EFFECT OF SPRAY DRYING ON CHEMICAL COMPOSITIONS AND BIOACTIVE COMPOUNDS OF *GANDERMA LUCIDUM* (LINGZHI) POWDER

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ABSTRACT

Ganoderma lucidum is one of the most famous medicinal mushroom species and has been used in traditional medicine in the Far East. The objective of this study was to optimize the conditions for the extraction and production of *G. lucidum* powder using spray drying. Two distinct strains of *G. lucidum*, identified as MG2 and G2, were analyzed. The extraction methods involved heat reflux extraction at temperatures of 80, 90, and 100°C, with extraction times of 1, 2.5, and 4 hours, as well as varying the *G. lucidum* to water ratios of 1:6, 1:9, and 1:12 to determine the optimum conditions for extraction. The results indicated that the optimum condition for heat reflux extraction was at 100°C for 4 hours with a *G. lucidum* to water ratio of 1:12. The yield was 12.72%, with ganoderic acid A content at 0.53 mg/100 mg and β-glucan at 4.04 mg/100 mg. These optimum conditions were then applied to pilot-scale extraction. The inlet temperature of the spray drying process at inlet temperatures of 140, 160, and 180°C to produce *G. lucidum* powder was investigated. The results indicated that 140°C was the optimum drying temperature due to its highest yield, ganoderic acid A, and β-glucan content. Pilot-scale water extraction and spray drying to produce *G. lucidum* powder from both strains MG2 and G2 were studied. The extraction was performed with and without maltodextrin. The strains of *G. lucidum* and the presence of maltodextrin influenced the proximate constituents, minerals, and germanium content of the *G. lucidum* powder. Analysis of MG2 and G2 powders revealed that MG2 had nutritional value as a good source of carbohydrates, protein, and minerals. The germanium content found in the strain MG2 more than that of G2. Furthermore, the species and presence of maltodextrin influenced the β-glucan, ganoderic acid A, and antioxidant activity of *G. lucidum* powder. Analysis showed β-glucan content ranged from 9.91% to 38.83%; additionally, ganoderic acid A levels varied from 0.85 mg/g to 0.92 mg/g. The MG2 had the highest total antioxidants, as measured by the FRAP and TEAC methods. In contrast, ABTS analysis indicated that G2 exhibited better antioxidant properties. The DPPH method demonstrated that extracts from G2, followed by MG2, offered antioxidant activity.

Keywords: *Ganoderma lucidum*, Antioxidant, β-glucan, Ganoderic acid A, Germanium, Mineral, Proximate analysis, Spray dry

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Phraeo Vijitrotai

TABLE OF CONTENTS

Page

TABLE OF CONTENTS (Cont.)

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

LIST OF ABBREVIATIONS

GE	*G. lucidum* extract
Ge	Germanium
MG2	*G. lucidum* MG2 ChiangMai, Thailand
G2	*G. lucidum* G2 imported from China
G	Ganoderma
HPLC	High Performance Liquid Chromatography
Y	Yield
ICP-OES	Inductively Coupled Plasma-Optical Emission Spectrometer
DPPH	1,1-Diphenyl-2-picrylhydrazyl
ABTS	2,2′-Azino-bis (3-Ethylbenzothiazoline-6-Sulfonic Acid)
ACN	Acetonitrile
FRAP	Ferric Reducing Antioxidant Power
TEAC	means Trolox equivalent antioxidant capacity mg/g sample
EC_{50}	means 50% Effective concentration g Sample/L

CHAPTER 1
INTRODUCTION

1.1 Introduction

G. lucidum, also known as Lingzhi, has gained popularity as a source of nutrients and bioactive secondary metabolites, often referred to as an elixir of life in East Asian countries for health and longevity. Its extracts contain bioactive triterpenes, polysaccharides, and proteins, which are primarily consumed for their pharmaceutical benefits rather than as food (Jeewanthi et al., 2017). Research has demonstrated that *G. lucidum* possesses immunomodulating, blood glucose-regulating, antibacterial, antiviral, anticancer, and antioxidant properties, confirmed through in vitro and animal studies (Ahmad, 2018; Zhang et al., 2019; Wang and Lin, 2019; Yang et al., 2020; Yalcin et al., 2020; Ren et al., 2021).

Traditionally, G. lucidum has been highly regarded for its medicinal properties (Sanodiya et al., 2009). Its fruiting bodies, mycelia, and spores are used to treat various health conditions, including chronic hepatitis, hypertension, and hyperglycemia. *G. lucidum* features a large, dark, sculptured structure with a varnished, woody texture (Bao et al., 2002). It is recognized for its nutritional value, being rich in proteins, vitamins, and minerals while low in fat and calories (Genccelep et al., 2009; Kuldo et al., 2014). However, due to their thick, rigid, and corky fruiting bodies, Ganoderma species are not typically considered edible despite their widespread medicinal use (Jonathan et al., 2010).

G. lucidum extracts contain various bioactive compounds, including carbohydrates, glycosides, triterpenoids, and phenolic compounds (Nguyen et al., 2009). It is also noted for its anti-hypertensive and anti-diabetic properties, along with microbiological activity against several pathogens. The species has been reported to possess anti-tumor, antibacterial, anti-inflammatory, and hepato-protective effects (Kuldo et al., 2014; Liu et al., 2019).

Lingzhi is notable for accumulating essential metals and metalloids, particularly germanium (Ge), with levels reported at approximately 1 mg/kg in Ganoderma species (Siwulski et al., 2017). Germanium exhibits various biological effects, including antibacterial and anticancer properties (Li et al., 2017). Despite high concentrations in *G. lucidum*, there is limited knowledge on the kinetics of germanium uptake and its effect on the mushroom's growth, morphology, and biological activity.

Bioactive polysaccharides from Ganoderma species, particularly β-glucans, are abundant yet inadequately characterized due to extraction challenges (Sakai and Chihara, 1995). Various methods, including organic solvent extraction, have been developed to improve yields and reduce costs (Kallithraka et al., 1997; Vernhet et al., 1996). A hot water extraction method is effective and can be automated to ensure

quality and remove impurities (Jarvis and Morgan, 1997). Triterpenoids also play a significant role in *G. lucidum's* pharmacological effects (Boh et al., 2007).

This study aims to determine the optimal temperature and time conditions for extracting *G. lucidum* using water as a solvent. Following this, the production of *G. lucidum* powder will be explored using the spray drying technique in a larger-scale process. Two strains of *G. lucidum*, MG2 and G2, will be utilized, and their antioxidant activity and bioactive compound content will be analyzed. This research is intended to contribute to the further development and utilization of *G. lucidum* in Thailand.

1.2 Objectives

1. To study the optimization condition of temperature and time for extraction *G. lucidum*.
2. To study the production of *G. lucidum* powder by spray drying technique
3. To investigate the impact of pilot-scale *G. lucidum* powder production on the antioxidant, bioactive compounds and its nutrition.

1.3 Scopes of research

This research focuses on optimizing extraction processes, refining production techniques, and analyzing the bioactive compounds and antioxidant properties of *G. lucidum*. The optimization of extraction conditions involves studying the effects of extraction temperature (80°C, 90°C, and 100°C), extraction time (1 hour, 2.5 hours, and 4 hours), and *G. lucidum*-to-water ratios (1:6, 1:9, and 1:12) on yield and bioactive compounds, specifically β-glucan and ganoderic acid A. Additionally, optimal conditions for producing *G. lucidum* powder through spray drying are investigated by varying inlet temperatures (140°C, 160°C, and 180°C) while keeping a constant outlet temperature of 95°C, assessing effects on yield and bioactive compounds. The study also includes developing pilot-scale production methods for *G. lucidum* and analyzing its bioactive compounds. Antioxidant assays, such as FRAP, DPPH, and ABTS, along with proximate and mineral analyses, are performed to evaluate the nutritional and antioxidant potential of *G. lucidum*. This research aims to establish efficient production and extraction methods while providing comprehensive insights into the bioactive properties of *G. lucidum*.

CHAPTER 2
LITERATURE REVIEWS

2.1 *Ganoderma lucidum*

Ganoderma is a genus of over 300 species of wood-decaying fungi in the family Ganoderma taceae. These fungi, found globally, can act as saprobes on dead wood or as parasites on live trees, including hardwoods, conifers, and palms. While some species are host-specific, most infect a broad range of plants and are significant as plant pathogens. Key features include large, tough, shelf-like caps (pilei) that vary in color (white, yellow, brown, red, or purple) and may exhibit a shiny, varnished surface. The fungi play an ecological role by decomposing lignin and cellulose but also cause severe damage to economically important plants like cacao, rubber, tea, coffee, and oil palm. Several species, notably *Ganoderma lucidum* (reishi or lingzhi), are valued in traditional Asian medicine for their potential health benefits, including cancer treatment, driving increased research interest.

Ganoderma lucidum (commonly known as Lingzhi in Chinese and Reishi in Japanese) has been recognized for over 2000 years as a medicinal mushroom with powerful health-promoting effects, particularly in traditional Chinese medicine. Known for its glossy exterior and woody texture, this mushroom is rare, typically found during the rainy season at the base of deciduous tree stumps. (Wasser, 2005). It has a long history of use in China, Japan, Korea, and other Asian countries for promoting health and longevity. *G. lucidum* is especially noted for its application as a complementary therapy in cancer treatments, being internationally recommended as an effective adjuvant therapy due to its potential therapeutic benefits. (Shamaki, et al. 2012) *G. lucidum* has been used popularly as a complementary treatment for cancer therapy in traditional Chinese medicine and is internationally recommended for its efficacy as an adjuvant in cancer treatments (Jin, et al., 2016).

Ganoderma lucidum (*Reishi*) is a versatile medicinal mushroom widely used to address immune deficiency diseases due to its broad immunostimulating, anti inflammatory, and antiallergenic properties. It contains over 100 oxygenated triterpenes, which enhance natural killer (NK) cell activity. *Reishi* is applied in treating

various infectious diseases, including bronchitis and viral hepatitis, by stimulating phagocytosis, increasing T-cell activity, and boosting CD4 cell counts in vivo. Additionally, it is recognized for its antidepressant effects, further underscoring its wide-ranging therapeutic applications.

Figure 2.1 The sample of *G. lucidum* cultivars; MG2
From: Functional nutrition for cancer. (2021)

Ganoderma lucidum (Reishi) has demonstrated efficacy in treating chronic conditions such as hepatopathy, hypertension, hyperglycemia, and neoplasia. The fruiting bodies, cultured mycelia, and spores of the fungus are particularly effective in these treatments. It polysaccharides have garnered significant attention for their anti-tumor and hypoglycemic properties, as documented in various studies, highlighting its potential in managing chronic diseases and enhancing health outcomes. (Miyazaki and Nishijima, 1981) (Wang, et al., 1997) and hypoglycemic

Ganoderma lucidum (Reishi), a revered fungus in traditional Chinese and Japanese medicine, is widely studied for its potent bioactive properties. It is known to improve bodily constitution, enhance healing, and is a key component in Chinese herbal medicine development. The fungus contains triterpenoids and polysaccharides, which have significant physiological and medicinal effects. Triterpenoids exhibit strong anti-cancer properties by inhibiting blood flow to cancer cells, depriving them of oxygen and nutrients. Polysaccharides, including β-glucans (β-1,3-d-glucopyranan with β-1,6-monoglucosyl side chains), are extensively studied for their therapeutic potential, including immune-modulating and anti-tumor effects. Efforts are ongoing to optimize the production and utilization of these functional molecules (triterpenoids, polysaccharides, sterols, lectins, and proteins) for developing therapeutic agents to treat cancer and other ailments.

2.2 Major bioactive components

Bioactive compounds are phytochemicals in foods that modulate metabolic processes, thereby promoting better health. They offer various beneficial effects, including, Antioxidant activity, protecting cells from oxidative damage. Enzyme modulation, through inhibition or induction. Receptor activity regulation, by inhibiting receptor functions. Gene expression regulation, through induction or inhibition. These properties make bioactive compounds integral to maintaining health and preventing diseases. (Correia, et. al., 2012). The bioaccessibility and bioavailability of bioactive compounds vary significantly. Importantly, the most abundant compounds in consumed fruits do not always result in the highest concentrations of active metabolites in target tissues. This highlights the complexity of absorption, metabolism, and utilization of these compounds in the human body. (Manach, et. al. 2005). This passage highlights the complexity of studying bioactive compounds, especially in terms of their bioavailability, which is the degree to which these compounds are absorbed and utilized by the body. The bioavailability of bioactive compounds is influenced by various factors, including (Carbonell-Capella, et. al., 2013). (Manach, et al., 2004). Chemical Structure: Bioactive compounds can be hydrophilic (water-soluble) or lipophilic (fat-soluble), which affects how they are absorbed and utilized in the body. Food Source, Different plant species contain varying concentrations of bioactive compounds, and these compounds may act differently depending on the food

source. Interactions with Other Biomolecules, Bioactive compounds often interact with other components in the food matrix, such as carbohydrates, lipids, and proteins. These interactions can impact their absorption. For example, antioxidants in fruits may be bound to macromolecules, which can affect their release and bioavailability during digestion. The structure of the food (its "matrix") can also impact how well bioactive compounds are absorbed. For instance, the physical form of the food (whether it's whole, processed, or cooked) can influence the bioavailability of antioxidants and other bioactive compounds. Concentration and Site of Action: Bioactive compounds are present in varying concentrations in foods and in the body, and they may have different sites of action, such as acting on different organs or tissues to exert their effects. In summary, understanding bioactive compounds and their role in health is complex, as it involves not only their inherent properties but also how they interact within the food matrix and the body, affecting their bioavailability and overall biological activity. This complexity explains why bioavailability is not always well understood. (Parada and Aguilera, 2007). (Carbonell-Capella, et al., 2014; Porrini and Riso, 2008).

