A STUDY ON BIOACTIVE FLUORESCENT COMPOUNDS FROM SELECTED MUSHROOMS AND ITS ANTIMICROBIAL ACTIVITY

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ABSTRACT

Mushrooms have long been appreciated for their flavor, texture, medicinal and nutraceutical attributes. The present study deals with fluorescent bioactive compounds from *Armillaria mellea* (MTCC 409) and *Omphalotus olearius* (MTCC 2790). The cultures were obtained from MTCC, Chandigarh and pure mycelial cultures were developed. Time scale studies for the production, extraction, and estimation of fluorescent dyes were carried out and evaluated for their antibacterial activities. *A. mellea* and *O. olearius* grown in potato dextrose broth recorded the maximum mycelial dry weight of 5.90 ± 1.12g/50mL and 0.57 ± 0.025 g/50mL respectively on 28th day. *A. mellea* and *O. olearius* mycelium (1000 mg) recorded maximum crude fluorescent dyes in acetone (25.5%) and methanol (55.3%) respectively. Acetone extracts of *A. mellea* and *O. olearius* recorded highest zone of inhibition (0.4 cm and 1cm) against *E. coli* and *P. aeruginosa*, respectively.

Keywords: *Armillaria mellea, Omphalotus olearius,* fluorescent dyes, antimicrobial activity

INTRODUCTION

Microorganisms are considered good source of valuable bioactive metabolites. The most important metabolites such as, penicillin, cyclosporine A, adriamycine antibacterial, antiviral, antitumor as well as anticoagulant properties have been reported in mushroom [1]. Fluorescent-labeled molecules are being used extensively for a wide range of applications in biological detection and diagnosis [2]. Bioluminescence has been widely exploited as marker system for detection and tracking of cells in the environment and as biosensors for the detection of pollutants [3]. Few of the fluorescent mushrooms in nature include *Armillaria fuscipes, A.gallica, A. mellea, A.tabescens, Dictyopanus foliicolus, D.pusillus, Filoboletu spallescens, F. yunnanensis, Mycena chlorophos, Neonothopanus gardneri, N. nimbi, Omphalotus illudens, O. japonicas, O. olearius, Panellus gloeocystidiatus,* and many have been evaluated for their medicinal, nutraceutical and fluorescent properties [4].

A. mellea contains the bioactive compounds such as polysaccharides and sesquiterpene aryl esters reported to have anti oxidation, immunopotentiation, anti-vertigo, anti-aging, anti-microbial and anti-bacterial activity [5, 6]. *O. illudens* contains the bioactive compounds as illudinic acid, illudin S and M, having antibacterial activity [7] and applications for treating cancer diseases [3]. *O. olearius* and *Lampteromyces japonicas* contain the bioactive compound which are like cytotoxic, tricyclic sesquiterpene and illudin S, those are reported to have anticancer activity [8]. Among the several fluorescent mushrooms, in this study *A. mellea* and *O. olearius* were utilized and fluorescent dyes were, extracted, estimated and tested for antimicrobial potential.

MATERIALS AND METHODS

Culture collection, maintenance and production of fluorescent dyes from *A. mellea* and *O. olearius*

Fluorescent mushrooms, *A. mellea* (MTCC 409) and *O. olearius* (MTCC 2790) were procured from microbial type culture collection (MTCC), Chandigarh, India. The pure culture mycelium was initiated and sub cultured at intervals of every 15 days. The cultures were maintained on the malt based media recommended by MTCC. Apart from the recommended media, the growth and metabolites production was also tested in potato dextrose broth.

Extraction of intracellular fluorescent dyes

Dried mycelium of *A. mellea* and *O. olearius* was taken and extracted by boiling for 4 hrs in 100 ml distilled water, acetone and methanol, respectively. The extracted dye were condensed and characterized.

Anti-bacterial activity of bioactive compounds

Loopful of bacterial culture was inoculated in the nutrient broth and incubated at 37 °C for 12-14 hrs to get the log phase. The fluorescent dyes extracted from selected mushrooms were tested for their efficacy towards selected bacteria by agar diffusion method [9]. Bacterial cultures, *Escherichia coli, Staphylococcus aureus, Salmonella typhi, Proteus vulgaris,* and *Pseudomonas aeruginosa* were used for the anti bacterial assays. Nutrient agar plates were prepared, bacterial cultures grown on nutrient broth was aseptically transferred using a sterilized cotton swab and swabbed on the surface of the NA plates and allowed to dry for 1h. Wells of size 0.5 cm in diameter was cut in the agar plates using a sterile cork borer. The extracts from fluorescent mushrooms (*A. mellea* and *O. olearius*) were added at concentrations of 25µl, 50µl, 75 µl and 100 µl into the wells of the inoculated plates. After 24 - 48 hrs of incubation at 37 °C these were observed for the development of zone of inhibition. Sizes of the inhibited zone were measured and recorded. All the assays were carried out in triplicates. Petriplates containing respective solvents (100 µl) in the wells with bacteria were maintained as blank.

