ANTIMICROBIAL ACTIVITY OF EDIBLE WILD MUSHROOM EXTRACTS

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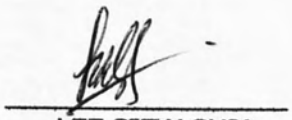

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ABSTRACT

ANTIMICROBIAL ACTIVITY OF EDIBLE WILD MUSHROOM EXTRACTS

The aim of this study was to examine the antimicrobial activity of *Schizophyllum sp.* which is an edible wild mushrooms widely available and consumed by the local community in Sabah, Malaysia. In vitro studies using disk diffusion screening test and macrobroth dilution method have demonstrated antimicrobial activity of various extracts of *Schizophyllum sp.* againts four foodborne pathogenic bacteria such as *Listeria monocytogenes* L55, *Salmonella typhimurium* S1000, *Escherichia coli* O157: H7 imre 91/02c and *Staphylococcus aureus* S277. The targeted extracts of *Schizophyllum sp.* were methanolic extract, phenolic extract, flavonoid extract, terpenoid extract and essential oils. All the extracts were showing inhibitory effects againts different types of bacteris except for the terpenoid extract which showed no antimicrobial activity againts all the bacteria tested. The methanolic extract of *Schizophyllum sp.* inhibited the growth of *S. aureus*, *L. monocytoges* and *E. coli*. The phenolic extract and flavonoid extracts were active against all the bacteria tested. Essential oils of *Schizophyllum sp.* only showed antimicrobial properties against *S. typhimurium* and *L. monocytogenes*. Further analysis was carried out on the essential oils using GC-MS has found out that 1-Octen-3-ol is the only compound present in the essential oils. The minimum inhibition (MIC) of various extract of Schizophyllum sp. were in the range of 90-264 mg/ml, where as the minimum bacteriocidal concentration (MBC) were in the range of 94-268 mg/ml. The decimal reduction time for MBC ranged between 0.89-5.35 h, where as the decimal reduction time for MIC ranged between 1.71-29.58 h for the various extracts tested against selected bacteria.

ABSTRAK

Tujuan kajian ini adalah untuk mengkaji aktiviti antimkrobiall bagi *Schizophyllum sp.* yang merupakan sejenis cendawan yang tumbuh liar dan boleh dimakan di mana ia adalah mudah diperolehi dan dinikmat sebagai makanani oleh penduduk tempatan di Sabah, Malaysia. Kajian *in vitro* meggunakan ujian screening *disk diffusion* dan kaedah *macrobroth dilution* telah menunjukkan bahawa pelbagai ekstraksi *Schizophyllum sp.* mempamerkan aktiviti antimikrobial terhadap empat jenis bakteria bawaan makanan iaitu, *Listeria monocytogenes* L55, *Salmonella typhimurium* S1000, *Escherichia coli* O157: H7 imre 91/02c dan *Staphylococcus aureus* S277. Ekstraksi dalam *Schizophyllum sp.* yang dijadikan sasaran bagi pengekstrakan termasuklah ekstraksi metanol, fenolik, flavonoid, terpenoid dan minyak pati. Semua ekstraksi ini menunjukkan aktiviti antimikrobial terhadap bakteria yang berbeza kecuali bagi ekstraksi terpenoid yang gagal menunjukkan sebarang aktiviti antimikrobial terhadap semua bakteria yang diuji. Ekstraksi metanol bagi *Schizophyllum sp.* merencatkan pertumbuhan S. *aureus*, *L. monocytoges* dan *E. coli*. Ekstraksi fenolik dan ekstraksi flavonoid pula adalah aktif terhadap semua bakteria yang diuji. Minyak pati daripada *Schizophyllum sp.* sekadar menunjukkan kesan antimikrobial terhadap *S. thyphimurium* dan *L. monocytogenes*. Kajian lanjutan yang dijalankan ke atas minyak pati dengan GC-MS telah mendapati bahawa 1-Octen-3-ol adalah kompaun tunggal yang hadir di dalam minyak pati. Kepekatan perencatan minima (MIC) pelbgai ekstraksi adalah dalam lingungan 90-264 mg.ml, manakala kepekatan bakteriasidal minima (MBC) adalah dalam lingkungan 94-268 mg/ml. *Decimal reduction time* bagi MBC adalah dalam lingkugan 0/89-5.35 jam, manakala *decimal reduction time* bagi MIC pula dalam lingkungan 1.71-29.58 h bagi pelbagai jenis ekstraksi terhdap bakteria tertentu.

