

1.0 INTRODUCTION

The term protein was derived from the Greek word 'proteios' which means 'primary' and therefore well chosen since protein is the first recognizable and distinctive expression of genetic information (Ananta and Rajib, 2008). Proteins are made up of linear polymers of amino acids in which the total number of amino acids residues are higher than fifty. They are classified based on their functions, properties and structures (Tayyab and Boyce, 2006).

Proteins are the most abundant organic molecules in the cells and play an important role performing a variety of functions in the living systems. These includes transportation of many low molecular weight compounds, catalyzing specific chemical reactions, helping in transmission of nerve impulses, performing a storage function during growth and development, providing mechanical support, involve in the immune response as defense mechanism and also as growth regulator in both prokaryotes and eukaryotes (Tayyab and Boyce, 2006).

Proteins have been isolated from various sources due to their therapeutic potentials and commonly investigated for their antifungal and antimicrobial properties. According to Moller *et al.* (2008), bioactive proteins and peptides are derived from animal and plant sources, as well as from mushrooms. Bioactive proteins have been detected directly or indirectly after release by hidrolsation or fermentation from many sources including bovine blood, gelatin, meat, eggs and various fish species like tuna, sardine, herring and salmon and also in wheat, maize, soy, rice, mushrooms, pumpkin and sorghum.

The mushroom is a macrofungus with a distinctive fruiting body which can be either epigeous or hypogeous and large enough to be seen with naked eye and to be picked

by hand (Chang and Miles, 1992). It can be divided into four categories : (a) those which are fleshy and edible fall into the edible mushroom category, e.g., *Agaricus bisporus*; (b) mushrooms which are considered to have medicinal applications, are referred to as medicinal mushrooms, e.g., *Ganoderma lucidum*; (c) those which are poisonous mushrooms, e.g., *Amanita phalloides*; (d) a various category which includes a large number of mushroom whose properties remain less well defined which grouped together as ‘other mushrooms’ (Chang, 1993).

Mushrooms constitute at least 14 000 and perhaps as many as 22 000 known species. The number of mushroom species on the earth is estimated to be 140 000, suggesting that only 10% are known. Assuming that the proportion of useful mushrooms among the undiscovered and unexamined mushrooms will be only 5%, which implies 7000 yet undiscovered species will be of possible benefit to mankind (Hawksworth, 2001). Even among the known species the proportion of well investigated mushrooms is very low.

Mushrooms have been traditionally used as medicine worldwide for centuries, as well consumed as nutritional foods. According to Buswell and Chang (1993), the medicinal aspect of mushrooms remains largely unexploited. It have been estimated that there are about 1800 mushrooms species with medicinal values (Chang and Miles, 2004). Recently, there have been increasing interest in traditional remedies for the treatment of various physiological disorder and numerous biologically active compounds have been reported in mushrooms as the result. Some medicinal properties of mushrooms with beneficial effects that have been recorded include anti-tumor, antiviral, hypocholesterolaemic, antibiotic activities and other biological activities (Pai *et al.*, 1990).

It is well known that mushrooms are rich in proteins. A large number of proteins have now been purified and characterized and many more remain to be isolated. Some of

these proteins have proven values. These biologically active proteins as recorded in the literature included protein with antifungal activity from the fungi *Pleurotus ostreatus* (Chu *et al.*, 2005), *Tricholoma giganteum* (Guo *et al.*, 2005), and *Lyophyllum shimeji* (Lam and Ng, 2001a); proteins with ribonuclease activity from *Pleurotus eryngii* (Ng and Wang, 2004a), *Pleurotus sajor caju* (Ngai and Ng, 2004), *Pleurotus pulmonarius* (Ye and Ng, 2002) and *Cantharellus cibarius* (Wang *et al.*, 2003); ribosome inactivating proteins from *Lyophyllum shimeji* (Lam and Ng, 2001a) and *Flammulina velutipes* (Ng and Wang, 2004c); lectin with mitogenic activity from *Boletus edulis* (Zheng *et al.*, 2007); proteins with HIV-1 reverse transcriptase inhibiting activities from *Lentinula edodes* (Ngai and Ng, 2003); and laccase from *Tricholoma giganteum* (Wang and Ng, 2004d) and *Ganoderma lucidum* (Wang and Ng, 2006a). Engineering of these proteins may lead to a higher potency of the desirable activities and minimization of the undesirable activities.

