Bioactive Compounds of the Wonder Medicinal Mushroom "*Ganoderma lucidum*" 64

Surya Sudheer, Ibrahim Alzorqi, Sivakumar Manickam, and Asgar Ali

Contents

S. Sudheer · I. Alzorqi · S. Manickam (✉)
Department of Chemical and Environmental Engineering, Faculty of Engineering, University of Nottingham, Semenyih, Malaysia
e-mail: soorya.m.sudheer@gmail.com; ibrahim.alzorqi@yahoo.com; Sivakumar.Manickam@nottingham.edu.my

A. Ali
Centre of Excellence for Postharvest Biotechnology (CEPB), School of Biosciences, University of Nottingham, Semenyih, Malaysia
e-mail: Asgar.Ali@nottingham.edu.my

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_45

Abstract

Ganoderma lucidum is a highly praised medicinal mushroom having high demand worldwide. Intense research activities are being carried out on the medicinal applications of this mushroom that include anticancer, antiallergic, antioxidant potential, etc. This wonder mushroom contains many bioactive compounds such as polysaccharides, triterpenes, polyphenols, proteins, amino acids, and organic germanium. Ancient Chinese use this mushroom in their medicinal preparations and declared good health and longevity. Health benefits of this mushroom are highly praised in ancient manuscripts. Many pharmaceutical and beauty products made from this mushroom are available in the markets and demands high price. Cultivation and production of this mushroom are limited to certain countries, which leads to an increase in its market value. This chapter describes the details of this special mushroom, including its taxonomy, morphology, ecological and economic status, cultivation, bioactive molecules, and its medicinal applications.

Keywords

Ganoderma lucidum · Bioactive compounds · Cultivation · Therapeutic applications · Polysaccharides · Triterpenes

Abbreviations

C:N	Carbon nitrogen ratio
EPS	Extracellular polysaccharides
G. lucidum	*Ganoderma lucidum*
GA	Ganoderic acid
GTS	Ganoderma triterpenes
HDP	Host Defense Potentiators
IPS	Intracellular polysaccharides
LSC	Liquid state cultivation
LSF	Liquid state fermentation
LZ	Lingzhi
PBP	Protein-bound polysaccharide
RIP	Ribosome inactivating proteins
ROS	Reactive oxygen species
SOD	Superoxide dismutase
SSC	Solid state cultivation
SSF	Solid state fermentation

1 Introduction

Mushrooms are progressively being evaluated in the East and West for their nutritional value. Many of the mushrooms are nutritionally considered as functional foods and some of which are purely evaluated as a source of medicinally beneficial compounds called "nutraceuticals." *Ganoderma lucidum* (Fig. 1) is such a popular mushroom worshipped for its medicinal use and the history of its usage goes back to many thousands of years in the orient. *Ganoderma lucidum* holds a prominent place in traditional Chinese medicine and is well documented in ancient scripts [1, 2]. It has a long record of use in promoting health and longevity in China, Japan, and other Asian countries. Its medicinal activity is usually localized, for example, moderating the immune system by reducing its activity when overstimulated, and proliferating the immune system by increasing the number of active cells. For Chinese people, *G. lucidum* symbolizes the combination of spiritual potency and essence of immortality and thus called as "herb of spiritual potency." Among the cultivated mushrooms, the nutritional and pharmaceutical values of *G. lucidum* are paramount.

Ganoderma lucidum is consumed as a herbal extract like concoctions of tea and tonics. A variety of Ganoderma products are available in the market in various forms such as powders, dietary supplements, soaps, ointments, antiseptic creams, and herbal soaps. The products of this mushroom are available in the market but are expensive as efforts and processes required for the cultivation of this mushroom are intensive. *Ganoderma lucidum* described as a fungal bio-factory by eminent scientists as well as medical practitioners. But the amount of this wild mushroom is not sufficient to meet its demand in the international market. In the mushroom industries through various trial and errors, cultivation of *G. lucidum* has been achieved a considerable progress in the last 40 years. For faster and efficient production, various methods are practiced in these days [3]. Traditional and modern cultivation methods are used for large-scale production. Traditional methods include bag, bottle, and wood log cultivation which acquire more time, whereas modern techniques such as solid and liquid state cultivation could be carried out within a limited period.

Fig. 1 *Ganoderma lucidum*

The benefits of *G. lucidum* are entrusted from generation to generation, as an anticancer, a symbol of divine power, good fortune, good health, and longevity. It is used for various ailments such as allergy, hypertension, cardiac diseases, cancer, viral (HIV), and bacterial infections. It is an annual mushroom belongs to the family of Ganodermataceae. All parts of the mushroom viz., fruiting body, mycelia, and spores, contain bioactive compounds and are useful for the above-mentioned treatments. Polysaccharides, triterpenoids, proteins, amino acids, nucleotides, alkaloids, steroids, lactones, fatty acids, and enzymes are the major components that are responsible for the wide array of bioactive properties shown by this mushroom [4]. A single chemical compound cannot be responsible for the bioactivity which in turn is brought about by a combination of two or more active compounds. Polysaccharides, triterpenoids, and polyphenols are the major active compounds in *G. lucidum* [5]. Polysaccharides are water-soluble fragments which can be extracted with hot water, whereas polyphenols and triterpenoids are more soluble in polar organic solvents such as ethanol and methanol. *Ganoderma lucidum* is considered to be a useful source of protein (7–8%), carbohydrates (3–5%), crude fat (3–5%), crude fiber (59%), ash (1.8%), and other trace elements on a dry weight basis [6, 7]. *Ganoderma lucidum* also contains a higher amount of chitin which makes it hard to chew and digest. Apart from the nutraceutical benefits, it also plays a key role in the environment as decomposers in nutrient cycle. *Ganoderma lucidum* decomposes dead wood of coniferous or hardwood species by producing enzymes such as laccase, manganese peroxidase, and lignin peroxidase which degrade the compounds of lignin and cellulose in the wood. These wood degrading enzymes are of special interest for industrial applications, i.e., bioremediation, biopulping, and biosorption of heavy metals [8].

2 History

The history of *G. lucidum* can be traced back to 2000 years. During ancient times, *G. lucidum* had been worshipped as one of the herbal medicines and believed to heal all kinds of diseases. It was first used by the emperor of China, Shih-Huang of Ch'in Dynasty (221-207 BC), who made the Great Wall of China. Special teas or concoctions made from this mushroom were used by the emperors of Great China and Japan for vitality and healthy life [2]. Those who presented this mushroom to the king were highly rewarded. *Ganoderma lucidum* is popular in folk medicine because of its promising medicinal properties including its potential to extend the lifetime. *Ganoderma lucidum* was first introduced by a famous Chinese medicinal practitioner *Shen Nongs*, who is known as the "Father of Chinese medicine." Medicinal value of this mushroom is well described in his book "Materia Medica" [9, 10]. One of the key features of this mushroom is the absence of side effects. Due to this, *G. lucidum* has attained a reputation as an ultimate health supplement. The popularity of this mushroom has given it a place in "American Pharmacopoeia and Therapeutic Compendium." In addition to its medicinal properties, people of ancient China had a spiritual belief of protecting the people and their homes from evil spirit [11].

3 Scientific Classification

Kingdom	Fungi
Phylum	Basidiomycota
Class	Agaricomycetes
Order	Polyporales
Family	Ganodermataceae
Genus	*Ganoderma*
Species	*lucidum*
Binomial name	*Ganoderma lucidum* (Curtis) P. Karst.

Petter Adolf Karsten in 1881 named the genus as Ganoderma. *Ganoderma lucidum* has several names based on its geographical location and its traditional and historical applications. In Japan, this mushroom is commonly known as Reishi, Mannentake, or 10,000 years mushroom, and symbol to happiness, fortune, immortality, and good health. It has been known as Lingzhi in the Korean and Chinese ancients for more than 4000 years. It has also been called as a "mushroom of herb and immortality." However, the concept of this mushroom as an herb of immortality was described in transcripts of the first ancient empire of China (221-207 B.C) and illustrated in their literature and art perspectives. Botanically, the Latin name is *Ganoderma lucidum*, where lucidum was derived from its shiny surface [2, 9]. There are around 6 species of Ganoderma which are similar to *G. lucidum*. They are *G. atrum, G. tsugae, G. tropicum, G. applanatum, G. capense*, and *G. sinensis*.

4 World Production of *G. lucidum*

Worldwide consumption of *G. lucidum* is several thousand tons, and the market is growing rapidly. A decade ago, more than 90 brands of *G. lucidum* products were registered and marketed internationally [12]. Numerous *G. lucidum* products prepared from various parts of the mushroom are currently available on the market [13]. The simplest type of product available in the market consists of intact fruiting bodies ground to powder and then processed to capsule form. Other products are prepared from the following sources: (1) dried and powdered mycelia harvested from submerged liquid cultures grown in fermentation tanks; (2) dried and powdered combinations of substrate, mycelia, and mushroom primordia, following inoculation and incubation of a semisolid medium with fungal mycelia; and (3) intact fungal spores or spores that have been broken by mechanical means or have had the spore walls removed. Preparations made of spore have been promoted in the recent years because of the faster medicinal effects. It is generally hard to remove the spore wall; therefore, modern techniques, such as ultrasonic extraction, enzyme-based extraction methods are used which represent an additional and often a costly step in the production, are still controversial. Other products that are available in the market are prepared with extracted bioactive compounds such as polysaccharides and triterpenes. These compounds are usually extracted with hot water or ethanol from fruiting bodies or mycelia and then evaporated to dryness and encapsulated either separately or integrated together in designated proportions. The adoption of supercritical CO_2 extraction technologies has enlarged the spectrum of extracted substances due to the low temperature required during processing [14]. Several other products have been prepared from antler, the deformed type mushroom fruiting body, which is claimed to be highly medicinal.

5 Morphological Characteristics

G. lucidum is a kidney-shaped mushroom with varnished and colored outer surface and has an elegant appearance. They produce pores on the underneath of mushroom cap instead of gills, thereby making them to be differentiated from other polypores which produce gills underneath. But similar to other annual mushrooms they do not grow frequently. This difference makes them rare and special among other mushrooms. The upper side of this mushroom is shiny and dark red. Sometimes it shows varying color depending on the natural habitats and environmental conditions. All the three parts of *G. lucidum* such as mycelium, fruiting body, and spores (Fig. 2a, b, and c) are very important source of bioactive compounds.

5.1 Mycelium

Mycelium of a mushroom is the branching vegetative network which can spread like a mat on the medium or wood logs. Ganoderma mycelium appears to contain many of the bioactive compounds as that of fruiting body [15]. The polysaccharides from the mycelia also have the similar efficacy as that of the fruiting body [16, 17]. The spores of *G. lucidum* shred from the fruiting bodies would land on dead, decaying woods, and under adequate conditions they germinate into mycelium. Mycelia undergo three developmental stages, from primary to secondary and then to tertiary mycelia and eventually the fruiting body will sprout out. In the late phase, formation of basidium and then basidiospores are produced.

