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CYTOTOXICITY AND CHEMICAL ANALYSIS OF SOME MEDICINAL MUSHROOMS

APARAJITH MANUPRIYA HETTIARACHCHI

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DECLARATION

I do hereby declare that the work reported in this project report/thesis was exclusively carried out by me under the supervision of Prof. A. M. Abeysekera. It describes the result of my own independent research except where due reference has been made in the text. No part of this project report/thesis has been submitted earlier or concurrently for the same or any other degree.

Date..... .....

Signature of the Candidate

Certified by:

1. Supervisor (Name): Prof. A. M. Abeysekera Date:.....

   (Signature):.....

2. Supervisor (Name): Dr. Asiri Perera Date:.....

   (Signature):.....

PGIS Stamp:

ABSTRACT

All mushrooms belong to the Kingdom Fungi. Most of the cultivated mushrooms belong to Phylum Basidiomycetes which produce spores which are called Basidia. Some of these Basidiomycete fungi are known to possess anti-cancerous, anti-bacterial, anti-fungal, anti-viral, hyper-cholestraemic, and hyperglycemic activities. In this study investigation of cytotoxicity of five different varieties of mushrooms, *Ganoderma lucidum, G. applanantum, Lentinus cretaceus, Calavatia Giuagantia* and *Agaricus bisporus* were carried out. To identify cytotoxicity Brine shrimp (*Artemia salina*) was used as the biological assay.

Freeze drying of the water extracted mushroom species *Ganoderma lucidum, Calvatia gigantia* and *Lentinus cretaceus* showed a high cytotoxicity, while *Agaricus bisporus* shows a medium cytotoxicity and *G. applanatum* did not show any cytototxicity, *G. applanatum* however was found to encourage the growth of Brine shrimp.

The ethanol extracts of all the species were positive for alkaloid and tested by Mayer's and Wagner's reagents. *Ganoderma lucidum* showed the presence of three alkaloids on TLC. Two of these were isolated by TLC and purified by preparative TLC.

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TLC	Thin Layer Chromatography
UV	Ultra violet
NMR Spectroscopy	Nuclear Magnetic Resonance Spectroscopy
^{1}H NMR	Proton Nuclear Magnetic Resonance
^{13}C NMR	Carbon Nuclear Magnetic Resonance
LC_{50}	Lethal Concentration 50

Chapter – 01

Introduction

1.1 A general description of mushroom

The organisms of the fungal lineage include mushrooms, rusts, smuts, puffballs, truffles, morels, molds, and yeasts, as well as many less well-known organisms. More than 70,000 species of fungi have been described; however, some estimates of total numbers suggest that 1.5 million species may exist. Only 64,000 species have been discovered so far, many species from tropical rain forests. [1]

All mushrooms belong to the Kingdom of Fungi, a group very distinct from plants, animals and bacteria. Like plants fungi have a distinct cellular structure. But most important feature in plants is the ability to use energy from the sun directly through chlorophyll. Thus fungi depend on other organisms for food, as do mankind and in fact all animals.

As the sister group of animals and part of the eukaryotic crown group that radiated about a billion years ago, the fungi constitute an independent group equal in rank to that of plants and animals. They share with animals the ability to export hydrolytic enzymes that break down biopolymers, which can be absorbed for nutrition. Rather than requiring a stomach to accomplish digestion, fungi live in their own food supply and simply grow into new food as the local environment becomes nutrient depleted. [1]

All fungi with the exception of yeasts form so-called hyphae, tiny threads that originate from the spores. These hyphae will branch out and form the mycelium. Although

the fungal filaments and spores are microscopic, the colony can be very large with individuals of some species rivaling the mass of the largest animals or plants. The larger spore – producing structures (bigger than about 1mm) are called mushroom. The major part of the living organism, however, is to be found under the ground or inside wood. [1]

Within their varied natural habitats fungi usually are the primary decomposer organisms present. Many species are free-living saprobes (users of carbon fixed by other organisms) in woody substrates, soils, leaf litter, dead animals, and animal exudates. The large cavities eaten out of living trees by wood-decaying fungi provide nest holes for a variety of animals. In some low nitrogen environments several independent groups of fungi have adaptations such as nooses and sticky knobs with which to trap and degrade nematodes and other small animals.

There are several key factors which affect the growth of mycelia, which are as follows:

- Temperature
- Water content and water activity
- pH [acidity] of the medium
- humidity
- Nutrients
- CO_2 concentration in the air
- Light
- Physical shock
- Microbial activity

The mushroom seed is generally referred to as spawn. In nature mushrooms use spores for generative multiplication. [1]

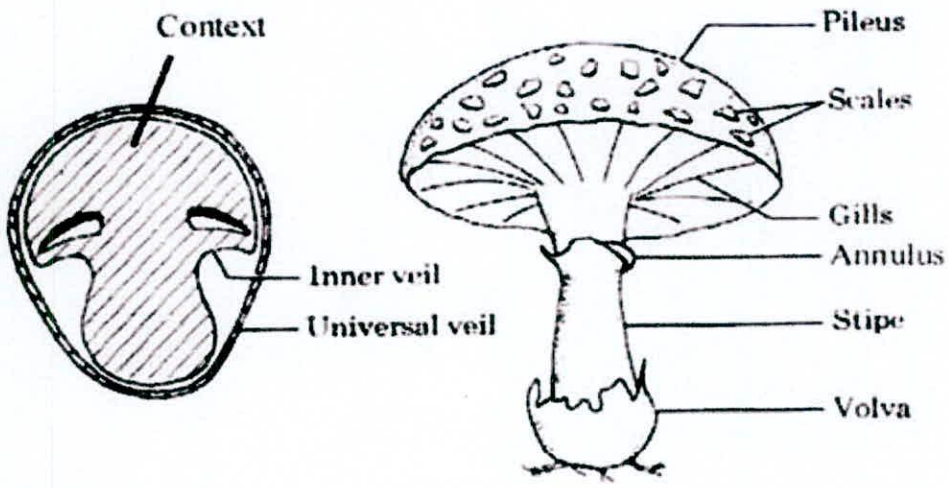

Figure 1.1 – Mushroom morphology

Morphological description of mushroom is shown in fig 1.1. Most of the cultivated mushrooms belong to the Sub Phylum Basidiomycetes, which produce their spores on so called Basidia. Another important Sub Phylum is the Ascomycetes, which produce their spores in Asci. [1]

1.2 Importance of mushroom

Some of these Basidiomycetes fungi have shown a variety of activities such as those listed below.

- Anti – cancerous
- Anti – bacterial
- Anti – fungal
- Anti – viral
- Hypercholesterolemic