2.2.1 Triterpenoids

This excerpt discusses the pharmacological properties of terpenoids found in *Ganoderma lucidum* (also known as reishi), a medicinal mushroom. Triterpenoids in *G. lucidum:* Terpenoids, particularly triterpenes, found in *G. lucidum* have attracted attention due to their efficacy in various pharmacological applications, especially in cancer treatment.Some of the important triterpenes isolated include ganoderiol, lucidenic acid, lucialdehyde, ganolucidinic acids, lanostanoids, and ganodermantriol. Triterpenoids are typically highly oxidized derivatives of lanostane and are characterized by complex structures, high molecular mass, and lipophilicity.These compounds may contain 30, 27, or 24 carbon atoms in their structure, and over 150 ganoderic acid derivatives have been identified. Ganoderic Acids: Triterpenoids with carboxyl groups are generally referred to as ganoderic acids, which play an important role in the pharmacological properties of *G. lucidum*. Triterpenoid Saponins: These compounds are glycosides with diverse bioactive properties, and are gaining prominence in cancer treatment due to their efficacy and safety.The review highlights the structural diversity and therapeutic potential of

triterpenoid saponins in chemoprevention and chemotherapy for cancer. Molecular Mechanisms: Triterpenoid saponins work by modulating several key cellular processes related to cancer, including: Cell proliferation, Apoptosis (programmed cell death), Autophagy (cellular degradation), Metastasis (spread of cancer), Angiogenesis (formation of new blood vessels), Inflammation, Oxidative stress, Multidrug resistance, Cancer stem cells, MicroRNAs. The review provides insights into the molecular basis of how triterpenoid saponins help prevent and treat cancer by targeting multiple signaling pathways involved in cancer development and progression. In summary, the research underscores the potential of triterpenoid saponins from *G. lucidum* as promising agents in cancer therapy, with their diverse biological activities and ability to modulate complex molecular mechanisms involved in cancer biology. (Ahmad, et al., 2018)

β-Glucan

β-glucan, a water-soluble dietary fiber with a wide range of sources and structural variations depending on its origin. Here are the key points: Sources of β-Glucan: β-Glucan is found in various organisms, including oats, barley, bacteria, yeast, algae, and mushrooms. The cell wall of baker's yeast (*Saccharomyces cerevisiae*) is particularly rich in β-glucan. Chemical Structure: β-Glucan consists of glucose units linked by β-glycosidic bonds. The linkages vary between sources: In bacteria and algae, glucose monomers are connected by β-(1→3) glycosidic bonds. In yeast and mushrooms, glucose monomers are linked by β-(1→3) and β-(1→6) glycosidic bonds. In oats and barley, the linkage is through β-(1→4) and β-(1→3) glycosidic bonds. Structure and Branching: β-Glucan obtained from bacteria and algae typically has a linear structure, whereas β-glucan from yeast, mushrooms, oats, and barley exhibits a branched structure. The synthesis of β-glucan in the cell wall is complex due to the variety of glucan types and the involvement of several enzyme classes. β-(1→6) glycosidic side chains connect the main β-(1→3) glucan chains, creating a rigid network. β-glucan and chitin are often linked by β-(1→4) linkages, contributing to the structural integrity of the cell wall. Water Solubility: There is no clear distinction between the insoluble and soluble fractions of β-glucan. The water solubility of β-glucan is influenced by its structural composition, with branched structures generally exhibiting greater solubility. β-glucan is a versatile polysaccharide

with varying structures depending on its source, and its water solubility is influenced by its specific chemical structure. The complex synthesis process and branching patterns contribute to its diverse biological effects and applications. (Chioru and chirsanova, 2023). The extraction conditions and properties of β-glucan, particularly its solubility, molecular weight, and viscosity, as well as its health effects, especially in lowering cholesterol and enhancing immune response. Here are the key points: Extraction and Solubility: The ratio of soluble to insoluble β-glucan fractions is influenced by the extraction conditions. β-Glucans with β-(1→3) linkages and high degrees of polymerization tend to be insoluble in water, facilitating interactions between glucan molecules and with water molecules. Viscosity and Cholesterol-Lowering Effect: The viscosity of β-glucan in the gut is primarily responsible for its cholesterol-lowering effects. Higher viscosity results from higher molecular weight and greater water solubility, which leads to greater serum cholesterol reduction. This effect occurs because the increased viscosity reduces bile acid reabsorption, leading to greater bile acid excretion. This, in turn, stimulates the synthesis of bile acids from cholesterol, which helps reduce LDL cholesterol in the blood. The molecular weight and concentration of β-glucan directly impact its viscosity, which is important for glycemic control. High molecular weight β-glucan forms viscous, pseudoplastic solutions even at low concentrations, while low molecular weight β-glucan forms softer gels at higher concentrations. The solubility, molecular weight, and viscosity of β-glucan play key roles in its cholesterol-lowering and immune-boosting effects. Its ability to form viscous solutions in the digestive tract reduces cholesterol absorption, while its interaction with macrophage receptors enhances immune function by activating phagocytes and leukocytes. (Hamad, et al., 209) (Chioru, 2023)

β-Glucan is a naturally occurring polysaccharide found in the cell walls of plants, bacteria, and fungi, including mushrooms. It has been shown to stimulate the function of innate immune cells, such as: macrophages, dendritic cells (DCs), granulocytes, natural killer (NK) cells. additionally, β-glucan augments adaptive immune responses, which can contribute to inhibiting tumor growth and metastasis. This immunostimulatory property makes it a promising candidate for enhancing the body's immune defense against cancer. (Lee and Kim, 2014; Yoon, Koppula, & Lee, 2013). β-Glucan has been approved as an immunoadjuvant therapeutic for cancer treatment in some countries. As an immunoadjuvant, it enhances the effectiveness of

cancer treatments by stimulating the immune system, particularly the innate immune cells. This approval highlights its potential as a supportive therapy to boost immune responses against tumors and improve the overall effectiveness of cancer treatments. (Ina, et al.,2013) (Wang, et al.,2012). β-glucan, a polysaccharide found in various edible and medicinal mushrooms, and its therapeutic potential. Here are the key points: β-Glucan and Antitumor Effects: β-glucan has been shown to exert multiple antitumor effects through its interaction with Dectin-1, a receptor involved in immune responses. Mushroom-Derived Polysaccharides: Various polysaccharides, such as lentinan (from *Lentinus edodes*), sonifilan (from *Schizophyllum commune*), PSK and PSP (both from *Coriolus versicolor*), have demonstrated anti-tumor and anticancer activities. Clinical Use: Several commercial polysaccharides (e.g., lentinan, PSK, PSP) have undergone clinical trials and are recognized as therapeutics in Japan and China. Structure and Composition: Lentinan: Contains a β-1,3 linkage backbone with 1,6 linkage branches. Sonifilan: Derived from *Schizophyllum commune*. PSK: Contains fucose. PSP: Contains rhamnose and arabinose, with a polypeptide chain abundant in glutamic and aspartic acids. Mechanisms of Antitumor Activity: The antitumor effects of these polysaccharides are mediated through several mechanisms, including: Stimulation of the host defense mechanism. Cytotoxicity against endothelial cells. Decreased production of IL-6 (a pro-inflammatory cytokine). Enhancement of apoptosis (programmed cell death). These polysaccharides, through various bioactive properties, help modulate immune responses, enhance cell death in tumors, and contribute to tumor suppression.

Ganoderic acid A

1. Ganoderic acid A (GA) is a bioactive compound with the molecular formula C30H44O7, derived from the medicinal mushroom *Ganoderma lucidum* (reishi mushroom). It is recognized for its anticancer, antioxidant, and anti-inflammatory properties, which make it highly valuable in biomedical applications. Pharmacological Properties of GA: Anticancer: GA has shown potential in combating cancer by various mechanisms, including inhibition of cancer cell growth. Antioxidant: It helps neutralize free radicals, reducing oxidative stress that can lead to various diseases. Anti-inflammatory: GA may reduce inflammation, contributing to its therapeutic effects in diseases

associated with chronic inflammation. Therapeutic Potential for Liver Injuries: GA has shown promise in treating liver injuries, including those caused by toxic compounds. The study focuses on the use of GA to address α-amanitin-induced liver injury. α-amanitin is a potent toxin that can cause significant liver damage. Metabolomics Analysis. The study will employ metabolomics to explore the mechanisms by which GA mitigates liver damage caused by α-amanitin. This approach aims to uncover the biochemical pathways involved, potentially providing insights into how GA can be developed as a therapeutic for liver toxicity.Drug Development Implications: The findings from this research could aid in drug development for treating liver injuries, potentially leading to new therapeutic options based on GA's protective properties. In summary, the study aims to elucidate the molecular mechanisms through which GA protects the liver from toxin-induced damage, which could ultimately inform the development of new treatments for liver injuries. (Gill, et al.,2018). Triterpenoids: Biological Activities: Triterpenoids isolated from *G. lucidum* have been studied for their wide-ranging activities. These include antibacterial, antiviral, antitumor, anti-HIV-1, antiosteoclastic differentiation, hepatoprotective, antioxidant, antihypertensive, cholesterol-lowering, and antiaggregation functions. Potential Therapeutic Benefits: The diverse effects suggest potential therapeutic applications in treating various diseases, such as infections, cancer, liver damage, cardiovascular issues, and oxidative stress-related conditions. Antiosteoclastogenesis: Potential for treating bone-related conditions by regulating bone resorption. Anti-asthma and Anti-hepatitis B: Their therapeutic role in conditions such as asthma and hepatitis B infection. The chapter emphasizes the multifunctional nature of ganoderic and lucidenic acids, highlighting their promise in combatting various human diseases, and underscores the importance of understanding their biosynthesis and pharmacokinetics for potential therapeutic use. (Hsu and Yen, 2014)

2.3 Proximate analysis

Mineral components

This elemental profile emphasizes the nutritional and medicinal potential of *G. lucidum* as a source of various essential minerals. However, the detection of heavy

metals underlines the importance of ensuring clean cultivation methods to avoid contamination, as heavy metal toxicity can adversely affect health. (Chen, et al., 1998). Mineral profile suggests that wild *Ganoderma* fruit bodies are a valuable source of essential minerals, which can contribute to health benefits related to bone health, cardiovascular function, and overall cellular processes. The higher mineral content (10.2%) may reflect the different growing conditions of wild *Ganoderma* compared to cultivated varieties. (Chiu, et al., 2000). The wild *Ganoderma* fruit bodies being free from harmful heavy metals like cadmium and mercury, combined with their significant selenium content and ability to transform inorganic selenium into bioactive forms, highlights their potential for health applications. (Du, et al., 2008). The presence of germanium in *Ganoderma lucidum* adds another layer of potential therapeutic benefit, as it has been studied for its immune-modulating, anti-inflammatory, and possible anticancer effects. Although germanium is not an essential element like vitamins or minerals, its presence in medicinal fungi like *G. lucidum* could offer synergistic benefits when combined with other bioactive compounds present in the mushroom. Given its prominent concentration among other detected minerals, germanium may contribute to the overall health-promoting properties of *G. lucidum*, although more research is needed to fully understand its mechanisms and potential therapeutic uses. (Chiu, et al. 2000). The germanium content in *Ganoderma lucidum* has garnered some attention due to its potential health benefits. Germanium, a trace element, is often associated with its antioxidant properties and its ability to enhance oxygen utilization in the body, which may contribute to improved energy levels and immune function. (Kolesnikova, et al., 1997). Although germanium has shown promise in certain studies for its immunopotentiating, antioxidant, antitumor, and antimutagenic activities, these effects have largely been observed in isolated studies or animal models. The overall health benefits of *G. lucidum* are more commonly attributed to other bioactive compounds, such as polysaccharides (notably beta-glucans) and triterpenoids, which have well-documented effects on immune modulation, antioxidant activity, and anti-inflammatory actionsbioactive compounds in *Ganoderma lucidum* (reishi mushroom), such as proteins and lectins, which may contribute to its medicinal properties. The protein content of dried *G. lucidum* is about 7–8%, which is lower than that of many other mushrooms. Additionally, a peptide preparation known as GLP, isolated from the fruiting bodies of *G. lucidum*, has been shown to possess hepatoprotective (liver-protecting) and antioxidant activities. (Wang,

2006) (Sun, He, and Xie 2004). (Chang and Buswell 1996; Mau, Lin, and Chen 2001). (Van Der Hem, et al., 1995); The presence of germanium in *G. lucidum* may contribute to its overall bioactive profile, but its direct role in promoting the health benefits commonly associated with the mushroom remains uncertain. More extensive research, including clinical studies, is needed to establish a clear connection between germanium content and the therapeutic effects attributed to *G. lucidum*. (Kawagishi, et al., 1997), (Chang and Buswell, 1996) (Mau, and Chen, 2001) including a novel 114-kDa hexameric lectin, which was revealed to be a glycoprotein having 9.3% neutral sugar and showing hemagglutinating activity on pronase-treated human erythrocytes (Sanodiya, et al., 2009). Lectins are indeed a diverse group of nonenzymatic proteins or glycoproteins that have the ability to bind specific carbohydrates, which is where the name "lectin" comes from, derived from the Latin word *legere*, meaning "to pick up" or "to choose." Their carbohydrate-binding ability gives them a wide range of biological functions, and they are produced by many species across the animal, plant, and microbial kingdoms. (Wang, 2006). (Iwata, et al., 2008). (Wasser, 2006). (Kim and Nho, 2004)

2.4 Antioxidant activity

Free radicals and their role in oxidative stress, along with the connection between free radicals and disease. The mention of exogenous and endogenous sources highlights the wide range of factors that contribute to free radical formation.