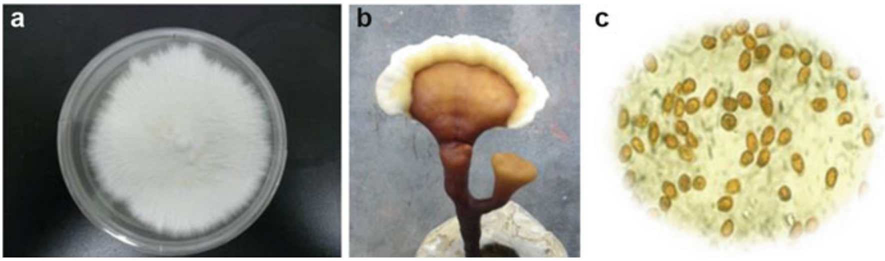

Fig. 2 Mycelium (**a**), fruiting body/basidiocarp (**b**) and spores (**c**) of *G. lucidum*

5.2 Fruiting Body

Basidiocarps or fruiting bodies of the mushroom vary from 2 to 10 cm in diameter. They are kidney-shaped and have shiny outer surface with yellow white striations at the margin which make them beautiful. In their initial stage of growth, they show yellow to white color. Later, with maturity they turn to reddish brown. The stipe (stem) is usually present, but sometimes it maybe with or without a reduced stipe. The length of stipe varies based on the conditions in which they grow as well as the nutrition available in the growth medium. The abaxial side of mushroom cap has several pores which are usually white. The matured mushroom shed spores through these tiny pores [18].

5.3 Spores

The spores of *G. lucidum* are slightly brown in the size range of 1–10 micron. The spore wall is composed of chitin. The spores are reported to contain higher amounts of bioactive compounds than the fruiting body. But the spores are hard to break due to the thicker chitin wall. Possessing a thicker chitin wall is the major characteristic of polypore mushroom as they protect the mushroom from adverse conditions. The sporoderm of *G. lucidum* is made-up of silica (19.01%), calcium (17%), and chitin (52.08–57.64%) [19]. The outer thick coating of sporoderm is resistant to acids giving it an extended shelf-life in the nature.

6 The Ecological Habitat of *Ganoderma lucidum*

This annual mushroom could be found growing on the decaying and dead bodies of a variety of trees. It grows on the decaying logs of deciduous trees like oak, maple, elm, willow, sweet gum, magnolia, and locust. Besides, it grows on the decaying logs of certain coniferous trees such as Larix, ptea, and pinus, in the subtropical regions such as Europe, Asia, and North and South America [20]. In the oriental countries, the mushroom has been found to grow mainly on the plum trees on the stumps near the soil surface and occasionally on soils arising from buried roots [2]. In temperate and subtropical regions of Asia, Europe, and South and North America, this mushroom can be found on the coniferous trees such as Larix, pinus, and picea. They cause white rot disease at the base of trees either alone or in a group. Its natural growth considerably takes longer time, which makes the mushroom very rare, treasured, and exclusive for the rich people and highly ranked powerful individuals. The above limitations push the scientists to find alternative artificial cultivation methods.

7 Nutrition and Growth

Ganoderma lucidum which is used as a folk medicine or nutrition is a saprophyte. They cannot carry out photosynthesis due to the absence of chlorophyll. Mycelia can directly absorb simple sugars, organic acids, and other carbohydrates that are present in the growth substrate. Mushroom mycelia cannot use inorganic carbon. The growth of mycelium requires the presence of nitrogen approximately 0.016–0.064%. For the production of fruiting body, the requirement of nitrogen is approximately 0.032–0.016%. Growth of mushroom needs the presence of minerals such as P, K, Ca, Mg, S, Fe, Mn, Zn, and other inorganic compounds. Temperature is another crucial factor which determines the growth, quality, and yield of the mushroom. Mushroom at various stages of growth and development requires different temperatures. For the germination of mycelia, the suitable temperature employed is in between 25 °C and 30 °C [21]. Primordia require 15–23 °C, whereas the fruiting bodies require 28–30 °C. Temperatures below 12 °C result in a slower growth, reduced stipe, and thicker flesh. Spores require 22–26 °C for their growth. All the stages of mushroom growth need the presence of water. For the mycelia growth, 45–65% of water is appropriate, but for the fruiting body development a relative humidity of 75–95% is necessary. Humidity more than 95% results in the chances for bacterial contamination. Presence of light is negligible for the growth of mushroom mycelia, whereas spore and primordia formation take place under low intensity of light. Similarly, light is very essential for the better development of fruiting body and its quality [22]. An optimum pH is the other limiting factor which affects the mushroom growth. Almost all the saprophytes including *G. lucidum* grow slightly under an acidic pH of 4–6.

8 Cultivation

Ganoderma lucidum cultivation was first introduced from China in 1980s. Currently there are a diverse range of cultivation methods based on the employed substrates. The three most common traditional methods of cultivation are wood pulp, wooden box, and natural wood log cultivation [23, 24]. Techniques such as wood log or wood chip cultivation are not applicable to produce *G. lucidum* in the commercial practice, as they raise ecological problems such as cutting and processing of trees, and thus there is a concern. It is therefore necessary to find an effective and a reliable way of cultivation as well as to produce in larger-scale to meet the market demands.

8.1 Wood Pulp Cultivation (Bottle Cultivation)

It is the simplest method of cultivation which involves placing the sterilized wood pulp in a glass bottle along with spawns of *G. lucidum*. Mushrooms along with the bottle are incubated for 3 months. This method usually yields smaller-sized mushrooms of relatively inferior quality.

8.2 Box Cultivation

This method involves grafting the fungi into drilled wooden logs (1 m), which are then placed in the wooden boxes. This method takes about 3–6 months for the generation of fruiting body. Mushrooms of medium-size and moderate quality are generally produced through this method.

8.3 The Natural Wood Log Method

Natural wood log cultivation could be carried out by the following two methods. One utilizes cultivation on longer unsterilized wood logs and the other uses shorter sterilized logs. First method was the common cultivation practice in China until the new development using short logs which was introduced in the late 1980s. Natural wood logs of 1 m were used for long woods without any sterilization. This method takes longer incubation periods (2–3 years) besides requiring more labor to produce matured fruiting bodies. Short wood log cultivation method was introduced from Japan. It is the common cultivation strategy adopted by natural log growers in China, Japan, Korea, and USA. This method offers the production of a larger basidiocarp with superior quality which involves the preparation of wood logs, especially broad-leaf hard woods of 15 cm in diameter with the length of 14–24 cm. The moisture content should be about 35–40%. The wood logs are then enclosed in polypropylene bags and sterilized. They are incubated with precultured mushroom spawns of 5–10 g. Mycelial run is usually carried out with less oxygen and under dark condition. Formation of mushroom primordia occurs during 50–60 days after spawning. The mushroom colonized wood logs are then buried under nutrient rich soil leaving the primordia above the soil in a green house. Regular monitoring is essential to ensure mushrooms of high quality. Harvesting of mushroom takes approximately 25–30 days after the formation of primordia [5].

8.4 Cultivation on Sawdust

The processing of wood logs is expensive and requires special kind of woods and expertise which make the farmers to think about using sawdust as a cultivating medium. Sawdust is the wood dust produced after processing of woods in the mills. In this method, *G. lucidum* is cultivated on sawdust filled in the polypropylene bags along with rice bran (10%) and $CaCO_3$ (3%). They are moistened to 60% and filled into polybags (700–1000 g). Finally, the bags are plugged with a cotton cap. Steaming of the bags is then carried out for 5 h at 95–100 °C. The bags are then kept at room temperature overnight and inoculated with mushroom spawns. About 3–4 weeks is an essential period for the mushroom mycelia to colonize on the substrate. The bags are then opened for the formation of primordia at 28 °C. The relative humidity is maintained at 85–90% during primordia development. Approximately after 2–3 months from the appearance of primordia, matured basidiocarps will be ready for harvest [25].

Following sawdust cultivation, new substrate compositions were proposed by various researchers. Chen [26] recommended a substrate media containing oak sawdust (80%), unprocessed wheat bran (18%), sucrose (1%), calcium carbonate

Table 1 Growth requirements for the cultivation of *G. lucidum*

Mycelial colonization
Temperature – 25 °C (optimum) Light – not needed Duration – 3–4 weeks
Primordia formation Temperature – 28–30 °C Relative humidity – ~90–95% Duration – 1 week after mycelia colonization Light – Reduced light (100 lux) CO_2 – >0.1%
Mushroom development
Temperature – 28–30 °C Relative humidity – 80–85% Duration – 25–30 days after primordia formation Light – 150–200 lux CO_2 – >0.1%

(1%), and 60–70% moisture for the cultivation of *G. lucidum*. Substrate media formulation using sunflower seed hulls [27], stillage grains [28] soy residue [29], etc., have been reported. Sawdust cultivation can be carried out in bags, trays or bed [30], bottles, and pots [31] based on the scale of production.

The growth of *G. lucidum* depends on cellulose and lignin that are present in the wood logs. They utilize these compounds by the production of enzymes which break down the bonds in the long cellulose chains into shorter chains so that the carbon become available for the growth of fungi. *Ganoderma lucidum* utilizes carbon resources from compounds such as sugar, starch, cellulose, hemicellulose, and lignin. Mycelia cultivation requires the presence of sugar and nitrogen sources. Compounds such as amino acids, urea, nitrogen can induce the mycelia growth [32]. Sawdust of broadleaf trees [33], cotton seed husk, corn cob, paddy straw, wheat and rice bran, corn powder, ammonium sulfate, and urea are used in the substrate media to produce fruiting body [34]. Carbon to nitrogen ratio is a key factor for the growth of mushroom as well as for the formation of fruiting body. Appropriate ratio of C:N required is 15–45:1 for mycelia and 30–40:1 for fruiting body [35, 36]. The growth requirement of *G. lucidum* on the solid substrates is given in the Table 1.

8.5 Modern Cultivation Practices

In these days, industries are looking forward for faster and cheaper practicing methods for the cultivation of *G. lucidum*. Both solid state fermentation (SSF) and liquid state fermentation (LSF) techniques offer scope for large-scale production of this mushroom using cheaply available agricultural residues. The details obtained from solid state cultivation (SSC) can be applied to study the liquid state cultivation

(LSC) [37, 38]. Cultivation of fruiting bodies of *G. lucidum* takes several months and proper environmental conditions; hence, mycelia-based and culture broth-based products have assumed immense importance due to increased quality control and year-round production [39]. SSF is nowadays used to upgrade the valuable food supplements, enzyme production, and the feed values of waste materials [40]. In submerged state cultivation method, mycelia culture can be standardized easily under controlled conditions and simple processes are required for downstream processing of the bioactive compounds such as polysaccharides and other compounds released into the culture medium. Several researchers have reported the cultivation of mycelia using submerged/liquid state cultivation. Standardizing the culture conditions and medium composition strongly influence mycelial growth and production of bioactive compounds in this mushroom [5, 39, 41]. A novel three-stage light irradiation strategy has been developed in submerged cultures of *G. lucidum* for the efficient production of polysaccharides and ganoderic acid [42].

Development of bidirectional SSF technology is the recent focus in the modern mushroom science. It is a new type of fermentation technique emerged from China in the 1990s. The key of this technology is that the medicinal mushroom strains are cultured in the special substrate, which consisted of Chinese medicinal herbs as substrate instead of the traditionally cultured nutritious substrate. The fermentation products are known as the medicinal fungal substances. During fermentation, these medicinal compounds in the substrate provide nutrients for the fungal growth. At the same time, the fungal strains produce enzymes which change the tissue and components in the substrate to new functional components. Thus, by bidirectional fermentation, medicinal strains produce bioactive compounds of higher quality [43, 44].

