Send Orders of Reprints at reprints@benthamscience.net

Recent Patents on Anti-Cancer Drug Discovery, **2013**, *8*, 255-287

Ganoderma lucidum: A Potential for Biotechnological Production of Anti-Cancer and Immunomodulatory Drugs

Bojana Boh*

University of Ljubljana, Faculty of Natural Sciences and Engineering, Askerceva 12, Ljubljana, Slovenia

Received: October 30, 2012; Accepted: November 26, 2012; Revised: December 06, 2012

Abstract: Based on the analysis of more than 270 patents and scientific articles, this state-of-the-art review presents *Ganoderma lucidum,* a medicinal basidiomycete mushroom with immunomodulatory and anti-cancer effects. Cultivation methods for the commercial production of *G. lucidum* fruit bodies and mycelia are summarized, with main active compounds of triterpenoids, polysaccharides, and proteins, often found in forms of proteoglycans or glycopeptides. Pharmacological effects with emphasis on anti-cancer and immunomodulatory functions are presented, separately for spores and dry mycelia, and for the groups of triterpenoids, polysaccharides, proteins and glycoproteins. Patents disclosing preparation methods of extracts and purified pharmaceutical isolates are reviewed, and examples of anti-cancer formulations, used as pharmaceuticals or nutraceuticals, are given. The review suggests that according to the present understanding, the anti-cancer activity of *G. lucidum* may be attributed to at least five groups of mechanisms: (1) activation/modulation of the immune response of the host, (2) direct cytotoxicity to cancer cells, (3) inhibition of tumor-induced angiogenesis, (4) inhibition of cancer cells proliferation and invasive metastasis behaviour, and (5) carcinogens deactivation with protection of cells. Although, the data from recent *in vitro* and *in vivo* studies demonstrate promising anti-cancer effects, a need is identified for further (1) isolation and purification of compounds, with deeper understanding of their individual and synergistic pharmacological effects, (2) molecular level studies of the antitumor and immuno-supportive mechanisms, (3) well designed *in vivo* tests and controlled clinical studies, and (4) standardisation and quality control for *G. lucidum* strains, cultivation processes, extracts and commercial formulations.

Keywords: Anti-cancer, *Ganoderma lucidum,* immunomodulation, polysaccharides, proteins, triterpenes.

1. INTRODUCTION

Ganoderma lucidum and other species of wood degrading basidiomycete mushrooms, used for centuries in Asian traditional medicine, were almost unknown in scientific research until three decades ago. Recently, scientific attention has been focused on *G. lucidum* polysaccharides and triterpenoids, which have been recognised as a promising natural source of immunomodulatory and anti-cancer compounds. Several *Ganoderma*-derived traditional and pharmaceutical products of different chemical composition, such as triterpenoids, polysaccharides, proteins, and polysaccharide-polypeptide complexes, have appeared on the market, either as raw extracts or in a purified form. Most attention is on the group of non-cellulosic water soluble β-D glucans with β-(1→3) linkages in the main chain of the glucan, and additional β-(1→6) branch points, that are characteristic for the antitumor and immunomodulating activity [1, 2].

In addition to an increasing number of scientific publications, new technological possibilities for commercial cultivation and use of *Ganoderma* mushrooms have given rise to numerous patents (Fig. (**1**)) [3, 4]. The inventions relate primarily to new methods of biotechnological cultivation of fruit bodies and/or mycelium biomass, extraction and isolation of active compounds, preparation of formulations, applications in pharmaceutical products or food supplements, and various methods for increasing or supporting the immunity of the organism by *G. lucidum* preparations alone or in combinations with other pharmaceuticals.

In Asian medicinal traditions, *G. lucidum* has been used for the prevention or treatment of a variety of diseases, including cancer. As Western medicine started to conduct research and accept some natural products from the traditional Asian medicines, the popularity of herbal and fungal therapies, such as *G. lucidum* for the treatment of cancer, have been increasing in Europe and the United States. Therefore, systematic research and testing, aiming towards a better understanding of the mechanisms, responsible for biological effects of *G. lucidum*, are much needed for clearer elucidation, validation and scientific justification of *G. lucidum* preparations [1, 2].

This article provides a comprehensive state-of-the-art review on *G. lucidum*, including both, scientific research articles and patent documents. Based on the analysis of more than 260 patents and scientific articles, the review presents and discusses: cultivation methods of *Ganoderma lucidum;* active compounds from the groups of triterpenoids, polysaccharides and glycoproteins; pharmacological effects with

*Address correspondence to this author at the University of Ljubljana, Faculty of Natural Sciences and Engineering, Askerceva 12, Ljubljana, Slovenia; Tel: +386 1 2514326 ext.208; Fax: +386 1 425 8684;
E-mail: bojana.boh@kii.ntf.uni-lj.si

2212-3970/13 $100.00+.00

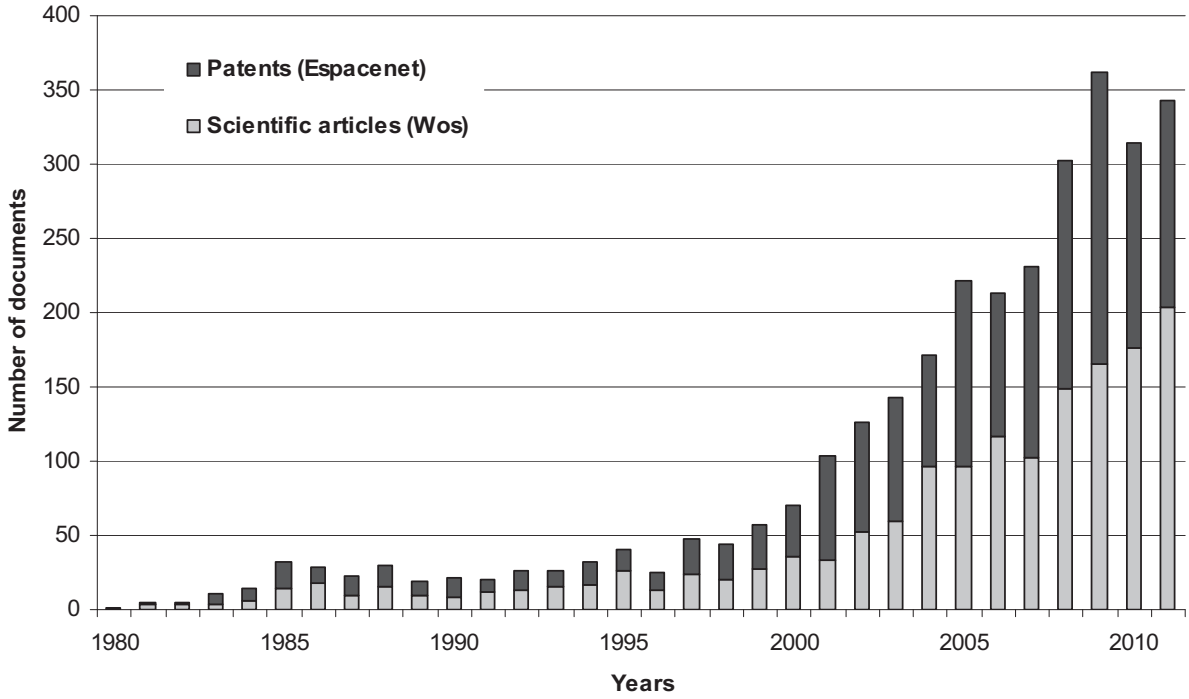

Fig. (1). Increasing number of new scientific articles and patents on *Ganoderma lucidum*, indicating a parallel growth of basic research and industrial innovation (scientific articles search in Web of Science database [3], patents search in Espacenet database [4]; search profile "Ganoderma lucidum" OR reishi OR "ling zhi")

emphasis on anti-cancer and immunomodulatory functions; downstream production processes of extracts and isolates; composition of anti-cancer formulations with *G. lucidum;* medical experience with *G. lucidum,* with examples of clinical trials; concluding remarks with an overview of *G. lucidum* anti-cancer mechanisms, and prospects for future research.