Functional foods and nutraceuticals are indeed growing areas of interest, especially as people become more health-conscious and look for ways to manage or prevent diseases through diet. Antioxidants in these foods can neutralize free radicals, potentially reducing the risk of chronic conditions like cardiovascular disease, cancer, and more. This approach is particularly appealing as complementary therapy, as it aligns with preventive health care and offers a natural means to enhance well-being.

The antioxidant activity of polysaccharides from *G. lucidum*, commonly known as reishi or lingzhi, has been extensively studied using various in vitro methods, including DPPH, ABTS, and superoxide radical scavenging assays. These assays assess the ability of polysaccharides to neutralize free radicals, which are implicated in oxidative stress and damage. While the antioxidant potential of these

polysaccharides is well established, the precise mechanisms by which they exert their effects remain incompletely understood. For instance. Radical Scavenging Activity: Polysaccharides from *G. lucidum* can donate electrons or hydrogen atoms to neutralize free radicals, such as DPPH and ABTS radicals. This reduces the oxidative stress in cells, potentially protecting against damage from reactive oxygen species (ROS). Chelating Activity: Some polysaccharides can bind metal ions like iron, preventing them from catalyzing the formation of free radicals through Fenton reactions. Enzyme Modulation: These polysaccharides may influence antioxidant enzymes (e.g., superoxide dismutase, catalase, and glutathione peroxidase), enhancing the body's natural defenses against oxidative stress. Hydroxyl and Superoxide Radical Scavenging: Hydroxyl radicals, the most reactive ROS, and superoxide radicals are particularly damaging. Polysaccharides from *G. lucidum* demonstrate strong scavenging potential, but the molecular pathways and interaction sites are not fully mapped out. (Wang, et al.,2017) The challenge now is to delineate the exact pathways by which these polysaccharides confer protection at the cellular and molecular levels. Researchers need to explore the interactions between these compounds and cellular components, such as lipids, proteins, and nucleic acids, to gain a clearer understanding of their antioxidant mechanism. Additionally, isolating specific active polysaccharide components and examining their structure-activity relationships could help further elucidate their mode of action.

The consumption of antioxidant-rich plants has long been associated with health benefits, particularly in the prevention of chronic diseases like cancer. Research suggests that antioxidants help protect cellular components from oxidative damage, which can reduce the risk of mutations and carcinogenesis (the formation of cancer). By protecting cells from oxidative stress, antioxidants also support immune function, enabling immune cells to maintain their surveillance and response to pathogens and abnormal cells, including cancer cells. (Wachtel-Galor 2004 and Collins 2005; Benzie). (Wu and Wang, 2009).

2.5High performance liquid chromatography (HPLC)

High-performance liquid chromatography (HPLC) has evolved significantly since its invention, and its various adaptations have made it one of the most widely

used analytical techniques in chemical and biochemical analysis. Advancement to High-Performance Liquid Chromatography: As manufacturers refined the technology and increased the efficiency of the devices, the name was changed to high-performance liquid chromatography. The abbreviation remained the same—HPLC—because the core concept of high-pressure liquid chromatography was maintained, but the performance of the devices improved due to technological advancements. Preparative vs. Analytical HPLC: Preparative HPLC is used for the purification of chemical compounds. In this method, a larger quantity of material is loaded onto the chromatographic column, and fractions of the separated compounds are collected. The primary purpose is to isolate pure compounds from a mixture, which can then be analyzed or used for further applications. Analytical HPLC is focused on characterizing chemical compounds. Rather than collecting fractions, the eluent (solution exiting the column) is monitored by a detector, often involving technologies like ultraviolet (UV) or visible light absorption, or more advanced methods like mass spectrometry (MS). The goal is to determine the structural and physical properties of the compounds, such as concentration, molecular weight, and purity. Analytical UHPLC is becoming increasingly important for characterizing compounds in complex mixtures and is frequently used in fields like pharmaceutical research, biotechnology, environmental testing, and forensic analysis. HPLC has evolved from its early roots as high-pressure liquid chromatography to the ultra-high-performance systems available today. Whether used for preparative or analytical purposes, normal-phase or reversed-phase configurations, HPLC continues to be a powerful tool in separating, characterizing, and purifying a wide range of chemical and biological compounds.

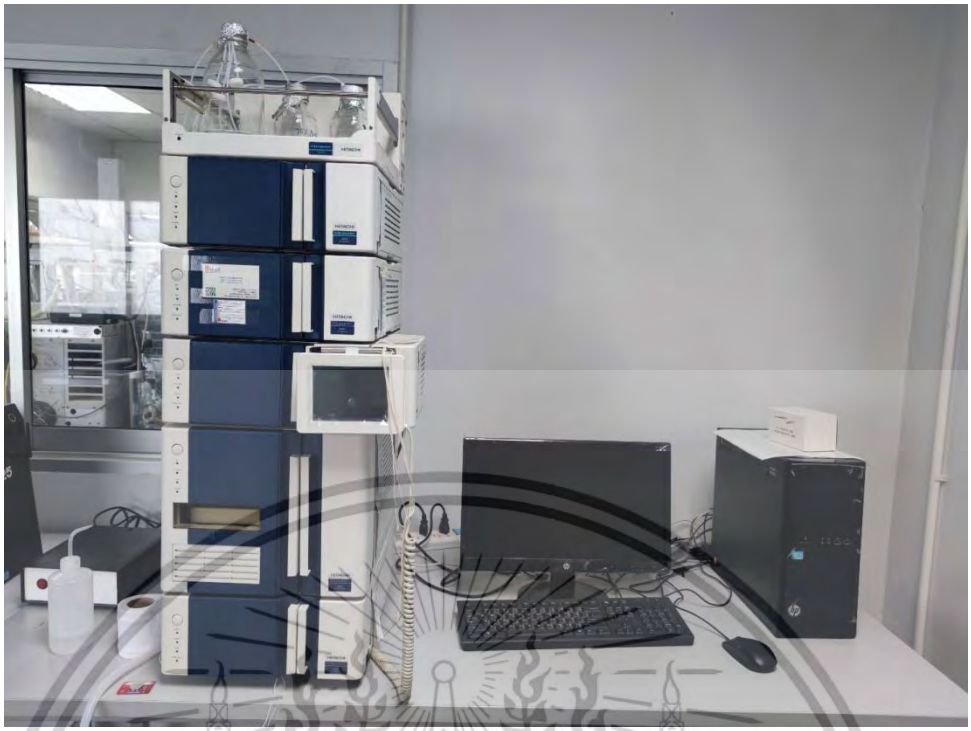

Figure 2.2 High Performance Liquid Chromatography (HPLC)

2.6 **Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES)**

Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). Here's a concise summary of the process. Sample Introduction: A sample solution is pumped under high pressure into a nebulizer, converting it into an aerosol. Spray Chamber: The aerosol passes through a spray chamber, which removes excess droplets and directs the sample into the torch. Plasma Generation: Argon gas is introduced into the torch, and a high-frequency electric current is applied to the torch coil, ionizing the argon gas to create plasma. Excitation and Emission: The plasma, which is at a high temperature (about 10,000K), excites the atoms of the sample. As these atoms return to lower energy states, they emit characteristic light (emission rays). Detection and Analysis: The emitted light is collected using optics, and the wavelengths of the emitted rays are analyzed by detectors to identify the elements present. The intensity of the emission provides quantitative data on the concentration of each element. Signal Processing: The results are processed by computers and signal processing units, allowing the analyst to determine both the element types and their concentrations in the sample. This process is commonly used for elemental analysis in various scientific and industrial fields.

2.7 Drying

Drying process, its importance, and various applications. Here's a concise summary. Definition of Drying: Drying involves using heat to remove liquid, typically water, from a material, which can be a wet solid or a liquid solution. The process requires a heat source and a sink to receive the produced vapor. Drying can also refer to the removal of water vapor from gases. Scientific Context: Drying is considered a mass transfer process, and extreme drying is known as desiccation. Importance in Food Preservation: Drying is crucial for preserving food, as it removes moisture that supports microbial growth and enzymatic activity. This slows down spoilage and deteriorative reactions, thus extending the shelf life of agricultural products. However, traditional drying methods can result in loss of sensory and nutritional qualities due to changes in texture and biochemistry. Applications: Drying has diverse applications, such as drying hair, candy, semiconductor wafers, wood, and food. Freeze drying, a specialized form of drying, is used for preserving pharmaceuticals, vaccines, blood, and certain foods. In essence, drying is a versatile and essential process for preservation, though it can affect the quality of the material being dried. (Watson and Harper, 1988).

Spray Dryer

Spray drying is a process that converts a solution, suspension, or emulsion into a dried powder in a single step. This is achieved by atomizing the liquid into small droplets and passing them through a high-temperature gaseous medium, typically air. The process quickly removes moisture, resulting in fine powder particles. Origin: Spray drying was first developed in the 1860s (Pency, 1872). Industrial Use: The dairy processing industry was the first to adopt spray drying technology, followed by its application in the pharmaceutical industry. Spray drying is widely used for producing powdered products in various industries due to its efficiency and ability to preserve the material's properties. (Schuck, et al., 2016) and food (Gharsallaoui, et al., 2007; Truong et al., 2005)

Figure 2.3 Spray Dryer

Spray drying, despite its technological challenges, has become essential in various industries such as pharmaceuticals, food, ceramics, and dairy. The process offers several advantages:Consistent Powder Quality: Provides uniformity in the final product throughout the drying process. Controllable Continuous Processing: Allows for steady, regulated production. Versatility in Dryer Design: Can be tailored to specific applications and capacities. Applicability to Both Heat-Sensitive and Heat-Resistant Materials: Useful for a wide range of materials. Suitable for Various Feedstocks: Works with slurries, emulsions, pastes, and melts. Cost-Effective and Scalable: Efficient for large-scale production and formulation. Amorphous Solid Dispersions: Ideal for preparing low solubility small molecule drugs.Time and Cost Savings: Offers a more efficient alternative to lyophilization for large biomolecule processing. Despite these advantages, applying spray drying to biologically complex, high-value therapeutic products like biopharmaceuticals requires ongoing research. This research must combine experimental protocols, statistical analysis, and advanced computational modeling to overcome the specific challenges involved. (Van and Taylor, 2011).

Spray drying is a highly efficient process that involves the intensive, continuous, and gradual application of heat to dry a liquid product. The technique works by atomizing the liquid into small droplets, which are then exposed to a hot gas stream, rapidly converting the liquid into powder. This process can generate nanoparticles with a narrow size distribution in a very short time. (Singh and Van, 2016).

Spray drying is a well-established method for producing solid formulations from a liquid solution or suspension, with significant applications in both the pharmaceutical and food industries. The process is a single-step technique that involves the following: Atomization: The liquid feed is atomized into fine droplets. Drying: These droplets are injected into a drying chamber containing hot air or nitrogen. The high temperature causes the droplets to instantly dry into solid particles. Collection: The dried particles are collected within the chamber. (Seville, 2007).

Spray drying is a highly effective single-step process used to produce dry powders, including respirable formulations for pulmonary delivery of vaccines. It allows for the production of dry powders that can be inhaled, offering several advantages in terms of controlling particle size, morphology, and formulation. Efficient Powder Production: The process involves atomizing a liquid (solution, suspension, or emulsion) into fine droplets using a spray nozzle atomizer. These droplets rapidly lose moisture and dry when they encounter a stream of hot gas in an insulated chamber. Control over Particle Size: The resulting powders typically have a mean particle size below 10 μm (preferably around 5 μm), which is ideal for effective lung delivery. Uniformity and Stability: The powder produced has consistent characteristics with minimal batch-to-batch variability, ensuring reproducible therapeutic effects upon inhalation. The dry powders are chemically and physically stable with minimal residual solvent. Excipients in Formulation: Excipients like lactose and l-leucine are often included in the formulation to prevent aggregation, inactivation, and to improve yield, processability, and storage stability. Applications in Vaccines and Drug Delivery: Spray drying is particularly suitable for vaccines and other therapeutic drugs intended for pulmonary delivery, offering a cost-effective and scalable method for producing inhalable dry powders.Spray drying plays a crucial role

in the development of inhalable pharmaceutical products, especially those requiring controlled particle properties and long-term stability.

Spray drying technology is a versatile method for producing nanoparticles with low and uniform sizes, and it can be easily scaled up for industrial use. The process typically involves the following steps. Drug and Polymer Dissolution: The drug and polymer are dissolved in an organic solvent. Atomization and Drying: The solution is atomized through a nozzle, creating droplets that are simultaneously dried in a hot gas stream. The resulting particles are nanoparticles. Particle Size Control: The size of the nanoparticles can be controlled by adjusting process variables, such as spray flow rate and inlet/outlet temperatures. (Beck et al., 2012).

