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HADIAH

**POTENSI AKTIVITI ANTIMIKROB EKSTRAK**

*Ganoderma applanatum*

**LIM CHIEW CHIN**

**DISERTASI YANG DIKEMUKAKAN UNTUK  
MEMENUHI SEBAHAGIAN DARIPADA SYARAT  
MEMPEROLEHI IJAZAH SARJANA MUDA SAINS  
DENGAN KEPUJIAN DALAM BIOLOGI  
PEMULIHARAAN**

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applanatum Extract

Ijazah: Sarjana Muda Sains

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## DECLARATION

The materials in this thesis are original except for quotations, excerpts, summaries and references, which has been duly acknowledged.

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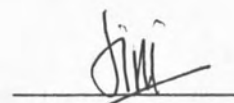


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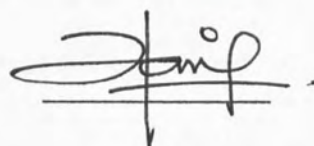
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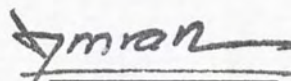
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## ABSTRAK

Potensi *Ganoderma applanatum* sebagai agen antimikrob telah dikaji. Objektif kajian ini adalah untuk menentukan keberkesanan ekstrak kasar dalam mengawal mikroorganisma patogen. Faktor-faktor seperti kepekatan, isipadu, suhu dan hari penyimpanan ekstrak telah dikaji. Ekstrak kasar ini telah diuji aktiviti antimikrobnya terhadap bakteria Gram-positif dan bakteria Gram-negatif serta sejenis kulat dengan menggunakan kaedah “disc diffusion”. Aktiviti antibakteria adalah lebih berkesan terhadap bakteria Gram-positif iaitu *Staphylococcus aureus* dan *Bacillus cereus*. Kepekatan, isipadu, suhu dan hari penyimpanan ekstrak boleh mempengaruhi saiz zon perencatan terhadap bakteria yang diuji. Ekstrak yang berkepekatan tinggi iaitu 500mg/ml dapat menghentikan percambahan spora *Candida albicans* dan mengurangkan pemanjangan tiub germa. Keputusan menunjukkan ekstrak adalah sangat stabil terhadap suhu penyimpanan tetapi hari penyimpanan telah mengurangkan keberkesanan ekstrak. Menurut kajian ini, boleh disimpulkan bahawa makrofungi mempunyai aktiviti antimikrob, dimana ia adalah berpotensi sebagai dadah antimikrob terhadap bakteria yang tertentu.



## ABSTRACT

The potential of *Ganoderma applanatum* as an agent antimicrobial was studied. The objective of this research was to determine the effectiveness of crude extract of the macrofungi in controlling the growth of pathogenic microbes. The factors like concentration, volume, and storage temperature and incubation period of the extract were investigated. The extracts were tested for their antimicrobial activity against several Gram-positive and Gram-negative bacteria and against one yeast species using disc diffusion method. Antibacterial activity was demonstrated especially against Gram-positive bacteria including *Staphylococcus aureus* and *Bacillus cereus*. Concentration, volume, temperature and incubation period of the extract can affect the size of the inhibition zone against test organisms. High concentration of *G. applanatum* extract such as 500mg/ml was found to be capable of stopping the germination of the *Candida albicans* spores and decreased the germ-tube length. The result showed that the extract was stable against storage temperature but the length of incubation period of the extract has slightly decreased the effectiveness of the extract. Based on the current findings, it can be concluded that this fungus has antimicrobial activity, which is as potent as standard antimicrobial drugs against certain microorganisms.



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**LIST OF SYMBOLS**

=	Equal
x	Times
/	Divide
+	Plus
-	Minus
±	Plus or minus from one amount
<	Smaller than
>	Bigger than
%	Percentage



**LIST OF UNITS**

°C	Degree Celsius
g	Gram
h	Hour
ml	Milliliter
mg/ml	Milligram per milliliter
mm	millimeter
l	Liter
μl	Microlitre
μm	Micrometer
%	Percentage
v/v	Volume per volume
w/v	Weight per volume



**LIST OF ABBREVIATION**

CFU	Colony Forming Unit
min	Minute
NA	Nutrient agar
OD	Optical density
PDA	Potato Dextrose Agar
psi	Pascal
r.p.m	Rotary per minute
sp./spp.	Species
T	Transmission
MWU	Mann-Whitney U Test
p	Significant value



## **CHAPTER 1**

### **INTRODUCTION**

#### **1.1 INTRODUCTION TO FUNGI**

Fungi are eukaryotes, and most are multicellular. Although fungi were once grouped with plants, they are unique organisms that generally differ from other eukaryotes in nutritional mode, structural organization, growth and reproduction. In fact, molecular studies indicate that animals, not plants, are the closest relatives of fungi (Campbell and Reece, 2002).