Bidirectional SSF was applied by Chen and Chen [45] in *G. lucidum*. They designed three types of medium; *G. lucidum* in an ordinary growth medium, Chinese materia medica (CMM) medium containing *Radix astragali*, and selenium rich CMM-containing medium. Fermentation products from the three types of media were checked at fixed time intervals. Polysaccharide contents of fermentation products from these culture media were 4.65%, 3.76%, and 4.50%, respectively. By observing the changes in the concentration of polysaccharide, protein, and total saponin in fermentation products from the CMM-containing medium at various times, they concluded that the 28th fermentation day was the time during which secondary metabolism was the most active. Similar studies were carried out using *G. lucidum* with different medicinal herbs [46].

Another application of *G. lucidum* is in the functional food research and development. For this, the amount of polysaccharides, reducing sugars, amino acids, and protein contents were determined. The outcome indicates that the optimum production needs specific conditions, i.e., size of the granule, temperature, fermentation time, and inoculum size. The amount of polysaccharide was found to be 21.97 mg/g under optimum culture conditions [47]. Further research in the production of polysaccharides from *G. lucidum* was reported by You [48]. Moreover, *G. lucidum* cultivation opens a door to process forest and agricultural wastes into useful by-products and thus reduces the environmental pollution.

9 Bioactive Compounds in *G. lucidum*

Approximately 400 different bioactive compounds are present in this mushroom. Polysaccharides, triterpenoids, organic germanium, ergosterol, nucleotides, sterols, amino acids, fatty acids, proteins, intracellular/extracellular enzymes, and trace elements [2, 49] are the major active compounds possessing biological activities. All parts of *G. lucidum* viz., fruiting body, mycelia, and spores, are reported to contain these compounds. Mushroom-derived polysaccharides and triterpenes have increased demand in the market. The extracts of this mushroom are given as a health supplement or medicine for anticancer, antiviral, immunomodulating, hepatoprotective, and hypocholesterolemic agents [50, 51]. Absence of side effects and presumed health benefits give reputation for this mushroom to use as herbal medicine [39].

The bioactive compounds from *G. lucidum* have a long history and have received considerable attention in recent years. Intensive work on the therapeutic effects of *G. lucidum* has been reported in the literature.

Ganoderma lucidum possesses unique bioactive molecules which include

- Polysaccharides [52–54]: A variety of polysaccharides that tend to be the components to interact with the immune system and are subdivided into β-1,3-glucans and polysaccharide peptides such as peptidoglycan.
  - Water-soluble polysaccharide peptides include GLPS peptide (GLPP) [55], GLPG [56], GLIS [57], PGY [58] and F3 [59].
  - β-1,3-Glucan (a subset of polysaccharides) sometimes called Curdlan [53] and other Glucan molecules [60].
- Triterpenoids: Over 120 triterpenoids [61] were revealed which can be separated into two classes. Those with a carboxylic side chain (Ganoderma acids) and those without (Ganoderma alcohols). Some are referred to as lucidenic acids [62].
- Nucleotide bases such as thymine, uridine, inosine, guanosine, and adenosine are present in this mushroom. The sum of all ranging from 303 to 1217 mcg/g in the mushroom cap and 22–334 mcg/g in the stem [63].
- Bioactive proteins such as LZ-8 (Lingzhi-8) [64], Ganodermin A and 114 kDa hexameric lectin, a glycoprotein with 9.3% sugar [65] are present.
- A reversible and a highly specific competitive alpha-glucosidase inhibitor known as SKG-3 with an IC_{50} of 4.6 mcg/mL [66].
- Ergostane sterols and ergosterol, also known as pro-vitamin D2 [67].
- C19 fatty acids (nonadecenoic acid and cis-9-nonadecenoic acid) [68, 69].
- Riboflavin.
- Vitamin C.
- Copper and Zinc [70].
- Selenium of up to 72 mcg/g dry weight and can transform selenium into selenium-containing proteins [71].
- Germanium of up to 489 mcg/g based on dry weight.

9.1 Structure and Properties

Polysaccharides, triterpenoids, and peptidoglycans are the main sources that are responsible for the bioactivity of *G. lucidum*. Activities from other molecules have not been studied significantly. Major triterpenoids responsible for the bioactivity are Ganodermic acids, Ganodermic alcohols, and Lucidenic acids.

9.1.1 Polysaccharides

Polysaccharide is a long chain of monosaccharides linked by glycosidic bonds. More than 100 types of polysaccharides have been reported to be present in *G. lucidum.* Most of them belong to a group of β-glucan which consists of a linear backbone of β-(1,3) linked D-glucopyranosyl groups with varying degree of branching from the C-6 position. They are considered as the active agents to fight against cancer. The molecular weight ranges from 4×10^5 to 1×10^6 in the primary structure [2]. β-Glucans of higher molecular mass are more effective than that of glucans with low molecular mass [72, 73]. β-Glucans also exist with heteropolysaccharide chains of xylose, mannose, galactose, uronic acids, and β, D-glucan-protein complexes which are present in the dry fruiting body (10–50%) [74, 75].

Polysaccharides prevent oncogenesis by not attacking the cancer cells directly, but by activating the immune response in the host. Fang and Zhong [76] indicated that active immunomodulatory polysaccharides are water-soluble (β-1,3 and β-1,6) glucans which can be precipitated by ethanol. Other polysaccharides such as glycopeptides [77, 78] and proteoglycans [79, 80] are also reported to have immunomodulatory activities. Purified mushroom polysaccharides have been used worldwide especially in China, Japan, and Korea for years for clinical purposes without any side effects. Polysaccharides are reported to increase the quality of life of cancer patients and offer promising survival rates [81]. Mushroom extracts are used as tonic for the immune system. Polysaccharides are reported to be very effective for wound healing [82].

9.1.2 Triterpenoids

Other class of bioactive compounds are also present abundantly in *G. lucidum*. Approximately 140 types of triterpenes have been identified in *G. lucidum* [49, 83, 84]. Most of them are lanostane type triterpenes. Extract of *G. lucidum* tastes bitter due to the presence of these triterpenoids, especially Ganoderic acid which is the major type of triterpenoid present in this mushroom. There are several types of Ganoderic acids such as, GA-A, B, C, and F. Previous studies indicated that triterpenoids are present more in the spores as compared to other parts of the mushroom. The production of bioactive compounds is also affected by the area and conditions in which they grow [85]. The basic structure of triterpenoid depends on the structure of lanosterol, an important intermediate for the biosynthesis of steroid and triterpene. Triterpenes are divided into three groups based on the number of carbon atoms and functional groups which are C30, C27, and C24 compounds [86]. Many of them find useful as chemotherapeutic agents.

9.1.3 Phenolic Compounds

Phenolic compounds are one of the important and the most commonly extracted bioactive compounds from *G. lucidum*. They can be classified as simple phenols and phenolic acids such as gallic acid, benzoic acid, syringic acid, chlorogenic acid, and polyphenols which are classified into many distinct groups such as flavonoids, tannins, stilbenes. Flavonoids are a group of polyphenolic compounds with known health-beneficial properties that include free radical scavenging, inhibition of hydrolytic and oxidative enzymes, and anti-inflammatory activity [87]. Research studies suggest that the biological activity of these compounds is related to their antioxidant activity. Phenolic compounds have significant biological and pharmacological properties, and some have demonstrated remarkable ability to alter sulfate conjugation. The bioactivity of phenolics may be related to their ability to chelate metals, inhibit lipoxygenase, and scavenge free radicals [88]. Methanolic extracts of *G. lucidum* have been reported to contain higher antioxidant activity [89, 90], and their radical scavenging mechanism has been revealed (c). Heleno et al. [91] revealed that the phenolic extracts of fruiting body and mycelia have higher antioxidant potential than their corresponding polysaccharide extracts, highlighting a higher contribution of free phenolic compounds than the antioxidants linked to polysaccharides.

9.1.4 Sterol and Ergosterol

Sterols are derivatives of triterpenoids. *Ganoderma lucidum* has been determined to contain ergosterol and 24-methylcholesta-7,22-trien-3-ol. 8,9-epoxyergosta-5,22-dien-3,15-diol from *G. lucidum* was reported as the first isolated free sterol [72].Hajjaj et al. [92] reported the isolation and identification of 26-oxygenosterols such as ganoderol A, ganoderol B, and ganoderic acid Y. They also determined that 26-oxygenosterols could lead to novel therapeutic agents that can lower the blood cholesterol.

Ergosterol is one of the important pharmaceutically relevant compounds. It is a vitamin D precursor. The integrity of fungal cell membrane is maintained by ergosterol. It also generates cellular energy. Measurement of Ergosterol is an important parameter in the biomass production. A new, highly oxygenated sterol, 22E, 24R-ergosta-7,22-diene-3beta, 5alpha, 6beta, 9alpha,14alpha-pentol was reported by Zang et al. [93]. Ergosterol was found to be higher in *G. lucidum* (median content, 705.0 μg/g; range, 189.1–1453.3 μg/g; $n = 19$) as compared to other species (median content, 80.1 μg/g; range, 16.0–409.8 μg/g; $n = 13$) [94].

9.1.5 Proteins

In addition to polysaccharides and triterpenoids, *G. lucidum* is a reservoir of proteins and peptides with biological activities [95]. LZ-8 is one of the proteins isolated from *G. lucidum* which appears to be related to an ancestral protein of the immunoglobulin superfamily. Confirmation of this protein was carried out by sequencing studies, and it resembled with the sequences and secondary structure of heavy chain region of immunoglobulin. The biological activities are almost similar to lectins which have mitogenic capacity [96] towards mouse spleen cells and human peripheral lymphocytes in vitro.

Other proteins are ribosome inactivating proteins (RIP), antimicrobial proteins, ribonucleases, and laccases. All these compounds play essential role in regulating the body's immune mechanism directly or indirectly. Among these proteins, FIP plays a vital role in antitumor, antiallergic, proliferation of lymphocytes, and transplant rejection activities [97]. Seven FIPs have been identified till now from varied species of Ganoderma. They are LZ-8 (*G. lucidum*), FIP-gts (*G. tsugae*), FIP-fve (*Flammulina velutipes*), FIP-vvo (*Volvoriella volvacea*), FIP-gmi (*G. japonicum*), and FIP-gsi (*G. sinensis*) [98, 99]. One molecule of FIP composed of 110 to 114 amino acids and their molecular weights are around 13 kDa. Among the FIP proteins, LZ-8 contains low levels of carbohydrates, but other FIPs are pure proteins without any carbohydrates. The presence of a lower amount of FIP in *G. lucidum* is a problem for recovery to meet its growing demand. Nowadays it is the major concern among the researchers, and thus the focus is on the genetic engineering to develop FIP protein through cloning FIP genes in eukaryotes and prokaryotes [100, 101].

9.1.6 Nucleotides and Nucleosides

These are the nitrogenous compounds which play important roles in the metabolism and stimulate hemopoieses. Nucleosides include adenosine and 5-deoxy-50 methylsulfinyladenosine. Adenosine of *G. lucidum* suppresses platelet aggregation and prevents heart attacks and thrombosis [102].

9.1.7 Lipids and Fatty Acids

Phosphatidic acids are not the abundant lipid constituents in the living organisms, but they play significant role in the membrane trafficking events and defense mechanisms against infection and tissue damage during inflammation. Presence of these lipids makes this mushroom important among the medicinal species [103]. The main fatty acids in *G. lucidum* are palmitic acid, linoleic acid, oleic acid, and stearic acid. Fatty acids in the spores could inhibit tumor cell proliferation [94]. Non-adecanoic acid is another fatty acid present in this mushroom which has the highest inhibitory activity, followed by heptadecanoic acid, stearic acid, and palmitic acid [69]. Palmitic acid and stearic acid are the strong apoptotic agents [104].

