UNIVERSITI PUTRA MALAYSIA

PRODUCTION AND PROPERTIES OF POLYSACCHARIDES FROM MYCELIA OF THREE *GANODERMA* SPECIES

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PRODUCTION AND PROPERTIES OF POLYSACCHARIDES FROM MYCELIA OF THREE *GANODERMA* SPECIES

By

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The grace of the Lord Jesus Christ and the love of God and the fellowship of the Holy Spirit be with you all.

(2 Corinthians 13:14)

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LIST OF ABBREVIATIONS

%	Percentage
/	Per
^oC	Degree Celsius
pH	Exponent of Hydrogen Ion
ppm	Part Per Million
cm	Centimeter
kg	Kilogram
g	Gram
mg	Milligram
µg	Microgram
L	Liter
ml	Milliliter
M	Molar
mM	Millimolar
N	Normality
h	Hour
min	Minute
nm	Nanometer
CTL	Control
CF	Culture Filtrate
H	Homogenized Mycelium
E	Hot Water Extraction
CHO	Total Carbohydrates
Ps	Polysaccharides
RM	Malaysian Ringgit
sp.	Species

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science.

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By

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September 1999

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Faculty : Science and Environmental Studies

Pharmacological research has shown that extract of certain *Ganoderma* species have medical effects including anti-tumor, anti-virus, lowering blood fat and regulating blood pressure.

One of the major ingredients of the water extract of *Ganoderma* fruit bodies is the polysaccharides and these are the polysaccharides which have been studied more extensively. However, new findings have recommended producing the *Ganoderma* mycelium instead of the fruiting bodies because of the several advantages in producing the fungal mycelium. Thus, this project was focused on the production and properties of polysaccharides from the *Ganoderma* mycelium.

Mycelia of three *Ganoderma* species (*G. lucidum*, *G. tropicum* and *G. tsugae*) were inoculated into five types of liquid growth media (barley extract, Fergus media, Mizuno media, PDB and soya bean extract) for 15 days at 25°C. Among these media tested, soya bean extract supported good growth of the fungal mycelium of all the three species of *Ganoderma*. Increasing the soya bean concentration resulted in a gradual increase in the growth of the fungal mycelium as well as the amount of the polysaccharides produced. Yield of total carbohydrates produced by the mycelium in all the three species reached its maximum level when incubated at 25°C for 15 days. A whole range of different polysaccharides with different molecular weights and linkage types were obtained from the culture filtrate, homogenized mycelium fraction as well as the hot water extraction of mycelium harvested at different periods of incubation. Polysaccharide with β-(1,3) linkages that were reported to have pharmaceutical value were identified. These polysaccharides were found to have a common molecular weight of 27,000 Da.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains.

PENGHASILAN DAN PEMBELAJARAN KE ATAS POLISAKARIDA DARI TIGA MISELIA *GANODERMA* SPESIS

Oleh

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Kajian farmakologikal telah menunjukkan bahawa ekstrak dari beberapa spesis *Ganoderma* mempunyai kesan perubatan termasuk anti-barah, anti-virus, penurunan lemak darah dan pengawalan tekanan darah.

Salah satu daripada kandungan utama dalam ekstrak cecair dari badan buah *Ganoderma* adalah polisakarida yang mana telah mendapat kajian yang lebih menyeluruh. Walau bagaimanapun, penemuan baru telah mencadangkan pertumbuhan miselia *Ganoderma* adalah lebih baik berbanding dengan badan buahnya kerana beberapa kelebihan dalam pertumbuhan miselia kulat. Oleh itu, projek ini telah menumpu kepada penghasilan dan pembelajaran ke atas polisakarida dari miselia *Ganoderma*.

