Ganoderma lucidum: A Potent Pharmacological Macrofungus

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Abstract: *Ganoderma lucidum* (Ling Zhi) is a basidiomycete white rot macrofungus which has been used extensively as "the mushroom of immortality" in China, Japan, Korea and other Asian countries for 2000 years. A great deal of work has been carried out on therapeutic potential of *Ganoderma lucidum*. The basidiocarp, mycelia and spores of *Ganoderma lucidum* contain approximately 400 different bioactive compounds, which mainly include triterpenoids, polysaccharides, nucleotides, sterols, steroids, fatty acids, proteins/peptides and trace elements which has been reported to have a number of pharmacological effects including immunomodulation, anti-atherosclerotic, anti-inflammatory, analgesic, chemo-preventive, antitumor, chemo and radio protective, sleep promoting, antibacterial, antiviral (including anti-HIV), hypolipidemic, anti-fibrotic, hepatoprotective, anti-diabetic, anti-androgenic, anti-angiogenic, anti-herpetic, antioxidative and radical-scavenging, anti-aging, hypoglycemic, estrogenic activity and anti-ulcer properties. *Ganoderma lucidum* has now become recognized as an alternative adjuvant in the treatment of leukemia, carcinoma, hepatitis and diabetes. The macrofungus is very rare in nature rather not sufficient for commercial exploitation for vital therapeutic emergencies, therefore, the cultivation on solid substrates, stationary liquid medium or by submerged cultivation has become an essential aspect to meet the driving force towards the increasing demands in the international market. Present review focuses on the pharmacological aspects, cultivation methods and bioactive metabolites playing a significant role in various therapeutic applications.

Keywords: *Ganoderma lucidum*, immune-modulation, anti-tumor, anti-HIV, submerged fermentation, polysaccharides, ganoderic acid.

1. INTRODUCTION

One of the most apparent influences modern times have brought to people is their realization of going back to the basics, to the natural and to the organic. The stress and pressure of modern society take a toll on the body's immune system, which is especially important for ill people, whose weakened immunity is more susceptible to infection and disease. Although the advances brought by technology has made life easier to people, many are still looking for better organic alternatives that are proven to be more effective in their most natural form, like *Ganoderma lucidum*, a fungus known by its many names like "Reishi," "Ling Zhi," and "Mannentake," for hundreds or even thousands of years, is recognized as powerful medicinal fungi because it has properties often associated with health and healing, long life, knowledge and happiness [1]. In fact, during the ancient time, it is believed that the *G. lucidum* in medicine was considered so promising that its medicinal value has been attested in a 2,000-year old Chinese medical text (*Shen Nong's Herbal Classic*) known as an authentic textbook of Oriental medical science [2, 3]. *G. lucidum* has been worshipped as a kind of herbal medicine, the emperors of the great Japanese and Chinese dynasties drank with their special teas and mushroom concoctions to achieve greater vitality and longer life. *G. lucidum* was also believed to be visible in the "elixir of eternal youth" that the ancient Taoists constantly searched for. Aside from contributing a lot to the treatment of various diseases, *G. lucidum* has also become popular because of its promising properties that might extend life span while increasing vigor and vitality [3].

Some actions and properties of *Ganoderma lucidum* include; antiallergin, antioxidant, analgesic, antifungal, anti-inflammatory, antitumor, antiviral, antiparasitic, cardiovascular, antidiabetic, immunomodulating, hepatoprotective, hypotensive and hypertensive, kidney and nerve tonic, sexual potentiator, bronchitis prevention and inhibits platelets aggregations, lowers: blood pressure, cholesterol and blood sugar [4-16]. The pharmacological effect of *G. lucidum* is based on their powerful immune-modulating action and immune potential capability, which support and enhance the over all immune function, due to the presence of more than 200 active elements which can be categorized into water soluble, organic soluble and volatile soluble compounds [11-17]. The major elements include polysaccharide, polysaccharide-peptide complex, β-glucans, lectins, organic germanium (Ge), adenosine, triterpenoids and nucleosides each having their own outstanding medicinal effects [9, 17, 18]. Because of its presumed health benefits and apparent absence of side-effects, it has attained a reputation in the east as the ultimate herbal substance. Ling Zhi has now been added to the American Herbal Pharmacopoeia and Therapeutic Compendium.

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2. THERAPEUTIC APPLICATIONS OF *GANODERMA LUCIDUM*

Ganoderma lucidum has been reported to have a number of pharmacological effects including immuno-modulating, anti-atherosclerotic, anti-inflammatory, analgesic, chemo-preventive, anti-tumor, radio-protective, sleep-promoting, anti-bacterial, anti-viral (including anti-HIV), hypo-lipidemic, anti-fibrotic, hepato-protective, anti-diabetic, antioxidative and radical-scavenging, anti-aging, hypoglycemic and antiulcer properties [4-13, 15, 16, 19]. Reishi has now become recognized as an alternative adjuvant in the treatment of leukemia, carcinoma, hepatitis and diabetes [6-11, 15, 16, 19-22]. Since the last decade clinical trials on the use of *G. lucidum* preparations to treat the cancer and other diseases have been reported in international peer-reviewed journals. A summary of the therapeutic effects and bioactive compounds of *G. lucidum* reported in the literature till 2009 has been presented in Table **1**.

2.1. Activity Against Human Immuno Virus (HIV)

Now there are an estimated 42 million people living with HIV or AIDS worldwide and more than 3 million die every year from AIDS-related illnesses. Acquired Immuno-Deficiency Syndrome (AIDS) caused by HIV infection has become an important social and medical problem [23]. Anti-HIV therapy by nucleoside analogues, such as 30'-azido-thymidine, is the major effective approach for the treatment of acquired immunodeficiency syndrome [23, 24]. These agents are potent inhibitors of HIV reverse transcriptase (RT) and protease [23, 25]. However, the emergence of drug-resistant variants of HIV and toxicities severely limits the long-term effectiveness of these drugs. Recent studies have indicated that many natural products are active as anti-HIV agents [23]. These compounds belong to a wide range of different structural classes, *viz.* coumarins, flavonoids, tannins, alkaloids, lignins, terpenes, naphtho- and anthraxquinones and polysaccharides [26].

Different *in vitro* studies indicated that various triterpenoids from *G. lucidum* had potent inhibitory activity against HIV. Lucidenic acid O and lucidenic lactone, isolated from the fruiting body of *G. lucidum*, not only inhibited the activities of calf DNA polymerase-α and rat DNA polymerase-β, but also those of HIV-1 RT [10]. Ganoderiol F and ganodermanontriol isolated from the fruiting bodies of *G. lucidum* are active against HIV-1 growth with an IC_{100} of 7.8 μg/ml [7, 8, 10, 27]. Ganoderic acid B and ganoderiol B showed potent inhibitory effect on HIV protease with an IC_{50} value of 0.17 mM. Other triterpenoids including ganoderic acid C1, 3β-5α-dihydroxy-6β-methoxyergosta-7, 22- diene, ganoderic acid-α, ganoderic acid H and ganoderiol A had moderate activity against HIV-1 protease with IC_{50} values of 0.17–0.23mM [7, 8, 10, 27, 28]. In addition, ganoderic acid-β, lucidumol B, ganodermanondiol, ganodermanontriol and ganolucidic acid A showed significant anti-HIV-1 protease activity with IC_{50} values of 20, 59, 90, 70 and 70 mM, respectively [29]. Ganoderic acid A, B and C1 had minor inhibitory activity against HIV protease with IC_{50} values of 140–430 mM. It appears that there is a structure- activity relationship for triterpenoid showing anti- HIV protease activity. The C3, C24, or C25 atoms are vital for the anti-HIV activity [29].

The aqueous low-molecular-weight fraction extracted from *G. lucidum* also exhibited anti-HIV activity using the XTT[2,3-bis (2-methoxy-4-nitro-5-sulfophenyl)- 5-[(phenyl-amino) carbonyl]-2H-tetrazolium hydroxide] antiviral assay, which can quantitatively measure cytopathic effects of HIV-1 on CEM cells, a human T lymphoblastoid cell line [30]. The IC_{50} and EC_{50} values were 125 and 11 μg/ml, respectively, resulting in a therapeutic index of 11.4. This aqueous low-molecular-weight extract was further fractionated to eight sub-fractions by methanol: GLA (methanolic extract), GLB (hexane soluble), GLC (acetic ether soluble), GLD (water soluble), GLE (neutral), GLF (acidic), GLG (alkaline) and GLH (amphoteric). All sub-fractions except GLD, GLF and GLH exhibited anti-HIV activity with IC_{50} and EC_{50} values of 22–44 μg/ml and 14–44 μg/ml, respectively. GLC and GLG inhibited HIV RT showing consistency. Incubation of GLC at 50 μg/ml or GLG (100 μg/ml) with Jurkat T cells gave 75% and 66% inhibition of HIV growth, respectively. Both low-molecular-weight and high-molecular-weight fractions from *G. lucidum* had negligible toxicities to CEM cells.

2.2. Immuno-Modulating effects of *Ganoderma lucidum*

Ganoderma lucidum has been reported to contain several major substances with potent immuno-modulating action. These include polysaccharides (β-D-glucan), proteins (Ling Zhi-8) and triterpenoids. The major immuno-modulating effects of these active substances derived from *G. lucidum* include mitogenicity and activation of immune effector cells, such as T cells, macrophages and natural killer cells, resulting in the production of cytokines, including interleukins, tumour necrosis factor-α (TNF-α) and interferons.

2.2.1. Mitogenic Activity

Polysaccharide fractions, methanolic extracts and protein LZ-8 of *G. lucidum* have mitogenic effect on mouse splenocytes and human peripheral blood mononuclear cells (PBMC) in the presence of various immuno-stimulating or immuno-suppressive agents such as phytohemagglutinin (PHA) and 12-O-tetradecanoylphorbol 13-acetate [30, 31-34]. Treatment of the PBMCs with cyclosporin A (CsA) led to blockage of the cell proliferation. The methanolic fraction from *G. lucidum* restored the CsA induced inhibition of the cell proliferation, which might be due to the inhibition of the protein kinase C signal pathway that accelerate the CsA signal pathway [30]. Recombinant Fan-jets had the same blast formation stimulatory activity to human peripheral blood lymphocytes as native Fip-gts [35]. Using deletion analysis, a sequence of about 10 amino acids of the N-terminal amphipathic alpha-helix domain of Fip-gts has been identified to be responsible for the immuno-modulatory activity.

2.2.2. Effect on Immune Effector Cells

T Cells

Extracts from *G. lucidum* are potent activator of T cells, inducing the production of a number of cytokines, in particular IL-2 [34, 36-38]. In human PBMC (primarily T cells) *in vitro*, the crude *G. lucidum* water-extract induced expression of cytokines such as IL-10, TNF-α, IL-1β, IL-6 and IL-

Table 1. Therapeutic Effects and Bioactive Compounds of *Ganoderma lucidum* Reported in the Literature Until 2009

Therapeutic Effects	Bioactive Compound	References
Immunomodulation: Mitogenic activity, Stimulation of immune effector cells and complement system	Protein LZ-8, β-D-glucan, Ganoderic acid	Kim *et al.*, [30]; Zhang *et al.*, [36]; Wang *et al.*, [38]; Won *et al.*, [41]; Lee *et al.*, [40]; Han *et al.*, [42]; Li *et al.*, [43]; Oh *et al.*, [44]; Tasaka *et al.*, [48]; Yang and Pai, [52]; Kino *et al.*, [50]
Anti-Cancer, Anti-Tumour, Chemo and Radio Prevention	β-D-glucans, heteropolysaccharides, glycoproteins, lanostanoid, 3β-hydroxyl-26-oxo-5α-lanosta-8,24-dien-11-one, and steroid, ergosta-7,22-diene-3β,3α,9α-triol	Miyazaki and Nishijima, [53]; Usui *et al.*, [54]; Usui *et al.*, [55]; Sone *et al.*, [56]; Kishida *et al.*, [57]; Zhang *et al.*, [58]; Cheong *et al.*, [59]; Wasser and Weis, [19]
Anti-HIV-1 And Anti-HIV-1-Protease	Triterpenoids: Lucidenic acid O; Lucidenic lactone; Ganoderiol; Ganodermanontriol and Ganoderic acid	Sahar, [27]; Hobbs, [7]; McKenna, [8]; Gao, [10]; Min, [29]
Anti-Diabetic	Glycans: Ganoderans B and D	Mohammad, *et al.*, [80]; Wasser, [4]; Hobbs, [7]; McKenna, [8]; Gao, [9]
Hepatoprotective	Ganoderic acids R and S and ganosporeric acid A	Hirotani, [81]; Chen, [82]; Wang, [83]; Gao, [11]
Anti-Inflammatory	Ganoderic Acid C, 3-oxo-5α-lanosta-8,24-dien-21-oic acid	Joseph *et al.*, [84]; Horng-Huey Ko *et al.*, [85]; Lin *et al.*, [86]
Anti-Allergic	ganoderic acids C and D	Zhou, [20]; Liu, [18]; Smith, [16]
Anti-Androgenic	ganoderol B	Liu *et al.*, [92]; Fujita *et al.*, [93]; Shimizu *et al.*, [94]
Anti-Angiogenic activity	Ethanol extract (Compound not reported)	Song, *et al.*, [95]
Anti-Herpetic	Acidic protein bound polysaccharides	Kim *et al.*, [96]; Eo *et al.*, [97]; Liu *et al.*, [98]; Oh *et al.*, [99]
Anti-Oxidant	Chloroform extract (Compound not reported)	Karaman *et al.*, [100]; Joseph *et al.*, [84]
Anti-Microbial: Anti-Viral, Anti-Bacterial, Anti-Fungal	Neutral protein bound polysaccharide, Acidic protein bound polysaccharide, ganodermin	Wasser, [4]; Stamets, [101]; Hobbs, [7]; McKenna, [8]; Gao, [10]; Smith, [16]; Suay, [102]; Yoon, [103]; Sugiura and Ito, [104]; Kim *et al.*, [105]; Eo *et al.*, 2000 [107]; Eo *et al.*, [106]; Wang and Ng, [108]
Estrogenic	Ethanol extract (Compound not reported)	Shimizu *et al.*, [94]
Anti-Mutagenic	Methanol extract (Compound not reported)	Lakshmi *et al.*, [109]
Anti-Ulcerogenic	Polysaccharides	Gao *et al.*, [12]
Anti-Proliferative activity	Ganoderic acid T	Hong, [110]; Jiang, [111]; Hu, [112]; Muller *et al.*, [113]; Tang *et al.*, [114]
Cardiovascular and Circulatory Functions	Powdered mycelium and water extract of mycelium (Compound not reported)	Kabir, [115]; Soo, [116]; Lee, [117]; Jin *et al.*, [118]