2. CULTIVATION METHODS

Ganoderma lucidum (W.Curt. :Fr.) P. Karst., family *Ganodermataceae*, order *Polyporales (*synonym *Ganoderma lucidum* (W.Curt.:Fr.) Lloyd, *Aphyllophoromycetideae*), is a white rot wood degrading basidiomycete with hard fruit bodies, known as *Ling-Zhi* in Chinese and *Reishi* in Japanese. *G. lucidum* is scarce in nature. Artificial cultivation has been introduced to meet the increasing demands for both, fruit bodies and mycelia biomass. A review of biotechnological cultivation methods for the commercial production of *G. lucidum* was published in our previous work [1], as well as in a recent review by Zhou *et al*. [2]. In short, cultivation technologies consist of the traditional growing of fruit bodies on wood logs or on sawdust-based substrates, and the modern cultivation of mycelia in bioreactors, either by solid-state or by submerged cultivation in liquid media (Fig. (**2**)).

Traditional Farming of Fruit Bodies

Traditional farming of *G. lucidum* fruit bodies on wood logs and in bags has been practiced especially in Asian countries, either outdoors as cultivation on hardwood stumps, buried logs or bed cultures, or indoors in bags or bottles filled with sawdust. Standard cultivation procedures have been published [5]. Rarely, patents report on *G. lucidum* fruit bodies cultivation on unusual agro-waste substrates, such as the residue of citrus fruit mixed with rice bran [6].

Biotechnological Production of Mycelia by Solid State Cultivation

There is scant information available on the biotechnological production of *G. lucidum* mycelium by solid state cultivation technologies (Table **1**) [7-12].

Production of Mycelia by Submerged Cultivation in a Liquid Medium

Most modern biotechnological processes for *G. lucidum* biomass production utilize submerged liquid substrate cultivations in bioreactors under controlled conditions. Substrates and process parameters have been described in detail in several scientific articles [13-23]. In addition, specific inventions on *Ganoderma* biomass cultivation in liquid media have been patented and disclosed in patent documents (Table **2**) [24-36]. The first patents on submerged liquid substrate cultivation of *G. lucidum* biomass originate from Asia, especially from Japan. Newer patents after the year 2000 described specific process improvement, additives and substrate compositions, and originate from a wider spectrum of countries.

Comparison and Discussion of Production Practices

Analysis of the literature suggests that fruit bodies and spores remain the basic raw materials in *G. lucidum* commercial products in traditional Asian medicines, complementary medicine, and dietary supplements. As the wild mushroom is scarce and difficult to collect in natural habitats, fruit

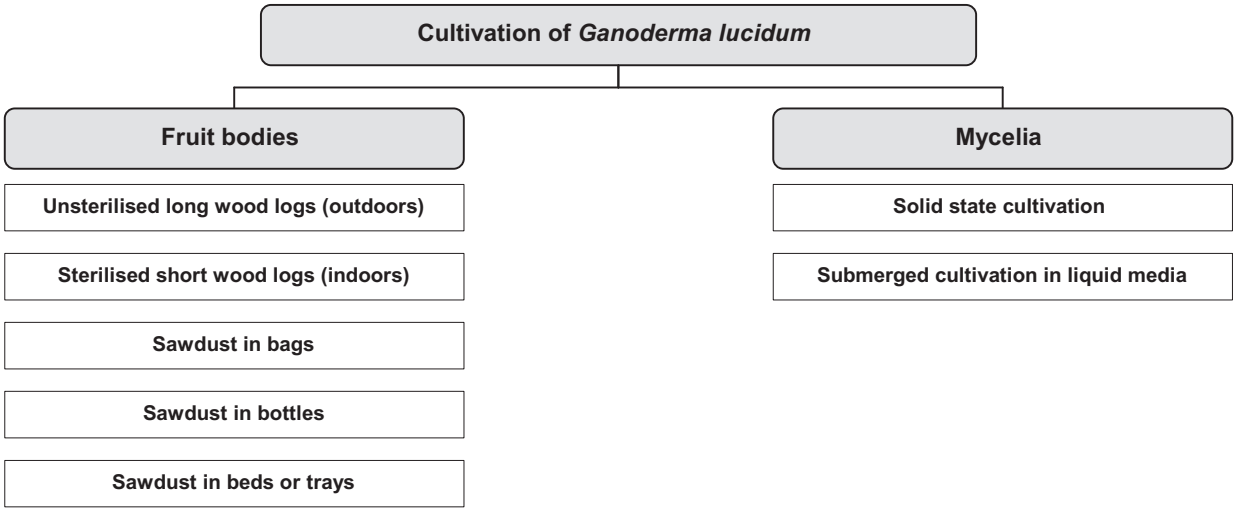

Fig. (2). Biotechnological cultivation methods for the commercial production of *G. lucidum* fruit bodies and mycelia.

Table 1. Patents on Solid State Biotechnological Production of *G. lucidum* Mycelia, Listed Chronologically.

Summary of the Invention	Patent, Year
Solid substrate containing bagasse fibres as a base material	[7] 1985
A low cost solid substrate composed of rice flour, powdered silkworm pupae, cane sugar, magnesium sulphate and potassium dihydrogen phosphate	[8] 1995
Glutinous rice and glucose substrate for the production of high purity *G. lucidum* mycelium	[9] 1998
A process of growing *G. lucidum* biomass in a horizontal stirred bioreactor on a solid substrate consisting 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. Large quantities of biomass can be produced, including the pharmaceutically active polysaccharides. Production of biomass and polysaccharides is dependent on substrate moistening	[10] 2000 [11] 2002
Substrate mixtures for solid state cultivation of several basidiomycetes, including *G. lucidum,* based on agars with additives, or based on grains, such as rice, corn, wheat, barley and oat	[12] 2003

bodies and spores of *G. lucidum* are produced in large quantities by cultivation methods in outdoor or indoor plantations on wood logs and in bags filled with sterilised wood chips or sawdust enriched with supplements. Breeding strategies of *Ganoderma* species, and quality control, including the DNA fingerprint, were reviewed and discussed recently by Zhou *et al*. [2]. The quality and content of physiologically active substances is difficult to control, and vary from strain to strain, depend on location, culture conditions, the growth stage of the fungus, the processing procedures, and formulation preparation [2, 37-39]. Setting up the fingerprint of good quality species, and the quality standards seems to be crucial for growers, researchers and consumers. Examples of recent publications on analytical methods that could facilitate the quality control include techniques such as FTIR microspectroscopy for the identification and distinction of broken cellular wall and unbroken *G. lucidum spores* [40], quantification of total polysaccharides and triterpenoids in *G. lucidum* by near infrared spectroscopy and chemometrics [41], and analysis of *Ganoderma lucidum* terpenoids by a direct ^{13}C NMR detection in HPLC hyphenation mode [42].

Cultivation of fruit bodies from spawn to cropping is a long process that takes approximately 90 to 150 days, or more. Therefore, for the production and isolation of pharmaceutically active substances from the fungal mycelia, much faster solid state and submerged liquid substrate biotechnological processes seem to be attractive. Compared to submerged cultivation in liquid media, a solid state culture has several advantages, such as lower investment costs and easier operations (a concentrated product can be obtained from a cheap substrate, such as an agricultural residue, with little pre-treatment or enrichment), and minimum waste production. However, there are some obvious disadvantages, including the longer cultivation time, and the difficulty of separating/isolating the biomass and active compounds from the solid undefined substrate, such as sawdust. For this reason, solid state cultivation of *G. lucidum* mycelia seems to be appropriate for the production of animal feed supplements, for which the whole overgrown substrate can be milled and used, and less suitable for pharmaceutical applications. Submerged liquid state cultivation of fungal biomass in industrial bioreactors has become the method of choice for large scale pharmaceutical applications of *G. lucidum* isolated products.