Miselia dari tiga *Ganoderma* spesis (*G. lucidum*, *G. tropicum* dan *G. tsugae*) telah ditumbuhkan dalam lima jenis medium pertumbuhan cecair (ekstrak barli, medium Fergus, medium Mizuno, PDB dan ekstrak kacang soya) selama 15 hari pada suhu 25°C. Di antara medium tersebut yang diuji, ekstrak kacang soya memberikan kadar pertumbuhan dan berat kering miselia yang baik kepada ketiga-tiga spesis *Ganoderma*. Penambahan kepekatan ekstrak kacang soya mengakibatkan penambahan kadar pertumbuhan miselia kulat dan juga kandungan polisakarida yang dihasilkan. Penghasilan jumlah karbohidrat yang dihasilkan pada ketiga-tiga spesis miselia mencapai tahap maksimal masing-masing ketika mereka dieramkan pada suhu 25°C selama 15 hari. Polisakarida yang berbeza berat molekul dan jenis ikatan telah diperolehi daripada medium pertumbuhan, hasil turasan homogenat serta ekstrak air panas miselia pada tempoh pengeraman yang berbeza. Polisakarida yang mengandungi ikatan β-(1,3)-glikosidik, yang dilaporkan mempunyai nilai farmaseutikal, didapati memperolehi berat molekul umumnya pada 27,000 Da.

CHAPTER I

INTRODUCTION

Mushrooms have long been treasured as a delicacy not only for their delicious flavor, high nutritive value and tantalizing texture but also for their therapeutic properties. The practice of using fungi as medicines was found in the tradition of many cultures in the past which persisted until today, such as China, Japan and Korea. The first Chinese book on medicinal substances, "The Seng Nung's Herbal", written some 2,000 years ago, recorded the beneficial effects of the various fungi. It was not until this century that the medicinal values of fungi first gained worldwide attention when antibiotics were obtained from *Penicillium*. It is now well documented that the major fungal groups produce antibiotic substances and quite a number of them have been shown to possess antitumor activities and other pharmacodynamic properties (Jong *et al.,* 1991).

Traditionally in the Orient, the fruiting body of *Ganoderma* had always been regarded by the Chinese to be of a high quality herbal medicine for many decades, the so called "elixir of life". It was believed that some *Ganoderma* could improve one's constitution, increase the body's healing ability and helped maintain good health (Tong and Chen, 1990). *Ganoderma* was not only eaten as a food item but they had a long history of use as traditional medicine in China and Japan. The virtues of its extracts had been handed down from generation to generation. It had been highly valued as a "cancer cure" (Ito *et al.*, 1977; Matsumoto *et al.*, 1978).

Ganoderma are species of *Basidiomycetes* that belongs to *Polyporaceae* (or *Ganodermataceae*) of *Aphyllophorales*. They differ from the ordinary mushrooms belonging to the order *Agaricales* in that they have pores rather than gills on the under surface of the fruiting bodies. Their fruiting bodies exhibit various changes in shape, lustre and color, flesh quality and bitterness, depending on the culture conditions such as temperature, light, moisture, carbon dioxide concentration and the production site. Such fruiting bodies are divided into Seishi (blue), Sekishi (red), Ohshi (yellow), Hakushi (white), Shishi (violet) and Kokushi (black) according to their lustre and color; or into Rokkakushi (deer antler Reishi), Gyukakushi (ox horn Reishi), Unshi (cloud-like Reishi) and Nikushi (meat-like Reishi) based on their shape (Wang *et al.*, 1984). *Ganoderma* fruiting body was called "Reishi" in Japanese. In China, it was called "Lingzhi" and was divided into four families: Ganoderma, Haddowia, Amauroderma, and Humphreya. These were further classified into 88 different species by Zhao (1989). Reishi was given other names such as Mannentake, Saiwaitake, Sakikusa, Saegusa, Kamishiba, Gyokurai, Kisshotake, Sankei, Fushiso and Zuishi (Mizuno *et al.*, 1995).

As early as the 1920's, researchers had carried out research to investigate its pathogenic and decay properties (Venkatarayan, 1936; Pirone, 1957; Naidu *et al.,* 1966; Kumari and Sirsi, 1971; Blanchette, 1984; Adaskaveg and Gilbertson, 1985), cultivation techniques (Bose, 1929; Menon, 1963), morphological and genetic characteristics (Banerjee and Sarkar, 1956; Sarkar, 1959), as well as its identification (Banerjee and Sarkar, 1958; Banerjee and Sarkar, 1985; Steyaert, 1961; Bazzalo and Wright, 1982; Adaskaveg and Gilbertson, 1986). However, intensive research and scientific evaluation on its pharmacological effect started only in 1960's. The medicinal value of *Ganoderma* was closely linked to the presence of the following compounds in the mushroom fruiting bodies: polysaccharides, triterpenoids, adenosine and organic germanium (Miyazaki *et al.*, 1983; Shimizu *et al.*, 1985; Morigawa *et al.*, 1986; Betty, 1987; Mizuno *et al.*, 1995; Tong, 1995).