2 [34]. Crude polysaccharide fractions isolated from fresh fruiting bodies of *G. lucidum* potentiated the release of interferon (IFN)-γ from human T cells [38]. A polysaccharide fraction (GL-B) from *G. lucidum* promoted the production of IL-2 in a dose-dependent manner and markedly enhanced the cytotoxicity of cytotoxic T lymphocytes [37]. GL-B also restored the mixed lymphocyte response to alloantigen, automatic proliferation and IL-2 production of splenocytes in aged mice [37]. Protein LZ-8 of *G. lucidum* is also a potent T cell activator, mediating its effects via cytokine regulation of integrin expression [32]. Stimulation of human peripheral blood lymphocytes with LZ-8 resulted in the production of IL-2 and a corresponding up-regulation of IL-2 receptor expression [32]. In addition to T cell proliferation, microscopic examination of LZ-8-stimulated peripheral blood lymphocytes revealed that LZ-8 induced cellular aggregate formation. The aggregate formation correlated with a dramatic rise in ICAM-1 expression and an increased production of IFN-γ, TNF-α and IL-1 beta, molecules associated with regulation of ICAM-1 expression. Both the aggregate formation and the

proliferative effects of LZ-8 were blocked by addition of monoclonal antibody to either CD18 or CD11a, the counter-receptor complex components for ICAM-1. Furthermore, addition of neutralising antibodies to both IL-2 receptor and TNF-α blocked aggregate formation, cellular proliferation and ICAM-1 expression.

Natural Killer (NK) Cells

A water-extracted polysaccharide fraction from *G. lucidum* enhanced the cytotoxicity of splenic NK cells in tumour-bearing mice [39, 40]. The water-extracted fractions from *G. tsugae* mycelium have been shown to activate NK cells in mice [41]. The water-soluble extract of *G. tsugae* and alcohol-insoluble subfraction, but not the alcohol-soluble subfraction, increased the splenic NK cytotoxic activity in a dose-dependent manner in mice. The alcohol-soluble subfraction also increased the serum IFN level in mice [41], which was reduced by either IFN-(α, β) antiserum or IFN-γ monoclonal antibody *in vitro*.

Macrophages

Macrophages constitute first line of defense in the body. Activation of macrophages by substances from *G. lucidum* resulted in the release of cytokines, nitric oxide (NO) and other mediators [38, 40, 42]. Polysaccharides from *G. lucidum*, in particular β-D-glucans, are potent stimulators of murine and human macrophages *in vitro* and *in vivo* [38, 40, 42, 43]. CR3 receptors on macrophages are bound by β-D-glucans and internalised, priming a series of molecular events. Crude water-extracted polysaccharides isolated from fresh fruiting bodies of *G. lucidum* potentiated the production of cytokines, including IL-1β, IL-6, IFN-γ and TNF-α by human macrophages, which were anti-proliferative, differentiation and apoptosis inductive to the HL-60 and the U937 leukemic cells [38, 40]. IFN-γ and TNF-α released from macrophages acted synergistically on the inhibition of growth of leukemic cells. GLB7, a *G. lucidum* polysaccharide, decreased the production of oxygen free radicals and antagonized the respiratory burst induced by PMA in murine peritoneal macrophages [43]. These observations suggest that GLB7-decreased production of oxygen free radicals in murine peritoneal macrophages further suggesting the anti-aging effect of Ganoderma polysaccharides.

Ganoderan (GAN), a β-D-glucan isolated from *G. lucidum*, enhanced the production of NO in the RAW 264.7 macrophages [42]. The cell proliferation of GAN-treated Raw 264.7 cell lines was inhibited. These results indicate that the β-glucan-related polysaccharides of the higher fungus activate macrophage and release NO, which is an important chemical messenger for the induction of many biological responses. A protein-polysaccharide fraction (GLB) from the growing tips of *G. lucidum* is a strong stimulator to the macrophages [44]. When analyzed using a flow cytometer, GLB (100 μg/ml) increased the phagocytic activity of the BALB/c mouse peritoneal macrophages as well as chicken macrophage BM2CL cells against FITC-labeled *C. albicans* by 55.2% and 21.2%, respectively. GLB also increased the spreading and the expression of MHC class II molecules of BM2CL cells as well as the mouse peritoneal macrophages.

Mast Cells

A water extract of the fruit body of *G. lucidum* had inhibitory activity on histamine release from rat peritoneal mast cells induced by compound 48/80 or antigen (egg white albumin)-antibody reaction and on passive cutaneous anaphylaxis reaction in guinea-pigs and rats [45, 46]. Two ganoderic acids (C and D) isolated from the fruit body of *G. lucidum* by methanol inhibited the histamine release from rat mast cells induced by compound 48/80 and concanavalin-A [47]. A chloroform extract from *G. lucidum* broth also significantly inhibited histamine release from rat peritoneal mast cells induced by A-23187 and compound 48/80 [48]. Further studies on the mechanism(s) for the inhibitory activity on histamine release from mast cells revealed that oleic acid present in the active fraction induced membrane stabilisation in model membrane systems [47]. Cyclooctasulfur extracted from the culture medium of *G. lucidum* decreased calcium uptake from the extracellular medium by a disulfide exchange reaction in the cell membrane, leading to inhibition of histamine release from mast cells [49].

In vivo, LZ-8 prevented the production of a systemic anaphylaxis reaction in mice, when it was administered repeatedly, which is thought to be due to reduced antibody formation [50].

2.2.3. Effects on Complement System

An alkali extract isolated from cultured mycelium of *G. lucidum* activated both classical and alternative pathways of complement [51]. This fraction also activated reticulo-endothelial system of mice in the carbon clearance test and increased hemolytic plaque forming cells of the spleen. The alkali extract consisted of 10% carbohydrate and 49% proteins respectively. Recently, a clinical study in old patients with insomnia and palpitation has shown that taking *G. lucidum* essence for 4-6 weeks increased their serum C3 levels [52].

2.3. Anti-Tumour Activity

Polysaccharides (β-D-glucans, heteropolysaccharides and glycoproteins) isolated from *G. lucidum* demonstrated anti-tumour activity against sarcoma 180 in mice [19, 53-59]. Triterpenoids, such as ganoderic acids T-Z isolated from *G. lucidum*, showed cytotoxic activity *in vitro* on hepatoma cells [60, 61]. A lanostanoid, 3β-hydroxyl-26-oxo-5α-lanosta-8,24-dien-11-one and a steroid, ergosta-7,22-diene-3β,3α,9α-triol, isolated from fruiting bodies of *G. lucidum* demonstrated potent inhibitory effects on KB cells and human PLC/PRF/5 cells *in vitro* [62]. The polysaccharide-mediated potentiation of immune function is thought to be the major mechanism of anti-tumor action by *G. lucidum*. Among the multiple polysaccharides, active β-D-glucans are responsible for the anti-tumor effect [53-57]. β-D-glucans appear to act by binding to leucocyte surfaces or serum-specific proteins, leading to activation of macrophage, T-helper, NK cells and other effector cells [63-66]. All of these increase the production of cytokines such as TNF-α, interleukins and interferons, nitric oxide and antibodies by the activated effector cells.

In addition to host defence potentiation, other mechanisms are also involved in the anti-tumour effect of *G. lucidum*. A compound from *G. lucidum* suppressed the growth of K562 leukemic cells in a dose- and time-dependent manner and induced their differentiation into more mature erythrocytic cells [67]. The conditioned medium from *G. lucidum* PS-stimulated human blood mononuclear cells (PSG-MNC-CM) significantly inhibited the growth of U937 cells and stimulated them to differentiate into mature monocytes/macrophages which had functions of phagocytosis and producing cytoplasmic superoxide [68, 69]. Inhibition of DNA polymerase and post-translational modification of oncoproteins are reported to contribute to the anti-tumour activity of *G. lucidum* [70-72]. The organic Germanium in *G. lucidum* may also contribute to its anti-tumour activity [73-76].

2.4. Chemo- and Radio-Prevention

The *G. lucidum* exhibited chemo- and radio-preventive effects which are attributed to its effects on immune system. Ganoderma polysaccharides restored the TNF-α production inhibited by cyclophosphamide to normal level in mice [43]. Both the *G. lucidum* extract and Krestin (protein-bound β-glucan isolated from *Coriolus versicolor* (L.:Fr.) Quél) were beneficially effective on the recovery of the cellular immunocompetence (measured by ^{3}H-thymidine incorporation with splenic cells stimulated through mitogens such as PHA and Con A) [77]. The *G. lucidum* extract (400 mg/day/kg body weight) appeared better than Krestin (500 mg/day/kg body weight) in repairing the damage of subset T-cells in the spleen of γ-irradiated mice, since the relative thymus weight and CD4 and CD8 splenocytes were higher in *G. lucidum* extract-treated mice compared with Krestin-treated mice [78].

In morphine-dependent mice, a polysaccharide peptide from *G. lucidum* could restore several immunologic parameters depressed by morphine treatment to or even beyond normal levels [79]. Both c-myb and c-myc mRNA expression in splenocytes of repetitive morphine-treated mice were significantly decreased and the polysaccharide peptide could induce the expression of these genes, indicating that the polysaccharide peptide from *G. lucidum* could be of potential application in controlling abuse of opiates-induced immunodeficiency.

2.5. Anti-Diabetic Effects

Animal studies have demonstrated that the polysaccharide fractions of *G. lucidum* have potential hypoglycemic and hypolipidemic activities. The aqueous extract (1000 mg/Kg) of *G. lucidum* normalized blood glucose levels in alloxan induced diabetes in Wistar rats [80]. A water extract of Reishi reduced the increase in blood glucose levels in rats following oral glucose test. Following adrenaline (i.v.) or oral glucose in rats, the mushroom inhibited increase in blood glucose without raising blood insulin levels. Glycans (ganoderans B and D) have shown significant hypoglycemic activity in mice. 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 (DM) was carried out [9]. Treatment with Ganopoly significantly decreased the mean HbA1c from 8.4% at baseline to 7.6% at 12 weeks. Significant changes in mean FPG and PPG levels at the last visit paralleled the changes in mean HbA1c levels. Changes in fasting insulin, 2-hr postprandial insulin, fasting C-peptide and 2-hr postprandial C-peptide were consistent with the between-group differences in these end points being significant at the last visit. Overall, Ganopoly was well tolerated. This study demonstrated that Ganopoly is efficacious and safe in lowering blood glucose concentrations [9]. A 2-month open label comparative clinical study of a reishi powder extract (1 g t.i.d.) for eight diabetic patients (four with NIDD and four with IDDM) found hypoglycemic effects comparable to those found in controls who were administered insulin (100 IU=ml for 60 days) or oral hypoglycemic agents (250 mg/day for 60 days) [4, 7-9].

2.6. Hepatoprotective Effect

Ganoderic acids R and S and ganosporeric acid A from *G. lucidum* showed *in vitro* anti-hepatotoxic activity in the galactosamine-induced cytotoxic test with primary cultured rat hepatocytes [81, 82]. Triterpenoids extract of *G. lucidum* (75% ethanol) can protect mice against hepatic necrosis induced by chloroform and d-galactosamine. The hepatoprotective effects were perhaps related to the ability to promote the activity of scavenging enzymes for hepatic free radicals in mice and thus to raise the ability of antioxidation in mice [83]. Ganopoly, the polysaccharide-containing preparation of *G. lucidum*, was proven to be hepatoprotective in a double-blind, randomized and multicentered study in patients with chronic hepatitis B. Within the 6 months study period, 33% (17/52) of treated patients had normal aminotransferase values and 13% (7/52) had cleared hepatitis B surface antigen from serum and the drug was well tolerated [11].

2.7. Anti-Inflammatory Property

Anti-inflammatory drugs make up about half of analgesics, remedying pain by reducing inflammation as opposed to opioids which affect the brain. Joseph *et al.*, [84] showed anti-inflammatory activity of the chloroform extract of *G. lucidum* in carrageenan induced acute and formalin induced chronic inflammatory models in mice. The extract showed remarkable anti-inflammatory activity in both models, comparable to the standard reference drug diclofenac. Horng-Huey Ko *et al.*, [85], studied the anti-inflammatory properties of triterpenoids and steroids from both *G. lucidum* and *G. tsugae*. Twelve compounds, including ergosta-7,22-dien-3β-ol (1), ergosta-7,22-dien-3β-yl palmitate (2), ergosta-7, 22-dien-3-one (3), ergosta-7,22-dien-2β,3α,9α-triol (4), 5α,8α-epidioxyergosta-6,22-dien-3β-ol (5), ganoderal A (6), ganoderal B (7), ganoderic aldehyde A (8), tsugaric acid A (9), 3-oxo-5α-lanosta-8,24-dien-21-oic acid (10), 3α-acetoxy-5α-lanosta-8,24-dien-21-oic acid ester β-D-glucoside (11) and tsugaric acid B (12), were assessed *in vitro* by determining their inhibitory effects on the chemical mediators released from mast cells, neutrophils and macrophages. Compound 10 showed a significant inhibitory effect on the release of β-glucuronidase from rat neutrophils stimulated with formyl-Met-Leu-Phe (fMLP)/cytochalasin B (CB) whereas compound 9 significantly inhibited superoxide anion formation in fMLP/CB-stimulated rat neutrophils. Com-

pound 10 also exhibited a potent inhibitory effect on NO production in lipopolysaccharide (LPS)/interferon-γ (IFN-γ)-stimulated N9 microglial cells. Moreover, compound 9 was also able to protect human keratinocytes against damage induced by ultraviolet B (UV B) light, which indicated compound 9 could protect keratinocytes from photodamage. Water extracts of reishi mushroom were shown to possess significant activity against carrageenin–induced paw oedema when administered subcutaneously to rats [86].