9.1.8 Amino Acids

Nutritional analysis of *G. lucidum* showed the presence of 16 amino acids (Table 2), where glutamic acid (120), aspartic acid (117), glycine, and alanine show the highest relative abundance, whereas methionine shows the least [77].

9.1.9 Alkaloids and Other Compounds

Generally, alkaloid content is relatively less in *G. lucidum*. The alkaloids such as choline and betaine were isolated from the spores of *G. lucidum* [72]. Studies have showed the presence of alkaloids and their chemical allies, i.e., saponins, flavonoids, and tannins [105] in *G. lucidum*.

Studies by Mizuno [106] showed that the extracts of *G. lucidum* (% dry weight) consist of folin-positive material (68.9%), protein (7.3%), glucose (11.1%), and metals such as K, Ca, Ge, and Mg. The outcome of this study agrees well with the reports of other investigations [20, 107]. Moreover, there is a difference in the qualitative and quantitative results in the chemical composition of *G. lucidum* extracts. It is mainly

Table 2 Amino acid composition in *G. lucidum*

Amino acid	Relative abundance
Glutamic acid	120
Aspartic acid	117
Glycine	108
Alanine	100
Threonine	66
Valine	61
Proline	60
Leucine	55
Serine	54
Isoleucine	36
Phenylalanine	28
Arginine	22
Lysine	21
Tyrosine	16
Histidine	12
Methionine	6

affected by the factors such as quality of the strain, origin, cultivation conditions, stages of harvesting, and extraction processes [83]. Elemental analysis of log-cultivated fruiting bodies of *G. lucidum* revealed phosphorus, silica, sulfur, potassium, calcium, and magnesium to be their main mineral components. Iron, sodium, zinc, copper, manganese, and strontium were also detected in lower amounts, as were the heavy metals such as lead, cadmium, and mercury [108]. *Ganoderma lucidum* also contains soluble proteins, oleic acid, cyclo-octasulfur which is an ergosterol peroxide (5,8-epidioxy-ergosta-6,22E-dien-3-ol), and cerebrosides ((4E0,8E)-N-D-20-hydroxystearoyl-1-Ob-D-glucopyranosyl-9-methyl-4-8-sphingadienine, and (4E,8E)-N-D-20-hydroxypamitoyl-1-O-b-D-glucopyranosyl-9-methyl-4-8-sphingadienine [49, 106, 109]. Compounds such as choline, betaine, tetracosanoic acid, stearic acid, palmitic acid, ergosta-22-dien-3-ol, nonadecanoic acid, behenic acid, tetracosane, hentriacontane, ergosterol, and β-sitosterol have been reported in the spores of *G. lucidum* [110].

Some attention has been given to the germanium content of *Ganoderma* spp. Germanium was the fifth highest in terms of concentration (489 μg/g) among the minerals detected in *G. lucidum* fruiting bodies collected from the wild [111]. Germanium is not an essential element at low doses. It has been credited with immunopotentiating, antitumor, antioxidant, and antimutagenic activities [112]. Other compounds that have been isolated from *G. lucidum* include enzymes such as metalloprotease, which delays clotting time, ergosterol (provitamin D_2), nucleosides, and nucleotides (adenosine and guanosine) [2, 50].

9.2 Extraction, Product Recovery, and Analysis of Bioactive Compounds

Ganoderma lucidum is enriched with active compounds such as polysaccharides, triterpenoids, nucleotides, organic germanium, fatty acids, proteins, amino acids, and sterols. Therefore, it is essential to develop a methodology for the effective recovery and easy analysis of these bioactive compounds. All these components have been reported to have pharmaceutical applications. Separation of individual components is difficult, time-consuming, and expensive; therefore, industries are looking for random isolation of compounds in the form of crude mushroom extract. In the past few years, it is being a challenge to develop a methodology for the elicitation, recovery, extraction, and analysis of bioactive compounds from *G. lucidum.* Triterpenoids appear to be hydrophobic and are present in ethanol or chloroform fractions, whereas the polysaccharides are water-soluble and are the major bioactive compounds in the water-soluble extract of Ganoderma.

9.2.1 Preparation of *G. lucidum* for the Extraction of Bioactive Compounds

Ganoderma lucidum should be first processed after collection. Preparing the raw materials for a process involves cleaning the material from dirt by washing or rinsing and removing the trunks either manually or by using a vegetable slicer. Thereafter, the material should be dried to reduce their moisture content, to avoid probability of degradation, and to facilitate the safe storage and transportation [113]. By removing the moisture content, the efficiency of extraction process will be enhanced remarkably.

9.2.2 Drying (Demoisturization)

Generally, many drying methods are used to dry the mushroom sample. These methods are classified into two groups: heating (baking, ovens, stoves, and infrared) and air-drying (drying chambers with air circulation) [113]. Classically, drying *G. lucidum* in an open air either under shading or direct sunshine is the most commonly used method due to its low-cost and simple preparation set-up. However, choosing the right drying method is limited by the type of desired bio-components and their physical properties. For example, for thermolabile components (thermally nonstable components), such as polyphenols and triterpenes, it is recommended to maintain the drying temperature below 40 °C to avoid thermal degradation of these components [114, 115]. Also, for some of the light sensitive components such as polyphenolics, it is important to keep the raw materials away from the sun light during the drying stage [115]. For industrial production, large-scale batches could be processed by hot air-drying method which is an economically cost-effective but physically may affect the products due to the probability of oxidation damage that can occur when exposed to air stream [116]. In general, to retain most of the bioactive components, a temperature range of 50–60 °C is the most commonly followed for drying the herbal materials [117–119].

Through an extended literature survey, different drying methods were reported to prepare the herbal materials for the extraction of bioactive compounds. *Ganoderma lucidum* under 60 °C for 24 h [120] is suitable to retain the bioactive compounds, while in many other reviews air drying chambers were used to dry *G. lucidum* under 105 °C [54, 121]. The flexibility of using different drying methods could be attributed to the ability of polysaccharides to withstand relatively a wider range of temperatures, i.e., 30 °C [122], 45 °C [123], 50 °C [124], 90 °C [125], and 105 °C [126], depending on the matrix of the mushroom.

9.2.3 Size Reduction of Herbal Materials (Grinding)

Size reduction is the second important preparatory stage for any extraction process. It was found that cutting the mushroom into small pieces or reducing the particle size of the raw materials before extraction is essential to increase the mass transfer rate of the entire process. Reducing the particle size leads to an increase in the contact surface area between the solvent and the extracted mass, which increases the diffusion of bio-components in the solvent and thus results into an increase in the overall mass transfer rate. The size reduction can be implemented by inserting the mushroom sample into a hammer mill or disc pulverizer provided with sieves in different ranges of particle permeation. Many factors affect the grinding of mushroom samples such as adjusting the speed of the rotor clearance between the hammers and the lining of the grinders, the discharge capacity of the mill, and the sieve sizing which is preferable to be in between 30 and 40 mesh as an optimal range for the extraction of mushroom samples.

For *G. lucidum*, to improve the extraction process of polysaccharides, the fruiting body is preferably to be used in the powder form [121]. A 32 range of particle size (2–5 mm) is reported by several studies on the ultrasonic-assisted extraction of polysaccharides from *G. lucidum*. The fruiting bodies of *G. lucidum* were crushed to pass 2 mm screen [73, 120, 127], 5 mm [118] or cutting into small pieces of 3 to 2 cm of 2 mm thickness [128, 129]. Generally different grinding machines (pulverisers) are used to prepare fine particles in the powder form. Usually these grinding machines are provided with shaker and sifting-sieves of 12–120 mesh [130], such as 70 mesh [131], 40 mesh [132], and 60 mesh [133].

9.2.4 Pretreatment with Aqueous Ethanol

Many studies showed that pretreating the herbal materials with polar organic solvents (ethanol, methanol), or an aqueous alcoholic mixture, helps to exclude large amount of constituents (monosaccharides, fatty acids, amino acids, phenols, and endogenous enzymes), other than macromolecules such as polysaccharides, nucleic acids, and proteins which could be extracted in a later stage by the polar solvent such as water. The aqueous mixture of ethanol, 60–90% (v/v), has been used for this purpose due to its ability to extract a wide range of components with different polarity. The most important stage in the pretreatment with aqueous ethanol is the prior removal of hydrophobic constituents, such as fats that greatly influence the extraction stage and limit the penetration of water into the solid structure [134]. In the extraction of polysaccharides from *G. lucidum,* the raw materials are treated first with 80% ethanol, under shaking, at 30 °C for 24 h, to remove free sugars (monosaccharides), polyphenolics, and lipophilic components [116, 127, 135]. Another composition of aqueous ethanol, i.e., 95%, has been reported in the pre-treatment of *G. lucidum* relatively at a higher temperature (70 °C), to improve the removal of endogenous enzymes and to reduce the long time required as compared to 80% ethanol at room temperature [120, 124].

9.3 Polysaccharides

They are the major contributors of bioactivity in *G. lucidum* with a wide range of physicochemical properties. Major class of polysaccharides are β-1,3 and β-1,6-D glucans. They exist in two forms, i.e., water-soluble and water-insoluble. Polysaccharides are of two types: extracellular (EPS) and intracellular (IPS). Exo-polysaccharides or extracellular polysaccharides are secreted by the microorganisms into the surrounding environment. Generally, IPS is extracted with 1 M NaOH at 60 °C for 1 h. Other solvents such as ammonium oxalate (1%) and NaOH (5%) could also be used for the extraction. EPS is usually extracted by precipitation with 3 to 4 volumes of ethanol of 95% [39]. To eliminate smaller molecules such as mono- and oligosaccharides, dialysis of the filtrate is sometimes preferred before applying precipitation using ethanol [76]. In case of solid-state fermentation (SSF), it is difficult to separate EPS from IPS as mycelia will be strongly bound with the substrate and EPS does not dissolve in the liquid phase. To recover the polysaccharides, the fermented substrate along with mycelia should be extracted with cold or hot water [41] for 5 h. However, such a method not only separates EPS and IPS produced by the organisms, but also some polysaccharides from the solid substrate. The resulted polysaccharides are analyzed by phenol-sulfuric acid method [136]. But it has been found out that the detection of polysaccharide by this method is not stable because along with polysaccharides the presence of some of the monosaccharides, oligosaccharides, and protein also give faulty positive result for phenol-sulfuric acid. Therefore, it is important to eliminate other molecules from the samples using techniques such as dialysis, gel exclusion chromatography, or column chromatography. Determination of proteins separately is also necessary to make sure the accuracy of phenol-sulfuric acid assay [39].

To isolate water-soluble polysaccharides from *G. lucidum*, the samples are extracted with hot water (95–100 °C). It is followed by three volumes of ethanol precipitation and the polysaccharide fractions are collected by centrifugation [77].Alkali-soluble polysaccharides are separated by 0.1–1.0 M sodium hydroxide [137, 138]. It has also been reported that to remove the lipids present in the sample, prior to the hot water or alkali extraction it is to be extracted first with ethanol under reflux [53]. This pretreatment also deactivates enzymes which hydrolyze the polysaccharides. Cheong et al. [75] reported that the biological activity of polysaccharide differs based on the types of extracting solvents as well as the fractionation methods employed.