3. ACTIVE COMPOUNDS

The main groups of active constituents of *Ganoderma* mushrooms are triterpenoids, polysaccharides, and proteins. The polysaccharide and peptide parts have often been found in natural forms of proteoglycans or glycopeptides.

Table 2. Patents on Submerged Liquid Cultivation of *G. lucidum* Mycelia, Listed Chronologically.

Summary of the Invention	Patent, Year
Novel water soluble high-molecular polysaccharide Ganodellan, produced by *G. lucidum* in a submerged cultivation process under aerobic conditions. The polysaccharide was described as a *β*-glucan with potential uses in food thickening and gelation, as well as an antineoplastic agent	[24] 1985
Submerged liquid cultivation process of *G. lucidum* and other medicinal basidiomycete mycelia, for the production of carcinostatic compounds. Spores or mycelium were used as an inoculum. The submerged process took place in aqueous liquid substrate, consisting of a soluble starch as a source of carbon, hydrogen and oxygen, a liquid nitrogen source, and a small amount of amylase. Aeration was provided by mixing	[25] 1986
G. lucidum glycoprotein with hemagglutination and immunosuppressive activities, produced by submerged culturing of *Ganoderma* mycelia in a liquid medium	[26] 1988
G. lucidum mycelium cultivation in a liquid substrate, consisting of glucose and yeast extract, glutamine or glutamic acid. The mycelium produced a viscous polysaccharide with *β*-1,4-glucosidic bonds, composed mainly of glucose, with some fructose and amino acids in side chains. The polysaccharide was soluble in water and insoluble in ethanol, and was used for increasing viscosity, as an emulsifier and humectant	[27] 1989
Process for *G .lucidum* submerged cultivation in a liquid medium for the production of ganoderic acids A and Z with antitumor and hypotensive activity. Seed mycelia were produced in a static culture on a dextrose agar medium, while the main production process took place in a shaken or agitated aqueous medium containing glucose and corn steep liquor	[28] 1992
A procedure of growing *G. lucidum* by submerged cultivation in a bioreactor. The inoculum was prepared on potato dextrose agar, and then transferred into a liquid cultivation medium, consisting of a filtrate of peeled cooked potatoes, glucose and olive oil in water. Detailed process parameters were described. The process was optimised for the production of *G. lucidum* biomass and its metabolites in a laboratory bioreactor, with a potential industrial scaling up	[29] 1998
Industrial large scale *G. lucidum* submerged cultivation. The 10 tons tank fermentation technology yielded 2.6 to 3.3 % of dried mycelium, with 6 to 8 % polysaccharide content in a dry biomass	[30] 1998
Biotechnological cultivation of medicinal basidiomycetes with β–glucosidase activity, such as *G. lucidum* or *Lentinus edodes*, in a medium containing isoflavones, such as genistein from soybean seed or arrowroot. The products synergistically combined physiological activities of the aglycone of isoflavones and the physiological activity of basidiomycetes, and had antitumor effects due to tumor angiogenesis inhibition, tumor cell growth suppression, and /or tumor cell apoptosis induction	[31] 2001
A multi-step method for *G. lucidum* mycelium production with a subsequent isolation of exo-polysaccharides from the mycelia culture. The biomass was produced in a synthetic medium at 25°C, with aeration at 100 to 150 rpm, for 5 to 15 days. After homogenizing the cultured medium in ice water, the content was used for inoculating the second synthetic medium, with culturing for 10 to 30 days at 20 to 35 °C, pH 4 to 7, aeration 50 to 150 rpm. The final culture broth was centrifugated, the supernatant was treated with ethanol, the precipitate dissolved in distilled water and dialysed	[32] 2003
Improvements of *G. lucidum* liquid submerged cultivation by the addition of rare earth elements, such as praseodymium Pr^{3+}, neodymium Nd^{3+} or lanthanum La^{3+}. Yields of triterpenoids and polysaccharides were increased	[33-34] 2010
Shortening of cultivation time from 2 - 3 weeks to 7 days was achieved. A two step process utilised different substrate compositions: a dense nutrient medium containing extracts of corn and wheat, with agar, and a second liquid substrate containing glucose, soya flour, potassium dihydrophosphate, magnesium sulphate and arachidonic acid in a vegetable oil. The submerged cultivation was used for the production of anticancer agents	[35] 2011
A two-step biotechnological process for cultivation of *G. lucidum* biomass in a liquid medium, resulting in spherical mycelium formations. After separation from the liquid medium by centrifugation, the biomass was dehydrated and conditioned as granules	[36] 2011

Triterpenoids

More than 130 triterpenoid compounds were found, isolated and structurally characterised in *Ganoderma* fruit bodies, spores, and mycelia biomass, such as ganoderic (highly oxygenated C_{30} lanostane-type triterpenoids), lucidenic, ganodermic, ganoderenic, ganolucidic and applanoxidic acids, lucidones, ganoderals and ganoderols. Their basic chemical structure is based on lanosterol, an intermediate in the steroid and triterpene biosynthesis. A series of scientific articles from the 1980 onward reported on new triterpenoid substances, their isolation procedures, structures and properties [43-59]. According to the number of carbon atoms, triterpenoids have been divided into three main groups: C30, C27 and C24 triterpenoid compounds. Similar structures with different functional groups have been marked with letters, such as ganoderic acids A, B, C, etc. to V, W, X, and Y [2]. Representative structural examples of *Ganoderma* triterpenoids are shown in Fig. (**3-11**).

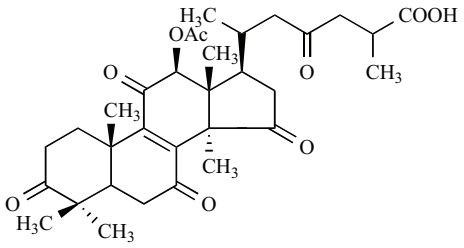

Fig. (3). Ganoderic acid F 12β-acetoxy-3,7,11,15,23-pentaoxo-5α-lanost-8-en-26-oic acid.

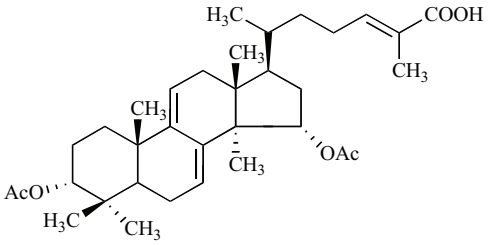

Fig. (4). Ganodermic acid R (24*E*)-3α,15 α-diacetoxy-5α-lanosta-7,9(11),24-triene-26-oic acid.

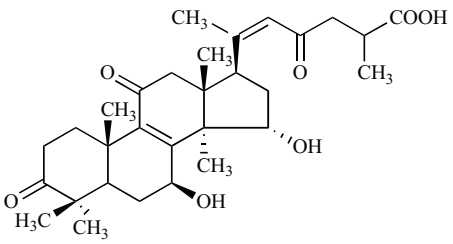

Fig. (5). Ganoderenic acid A (20*E*)-7β,15α-dihydroxy-3,11,23-trioxo-5α-lanosta-8,20-dien-26-oic acid.