More than 100 different highly oxygenated lanostanoid triterpenes have been identified in reishi mushrooms. The predominant triterpenes are ganoderic acids A-Z. Ganoderic Acid C, isolated by careful fractionation of a non-polar solvent extract of *G. lucidum*, was found to account for most of the anti-inflammatory activity as determined by *in vitro* tests, such as histamine release from mast cells. An ethyl acetate extract rich in ganoderic acids was later found, by another group of researchers, to exhibit both systemic and topical anti-inflammatory activity in standard animal models, such as the croton oil-induced mouse ear inflammation test [87].

2.8. Anti-Allergic Property

Allergy and asthma are two examples of histamine mediated allergic response. Both conditions are increasing in frequency and are difficult to target with modern medicine. The modern approach to ant-allergic drug research is target-specific and does not consider the natural defence mechanisms of the body or the causative factors (the shift of cytokine TH1 to a predominantly TH2 cytokine) underlying histamine-mediated allergic responses. The fruiting bodies *G. lucidum* have been traditionally used as anti-inflammatory agents for the treatment of asthma or allergy. *G. lucidum* as immunonutraceutical [88], with its unique array of compounds working in concert, could play a major role in treatment of histamine-mediated allergic responses. Powell [87] stated that *G. lucidum* is an effective agent to restore the normal balance between the cytokines TH1 and TH2 immune states in patients with histamine-mediated allergic responses. Such an approach treats the underlying cause for the TH2 condition. In a case study of hay fever patients, Powell [87] found that patients (male) of age 5 and 39 with different doses viz. 2 tablets x 500 mg a day and 6 tablets x 500 mg per day of *G. lucidum* respectively, a marked decrease in drowsiness, itchiness and sneezing was found after 2 and 10 days respectively. In the course of a screening test for the inhibition of histamine release from rat mast cells, it was found for the first time that ganoderic acids C and D inhibited histamine release from rat mast cells [45-49]. Other than the triterpenoid compounds, cyclo-octasulfur from this fungus also effectively inhibited histamine release from rat peritoneal mast cells and interacted with membrane proteins to inhibit Ca uptake causing a blockade of histamine release [16, 18, 20, 45-49].

2.9. Anti-Androgenic Activity

Nowadays androgen-mediated diseases such as prostate cancer, hirsutism, acne androgenic alopecia and benign prostatic hyperplasia (BPH) are considered to be serious problems [19, 89]. Above all, BPH is one of the most common symptoms seen in older men and 40% of men of 50–60 years of age group and 90% of those 80–90 years of age group are diagnosed with BPH. The principal prostatic androgen is dihydrotestosterone (DHT), which is synthesized by steroid enzyme 5α-reductase from its substrate testosterone [90]. Since the weight of the seminal vesicles depends on the 5α -reduced androgens, it is important to maintain adequate levels of the DHT. Two isoforms of 5α -reductase have been cloned, expressed and characterized (types 1 and 2) that display different tissue expression patterns, enzyme kinetic parameters and chromosomal localization [91]. Liu *et al.,* [92] observed that the ethanol extract of *G. lucidum* showed inhibitory activity on both isozymes (types 1 and 2) of 5α-reductase and ventral prostate growth induced by testosterone. Activity-guided fractionation and TLC analysis suggested that the active principles *in vivo* were triterpenoids indicating that the triterpenoids fraction of *G. lucidum* might be a useful ingredient in the treatment of benign prostatic hyperplasia (BPH). Liu *et al.,* [92] also showed a significant research work on anti-androgen effect of Ganoderol B isolated from the fruiting body of *G. lucidum*. Ganoderol B with 5α-reductase inhibitory activity and the ability to bind to androgen receptor (AR) can inhibit androgen- induced LNCaP cell growth and suppress re-growth of the ventral prostate induced by testosterone in rats. The down-regulation of AR signaling by ganoderol B provides an important mechanism for its anti-androgenic activity. The result suggests that ganoderol B might be useful in prostate cancer and benign prostatic hyperplasia therapy. Fujita *et al.,* [93] reported that the extract of *G. lucidum* showed the strongest 5α-reductase inhibitory activity. Shimizu *et al.,* [94] also reported the preventative effect of benign prostatic hyperplasia by *G. lucidum*.

2.10. Anti-Angiogenic Activity

Angiogenesis is a physiological process involving the growth of new blood vessels from pre-existing vessels. It is a normal process in growth and development, as well as in wound healing. However, it is also a fundamental step in the transition of tumors from a dormant state to a malignant one. Anti-angiogenic therapy may be an important component of treatment regimens for cancer patients. *G. lucidum*, showed anti-angiogenic activity and its inhibition of the inducible nitric oxide production. 70% ethanol extract of fresh fruiting bodies of *G. lucidum* showed significant anti-angiogenic activity [95]. *G. lucidum* significantly inhibited LPS-induced NO production in RAW 264.7 macrophages. Hence, *G. lucidum* can be an excellent agent for controlling angiogenesis.

2.11. Anti-Herpetic Activity

Herpes simplex is a viral disease caused by herpes simplex viruses viz., herpes simplex virus 1 (HSV-1) and herpes simplex virus 2 (HSV-2). *G. lucidum* was proved to be a potent inhibitor for both herpes simplex virus 1 (HSV-1) and herpes simplex virus 2 (HSV-2). Kim *et al.,* [96] reported anti-herpetic activities of acidic protein bound polysaccharide (APBP) isolated from *G. lucidum.* APBP showed potent antiviral activity against HSV-1 and HSV-2 in Vero cells at its 50% effective concentration (EC_{50}) of 300 and 440 μg/ml, respectively. APBP had no cytotoxicity on Vero cells at a concentration of $1x10^4$ μg/ml. The combinations of APBP with IFN alpha on HSV-1 or HSV-2 showed more

potent synergistic effects with CI values of 0.30–0.62 for 50–90% effective levels than those of APBP with IFN gamma with CI values of 0.65–1.10. These results suggest the possibility of developing APBP as a new anti-herpetic agent. Various protein bound polysaccharides viz., GLhw, GLhw-01, GLhw-02 and GLhw-03 with anti-herpetic activities were isolated from *G. lucidum* [97]. Among them, the acidic protein bound polysaccharide, GLhw-02, exhibited the most potent anti-herpetic activity suggesting that GLhw-02 possesses the possibility of being a new antiherpetic agent. Liu *et al.,* [98] showed possible mode of action of anti-herpetic activities of a proteoglycan isolated from the mycelia of *G. lucidum in vitro*. A bioactive fraction, *G. lucidum* proteoglycan (GLPG) extracted and purified from the mycelia of *G. lucidum* inhibited viral replication by interfering with the early events of viral adsorption and entry into target cells. Thus, this proteoglycan seems to be a potential candidate for anti-HSV agents. Oh *et al.,* [99] reported anti-herpetic activities of acidic protein bound polysaccharide (APBP) isolated from *G. lucidum* alone and in combinations with acyclovir (ACV) and vidarabine (ara-A). APBP showed potent antiviral activity against HSV-1 and HSV-2 in Vero cells. APBP had no cytotoxicity on Vero cells at a concentration of $1x10^4$ μg/ml. APBP exhibited a potent antiviral activity with selectivity index (SI) of more than 22.73. The combinations of APBP and ACV on HSV-1 and HSV-2 showed potent synergistic effects and these results suggest that the possibility of developing APBP as a new antiherpetic agent.

Hence, it appears that *G. lucidum* act as a potent inhibitor for both herpes simplex virus 1 (HSV-1) and herpes simplex virus 2 (HSV-2) and has the potential as a anti-herpetic agent.

2.12. Anti-Oxidant Property

Studies on fungal biomolecules as potential natural antioxidants are scanty and *G. lucidum* was shown to possess marked free radical scavenging and lipid peroxidation inhibition properties [84, 100]. The MeOH and chloroform extracts of *G. lucidum* exhibited antioxidative activities. The chloroform extract showed significant superoxide scavenging activity (IC_{50}: 144.6 ± 1.5 μg/ml) and is of potential interest as source of strong natural antioxidants in the food and cosmetics industries.

2.13. Anti-Bacterial Activity

Several studies demonstrated that *G. lucidum* contained antibacterial constituents that are able to inhibit gram-positive and/or gram-negative bacteria [4, 7, 8, 10, 16, 101, 102]. The aqueous extract of the carpophores of *G. lucidum* inhibited 15 types of gram-positive and gram-negative bacteria. The studies further indicated that combinations of *G. lucidum* extract with four antibiotics (ampicillin, cefazolin, oxytetracycline and chloramphenicol) resulted in additive effects in most instances: synergism in two instances when combined with cefazolin against *Bacillus subtilis* and *Klebsiella oxytoca* and antagonism in two instances [103].

It appears that some constituents such as ganomycin, triterpenoids and aqueous extracts from Ganoderma species have a broad spectrum of *in vitro* antibacterial activity against gram-positive and gram-negative bacteria and *Helicobacter pylori*. Thus, it is possible that the antibacterial activity of Ganoderma species may be beneficial for those patients with chronic infection (e.g., chronic bronchitis) and those with *H. pylori*-positive peptic ulcer diseases, though clinical studies are required to confirm this.

2.14. Anti-Viral Activity

The goal of antiviral chemotherapy is the discovery of antiviral agents that are specific for the inhibition of viral multiplication without affecting normal cell division. It is necessary to identify and develop new antiviral agents without adverse side effects and viral resistance. *G. lucidum* appears to be very safe because oral administration of the extract did not display any toxicity [104, 105]. Eo *et al.,* [106] showed antiviral activities of various water and methanol soluble substances isolated from *G. lucidum*. Five substances, from carpophores of *G. lucidum* GLhw, GLMe-1, -2, -4 and -7 significantly inhibited the cytopathic effects of HSV and VSV. In the plaque reduction assay, GLhw inhibited plaque formation of HSV-2 with 50% effective concentrations (EC_{50}) of 590 and 580 μg/ml in Vero and HEp-2 cells and its selectivity indices (SI) were 13.32 and 16.26. GLMe-4 did not exhibit cytotoxicity up to 1000 μg/ml, while it exhibited potent antiviral activity on the VSV New Jersey strain with an SI of more than 5.43. Eo *et al.,* [107] in further studies reported possible mode of antiviral activity of acidic protein bound polysaccharides viz., a neutral protein bound polysaccharide (NPBP) and an acidic protein bound polysaccharide (APBP) isolated from *G. lucidum* on herpes simplex viruses. APBP exhibited more potent HSV-1 and HSV-2 antiviral activity than NPBP with 50% effective concentration (EC_{50}) of 300–520 μg/ml. In order to examine the possible mode of the antiviral activity of APBP its virucidal effect, antiviral activity in preincubation, attachment and penetration assay were tested with HSV-1 and HSV-2. APBP was found to have a direct virucidal effect on HSV-1 and HSV-2. APBP did not induce IFN or IFN-like materials *in vitro* and is not expected to induce a change from a normal state to an antiviral state. APBP in concentrations of 100 and 90μg/ml inhibited up to 50% of the attachment of HSV-1 and HSV-2 to Vero cells and was also found to prevent penetration of both types of HSV into Vero cells. These results show that the antiherpetic activity of APBP seems to be related to its binding with HSV-specific glycoproteins responsible for the attachment and penetration and APBP impedes the complex interactions of viruses with cell plasma membranes.

2.15. Anti-Fungal Activity

Several thousand species of fungi have been described, but fewer than 100 are routinely associated with invasive diseases of humans. In general, healthy humans have a very high level of natural immunity to fungi and most fungal infections are mild and self-limiting. Intact skin and mucosal surfaces and a functional immune system serve as the primary barriers to colonization by these ubiquitous organisms, but these barriers are sometimes breached. Few anti-fungal principles have been isolated from *G. lucidum*. A 15-kDa antifungal protein, ganodermin, was isolated from the fruiting body of *G. lucidum* [108]. Ganodermin inhibited the mycelial 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. It was devoid of hemagglutinating, deoxyribonuclease, ribonuclease and protease inhibitory activities.

2.16. Estrogenic Activity

Osteoporosis is a disease of bone that leads to an increased risk of fracture. In osteoporosis the bone mineral density (BMD) is reduced, bone microarchitecture is disrupted and the amount and variety of non-collagenous proteins in bone is altered. Hormonal factors strongly determine the rate of bone resorption; lack of estrogen (e.g. as a result of menopause) increases bone resorption as well as decreasing the deposition of new bone that normally takes place in weight-bearing bones. The amount of estrogen needed to suppress this process is lower than that normally needed to stimulate the uterus and breast gland. Shimizu *et al.,* [94] reported the preventative effect of Osteoporosis by *G. lucidum*. They found that the ethanol extract from the fruiting body of *G. lucidum* showed significant effects on the proliferation of MCF-7 cells (human breast cancer). This proliferation effect is related to the estrogenic activity of *G. lucidum*, because this proliferation activity was inhibited by the addition of the anti-estrogenic compound **ICI**182, 780. The ethanol extract of *G. lucidum* prevented ovariectomy-induced bone loss and decreased the concentration of osteocalcin in the blood serum, similar to the action of 17β- estradiol. The study provided evidence that the ethanol extract of *G. lucidum* protects against bone loss caused by estrogen deficiency, without a substantial effect on the uterus. These results showed that *G. lucidum* might be a useful ingredient for the treatment of osteoporosis.