Spray drying is widely used to produce redispersible nanoparticles (PNPs) by converting them into solid powders through a dehydration process. The key objective is to create powders that can be easily redispersed, maintaining their nanoscale particle size after drying. Here's how the process works. Preparation: A solution containing a solvent and polymer as a solute is prepared. Atomization: The solution is atomized into tiny droplets, creating a spray. Drying: These droplets are introduced into a hot environment where the solvent evaporates, causing the polymer to precipitate and form solid particles. Particle Formation: Typically, one droplet forms one nanoparticle, with the polymer precipitating on the surface of the droplets to create solid nanoparticles. (Eslamian and Shekarriz, 2009).

2.8Reflux extract

eflux extraction is a process where extraction and concentration are performed simultaneously. Here's how the process works and its advantages. Extraction and Concentration: The extract in the extraction tank is continuously pumped out and concentrated in a separate tank during the extraction. Solvent Recycling: The evaporated solvent is condensed and pumped back into the extraction tank, effectively recycling the solvent during the process. Advantages of Solvent Recycling Reflux Extraction. Increased Mass Transfer: By renewing the solvent during extraction, the mass transfer driving force is enhanced, leading to a faster extraction process. Reduced Solvent Use: Since the solvent is recycled, less solvent is required, which makes the process more efficient and cost-effective. No Need for a Storage Tank: Unlike

conventional extraction methods, this process doesn't require a separate storage tank before concentration, which reduces capital investment. Higher Efficiency: The solvent recycling reflux extraction has been shown to be more efficient, as demonstrated in the extraction of polysaccharides from *Grifola rondose*. Solvent recycling reflux extraction offers a more efficient, cost-effective, and environmentally friendly alternative to traditional extraction methods, particularly useful for applications like extracting bioactive compounds from natural sources. (Li, et al.,2009). (Zhao, et al., 2011). and the preparation of Jianwei Xiaozhang pills (Wu, et al., 2013) Xinmaikang, and solvent recycling reflux extraction is gaining popularity in botanical medicine factories due to its ability to reduce costs while efficiently extracting valuable botanical components. The process enhances the overall extraction efficiency by recycling the solvent, which lowers solvent consumption and reduces the need for additional storage tanks, thus lowering operational costs. This method is increasingly applied to extract bioactive compounds from plants, improving the sustainability and cost-effectiveness of producing botanical extracts for medicinal use. (Chen, et al., 2013).

2.9 Hi-speed Extractor

The Hi-speed Extractor is an advanced extraction method that uses high temperature and high-pressure water to extract bioactive compounds from ground herbs. This technology is especially useful in botanical medicine production, where it can increase efficiency, reduce processing time, and minimize waste while maintaining high extract quality.

Figure 2.4 Advantages of falling film evaporators

From: Eurobest. (2024).

Falling film evaporators are widely preferred in many industrial applications due to their several advantages over flooded and vertical tube evaporators. Below are the key benefits of falling film evaporators. Advantages Over Flooded Evaporators:

1. Compact Design: Improved heat transfer allows for a more compact design, saving space and reducing equipment size.

2. Uniform Heat Transfer: The improved wettability across the tubes ensures consistent heat transfer properties.

3. Higher Evaporation Rate: Falling film evaporators have two to three times the evaporation rate of flooded evaporators, making them more efficient.

4. Faster Operation: The short contact time for the working fluid leads to faster operation, which is particularly beneficial in the food industry.

5. Reduced Fouling: The falling film helps wash away deposits on the tubes, minimizing the chances of fouling and enhancing efficiency.

Falling film evaporators offer significant advantages in terms of compactness, efficiency, and reduced fouling compared to other types of evaporators, particularly in industries like food processing. However, more research is needed to fully understand their performance at lower temperatures.

2.10 Maltodextrin

Maltodextrins are a special class of starch hydrolysates, typically produced through acid or enzyme hydrolysis of starch, and are differentiated from corn syrups by having a lower Dextrose Equivalent (D.E.) of less than 20. Below are key characteristics and applications of maltodextrin. Low Sweetness: Maltodextrins have little to no sweetness, which makes them ideal for applications where sweetness needs to be minimized. High Molecular Weight: This gives them low osmotic pressure and high viscosity in solutions, which can help to improve the texture and mouthfeel of products without increasing sweetness. Low Reducing Power: Maltodextrins participate minimally in browning reactions, making them useful in products that require stability and minimal color changes during processing. Water Solubility: Maltodextrin is highly water-soluble, making it a versatile ingredient for various food, beverage, and industrial applications. Food & Beverage Applications.Thickener & Stabilizer: Maltodextrin is widely used in food products like ice cream, beer, frozen foods, and salad dressings as a thickener and stabilizer. It helps improve texture and consistency without increasing sweetness. Flavor & Mouthfeel: In beverages like fruit drinks, maltodextrin improves mouthfeel and adds body without altering the flavor profile. It is also used to suspend solids in liquids, such as in tomato cocktails. Sweetener in Blends: Maltodextrin is used in combination with sweeteners like sucralose and stevia to improve texture and reduce sweetness. Low Sweetness Option: Unlike sucrose, maltodextrin is not a potent sweetener but has similar calorie content to sugar, making it useful for controlling the sweetness in products like fruit juices and snack foods. Health & Personal Care Applications. Cosmetics: Maltodextrin serves as an absorbent, binding agent, emulsion stabilizer, and film-forming agent in cosmetics and personal care products. It is used in products like baby care items, cleansing products, and hair and skin care formulations. Skin Conditioning: It enhances the texture and skin conditioning properties of cosmetic formulations, making it suitable for use in products like lotions and creams. Health Considerations: Caloric Content: Although maltodextrin is not as sweet as sucrose, it provides similar calories, which is important for individuals with obesity or diabetes to monitor. It is listed as an ingredient on food labels for consumers' awareness. Maltodextrin is a versatile, water-soluble polysaccharide that serves many functions, from a food thickener and stabilizer

to a component in personal care products and animal feed. It is widely used due to its cost-effectiveness, safety, and functional properties in a variety of industries.

Maltodextrin plays a significant role in the food industry due to its versatility in enhancing texture, taste, and functionality in various food products. Below are its primary uses and benefits in food manufacturing: Uses in the Food Industry: Fat Substitute in Low-Fat Foods: Maltodextrin serves as a fat replacer in low-fat products by forming gels that mimic fat emulsions. This reduces the calorie content while maintaining the desired texture and consistency of the product. Emulsifier and Viscosity Enhancer: It acts as an emulsifier in products like ice cream and beverages, helping to stabilize mixtures of fat and water. Additionally, maltodextrin improves the viscosity of yogurts and juices, giving them a creamier texture without adding sweetness. Instant Products: In instant soups, sauces, and other convenience foods, maltodextrin aids in the quick dissolution of powdered products in water. It also serves as a carrier for flavors, enhancing the overall taste experience. Thickening and Filling Agent Maltodextrin is often used as a thickener or filler to increase the volume of processed foods, such as soups, sauces, and gravies, providing a more substantial texture without adding significant calories or sweetness. Preservative and Shelf-Life Extension: By absorbing moisture, maltodextrin helps extend the shelf life of packaged foods. It acts as a stabilizer, preventing spoilage and maintaining food quality during storage. Chemical Properties: Maltodextrin is a mixture of saccharides consisting of d-glucose units linked by α-1-4 bonds, with a typical degree of polymerization (DP) of 3 to 20 glucose units and a Dextrose Equivalent (DE) of less than 20. The manufacturing process involves the partial hydrolysis of starch, typically from corn (in the U.S.) or wheat (in Europe). The process may use acid-catalyzed hydrolysis followed by enzyme treatment (usually amylase), producing maltodextrins with improved properties like low hygroscopicity and high-water solubility. Maltodextrin is an essential ingredient in modern food production due to its ability to improve texture, control viscosity, extend shelf life, and reduce fat content in a wide range of products. Its versatility and ease of use in food processing continue to make it a preferred choice in creating healthier, more convenient food options.

CHAPTER 3
METHODOLOGY

3.1 Material

In this study, two strains of *Ganoderma lucidum*, MG2 and G2, were utilized. The strain MG2 was sourced locally from Chiang Mai, Thailand, while the strain G2 was imported from China. The dried *G. lucidum* was ground to a particle size of 4 mm using both a cutting mill and an ultra-centrifugal mill. After processing, the ground material was stored at room temperature in an airtight black polythene bag to maintain its quality until further use.

Figure 3.1 The sample of *G.lucidum* , MG2 (Thailand)

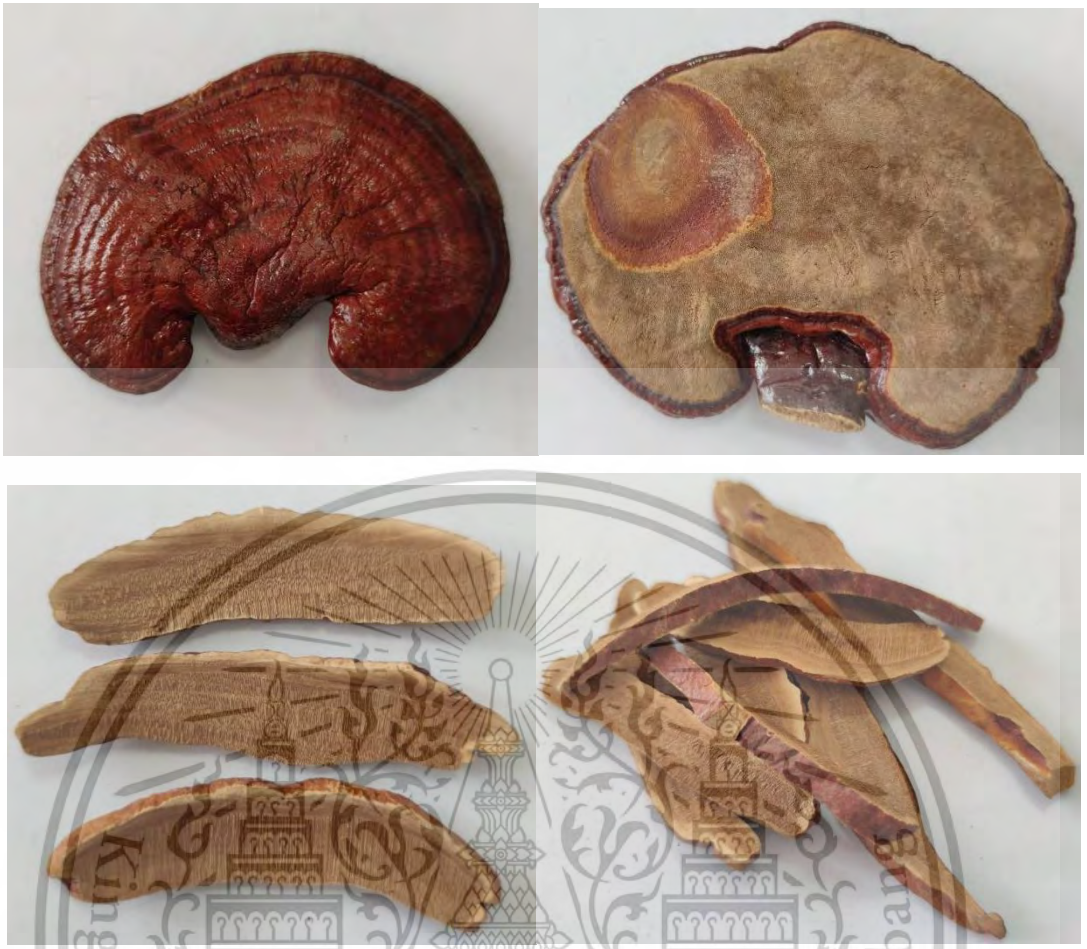

Figure 3.2 The sample of *G.lucidum* , G. *lucidum* G2 (China)

3.2 Chemicals

3.2.1 Acetonitrile (ACN) (RCI Labscan, Thailand)

3.2.2 Beta-Glucan CFA Standard (Megazyme, Ireland)

3.2.3 Methanol (Q RëC™, New Zealand)

3.2.4 Trifluoroacetic acid (Merck, India)

3.2.5 ABTS (2,2 azino bis (3-etheylbenzothiazoline-6-sulphonicacid) (Sigma-Aldrich)

3.2.6 Ganoderic acid A (Biopurify, China)

3.2.7 CHCl3 (RCI Labscan, Thailand)

3.2.8 FeSO4·7H2O (Qrec, Newzealand)

3.2.9 DPPH free radicals (Aldrich, US)

3.2.10 Ascorbic acid (Merck, Germany)

3.3 Instruments and equipment

3.3.1 High Performance Liquid Chromatography (Hitachi High-Tech /Japan)

3.3.2 Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) (Teledyne Leeman Labs USA - New Hampshire)

3.3.3 Rotary Evaporators (IKA Works, Thailand)

3.3.4 Spray dryer (SDE-5 Euro Best, Thailand)

3.3.5 Reflux extract (Shanghai, China)

3.3.6 Hi-speed Extractor (Model HX-10, Euro Best Thailand)

3.3.7 Falling film evaporators (VPF-100/1F, Euro Best Thailand)

3.3.8 Spray Dryer (SDE-100 EURO 2, Euro Best Thailand)

3.3.9 3.16 UV-visible spectrophotometer UV1800 (Shimadzu, USA)

3.3.10 Hot air oven (UM500, Memmret)

3.3.11 Vortex mixer (Genie 2, USA)

3.3.12 Crude protein kjeltecTM2200 (Foss, Netherlands)

3.3.13 Ether extract SOX 416 (Gerhardt, Germany)

3.3.14 Spectrophotometer UV-1601 (Shimadzu, USA)

3.4 Research Methodology

3.4.1 To study optimization condition of *G. lucidum* extraction.

The first part of the research methodology focused on optimizing the extraction of G. lucidum G2 using heat reflux extraction techniques. The conditions include extraction temperature from 80, 90, and 100°C, extraction time from 1, 2.5, and 4 hours, and water ratio from 1:6, 1:9, and 1:12, respectively. The conditions for extraction of *G. lucidum G2* on yield (Y), β-glucan, and Ganoderic acid A used to be optimized. The analysis of ganoderic acid A, β-glucan, and yield (Y) was conducted as follows:

3.4.1.1 Preparation of *G. lucidum* extracted Ganoderic acid A and β-glucan.

The extracted solution was filtrated through analytical filter paper and the filtrate was evaporated to dryness using a rotary evaporator 60°C. The dry extract was dissolved 100 mg in HPLC water 25 mL using temperatures from 150°C for 15

minute and filtrated through a 0.45 μm membrane filter unit. Then, each sample solution was analysed by HPLC.

