

POTENTIAL ANTIMICROBIAL ACTIVITIES OF
Ganoderma boninense EXTRACT

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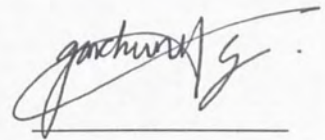
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ABSTRACT

Ganoderma boninense was evaluated for their antimicrobial and antifungal activities against bacteria *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus* and fungus *Candida albicans*. The fruiting bodies were extracted with 95% ethanol and the crude extract was used to test in paper disc diffusion method and Germ Tube test. In antimicrobial activities test, volumes, temperatures, concentrations and periods of storage crude extract were considered and those factors may affect the size of diameter inhibition zone. The results revealed that the ethanolic extracts of *G. boninense* inhibited effectively against growth of *S. aureus*, following by *B. cereus* and *E. coli*. However, temperature gave no significant effect against studied bacteria. In antifungal activities, high concentration of *G. boninense* crude extract was found to be capable of decrease the germination and germ-tube length of the *C. albicans* spores. Based on the current findings, it can be concluded that *G. boninense* has antimicrobial and antifungal activities, which were potent as antimicrobial agents.



ABSTRAK

Ganoderma boninense dinilai untuk menentukan aktiviti antimikrob and antifungal terhadap bakteria *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus* dan fungus *Candida albicans*. *Ganoderma boninense* diekstrak dengan menggunakan 95% ethanol dan ekstrak kasar tersebut diuji dengan kaedah “disc diffusion” dan ujian “germ tube”. Dalam aktiviti antimikrob, isipadu, suhu, kepekatan dan masa penyimpanan ekstrak adalah dipertimbangkan dan faktor-faktor tersebut akan mempengaruhi saiz diameter zon perencatan. Keputusan menunjukkan ekstrak ethanol *G. boninense* menghalang pertumbuhan *S. aureus* secara efektif, seterusnya ialah *B. cereus* dan *E. coli*. Walaubagaimanapun, suhu tidak menunjukkan kesan nyata terhadap bakteria. Dalam aktiviti antifungal, ekstrak *G. boninense* dengan kepekatan tinggi mempunyai keupayaan mengurangkan penunasan dan panjang tiub tunas spora *C. albicans*. Hasil kajian yang dijalankan, boleh disimpulkan bahawa *G. boninense* mempunyai kesan dalam aktiviti antimikrob dan antifungal dan berpotensi menjadi agen antimikrob.



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LIST OF ABBREVIATION

ANOVA	Analysis of Variants
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



LIST OF UNITS

°C	Degree Celsius
g	Gram
h	Hour
km	Kilometer
ml	Milliliter
mg/ml	Milligram per milliliter
mm	milimeter
μl	Microlitre
μm	Micrometer
pH	Hydrogen ion concentration
v/v	Volume per volume
w/v	Weight per volume



LIST OF SYMBOLS

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



CHAPTER 1

INTRODUCTION

1.1 Background

Generally, fungi are eukaryotes organism in fungi kingdom which contain nucleus with cell's genetic material (DNA), surrounded by a special envelope called the nuclear membrane. It can appear into two forms, either unicellular such as yeasts or multicellular such as mushrooms (Madigan *et al.*, 2003). Mushrooms were known and consumed as food in prehistorically times. Throughout history, many mushrooms have been used in different cultures and societies for their medicinal and tonic property (Cimerman, 1999).

Medicinal compound from mushroom plays an important role in medical realm because of their antimicrobial traits. Recently, many studies found that mushroom have its own medicinal value and being observed and extracted the medicinal compound as antibiotic or antimicrobial to use in public health. However, many antimicrobial has not been discovered yet. Fungi are not only considered for their



pathogenic, on the other hand, they also play a very important role in antibiotic and antimicrobial production. Since their discovery, antimicrobial drugs have been proved remarkably effective for controlling bacterial infections. However, it was soon evident that bacterial pathogens are unlikely to surrender unconditionally because some pathogens rapidly become resistant to the first discovered effective drugs (Barbour, 2004). Fortunately, of more than 100,000 species of fungi, only about 100 are pathogenic to human and animals (Tortora *et al.*, 2002). It is because all fungi are chemoheterotrophs, requiring organic compound for energy and carbon (Tortora *et al.*, 2002).

One of the important disease problems that has gained much attention lately is the Basal Stem Rot (BSR) caused by *Ganoderma* spp. The disease is found to occur quite often in areas which were previously planted with coconut or oil palm. Much effort, money and time have been diverted to solve this problem, yet today studies are still far from achieving a good control against *Ganoderma boninense* (Sariah and Mohamad, 1990). By throwing or burning *G. boninense*, it is better to investigate the usefulness of *G. boninense* because *Ganoderma* spp. has good effect in antimicrobial activities.

Moreover, bacteria exist in our environment all the time. For bacteria, they are classified as prokaryotes microorganism in monera kingdom which are very small, simple, unicellular and contain genetic material with no nuclear membrane. According



to Bergey Manual of Systematic Bacteriology, bacteria can be divided into four division based on its cell walls. There are thin cell wall of gram negative, thick cell wall of gram positive, prokaryotes without cell wall and extra ordinary cell wall. Bacteria include all of the pathogenic prokaryotes and many of the nonpathogenic prokaryotes. People might gain diseases when their immune systems are in bad condition.

Taxonomically, the genus *G. boninense* is categorized as a higher fungus, a polyporoid genus, within the family: Ganodermataceae. Order is under Aphyllophoraales, class is Basidiomycetes and subclass is Hymenomycetes (Idris and Ariffin, 2004).