2.17. Anti-Mutagenic Effect

The anti-mutagenic activity of methanolic extract of *G. lucidum* on hepatic damage caused by benzo[*a*]pyrene was investigated [109]. The activity was assayed by Ames *Salmonella* mutagenicity test using histidine mutants of *Salmonella typhimurium* tester strains, TA98, TA100 and TA102. The methanolic extract of the mushroom significantly inhibited ($P < 0.001$) the *in vitro* sodium azide (NaN_3), *N*-methyl-*N* ˡ-nitro-*N*-nitrosoguanidine (MNNG) and 4-nitro-*o*-phenylenediamine (NPD) and benzo[*a*]pyrene (B[*a*]P) induced his+ revertants in a dose dependent manner. *In vivo* anti-mutagenic activity of extract was also assayed by determining the mutagenicity of the urine of rats administrated with B[*a*]P as a mutagen. The prior administration of extract markedly inhibited mutagenicity induced by B[*a*]P. The results indicated that the methanolic extract of *G. lucidum* occurring in South India possessed significant anti-mutagenic activity. The effect of B[*a*]P on hepatic enzymes, such as serum glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and alkaline phosphtase (ALP), were also evaluated. The extract prevented the increase of SGOT, SGPT and ALP activities consequent to B[*a*]P challenge and enhanced the levels of reduced glutathione (GSH) and activities of glutathione peroxidase (GPx), glutathione-*S*-transferase (GST), superoxide dismutase (SOD) and catalase (CAT). The extract also profoundly inhibited lipid peroxidation induced by B[*a*]P. The results revealed that *G. lucidum* extract restored antioxidant defense and prevented hepatic damage consequent to the challenge by B[*a*]P.

2.18. Anti-Ulcerogenic Effect

Ulceration is a condition where discontinuation of skin is found. There are various kinds of ulcers found viz. oral, peptic, corneal, venous and genital. *G. lucidum* appears to be one of the options for curing this disorder. Similarly, Gao *et al.,* [12] investigated whether *G. lucidum*'s polysaccharides (GLPS) had a direct mucosal healing effect in the indomethacin-treated rat and explored the possible mechanisms by determining the gastric mucosal mRNA and protein levels of TNF-α and ornithine decarboxylase (ODC) activity. In addition, the effects of GLPS on the cellular proliferation, ODC and c-Myc protein expression and mucus synthesis in the rat gastric cell culture (RGM-1) were examined in the study. The study demonstrated that GLPS at 250 and 500 mg/kg by intragastric input caused ulcer-healing effect in the rat; this was accompanied with a significant suppression of TNF-α gene expression, but with an increased ODC activity. In RGM-1 cells, GLPS at 0.05, 0.25 and 1.0 mg/ml concentrations significantly enhanced [3H] thymidine incorporation and ODC activity in a concentration-dependent manner. However, these effects were abrogated by the addition of the ODC inhibitor, DL-a-difluoromethyl-ornithine (DFMO). GLPS at 0.25–1.0 mg/ml also increased mucus synthesis, as indicated by the increased D-[6-^{3}H] glucosamine incorporation in RGM-1cells. Furthermore, GLPS at 0.05–1.0 mg/ml conc. increased the c-Myc protein expression. These findings indicated that GLPS produced a mucosal healing effect in the rat model, perhaps due partly to the suppression of TNF-α and induction of c-myc and ODC gene. Therefore, it can be clearly understood that *G. lucidum* shows an anti-ulcerogenic effect.

2.19. Anti-Proliferative Activity

Ganoderma in a dose- and time-dependent manner inhibited the cell proliferation and induced apoptosis of HT-29, a human colon carcinoma cell line and MDA-MB-231 and MCF-7 breast cancer cell lines [110-112]. *G. lucidum* causes apoptosis in leukemia, lymphoma and in multiple myeloma cells [113]. When *G. lucidum* extract was screened for its anti-proliferative activity using a panel of 26 human cancer cell lines, six hematologic cell lines were found to be most sensitive and they included: HL-60 (ED50 26 μg/ml), U937 (63μg/ml), K562 (50 μg/ml), Blin-1 (38μg/ml), Nalm-6 (30 μg/ml) and RPMI8226 (40 μg/ml). Cell cycle analyses revealed a G2/M arrest, most prominently in HL-60 cells. Four hematopoietic cell lines (HL-60, Blin-1, U937, RPMI 8226) were examined for apoptosis, which ranged between 21 and 92%. After exposure to *G. lucidum* extract, HL-60 cells became multinucleated with an increased DNA content. These results indicate that *G. lucidum* extract has a profound activity against leukemia, lymphoma and multiple myeloma cells and may be a novel adjunctive therapy for the treatment of hematologic malignancies. Tang *et al.,* [114] showed Ganoderic acid T (GA-T), which is a lanostane triterpenoid purified from methanol extract of *G. lucidum* mycelia, was found to exert cytotoxicity on various human carcinoma cell lines in a dose-dependent manner, while it was less toxic to normal human cell lines. Animal experiments *in vivo* also

showed that GA-T suppressed the growth of human solid tumor in athymic mice. It markedly inhibited the proliferation of a highly metastatic lung cancer cell line (95-D) by apoptosis induction and cell cycle arrest at G1 phase. Moreover, reduction of mitochondria membrane potential ($\Delta\psi_m$) and release of cytochrome c were observed during the induced apoptosis. All these findings demonstrate that GA-T induced apoptosis of metastatic lung tumor cells through intrinsic pathway related to mitochondrial dysfunction and p53 expression and it may be a potentially useful chemotherapeutic agent.

2.20. Cardiovascular and Circulatory Functions

Cholesterol and Lipid Metabolisms

The powdered mycelium of *G. lucidum*, at 5% of the diet of rats for 4 weeks, caused plasma total cholesterol to decrease significantly (by 18.6%) compared to controls. Total liver triglyceride and total liver cholesterol levels were also significantly lower in the Reishi-fed group (by approximately 46% and 56%, respectively) [115, 116].

Hypertension

A water extract of the mycelium administered to rats and rabbits (3–30 mg/kg i.v.) produced significant hypotensive effects; an activity the researchers suggested is secondary to the primary effect that suppresses sympathetic outflow of the central nervous system [117]. The powdered mycelium of Reishi, at 5% of the diet of spontaneously hypertensive rats for 4 weeks, caused systolic blood pressure to be significantly lower (approximately 10 mmHg) without causing a significant difference in the heart rate [115]. Jin *et al.*, [118] conducted a double-blind, placebo-controlled clinical study of *G. lucidum* in 54 patients with primary stage-II hypertension who had not responded to previous drug treatment (captopril 25 mg t.i.d. or nomodipine 20 mg t.i.d.). In the group which was administrated *G. lucidum* extract tablets (2 tablets b.i.d. or 220 mg/day), systemic blood pressure significantly improved in 82.5%, with capillary and arterial blood pressure showing significant improvements in as little as 14 days. No changes of any significance were found in the placebo group. According to Soo [116] in treating hypertension, *G. lucidum* was shown to be highly effective in a very large number of treated cases. In the more successful cases, blood pressure was back to normal within 2 months and in some cases, within 2 weeks.

3. OTHER BENEFICIAL ASPECTS OF *GANODERMA LUCIDUM*

3.1. Decomposer or Wood-Rotting Macrofungi

G. lucidum is economically and ecologically important not only as a source of medicinal and neutraceutical products but its role in nutrient cycling as decomposers of dead wood with a cosmopolitan distribution and can grow on both coniferous and hardwood species. *Ganoderma* are white-rot fungi and have enzymes that allow them to break down wood components such as lignin and cellulose. There is significant commercial interest in harnessing the power of these wood-degrading enzymes for industrial applications such as biopulping or bioremediation [119].

3.2. Biosorption of Heavy Metals

Biosorption, in which viable or nonviable biomass, is directed to accumulate toxic heavy metals from aqueous stream, is attracting wide attention as an alternate wastewater treatment technology. Different studies reports screening and evaluation of non-edible woodrotting macrofungi (mushrooms), which occur abundantly in tropical forests, for metal binding potential. *G. lucidum* exhibited the highest binding potential for copper (II) uptake. No specific pre-treatment was required for its potential application. Simple gravity settling could be used for separating the sorbent from the aqueous stream. *G. lucidum* could bind many other metallic cations. Systematic study on Cu and La indicated ion exchange/ complexation to be the major mechanism for metal sorption [120].

4. THE MACROFUNGI

4.1. General Description

In China and Korea *Ganoderma lucidum* known as Ling Zhi which means "herb of spiritual potency" whereas the Japanese call this mushroom Reishi or mannentake (10,000 year mushroom) [4, 121]. In Latin, lucidum means shiny or brilliant and aptly describes this mushroom's fruiting body, which has a modeled, sculptured, varnished appearance. *G. lucidum* occurs in six different colors, but the red variety is most commonly used and commercially cultivated in North America, China, Taiwan, Japan and Korea. *G. lucidum* is one of the most beautiful mushrooms in the world. When very young its varnished surface is Chinese red, bright yellow and white. Later the white and yellow shades disappear, but the resulting varnished, reddish to reddish brown surface is still quite beautiful and distinctive (Fig. (**1**)). The virtues of *G. lucidum* extracts, handed down from generation to generation, include it as a ''cancer cure'' and a symbol of happy augury, good fortune, good health, longevity and even immortality [7]. Beginning with the Yuan Dynasty (1280–1368 A.D.), *G. lucidum* has been endlessly represented in art—in paintings, carvings of jade and deer's antlers, furniture and carpet designs, balustrades, jewelry, women's hair combs, perfume bottles—in short, wherever an artistic urge found an outlet. The earliest mention of Ling Zhi was in the era of the first emperor of China, Shinghuang of the Ch'in Dynasty (221–207 B.C.) [1, 4, 7]. Subsequently, depictions of this fungus proliferated through Chinese literature and art. *G. lucidum* is annual and does not actually grow more each year like some polypores, its fruiting body is quite tough and can last for months [101, 122]. This magical mushroom is now effectively cultivated and is readily available [101]. The authors performed artificial cultivation of this magical macrofungi by solid substrate and submerged fermentation technology and studies on the production and characterization of immunomodulating bioactive metabolites of *G. lucidum* are ongoing at the author's laboratory.

4.2. Classification, Origin and Distribution

The family of Ganodermataceae consists of a large group of tree fungi of the genus Ganoderma. The *Ganoderma lucidum* has been classified into Kingdom- Fungi, Phylum-Basidiomycota, Class- Basidiomycetes, Sub-class- Homobasidiomycetes, Order- Polyporales, Family- Ganodermataceae, Genus- *Ganoderma* and Species- *lucidum* [101,

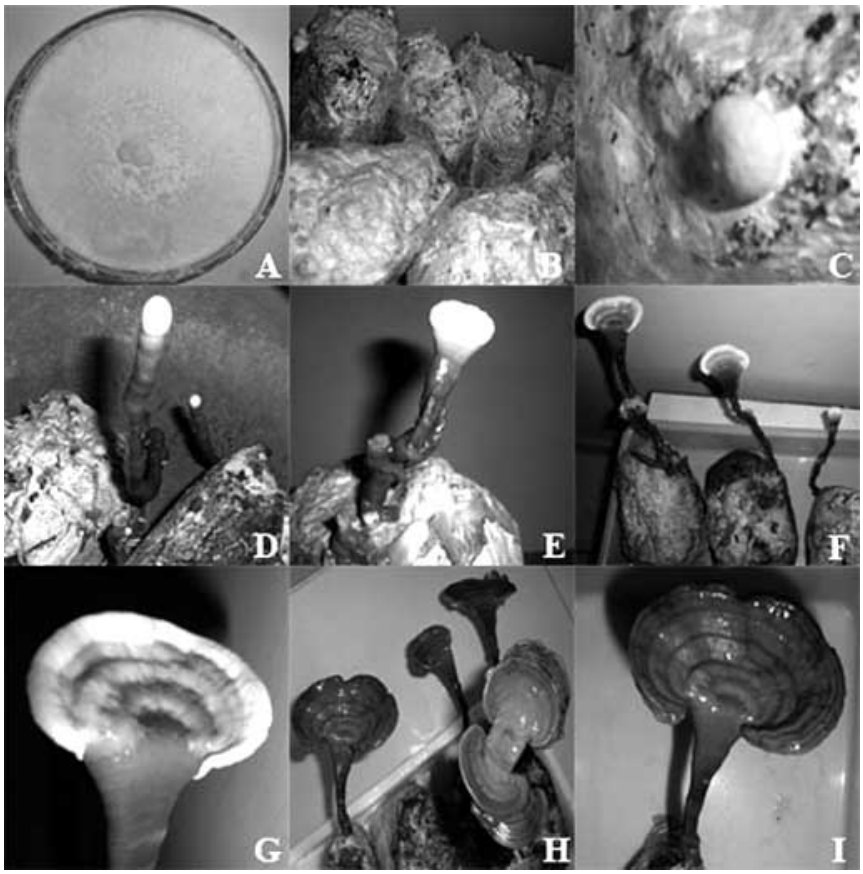

Fig. (1). Different Growth stages of *Ganoderma lucidum* during artificial cultivation; A, Culture plate (Growing in Potato Dextrose Agar Medium); B, Mycelial colonization on solid substrate; C, Primordia formation; D, Elongation of primordia; E, Cap formation; F, Flattening and growth of cap; G, Thickening of cap; H, Maturation of fruitbody; I, Mature fruit body.

122, 123]. *G. lucidum* is mainly found in tropical and subtropical areas. It is grows on a wide variety of dead or dying trees, e.g., deciduous trees especially oak, maple, elm, willow, sweet gum, magnolia and locust. *G. lucidum* is less frequently found on coniferous trees (e.g., Larix, Picea, Pinus) in Europe, Asia and North and South America (in temperate rather than subtropical regions). In the Orient, it grows primarily on plum trees. It is also found on stumps, generally near the soil surface and occasionally on soils arising from buried roots. *G. lucidum* generally occurs in two growth forms, one, found in North America, is sessile and rather large with only a small or no stalk, while the other is smaller and has a long, narrow stalk and is found mainly in the tropics [101, 122].