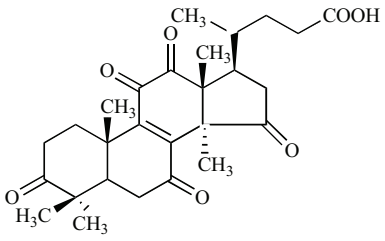

Fig. (6). Lucidenic acid D1 4,4,14α-trimethyl-3,7,11,12,15-pentaoxo-5α-chol-8-en-24-oic acid.

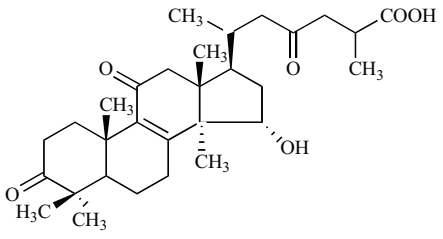

Fig. (7). Ganolucidic acid A 15α -hydroxy-3,11,23-trioxo-5α -lanost-8-en-26-oic acid.

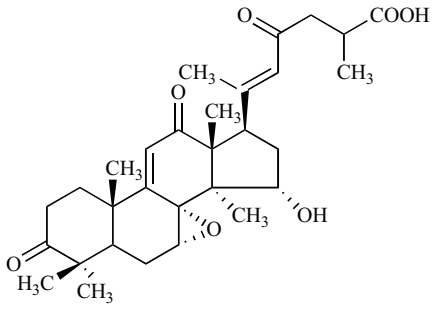

Fig. (8). Applanoxidic acid A (20E)-15α-hydroxy-7α,8α-epoxy-3,11,23-trioxo-5α-lanosta-9(11),20-dien-26-oic acid.

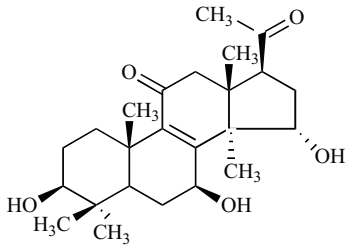

Fig. (9). Lucidone C 3β,7β -dihydroxy-4,4,14α-trimethyl-11,15,20-trioxo-5α -pregn-8-ene.

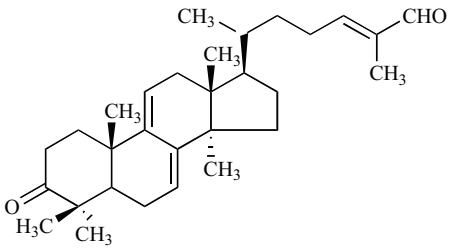

Fig. (10). Ganoderal A (24*E*)-3-oxo-5α -lanosta-7,9(11),24-triene-26-al.

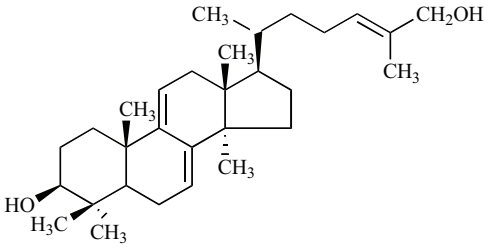

Fig. (11). Ganoderol B ganodermadiol-5α -lanosta-7,9(11),24-triene-3β,26-diol.

Descriptions of triterpenoid extraction procedures in scientific articles vary; the process usually starts with methanol or ethanol extraction, rarely with chloroform or ether. The second stage depends on the characteristics of the desired triterpenoid fraction. For instance, in the isolation of ganoderic acids, which contain the carboxylic group, the total acid fraction can be separated by altering the pH, i.e. by alkali treatment and acidification in aqueous solution, followed by a liquid-liquid extraction into an organic solvent, such as chloroform or ethyl acetate [43-44, 46, 53]. Specific further separation and purification steps, including chromatographic

techniques, can be applied for final isolation of individual triterpene compounds [43, 48, 50-51, 54, 56]. Recently, Cheng reported on a fast and convenient preparative method for separation of *G. lucidum* triterpenoids, based on a counter-current chromatography technique [59].

In addition to scientific articles, some patents [28, 60-63] address *Ganoderma* triterpenoids, their biotechnological production, isolation, and activities. Tsujikura *et al*. [28] described cultivation of *G. lucidum* mycelia in a liquid medium, and the process for isolation of ganoderic acids, based on extraction with chloroform. A patent by Hattori *et al*. [60] proposed an extraction of triterpenoids from *G. lucidum* using 70 - 99 % ethanol as a solvent, at low temperature. Compared to extraction with hot alcohol, the low temperature process had a higher yield of triterpenoid compounds with inhibitory effects against HIV. Yang [61] disclosed an extraction method for the isolation of *G. lucidum* triterpenes with antineoplastic effects, based on supersonic extraction with recycling of solvents. The process, distinguished by lower solvent consumption and a quicker extraction time, consisted of supersonic leaching, dissolution in chloroform, supersonic alkali extraction, acidification with hydrochloric acid, supersonic extraction, and decompression evaporation. On a large-scale production level, Xu [62] patented a technique and technology for industrial extraction of triterpenes from *G. lucidum* mycelia, produced by submerged cultivation. With an improved *G. lucidum* strain, the reported yield of extracted triterpenes reached 5 - 7g100g^{-1}of dry mycelium.

Besides the extraction and isolation of natural triterpenes from *G. lucidum* fruit bodies, spores and mycelia, attempts have been made to prepare semi-synthetic and synthetic pharmaceutically active triterpenoid analogues. A recent patent application by Minto and Kennedy [63] disclosed a series of pharmaceutical triterpenoids, such as delta-7,9(11) steroids and related triterpenoid compounds from *G. lucidum*, and a synthesis processes for preparing derivatives with different combinations of substituted groups. As an example, a process for preparing ganodermanontriol was disclosed, which provided ganodermanontriol in an overall yield of 15.3 % over 9 steps. Processes for preparing stereoisomeric triols in overall yields of 11.3-14.5 % were also described. The compounds were chemically characterised and tested in cell proliferation assays on human cancer cell lines.

Polysaccharides

Studies [64-67] have shown that the most active immunomodulatory polysaccharides are water-soluble β-1,3-D and β-1,6-D glucans, which can be precipitated from an aqueous solution by ethanol. Their prevailing structure is β-1,3 D-glucopyronan with 1-15 units of β-1,6 monoglucosyl side chains (Fig. (**12**)). Their 1,3-linked backbone, relatively small side chains, and an organized helical structure have been recognised as beneficial for promoting immunostimulation effects [64, 66-67].

However, in addition to β-1,3-D and β-1,6-D glucans, other *G. lucidum* glucans and heteropolysaccharides with different composition and structure have been reported. Within the group of glucans, a water-soluble branched glucan (named GSG) with several different linkages was isolated from spores [68]. A linear water-insoluble β-1-3-D glucan (named GL-IV-I) was obtained from fruit bodies by NaOH aqueous solution extraction. Its water soluble derivatives were prepared by sulphation, carboxy-methylation, hydroxyethylation, hydroxypropylation, and methylation [69]. A neutral β-D-glucan (named GLSA50-1B) was recently isolated from spores of *G. lucidum*, by hot-water extraction, graded ethanol precipitation, anion-exchange chromatography, and gel permeation chromatography. Its 1,6-linked β-D-glucose backbone had different length of branches consisting of terminal and 1,4-linked glucose residues, attached to O-4 of alternative glucose residues in the backbone [70].