3.4.1.2 Preparation of standard ganoderic acid A process

Chromatography (HPLC) The samples and standard of ganoderic acid A were analyzed by HPLC separation with a Luna C_{18} column (150 x 2.00 mm) with a 5μm internal diameter. The mobile phase A consisted of 0.1% trifluoroacetic acid in Mili-Q water and the mobile phase B was 5% acetonitrile for 5 minutes, then increasing to 95% acetonitrile over 20 minutes, before holding at 95% for 5 minutes with a flow rate of 0.4 ml/min. The injection volume for the sample and standard solution was 10μL. The detection occurred at UV light at 250 nm wavelength. (Li, et al., 2006).

3.4.1.3 Preparation of standard of β-glucan

β-glucan standard: Supplied as a freeze-dried powder with a bulking agent. Take one 25 mL vial containing 40 mg of β-glucan and add 20 mL of water to dissolve it. Dilute the solution to achieve a final concentration of 400 μg/mL of β-glucan. Add a magnetic stirrer bar (5 x 15 mm) to the vial. Transfer the entire contents into a 100 mL volumetric flask for further use. (Chen, et al., 2008)

3.4.1.4 HPLC instrumentation and chromatographic conditions

Analysis of ganoderic acid A by high-performance liquid chromatography (HPLC) The samples and standard of ganoderic acid A were analyzed by HPLC separation with a Luna C_{18} column (150 x 2.00 mm) with a 5 μm internal diameter. The mobile phase A consisted of 0.1% trifluoroacetic acid in Mili-Q water and the mobile phase B was 5% acetonitrile for 5 minutes, then increasing to 95 % acetonitrile over 20 minutes, before holding at 95% for 5 minutes with a flow rate of 0.4 ml/min. The injection volume for the sample and standard solution was 2.0-10 μL. The detection occurred at UV light at 250 nm. wavelength. (Li, et al., 2006).

Analysis of β-glucan by high-performance liquid chromatography (HPLC) The samples and standard of β-glucan was analyzed by HPLC separation with a Luna C_{18} column (250 x 4.6 mm) with a 5 μm internal diameter. The mobile phase was acetonitrile (ACN) 100% with flow rate of 0.5

ml/min. The injection volume for the sample and standard solution was 10 µL. The detection occurred at UV light at 305 nm. (Chen, et al., 2008)

3.4.1.5 *Yield*

Yield (%) and product recovery were calculated using equations.

$$\text{yield} = \frac{\text{sample weight (g) after drying}}{\text{sample weight (g) before drying}} \times 100$$

3.4.2 The production of *G. lucidum* powder using spray dry process

The use of *G. lucidum* water extracts from 3.1 to study the production of *G. lucidum* powder and then spray drying of inlet temperature range of 140,160 and 180°C and outlet temperature of 95°C, to produce *G. lucidum* powder rate of liquid pump 4.0 ml/min to comparison yield and β-glucan and ganoderic acid A. The method for analyzing ganoderic acid A, β-glucan, and yield (Y) was the same as in 3.4.1.

3.4.3 The production of *G. lucidum* on pilot scale.

The preparation of *G. lucidum* powder was carried out by extracting ground *G. lucidum* at a ratio of 20 kg per 1000 kg of water using a commercial high-speed extractor. The extraction process was conducted under controlled temperatures. The resulting extracted solution was then filtered using a falling film evaporator at 60°C. Both *G. lucidum* MG2 and G2 strains underwent extraction, followed by evaporation. Afterward, the extracts were subjected to spray drying using a spray dryer with an inlet temperature of 140°C and an outlet temperature of 80°C. Additionally, the process was carried out both with and without the inclusion of maltodextrin, to produce the final *G. lucidum* powder. The method for analyzing ganoderic acid A, β-glucan, and yield (Y) was the same as in 3.4.1. The analysis of Antioxidant assay, Proximate analysis, and Mineral Analysis was conducted as follows:

3.4.3.1 Antioxidant assay

3.4.3.1.1 FRAP

The Ferric Reducing Antioxidant Power (FRAP) assay of the hot water extract (HWE) of *Ganoderma lucidum* was conducted following the modified

method of Benzie and Strain (1999). The FRAP reagent was prepared by mixing 1 unit of $FeCl_3 \cdot 6H_2O$ solution (20 mM), 1 unit of TPTZ (2,4,6-tripyridyl-s-triazine) solution, and 10 units of acetate buffer (0.5 M, pH 3.6). The reagent was warmed to 37°C. To measure the reducing power, 20 µL of the *G. lucidum* sample was added to 980 µL of the prepared FRAP reagent. The absorbance was measured at 593 nm both immediately after mixing and after 90 minutes of reaction to assess the sample's ability to reduce the ferric ions.

3.4.3.1.2 DPPH

The antioxidant ability of the hot water extract (HWE) of *Ganoderma lucidum* to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals was determined using the method of Brand-Williams et al. (1995) with minor modifications. In the DPPH assay, 10 µL of each extract or standard compound was added to 60 µL of DPPH reagent and mixed with 180 µL of methanol. After allowing the reaction to occur for 30 minutes, the absorbance was measured spectrophotometrically at 515 nm. The antioxidant activity was quantified by calculating the concentration of the sample required to inhibit 50% of the DPPH radical formation (EC_{50}). The experiment was performed in triplicate for accuracy.

3.4.3.1.3 ABTS

The ABTS assay was used to evaluate the radical scavenging activity of the hot water extract (HWE) of *Ganoderma lucidum* and the standard compound, propyl gallate (PG). For the assay, 10 mL of each extract or standard compound (including Trolox, a vitamin E analog) was mixed with 290 µL of 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) solution. For the control group, distilled water was used in place of the sample. After 30 minutes of reaction, the absorbance was measured spectrophotometrically at 515 nm. The procedure was repeated using PG as a positive control, with absorbance measured at 734 nm after 5 minutes of reaction. The results were expressed as milligrams of Trolox equivalents (TE) per gram of dry weight (mg TE/g d.w.), based on the standard curve, and all samples were analyzed in triplicate. (Arnao et al., 2001)

3.4.3.4 Proximate analysis of *G. lucidum* powder

The proximate nutritional analysis of *Ganoderma lucidum* powder was conducted using methods from the Association of Official Analytical

Chemists (AOAC, 1995). The analysis included the following steps: Moisture Content: 2g of the test sample was dried at 105°C for 16 hours in a hot air oven (UM500, Memmret). Ash Content: 2g of the *G. lucidum* powder was combusted in an electric furnace at 505°C for two hours to measure ash content. Protein Content: The protein content was estimated using the Kjeldahl method, with nitrogen content multiplied by a conversion factor of 4.38 (Fujihara et al., 1995). Lipids: Lipid content was determined by the Soxtec 20055 Extraction unit (Foss Tecator) using petroleum ether.Carbohydrate (%) = 100 - (g moisture + g ash + g lipid + g protein + g fiber) (Rizal, et al., 2015). Energy (kJ) = 3.25 × (g protein) + 4.2 × (g carbohydrate) + 9.10 × (g fat) respectively (Kumari and Atri 2014). All experiments were carried out in triplicate. The energy was calculated according to the following equation: Energy (kcal)=4× (g protein+g carbohydrate) +9× (g fat) (Leal, et al., 2013) (Leal et al., 2013).

3.4.3.5 Mineral Analysis of *G. lucidum* powder

Used for mineral analysis using an ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry) system. This method is effective for accurately determining the concentration of various minerals in a sample.ICP-OES Instrument: Perkin Elmer Optima 4300 DV (USA). RF Generator: 40 MHz (solid-state, free-running). Gas Flow Rates: 15 L/min for plasma gas, 0.8 L/min for auxiliary gas. Reading Time: 3 seconds per measurement. Detection Limit: Defined as three times the standard deviation of ten blanks. Calibrations and Analysis. The calibration was performed using commercially available working standards. Measurements were taken within the linear range of these standards, ensuring reliable readings. Appropriate wavelengths and ICP-OES parameters for each metal or mineral (K, P, Mg, Ca, Mn, Fe, Cu, Zn, Ge) All mineral concentrations were reported in milligrams per 100 grams of dry sample weight. (Mallikarjuna, et al., 2013).

3.4.4. Statistical Analysis

SPSS V.16 software to perform comprehensive statistical analyses on experimental data. I applied One-Way Analysis of Variance (ANOVA) and Least Significant Difference (LSD) tests to determine statistical differences across various methods, ensuring the robustness of results. All data were presented as mean ± SEM, and statistical significance was set at $P \leq 0.05$.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Optimization condition of *G. lucidum* extraction.

The study of the temperature and time used during the experiment, suitable to extraction *G.lucidum* using temperature in the range of 80, 90 and 100°C, extraction time 1, 2.5 and 4 hrs. and ratio of water . Shown that in table 4.1

Yield value *G.lucidum* time used in extraction showed that the duration with different results there were significant different ($P<0.05$). The maximum yield was 4 hours, 12.94 followed by 2.5 times hours 12.60 percent, respectively.

β-glucan value in *G.lucidum* the time spent in extraction showed that in Table 4.1 different times caused the values β-glucan value there were significant differences ($p<0.05$). The maximum amount of β-glucan is 4 hours, which is 0.4322 mg/100mg. That the longer the extraction time, the higher the amount. β-glucan are increasing. The extraction time was 2.5-4 hrs. with the highest received β-glucan, there was no difference between the two rates. The extraction time of 2.5 and 4 hrs.

Ganoderic acids A in *G.lucidum* . The time in extraction showed no significant difference ($p> 0.05$). The highest ganoderic acids content was 4 hours, 0.4322% which ganoderic acids A is an important substance in the group Ganoderic acids A in *G.lucidum* per extract was found to be significantly different ($p> 0.05$).

The result showed that extraction time significantly influenced both yield and β-glucan content. However, it had no measurable impact on the concentration of Ganoderic acid A in the extracts. Extraction durations of 2.5 and 4 hours yielded comparable results in terms of both yield and β-glucan content. Notably, extending the extraction time further enhanced both the yield and β-glucan concentration.

Table 4.1 Effect of extraction time on yield, Ganoderic acid A and β-glucan of *G. lucidum* extract.

Extraction Time (hrs.)	**Yield (%)**	**Ganoderic acid A^{ns}** (mg/100mg)	**β-glucan** (mg/100mg)
1	10.65±0.33^{b}	0.40±0.09	3.91±0.50^{b}
2.5	12.60±0.64^{a}	0.38±0.06	4.15±0.43^{a}
4	12.94±0.35^{a}	0.43±0.10	4.06±0.41^{a}

Results are expressed as mean±SEM. Mean values with different lower case superscripts (a–c) represent statistically significant difference at 95% level (P ≤ 0.05) with post hoc least significance difference (LSD) test.

Yield value *G.lucidum* temperature the highest yield was 12.52%, 100°C followed by 12.24%, 90°C, respectively. Increased temperature makes the speed of molecular movement faster. Infiltration, diffusion, and speed of dissolution will accelerate, but the temperature is high will cause some parts of the active ingredients to be damaged. In general, the heat is about 60°C with the most suitable should not exceed 100°C. Shown that table 4.2.

The results show that table 4.2 of β-glucan in *G.lucidum* per extraction temperature showed no significant difference (p> 0.05). The highest amount of β-glucan was 80 and 90°C which was 4.06%. The temperature of 100 and 80°C the least amount of β-glucan with study of the amount of β-glucan in *G.lucidum G2.* 3.43(Cho, et al., 2013). Show that table 4.2

Ganoderic acids A in *G.lucidum* . Show that table 4.2 The highest amount of ganoderic acids A was 100 and 90°C. 4.06%, followed by 90°C 0.81 mg. indicating that heat does not affect the amount of extracts.

The effect of temperature on the extraction of *G.lucidum* extract using the hot water distillation method indicated that varying the extraction temperature did not influence the yield, β-glucan content, or Ganoderic acid A content, as shown in Table 4.2 However, the results revealed a trend similar to that observed with extraction time which is increasing the temperature generally led to higher yield, β-glucan, and Ganoderic acid A content.

Table 4.2 Effect of extraction temperature on yield, Ganoderic acid A and β-glucan of *G. lucidum* extract.

Extraction Temperature (°C)	Yield (%)^{ns}	Ganoderic acid A^{ns} (mg/100mg)	β-glucan (mg/100mg)
80	11.86±1.33	0.39±0.10	3.43±0.62^{b}
90	12.24±0.89	0.40±0.06	3.81±0.64^{ab}
100	12.52±1.24	0.44±0.11	4.06±0.91^{a}

Results are expressed as mean±SEM. Mean values with different lower case superscripts (a–c) represent statistically significant difference at 95% level ($P \leq 0.05$) with post hoc least significance difference (LSD) test.
^{ns}Values in the same column followed by different letters differ significantly ($p<0.05$)

Table 4.3 demonstrates that using different mushroom-to-water ratio did not significantly affect the yield or β-glucan content, but it did have an impact on the Ganoderic acid A content. A higher water ratio resulted in a greater amount of Ganoderic acid A.