4.3. Related Species of Ganoderma

Genus Ganoderma encompasses several species, which are widely used for medicinal purposes, e.g., *Ganoderma lucidum*, *Ganoderma luteum* Steyaert, *Ganoderma atrum* Zhao, Xu and Zhang, *Ganoderma tsugae* Murrill, *Ganoderma applanatum* (Pers.: Wallr.) Pat., *Ganoderma australe* (Fr.) Pat., *Ganoderma capense* (Lloyd) Teng, *Ganoderma tropicum* (Jungh.) Bres., *Ganoderma tenue* Zhao, Xu and Zhang and *Ganoderma sinense* Zhao, Xu and Zhang. According to two famous Chinese plant medical books, Shen Nong Ben Cao Jing (25–220 A.D., Eastern Han Dynasty) and Ben Cao Gang Mil by Li Shi-Zhen (1590 A.D., Ming Dynasty), six Ling Zhi species/varieties were known in China at that time. Worldwide, more than 250 Ganoderma species have been described [4, 124]. However, in therapeutic practices and literature citations, Ganoderma usually refers to the species of *Ganoderma lucidum*. Besides being treasured for its medicinal value in China for more than 1000 years, the lack of availability of *G. lucidum* was also largely responsible for it being so highly cherished and expensive. During ancient times in China, any person who picked the mushroom from the natural environment and presented it to a high-ranking official was usually well rewarded. Even in the early 1950s, it was presented to Chinese leaders in Mainland China and Taiwan, following the occasional discovery in the wild. In the past, *G. lucidum* grew in small quantities only in the wild; therefore, it was very expensive.

4.4. Cultivation and Production of *Ganoderma lucidum*

G. lucidum has been the focus of great interest due to its potential as a source of bioactive substances and pharmaceuticals [6, 125]. A large and diverse spectrum of chemical compounds with a pharmacological activity has been isolated from the mycelium, fruiting bodies and sclerotia of *G. lucidum*: triterpenoids, polysaccharides, proteins, amino acids, nucleotides, alkaloids, steroids, lactones, fatty acids and enzymes [126]. Polysaccharides isolated from *G. lucidum* are the major components responsible for its medicinal properties. Till now, polysaccharides, Ganoderic acid and other metabolites of *G. lucidum* are mainly extracted from solid state cultivated fruitbody and submerged cultured biomass and broth.

As *G. lucidum* is very rare in nature, the amount of wild mushroom is not sufficient for commercial exploitation. Its cultivation on solid substrates, stationary liquid medium or by submerged cultivation has become essential to meet the increasing demands in the international markets. So in the present review the aforesaid cultivation methods are eminently described, keeping in mind that *G. lucidum* is a therapeutic fungal biofactory.

4.4.1. Solid State/Substrate Cultivation of Ganoderma lucidum

The information about the production of *G. lucidum* basidiocarp (fruitbody) and mycelium in solid state or solid substrate cultivation/fermentation is not much established but it can be capitalized for small and large scale industries to meet the increasing demand and supply of present status. Evidently, there are only few reports available for the solid state cultivation of *G. lucidum.*

Though there is very little information about the production of *G. lucidum* in solid state fermentation but Habijanic and Berovic [127] reported a unique work which was carried out in a horizontal stirred tank reactor with a total volume of 30L with suitable conditions which gave satisfactory rates of cell growth and exopolysaccharide production. Their eminent work showed a positive aspect for satisfactory rates of growth and exopolysaccharide production by favourable conditions such as: a temperature of 30°C, an air flow of 2L/min and an agitation of 80 rpm for 2min every second day during the first 7 days and every day during the latter stages of the cultivation and with a prime factor of initial moisture content. The substrate used in this study was composed of beech sawdust, olive oil, $(NH_4)_2SO_4$, KH_2PO_4, $CaCl_2{\cdot}2H_2O$, $MgSO_4{\cdot}7H_2O$, $FeSO_4{\cdot}7H_2O$ and distilled water which proved the most appropriate substrate for the production of immunostimulatory animal feed supplements, because in this study the whole fermented substrate is used as the product.

Yang *et al.*, [128] used stillage grain from a rice-spirit distillery in the solid state fermentation of *G. lucidum*. In their study, cultures were carried out in test tubes or propylene bags and *G. lucidum* was cultivated on stillage grain supplemented with wheat bran, ground rice or sawdust at different ratios. The effect of mixed ratios on the production of mycelia and fruit body was investigated. The compositions of fruiting body were also analyzed and compared. The additions of $NH_4H_2PO_4$ and $CaCO_3$ favored mycelial growth in the test-tube cultures. Due to its high content of carbohydrate and nitrogen, stillage grain was considered as a nutritive substrate for mycelial growth. The results obtained from propylene-bag cultures reveal that the substrate type altered not only the shape of fruiting bodies but also their constituents. Sawdust supplemented with stillage grain at a ratio 4:1 at a water content of 60% was optimal for the production of fruiting body.

Hsieh and Yang [129], used soy residue for the solid-state fermentation of *G. lucidum.* In their study, the solid-state fermentation was conducted in three types of containers: test tube, 500-ml flask and sterilize-able polypropylene plastic bag. The highest rate of mycelial growth of 6 mm/day was observed in the medium of carbon/nitrogen (C/N) ratio of 80 using test tubes. However, a growth rate of 7.5 mm/day was found at the C/N ratio of 70–80 in the 500-ml flasks. In the tests using plastic bags, the fruiting bodies were fully developed only for the C/N ratios of 70 and 80. The components of fruiting bodies obtained from different media were also analyzed and compared. The contents of ash, polysaccharides and crude protein of fruiting bodies were found higher in the media of C/N ratio of 80.

Veena and Pandey [130], evaluated agriculture and forestry wastes such as saw dust and wood chips alone and in combination with rice bran, wheat bran and finger millet or ragi (*Eleusine coracana*) powder. In their study, the optimum temperature for mycelial growth was 30°C and preferred pH was 4.0 to 6.5. Addition of rice bran, wheat bran and ragi powder reduced the spawn running and improved the biological efficiency. Consistently a high biological efficiency (> 20%) was obtained with three combinations viz. saw dust (90%) with rice bran (10%) or wheat bran (10%), saw dust 80% + rice bran 18% + $CaCO_3$ 1% + sucrose 1%.

Peksen and Yakupoglu [131] were investigated, Tea waste (TW) as a new supplement for substrate mixtures in *G. lucidum* cultivation. In this study they determined the effects of sawdust (S) based substrates supplemented with TW at the various levels (75S:25TW, 80S:20TW, 85S:15TW and 90S:10TW) and *G. lucidum* strains on yield, biological efficiency (BE) and the chemical composition of fruiting bodies in solid-state fermentation. Significant differences were found among substrates regarding yield and BE, while yield and BE of the strains were not different. The substrate formulations producing highest yield and BE were 80S:20TW (87.98 g/kg substrate and 34.90%) and 75S:25TW (82.30 g/kg substrate and 31%). Yield and BE of substrates containing TW were generally higher than that of the control (80sawdust:18wheat bran:1sucrose:1CaCO3). Nitrogen, potassium, iron and manganese contents and C:N ratios of substrates were strongly correlated with yield. BE showed positive and significant correlations with potassium, iron and manganese. Moisture content, potassium, magnesium, calcium, iron and zinc contents of the fruiting bodies were affected by both strain and substrate. It was concluded that TW can be used as a supplement for substrate preparation in *G. lucidum* cultivation.

Experimental Evidence

In the present study, authors studied the process of solid state fermentation of *G. lucidum* and evaluated the locally available substrate for the cultivation of an indigenous isolate alongwith estimation of intracellular polysaccharides (IPS) from fruitbody of *G. lucidum*. In this study authors used saw dust of the broad leaf plant *Tectona grandis* (Teak) and narrow leaf plant *Acacia spp.* as substrate to get more appropriate results. Substrates were supplemented with wheat bran and rice bran. $CaSO_4$ and $CaCO_3$ at different ratio were also added to substrate to maintain the pH 4.5. The substrate prepared was as written: saw dust (teak) + 20% wheat bran + 2% $CaSO_4$ + 0.6% $CaCO_3$; saw dust (teak) + 20% rice bran + 2% $CaSO_4$ + 0.6% $CaCO_3$; saw dust (*Acacia spp.*) + 20% wheat bran + 1.5% $CaSO_4$ + 0.5% $CaCO_3$; saw dust (*Acacia spp.*) + 20% rice bran + 1.5% $CaSO_4$ + 0.5% $CaCO_3$ and were named as substrate I, II, III and IV respectively. The moisture level of the substrate was maintained at 65% and Bag system was adopted for cultivation. 500 gm

substrate was filled in polypropylene bags and plugged with non-absorbent cotton after putting a plastic (PVC) ring at the neck. Successive sterilization of substrate was done in three days at 15 psi for 60 min each day. Upon cooling the sterilized bags were inoculated with wheat grain spawn at the rate of 4% and incubated in dark. After complete mycelial colonization (bags were white all over) the bags were opened and rolled back to expose upper surface and proper conditions (temperature, R.H. and light intensity) for different stages of primordia and fruit body formation were optimized and provided (Fig. (**1**)). Fruit body was oven dried at <45 ^{o}C after maturation. The fruit body was crushed and extracted with 85% ethanol to eliminate low-molecular components. Then, the IPS was extracted with 1% ammonium oxalate solution (Temp. 98°C, 6 h) from which polysaccharides were precipitated by acetone. IPS content of fruit body was estimated by Phenol sulfuric acid assay [132]. After harvesting the first flush, conditions for primordia formation were again switched on (i.e. temp.- 28±2 ^{o}C, R.H.- 95%, light- 800 lux) for staring and completing the second flush and the same procedure was been repeated for the third flush.

The optimum temperature for mycelial colonization was 30±2 ^{o}C. For the complete mycelial colonization substrate- I, took 18 days; substrate- II, 16 days; substrate- III, 26 days and substrate- IV, 23 days. Further primordia formation took in 12-14, 10-12, 16-19 and 14-16 days in substrate-I, substrate- II, substrate- III and substrate- IV respectively, soon after mycelial colonization of *G. lucidum*. The optimum light intensity (800 lux), R.H. (95%) and temperature (30±2 ^{o}C) were found after standardization for primordia formation. Similarly the time period and optimum conditions were standardized for different stages of fruit body formation and IPS production from fruit bodies obtained from different substrate are shown in Table **2**.

Conclusion

According to the present investigation, it was clearly observed that teak saw dust and rice bran was found to be most favourable substrate for the cultivation and production of *G. lucidum* and its IPS. Though experimental evidences showed good results for acacia saw dust and rice bran but in comparison teak saw dust and rice bran showed a promising result. Here in this study wheat bran taken as the supplement showed low efficiency in compare to rice bran. Hence, it is clearly judged that the present study revealed an excellent result in comparison to other works or studies done before.

4.4.2. Submerged Cultivation of Ganoderma lucidum

Polysaccharides and ganoderic acid of *G. lucidum* are mainly extracted from solid cultivated fruiting bodies which is a time-consuming and quality-fluctuating process [133]. Submerged culture by fermentation is an alternative approach for efficient production of polysaccharides and ganoderic acid of *G. lucidum.* There are several advantages of submerged culture over solid culture for polysaccharides and ganoderic acid production viz. high productivity, low costs, availability of convenient control and easy downstream processing [134]. The production of fruitbody takes at least 3-5 months, while reasonable amount of product of interest can be obtained by submerged fermentation after only 2-3 weeks [135].

Wagner *et al.*, [136] reviewed excellently by summarizing the published data on submerged fermentations with *Ganoderma* (Table **3**). They described only one report of a large scale fermentation, in which *G. tsugae* was cultivated in tanks with a volume of 20 m^3. All reported studies of fermentation with *G. lucidum* were done in volumes of 10 L or less. Roughly half the studies were undertaken in Erlenmeyer flasks and half in bioreactors. The advantage of using bioreactors is that it is easier to control environmental conditions such as temperature, dissolved oxygen and pH. These fermentations were done with different objectives. Some aimed simply to produce biomass, with no concern for its composition. Others aimed to maximize the production of either ganoderic acids or polysaccharides and to understand how different variables affect their production. The best yields reported till date are 22.1 g/L for biomass [134], 1.71 g/L for **EPS** [137], 2.49 g/L for **IPS** [135] and 582 mg/L for **GA** [138].

Table 2. Solid State Fermentation of *Ganoderma lucidum*

Substrate	Days for Mycelial Colonization	Days for Premordia Formation	Days for Fruit Body Formation			Fruitbody gm/kg Substrate (Dry Weight Basis)	IPS gm/100gm Fruitbody (Dry Weight Basis)
			Cap Formation and Growth (Whitening)	Thickening of Cap (Yellowing)	Maturation of Fruit Body (Redning)		
substrate-I	18-20	12-14	10-11	15-17	14-17	178±0.12	2.36±0.08
substrate-II	16-19	10-12	9-11	13-15	13-15	196±0.19	2.54±0.13
substrate-III	26-30	16-19	11-12	15-18	15-19	120±0.29	2.02±0.22
substrate-IV	23-29	14-16	10-11	14-16	14-17	129±0.11	2.03±0.14
Optimum conditions	temp.- 30±2 ^{o}C, dark and high CO_2	temp.- 28±2 ^{o}C, R.H.- 95%, light- 800 lux, CO_2- 1500 ppm	temp.- 28±2 ^{o}C, R.H.- 80%, light- 800 lux, CO_2- 1000 ppm	temp.- 25±1 ^{o}C, R.H.- 60%, light- 800 lux, CO_2- 1000 ppm	temp.- 25±1 ^{o}C, R.H.- 60%, light- 800 lux, CO_2- 1000 ppm		

Value of the yield of fruitbody and IPS content is the mean± standard error of the mean of 10 replicate analyses.