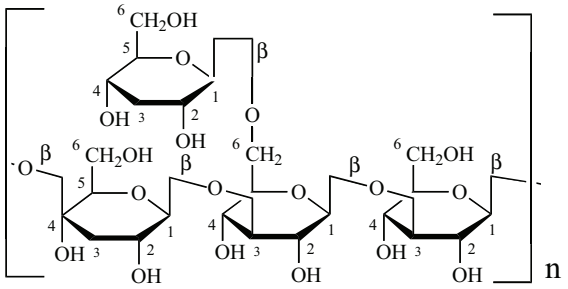

Fig. (12). Typical structure of a β-1,3-D glucan with β-1,6 side chains (1,6-monoglucosyl-branched 1,3-β-D-glucan).

Within the group of heteropolysaccharides, a galactose rich extracellular polysaccharide (named GLP-2), composed of galactose, mannose, glucose, arabinose and rhamnose in the molar ratios 103:17:12:10:3, isolated from submerged culture broth, was reported by [71]. A water-soluble heteropolysaccharide (named LZ-C-1) with a molecular weight of 7 kDa, composed of L-fucose, D-glucose and D-galactose, was isolated from the fruit bodies [72]. Five water-soluble heteropolysaccharides (GL-I to GL-V) were isolated from fruit bodies, with monomer structure of glucose, galactose, mannose, arabinose, with relatively low molecular weights. The chain branching decreased from GL-I as the most branched heteropolysaccharide with a 27.0 % degree of branching, to GL-V as a mainly linear glucan [73]. Recently, a neutral hetero-polysaccharide with antihyperglycemic properties, named FYGL-1, was isolated from fruiting bodies, with a molecular weight of 78 kDa. The structure characterization revealed the main monomers of galactose, rhamnose and glucose in mole ratio of 1.00 : 1.15 : 3.22 [74].

Isolation procedures of intracellular polysaccharides from the fruit bodies or mycelia usually begin with hot water extraction, sometimes also with alkaline or acidic aqueous solution extraction. Extracellular polysaccharides, produced by submerged liquid cultivation, can be obtained directly from the aqueous phase of the broth, after removal of mycelia by filtration. In both cases, polysaccharides are precipitated from the aqueous phase by concentrated ethanol, e.g. by 3-fold volume of 96 % ethanol at 0-4ºC, for 12 hours [75]. Further purification methods can employ column chromatography, such as ion-exchange, gel and affinity chromatographies [76]. A patent by Song *et al*. [32] presented a

method for producing water-soluble extracellular polysaccharides from the submerged mycelia culture of *G. lucidum*. After the cultivation stage, the broth was centrifuged, and the supernatant containing raw extracellular polysaccharides was treated with ethanol. The polysaccharide precipitate was redissolved in distilled water, centrifuged, and the supernatant with the water-soluble extracellular polysaccharide fraction was dialyzed.

Proteins

One of the best known and most studied proteins isolated from *G. lucidum* is the immunomodulatory protein ling zhi-8, also known as LZ-8. The isolation and characterization of LZ8 was done by Kino *et al*. in 1989 [77], and its complete amino acid sequence was published by Tanaka and coworkers [78]. LZ-8 was described as a small protein of 110 amino acid residues, with a molecular weight of 12.4 KDa and an isoelectric point at pH 4.4. Its immunomodulatory effects were confirmed by several research groups [77, 79-81].

With the development of recombinant technologies, recombinant LZ-8 protein (rLZ-8) became available for pharmaceutical applications. Patent application by Sun *et al*. [82] claimed pharmaceutical compositions based on rLZ-8, and its uses for prevention or treatment of thrombocytopenia. Sun and Zhang [83] patented pharmaceutical compositions comprising rLZ-8, and pharmaceutical uses for antitumor treatment, leukocyte increase, and inhibition of immunological rejection. The rLZ-8 protein was expressed in Pichia yeast. Zhou *et al*. [84] disclosed a gene and a nucleotide sequence of an immunomodulating *G. lucidum* fungal immunomodulatory protein (FIP). According to their invention, the FIP gene was expressed in *E. coli* for potential large scale production of recombinant FIP for pharmaceutical applications.

Examples of other proteins, isolated from *G. lucidum*, include a ribonuclease with a molecular mass of 42 kDa [85], a proteinase A inhibitor with a molecular mass of 38 kDa and with a remarkable heat stability [86], and ganodermin, a 15 kDa protein with antifungal activity [87].

Glycopeptides and Proteoglycans

G. lucidum polysaccharides and proteins often form natural complexes. Scientific articles report on the isolation of several glycopeptides and proteoglycans [88, 89], such as a proteoglycan with a carbohydrate : protein ratio of 10.4 : 1, possessing an antiherpetic activity [90], a 114 kDa hexameric glycoprotein lectin with 9.3 % neutral saccharide, stable at pH 5-9 and a temperature up to 50°C, with hemagglutinating activity [91], a 12 kDa water-soluble glycopeptide (GLPCW-II), consisting of 90 % carbohydrate and 8 % protein [92], several low molecular weight peptides with hepatoprotective effects [93], and a water-soluble glycopeptide named LZ-B-1 [94].

Additional information on *G. lucidum* glycopeptides and proteoglycans come from patents. Murata *et al*. [27] disclosed a mucilaginous water-soluble extracellular polysaccharide with *β*-1,4 bonds, composed of glucose and fructose, with an amino acid part consisting of serine, glycine, alanine and other amino acids. The polysaccharide was produced in a submerged liquid culture and was isolated for its potential uses in viscosity control, emulsification and humecting. Patent applications by Lee *et al*. [95, 96] described a *G. lucidum* proteoglycan G 009 with antitumor and immunostimulating effects. Its main monosaccharide units were identified as β-glucose, α-glucose, galactose, α-mannose and fructose, and as amino acid components glycine, alanine, histidine, arginine, valine, aspartic acid, threonine, isoleucine, serine, leucine, glutamic acid, tyrosine, proline, phenylalanine and methionine. Patent applications by Tsunoo *et al*. [97, 98] disclosed a glycoprotein, isolated from *G. lucidum* mycelia, with immunosuppressive activity, with no human hemagglutination effects. The glycoprotein had two amino acid sequences in its proteinaceous structural unit, and 0.3-3.0 wt. % saccharide content, with galactose, mannose and hexosamine as the main monosaccharides. Its molecular weight was between 12,000 - 18,000 g mol^{-1}, and the isoelectric point at pH 4.4 - 4.6. A patent by Lin *et al* [99] focussed on *G. lucidum* peptides, in particular on a high molecular weight peptidoglycan, named polysaccharide protein G1-PP, which was isolated from the glossy *G. lucidum* by extraction, condensation, and ultrafiltration or dialysis. The patent revealed a process for industrial production of polysaccharide protein G1-PP and its use in medicaments with antineoplastic action. A patent application by Yu *et al.* [100] described an immunostimulatory fucose-containing glycoprotein fraction, containing 10-20 % protein and 80-90 % polysaccharide, with 7.1 % part of deoxy sugar L-fucose. The product was obtained from an alkaline *G. lucidum* extract, specifically by 0.1N NaOH extraction, neutralisation, ethanol precipitation, and purification by Sephacryl S-500 column chromatography. Two patent applications by Yu *et al*. [101] and Liang *et al*. [102] described *G. lucidum* fractions, named F3, containing polysaccharides and glycopeptides with terminal fucose residues. The total sugar content of subfraction F301 was 37 %, with the following monosaccharide parts (%): glucose 74, galactose 9, mannose 9, glucosamine 5, and fucose 4. The total sugar content of subfraction F331 was 19 %, with the monosaccharide composition of (%): glucose 59, glucosamine 18, mannose 13, galactose 7, and fucose 3. F3 fractions induced immunomodulatory, hematopoietic and tumor-inhibiting phenotypic changes in eukaryotic cells, and also exhibited anti-viral properties. According to a patent by Wang *et al*. [103], a crude *G. lucidum* extract, prepared by 0.1 N NaOH alkaline extraction, neutralization and ethanol precipitation, was analysed, and the following monosaccharide composition was determined (%): 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. The reported amino acid composition of crude extract, expressed as relative abundance, was: Glu 120, Asp 117, Gly 108, Ala 100, Thr 66, Val 61, Pro 60, Leu 55, Ser 54, Ile 36, Phe 28, Arg 22, Lys 21, Tyr 16, His 12, and Met 6. The crude extract was further purified by gel filtration chromatography on Sephacryl S-500 column. The purified fraction F3 (terminal fucose-containing glycoprotein fraction) and its subsequent three subfractions (F3G1, F3G2, F3G3) contained the same monosaccharides, although in different ratios. The glycoproteins stimulated spleen-cell proliferation and expression of cytokines. The results showed that the saccharide moiety was responsible

for the activity; furthermore, the presence of fucose in the saccharide was required.