Table 4.3 Effect of *G.lucidum* to water ratio on yield, ganoderic acid A and β-glucan on *G. lucidum* extract.

G. lucidum :Water	Yield^{ns} (%)	Ganoderic acid A (mg/100mg)	β-glucan^{ns} (mg/100mg)
1:6	11.86±1.13	0.29±0.02^{b}	3.76±0.65
1:9	12.22±1.07	0.38±0.09^{ab}	3.52±0.61
1:12	12.58±1.01	0.40±0.05^{a}	3.79±0.96

Results are expressed as mean±SEM. Mean values with different lower case superscripts (a–c) represent statistically significant difference at 95% level ($P \leq 0.05$) with post hoc least significance difference (LSD) test.
^{ns}Values in the same column followed by different letters differ significantly ($p<0.05$)

Based on these experimental results, the optimal extraction conditions were determined to be an extraction temperature of 100°C, an extraction time of 4 hours, and a *G. lucidum* -to-water ratio of 1:12 to be used in the next step of testing.

4.2 The study on spray drying condition of *G. lucidum*

The use of *G. lucidum G2* solution extracts from suitable condition from topic 4.1 was study on the spray drying conditions to find suitable conditions for producing *G. lucidum G2* extract powder. In the experiment, the spray drying condition was studied under 3 different inlet temperatures at 140, 160, and 180 °C with 30% maltodextrins at each condition. The ganoderic acids A, β-glucan and yield were analysis in *G. lucidum* powder as shown in Table 4.3.

Table 4.4 Effect of inlet temperature on Ganoderic acids A, β-glucan content and yield *G.lucidum* powder.

Compounds	**Inlet Temperature (°C)**		
	140	**160**	**180**
Ganoderic acids A [ns]	0.0231±0.04	0.0212±0.05	0.0201±0.02
β-glucan[ns]	5.4754±0.05	5.4703±0.03	4.7398±0.02
yield (Y)	15.0608±0.08[c]	16.6624±0.14[b]	18.7801±0.10[a]

Results are expressed as mean±SEM. Mean values with different lower case superscripts (a–c) represent statistically significant difference at 95% level ($P \leq 0.05$) with post hoc least significance difference (LSD) test.
[ns]Values in the same column followed by different letters differ significantly ($p<0.05$)

Ganoderic acids A yield in *G. lucidum* extract used different inlet temperatures resulted in different ganoderic acids A contents but without statistical significance ($p>0.05$). The highest content of ganoderic acides A was 0.0231 mg /100mg at the inlet temperature of 140°C, followed by 0.0212 mg/100 mg at 160°C respectively. Ganoderic acids A is an important substance in the triterpenoids group with a wide range of pharmacological effect, especially anti-cancer effect. *G. lucidum* has been processed into various forms of commercial products (Teekachunhatean et al., 2010) whereas triterpenoids can be extracted into several substances such as Ganoderic acid C, Ganoder acid B and D, Ganoder acid T – Z, (Eiadthong) and substance content of the triterpenoids (Soonthornchareonnon, 2014).

β-glucan content in *G. lucidum* extract used different inlet temperatures resulted in different β-glucan content but without statistical significance ($p>0.05$). The highest content of β-glucan was 5.4754 mg /100mg at the inlet temperature of 140 °C, followed by 5.4703 mg /100mg at 160 °C respectively. It was observed that the higher the temperature was, the lower the Polysaccharides content was.

The yield of *G. lucidum* extract used different inlet temperatures resulted in different yields but without statistical significance ($p>0.05$). The highest yield was 18.7801 at the inlet temperature of 180 °C, followed by 16.6624 at 160 °C respectively. It was observed that the higher the temperature was, the higher the yield was.

Different inlet temperatures resulted in different contents of ganoderic acids A, β-glucan, and yield but without statistical significance ($p>0.05$). Therefore, suitable temperature for drying is 140°C because it can generate the highest content of important substances. Ganoderic acids A and B-glucan are important substances with various pharmacological effects, especially anti-caner effect and antioxidant in *G.lucidum* extract in the next step.

4.3 The production of *G. lucidum* on the pilot scale

The preparation of *G. lucidum* powder involved grinding *G. lucidum* at a ratio of 20 kg per 1000 kg of water, followed by extraction using a commercial high-speed extractor. The extraction was conducted at a temperature of 100°C for 76 minutes to ensure optimal extraction of bioactive compounds. The resulting solution was then filtered using a falling film evaporator model (VPF200L) at 60°C, allowing the solution to evaporate until completely dried. Subsequently, *G. lucidum* MG2 and G2 extracts were further processed through evaporation, followed by spray drying using a spray drier model SDG100 NF, with the inlet temperature set at 140°C. This procedure ensured the production of a concentrated *G. lucidum* powder for further analysis. (Based on the results of experiment 4.2) and outlet temperature was 80°C with and without maltodextrin to produce *G. lucidum* powder that has been analysis method Antioxidant and the bioactive compounds of *G. lucidum* extract. Extraction time is shorter because pilot scale extraction allows for quick and time-saving extraction. Obtained next step.

4.3.1.1 β-glucan content

β-glucan extraction from the dried of two separate *G. lucidum* sources were used in this study: *G. lucidum* MG2 obtained from a local market in Chiang Mai, Thailand and *G. lucidum* G2 imported from China were examined via HPLC chromatogram of β-glucan. The amounts of eta glucan content in the two separate *G. lucidum* species are shown in Table 4.4. The results showed that the values of β-glucan

content exhibited significant differences ($p \leq 0.05$) The difference between *G. lucidum* MG2 and G2 with maltodextrin added during the spray drying process was significant ($p \leq 0.05$), while the β-glucan content ranged from 9.9149 to 38.8291%. It has been reported that Ganoderma is number one among medicinal mushrooms, and it has been considered the "king of medicinal mushrooms". *G. lucidum* belongs to a large group of polypore fungi and has been used as a traditional therapeutic mushroom for over 4,000 years (Zhang, et al., 2016). anoderma, particularly those with 1,6-branched structures containing 1,3-β-glucans, show significant efficacy in inhibiting tumor growth. These compounds work by stimulating the immune system, specifically through the activation of macrophages, which play a crucial role in immune defense. It is widely known that the cell walls of mushrooms contain a high number of polysaccharides, including B-glucan. Most of the bioactive B-glucan in mushrooms has been found to be complex with peptides. Hot water is the most popular B-glucan extraction technique, since it fractionates only water-soluble B-glucan into the aqueous extract. The water-insoluble BG has therefore been isolated by other extraction techniques. Previous authors (Toledo, 2013) In the current study, 1,3-β-glucan in the dehydrated *G. lucidum* powder ranged from 89.24 to 94.22 mg/g, which are higher than those reported in previous research by Shao et al. (2013), where the concentrations varied between 27.0 to 89.0 mg/g in dehydrated mushrooms. The differences in 1,3-β-glucan concentrations could be attributed to variations in the strains of *G. lucidum* used, as different strains tend to produce varying amounts of 1,3-β-glucan. Additionally, environmental factors such as vegetation and soil composition could influence the bioactive compound profiles of the mushrooms. Furthermore, Shao et al. (2013).

4.3.1.2 Ganoderic acid A content

HPLC chromatogram of ganoderic acid A. The validated analytical method was applied to the dried mycelia of two separate *G. lucidum* species sources which were used in this study via HPLC. Table 4.4 shows details of the extracts of *G. lucidum.* It was found that the concentration of ganoderic acid A varied from 0.8502 – 0.9241 mg/g in dehydrated mushrooms. The quantitative results are summarized in Table 4.4. It can be seen that fruiting bodies of *G. lucidum* were the best sources of the ganoderic acids A. For the *G. lucidum* sources used in this study, *G. lucidum* MG2 obtained from a local market in Chiang Mai, Thailand, and *G.*

lucidum G2 imported from China, the amount of each ganoderic acid ranged from 0.8502 to 0.9241 mg/100 mg, as shown in Table 4.4. For example, (Wang et al., 2019). Spray drying using Maltodextrin showed a minor deviation when maltodextrin was used. Revealing those flavonoids due to the different structures of these phytochemicals and their interactions with the maltodextrin a deviation in the fitting. when maltodextrin was used. These microparticles may be used as functional ingredients enriched in bioactive compounds and increase the nutritional value of functional foods.

Table 4.5 β-Glucans and Ganoderic acid A of *G. lucidum* powder with and without maltodextrin.

Samples	**β-glucan (mg/100mg)**	**Ganoderic acid A** [ns] **(mg/100mg)**
G.lucidum MG2	27.4867±4.08[b]	0.9241 ±0.15
G.lucidum G2	16.1796±0.48[bc]	0.8502 ±0.01
G.lucidum MG2 maltodextrin	38.8291±4.43[a]	0.8650 ±0.01
G.lucidum G2 maltodextrin	9.9149±1.29[c]	0.8720 ±0.00

Results are expressed as mean±SEM. Mean values with different lower case superscripts (a–c) represent statistically significant difference at 95% level ($P \leq 0.05$) with post hoc least significance difference (LSD) test.
[ns]Values in the same column followed by different letters differ significantly ($p<0.05$)

4.3.2 Antioxidant activity

4.3.2.1 ABTS

Free radical quantitative analysis was conducted with samples *from G. lucidum* MG2 and G2 with maltodextrin added to lingzhi via spray drying. The findings are shown in Table 4.5 ABTS sought to determine the EC_{50} value of *G.lucidum* extract powder to assess the differences between the MG2 and G2 species, both with and without maltodextrin. It was found that *G. lucidum* G2 0.10 (mg/mL) had the best antioxidation activity, followed by *G. lucidum* MG2 0.13 (mg/mL) Compared to the curve. The standard of Trolox from the ABTS method is shown in Table 4.5. (Wang, et al, 2019). The EC50 value is a key indicator of antioxidant activity, with lower values signifying stronger antioxidant potential. In this study, lingzhine E and lingzhine F exhibited notable ABTS.+ scavenging effects, with EC50 values of 0.59 ± 0.15 mM and 0.27 ± 0.05 mM, respectively. These values are comparable to the positive control (trolox), which had an EC50 value of 0.42 ± 0.03

mM, demonstrating that both lingzhine E and lingzhine F have strong antioxidant capabilities, similar to the widely recognized antioxidant trolox.

4.3.2.2 DPPH

Antioxidant activity is one of the functions provided by various bioactive components of *G. lucidum* MG2 and G2. The activity assay assesses the ability of the spray dried powder to scavenge DPPH free radicals and considers the effects of different species and the use of maltodextrin. From Table 4.5, the study of antioxidant activity through in vitro free radical binding capacity by DPPH method showed that extracts from *G. lucidum* G2 had the greater antioxidant activity, followed by *G. lucidum* MG2. The concentration of the substance was able to reduce the concentration of DPPH by 50%, giving the value (EC_{50}) of 0.57 to 0.58, respectively (Table 4.5). *G. lucidum* G2 with maltodextrin and *G. lucidum* MG2 with maltodextrin were found to be antioxidative but could not be quantified with this method, because when the reaction occurs, the solution is not clear. Furthermore, when testing the antioxidant activity of DPPH, it was found that *G. lucidum* MG2 had the highest free radical scavenging ability, followed by *G. lucidum* G2. When testing DPPH of the extracts compared with the standard alpha-tocopherol, this was shown in the form of Trolox equivalent antioxidant capacity (TEAC)/g sample extract. It was also found that DPPH scavenging was the highest with TEAC values of 12.65 and 12.64 molar, respectively, which may be caused by various factors, such as the cable characteristics. Varieties, temperature, geography, and minerals used for cultivation also contribute to the differences. Moreover, the extraction of *G. lucidum* along with maltodextrin may lead to the DPPH antioxidant activity assay solution becoming cloudy. At the time of analysis, *G. lucidum* MG2 was a mature mushroom yielding strain, with high spores and content. (Anita et al, 2011). Reported that the reducing power and chelating effect on ferrous ions of mushroom extracts were evaluated and expressed as EC50 (mg/ml) values, which represent the effective concentration needed to achieve 50% of antioxidant properties. A lower EC_{50} value indicates stronger antioxidant activity. G. lucidum extract demonstrated excellent antioxidant activity, with a low EC_{50} value of 1.2 ± 0.0 mg/ml, confirming its high efficacy in antioxidant properties when compared to other extracts.