In Malaysia, studies on the cultivation techniques of a suitable strain of *Ganoderma* which was well adapted to the local climatic conditions (Tong and Chen, 1990), its growth characteristics, Ge uptake by the mycelium (Tong *et al.,* 1994a) as well as the fruiting bodies of the fungus (Tong *et al.,* 1994b) have been carried out.

So far, most of the commercial products prepared from *Ganoderma* and sold as health supplements were imported and extracted from its fruiting bodies. Previous studies (Tong and Chong, 1996) had shown that there were many advantages in producing these products from the fungal mycelium instead of its fruiting bodies. The fungal mycelium assimilated a much higher level (7 times) of organic germanium and had a much shorter growing period (20 days) compared to its fruiting bodies (120 days on commercial basis). Furthermore, the growth of mycelium was relatively more simple, economical, less problematic and easier to manipulate.

Currently, Malaysia imports about RM45 million worth of *Ganoderma* products annually. Following the success in growing the *Ganoderma* in Malaysia (Tong and Chen, 1990), the technology was transferred to the general public which attracted a tremendous amount of interest from local companies wanting to commercialize the production of *Ganoderma* in Malaysia. At present, the Malaysian consumers are paying exorbitantly (RM150 per 50 capsules) for these imported *Ganoderma*. It is high time that Malaysia produces these products locally not only to save import revenues but earn foreign exchange as well by exporting these health-benefiting supplements.

Therefore, the search for suitable substrates for the production of bioactive therapeutic compounds by *Ganoderma* species become imperative. This project was undertaken to focus on polysaccharides from the water extract of the *Ganoderma* mycelium with the following objectives:

a) to study the growth characteristics of *Ganoderma* in various locally available substrates.

b) to grow the *Ganoderma* mycelia in sufficient quantity in selected liquid medium for the extraction of polysaccharides.

c) to purify the various types of polysaccharides isolated and determine their purity, molecular weight and linkage type.

d) to study the effect of different growth conditions on the production of these polysaccharides.

CHAPTER II

LITERATURE REVIEW

Ganoderma had a history of more than 5,000 years. In ancient times, *Ganoderma* had been noted as the star among all the healing herbs in China. The book, which was known in Japan as "Shinnoh Honsohkyo" (Shen Nung's Herbal Medicine), is now recognized as being the original textbook of Oriental medical science. In it, *Ganoderma* was categorized as a "superior" medicine, ranked number one ahead of ginseng among the 365 recognized herbs.

According to "Ben Cao Gang Mu" (Outline of Herbal Medicine), the most comprehensive work of ancient Chinese herbal books compiled by Li Shi-Zhen of Ming dynasty (1590 AD) showed that *Ganoderma* was regarded as an elixir for longevity (Zhao and Zhang, 1994).

Traditionally, *Ganoderma* had served as a tonic to prevent illnesses. Emperors of the great Chinese dynasties and Japanese royalty drank teas and concoctions of the mushroom for vitality and long life.

Today, *Ganoderma* is consumed extensively as an "Adaptogen" – a food supplement that does not cure but enable the body to help itself. Adaptogen differs from drugs in many ways: (1) it requires no prescriptions (2) it is user-friendly, no high-tech equipment, needles, syringes or professional expertise is required for administration (3) it is less costly than most drugs (4) non-habiting forming (5) a substance that continues to work even after a state of normalcy is achieved.