In practice, *G. lucidum* polysaccharide preparations from different isolation procedures possibly contain residues of LZ-8 and other polypeptides. To better understand specific pharmacological effects, targets and regulating functions, some researchers removed proteins from polysaccharides by deproteinisation with trichloroacetic acid (TCA), to obtain relatively pure forms of polysaccharides. For instance, Yeh *et al*. [81] studied deproteinised polysaccharides in comparison with LZ-8 and concluded that LZ-8 could activate murine macrophages and T lymphocytes, but a deproteinised polysaccharide was merely the activator for macrophages, which suggested their diverse roles in activating the innate and adaptive immunity.

Other Compounds

Although triterpenes, polysaccharides, proteins, and peptidoglycans have been most thoroughly investigated, other active compounds from *G. lucidum* have been described, such as adenosine with anti platelet aggregation effect, fatty acids with potential effect of tumor cell proliferation inhibition, alkaloids, vitamins and essential minerals, hydrocarbons, monoterpenes and sesquiterpenes [104-106].

4. PHARMACOLOGICAL EFFECTS

When powdered fruit bodies, spores or crude extracts have been applied and pharmacologically tested, the relations between specific chemical compounds of *G. lucidum* and the observed effects could not be clearly attributed nor explained. However, an increasing number of studies aimed to elucidate the individual target effects of triterpenoids, polysaccharides and protein preparations in regard to their modes of action on particular diseases, including cancer. In more recent studies, purified substances isolated from *G. lucidum* have been researched to unveil the molecular mechanisms responsible for the antitumor and immunosupportive activities, such as genome analysis, gene expression, biosynthesis of regulatory molecules and enzymes, their role and function in biochemical pathways, and physiological responses in different tissues, organs and organisms. From the viewpoint of potential industrial applications, *G. lucidum* products, production methods, proposed applications and formulations have been claimed and disclosed in patent documents. The results of patent analyses are presented in Tables **3**-**7**.

Pharmacological Effects of Spores and Mycelia

Scientific evidence of pharmacological and particularly antitumor activities of *G. lucidum* spores has been documented by several research groups [107-111]. For instance, spores inhibited tumour cell proliferation and induced tumour cell death [108], elicited antitumor immune responses [110], and improved cancer-related fatigue in breast cancer patients [111].

In addition, patent documents disclosed the products based on *G. lucidum* spores or dry pulverised mycelia (Table **3**), with their pharmacological effects, examples of testing and proposed pharmaceutical applications in treatment of cancer, related to mechanisms of tumor growth suppression [112], immunomodulatory effects [113], and inhibition of angiogenesis [114].

Pharmacological Effects of Triterpenoids

Pharmacological effects of triterpenoids have been reviewed previously [1, 2, 115]. Analysis of extensive scientific research on *G. lucidum* triterpenoid extracts and purified triterpenoid compounds has demonstrated the following pharmacological effects, documented in scientific publications:

- Anti-cancer effects [56, 57, 116-132], which were attributed to and described as:
    - triterpenoid cytotoxicity against cancer cells [56, 116, 117, 125]
    - inhibition of cell growth [57, 118-121],
    - induction of cell apoptosis [123], recognised specifically as mitochondria mediated apoptosis [124, 130-131], or as induction of DNA damage, G1 cell cycle arrest and apoptosis [132]
    - inhibition of tumor invasion through down-regulating matrix metalloproteinase gene expression [127], and
    - antitumor or antimetastatic activities due to the inhibition of tumor-induced angiogenesis [119, 122].
- Anti-inflammatory effects [133-135], observed as suppression of the inflammatory response [135].
- Antihistaminic effects [136], documented as suppression of histamine release.
- Anti-viral properties, such as anti-HIV effect observed as an inhibitory activity against HIV-1 protease [137-139], and inhibitory effect on Epstein-Barr virus activation [139].
- Cell protective effect, such as antimutagenic and hepatoprotective activity in case of hepatic damage caused by mutagens [140], and nephroprotective effect in case of cisplatin induced nephrotoxicity [141].
- Cholesterol synthesis inhibition [142-143], e.g. by inhibition of cholesterol esterase [143].
- Platelet aggregation effects [144-146], attributed to thromboxane A2-dependent pathway [145], or prostaglandin E(1)-induced cyclic AMP elevation [146].
- Anti-androgenic activity in benign prostatic hyperplasia and prostate cancer [147, 148], by suppressing the function of androgen and its receptor, thus reducing growth of the prostate induced by testosterone.

In patent documents, listed chronologically in Table **4**, triterpenoid products have been disclosed mainly in relation to the inhibition of cancer cell proliferation [61, 63]; inhibition of angiogenesis [149, 150], and inhibition of HIV [60].

Table 3. Patent Documents on *G. lucidum* Spores and Dry Pulverised Mycelia.

Product	Production Method	Effects /Activity	Examples of Testing	Proposed Applications / Formulations	Patent, Year
Germination activated sporoderm-broken spores, collected from *G. lucidum* red fruit bodies	Three-staged process: (1) induction of spore germination by soaking and culturing, (2) treating spores with cell wall breaking enzymes and/or by mechanical force, (3) drying of whole spores or extraction of bioactive substances	Effects on immunological disorders, cancer, AIDS, hepatitis, diabetes, cardiovascular diseases, inhibition of free radical oxidation, prevention of hepatotoxic effects	(1) Tumor suppressing effect of *G. lucidum* sporoderm broken spores on mouse transplant sarcoma and hepatoma tumors (2) Toxicology tests on mice and rats (3) Clinical examples: chronic hepatitis B and hepatoma patients	Pharmaceutical products, dosed by any convenient method	[112] 2001
Germination activated *G. lucidum* spores	Two stage process: (1) soaking spores to induce germination, (2) breaking the sporoderm by mechanical and/or enzymatic methods	Immunological / immunomodulatory effects. A corticosteroid, e.g. prednisolone, can be co-administered for a synergistic effect	(1) *In vivo* tests on mice: immunoregulatory effects, delayed allergic reaction, serum haemolysin titre, carbon clearance test (2) Toxicity and mutagenicity tests on rats: LD_{50}, bone marrow micronucleus test, sperm deformation test, Ames test (3) Effects on systemic lupus erythematosus on mice	Pharmaceutical oral compositions for treating immunological disorders, particularly autoimmune diseases, e.g. systemic lupus erythematosus	[113] 2003
Dry pulverised cultivation product, containing mycelium of *G. lucidum* or *Lentinus edodes*	Mycelium is cultivated in a production medium with additives containing isoflavones, e.g. soybean seeds, their products, or arrowroot. Most typical aglycone of isoflavone is genistein	Antitumor effects: tumor angiogenesis inhibition effect, tumor cell growth suppression effect, tumor cell apoptosis induction effect Physiological activity of the aglycone of isoflavone and of the basidiomycetes was synergistically increased	(1) Chemical composition of dry pulverised material (2) Anti-cancer tumor cell growth suppression effect on mouse melanoma cells, mouse colorectal cancer cells, mouse lung cancer cells, mouse angioendothelioma cells, rat breast cancer cells, human prostatic cancer cells, human bladder cancer cells (3) Tumor angiogenesis suppression tests on : mouse tumor cells and ex ovo tests using vitelline membrane	Health care food compositions. Feedstuffs for animals or fish cultures. Pharmaceutical antitumor agents	[114] 2003