4.3.2.3 FRAP

Reducing efficiency of a *G. lucidum* extracts were observed using the FRAP method, which is a method for analyzing the electron transport/electron

capacity of *G. lucidum* coarse union samples as electron carriers of the samples by the extract. Of the two species of *G. lucidum*, MG2 acts as an electron donor to the [Fe3+ -TPTZ] complex, causing the iron atoms to be reduced and converted to the hexagonal compound of [Fe2+ -TPTZ]. The reducing efficiency values of crude extracts from the two species of *G. lucidum* and extraction methods with the addition of maltodextrin are shown as follows in Table 4.5. It was found that *G. lucidum* MG2 had the highest total amount of antioxidants by the FRAP and TEAC methods, and the total amount of phenolic compounds was 13.49 (TEmM/g FW), while for *G. lucidum* G2 the value was 12.11. With maltodextrin the value for *G. lucidum* G2 was 5.11 while for *G. lucidum* MG2 with maltodextrin the value was 0.36 Table 4.5. These differences may be attributed to various factors including the species and the environment. (Pecić et al, 2006). Found that ethanol extracts of *G. lucidum* exhibited significantly higher antioxidant activity, with values ranging from 4.32 to 6.65 FRAP units and 2.52 to 6.00 mmol Trolox Equivalent. In contrast, a special brandy infused with the same amount of *G. lucidum* (40 g/L) showed much lower antioxidant activity, measuring at 0.432 FRAP units and 1.043 mmol Trolox Equivalent. the extraction process concentrates the bioactive compounds, leading to a significant increase in antioxidant capacity in the ethanol extracts compared to the brandy.

Table 4.6 Antioxidant assay of *G. lucidum* powder from strains MG2 and G2

Antioxidant assay	Samples	EC_{50}	TEAC
ABTS	*G.lucidum* MG2	0.13±0.01	22.38±0.61
	G.lucidum MG2 maltodextrin	1.03±0.02	3.02±0.06
	G.lucidum G2	0.10±0.02	30.77±2.32
	G.lucidum G2 maltodextrin	0.63±0.03	5.01±0.20
DPPH	*G.lucidum* MG2	0.57±0.01	12.65±0.29
	G.lucidum MG2 maltodextrin	a	a
	G.lucidum G2	0.58±0.05	12.64±0.97
	G.lucidum G2 maltodextrin	a	a
FRAP	*G.lucidum* MG2	ND	13.49±2.02
	G.lucidum MG2 maltodextrin	ND	0.36±0.43
	G.lucidum G2	ND	12.11±0.82
	G.lucidum G2 maltodextrin	ND	5.11±0.12

EC50: means half maximal "Effective Concentration 50% Effective concentration g Sample/L
TEAC: means Trolox equivalent antioxidant capacity mg/g sample
a: ability to resist oxidation but cannot be quantified because when the solution is turbid after reaction
ND: Not detect Values are given as mean ± SD from triplicate determination.

4.3.8 Proximate composition of *G. lucidum* powder MG2 and G2

The chemical composition of moisture, protein, fat, calcium, ash, and energy value is used to calculate the proximate composition values. The Ganoderma sample consisted of two separate *G. lucidum* species: *G. lucidum* MG2 and G2. Table 4.6 shows the results of a study.

Carbohydrates

The carbohydrate content of *G. lucidum* MG2 and G2 mushrooms with maltodextrin added during the spray drying process was significantly different ($p \leq 0.05$). The highest carbohydrate content was observed to range from 93.96% to 93.12%, as shown in Table 4.6, which details the carbohydrate analysis of various *G. lucidum* sources. Maltodextrin, a polysaccharide carbohydrate obtained by digesting starch molecules, was used as an additive during the spray drying process. It is a white, flavorless powder or flake and is widely utilized in the food industry for various purposes, including as an anticaking agent (Soonthorncharoennon, 2009). Its use in this study helped enhance the carbohydrate content in the mushroom extracts, which may contribute to the overall stability and bioaccessibility of the bioactive compounds.

Protein

As shown in Table 4.6, *G. lucidum* MG2 exhibited the highest protein composition at 28.49% (DW), followed by *G. lucidum* G2 with 18.74% (DW). The protein content in *G. lucidum* MG with maltodextrin was significantly lower, at 2.30% (DW), while *G. lucidum* MG2 with maltodextrin showed the lowest protein content at 1.84% (DW). The variance in protein content across the different *G. lucidum* samples can be attributed to several factors, including the type of mushroom, growth stage, component samples, nitrogen availability, and the substrate or habitat in which the mushrooms were cultivated (Zahid et al., 2010). These variables play a significant role in influencing the nutritional composition of the mushrooms.

Fat

The fat content of *G. lucidum* powders varied across the samples. The lowest fat content, at 0.10% (DW), was observed in *G. lucidum* G2 samples, both with and without maltodextrin. The highest fat content was found in *G. lucidum* MG2, which had 0.40% (DW) fat. A significant difference ($P \leq 0.05$) was noted between the *G. lucidum* MG2 and *G. lucidum* G2 samples, indicating that *G. lucidum* MG2 has a higher fat content compared to *G. lucidum* G2. The differences in fat content, the

overall fat levels in both *G. lucidum* MG2 and G2 are relatively low, which is beneficial from a nutritional standpoint. Low fat content makes these mushrooms a healthy dietary option, particularly suitable for products intended to promote wellness. As shown in Table 4.6, the fat analysis of different *G. lucidum* sources confirms these findings. (Zahid et al., 2010).

Ash

The ash content of the various *G. lucidum* samples is summarized in Table 4.6. *G. lucidum* MG2 exhibited the highest ash content at 29.04% (DW), followed by *G. lucidum* G2, which contained 21.45% (DW). However, the addition of maltodextrin to the powders significantly reduced the ash content, with *G. lucidum* G2 with maltodextrin and *G. lucidum* MG2 with maltodextrin showing ash levels of only 2.38% (DW) and 1.62% (DW), respectively. The ash content reflects the mineral composition of the mushrooms, which can vary based on factors such as the raw materials, spores, and processing methods used for lingzhi products (Soonthorncharoennon, 2000). Notably, *G. lucidum* MG2 showed the highest ash content, indicating its superior mineral profile. These results align with findings from the Muang Ngai Agricultural Park Special Project, which reported similar trends in lingzhi mushroom production, identifying *G. lucidum* MG2 as an excellent strain for commercial cultivation due to its high yield and nutrient profile. Furthermore, Zahid et al. (2010) highlighted that the total yield and nutritional composition of *G. lucidum* MG2 make it a top choice for large-scale lingzhi mushroom production, emphasizing its potential for commercial applications.

Moisture

The moisture content of *G. lucidum* G2 and MG2 powders without the addition of maltodextrin during the spray drying process was found to be the highest. The addition of maltodextrin significantly reduced the moisture levels, as maltodextrin contributes as a solid component to the mixture, effectively reducing free water activity. This reduction in moisture also prevents caking, aided by the use of anti-caking agents (Sornsomboonsuk et al., 2018). In terms of calorie content, differences were observed between *G. lucidum* MG2 and G2 during the spray drying process. The powders with maltodextrin added exhibited higher calorie content, attributed to the properties of maltodextrin. As a polysaccharide carbohydrate, maltodextrin is derived from the partial digestion of starch molecules, breaking them into shorter glucose chains, which contribute to increased caloric value (Sornsomboonsuk et al., 2018).

Calorie

Calorie Content in *G. lucidum* Significant differences in calorie content ($P \leq 0.05$) were observed between *G. lucidum* MG2 and G2, as detailed in Table 4.6. *G. lucidum* MG2 exhibited a higher caloric value of 3902.4 Kcal/kg, compared to *G. lucidum* G2 at 3859.7 Kcal/kg. During the spray drying process, the addition of maltodextrin further increased the calorie content of the samples. This is due to the properties of maltodextrin, a polysaccharide carbohydrate that is partially hydrolyzed from starch into shorter chains of glucose. These glucose chains contribute to the increased caloric density of the product (Siriporn, 2018).

Table 4.7 Proximate analysis of *G. lucidum* powder from strains MG2 and G2 (%dry weight).

Proximate composition	***G.lucidum* MG2**	***G.lucidum* G2**	***G.lucidum* MG2 maltodextrin**	***G.lucidum* G2 maltodextrin**
Carbohydrate	37.42±0.32^{c}	55.11±0.13^{b}	93.96±0.12^{a}	93.12±0.14^{a}
Crude protein	28.49±0.32^{a}	18.74±0.13^{b}	1.84±0.09^{d}	2.30±0.07^{c}
Crude lipid	0.34±0.32^{b}	0.10±0.13^{c}	0.40±0.00^{a}	0.10±0.00^{c}
Ash	29.04±0.05^{a}	21.45±0.02^{b}	1.62+0.06^{c}	2.38±0.11^{d}
Moisture	4.71±0.33^{a}	4.60±0.09^{b}	2.19±0.12^{d}	2.20±0.15^{c}
Calorie (Kcal/kg)	3142.2±0.03^{c}	3427.1±0.13^{b}	3902.4±0.00^{a}	3859.7±0.13^{a}

Results are expressed as mean±SEM. Mean values with different lower case superscripts (a–c) represent statistically significant difference at 95% level ($P \leq 0.05$) with post hoc least significance difference (LSD) test.
^{ns}Values in the same column followed by different letters differ significantly ($p<0.05$)

4.3.9 Mineral Analysis of *G. lucidum* MG2 and G2

The mineral concentrations of *G. lucidum* MG2 and G2 are presented in Table 2. Analysis revealed that *G. lucidum* MG2 exhibited the highest mineral content across all measured parameters compared to G2. The concentrations of key minerals in *G. lucidum* MG2 were as follows, Phosphorus (P): 1.94% Potassium (K): 3.66% Magnesium (Mg): 1.02%, Calcium (Ca): 1.94%, Manganese (Mn): 57.3 ppm, Ferrous (Fe): 165 ppmm Copper (Cu): 46.7 ppm, Zinc (Zn): 112 ppm, Germanium (Ge): 18.24

ppm. The results highlight *G. lucidum* MG2 as a rich source of essential minerals, making it a valuable strain for nutritional and medicinal applications. The high levels of potassium, magnesium, calcium, and zinc, as well as the presence of germanium, underscore its potential for promoting health and supporting metabolic functions.

Minerals in various *G. lucidum* are affected by a range of environmental conditions, including: Food sources are part of the breeding environment. Terrain, climate, and other conditions that affect the species of Lingzhi mushrooms, such as insects and pests, are all consistent with study (Soonthorncharoennon, 2000). The results of a study on the production of lingzhi and spores in accordance with the appropriate agricultural guidelines (GAP) at the Muang Ngai Agricultural Park Special Project, as well as the results of a study on the production of lingzhi and spores in accordance with the appropriate agricultural guidelines (GAP), revealed that *G. lucidum* MG2 was the most important substance content. Because of their entire flower yield and thick spores, *G. lucidum* MG2 was found to be the most suited species for commercial lingzhi mushroom cultivation.

Table 4.8 Mineral concentrations of *G. lucidum* powder from strains MG2 and G2

Minerals	**Unit**	***G.lucidum* MG2**	***G.lucidum* G2**	***G.lucidum* MG2 maltodextrin**	***G.lucidum* G2 maltodextrin**
P	%	2.30±0.05^{a}	1.56±0.00^{b}	0.13±0.05^{c}	0.17±0.00^{c}
K	%	3.66±0.10^{a}	3.55±0.00^{a}	0.18±0.08^{c}	0.38±0.00^{b}
Ca	%	1.94±0.32^{a}	1.35±0.07^{b}	0.11±0.03^{d}	0.17±0.00^{c}
Mg	%	1.02±0.17 ^{a}	0.79±0.03^{b}	0.06±0.16^{d}	0.09±0.00^{c}
Fe	ppm	165.00±7.33^{a}	58.60±3.03^{b}	16.90±1.13^{c}	10.60±4.41^{c}
Mn	ppm	57.30±0.90^{a}	46.90±0.10^{b}	3.89±0.58^{d}	16.80±0.19^{c}
Cu	ppm	46.70±4.42^{a}	44.70±0.62^{a}	5.98±1.21^{b}	10.70±1.07^{b}
Zn	ppm	112.00±1.27^{a}	84.90±0.87^{b}	9.49±1.53^{c}	8.46±0.49^{c}
Ge	ppm	18.24±0.98^{a}	13.80±0.48^{b}	5.11±0.16^{c}	4.85±0.81^{c}

Results are expressed as mean±SEM. Mean values with different lower case superscripts (a–c) represent statistically significant difference at 95% level ($P \leq 0.05$) with post hoc least significance difference (LSD) test.

CHAPTER 5
CONCLUSIONS

5.1 Conclusions

The optimal extraction conditions were determined to be an extraction temperature of 100°C, an extraction time of 4 hours, and a *G. lucidum* to water ratio of 1:12. Therefore, suitable temperature for spray drying was 140ºC.

The production of *G. lucidum* on the pilot scale found that maltodextrin in the spray drying process of *G. lucidum* powder has an impact on its antioxidant activity. Analysis of the antioxidant properties of *G. lucidum* strains MG2 and G2, after spray drying, showed that the MG2 strain had the highest antioxidant levels according to the FRAP (Ferric Reducing Antioxidant Power) and TEAC (Trolox Equivalent Antioxidant Capacity) assays, with MG2 outperforming G2. In contrast, the ABTS assay indicated that the G2 strain demonstrated superior antioxidant properties, while the DPPH assay found *G. lucidum* G2 to have higher antioxidant activity, followed by MG2. To further differentiate the bioactive properties of these strains, high-performance liquid chromatography (HPLC) analysis was conducted to measure β-glucan content a significant compound in *Ganoderma* species. The analysis revealed that β-glucan content ranged from 9.91% to 38.83%. Additionally, ganoderic acid A was detected in amounts between 0.85 and 0.92 mg/g, underscoring the role of local medicinal mushrooms in enhancing bioactive compounds. These results suggest that compounds like 1,3-β-glucan significantly contribute to the nutraceutical value of *G. lucidum* powder.

5.2 Recommendations

The following recommendations are made for further study:

1. The heavy metal substances contained in *G. lucidum* should be analyzed to see if the amount exceeds the public health limit or not.