Cultivation of *Ganoderma*

The natural source of wild *Ganoderma* is extremely scare and its quality fluctuates. Artificial culture and cultivation of *Ganoderma* were attempted initially by Hemni *et al.*, in 1937 (cited in Mizuno *et al.*, 1995). In 1971, Naoi, a notable food scientist of the Kyoto University, initiated the culturing of *Ganoderma* in mass production by cultivating the spawn using pots containing sawdust (cited in Mizuno *et al.*, 1995). Since then, the use of bed logs or sawdust had become an established practice. His success soon promoted an extensive research and clinical study and eventually led to the advent of potent *Ganoderma* products which were scientifically processed through advanced technology.

With an increase in knowledge of the climates that it thrived in and the correct amounts of oxygen and moisture needed for the spores to grow into the *Ganoderma* fruiting bodies, *Ganoderma* was being produced on a large scale for pharmacological and clinical studies by artificial cultivation and submerged fermentation (Liu *et al.*, 1979a; Zhang, 1980). In China, studies on submerged cultures of *Ganoderma lucidum* growing on soya bean protein produced a kind of protein complex drink (Xiong *et al.*, 1994).

Today, *Ganoderma* cultivation had prospered not only in China and Japan, but Taiwan, Korea, and Thailand as well.

Fruiting Body of *Ganoderma*

Structurally, *Ganoderma* fruiting body can be divided into the top (hymenophore) and the stalk (prosthecae). The essential parts of the top are the pileus and hymenium. Qualitative and quantitative analysis on the pileus, hymenophore, spores and prosthecae revealed the uneven distribution of the therapeutic content of *Ganoderma* (Figure 1), of which the hymenophore or the top was the most valuable and potent part of this herb (extracted from "Win Reishi", 1995).

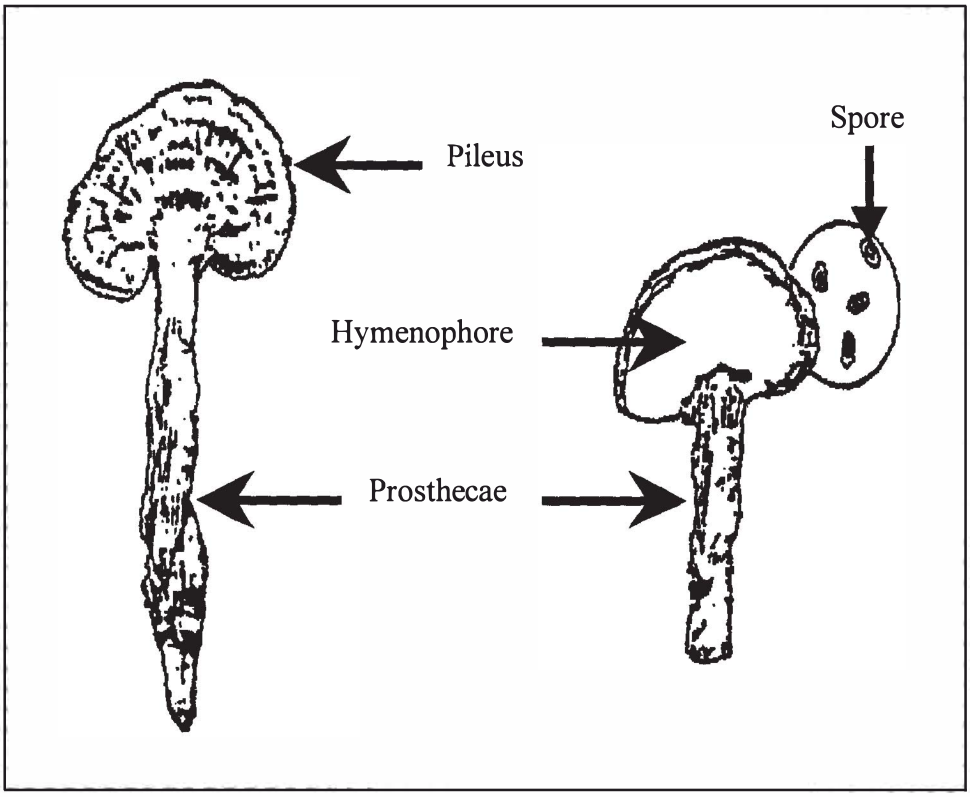

Figure 1: Fruiting body of *Ganoderma*.