The extracted polysaccharides are further isolated and purified by fractional precipitation, acidic precipitation, ion exchange chromatography, gel filtration, affinity chromatography, and TLC. Electrophoresis and gel filtration chromatography techniques are used as effective tools for determining the homogeneity and molecular weight. Paper chromatography, TLC, GC-MS, and HPLC are used for compositional analysis [72].

9.4 Triterpenoids

More than 130 oxygenated triterpenes have been isolated from *G. lucidum* [139] and most of them are lanostane-type triterpenes. There are two types of extraction methods for the isolation of triterpenes from *G. lucidum*. One involves the extraction of total triterpenes using the organic solvents and water. Min et al. [85, 140] extracted the triterpenes from spores of *G. lucidum* by refluxing with methanol. Extraction of triterpenes is usually done by means of methanol, ethanol, acetone, chloroform, ether, or a mixture of these solvents.

The second approach is the selective isolation of acidic triterpenes from the fraction of total triterpene. Extraction of fruiting bodies with 95% aqueous ethanol under reflux has been reported [141]. The obtained ethanol fraction was evaporated under reduced pressure to obtain a residue which was then suspended in water followed by extraction with chloroform. 5% $NaHCO_3$ was added to the chloroform extract and the water phase was collected which was again extracted with chloroform after acidified with HCl (6 N) to a pH less than 3 [142]. The collected residue after chloroform evaporation was suspended in absolute ethanol and spectrophotometric measurements were made at 245 nm to determine the Ganoderic acid [143]. Triterpenes could be further purified by silica gel column chromatography and preparative HPLC [144, 145].

10 Therapeutic Effects of Bioactive Compounds of *G. lucidum*

Being worshipped as a herbal medicine, a great deal of work has been carried out on the therapeutic applications of this special mushroom [14]. Medicinal use of *G. lucidum* has been recorded in ancient Chinese manuscripts. Extracts of this mushroom have been included in the treatment of insomnia, dizziness, chronic hepatitis, coronary heart diseases, hypertension, carcinoma, and bronchial cough. One of the promising properties shown by this wonder medicine is the extended lifespan while increasing the vitality. *G. lucidum* has been used in the fields of antiaging, male sexual dysfunction, hypercholesterolemia, chemotherapy induced toxicity, anticarcinogenic, narcotic-induced immunosuppression, antitumor activity, radioprotective, sleep promoting, antiviral, antifibrotic, antiulcer, radical-scavenging, and in the immunostimulation [2]. It is widely used as an active adjuvant in the treatment of carcinoma and related symptoms.

Sliva [146] indicated that in Western medicine one of the major obstacles in accepting the mushroom based natural products is their complexity. Alternatively, this complexity can also bring significant advantages. Some of the components in the natural products could reduce the cytotoxicity of cells, and the interaction between some of the biologically active components may be responsible for their in vivo effects. Besides, different compounds could modulate the unrelated signaling and thereby exhibiting a synergistic effect. The triterpenes of *G. lucidum* directly suppress the growth and invasive behavior of cancer cells, whereas polysaccharides of *G. lucidum* stimulate the immune system resulting in the production of cytokines and activation of anticancer activities of immune cells. The data obtained from the research studies demonstrate the effect of *G. lucidum* only on the molecular level, and thus preclinical and clinical studies are necessary for the validation of this natural product in the prevention and/or therapy of cancer. The important medicinal applications of *G. lucidum* are described below.

10.1 Anticancer Studies

Cancer is one the most concerned medical conditions among the human population and effective treatments are always sought which have least or no side effects. *Ganoderma lucidum* has been a popular supplement taken by healthy individuals to boost their immune system or by the cancer patients to reduce the side effects of chemotherapy. *Ganoderma lucidum* is considered to be a factory for bioactive compounds which can reduce the lethal effects of cancer. Fruiting body, mycelia, or spores are reported to contain these bioactive compounds. Polysaccharides and triterpenes are the two major classes of components in this mushroom which exhibit chemopreventive and/or tumoricidal effects as proved by numerous in vitro and in vivo studies [147]. Tumor implanted animal models have shown inhibitory effects on angiogenesis and metastasis. However, evidence from well-designed human trials is still scarce. Tomasi et al. [148] tested 58 basidiomycetes mushrooms, of which *G. lucidum* was shown to be the most effective in killing the cancer cells. *Ganoderma lucidum* induced cell-cycle arrest and apoptosis in various human and rodent tumor cells, including murine lymphocytic leukemia L1210 and Lewis lung carcinoma, have been reported [148].

Ganoderma lucidum also exhibits chemo-and radio preventive effects which are attributed to its effects on the immune system. *Ganoderma lucidum* extract showed better effect than Krestin (protein bound β-glucan isolated from *Coriolus versicolor*) in repairing the damage of subset T-cells in the spleen of irradiated mice [149]. One of the polysaccharide peptides from *G. lucidum* reported to restore the immunologic parameters depressed by morphine treatment beyond normal levels [150].

10.2 Antioxidant Activity

Antioxidants are natural or man-made substances which can prevent the activity of other chemicals known as free radicals that cause damage to the cells. *Ganoderma lucidum* is one such mushroom widely used due to its antioxidant activity. Consumption of antioxidants may help prevent cancer and other chronic diseases [151].Antioxidants protect cellular components from oxidative damage, decrease risk of mutations and carcinogenesis, and protect immune cells, allowing them to maintain immune surveillance and responses. The bioactive compounds such as polysaccharides and triterpenoids show antioxidant activity in vitro [58, 152, 153]. Antioxidants from *G. lucidum* were found to be absorbed quickly after ingestion, resulting in an increase in plasma total antioxidant activity [154].

Ooi and Liu [155] reported that protein-bound polysaccharide (PBP) and polysaccharide peptide could mimic the endogenous antioxidant superoxide dismutase (SOD) in cancer-bearing animals in vivo. These polysaccharides were also reported to protect the immune cells from oxidative damage. The protective effects of *G. lucidum* on DNA strand scission induced by a metal-catalyzed Fenton reaction, ultraviolet irradiation, and hydroxyl radical attack were shown in agarose gel electrophoresis in vitro [156]. Hot water extracts of *G. lucidum* significantly protected Raji cells from hydrogen peroxide (H_2O_2)-induced DNA damage [157]. The aqueous extract protected cellular DNA from oxidative damage, whereas the ethanolic extract damaged the cellular DNA with increased H_2O_2 production and significant cell-killing effects were observed. The results suggested that different effects of *G. lucidum* could be exhibited by different components of extract in bladder chemoprevention. Methanol extracts of *G. lucidum* were reported to prevent kidney damage (induced by the anticancer drug cisplatin) through restoration of the renal antioxidant defense system [158]. In contrast, a fraction of Ganoderma triterpenes (GTS) was found to enhance the intracellular reactive oxygen species (ROS) in HeLa cells, leading to more DNA damage and apoptosis, whereas such a synergism was inhibited by a ROS scavenger [159]. In an animal study (diabetic rats), nonenzymic and enzymic antioxidant levels increased and lipid peroxidation levels decreased with *G. lucidum* treatment [160]. However, a direct link has not been established between the antioxidant properties of *G. lucidum* and its immunomodulatory and anticancer effects.

10.3 Antiallergic Property

Another important application of the fruiting bodies of *G. lucidum* is their application as anti-inflammatory agents for the treatment of asthma or allergy. *Ganoderma lucidum* has unique array of compounds called immunonutraceuticals, which play a leading role in the treatment of histamine-mediated allergic responses [161]. *Ganoderma lucidum* is reported to be as an effective agent to restore the normal balance between the cytokines TH1 and TH2 immune states in patients with histamine-mediated allergic responses [162]. In a case study of hay fever patients, Powell found that patients (male) of age 5 and 39 with different doses viz. 2 tablets × 500 mg a day and 6 tablets × 500 mg per day of *G. lucidum* resulted in a decrease in drowsiness, itchiness and sneezing.

10.4 Antibacterial and Antiviral Activity

Ganoderma lucidum has been widely reported as a good antibacterial agent. Aqueous as well as methanolic extracts of *G. lucidum* inhibited several types of gram-positive and gram-negative bacteria. Studies indicated that a combination of *G. lucidum* extract with different antibiotics has resulted in an additive effect in most of the instances [163]. Active compounds such as triterpenes, ganomycein, and other aqueous extracts have broad spectrum antibacterial activity in vitro [39]. A 15-kDa antifungal protein ganodermin isolated from *G. lucidum* is known to inhibit the mycelia growth of *Botrytis cinerea*, *Fusarium oxysporum*, and *Physalospora piricola* with an IC 50 value of 15.2 μM, 12.4 μM, and 18.1 μM, respectively [65].

10.5 Antidiabetic Effect

Few animal studies of polysaccharide fractions of *G. lucidum* demonstrated that they have the potential hypoglycemic and hypolipidemic activities. The aqueous extract (1000 mg/kg) of *G. lucidum* normalized the blood glucose levels in alloxan-induced diabetes in Wistar rats [164]. Water extract of *G. lucidum* reduced the increase in blood glucose levels in rats following oral glucose test. A clinical study aimed at evaluating the antidiabetic efficacy and safety of polysaccharide fractions extracted from *G. lucidum* (Ganopoly) by a patented technique in 71 patients with confirmed type II diabetes mellitus has been reported by Gao et al. [109]. Treatment with Ganopoly significantly decreased the mean HbA1c from 8.4% at baseline to 7.6% at 12 weeks which demonstrated that Ganopoly is efficacious and safe in lowering the concentration of blood glucose.

11 Conclusions

Ganoderma lucidum is a well-known medicine with remarkable range of applications. Though the global consumption of *G. lucidum* is high, the production rate is not satisfactory. The bioactive compounds in *G. lucidum* make it very special and are classified under Host Defense Potentiators (HDP) which can have immune system enhancement properties. Various products such as capsules, creams, tonics, and syrups are available in the market, which offer high health benefits. Many researches have been performed in this mushrooms on its cultivational and medicinal aspects. Its positive health benefits include anticancer effects, antioxidant, antibacterial, blood glucose regulation, and antiviral effects and protection against liver and gastric injury. Though many studies are performed on animal models, successful studies on human cell models are least reported. Human experimental studies have often been small, and the results are not always supportive of the in vitro findings. Therefore, Ganoderma research on its clinical aspects need more supportive clarifications for the dosage and side effects in the human beings. Similarly, the mechanism of action of different bioactive molecules isolated from this mushroom is yet to be elucidated.

