



Bioassay guided fractionation of
Pleurotus cystidiosus to investigate the
antimicrobial activity,
anti-hypercholesterolemic effects,
antioxidant and cytotoxic properties.

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ABSTRACT

Mushrooms have recently become attractive as a source with various medicinal values. Separation and isolation of compounds/fractions of *Pleurotus cystidiosus* mushroom, responsible for antimicrobial activity, anti-hypercholesterolemic activity, antioxidative activity and cytotoxicity against *Hep-2* cancer cells were examined during this study.

Acetone (A), dichloromethane (D) and hexane (H) extracts of *P. cystidiosus* were obtained by a sequential extraction method and analyzed for antibacterial activity. All three extracts (20,000 ppm) did not show antibacterial activity against *E. coli*, *Staphylococcus* or *Klebsiella*. Antifungal assay was also carried out with bioassay guided fractionation method and all the test solutions were in the concentration of 20,000 ppm. Antifungal activity of acetone extract against *R. solani*, *Curvularia* spp., *Alternaria* spp., *C. acutatum* and *C. gloeosporioides* resulted in the highest inhibitory activity (12%) against *C. gloeosporioides*. Antifungal assay guided fractionation of acetone extract (A) resulted in four fractions, A1, A2, A3 and A4. Fractions A2 and A4 were further separated as those two fractions showed 22% and 17% inhibitory activities. Out of the four fractions A2-1, A2-2, A2-3 and A2-4, fraction A2-3 showed the highest inhibitory (26%) activity. Inhibition of *C. gloeosporioides* by the three fractions A4-1, A4-2 and A4-3 was not satisfactory. Further separation of fraction A2-3 resulted the isolation of 3 β ,5 α ,6 β -trihydroxyergosta-7,22-diene with 41% inhibitory activity.

Two mushroom species (X and Y) were used to study blood cholesterol lowering properties using rat models. The groups treated with each freeze dried mushroom powder (1g/kg body weight) decreased blood cholesterol and LDL cholesterol significantly. The cholesterol lowering property of mushroom X was slightly higher than that of mushroom Y. The increased dose of 2g/kg body weight of mushroom X freeze dried powder decreased blood cholesterol level more than that of the rats treated with the dose of 1g/kg body weight of mushroom X. The acetone extract (1g/kg body weight) and dichloromethane extract (0.5g/kg body weight) did not show significant decrease in hypocholesterolemic activity. Tablets produced using the freeze dried powder of mushroom X also showed significant cholesterol lowering properties. Acetone and dichloromethane extracts were further fractionated and ¹H & ¹³C NMR spectra were obtained and compared with those of lovastatin separated from a commercially available tablet used for lowering of cholesterol. The NMR spectra did not match. Literature reveals that dietary fiber can lower blood cholesterol levels. High fiber content of mushroom X and Y (5.7% and 5.0% respectively) may also play a role in lowering blood cholesterol levels. Estimation of the cholesterol content in rat fecal matter proved that freeze dried powder of mushroom X increases the cholesterol excretion.

All water soluble extract/fractions of *P. cystidiosus* A, A4-1, A4-2 and A4-3 showed significant antioxidant activity in DPPH radical assay. Fraction A4-2 and A4-3 showed higher NO radical scavenging activities. The respective IC₅₀ values obtained for DPPH radical scavenging assay were 1.12, 1.13, 0.87, 0.81 and 0.82 mg/mL. IC₅₀ values of nitric oxide radical scavenging assay were 4.81, 3.82, 5.38, 0.87 and 0.61 mg/mL. Fraction A4-2 and A4-3 also showed higher antiproliferative and cytotoxic activities against *Hep-2* cancer cells, in MTT and LDH assays. Cell morphological changes observed microscopically gave further evidence that those two fractions have cytotoxic activities. It is concluded that *P. cystidiosus* shows antifungal, cholesterol lowering, antioxidant and cytotoxic activities.