Experimental Evidence

In the present investigation, authors studied the process of submerged fermentation of an indigenous strain of *G. lucidum* for hyperproduction of biomass, polysaccharides and ganoderic acid. As mentioned in Table **3**, various researchers had carried out the same work but the quality and content of physiologically active substances vary from strain to strain and also depend on location, culture conditions [139] and growth of the mushroom [140]. Though, we know various eminent scientists such as Y. J. Tang, J. J. Zhong, F. C. Yang, C. B. Liau, Q. H. Fang, J. Habijanic, M. Berovic, B. Wraber, D. Hodzar, B. Boh, R. Wagner and others had contributed their constant and deliberate research work in context with the production of the bioactive metabolites- Ganoderic acid and Polysaccharide from *G. lucidum*. Similarly, in the present review authors reviewed and keenly observed various statistics cum methods from the research work undergone earlier for the similar field and significantly carried out a research investigation by following the best work of aforesaid scientists. The media selected for the similar work in the present study was as follows;

Medium1. $KH_2PO_4 \cdot H_2O$ 1gm/l; $MgSO_4 \cdot 7H_2O$ 0.5gm/l; vitamin B1 0.05gm/l; lactose 35 gm/l; Yeast Extract 5 gm/l and Peptone 5 gm/l [134],

Medium 2. Glucose 50 gm/l; K_2HPO_4 0.5 gm/l; KH_2PO_4 0.5 gm/l; $MgSO_4 \cdot 7H_2O$ 0.5 gm/l; Yeast Extract 1 gm/l; NH_4Cl 4gm/l [137],

Medium 3. 3 kg of peeled potatoes autoclaved and made up to 10 L with water; Glucose 20 g/L; olive oil 2 % [139].

All the experiment using above three media were performed in 250 ml Erlenmeyer flasks in triplicates. The experiment was carried out in optimum cultivation conditions such as temperature, T =30°C, shaking 120 rpm, pH= 4.5. Mycelium was separated from the cultivation broth by vacuum filtration and filtered cultured medium was concentrated $1/4^{th}$ at 50°C. Extracellular polysaccharides (EPS) were precipitated from the concentrated medium by 96% ethanol, filtered, washed with acetone and dried. The mycelium was extracted with 85% ethanol to eliminate low-molecular components. Then, the intracellular polysaccharides (IPS) were extracted with 1% ammonium oxalate solution (Temp. 98°C, 6 h) from which polysaccharides were precipitated by acetone. Quantitative estimation of polysaccharide content was done by Phenol sulfuric acid assay [132] and for the determination of ganoderic acid (GA) content the dried mycelia were extracted by 50% (v/v) ethanol for one week (twice). After removal of mycelia by centrifugation, the supernatants were dried at 50°C under vacuum. The residues were suspended by water and later extracted with chloroform. The ganoderic acid in chloroform was extracted by 5% (w/v) $NaHCO_3$. After adding 2M HCl to adjust the pH of the $NaHCO_3$ layer to be lower than 3, the ganoderic acid in the $NaHCO_3$ layer was extracted with chloroform. After removal of chloroform by evaporation at 40°C, ganoderic acid was dissolved in absolute ethanol and its absorbency was detected at 245 nm. The result of the study comparing biomass, EPS, IPS and GA production for selected mediums is depicted in Table **4**.

Finally, it was concluded that olive oil which was used as antifoam agent in medium 3, stimulated growth and was found to be beneficial in the biomass production of *G. lucidum* and it was assumed that such stimulation is due to a partial incorporation of lipids in the cell membrane, thereby facilitating the uptake of nutrients from the medium. While polysaccharides and ganoderic acid production was influenced by the concentration of sugar (Glucose, lactose).

4.5. Fermentation Strategies

Five main strategies have been used for submerged fermentation of *G. lucidum*. The ideas for these strategies arose from the behavior of *Ganoderma* under different environmental conditions. All of the fermentation techniques described below was carried out at laboratory scale and the knowledge generated from each of them will contribute to the scaling-up of the process.

4.5.1. Batch Fermentation

Batch fermentation is the simplest technique reported. Most of the studies have been done in Erlenmeyer flasks [133-135, 137, 138, 141-144], although some have been done in bioreactors [134, 137, 139, 145-147].

4.5.2. Fed-Batch Fermentation

The strategy of fed-batch fermentation is to add one or more of the nutrients during the fermentation, based on the possibility that the high concentrations required for high final growth and product yields might inhibit growth if added in total at the start of the fermentation. Potentially, growth and product formation can be extended for long periods compared to normal batch fermentation. Tang and Zhong [114] investigated fed-batch fermentation with *G. lucidum*, motivated by the observation that lactose concentrations above 35 g/L had diminished the production of ganoderic acid. The fermentation was started at 35 g/L. The lactose level was monitored off-line and when it fell to between 5 and 10 g/L, sufficient lactose was added to increase its concentration by 15 g/L, in a volume equal to 10 % of the working volume of the bioreactor [134, 135]. The production of biomass, polysaccharides and ganoderic acid were improved. The final yields of GA, EPS and IPS were 11, 43 and 48 % higher, respectively, in fed-batch than in batch fermentation [146]. Therefore lactose feeding stimulates the production of polysaccharides more than it stimulates the production of ganoderic acid. In Erlenmeyer flasks with glucose as the carbon source, the production of EPS was 124% higher for a fermentation in which glucose was added after 8 days of fermentation. At this time, the original glucose had fallen to below 2 g/L and the addition increased its concentration by 10 g/L [144]. No data was provided concerning ganoderic acid and IPS production in this case.

4.5.3. Bistage Control of pH

Based on the observations that while a constant pH of 3.0 improved growth, a constant pH of 6.0 favored exopolysaccharide production, a fermentation with bistage pH control was proposed, with the pH commencing at 3.0 and being changed to 6.0 after 2 days [145]. The production of EPS was the highest yet reported in the literature (20.04 g/L on

day 6). However, given that the polysaccharides were dried by heat and not freeze-dried as in the other works and also that they were quantified by a gravimetric method, the results must be considered preliminary and must be confirmed by more accurate and specific methods.

4.5.4. Two-Stage Culture Processes

The fact that ganoderic acid production was favored at a low oxygen tension led [133] to undertake a two-stage process in Erlenmeyer flasks. The first stage was realized with agitation in a rotary shaker. After 4, 8 or 12 days the agitation was stopped and the culture then remained static until the 24^{th} day. A control culture was shaken for the whole time. In static culture glucose was consumed at a slower rate and converted to biomass more efficiently. The highest biomass density was obtained with 4 days of agitation followed by 12 days of static culture. Unfortunately, no fermentation was left static from the time of inoculation. Results from such an experiment would contribute to a better understanding of the effects of agitation on the growth of *G. lucidum*. The highest production of ganoderic acid that has yet been reported (582 mg/L at day 12) was obtained in the culture that was agitated for only the first 4 days. The production of ganoderic acid was almost double that in the continuously-shaken control culture. A thick layer of mycelium was noted in this culture and, although the authors did not say whether it was at the surface of the medium or submerged, its large thickness would have restricted oxygen diffusion into the layer and low oxygen availability appears to stimulate GA production. No thick mycelial layer was found for the culture agitated during the first 12 days and then left static for further 12 days. In this case the production of GA did not increase significantly compared to a shaking culture without any static stage.

4.5.5. Immobilized Culture

Yang *et al.,* [142] introduced a polyurethane foam sheet into the medium of a submerged fermentation in an Erlenmeyer flask. The mycelium adhered to the surface of the foam matrix with almost no mycelia remaining free in the bulk liquid. The biomass density and the amount of EPS obtained were both markedly higher in this culture than in freely suspended cultures, indicating that the mycelial morphology adopted on a solid support was more favorable for both cell growth and polysaccharide formation. The polysaccharide secretion occurred at a slow rate and after 2–3 weeks of fermentation a large portion of polysaccharide was adsorbed on the support. As suggested by the authors, this may enable an alternative strategy for product and biomass recovery in which the support can simply be removed and pressed. There is no report and scientific data available on ganoderic acid production in immobilized cultures.

4.6. Techniques of Product Recovery and Analysis

This section presents and discusses the main methodologies used with *G. lucidum* for the recovery and quantification of products and biomass.

4.6.1. Biomass

In submerged fermentation biomass is generally recovered by filtration under suction or centrifugation. When it is intended to quantify the biomass of a sample, it is generally filtered through a pre-weighed filter paper (GF/C Whatman) [142] or a membrane with a standardized pore size [138, 143, 147]. Some authors filter the biomass, wash it off the filter with distilled water, recover it from the washings by centrifugation and then dry it until constant weight at temperatures ranging from 50 to 105 °C. No studies have been done about the effect of drying temperature on charring of the biomass or the loss of volatile cell components other than water.

The recovery of the biomass obtained by SSF is a difficult task because the mycelium binds tightly to the solid particles of the substrate. Therefore, it is normally impossible to determine the dry weight directly by the gravimetric method. Alternative methods for accompanying mycelium growth are based on indirect measurements of biomass components, although these are problematic because the level of the component in the biomass may vary during the growth cycle [148].

4.6.2. Polysaccharides

The filtered broth obtained from submerged fermentation contains water soluble exopolysaccharides that can be recovered by precipitation with 3–4 volumes of ethanol 95–96 %. The use of 2 volumes of acetone has also been reported [145]. Some workers dialyze the filtered broth before adding it to the ethanol solution [56, 138, 143], to eliminate smaller molecules, such as oligo and monosaccharides, which might interfere with the quantification of polysaccharides. Yang and Liau [141] ultrasonicated the mycelium with medium for 2 hours, with the intention to liberate cell-bound polysaccharide. However, they did not show that this treatment did not disrupt the cell, so it is impossible to affirm that IPS and EPS were quantified individually in this case. IPS is usually extracted with a solution of 1 M NaOH at 60 °C for 1 h. However, this is not the only fraction of IPS that can be obtained. Other solvents can be used for extraction, such as hot water, ammonium oxalate 1 % and NaOH 5 % (20). In SSF the EPS fraction is not dissolved in a liquid phase. In order to recover it, the fermented substrate, which also contains mycelium, must be extracted with cold water. The extraction of polysaccharides in boiling water for 5 h was reported in SSF [127], however, such a procedure is likely to extract not only the IPS and EPS produced by the organism, but also some of the polysaccharides from the solid substrate itself. The mainmethod used to quantify polysaccharides is the phenol-sulfuric method [132]. However, molecules other than polysaccharides, such as monosaccharides, oligosaccharides and proteins containing amino acids with phenolic groups, give false positive responses in this assay. The strategy of dialyzing the filtered broth before precipitating the polysaccharide fraction or washing the precipitate with ethanol eliminates smaller molecules. Proteins, however, will remain together with the polysaccharides and will contribute to the absorbance measured in the assay. Therefore, it is very important to determine the protein content by a specific method in order to assess the accuracy of the Phenol-sulfuric assay. Most of the reports do not mention the protein content of the recovered polysaccharide fraction. In fact, many workers do not acknowledge that precipitation with ethanol or acetone does not give a pure polysaccharide fraction. Often, the dried

Table 3. Fermentation Experiments Undertaken with Ganoderma and Key Results Obtained [136]

Ref.	Media with Components in g/L Unless otherwise Specified	Working Volume, Bioreactor and Conditions. Inoculum (Density of the Inoculum Culture)	Maximum Concentration Obtained (Day)				Linear Production Rate [R2] (Interval Used, Day)			
			γ(X)/g/L	γ(EPS)/g/L	γ(IPS)/g/L	γ(GA)/mg/L	ρ(X)/g/(L.d)	ρ(EPS)/g/(L.d)	ρ(IPS)/g/(L.d)	ρ(GA)/mg/(L.d)
141	Medium A	100 mL in 500-mL flask; initial pH=4.0; 100 rpm on rotary shaker; 30 °C. Disk inoculum.	5.5 (14)	-	-	-	-	-	-	-
137	Medium A	100 mL in 500-mL flask; initial pH=4.0; 100 rpm on rotary shaker; 30 °C. Inoculum*	-	1.71 (7)	-	-	-	-	-	-
137	Medium A	1 L in 2-L bioreactor; 6-blade impeller; 200 rpm; initial pH=4.0; 30 °C; 1 vvm; Inoculum 10 %	-	1.26 (7)	-	-	-	0.17 [0.85] (2-7)	-	-
137	Medium A	As previous entry but agitation 400 rpm. Inoculum 10 %	-	1.48 (3)	-	-	-	-	-	-
137	Medium A	3 L in 5-L bioreactor; pH=4.0; 30 °C; 100 rpm; 1 vvm; 7 days. Inoculum*	-	-	-	-	-	-	-	-
142	Medium A	100 mL in 250-mL flask; initial pH=4.0; 100 rpm on rotary shaker; 30 °C. Disk inoculum.	2.3 (8)	0.13 (8)	-	-	0.3 [0.84] (2-8)	0.03 [1.00] (4-8)	-	-
142	Medium A + olive oil 1%	As previous entry. Disk inoculum.	3.28 (7)	0.16 (7)	-	-	-	-		-
142	Medium A + safflower oil 1 %	As previous entry. Disk inoculum.	2.78 (7)	0.18 (7)	-	-	-	-	-	-
142	Medium A + oleic acid 1.5	As previous entry. Disk inoculum.	4.6 (7)	0.16 (7)	-	-	-	-	-	-
142	Medium A + palmitic acid 1	As previous entry. Disk inoculum.	4.4 (8)	0.16 (8)	-	-	0.6 [0.94] (2-8)	0.03 [0.97] (2-8)	-	-
142	Medium A + palmitic acid 2.5	As previous entry. Disk inoculum.	3.4 (7)	0.21 (7)	-	-	-	-	-	-
142	Medium A + oleic acid 1.5	100 mL in 250-mL flask; initial pH=4.0; rotary shaker 100 rpm; 30 °C. Polyurethane foam sheet as inert support. Disk inoculum.	8.7 (7)	0.27 (7)	-	-		-	-	-

(Table 3) contd….