RESULTS AND DISCUSSION

Among the two media, potato dextrose broth showed maximum mycelial dry weight of 5.90 ± 1.12g/50ml and 0.57 ± 0.025 g/50 ml for *A. mellea* and *O. olearius* respectively on 28th day.

Extraction of intracellular fluorescent dyes from *A. mellea*

Mycelium of *A.mellea* and *O. olearius* yielded good amount of crude fluorescent dyes from distilled water and different solvents and the extracts were processed for the determination of antibacterial activity. *A. mellea* and *O. olearius* mycelium (1000 mg) recorded maximum crude fluorescent dyes in acetone (255 mg) and methanol (553mg) respectively.

Antibacterial activity for *A. mellea* and *O. olearius*

A. mellea recorded zone of inhibition against both gram positive (*S.aureus*) and gram negative bacteria (*E. coli, P. aeruginosa* and *P. vulgaris*).

Acetone extract of *A. mellea* recorded the minimal zone of inhibition against *E. coli, S. aureus, P. vulgaris, P. aeruginosa* and *S. typhi.* Methanol extracts (Mycelia) of *A. mellea* recorded the zone of inhibition against *E. coli* (0.4 cm), *S. typhi* (0.3 cm), *S. aureus* (0.3 cm), *P. aeruginosa* (0.3 cm) and *P. vulgaris* (0.3 cm). Water extracts did not record any anti-bacterial activity.

Acetone extracts of *O. olearius* recorded the zone of inhibition against *E.coli* (0.4 cm), *S. typhi* (0.5 cm), *S. aureus* (0.6 cm), *P. aeruginosa* (1 cm) and *P. vulgaris* (0.6 cm), methanol extracts showed activity against *E. coli* (0.5 cm), *S. typhi* (0.3 cm) and *P. aeruginosa* (0.3 cm). Water extracts recorded the zone of inhibition against *S. typhi* (0.3 cm). Donnelly *et al.* [10,11] isolated two new sesquiterpene aryl esters, 4-o-methylmelleolide and judeol, both having strong antibacterial activity against gram positive bacteria. Armillaric acid also exhibited marked inhibitory activity against gram positive bacteria and yeast [10]. Momose *et al*. [13] isolated three compounds, melleolides K, L and M. Melleolides K had of antimicrobial activity against gram positive bacteria, yeast and fungi.

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REFERENCES

[1] Alfonso J Alanis. (2005). Resistance to Antibiotics: Are We in the Post-Antibiotic Era. *Archives of Medical Research*. 36: 697–705.

[2] Wei Lian *et al.* (2004). Ultrasensitive detection of biomolecules with Xuorescent dye-doped Nanoparticles. *Analytical Biochemistry.* 334: 135–144.

[3] Dao Thi van. (2006). The successful cultivation of a new luminous mushroom *O. af. illudent*. University of Natural Sciences, Ho Chi Minh City, VietNam.

[4] Chang ST and Buswell JA. (1996). Mushroom Nutraceuticals. *World J. Microb. Biotechnology*. 12: 473-476.

[5] Yang LH *et al.* (2007). Isolation and identification of polysaccharides from *Armillaria mellea* and their effects on scavenging oxygen free radicals. *Food Sci*. 28: 309-313.

[6] Sun Y *et al.* (2009). Structural elucidation and immunological activity of a polysaccharide from the fruiting body of *Armillaria mellea*. *Bioresource Technology.* 100: 1860–1863.

[7] Dufresne C *et al.* (1997). A novel illudane sesquiterpine antibiotic. *J. Nat. Prod.* 60: 188-190.

[8] Dandopani Chatterjee *et al.* (2011). Evaluation of Antitumor Activity of *Cuscuta reflexa* Roxb (Cuscutaceae) against Ehrlich Ascites Carcinoma in Swiss Albino Mice. *Tropical Journal of Pharmaceutical Research.* 10: 447-454.

[9] Erturk O *et al.* (2003). Antimicrobial activity of *Viscum album* L. subsp. *abietis* (Wiesb). *Turk. J. Biol*. 27: 255–258.

[10] Donnelly DMX *et al.* (1985b). Melledonal and melledonolxsesquiterpene esters from *Armillaria mellea*. *Tetrahedron Lett.* 26: 5343.

[11] Donnelly DMX *et al.* (1985a). Antibacterial sesquiterpene aryl ester from *Armillaria mellea*. *J. Nat. Prod*. 48: 10-16.

[12] Obuchi T *et al.* (1990). Armillaric acid, a new antibiotic produced by *A. mellea*. *Planta Med*. 56: 198-201.

[13] Momose I *et al.* (2000). Melleolides K, L and M, new melleolides from *Armillaria mellea*. *J Antibiot* (Tokyo). 53: 137–143.