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LIST OF SYMBOLS/ ABBREVIATIONS

°C	=	degree Celsius
kcl/g	=	Kilo Calories per gram
%	=	Percentage
% DV	=	percentage dairy value
mg	=	milligram
mcg	=	microgram
cm	=	centimeter
µm	=	micrometer
µl	=	microliter
mm	=	millimeter
kg	=	kilogram
RM	=	Ringgit Malaysia
/	=	per
$	=	dollar
h	=	hour
>	=	more than
±	=	plus minus
CFU/g	=	Colony forming units per gram
ml	=	milliliter
v/v	=	volume per volume
H_2O	=	water

MeOH = methanol

$CHCl_3$ = Chloroform

µl/ml = microliter per mililiter

MIC = Minimum Inhibitory Concentratio

GRAS = General Regard As Safe

LAB = Lacto Acid Bacteria

RDA = Recomanded Daily Allowence

DRI = Dietary Reference Intakes

B.C = before Christ

US = United States

CDC = Centers for Disease Control and Prevention

WHO = The World Health Organization

SFP = Staphylococcal food poisoning

CFU = Colony Forming Units

SA = *S. aureus*

ST = *S. thyphimurium*

LM = *L. monocytogenes*

EC = *E. coli* 0157: H7

ICUs = intensive care units

GC-MS = gas chromatography mass spectroscopy

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CHAPTER 1

INTRODUCTION

The use of mushrooms as a food is probably as old as civilization. In ancient time, mushrooms were consumed only for their culinary characteristics while the nutritive value of mushrooms was not known. Fruiting bodies of mushrooms are appreciated not only for texture and flavor but also for their chemical, nutritional properties and also their ability to provide specific benefits above and beyond their basic nutritional values. Edible mushrooms are highly nutritious, their nutritional values are favorably compare with that of meat, eggs and milk (Stephen *et al.*, 2004). Besides that, several edible mushrooms species act as source of physiological agents for medicinal applications, such as possessing antitumour action (Jong & Birmingham, 1992; Stephen *et al.*, 2004), cardiovascular system regulation (Jong & Birmingham, 1992; Stephen *et al.*, 2004,), antioxidant activity (Cheung & Cheung, 2005; Sun *et al.*, 2004) and antimicrobial activity (Stephen *et al.*, 2004; Smania *et al.*, 2003).

Mushrooms are eaten fresh (raw in salads), cooked, pickled or salted, and sometimes as condiments. There are marketed fresh, dried, frozen and canned. In canned mushrooms, there can be canned in many forms such as consist of the whole mushrooms, cut into four approximate equal parts, sliced into slices, randomly sliced and in pieces of caps and stems with irregular shapes and sizes (FDA, 2005). There are more than 2,000 species recorded in literature as edible mushroom where nearly 300 edible species were from India alone (Aindrila, 1989). White button mushroom

300 edible species were from India alone (Aindrila, 1989). White button mushroom (*Agaricus bisporus*), Crimini (*Agaricus bisporus*), Portabella (*Agaricus bisporus*), maitake (*Grifola frondosa*), shitake (*Lentinus edodes*), enoki (*Flammulina velutipes* and *Flammulina populicola*) and oyster (*Pleurotus sajor-caju* and *Pleurotus florida*) mushroom are mushrooms which are popular among the mushrooms eaters around the world. In Malaysia, mushrooms which are commonly cultivated including grey oyster (*Pleurotus sajor-caju*), Reishi/ Ling Zhi (*Ganoderma lucidum*), abalone mushroom (*Pleurotus cystidiosus*), white oyster (*Pleurotus florida*), black jelly (*Auricularia auricular-judae*), spilt-grill mushroom (*Schizophyllum spp.*), Yamabushitake/ Lion's Mane (*Hericium erinaceum*) dan Sweat mushroom (*Clitocybe sp.*) (Wan Kelthom & Normah, 2005).

