ANTIMICROBIAL POTENTIAL OF *Lepiota* sp. EXTRACT

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ABSTRAK

Kajian telah dijalankan ke atas *Lepiota* sp. untuk menentukan potensi antimikrob cendawan tersebut dalam mengawal pertumbuhan mikrob pathogen yang menyebabkan penyakit manusia khususnya penyakit kulit. Antara mikrob yang digunakan dalam ujian ini ialah *Staphylococcus aureus*, *Escherichia coli* dan *Candida albicans*. Ujian ini bertujuan untuk menentukan sama ada ekstrak *Lepiota* sp. mempunyai kesan antimikrob terhadap *Staphylococcus aureus*, *Escherichia coli* dan *Candida albicans*. Ujian ini juga bertujuan untuk menentukan faktor-faktor yang mempengaruhi keberkesanan ektrak cendawan ini dalam aktiviti antimikrob. Antara faktor-faktor yang dikaji ialah kepekatan ekstrak, suhu penyimpanan ekstrak, tempoh masa penyimpanan ekstrak dan masa tindakan ekstrak dengan mikroorganisma. Ujian antimikrob dijalankan dengan menentukan Unit Pembentukan Koloni (UPK) mikroorganisma yang diuji. Hasil kajian yang didapati menunjukkan bahawa *Lepiota* sp. mempunyai potensi antimikrob dalam mengawal pertumbuhan *S. aureus*, *E. coli* dan *C. albicans*. Keberkesanan ekstrak dapat dilihat melalui pengurangan dalam nilai UPK mikroorganisma. Kepekatan ekstrak 350 mg/ml didapati paling berkesan dalam aktiviti antimikrob. Kesan suhu penyimpanan dan tempoh masa penyimpanan memberi kesan yang berbeza terhadap mikroorganisma yang berlainan. Dalam ujian antifungi pula, ekstrak *Lepiota* sp. dibuktikan dapat menghadkan percambahan *C. albicans*. Peratusan spora yang bercambah akan dikurangkan apabila kepekatan ekstrak bertambah. Kepekatan ekstrak tidak memberi kesan yang signifikasi dalam mengawal pemanjangan tiub germa *C. albicans*.

ABSTRACT

The antimicrobial potential of *Lepiota* sp. extract in inhibiting the growth of pathogenic microbes isolated from human disease has been studied. *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* are the three microorganisms that were selected for this study. The objective of this study is to test for the antimicrobial activities of *Lepiota* sp. against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. This study also aimed to compare the factors that affect the effectiveness of the extract in antimicrobial activities. The factors which were considered in study are the concentrations of the extract, temperature of storage, period of storage and the mix time between the extract and the bacteria suspension. The value of Colony Forming Unit (CFU) was determined in the antimicrobial test. The results showed that *Lepiota* sp possessed antimicrobial potential in inhibiting the growth of *S. aureus*, *E. coli* and *C. albicans*. The extract was effective in decreasing the value of CFU of the microorganisms. The extract concentration of 350 mg/ml was proved to be the most effective in antimicrobial activities. The storage temperature and period of storage of the extract gave different effect to different microorganisms. In the antifungal test, *Lepiota* sp. extract gave favorable results in inhibiting the spore germination of *C. albicans*. The percentage of spore germination was reduced when the extract concentration was increased. Extract concentration has no significant effect on germ tube elongation.

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CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

Fungi are a diverse group of organisms that are classified within their own kingdom as they are neither plant nor animal (Lessoe and Conte, 1996). There are estimated to be over 100,000 different fungi, most of which form only tiny threads (hypha) that can only be seen through a microscope. Fungi are thallophytes that have no chlorophyll (Svrcek, 1996). This means that they cannot manufacture their own organic food such as sugars, starches, celluloses, proteins and fats, as the green plants do (Christensen, 1972).

As presently delimited, the kingdom Fungi is believed to constitute a monophyletic group that shares some characters with animals such as chitinous structures, storage of glycogen, and mitochondrial UGA coding for tryptophan (Baier, 1991).

Being unable to produce energy through photosynthesis, fungi acquire their organic matter and energy from the decomposition of dead or live matter of other organisms. This method of getting nourishment is called heterotrophic (Baier, 1991). Fungi play a key role in the natural cycle as decomposers, returning nutrients to the soil (Lessoe and Conte, 1996).