References

1. Wachtel-Galor S, Buswell JA, Tomlinson B, Benzie IFF (2004a) Lingzhi polyphorous fungus. In: Herbal and traditional medicine: molecular aspects of health. Marcel Dekker, New York, pp 179–228
2. Wasser SP (2005) Reishi or Lingzhi (*G. lucidum*). In: Coates P, Blackman MR, Cragg G, Levine M, Moss J, White J (eds) Encyclopaedia of dietary supplements. Marcel Dekker, New York, pp 603–622
3. Hou RH, Liao ST (2009) Research development of the artificial cultivation of *G. lucidum* in China. Guangdong Agric Sci 11:29–32. (In Chinese)
4. Hirose K, Niimura K, Ohara M, Oguchi Y, Matsunaga K, Kadochi J, Sugita N, Furushu T, Yoshikumi C (1988) Antiviral agent. J. P. Patent No.63316734

5. Boh B, Berovic M, Zhang J, Zhi-Bin L (2007) *G. lucidum* and its pharmaceutically active compounds. Biotechnol Annu Rev 13:265–301
6. Borchers AT, Stern JS, Hackman RM, Keen CL, Gershwin ME (1999) Mushrooms, tumours, and immunity. Proc Soc Exp Biol Med 221(4):281–293
7. Mau JL, Lin HC, Ma JT, Song SF (2001a) Non-volatile taste components of several specialty mushrooms. Food Chem 73:461–466
8. Joo SS, Ryu IW, Park JK, Yoo YM, Lee DH, Hwang KW, Choi HT, Lim CJ, Lee DI, Kim K (2008) Molecular cloning and expression of a laccase from *G. lucidum*, and its antioxidative properties. Mol Cell 25(1):112–118
9. Leung SWS, Yeung KY, Ricky YLS, Man YK (2002) Lingzhi (Ganoderma) research the past, present and future perspectives. In: Lin ZB (ed) Ganoderma: genetics, chemistry, pharmacology and therapeutics. Beijing medical University Press, Beijing, pp 1–9
10. Kim HW, Kim B (2002) Recent advances on the biologically active triterpenoids of *G. lucidum*. In: Zhi-Bin Lin (ed) Ganoderma: genetics, chemistry, pharmacology and therapeutics. Proceedings of international symposium on Ganoderma research, Shanghai, 21–23 Oct, Beijing, Medical University Press, pp 10–19
11. Chang ST, Buswell J (1999) *G. lucidum* (Curt.: Fr.) P. Karst. (Amphyllophoromycetidae) – a mushrooming medicinal mushroom. Int J Med Mush 1(2):139–146
12. Lin SC (2000) Medicinal fungi of China-production and products development. Chinese Agricultural Press, Beijing
13. Chang ST, Buswell JA (2008) Safety quality control and regulational aspects relating to mushroom nutriceuticals. In: Proceedings of 6th international conference on mushroom biology and mushroom products, GAMU Gmbh, Krefeld, pp 188–95
14. Wachtel-Galor S, Yuen J, Buswell JA, Benzie IFF (2011) Chapter 9: *G. lucidum* (Lingzhi or Reishi) a medicinal mushroom. In: Herbal medicine: biomolecular and clinical aspects, 2nd edn. CRC Press, Boca Raton
15. Wang JL, Li YB, Liu RM (2010) A new ganoderic acid from *G. lucidum* mycelia. J Asian Nat Prod Res 12(8):727–730
16. Hanaoka R, Ueno Y, Tanaka S (2011) The water-soluble extract from cultured medium of *G. lucidum* (Reishi) mycelia (Designated as MAK) ameliorates murine colitis induced by tri-nitrobenzene sulphonic acid. Scand J Immunol 74(5):454–462
17. Yang XJ, Liu J, Ye LB, Yang F, Ye L, Gao JR, Wu ZH (2006) In vitro and in vivo protective effects of proteoglycan isolated from mycelia of *G. lucidum* on carbon tetrachloride induced liver injury. World J Gastroenterol 12(9):1379–1385
18. Arora D (1986) Mushrooms demystified, 2nd edn. Ten Speed Press, Berkeley, pp 169–164. ISBN 0-89815. Available at http://www.mushworld.com/sub_en.html
19. Jungjing MA, Zhenjyi FU, Peiyan MA, Yanli SU, Qingjie Z (2007) Breaking and characteristics of *G. lucidum* spores by high speed centrifugal shearing pulveriser. J Wuhan Univ Technol Mat Sci Edit 22:617–621
20. Chen AW (1999) Cultivation of the medicinal mushroom *G. lucidum* (Curtis: Fr.) P. Karst (Reishi) in North America. Int J Med Mush 1(3):263–282
21. Jayasinghe C, Imtiaj A, Hur H, Lee GW, Lee TS, Lee UY (2008) Favourable culture conditions for mycelial growth of Korean wild strains in *G. lucidum*. Mycobiology 36(1):28–33
22. Magday JC Jr, Bungihan ME, Dulay RMR (2014) Optimization of mycelial growth and cultivation of fruiting body of Philippine wild strain of *G. lucidum*. Curr Res Environ Appl Mycol 4(2):162–172
23. Han JR, An CH, Yuan JM (2005) Solid-state fermentation of cornmeal with the basidiomycete *G. lucidum* for degrading starch and upgrading nutritional value. J Appl Microbiol 99(4):910–915
24. Erkel EI (2009) The effect of different substrate mediums on yield of *G. lucidum* (Fr.) Karst. J Food Agric Environ 7:841–844
25. Royse DJ (1996) Specialty mushrooms. In: Janick J (ed) Progress in new crops. ASHS Press, Arlington, pp 464–475

26. Chen AW (2003) A fresh look at an ancient mushroom *G. lucidum* (Reishi). MushWorld – Cultivation, 18 Mar 2003. Available at http://www.mushworld.com
27. Gonzalez-Matute R, Figlas D, Devalis R, Delmastro S, Curvetto N (2002) Sunflower seed hulls as a main nutrient source for cultivating *G. lucidum*. Micol Apl Int 14:19–24
28. Yang FC, Hsieh C, Chen HM (2003a) Use of stillage grain from a rice-spirit distillery in the solid state fermentation of *G. lucidum*. Process Biochem 39:21–26
29. Hsieh C, Yang F (2004) Reusing soy residue for the solid-state fermentation of *G. lucidum*. Bioresour Technol 91(1):105–109
30. Chen AW (2004) Growing *Ganoderma* mushrooms. Chapter 11. Mushrooms for the tropics. Part III. Mushroom worldwide. In: Mushroom grower's handbook 1, Oyster Mushroom Cultivation. pp 224–234. Available at https://www.scribd.com/document/265831668/mushroom-growers-handbook-1-mushworld-com-chapter-11-pdf
31. Kim HK (2001) Comparison of characteristics of *G. lucidum* according to geographical origins: consideration of growth characteristics. World Mushroom. Available at http://www.mushworld.com/sub_en.html
32. Zhou XW, Siu K, Zhang YM (2012) Applied modern biotechnology for cultivation of Ganoderma and development of their products. Appl Microbiol Biotechnol 93:941–963
33. Xia ZN, Jiang JA, He CZ, Liu DY (2003) Preliminary researchers on high yield cultivation techniques of Ganoderma. Hunan Agric Sci Technol 6:56–58
34. Yan MH (2000) Cultivation of *G. lucidum* using oak leaf and waste tea. Edible Fungi 2:22–23
35. Han XH, Wang HZ, He B (2003) A preliminary study on nutritional conditions for the strain mycelium growth of several cultivars of Ganoderma. J Hainan Univ 16:88–92
36. Wu BF, Liu LL, Fang ZH, Liu XY (2008) Effect of nutrition factors on mycelium growth of 51427 in *G. lucidum*. Anhui Agric Sci Bull 14:57–58
37. Maldonado MC, Saad AMS (1998) Production of pectinesterase and polygalacturonase by *Aspergillus niger* in submerged and solid state systems. J Ind Microbiol Biotechnol 20:34–38
38. Mahapatra S, Banerjee D (2009) Extracellular tannase production by endophytic Hyalopus sp. J Gen Appl Microbiol 55:255–259
39. Sanodiya BS, Takur GS, Baghel RK, Prasad GB, Bisen PS (2009) *G. lucidum*: a potent pharmacological macrofungus. Curr Pharm Biotechnol 10(8):717–742
40. Pandey A, Soccol CR, Mitchell D (2000) New developments in solid state fermentation: I- bioprocesses and products. Process Biochem 35:1153–1169
41. Habijanic J, Berovic M (2000) The relevance of solid-state substrate moisturing on *G. lucidum* biomass cultivation. Food Technol Biotechnol 38(3):225–228
42. Zhang W, Tang YJA (2008) Novel three-stage light irradiation strategy in the submerged fermentation of medicinal mushroom *G. lucidum* for the efficient production of ganoderic acid and Ganoderma polysaccharides. Biotechnol Prog 24:1249–1261
43. Zhuang Y, Hong J (2006) Two-way pattern solid fermentation engineering in medicinal mushrooms and further development of Chinese medicine residue. Zhong Yao Cai 31:1918–1919
44. Zhuang Y, Pan Y, Xie XM, Zhang LY (2007) The origin, development and its advantage and potential of the bi-directional solid fermentation for medicinal fungi. Edible Fungi China 26:3–6
45. Chen HZ, Chen J (2004) A preliminary report on solid-state fermentation of *G. lucidum* with *Radix astragali* containing medium. Chin J Integr Med 2:216–218
46. Gu SR, Qin JZ, Chen H (2005) New solid fermentation of 14 Ganoderma strains and the study on polysaccharose content of the fermentation product. Lishizhen Med Mater Med Res 16:313–314
47. Gao Y, Gao H, Chan E, Tang W, Xu A, Yang H, Huang M, Lan J, Li X, Duan W, Xu C, Zhou S (2005) Antitumor activity and underlying mechanisms of ganopoly, the refined polysaccharides extracted from *G. lucidum* in mice. Immunol Investig 34:171–198
48. You JJ (2009) The present status and future aspect of medicinal fungi in China. Edible Fungi Chin 28:3–5