Among the medicinal mushrooms reported, *Ganoderma* and especially *Ganoderma lucidum* is the most studied because of its great potential as a source of bioactive compounds. The fruiting body, mycelia, and spores of *G. lucidum* contain approximately 400 different bioactive compounds, which include triterpenoids, polysaccharides, nucleotides, sterols, steroids, fatty acids, proteins or peptides, and trace elements (Wasser, 2005).

According to Gao *et al.* (2003), *Ganoderma* spp. have been investigated as anti-tumor, antiviral agents and less so as anti-bacterial agents. Anti-bacterial activity has been observed against Gram-positive bacteria from the basidiocarp extracts of *G. lucidum* (Kim *et al.*, 1993) and *G. orgonense* (Brian, 1951). Steroids like 5 α -ergosta-7,22-dien-3 β -ol or 5,8-epidioxy-5 α ,8 α -ergosta-6,22-dien-3 β -ol, isolated from *G. applanatum* (Pers.) Pat. were proved to be weakly active against several Gram-positive and Gram-negative

microorganisms (Smania Jr *et al.*, 1999). Moreover, Sudirman and Mujiyati (1997) observed that seven Indonesian *Ganoderma* species inhibited the growth of *Bacillus subtilis*. In Malaysia, there is a paucity of studies on the indigenous *Ganoderma* spp. or their medicinal properties.

There is ongoing study, to document, the utilization of mushrooms as natural resources as food and medicines by Orang Asli communities in Selangor, Malaysia, at Mushroom Research Centre, University Malaya (Vikineswary *et al.*, FR168/2007A, unpublished data). Based on interviews carried out among the Orang Asli communities, *Ganoderma australe* were utilized as food/infusion by four communities and as medicine by only a group. *Ganoderma australe* which known as ‘cendawan birung’ by the ‘Mah Meri’, an indigenous community from Sungai Judah Village of Carey Island, is used as medicine for skin problem (dermatitis) known as shingles. Meanwhile, the ‘Temuan’ from Broga Village, Ulu Langat called this mushroom as ‘cendawan certing’ and used them for lung pain. The mushroom was soaked and the water applied to the chest area.

Ganoderma australe (Fr.) Pat (1890) is non-laccate species of *Ganoderma* and often found in tropical and sub-tropical regions (Fig. 1.1). In Malaysia, these species generally found as wood degrading fungi in forest reserves and plantation forests (Zakaria *et al.*, 2009). Literature is scarce on the chemical composition of this species and also on studies relating substances produced by *G. australe* and their biological activity. Studies had been done on their triterpenes and sterols with antibacterial activity against *Bacillus cereus* and *Staphylococcus aureus* (Gerber *et al.*, 2000) while methyl australate with antimicrobial activity has been reported from this fungus (Smania *et al.*, 2007) in Brazil. To the best of our knowledge, no studies had been done on isolation of proteins from the indigenous species of *Ganoderma australe*.



Figure 1.1
Ganoderma australe from
Bishop Trail, Fraser Hill,
Malaysia (Hasnol, 2007).

Recently, the study of peptides and proteins has become one of the most active areas of current research because many of them possess potent pharmacological potential. This research focused on isolation of bioactive proteins from indigenous strains of *Ganoderma australe*.

The main objectives of this study were to:

- a) screen for antimicrobial activities from selected indigenous *Ganoderma australe* strains.
- b) isolate and purify functional protein/s active against pathogenic bacteria and fungi from *Ganoderma australe*.
- c) test for selected medicinal properties of the isolated protein/s.
- d) characterize the functional protein/s isolated.