2. Samples should be collected during the same season and inspected before testing.

3. There should be a study of the shelf life of *G. lucidum* powder products.

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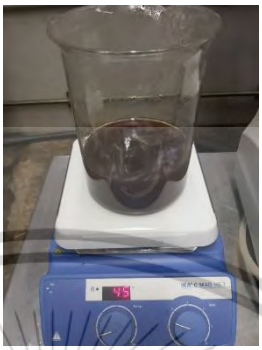
APPENDIX

Appendix A

Process of *Ganoderma lucidum* powder by spray dryer

Ganoderma lucidum Extract

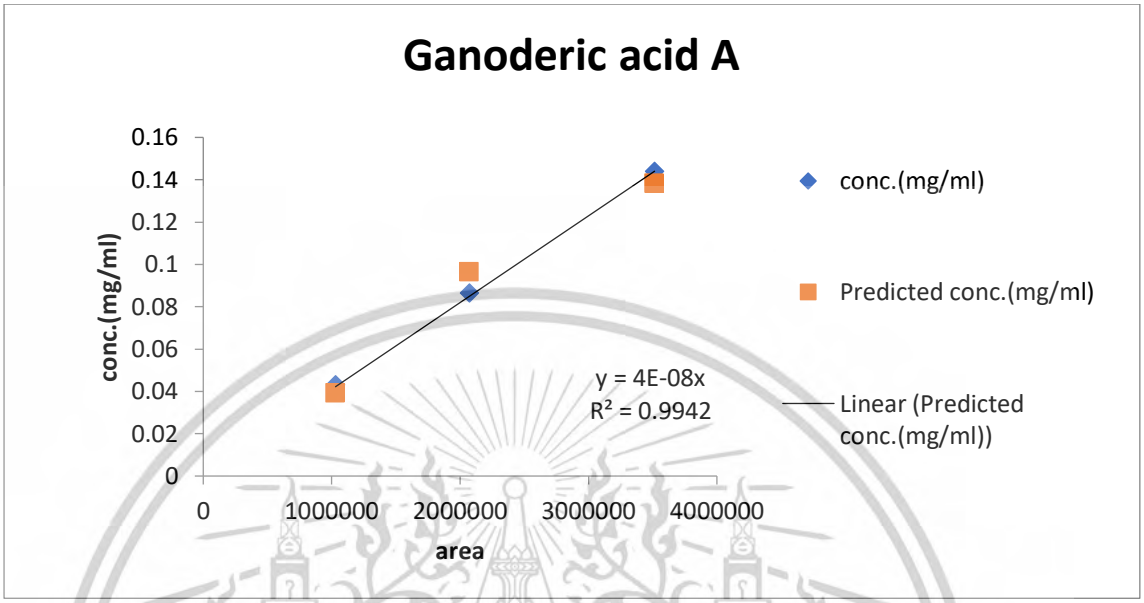


Temperature 140°C

Ganoderma lucidum powder

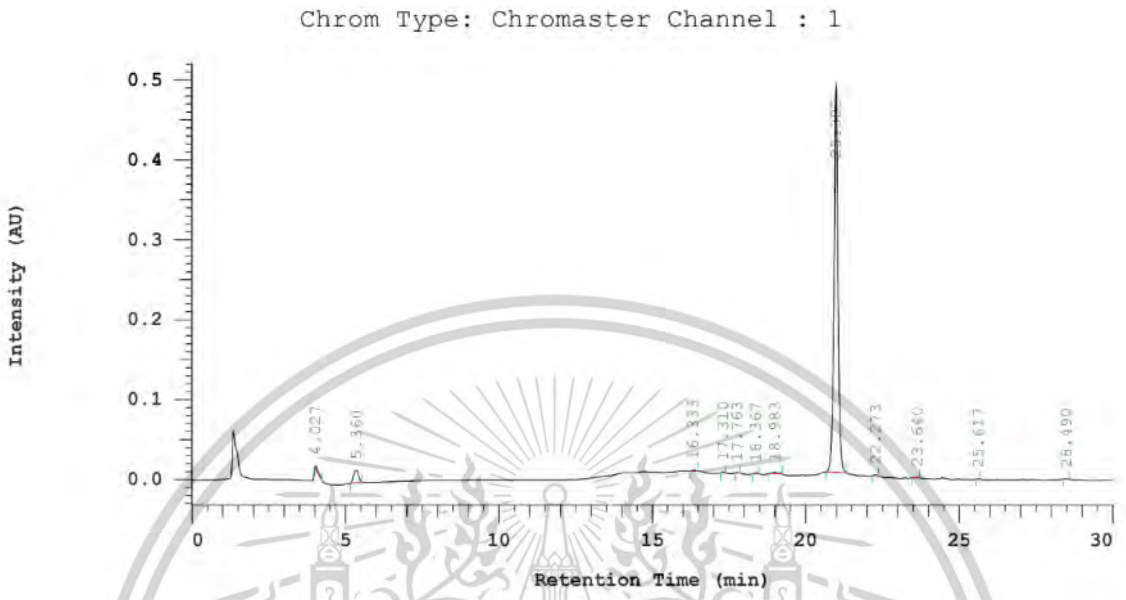
Appendix B

Calibration curve of ganoderic acid A

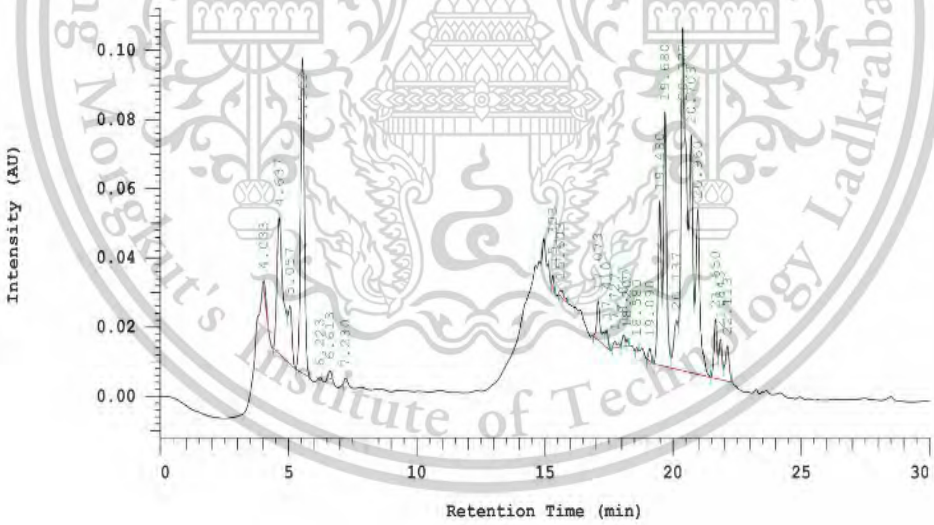


Appendix C

HPLC chromatograms for ganoderic acid A



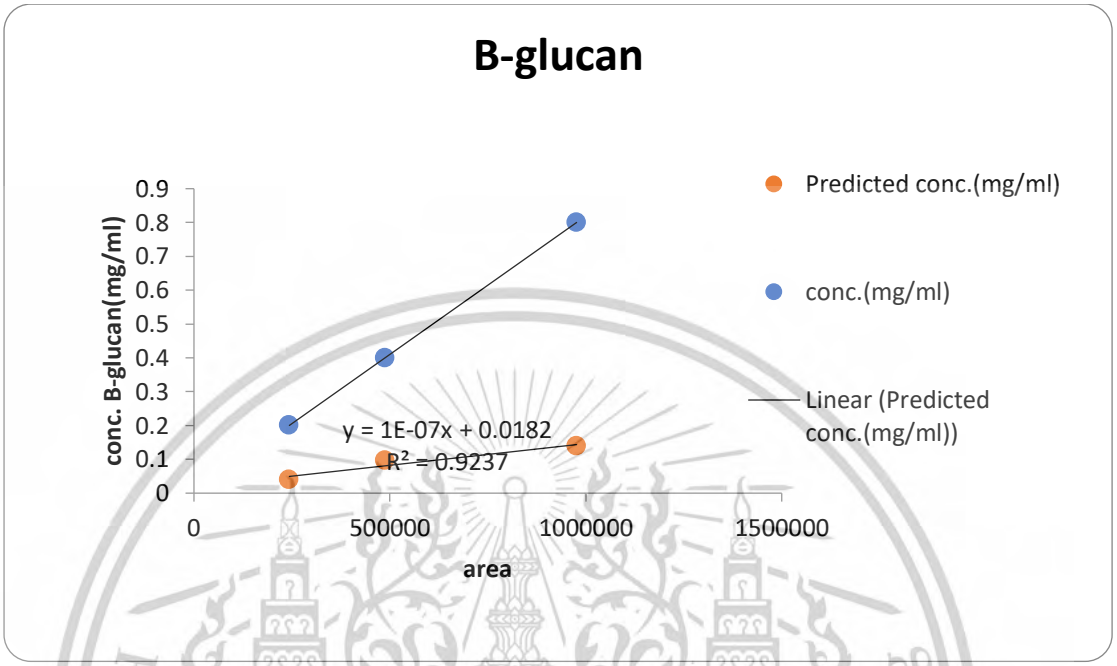
HPLC chromatograms of ganoderic acid A standard.



HPLC chromatogram of ganoderic acid A for *Ganoderma lucidum* MG2

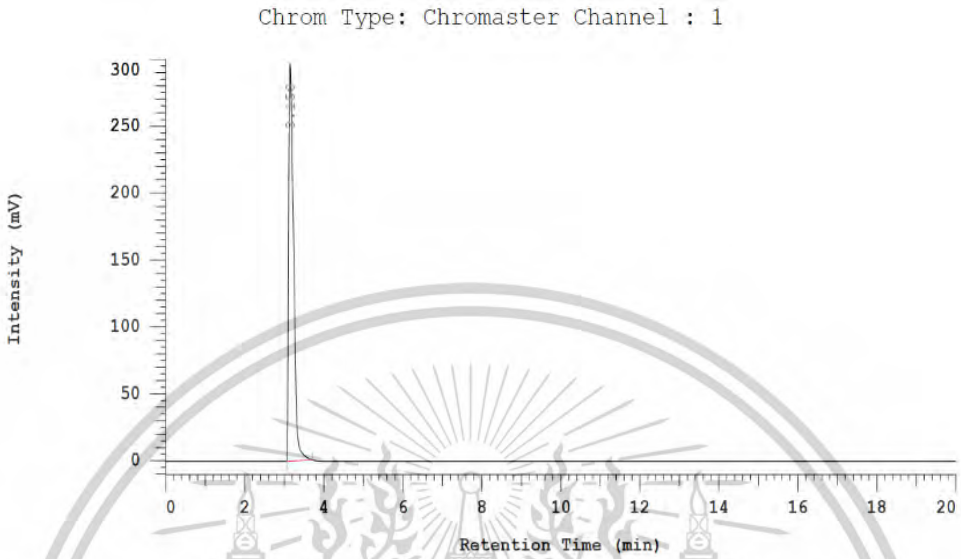
Appendix D

Calibration curve of B-glucan

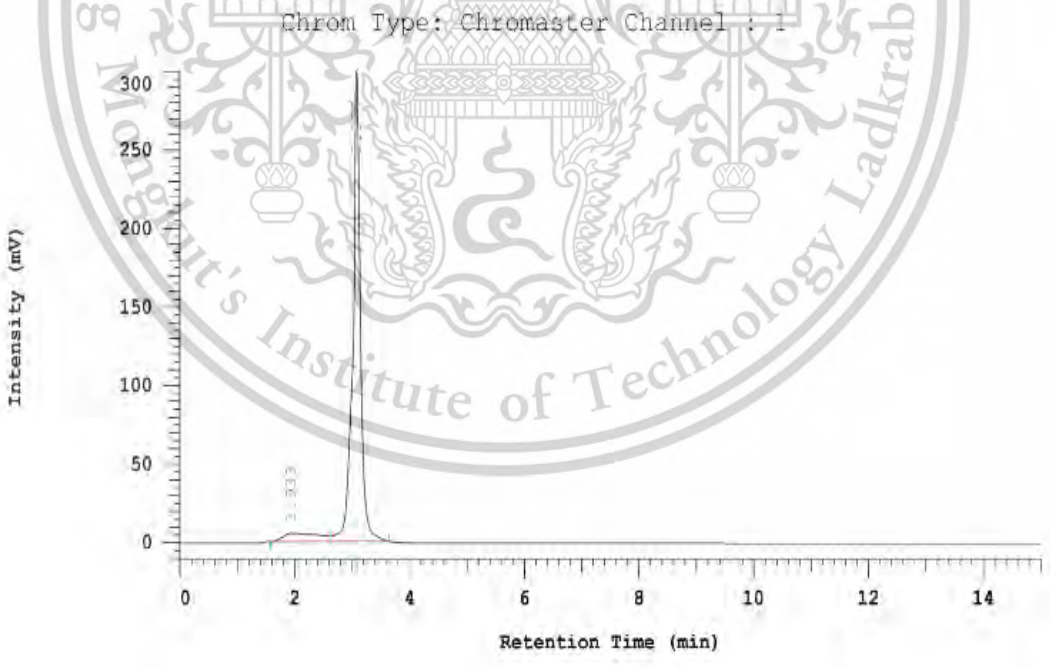


Appendix E

HPLC chromatograms for the β-glucan



HPLC chromatogram of β-glucan standard.



HPLC chromatogram of β-glucan for *Ganoderma lucidum* MG2

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