49. Mckenna DJ, Jones K, Hughes K (2002) Reishi, botanical medicines. The desk reference for major herbal supplements, 2nd edn. The Haworth Herbal Press, New York/London/Oxford, pp 825–855
50. Paterson RRM (2006) Ganoderma – a therapeutic fungal biofactory. Phytochemistry 67(18):1985–2001
51. Zheng WF (2011) Drug discovery from fungal metabolites: a review of the papers in this monographical issue of mycosystema concerned with the natural resources, problems and strategies. Mycosystema 30:151–157
52. Bao XF, Dong Q, Fang JN (2000) Structure and conformation behaviour of a glucan from spores of *G. lucidum* (Fr.) Karst. Sheng Wu Hua Xue Yu Sheng Wu Wu Li Xue Bao (Shanghai) 32(6):557–561
53. Bao XF, Liu CP, Fang JN, Li XY (2001) Structural and immunological studies of a major polysaccharide from spores of *G. lucidum* (Fr.) Karst. Carbohydr Res 332:67–74
54. Bao XF, Wang XS, Dong Q (2002) Structural features of immunologically active polysaccharides from *G. lucidum*. Phytochemistry 59(2):175–181
55. Ho YW, Yeung JS, Chiu PK, Tang WM, Lin ZB, Man RY, Lau CS (2007) *G. lucidum* polysaccharide peptide reduced the production of proinflammatory cytokines in activated rheumatoid synovial fibroblast. Mol Cell Biochem 301(1–2):173–179
56. Li Z, Liu J, Zhao Y (2005) Possible mechanism underlying the antiherpetic activity of a proteoglycan isolated from the mycelia of *G. lucidum* in vitro. J Biochem Mol Biol 38(1):34–40
57. Ji Z, Tang Q, Zhang J, Yang Y, Jia W, Pan Y (2007) Immunomodulation of RAW264.7 macrophages by GLIS, a proteopolysaccharide from *G. lucidum*. J Ethnopharmacol 112(3):445–450
58. Wu Y, Wang D (2009) A new class of natural glycopeptides with sugar moiety-dependent antioxidant activities derived from *G. lucidum* fruiting bodies. J Proteome Res 8:436–442
59. Chien CM, Cheng JL, Chang WT (2004) Polysaccharides of G. lucidum alter cell immunophenotypic expression and enhance CD56+ NK-cell cytotoxicity in cord blood. Bioorg Med Chem 12:5603–5609
60. Dong SF, Chen JM, Zhang W, Sun SH, Wang J, Gu JX, Boraschi D, Qu D (2007) Specific immune response to HBsAg is enhanced by β-glucan oligosaccharide containing α-(1→3)-linked bond and biased towards M2/Th2. Int Immunopharmacol 7:725–733
61. Fatmawati S, Kondo R (2011) Structure-activity relationships of ganoderma acids from *G. lucidum* as aldose reductase inhibitors. Bioorg Med Chem Lett 21(24):7295–7297
62. Weng CJ, Chau CF, Chen KD, Chen DH, Yen GC (2007) The anti-invasive effect of lucidenic acids isolated from a new *G. lucidum* strain. Mol Nutr Food Res 51:1472–1477
63. Gao JI, Leung KS, Wang YT, Lai CM, Li SP, Hu LF, Lu GH, Jiang ZH, Yu ZL (2007) Qualitative and quantitative analyses of nucleosides and nucleobases in Ganoderma spp. by HPLC-DAD-MS. J Pharm Biomed Anal 44(3):807–811
64. Van Der Hem LG, Van Der Vliet JA, Bocken CF, Kino K, Hoitsma AJ, Tax WJ (1995) Lingzhi-8: studies of a new immunomodulating agent. Transplantation 60:438–443
65. Wang H, Ng TB (2006) Ganodermin, an antifungal protein from fruiting bodies of the medicinal mushroom *G. lucidum*. Peptides 27(1):27–30
66. Kim SD, Nho HJ (2004) Isolation and characterization of alpha-glucosidase inhibitor from the fungus *G. lucidum*. J Microbiol 42(3):223–227
67. Liu JJ, Huang WH, Lv ML, Si JP, Guo BL, Li SJ (2011) Determination of Ergosterol in *G. lucidum* from different varieties and cultured tree species by HPLC. Zhong Yao Cai 34:187–190
68. Gao P, Hirano T, Chen Z, Yasuhara T, Nakata Y, Sugimoto A (2012) Isolation and identification of C-19 fatty acids with anti-tumor activity from the spores of *G. lucidum* (Reishi mushroom). Fitoterapia 83:490–499
69. Fukuzawa M, Hide I, Chen Z, Hirai Y, Sugimoto A, Yasuhara T, Nakata Y (2008) Possible involvement of long chain fatty acids in the spores of *G. lucidum* (Reishi Houshi) to its anti-tumor activity. Biol Pharm Bull 31:1933–1937

70. Matute RG, Serra A, Figlas D, Curvetto N (2011) Copper and zinc bioaccumulation and bioavailability of *G. lucidum*. J Med Food 14(10):1273–1279
71. Falandysz J (2008) Selenium in edible mushrooms. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 26(3):256–299
72. Huie CW, Di X (2004) Chromatographic and electrophoretic methods for Lingzhi pharmacologically active components. J Chromatogr B 812:241–257
73. Chang YW, Lu T (2004) Molecular characterization of polysaccharides in hot water extracts of *G. lucidum* fruiting bodies. J Food Drug Anal 12:59–67
74. Gao Y, Zhou S, Chen G, Dai X, Ye J, Gao H (2002) A phase I/II study of a *G. lucidum* (Curt.: Fr.) P. Karst. (Lingzhi, Reishi mushroom) extract in patients with chronic hepatitis B. Int J Med Mush 4:321–327
75. Cheong J, Jung W, Park W (1999) Characterization of an alkali-extracted peptidoglycan from Korean *G. lucidum*. Arch Pharm Res 22:515–519
76. Fang QH, Zhong JJ (2002a) Effect of initial pH on production of ganoderic acid and polysaccharide by submerged fermentation of *G. lucidum*. Process Biochem 37:769–774
77. Wang YY, Chen ST, Lin CC, Wong CH, Lin CH (2002) Studies on the immunomodulation and antitumor activities of *G. lucidum* (Reishi) polysaccharides: functional and proteomic analysis of fucose-containing glycoprotein fraction responsible for the activities. Bioorg Med Chem 10:1057–1062
78. Lin SQ, Wang SZ, Lin ZB, Lin YX (2002) Purification and identification of glycopeptides from *G. lucidum* fruit bodies cultivated with grass and wood log. In: Zhi-Bin Lin (ed) Ganoderma: genetics, chemistry, pharmacology and therapeutics. Proceedings of international symposium on Ganoderma research, Shanghai, 21–23 Oct, Beijing, Medical University Press, pp 109–114
79. Zhang QH, Lin ZB (1999) Effect of G. lucidum polysaccharides B on TNF-a and INF-g production and their mRNA expression. Beijing Yi Ke Da Xue Xue Bao 31:179–183
80. Ooi LSM, Ooi VEC, Fung MC (2002) Induction of gene expression of immunomodulatory cytokines in the mouse by a polysaccharide from *G. lucidum* (Curt.: Fr.) P. Karst. (Aphyllophoromycetideae). Int J Med Mush 4:27–35
81. Kidd PH (2000) The use of mushroom glucans and proteoglycans in cancer treatment. Altern Med Rev 5:4–27
82. Kang YK, Yoon SI, Lee SY, Kim DD, Lee SJ, Park WH, Hudson SM (2010) Chitosan-coated poly (vinyl alcohol) nanofibers for wound dressings. J Biomed Mater Res Part B Appl Biomater 92(2):568–576
83. Wasser SP, Weis AL (1997) Reishi mushroom [*G. lucidum* (Curt.: Fr.) P. Karst.] In: Nevo E (ed) Medicinal mushrooms. Peledfus, Haifa, p 39
84. Hobbs CH (1995) Medicinal mushrooms: an exploration of tradition, healing and culture, 2nd edn. Botanica Press, Santa Cruz, p 252
85. Min BS, Gao JJ, Hattori M, Lee HK, Kim YH (2001) Anticomplement activity of terpenoids from the spores of *G. lucidum*. Planta Med 67(9):811–814
86. Luo J, Lin ZB (2002) Advances of pharmacological effects of triterpenes from *G. lucidum*. Acta Pharm Sin B 37:574–578
87. Frankel EN (1997) Nutritional benefits of flavonoids. In: Ohigashi H, Osawa T, Terao J, Watanabe S, Yoshikawa T (eds) Food factors for cancer prevention. Springer, Tokyo, pp 6l3–616
88. Decker EA (1997) Phenolics: prooxidants or antioxidants? Nutr Rev 55:396–407
89. Mau JL, Lin HC, Song SF (2002) Antioxidant properties of several specialty mushrooms. Food Res Int 35(6):519–526
90. Kumari K, Prakash V, Rana S, Sagar A (2016) In vitro antioxidant activity of methanolic extract of *G. lucidum* (Curt.) P. Karst. IJASR 1(5):51–54
91. Heleno SA, Barros L, Martins A, Ferreira ICFR (2012) Fruiting body spores and in vitro produced mycelium of *G. lucidum* from Northeast Portugal: a comparative study of the antioxidant potential of phenolic and polysaccharidic extracts. Food Res Int 46:135–140

92. Hajjaj H, Mace M, Roberts M, Niederberger P, Fay LB (2005) Effect of 26-oxygenosterols from *G. lucidum* and their activity as cholesterol synthesis inhibitors. Appl Environ Microbiol 71:3653–3658
93. Zhang CR, Yang SP, Yue JM (2008) Sterols and triterpenoids from the spores of *G. lucidum*. Nat Prod Res 22(13):1137–1142
94. Lv GP, Zhao J, Duan JA, Tang YP, Li SP (2012) Comparison of sterols and fatty acids in two species of Ganoderma. Chem Cent J 6(1):10
95. Xu XF, Yan HD, Chen J, Zhang XW (2011) Bioactive proteins from mushrooms. Biotechnol Adv 29:667–674
96. Kawagishi H, Yamawaki M, Ido M, Shimada A, Kinoshita T, Murata T, Usui T, Kimura A, Chiba S (1997) A lectin from mycelia of the fungus *G. lucidum*. J Phytochem 44:7–10
97. Li YG, Ji DF, Zhong S, Zhu JX, Chen S, Hu GY (2011a) Anti-tumor effects of proteoglycan from *Phellinus linteus* by immunomodulating and inhibiting Reg IV/EGFR/Akt signalling pathway in colorectal carcinoma. Int J Biol Macromol 48:511–517
98. Kino K, Yamashita A, Yamaoka K, Watanabe J, Tanaka S, Ko K, Shimizu K, Tsunoo H (1989) Isolated and characterization of a new immunomodulatory protein Lingzhi-8 (LZ-8), from *G. lucidum*. J Biol Chem 264:472–478
99. Li QZ, Wang XF, Zhou XW (2011b) Recent status and prospects of the fungal immunomodulatory protein family. Crit Rev Biotechnol 31:365–375
100. Tsai LL (2007) Immunomodulatory protein cloned from *G. microsporum*. US Patent US 7601808 B2
101. Huang J, Zhang LX, Hu SN, Yu LJ (2008) Optimizing expression of LZ-8 protein and primary assaying of its immunomodulatory activity. Zhejiang Univ (Agric & Life Sci) 34:7–12
102. Shimizu A, Yano T, Saito Y, Inada Y (1985) Isolation of an inhibitor of platelet aggregation from a fungus, *G. lucidum*. Biol Pharm Bull 33(7):3012–3015
103. Gao Y, Zhou SH, Huang M, Xu A (2003) Antibacterial and antiviral value of the genus Ganoderma P. Karst. species (Aphyllophoromycetideae): a review. Int J Med Mush 5(3):235–246
104. Hardy S, Przybytkowski E, Joly E, Prentki M, Langelier Y (2003) Saturated fatty acid-induced apoptosis in MDA-MB-231 breast cancer cells. A role for cardiolipin. J Biol Chem 278:31861–31870
105. Ihayere CA, Oghenekaro AO, Osemwegie OO, Okhuoya JA (2010) Chemical nature of *G. lucidum* (Curtis) Karsten from woodlands of Edo state, Nigeria. C J Biol Sci 3:8–15
106. Mizuno T (1995) *G. lucidum* and *G. tsugae*: bioactive substances and medicinal effects. Food Rev Int 11(1):151–166
107. Stamets P (2000) Growing gourmet and medicinal mushrooms, 3rd edn. Ten Speed Press, Berkeley, p 339
108. Chen DH, Shiou WY, Wang KC, Hwang SY, Shie YT, Tsai CM, Shie JF, Chen KD (1999) Chemotaxonomy of triterpenoid pattern of HPLC of *G. lucidum* and *G. tsugae*. J Chin Inst Chem 46:47–51
109. Gao JJ, Nakamura N, Min BS, Hirakawa A, Zuo F, Hattori M (2004) Quantitative determination of bitter principles in specimens of *G. lucidum* using high-performance liquid chromatography and its application to the evaluation of Ganoderma products. Chem Pharm Bull 52(6):688–695
110. Liu GT (1999) Recent advances in research of pharmacology and clinical applications of Ganoderma P. Karst. species (Aphyllophoromycetideae) in China. Int J Med Mush 1(1):63–68
111. Chiu SW, Wang ZM, Leung TM, Moore D (2000) Nutritional value of Ganoderma extract and assessment of its genotoxicity and antigenotoxicity using comet assays of mouse lymphocytes. Food Chem Toxicol 38:173–178
112. Kolesnikova OP, Tuzova MN, Kozlov VA (1997) Screening of immunoactive properties of alkanecarbonic acid derivatives and germanium-organic compounds in vivo. Immunologiya 10:36–38
113. Li P, Yi L, Liu HJ (2011) Chapter 2: Collection and identification of raw herbal materials. In: Liu JH (ed) Traditional herbal medicine research methods: identification, analysis,

bioassay, and pharmaceutical and clinical studies. John Wiley & Sons, Inc., Hoboken, New Jersey, pp 27–79