Antimicrobial substance is harmful to microorganisms by either killing or inhibiting microbial growth. An antimicrobial agent is a natural product from other organism that can kill or inhibit the growth of microorganisms. They are either bactericidal which is used to kill microbes directly or bacteriostatic which prevent microbes from growing. In bacteriostatic, the host's own his defenses, such as phagocytosis and antibody production (Tortora *et al.*, 2002). Every antimicrobial agent such as antiseptic, disinfectant, sterilants or antibiotic is very popular using in mycology, pharmacology and bacteriology. The same concept of fungicidal and fungistatic is also used for fungi.



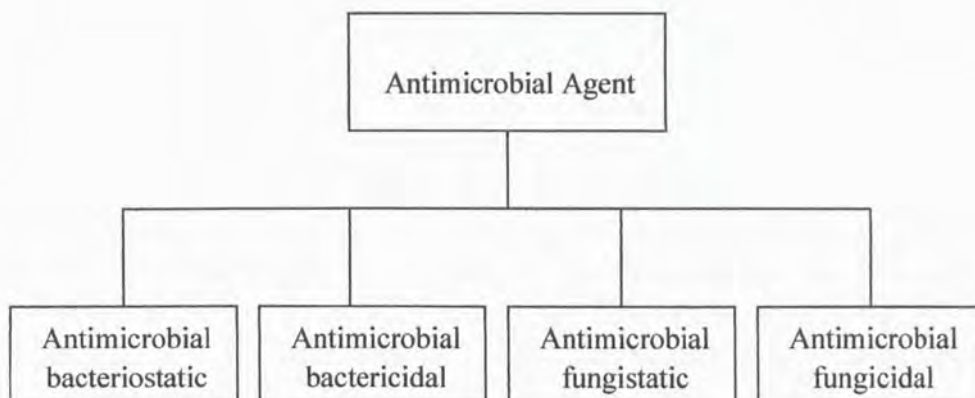


Figure 1.1 Types of antimicrobial agent.

In this case of study, aqueous, methanolic and ethanolic extractions are the most common method to obtain the crude extract from the fruiting body of mushroom. But, water and methanol extract were not good solvents of extracting mushroom especially *Lycoperdon pusillum* and *Lycoperdon giganteum* fruit-bodies because they could not be compared with the effectiveness of ethanol (Jonathan and Fasidi, 2003).

Furthermore, the most frequently used method of measuring bacterial populations is the plate count. Plate counts assume that each live bacterium grows and divides to produce a single colony. This is not always true because bacteria frequently grow linked in chains or as clumps. Therefore, a colony often results, not from a single bacterium, but from short segments of chain or from bacterial clump. To reflect this reality, plate counts are often reported as colony-forming units (CFU) (Tortora *et al.*, 2002).

1.2 Objectives

- 1) To extract antimicrobial compound from *Ganoderma boninense*.
- 2) To determine and compare antimicrobial activity of crude extract from *Ganoderma boninense* against bacteria *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus* and fungus *Candida albicans*.

1.3 Study Scope

To test and determine antimicrobial activities of *Ganoderma boninense* to bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus* and fungus *Candida albicans* based on the volumes, concentrations, temperatures and periods storage of crude extract.

1.4 Hypothesis

H_0 : *Ganoderma boninense* has found negative effect on bacteria and fungus.

H_a : *Ganoderma boninense* has found positive effect on bacteria and fungus.



CHAPTER 2

LITERATURE REVIEW

2.1 Introduction of Fungi

Fungi are absorptive where they obtain their food by absorbing soluble nutrient from their environment. On the other hand, Fungi are also heterotrophs and lacking of chlorophyll. They use organic matter of plants and animal as their food because they cannot produce food by themselves. The carbohydrates stored by fungi in fats, glycogen, oils and other important product forms of fungi. The enzymes are produced by fungi have been along the line of physiology specialization.

As fungi are achlorophyllous, they obtain nutrition by heterotrophic mode which is saprophytic mode and parasitic method (Rajan, 2000). Fungi may live either as saprobes on dead plants or animals and decay organic remain. There are two types of saprophytes which are obligate saprophytes and facultative saprophytes. Fungi which live on dead organic matter and are incapable of infecting living organisms are called obligate saprobes; those capable of causing disease on a living host or living on



dead organic matter depending on the situation are called facultative saprobes. Parasites live on other organisms (plants, animal and human beings) and obtain their nutrition from a living host. In the process they may cause diseases to the host. Parasites may be ectoparasites or endoparasites depending on their location in the body of the host. Ectoparasites live on the body of the host, while endoparasites live inside the tissues of the host. Parasites may also be classified into obligate parasites and facultative parasites. Obligate parasites are those which can live only on living protoplasm, while facultative parasites depending on the circumstances may live on dead organic matter or living host (Rajan, 2000).

In nutritional adaptations, most fungi will grow between 0° and 35°C, but the optimum temperature is in the range of 20-30°C (Alexopoulos *et al*, 1996). Fungi are generally adapted to the environment which pH is as low as about 5 and survived in temperature up to 62°C (Madigan *et al.*, 2003). Most fungi are resistant to osmotic pressure than bacteria; most can therefore grow in relatively high sugar or salt concentration (Tortora *et al.*, 2002).

2.2 General Feature of Fungi

Macrofungi can be divided into few parts; there are cap, gill, ring, stalk and mycelial connection. Macrofungi may produce basidium from the gill surface or plat (lamella).



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