The Fungi play an important role in many aspects of our daily lives. There are a number of human ailments and complaints caused by fungi, such as ringworm, 'athletes foot' and other skin diseases. On the other hand, certain moulds are extensively utilized to fight disease and infections, and this has resulted in the development of the antibiotics industries (Pegler, 1997).





Fungi are a more important antibiotic-producing group (approximately 20% of described antibiotics derive from fungi). Chemotherapeutically useful antibiotics have been found only among order *Aspergillales* (a group of filamentous spore-forming moulds) and include the penicilins, the cephalosporins and fusidic acid (Stephen *et al.*, 1978).

Although most healthcare professional skilled in the art of botanical medicine are aware of the immune enhancing properties of certain mushrooms and other fungi, few may realize that mushroom are rich source of natural antibiotics. In these, the cell wall glucans are well-known for their immunomodulatory properties, but few medical practitioners are aware that many of the externalized secondary metabolites- extra cellular secretions by the mycelium - combat bacteria and viruses. Additionally, the exudates from mushroom mycelia are active against protozoa such as the parasites that cause malaria, *Plasmodium falciparum*, and other microorganisms. It is not surprising our most significant anti-bacterial antibiotics have been derived from fungi (Hardman and Limbid, 2001).

In a recent *in vitro* study, extracts of more than 75 percent of polypore mushrooms species surveyed showed antimicrobials activity and 45 percent of 204 mushroom species (polypore and gilled mushrooms alike) inhibited growth of a wide variety of microorganisms (Suay *et al.*, 2000). In particular, this study showed that species in the polypore genus *Ganoderma* such as reishi (*G. lucidum*), *G. pfeifferi*, and *G. resinaceum*, all of the family Ganodermataceae, were specifically effective against



bacillus (*Bacillus subtilis*). Another studies showed that the artist conk (*Ganoderma applanatum*) demonstrated antimicrobial against Gram-positive *Bacillus cereus*, *Staphylococcus aureus*, and less activity against Gram-negative *E. coli*, and *P. aeruginosa* (Smania *et al.*, 2001)

*In vitro* studies of 26 proprietary cultures of basidiomycetous mushrooms provided by the author found that four species “completely” inhibited *E.coli*, stopping bacterial growth well in advance of the encroaching mycelia, suggesting an extracellular antibiotic (Thomas *et al.*, 1999). Of these four species totally inhibiting *E. coli*, three were polypores clones by the author from the Old Growth forest of the Pacific North America: *Ganoderma oregonense* Murr., artist conk (*G. applanatum*), and the tinder fungus (*F. fomentarius*).

## 1.2 WHAT IS *Ganoderma applanatum* ?

Ganodermataceae is comprised of poroid species that have distinctive basidiospores and often a pileus surfaces that appears varnished. The basidiospores are ovoid, usually truncate at one end, and golden brown in color. The two walls of spore are separated by columns. Because the outer walls sags between the supporting columns, the spore surface appears punctuate. The genus *Ganoderma* is common in temperate regions where species are important root and heart rot fungi that are pathogenic under some conditions (Alexopoulos *et al.*, 1996).



The genus *Ganoderma* consists 50 species which are especially found in tropical parts of the world. The species included now in *Ganoderma* were formerly referred to *Fomes* from which it differs by the presence of hard, often shining crust, and by the peculiar spores with their double wall and truncate apex (Mehrotra and Aneja, 1990).

Originally, *Ganoderma* (Photo 1.1) was an herbal medicine used in China. Lately it has been largely accepted worldwide as one of a health-maintaining food, especially in countries of the Pacific regions, like Japan, China, Korea, Taiwan, Thailand, Malaysia, Vietnam, Indonesia, and the United States. More and more individuals became aware of its effects of strengthening the constitution and maintaining health.



**Photo 1.1** *Ganoderma applanatum*



### 1.3 OBJECTIVES

The main objectives of this research is to test and determine the antimicrobial activities of *Ganoderma applanatum* to certain pathogenic bacteria such as *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and fungus *Candida albicans* base on the concentrations, volume, storage temperatures and storage period of the extract.

1. To screen the antimicrobial activities from fungus, *Ganoderma applanatum*.
2. To determine the effect of different concentrations of *G. applanatum* extract to microbes.
3. To determine the effect of different volume of *G. applanatum* extract to microbes.
4. To determine the effect of different incubation period of the extract to killing capability of extract to microbes.
5. To determine the effect of different temperature in keeping the extract to killing capability of extract to microbes.

### 1.4 HYPOTHESIS

Higher bacteriostatic and fungistatic effects of the extract are due to the larger inhibition zone, lower percentage of germinated spores and shorter the germ tube length.





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