The microbiological safety of food continues to be a major concern to consumers, regulatory agencies, and food industries throughout the world. Microbial activity is a primary mode of deterioration of many foods and is often responsible for the loss of quality and safety. Concern over pathogenic and spoilage microorganisms in foods is increasing due to increases in foodborne illness outbreaks and new evolving strains of foodborne pathogens. Microorganisms such as *Escherichia coli* 0157:H7, *Listeria monocytogenes*, *Salmonella typhimurium* and *Staphylococcus aureus* continue to be involved in foodborne disease and economic losses due to regulatory recalls and medications (Keun, Davidson & Chung, 2001). It has been estimated that as many as 30% of people in industrialized countries suffer from foodborne disease each year and in 2000 at least two million people died from diarrhoeal disease worldwide (Burt, 2004).

The inactivation or inhibition of these microorganisms using physical and chemical food preservation methods is important to the maintenance of safe food and longer shelf-life. These are traditional methods which have been use for long time but however in some cases, physical preservation methods such as heat, cold, or irradiation are not always desirable (Kim *et al.*, 2001). Chemical food antimicrobials are synthetic preservatives which have been regulatory approved for their use in foods as one of the alternative to preserve food. These traditional antimicrobials include the organic acids (acetic acid, benzoic acid, lactic acid, propionic acid and sorbic acid) and nitrite and sulfites (Kim *et al.*, 2001). These compounds have been used for years to control growth of microorganisms in foods. The sage of synthetic preservatives, however, is limited because of the concern that chemical residues could potentially cause side effects and health problems (Aun *et al.*, 2004).

Due to the changes of consumer consumption pattern, the consumers are more conscious about the healthiness and safety of food they consume. As a result, there has been a growing interest in the application of natural antimicrobials in foods to control pathogenic organisms recently. Driving forces behind this increased interest including desire in the food industry to expand the selection and spectrum of available international regulatory agencies approved food antimicrobials which are very limited for used in foods today, where those available are often useful only in low pH foods (Kim *et al.*, 2001). To date, nisin is the only antimicrobial peptide (bacteriocin) used commercially as biopreservative in processed cheese, dairy products, milk and canned foods (Appendini & Hotchkiss, 2000). Furthermore, consumer demand for minimally processed and natural food products has renewed interest in the use of natural food antimicrobial agents for food preservation (Kim

that have been known to inhibit microbial growth and already been well studied in cultured media such as extract of cloves (Hao *et al.*, 1998; Stecchini *et al.*, 1993), rosemary (Campo *et al.*, 2000; Pandit *et al.*, 1994; Shelef *et al.*, 1980), sage (shelef *et al.*, 1984), garlic (Kim *et al.*, 2004; Azzouz & Bullerman, 1982), Onion (Kim, Kim & Kyung, 2004), hops (Larson *et al.*, 1996), thyme (Juven *et al.*, 1994) and coriander (Aun *et al.*, 2004).

Mushrooms are recognized as a special food due to their therapeutic value as well as an important source of biologically active compounds of medicinal value (Breene, 1990). Mushroom accumulate a variety of secondary metabolites, including phenolic compounds, polyketides, terpenes and steroids (Cheung *et al.*, 2003) which may be potential to be used as natural antimicrobial compound to preserve foods in the future, which will benefit consumers as well as food industries. Local edible wild mushrooms are mainly consumed by rural communities where residents of other country may categorize it as inedible mushrooms due to their flavor and texture properties. It is true that very few species are universally accepted as an item of food, as a species eaten in one country may not be deemed edible in other part of the world. Besides, mushroom-eating habits vary from country to country (Aindrila, 1989).

Local edible wild mushrooms can be found abundant in the local market during the booming seasons and widely consumed by rural communities. However, there is still lack of studies that have been done on local edible wild mushrooms in our tropical rain forest including their antimicrobial properties. Although many species of mushrooms especially the cultivated mushrooms have been well studied in many disciplines, locally available edible wild mushrooms may be different on composition

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