Pharmacological Effects of Polysaccharides

The analyses of a large corpus of scientific research articles on *G. lucidum* purified polysaccharide fractions and polysaccharide extracts provided evidence of anticancer effects, and suggested that the *in vivo* antitumor activity of polysaccharides may be mediated by different mechanisms, particularly through modulations of the immune system, and the inhibition of tumor-induced neovascularisation processes [115, 151, 152]. Results of individual *in vitro* and *in vivo* anticancer studies with *G. lucidum* polysaccharides supported the following mechanisms:

- Anticancer effects measured as the cancerous cell and tumor growth inhibition [153-165],
- Anticancer effects attributed to the stimulation of immune functions of macrophages and lymphocytes [81, 88, 104, 166-169],
- Anticancer effects measured as cytokine release, such as tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ) and interleukins (IL-1, IL-2, IL-3, IL-6) [75-76, 166, 170-180],
- Anticancer effects due to the anti-angiogenic activity 171-173 [181-183].

Recent studies [163-165] provide evidence that polysaccharides may also directly contibute to apoptosis of cancer cell. In a study by Liu *et al*. [165] intracellular polysaccharides from submerged cultivation of *G. lucidum* were investigated on human liver cancer cells. Polysaccharides inhibited human hepatocarcinoma cells HepG2 during the earlier phase with a lower dosage, but became less functional in a later phase, regardless of the dosage applied. However, in a later incubation phase with a high dosage of polysaccharides, apoptosis of the HepG2 cells appeared, and was enhanced by a supplemental dose of polysaccharides. Polysaccharides

Table 4. Examples of Patents on *G. lucidum* Triterpenes.

Triterpene Product	Production Method	Effects /Activity	Patent, Year
G. lucidum triterpene extract	Supersonic extraction with recycling of solvents	Antineoplastic effects	**[61]** 2002
G. lucidum triterpene extract	Extraction of triterpenoids by 70 - 99 % ethanol as a solvent, at low temperature	Inhibition of HIV or HIV-protease	**[60]** 2003
Neovascularisation inhibitor comprising Ganoderic acid F	Extraction from fruit body of *G. lucidum* or chemical synthesis	Suppression of neovascularisation	**[149]** 2004
Mixture of *G. lucidum* triterpene extract and polysaccharide extract	Triterpene extraction with ethanol, followed by partitioning from petroleum ether to dichloromethane Polysaccharide extraction with water, precipitation by ethanol, proteins removal with trichloroacetic acid, dialysis	(1) Inhibition of cancer cell proliferation. (2) Angiogenesis inhibition	**[150]** 2009
Triterpenoid analogues, e.g. delta-7,9(11) steroids, and their salts	Synthesis of derivatives with different combinations of substituted groups, e.g. ganodermanontriol	(1) Inhibition of cancer cell proliferation, invasion and metathesis (2) Antiandrogenic, anticomplement, antihistamine, anti inflammatory, antioxidant, or hypocholesterolemic activity	**[63]** 2012

inhibited also other human hepatocarcinoma cells (BEL-7402 and Huh-7). However, normal human liver cells L02 were stimulated by polysaccharides in a positive dose- and time-dependent manner.

In addition to anticancer activity, other medicinal effects of *G. lucidum* polysaccharides have been reported, as follows:

- Protection of living cells, such as genoprotection achieved through protecting cellular DNA from oxidative or radiation damage [126, 184-185]; protective effects on macrophages [186], pancreatic islets [187], and preservation of the synaptic density protein synaptophysin, which is exterminated in Alzheimer's disease [188].
- Other functions related to the immune system, such as reduction of the immunosuppressive response, induced by anticancer drugs [189, 190]; enhancement of unspecific immune functions [191]; induction of gene expression patterns that could reverse resistance to chemotherapeutic drugs [192]; abatement of allergies and autoimmune diseases by decreasing levels of IgE and/or IgG2 [193]; anti-inflammatory activity and treatment of psoriasis by inducing increased IL-10 and/or IL-1Ra expression [194].
- Antibacterial / antibiotic activity, e.g. against human pathogenic bacteria *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* [195], *Staphylococcus* sp., including methicillin resistant *Staphylococcus aureus* [196], or in compositions for reducing minimal inhibitory concentration of antibiotics [197].
- Anti-viral activity [198-199],
- Analgesic and anti-arthritic effects [200],
- Hypoglycaemic / antidiabetic effects [201-202],
- Antioxidant activity and free radical scavenging [203-206],
- Neuroprotective effect, observed as a reduction of neural inflammation and reduced production of TNF-α and IL-18, in treatment of degenerative neurological disorders [207].

Further insights into the polysaccharide induced anticancer effects are provided by the analysis of patent documents on *G. lucidum* polysaccharides, their effects, *in vivo* and *in vitro* animal testing, and suggested applications (Table **5**). Pharmacological activities of polysaccharides have been claimed and/or described as antitumor effects achieved by tumor cell growth suppression, tumor cell apoptosis, angiogenesis inhibition, and activation of the immune system to produce cytokines [31, 208-211].

Pharmacological Effects of Proteins, Glycopolysaccharides and Peptidoglycans

Water-based extracts and their fractions contain polysaccharides with polypeptide parts that may be residues of LZ-8 and other proteins, and/or amino acids bound to polysaccharide chains in different ratios, thus forming glycopolysaccharides and peptidoglycans. To determine the specific targets and regulating functions of protein and polysaccharide parts, some researchers used the technique of protein removal by trichloroacetic acid (TCA) to obtain pure deproteinised polysaccharide fractions, and the recombinant techniques to produce proteins, such as a pure LZ-8 protein without glycosylation [81]. However, in most cases the pharmacological

Table 5. Examples of Patents on *Ganoderma* Polysaccharides, Sorted Chronologically.