Ref.	Media with Components in g/L Unless otherwise Specified	Working Volume, Bioreactor and Conditions. Inoculum (Density of the Inoculum Culture)	Maximum Concentration Obtained (Day)				Linear Production Rate [R2] (Interval Used, Day)			
			γ(X)/g/L	γ(EPS)/g/L	γ(IPS)/g/L	γ(GA)/mg/L	ρ(X)/g/(L.d)	ρ(EPS)/g/(L.d)	ρ(IPS)/g/(L.d)	ρ(GA)/mg/(L.d)
142	Medium A + palmitic acid 1	As previous entry. Disk inoculum.	6.1 (7)	0.32 (7)	-	-	-	-	-	-
133	Medium B + Glc 35; YE 10; P 5	50 mL in 250-mL flask; initial pH=5.5; rotary shaker 120 rpm; 30 °C. Inoculum 10 % (ρ (X)=325 mg/L)	15.8 (8)	0.62 (8)	0.86 (8)	150 (8)	-	-	-	-
133	Medium B + Glc 35; YE 5; P 5	As previous entry. Inoculum 10 % (ρ(X)=325 mg/L)	15.4 (8)	0.81 (8)	0.81 (8)	171 (8)	-	-	-	-
133	Medium B + Glc 65; YE 5; P 5	As previous entry. Inoculum 10 % (ρ (X)=325 mg/L)	12.8 (10)	1.08 (10)	0.95 (10)	159 (10)	-	-	-	-
133	Medium B + Glc 50; YE 5; P 5	As previous entry. Inoculum 10 % (ρ (X)=325 mg/L)	16.7 (10)	0.85 (10)	1.19 (10)	212 (10)	-	-	-	-
143	Medium B + Glc 35; YE 5; P 5	As previous entry but initial pH=3.5 Inoculum 10 % (ρ (X)=325 mg/L)	13.8 (8)	1.02 (14)	0.94 (8)	155 (8)	-	0.08 [0.97] (1-12)	-	-
143	As previous entry	As previous entry but initial pH=5.5 Inoculum 10 % (ρ (X)=325 mg/L)	15.5 (8)	0.63 (8)	1.06 (8)	193 (8)	-	-	0.15 [0.99] (1–8)	-
143	As previous entry	As previous entry but initial pH= 6.5 Inoculum 10 % (ρ (X)=325 mg/L)	17.3 (8)	0.59 (8)	1.05 (8)	208 (8)	2.3 [0.97] (1–8)	-	-	27 [0.98] (2–8)
143	As previous entry	50 mL in 250-mL flask; 120 rpm on rotary shaker; 30 °C; 10 g/L of Glc added on day 8. Inoculum 10 % (ρ (X)=325 mg/L)	-	1.41 (14)	-	-	-	0.19 [0.98] (8–12)	-	-
138	*	50 mL in 250-mL flask; rotary shaker 120 rpm; 30 °C; static after 4 days. Inoculum 10 % (ρ(X)=325 mg/L)	20.9 (16)	-	-	582 (12)	0.9 [0.99] (4–16)	-	-	56 [0.97] (4–12)
144	*	Bioreactor*. Inoculum ρ(X)=170 mg/L	15.3 (8)	0.71 (8)	0.79 (8)	267 (8)	-	-	-	36 [1.00] (2–8)
144	*	Bioreactor*. Inoculum ρ(X)=330 mg/L	15.7 (8)	0.79 (8)	1.08 (8)	214 (8)	2.8 [0.99] (1–6)	-	-	-

(Table 3) contd….

Ref.	Media with Components in g/L Unless otherwise Specified	Working Volume, Bioreactor and Conditions. Inoculum (Density of the Inoculum Culture)	Maximum Concentration Obtained (Day)				Linear Production Rate [R2] (Interval Used, Day)			
			γ(X)/g/L	γ(EPS)/g/L	γ(IPS)/g/L	γ(GA)/mg/L	ρ(X)/g/(L.d)	ρ(EPS)/g/(L.d)	ρ(IPS)/g/(L.d)	ρ(GA)/mg/(L.d)
144	*	Bioreactor*. Inoculum ρ(X)=670 mg/L	14.2 (8)	0.88 (8)	1.22 (8)	180 (8)	-	0.11 [0.87] (1–8)	0.16 [0.97] (1–8)	-
135	Medium B + sucrose 35; YE 5; P 5.	50 mL in 250-mL flask; initial pH=5.5; rotary shaker 120 rpm; 30 °C. Inoculum 10 % (ρ(X)=625 mg/L)	3.5 (2)	0.63 (day*)	0.25 (day*)	40 (day*)	-	-	-	-
135	Medium B + Glc 35; YE 5; P 5.	As previous entry. Inoculum 10 % (ρ (X)=625 mg/L)	10.1 (10)	0.47 (day*)	0.97 (day*)	144 (day*)	-	-	-	-
135	Medium B + maltose 35; YE 5; P 5.	As previous entry. Inoculum 10 % (ρ (X)=625 mg/L)	12.2 (10)	0.57 (day*)	1.16 (day*)	119 (day*)	-	-	-	-
135	Medium B + lactose 35; YE 5; P 5.	As previous entry. Inoculum 10 % (ρ (X)=625 mg/L)	12.3 (12)	0.53 (day*)	1.43 (day*)	180 (day*)	-	-	-	-
135	Medium B + lactose 50; YE 5; P 5.	As previous entry. Inoculum 10 % (ρ (X)=625 mg/L)	13.8 (14)	0.79 (14)	1.65 (12)	110 (14)	-	-	0.11 [0.97] (2–12)	-
135	Medium B + lactose 65; YE 5; P 5.	As previous entry. Inoculum 10 % (ρ (X)=625 mg/L)	14.4 (14)	0.94 (14)	1.41 (14)	92 (14)	0.81 [0.95] (2–14)	0.06 [0.98] (2–14)	-	-
135	Medium B + lactose 35; YE 5; P 5.	50 mL in 250-mL flask; rotary shaker 120 rpm; 30 °C; lactose added on day 8. Inoculum 10 % (ρ(X)=625 mg/L)	20 (12)	0.96 (20)	1.97 (16	258 (20)	-	0.49 [0.98] (8–20)	-	-
135	As previous entry	As previous entry but lactose added on day 10. Inoculum 10 % (ρ(X)=625 mg/L)	20 (14)	0.80 (22)	2.01 (14)	334 (20)	-	-	0.24 [0.96] (10–14)	20 [1.00] (10–20)
135	As previous entry	As previous entry but lactose added on day 12. Inoculum 10 % (ρ(X)=625 mg/L)	20 (14)	0.76 (18)	1.78 (18)	270 (20)	-	-	-	-
135	As previous entry	2 L in agitated bioreactor, 2 x 6-blade impellers; 100-180 rpm; 0.25-0.5 vvm. Inoculum %* (ρ(X)=625 mg/L)	16.7 (10)	0.61 (12)	1.68 (20)	178 (10)	-	0.03 [0.96] (4–12)	0.10 [0.95] (4–12)	-

(Table 3) contd….

Ref.	Media with Components in g/L Unless otherwise Specified	Working Volume, Bioreactor and Conditions. Inoculum (Density of the Inoculum Culture)	Maximum Concentration Obtained (Day)				Linear Production Rate [R2] (Interval Used, Day)			
			γ(X)/g/L	γ(EPS)/g/L	γ(IPS)/g/L	γ(GA)/mg/L	ρ(X)/g/(L.d)	ρ(EPS)/g/(L.d)	ρ(IPS)/g/(L.d)	ρ(GA)/mg/(L.d)
135	As previous entry	Bioreactor and operation as previous entry. lactose increased 15 g/L on day 10. Inoculum %* (ρ(X)=625 mg/L)	21.9 (12)	0.87 (20)	2.49 (22)	367 (12)	-	0.02 [1.00] (12–20)	0.06 [0.86] (12–22)	-
134	As previous entry	3.5 L in stirred bioreactor; 100–250 rpm; 30 °C; 0.25–1.0 vvm; initial pH 5.5. Inoculum 10 %	18.4 (10)	0.81 (14)	-	-	1.7 [0.98] (4–10)	0.05 [0.99] (4–14)	-	-
134	As previous entry	Bioreactor and operation as previous entry but lactose fed on day 10. Inoculum 10 %	22.1 (12)	1.25 (16)	-	-	-	0.12 [0.99] (10–16)	-	-
146	Medium B + lactose 35; YE 2.5; P 5	3.5 L in bioreactor with 2 6-bladed turbines (6.5 cm i.d.). 30 °C. DOT maintained at 10 %. Inoculum*	4.1 (12)	0.70 (12)	0.60 (12)	147 (9)	-	0.05 [1.00] (4–12)	0.05 [0.99] (4–12)	-
146	As previous entry	As previous entry but DOT maintained at 25 %. Inoculum*	14.7 (10)	0.60 (12)	1.56 (10)	340 (10)	1.4 [1.00] (4–10)	0.05 [0.99] (4–12)	0.20 [0.98] (4–10)	34 [0.87] (4–10)
146	As previous entry	As previous entry but agitation 200 rpm, Q(air)=0.22 L/min (kLa = 16.4 h–1). Inoculum*	12.1 (8)	0.97 (13)	1.91 (10)	245 (10)	-	0.06 [0.99] (2–12)	-	-
146	As previous entry	As previous entry but agitation 200 rpm, Q(air)=1.75 L/min (kLa = 78.2 h–1). Inoculum*	15.6 (10)	0.92 (13)	2.19 (10)	338 (10)	2.1 [1.00] (2–8)	-	0.25 [0.98] (2–10)	-
146	As previous entry	As previous entry but agitation 200 rpm, Q(air)=3.5 L/min (kLa = 96.0 h–1). Inoculum*	13.9 (14)	0.92 (13)	2.09 (10)	450 (10)	-	-	-	53 [0.99] (2–10)
145	Glc 60; KH2PO4 0.5; YE 6; (NH4)2HPO4 5; Sigma antifoam 289 0.5 %	2 L in 3-L air-lift fermenter; 25 °C; 2.5 vvm.; initial pH at 6.0 (not controlled). Inoculum 5 %	7.9 (8)	4.1 (8)	-	-	1.0 [0.96] (1–7)	0.6 [1.00] (1–7)	-	-
145	As previous entry	As previous entry but pH controlled at 3.0. Inoculum 5 %	12.9 (6)	6.4** (8);	-	-	2.4 [1.00] (1–6)	0.7 [0.96] (1–8	-	-

(Table 3) contd….

Ref.	Media with Components in g/L Unless otherwise Specified	Working Volume, Bioreactor and Conditions. Inoculum (Density of the Inoculum Culture)	Maximum Concentration Obtained (Day)				Linear Production Rate [R2] (Interval Used, Day)			
			γ(X)/g/L	γ(EPS)/g/L	γ(IPS)/g/L	γ(GA)/mg/L	ρ(X)/g/(L.d)	ρ(EPS)/g/(L.d)	ρ(IPS)/g/(L.d)	ρ(GA)/mg/(L.d)
145	As previous entry	As previous entry but pH controlled at 6.0. Inoculum 5 %	5.7 (4)	13.6** (7);	-	-	1.7 [0.99] (1–4)	2.2 [0.99] (1–8)	-	-
145	As previous entry	As previous entry but bistage control of pH=3.0 initially and 6.0 later. Inoculum 5 %	8.2 (5)	20.0** (6)	-	-	1.7 [0.99] (1–5)	4.2 [1.00] (2–6)	-	-
147	Glc 20; YE 2; P 2; K2HPO4 1; KH2PO4 0.46; MgSO4·7H2O 0.5	3 L in 5-L Jar fermenter; initial pH=5.0; 25 °C; 150 rpm; 2 vvm. Inoculum 4 %	6.9 (15)	1.16** (15)	-	-	-	-	-	-
147	Glc 10; ME 3; P 5; YE 3	As previous entry. Inoculum 4 %	5.7 (15)	0.66** (10)	-	-	-	-	-	-
147	PDB 24; ME 10; P 1	As previous entry. Inoculum 4 %	16.3 (11)	6.5** (12)	-	-	1.52 [0.95] (1–11)	0.60 [0.98] (1–12)	-	-
56	Glc 50; ME 40; YE 1; KH2PO4 0.5; K2HPO4 0.5; MgSO4·7H2O 0.5	Working volume*. Shaking culture at 28 °C. Inoculum*		0.85** (7)	-	-	-	-	-	-
139	3 kg of peeled potatoes autoclaved and made up to 10 L with water; Glc 20 g/L; olive oil 2 %	Working volume*. 10-L reactor, 3 Rushton turbines; 300 rpm; initial pH=5.8; 30 °C; p(O) 2 =70–80 %; redox potential E=100–450 mV; Q(air)=30 L/min. Inoculum 17 % wet weight of 6-day shake flask culture.	5.4 (8)	-	-	-	0.9 [0.99] (1–5)	-	-	-
139	As previous entry	As previous entry but after 8 days 7 L of broth substituted with fresh sterile medium. Inoculum as previous entry	9.6 (15)	-	-	-	1.1 [0.95] (10–14)	-	-	-
58 ***	Glc 30; P 3; K2HPO4 1.5; MgSO4 0.7; vit. B1 0.01	Working volume*, 20 t tank; t=(26+1) °C; 7–10 days. Inoculum*	25 kg total	-	-	-	-	-	-	-

*not possible to determine from the article; **determined by the gravimetric method; ***the fungus used was *Ganoderma tsugae.*

Medium A = Glc_{50}; K_2HPO_4 0.5; KH_2PO_4 0.5; $MgSO_4 \cdot 7H_2O$ 0.5; YE_1; NH_4Cl_4; Medium B = $KH_2PO_4 \cdot H_2O1$; $MgSO_4 \cdot 7H_2O$ 0.5; vitamin B1 0.05.