114. Krishnaswamy N (2010) Chemistry of natural products: the terpenes. Taylor & Francis Group, New York, pp 1–10
115. Kim DO, Lee CY (2003). Extraction and isolation of polyphenolics. In: Current protocols in food analytical chemistry, I:I1:I1.2 (Online)
116. Fan L, Li J, Deng K, Ai L (2012) Effects of drying methods on the antioxidant activities of polysaccharides extracted from *G. lucidum*. Carbohydr Polym 87(2):1849–1854
117. Liu GQ, Zhao Y, Wang XL, Zhu CY (2011) Response surface methodology for optimization of polysaccharides extraction by mild alkaline hydrolysis from fruiting body of medicinal mushroom, *G. lucidum*. J Med Plant Res 5(10):2064–2070
118. Chin SK, Law C, Cheng P (2011) Effect of drying on crude ganoderic acids and water soluble polysaccharides content in *G. lucidum*. Int J Pharm Pharm Sci 3:38–43
119. Chin SK, Law CL, Supramaniam CV, Cheng PG (2009) Thin-layer drying characteristics and quality evaluation of air-dried *Ganoderma tsugae* Murrill. Dry Technol 27(9):975–984
120. Huang SQ, Ning ZX (2010) Extraction of polysaccharide from *Ganoderma lucidum* and its immune enhancement activity. Int J Biol Macromol 47(3):336–341
121. Liu W, Xu J, Jing P, Yao W, Gao X, Yu L (2010) Preparation of a hydroxypropyl *G. lucidum* polysaccharide and its physicochemical properties. Food Chem 122(4):965–971
122. Chen W, Wang WP, Zhang HS, Huang Q (2012) Optimization of ultrasonic-assisted extraction of water-soluble polysaccharides from *Boletus edulis* mycelia using response surface methodology. Carbohydr Polym 87(1):614–619
123. Wen L, Lin L, You L, Yang B, Jiang G, Zhao M (2011) Ultrasound-assisted extraction and structural identification of polysaccharides from *Isodon lophanthoides* var. gerardianus (Bentham) H. Hara. Carbohydr Polym 85(3):541–547
124. Zhao Q, Kennedy JF, Wang X, Yuan X, Zhao B, Peng Y et al (2011) Optimization of ultrasonic circulating extraction of polysaccharides from *Asparagus officinalis* using response surface methodology. Int J Biol Macromol 49(2):181–187
125. Chen X, Wang W, Li S, Xue J, Fan L, Sheng Z et al (2010) Optimization of ultrasound-assisted extraction of Lingzhi polysaccharides using response surface methodology and its inhibitory effect on cervical cancer cells. Carbohydr Polym 80(3):944–948
126. Hromadkova Z, Ebringerova A, Valachovič P (1999) Comparison of classical and ultrasound-assisted extraction of polysaccharides from *Salvia officinalis L*. Ultrason Sonochem 5(4):163–168
127. Huang SQ, Li JW, Wang Z, Pan HX, Chen JX, Ning ZX (2010) Optimization of alkaline extraction of polysaccharides from *G. lucidum* and their effect on immune function in mice. Molecules 15(5):3694–3708
128. Xu DJ, Xia Q, Wang JJ, Wang PP (2008) Molecular weight and monosaccharide composition of *Astragalus polysaccharides*. Molecules 13(10):2408–2415
129. Zhao L, Dong Y, Chen G, Hu Q (2010) Extraction, purification characterization and antitumor activity of polysaccharides from *G. lucidum*. Carbohydr Polym 80:783–789
130. Wakte P, Sachin B, Patil A, Mohato D, Band T, Shinde D (2011) Optimization of microwave, ultra-sonic and supercritical carbon dioxide assisted extraction techniques for curcumin from *Curcuma longa*. Sep Purif Technol 79(1):50–55
131. Claver IP, Zhang H, Li Qin Z, Huiming Z (2010) Optimization of ultrasonic extraction of polysaccharides from Chinese malted sorghum using response surface methodology. Pak J Nutr 9(4):336–342
132. Kozarski M, Klaus A, Nikšić M, Vrvić MM, Todorović N, Jakovljević D, Van Griensven LJ (2012) Antioxidative activities and chemical characterization of polysaccharide extracts from the widely used mushrooms *G. applanatum*, *G. lucidum*, *Lentinus edodes* and *Trametes versicolor*. J Food Compos Anal 26(1):144–153
133. Cheung YC, Siu KC, Liu YS, Wu JY (2012) Molecular properties and antioxidant activities of polysaccharide–protein complexes from selected mushrooms by ultrasound-assisted extraction. Process Biochem 47(5):892–895

134. Azmi AF, Mustafa S, Hashim DM, Manap YA (2012) Prebiotic activity of polysaccharides extracted from *Gigantochloa levis* (Buluh beting) shoots. Molecules 17(2):1635–1651
135. Li YQ, Fang L, Zhang KC (2007) Structure and bioactivities of a galactose rich extracellular polysaccharide from submergedly cultured *G. lucidum*. Carbohydr Polym 68(2):323–328
136. Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350–356
137. Miyazaki T, Nishijima M (1982) Structural examination of an alkali-extracted, water-soluble hetero glycan of the fungus *G. lucidum*. Carbohydr Res 109(1):290–294
138. Sone Y, Okuda R, Wada N, Kishida E, Misaki A (1985) Structure and antitumor activities of the polysaccharide isolated from fruiting body and the growing culture of mycelium of *G. lucidum*. Agric Biol Chem 49:2641–2653
139. Kim HW, Kim BK (1999) Biomedical triterpenoids of *G. lucidum* (Curt.: Fr.) P. Karst. (Aphyllophoromycetideae). Int J Med Mush 1(2):121–138
140. Min BS, Nakamura N, Miyashiro H et al (1998) Triterpenes from the spores of G lucidum and their inhibitory activity against HIV-1 protease. Chem Pharm Bull 46(10):1607–1612
141. Fang QH, Zhong JJ (2002b) Submerged fermentation of higher fungus *G. lucidum* for production of valuable bioactive metabolites – ganoderic acid and polysaccharide. Biochem Eng J 10:61–65
142. Ma J, Ye Q, Hua Y, Zhang D, Cooper R, Chang MN, Chang JY, Sun HH (2002) New lanostanoids from the mushroom *G. lucidum*. J Nat Prod 65:72–75
143. Tang YJ, Zhong JJ (2002) Exopolysaccharide biosynthesis and related enzyme activities of the medicinal fungus, *G. lucidum*, grown on lactose in a bioreactor. Biotechnol Lett 24:1023–1026
144. Kikuchi T, Kanomi S, Kadota S, Murai Y, Tsubono K, Ogita Z (1986) Constituents of the fungus *G. lucidum* (Fr.) Karst. I. Structures of ganoderic acids C2, E, I, and K, lucidenic acid F and related compounds. Chem Pharm Bull 34:3695–3712
145. Hirotani MA, Furaya T, Shiro M (1985) A ganoderic acid derivative, a highly oxygenated lanostane-type triterpenoid from *G. lucidum*. Phytochemistry 24(9):2055–2061
146. Sliva D (2004) Cellular and physiological effects of *G. lucidum* (Reishi). Mini-Rev Med Chem 4:873–879
147. Yuen JW, Gohel MD (2005) Anticancer effects of *G. lucidum*: a review of scientific evidence. Nutr Cancer 53:11–17
148. Tomasi S, Lohezic-Le DF, Sauleau P, Bezivin C, Boustie J (2004) Cytotoxic activity of methanol extracts from Basidiomycete mushrooms on murine cancer cell lines. Pharmazie 59:290–293
149. Chen WC, Hau DM, Wang CC, Lin IH, Lee SS (1995) Effects of *G. lucidum* and krestin on subset T-cell in spleen of gamma-irradiated mice. Am J Chin Med 23:289–298
150. Lu ZW (1995) Psycho-neuroimmunological effects of morphine and the immunoprotection of Ganoderma polysaccharides peptide in morphine dependent mice. Chin J Phys 26:45–49
151. Benzie IFF, Wachtel-Galor S (2009) Biomarkers of long-term vegetarian diets. Adv Clin Chem 47:169–220
152. Yuen JW, Gohel MD (2008) The dual roles of Ganoderma antioxidants on urothelial cell DNA under carcinogenic attack. J Ethnopharmacol 118:324–330
153. Saltarelli R, Ceccaroli P, Lotti M, Zambonelli A, Buffalini M, Casadei L, Vallorani L, Stocchi V (2009) Biochemical characterisation and antioxidant activity of mycelium of *G. lucidum* from Central Italy. Food Chem 116:143–151
154. Wachtel-Galor S, Szeto YT, Tomlinson B, Benzie FIF (2004b) *G. lucidum* (Lingzhi): acute and short-term biomarker response to supplementation. Int J Food Sci Nutr 1:75–83
155. Ooi VEC, Liu F (2000) Immunomodulation and anti-cancer activity of polysaccharide–protein complexes. Curr Med Chem 7:715–729
156. Lee JM, Kwon H, Jeong H, Lee JW, Lee SY, Baek SJ, Surh YJ (2001) Inhibition of lipid peroxidation and oxidative DNA damage by *G. lucidum*. Phytother Res 15:245–249

157. Shi YL, James AE, Benzie IF, Buswell JA (2002) Mushroom-derived preparations in the prevention of H_2O_2-induced oxidative damage to cellular DNA. Teratog Carcinog Mutagen 22:103–111
158. Sheena M, Ajith A, Janardhanan K (2003) Prevention of nephrotoxicity induced by the anticancer drug cisplatin, using *G. lucidum*, a medicinal mushroom occurring in South India. Curr Sci 85:478–482
159. Yue QX, Xie FB, Guan SH, Ma C, Yang M, Jiang BH, Liu X, Guo DA (2008) Interaction of Ganoderma triterpenes with doxorubicin and proteomic characterization of the possible molecular targets of Ganoderma triterpenes. Cancer Sci 99:1461–1470
160. Jia J, Zhang XI, Hu YS, Wu Y, Wang QZ, Li NN, Guo QC, Dong XC (2009) Evaluation of in vivo antioxidant activities of *G. lucidum* polysaccharides in STZ-diabetic rats. Food Chem 115:32–36
161. Calder PC (2003) Immunonutrition: may have beneficial effects in surgical patients. BMJ 327(7407):117–118
162. Powell M (2006) *G. lucidum* (Reishi) in the management of histamine-mediated allergic responses. Townsend Letter pp 78–81
163. Yoon SY, Eo SK, Kim YS, Lee CK, Han SS (1994) Antimicrobial activity of *G. lucidum* extract alone and in combination with some antibiotics. Arch Pharm Res 17:438–442
164. Mohammed A, Adelaiye AB, Abubakar MS, Abdurahman EM (2007) Effects of aqueous extract of *G. lucidum* on blood glucose levels of normoglycemic and alloxan induced diabetic wistar rats. J Med Plant Res 1:34–37