play major roles in stabilizing the synthesized AgNPs. Transmission electron microscopy (TEM) analysis showed spherical shape of AgNPs with size ranges from 14.5 nm to 23.3 nm. Antibacterial studies against *Staphylococcus aureus*, *Salmonella enterica*, *Bacillus cereus*, and *Escherichia coli* through disc diffusion method revealed 20 mm (for 40 μg/ml) of inhibition of zone especially for *S. enterica* and exhibited excellent synergistic effect when combined with moxifloxacin and streptomycin. Further, in anticancer studies, these nanoparticles demonstrated good anticancer effect against HEp-2 cancer cell line with IC50 value at 30 μg/ml through MTT assay.

Keywords: *Penicillium italicum*; FTIR; TEM; Antibacterial activity; Anticancer activity

1. Introduction

Nanotechnology is a branch of science that embodies biological, chemical, physical, and electrical and electronics engineering. The field offers a promising way to improve the properties of metals by transforming them into nanoparticles with in a size range of 1−100 nm [1,2]. Inorganic metal and metal oxide nanoparticles have paved a way to discover promising antimicrobial and anticancer candidates in recent years. Bacterial resistance towards antibiotics has emerged as a global issue nowadays due to excessive use of antibacterial agents. The bacterial resistance facilitates the re-emergence of diseases and is known as superbugs. Metallic nanoparticles have

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always acted as novel antimicrobial agents to resolve antibiotic resistance issues [3].

Various physical and chemical methods are used to prepare metallic nanoparticles [4]. However, these methods offer considerable toxic wastes and not environmental friendly. Further, the properties of nanoparticles can be enhanced when synthesized in a greener route since no toxic chemicals are used during the process. Green synthesis of nanoparticles is beneficial compared to chemical and physical approaches as the nanoparticles produced are nontoxic, economical and more stable [5,6].

In this study, the cell filtrate of *Penicillium italicum* was used for the production of silver nanoparticles (AgNPs). The nanoparticles were characterized by using a UV–Visible spectrophotometer, FTIR, and TEM analysis to confirm their particles size, shape, distribution and stability. The synthesized AgNPs were further evaluated for their antibacterial and anticancer activities. The antibacterial activity was performed using disc diffusion method against selected bacterial pathogens followed by studies on the synergistic effect with standard antibiotics such as moxifloxacin and streptomycin respectively. Evaluation of anticancer activity was carried out by HEp-2 cell line by MTT assay.

2. Material and methods

2.1. Soil sample collection

The soil sample was collected from Universiti Kuala Lumpur Royal College of Medicine Perak (UniKL RCMP), Tasek Premise. The soil sample was collected from 2 cm to 3 cm depth by using a sterile spatula and was transferred to the sterile plate with a cover. Then, the covered plate was placed at Research and Development Laboratory of UniKL RCMP, Tasek Premise and incubated at room temperature to dry the soil sample for 3–4 days for further process.

2.2. Isolation of fungal culture

Isolation of fungal culture was carried out by using serial dilution technique and spread plate method. About 1 g of dried soil sample was diluted in 10 mL of sterilized distilled water. Then, the solution was serially diluted to prepare a dilution with concentration range from 10^{-1} mL until 10^{-5} mL. 0.5 mL volume of solution from each concentration range of 10^{-3} mL until 10^{-5} mL was transferred aseptically onto PDA plates and was spread respectively to the concentration. The plates were then placed in a dark environment and incubated at room temperature for 3 days. Then, for the preparation of a pure culture of *Penicillium italicum* the isolated fungal were sub-cultured on PDA plates. The purely isolated *Penicillium italicum* was maintained at 4 °C for further studies [7].

2.3. Microscopic and colony characterization

The author observed *Penicillium italicum* in mycology, and the colony morphology was recorded concerning colour, size, shape, and nature of colony.

2.4. Biosynthesis of AgNPs

The purely isolated *Penicillium italicum* was used to synthesis the AgNPs by extracellular biosynthesis. Fungal biomass was grown aerobically by adding the fungal spore in a liquid nutrient media and incubated at 30 °C on an orbital shaker at 160 rpm for 72 h. After 72 h, the produced fungal biomass was filtered by using Whatman filter paper No. 1 and thoroughly washed 2–3 times by using distilled water to remove the residual media part and other debris. The fungal biomass was put into a conical flask which contains 100 mL of double distilled water and incubated at 25 °C on rotary shaker at 160 rpm for another 72 h. After 72 h of incubation on the rotary shaker, the biomass was filtered by using Whatman filter paper No. 1 and washed thoroughly with distilled water 2–3 times to remove a residual part until a clean biomass was produced. The fresh, clean, clear and cell-free extract was taken into the clean conical flask for further study purpose. 1 mM of $AgNO_3$ was added into the conical flask containing the clear cell-free extract and kept at 25 °C on a rotary shaker at 160 rpm for 72 h in a dark condition.

2.5. Characterization of AgNPs

The colour of the solution becomes more darken and cloudy after 72 h of incubation on the rotary shaker 160 rpm at 25 °C indicated the formation of silver nanoparticles. These AgNPs were further investigated by using UV–Visible Spectrophotometer, and analysis was carried out from the wave length 300–600 nm to check the maximum absorbance (λ_{max}). FTIR analysis was used to reveal the proteins, and functional group contained in the AgNPs which responsible for the stability of the nanoparticles and FTIR powder form of the sample was mixed with potassium bromide and observed the spectra by FTIR spectroscopy. The

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