Abbreviations: EPS = exopolysaccharide; GA = ganoderic acid; IPS = intracellular polysaccharide; Glc = glucose; vvm = volumes of air per working volume per minute; DOT = dissolved oxygen tension; P = peptone; ME = malt extract; PDB = potato dextrose broth; YE = yeast extract; Disk inoculum = several 5 x 5 mm disks of agar with mycelium.

Table 4. Submerged Fermentation of *Ganoderma lucidum* for Biomass, EPS, IPS and GA Production

Medium	Biomass gm/l	EPS gm/l	IPS gm/l	GA gm/l
Medium 1	14.023±0.21	1.22±0.23	2.55±0.01	0.285±0.08
Medium 2	15.15±0.21	1.76±0.11	2.52±0.22	0.215±0.14
Medium 3	19.029±0.17	1.21±0.26	2.33±0.19	0.192±0.12

Value of the yield of biomass, EPS, IPS and GA content is the mean± standard error of the mean of 3 replicate analyses.

precipitate is weighed and reported as polysaccharide, with the implicit assumption that it is free of contaminants. Particular care should be taken when an undefined medium is used. Components such as yeast extract and peptone, which are often used in liquid culture and solid residues used in SSF may add non-polysaccharide components that not only might be extracted together with the polysaccharide produced by the organism but also might be counted as polysaccharide by the analytical method used.

4.6.3. Ganoderic acid

Ganoderic acid extraction and determination is a multistep process. First the dried mycelium is extracted twice with ethanol 50 %, each extraction lasting for one week. The supernatant is then dried and the residue suspended in water. The aqueous solution is then extracted with chloroform and a solution of 5% $NaHCO_3$ is then added to this organic phase. The aqueous phase is then collected and acidified with HCl to a pH less than 3. The ganoderic acid may be extracted again with fresh chloroform. The chloroform fraction is then evaporated. The resulting residue is suspended in absolute ethanol and the ganoderic acid is measured at 245 nm [133, 134, 138, 143, 144]. The authors do not provide proof that the fraction obtained contains only ganoderic acids. They only say that the GA present in mycelia of *G. lucidum* are a complicated mixture and usually have α, β-unsaturated carbonyl groups, whose absorbance is maximal between λ=230–260 nm [146]. Other organic acids may be extracted and purified together with the GA, although they may not necessarily absorb significantly in this wavelength range.

4.7. General Nutritional Components of *Ganoderma lucidum*

G. lucidum contains mainly protein, fat, carbohydrate (Table **5**) [149] and fiber. Artificially cultivated variety has similar contents of nutritional components compared with wild types and the extraction significantly increases the amounts of crude protein and carbohydrates and deleted crude fiber. Mizuno [17] reported the composition of *G. lucidum* extract (% of dry weight), which consisted of folin-positive material (68.9%), glucose (11.1%), protein (7.3%) and metals (10.2%) (K, Mg and Ca are the major components with Ge having the 5th highest metal concentration at 489 μg/g). These results generally agree with those reported by other authors [7, 101, 122]. However, there are qualitative and quantitative differences in the chemical composition of *G. lucidum* products depending on the strain, origin, extracting process and cultivation conditions [4, 7, 8, 17, 101].

4.8. Major Bioactive Constituents

Over 300 reports have been published concerning the chemical constituents of *G. lucidum* and related species. The fruiting body, mycelia and spores of *G. lucidum* contain approximately 400 different bioactive compounds, which mainly include triterpenoids, polysaccharides, nucleotides, sterols, steroids, fatty acids, proteins/peptides and trace elements [8, 11, 16, 17, 150].

Table 5. Carbohydrate Compositions of Crude *Ganoderma lucidum* Extract [149]

Sugar Components	Percentage (%)
d-Glucose	58.0
d-Mannose	15.5
l-Fucose	9.7
d-Galactose	9.3
d-Xylose	5.4
d-GlcNAc	1.0
l-Rhamnose	0.5

4.8.1. Terpenoid Compounds

Triterpenes

At least 140 different triterpenes have been identified in *G. lucidum* [4, 7, 8, 17, 150]. The majority is bitter tasting and largely occurs as ganoderic acid [150]. A new triterpenoid, named ganosporeric acid A, was recently isolated from the ether-soluble fraction of the spores [29]. Min *et al.*, [151] reported the isolation of six new lanostane type triterpenes and also from the spores (ganoderic acids γ, δ, ε, ζ, η and θ). Preliminary studies indicate that the spores contain considerably higher contents of ganoderic acids than other parts of the fungus and that triterpene composition of the fruit body varies according to the area in which it is grown [29]. The spores also contain triterpene lactones [150]. Fig. (**2**) represents the structure of all ganoderic acid and triterpenoids reported as in the literature.

4.8.2. Carbohydrates

Polysaccharides

More than 100 types of polysaccharides have been isolated from the fruiting body, spores and mycelia, or separated from the broth of a submerged liquid culture of *G. lucidum*. Most have a molecular weight ranging from 4 x 10^5 to 1 x 10^6 in the primary structure. They comprise one of the major sources of *G. lucidum*'s pharmacologically active

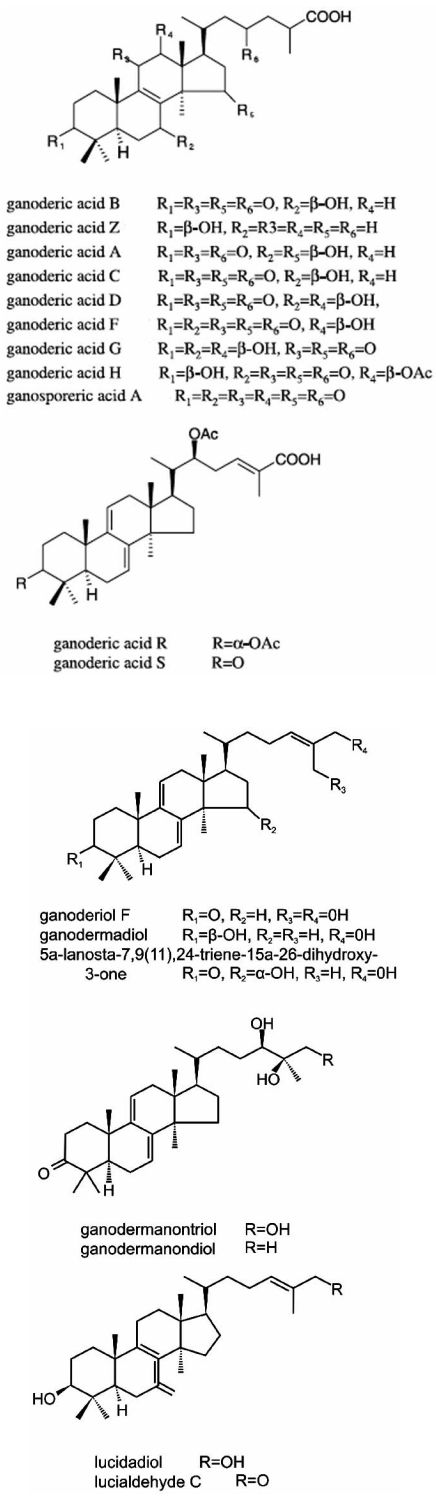

Fig. (2). Structure of Triterpenoids of *Ganoderma lucidum.*

compounds. *G. lucidum* polysaccharides such as β-D-glucans, heteropolysaccharides and glycoprotein have been isolated and characterized and are considered the major contributors of bioactivity of the mushroom β-D-glucans consist of a linear backbone of β-(1→3)-linked D-glucopyranosyl groups with varying degrees of branching from the C6 position (Fig. (**3**)). In addition to water-soluble β-D-glucans, β-D-glucans also exist with heteropolysaccharide chains of xylose, mannose, galactose, uronic acid and β-D-glucans–protein complexes that are present at 10–50% in dry *G. lucidum* [11, 14, 59, 152]. Some protein-bound polysaccharides and fucose containing glycoprotein with bioactivity have been isolated [9, 149, 153].

4.8.3. Proteins

Some proteins with bioactivity have also been isolated from *G. lucidum*. The LZ-8 is one such protein isolated from *G. lucidum*, which was shown, by sequencing studies, to be similar to the variable region of the immunoglobulin heavy chain in its sequence and in its predicted secondary structure. Major biological activities of LZ-8 resemble those of lectins, with mitogenic capacity toward mouse spleen cells and human peripheral lymphocytes and agglutination of sheep red blood cells *in vitro*. Neither was inhibited by the mono- or dimeric sugars examined, indicating that LZ-8 is not a lectin per se. It did not agglutinate human red blood cells but could function as a potent suppressor of bovine serum albumin-induced anaphylaxis in CFW mice *in vitro*. It appears to be related to an ancestral protein of the immunoglobulin superfamily [154].

4.8.4. Nitrogenous Compounds

Nucleotides and Nucleosides

Nucleosides include adenosine and 5-deoxy-50 methylsulfinylad- nosine [17].

4.9. Other Constituents

Reishi also contains sterols, amino acids (Table **6**) [149], soluble proteins, oleic acid, cyclo-octasulfur, an ergosterol peroxide (5,8-epidioxy-ergosta-6,22E-dien-3-ol) and the cerebrosides (*4E'*,8E)-N-D-2'-hydroxystearoyl-1-Oβ- D-glucopyranosyl-9-methyl-4-8-sphingadienine and (4*E*,8*E*)-N-D-2'-hydroxypamitoyl-1-O-β-D-glucopyranosyl-9-methyl-4-8-sphingadienine [4, 8-10, 17]. Regarding the inorganic ions, the mushroom contains Mg, Ca, Zn, Mn, Fe, Cu and Ge. The spores themselves contain choline, betaine, tetracosanoic acid, stearic acid, palmitic acid, ergosta-7, 22-dien-3-ol, nonadecanoic acid, behenic acid, tetracosane, hentriacontane, ergosterol and β-sitosterol. One of the lipids isolated from *G. lucidum* is pyrophosphatidic acid [10, 17].

5. COMMERCIAL ASPECT OF *GANODERMA LUCIDUM*

Today, present need in every aspect has increased so fast that every research work indicating commercialization requires major modifications to meet the upcoming challenges and obstructions. Similarly, the present review is an attempt to create an understanding and make aware about the vital importance of the macrofungi "*Ganoderma lucidum*". Under the names *Ling zhi* or *Reishi*, a number of *G. lucidum* products are sold as over-the-counter products in the forms of health drinks, powders and dietary supplement as well as specific functional agents as mycelial or fruit body extracts, intracellular polysaccharides, extracellular polysaccharides, spores, etc [6, 76]. In 2008, the worldwide production of *G.*

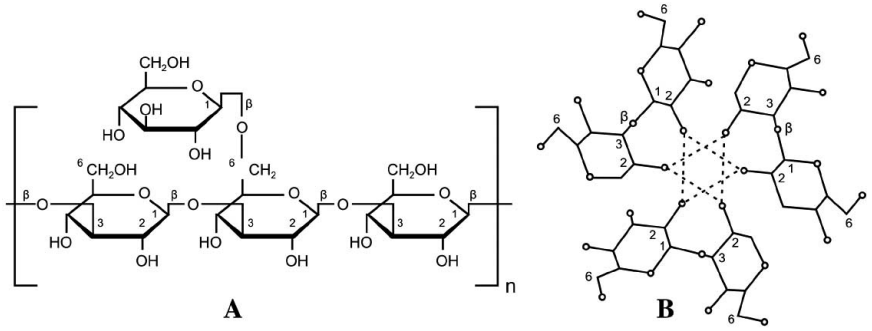

Fig. (3). Structure of β-D-glucans of *Ganoderma lucidum,* A, Primary molecular diagram; B, Higher level molecular diagram.

Table 6. Amino Acid Analysis of *Ganoderma lucidum* Extract [149]

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

lucidum was approximately 9500 tonnes, of which China contributed 6000 tonnes. *G. lucidum* is mainly used as a tonic and a remedy for the treatment of a variety of diseases, including chronic hepatopathy, hypertension, neurasthenia, insomnia, bronchitis, gastric ulcer, diabetes, hyperglycemia and cancer [3, 76]. Hence, it may be noted that *G. lucidum* is an excellent source of medicine which is enabling the present need of human mankind in every aspect.

6. CONCLUSION

Ganoderma lucidum, a mushroom of biomedical importance, contains a number of bioactive components, many of them biological response modifiers which activate our immune systems for a multitude of defensive functions. The immuno-modulating effects of *G. lucidum* are associated with its anti-tumour activity. Like synthetic biological response modifiers, *G. lucidum* has not demonstrated significant direct anti-cancer efficacy like cytotoxic chemotherapeutic agents. However, *G. lucidum* appears to serve as an effective adjuvant role in the treatment of cancer. The other possible applications of *G. lucidum* as immuno-modulator are in the field of infectious diseases, especially when such infectious episodes occur in individual whose immune system does not function optimally: young children, the elderly, patients submitted to major anaesthetic and surgical procedures. The identified bioactive compounds in *G. lucidum* of known molecular structures account for a wide range of beneficial biomedical effects, most notably in prevention of diverse physiological disorders and diseases. The *G. lucidum* is a golden medicinal fungus and is yet to be exploited commercially. In fact, the reason that some of the Ganoderma preparations are not yet available as medicines may be due to difficulties relating to mass production, hence, standardization of liquid media for large scale cultivation of *G. lucidum* should be undertaken. In the search for active compounds from *G. lucidum*, the majority of research had been focussed on extracts from the fruiting body and there have been fewer studies on extracts from the liquid cultivated mycelium. It appears that there are a number of biologically active compounds to be explored in the mycelium and the future research may be oriented in that direction.

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