The structures of fungi are microscopic and not visible to the naked eye. Some are unicellular like yeast, but most string their cells together in long, thread-like strands called hypha. The hypha is essentially a tube with a rigid wall, containing a moving slug of protoplasm (Deacon, 1997). The shape and size of these hyphae varies in different groups of fungi, 1.5-12μm in diameter and from a few up to 100μm in length. The walls of hyphae are often strengthened with chitin, a polymer of N-acetylglucosamine. Most fungi produce an extensive system of hyphae, which may be visible when growing thickly in a mass called mycelium (commonly referred to as mould).

Hyphae can be either continuous tubes or divided by septa (walls) into individual cells, arranged in an end-to-end order. These septa are however not entirely closed; single or several openings forming a multi-perforate structure allow protoplasmic streaming. The protoplasm which fills the cells at the growing tips of the mycelium manufactures dozens of different kinds of enzymes and a number of simple and not so simple organic acids (Christensen, 1972). Mycelium can be of any size from tiny clusters to massive acre wide systems, which effectively form the feeding and growing body of the fungus.

Fungi also posses a true nucleus (Scvrek, 1996) and the reproductive unit in fungi is primarily represented by the spore. Spores may be uni or pluricellular, of many different colours and shapes, depending on the species and growth conditions. In size, spores vary from 5-20μm in diameter (Florian, 1993). Spores are the results of either sexual or asexual reproduction.

Asexual spore formation is often quick and profuse, making possible rapid dissemination of the fungus during favourable conditions. Sexual spore formation is a considerably more complex process, and is believed to be far more resistant since they mature more slowly and are both sexually and asexually produced spores can, if environmental conditions do not allow immediate germination, go into a state of dormancy, stasis, with a hold-over function, in which the water contents of the spore is lowered, and the metabolism inactivated but reversible. When dormancy of a spore is broken through activation, germination starts with the formation of a germ tube. Long, branching, threadlike structures, hyphae, develop from these germ tubes, eventually forming a complex mycelium.

When sporulation begins, the mature spores are very easily separated from their fruiting bodies, and due to their low weight, spreading and distribution can take place by a minute current of air. Spores can remain dispersed in air for very long time, are easily spread and therefore ubiquitous on our planet. The fungi may impose a potential health-hazard to humans causing mycoses, mycoallergies and mycotoxicoses. These diseases include invasions of the skin, lungs, eyes and brain.

All species of fungi have specific requirements of moisture and temperature before they will produce a fruit. Some mushrooms have a very restrictive set of requirements while others have very broad sets of requirements. Even under the proper climatic conditions, mushrooms may not develop if the mycelium lacks the necessary food energy. Many fungi will not fruit for several years; then one year when everything is just right, the woods will seem full of them. Other fungi with a broader range of temperature and moisture requirements appear annually.

The organisms of the fungal lineage include mushrooms, rusts, smuts, puffballs, truffles, morels, molds, and yeasts, as well as many less well-known organisms (Alexopoulos *et al*., 1996). About 70,000 species of fungi have been described; however, some estimates of total numbers suggest that 1.5 million species may exist (Hawksworth, 1991; Hawksworth *et al*., 1995). The mycota of Borneo has much in common with the mycotas found throughout Southeast Asia and Australia, and much less with that of Europe. The true fungi form a separate kingdom, which includes three phyla, which are Ascomycota (Cup fungi and allies), Basidiomycota (Mushrooms and allies), Zygomycota (Pin-moulds and allies), and Chytrids are often included but are not true fungi (Pegler, 1992). Placement into a division is based on the way in which the fungus reproduces sexually. The shape and internal structure of the sporangia, which produce the spores, are the most useful character for identifying these various major groups.

Fungi that will be studied in this paper have the mycological data as follows:

Kingdom: Fungi

Phylum: Basidiomycota

Class: Holobasidiomycetes

Order: Agaricales

Family: Lepiotaceae

Genus: *Lepiota*

Photograph 1.1 *Lepiota* sp.

The Lepiotaceae was formerly considered to consist of a single genus, *Lepiota*, but some mycologists have felt that a number of changes were needed. First, there was the matter of spore colour, because one species had green spores, while the rest have white, cream, or pale buff spores. It was generally conceded that the green-spore member belonged in a separate genus, and it is now called *Chlorophylum molybdites*..

Mushrooms in this group have white spore prints (greenish in the case of *Chlorophyllum molybdites*), free gills that are white to yellow and are a central stipe easily separable from the pileus , partial veils that typically leave a ring on the stem, and dry caps that are often scaly but do not have patches or warts. There is no volva, though one species has a rimmed basal bulb (Orr and Orr, 1979). Although there was no report on the antimicrobial activities of *Lepiota* sp, this study is still carried on to determined the antimicrobial potential of the studied fungi.

The Basidiomycota or club fungi are those fungi in which the sexual spores (basidiospores) are borne externally on special club-shaped cells, called basidia, which may develop singly or in layers known as hymenial layers (Gray, 1959). They range from common mushroom forms to some of the most important plant pathogens. In accordance with their way of life, mushrooms are divided into three basic categories:

i) Symbiotic fungi (mycorhizal fungi)

ii) Fungi subsisting on dead or decaying matter (saprophytic fungi)

iii) Fungi feeding on living organisms (parasitic fungi)

The objectives of this research are to determine the antimicrobial potential of mushroom against *Staphlococcus aureus*, *Escherichia coli* and *Candida albicans* in human disease especially skin disease.

1.2 OBJECTIVES

The objectives that are aimed to achieved are as follows:

1. To compare the antimicrobial activities in different concentrations of *Lepiota* sp. extract against *Staphlococcus aureus*, *Escherichia coli* and *Candida albicans*.

2. To compare the effect of storage temperature and period of storage of *Lepiota* sp. in antimicrobial activities.

3. To test for the effect of extract concentrations to spore germination and germ tube elongation of *C. albicans*.

1.3 HYPOTHESES EXPECTED

The hypotheses that are expected are as follows:

1. The higher concentrations of mushroom extract will have a greater antimicrobial potential.

2. For antimicrobial test, the shorter period and lower temperature of storage of the extract is more effective and the colony formed is lesser.

3. For antifungal test, higher concentrations of the extract will inhibit the spore germination and germ tube elongation of *C. albicans* more effectively.

CHAPTER 2

LITERATURE REVIEW

2.1 MEDICINAL USE OF MUSHROOMS

For hundreds of years mushrooms have excited both the minds and stomachs of millions of people. They have been used for food, medicine, religious ceremonies, and for poisoning enemies. Mushrooms have been an important part of many cultures around the world. In California some Native Americans have made soups out of shredded mushrooms. The number of mushrooms on Earth is estimated at 140,000, yet maybe only 10% (approximately 14,000 named species) are known (Christensen, 1972).

Mushrooms comprise a vast and yet largely untapped source of powerful new pharmaceutical products. In particular, and most importantly for modern medicine, they represent an unlimited source of polysaccharides with antitumor and immunostimulating properties. Several classes of compounds, such as proteins, peptides, lipopolysaccharides, glycoproteins, and lipid derivatives, have all been classified as molecules that have potent effects on the immune system (Tzianabos, 2000). Some of these compounds exert immunomodulatory and antitumor actions and are marketed drugs. Extensive studies have revealed that a number of mushroom species are of value in the prevention a treatment of cancer, viral diseases, hypercholesterolemia, and hypertension.

Since the introduction of penicillin into therapeutics more than 50 years ago, numerous drugs with antibacterial activity have been obtained from the Actinomycetes and Bacillus (Smania *et al.,* 1995). Many of the Basidiomycetes mushrooms contain biologically active polysaccharides in fruit bodies, cultured mycelium, culture broth (Wasser, 2002). However, the resistance effect from prolonged usage of antibiotics were caused scientist to search for new source of antibiotics from various organisms including fungi. Commonly known mushrooms that are both edible and have functional properties include *Auricularia* (Mu-er), *Flammulina* (Enokitake), *Grifola* (Maitake), *Hericium*, *Lentinus* (Shiitake), *Pleurotus* (Oyster), and *Tremella* (Yiner).

Recently, many species of fungi have been found to be highly potent immune system enhancers, potentiating animals and human immunity against cancer (Feng *et al.,* 2001). Fungi such as Shittake and Agaricus have been used widely as medicinal mushroom to cure several of diseases and to enhance body immune system. Researches also show that many mushroom extractions contain antibacterial and antiviral effect against microorganisms. For example, recent study on *Pycnoporus sanguineus* has demonstrated that its extract was shown to contain a compound with biological activity against strains of *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi, Staphylococcus aureus,* and others bacteria (Smania *et al.,* 1995).

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