Polysaccharide Product / Production	Effects/Activity	Testing	Applicability/ Formulations /	Patent, Year
Polysaccharide from *G. lucidum* or *Lentinus edodes*, with an anaglycone of isoflavone from soybean seed or arrowroot (e.g. genistein). Production by submerged cultivation in a liquid medium with isoflavones-containing materials	Claimed anti-tumor effects by tumor angiogenesis inhibition, tumor cell growth inhibition, tumor cell apoptosis induction, or by immune system enhancement	(1) Tumor cell growth suppression tests on mouse melanoma and colorectal cancer cells, on rat breast cancer cells, on human bladder cancer and prostatic cancer cells (2) Angiogenesis suppression tests: hemoendothelial cell growth suppression tests on mouse brain hemoendothelial cells; tumor angiogenesis suppression tests on mouse colorectal cancer cells and mouse brain hemoendothelial cells (3) Determination of growth suppression concentrations (IC_{50}) for tumor cells (4) *In vivo* tumor cell apoptosis induction test on rats (5) *In vivo* tumor angiogenesis suppression tests on mice (6) *In vivo* angiogenesis suppression tests and anti-tumor tests on cancer-carrying mice	(1) Pharmaceutical therapeutic and/or preventive products for oral administration in treatment of cancer, osteoporosis and as an immunopotentiating agent (2) Health-care foods (3) Animal feedstuff compositions	[31] 2001
Polysaccharide with α-1,3-glucoside linkage from mycelia of *G. lucidum, Shizophyllum commune, Coriolus versicolor, Lentinula edodes*	Selective action on receptor P1 to activate natural killer T cells; induction of mass production of cytokines (IFN-γ, IL-12)	Clinical studies: 37 cancer patients, dosage 6 g per day for 3 months. Measurement of activation of NKT and increase in number of NKT cells in cancer patient blood; measurement of IL-12, IL-10, IFN-γ. Of 37 cases, 6 patients showed complete recovery, 14 partial recoveries, 14 no progress of cancer, in 3 cases ineffective	(1) Anticancer pharmaceuticals for oral administration (dosage 3-6 g per day for 3 months) (2) Health-care auxiliary food preparation for oral uptake	[208] 2003
Water soluble α- 1→3 polysaccharide from cultured mycelium of *G. boninense*	Induction of the production of interleukin 12 (IL-12)	(1) *In vivo* on cancer bearing mice: increased production of IL-12, increased survival rate, suppression of tumor size (2) Eight clinical examples of individual patients with advanced cancer, taking oral composition of *G. boninense*. Levels of IL-12 increased, various tumor markers decreased due to potentiation of the immunity	(1) Pharmaceutical anticancer composition for oral administration (administration dose 100 mg - 2,000 mg kg^{-1} body weight per day)	[209] 2003
Deproteinised polysaccharide extracts from *Ganoderma* fruit bodies	Immunopotentiating and anticancer activity	*In vivo* tests on mice: Proliferative response of lymphocytes to Concanavalin A. Natural killer cytotoxicity assay on mouse lymphoma cell line. Granulocyte/ macrophage colony stimulating activity. Enhancement of antibody response to pneumococcal vaccine. Antitumor activity against mouse lung carcinoma	Oral pharmaceutical compositions with immunopotentiating and antitumor effects	[210] 2003
Polysaccharide from *G. lucidum* or *Lentinus edodes*, with an anaglycone of isoflavone from soybean seed or arrowroot (e.g. genistein). Production by submerged cultivation in a liquid medium with isoflavones-containing materials	Claimed antitumor effects by tumor angiogenesis inhibition, tumor cell growth inhibition, tumor cell apoptosis induction, or by immune system enhancement	(1) Tumor cell growth suppression tests on mouse melanoma and colorectal cancer cells, on rat breast cancer cells, on human bladder cancer and prostatic cancer cells (2) Angiogenesis suppression tests: hemoendothelial cell growth suppression tests on mouse brain hemoendothelial cells; tumor angiogenesis suppression tests on mouse colorectal cancer cells and mouse brain hemoendothelial cells (3) Determination of growth suppression concentrations (IC_{50}) for tumor cells (4) *In vivo* tumor cell apoptosis induction test on rats (5) *In vivo* tumor angiogenesis suppression tests on mice (6) *In vivo* angiogenesis suppression tests and antitumor tests on cancer-carrying mice	(1) Pharmaceutical therapeutic and/or preventive products for oral administration in treatment of cancer, osteoporosis and as an immunopotentiating agent (2) Health-care foods (3) Animal feedstuff compositions	[211] 2011

effects have been studied with natural non-purified isolates, which contain polysaccharide and polypeptide parts in different ratios. Therefore, the results of pharmacological studies of proteins, glycopolysaccharides, peptidoglycans and polysaccharides may overlap.

Scientific articles and patents reported on the following groups of medicinal effects of *G. lucidum* proteins, glycopolysaccharides and peptidoglycans:

- Anticancer effects attributed to the antitumor immunomodulation mechanisms [89, 95, 96, 162, 212]. A specific and thoroughly researched example is the immunomodulatory protein LZ-8 [77, 78, 81], although other proteins and peptidoglycans have expressed similar antitumor immunomodulatory effects [88, 213, 214].
- Anticancer effects due to the anti-angiogenic activity, by inhibiting the growth of vascular endothelial cells [181, 215].
- Anticancer effects attributed to endoplasmic reticulum stress-mediated autophagic death of cancer cells [216].
- Immunosuppressive activity, found in some glycoproteins [97, 98].
- Anti-arthritic effects by reduced production of proinflammatory cytokines [217].
- Anti-viral activity, e.g. of a protein bound polysaccharide [218], proteoglycan isolated from the mycelia with antiherpetic activity [90], and a fucose-containing glycoprotein for treatment of viral infections, particularly influenza type A virus [101].
- Hepatoprotective effect, observed by *G. lucidum* peptides against D-galactosamine-induced liver injury [93] and carbon tetrachloride-induced liver injury in mice [219].
- Antioxidant activity and free radical scavenging, contributing to cell protection against oxidative stress and damage [186, 220-222].
- Thrombocytopenia prevention or treatment, e.g. by a recombinant LZ-8 protein [82].
- Dermatocosmetic beneficial effects [223].

Analysis of patent documents on *G. lucidum* proteins, their pharmacological effects, testing, and pharmaceutical applications is presented in Table **6**. Results of case studies provide evidence for antitumor effects, induced by the inhibition of angiogenesis [224], or by enhancement of innate and adaptive immunity by activating dendritic cells and macrophages, to produce cytokines and chemokines, and maturation of dendritic cells achieved through the activation of a mitogen-activated protein kinase pathway or NF-κB [225]. A recent patent by Chu *et al*. [226] porposed inclusion of recombinant LZ-8 protein into vaccines, to support the immunization process, and augment the elimination of pathogens and neoplastic cells.

Similar effects are described in patents on *G. lucidum* glycopolysaccharides and peptidoglycans (Table **7**), applied in pharmaceutical preparations with antitumor and immunostimulating effects [96], specifically associated with gene expression of inflammatory cytokine interleukin-1 (IL-1) and/or precursor protein pro-IL-1, and/or IL-1 converting enzyme [227], stimulation of a protective immune response to pathogens and cancer antigens [100], induction of immunomodulatory, hematopoietic and tumor-inhibiting phenotypic changes in dendritic, epithelial and monocyte cells, and cellular precursors, mediated through toll-like receptor TLR and other transmembrane receptors, resulting in an increased production of cytokines, such as IL-6, IL-8, and TNF-α [102], and reduction of migration and invasion of cancer cells and /or modulation of epithelial-mesenchymal transition (EMT), affecting cell markers, cell polarity and cell-junction proteins, up- or down-regulation of cell markers expression, and conversion of cells [228]. Other medical applications, mentioned in patents on *G. lucidum* glycopolysaccharides and peptidoglycans, include anti-viral activity, particularly on H1N1 influenza virus infection [101].

5. PREPARATION OF EXTRACTS AND ISOLATES

Preparation methods of *G. lucidum* extracts and isolates have been described in research papers 38, 50, 111, 147, 166, 194 [38, 53, 153, 174, 204], including specific procedures for extracting and isolating water-soluble polysaccharides 64, 150, 153, 158 [69, 156, 159, 167], proteins [77], triterpenoids 54, 113, 114 [42, 57, 118, 120], and organic solvent extracts [140]. Patent documents disclose inventions related to extraction methods, technological processes, and compositions of extracts or isolates 7, 56-58, 84, 92, 96, 144, 185, 201 [7, 60-62, 90, 98, 101, 159, 185].

Examples of detailed patent descriptions of triterpene isolation methods are given in Table **8**, applying extraction media of methanol, ethanol [229], or supercritical carbon dioxide extraction [230] to produce triterpene pharmaceutical and nutraceutical compositions.

In case of *G. lucidum* purified polysaccharides (Table **9**), patent descriptions of isolation and production methods combine consecutive steps of water extraction, precipitation by ethanol, and additional purifications methods, such as complexation with hydrolysable tannins [231], deproteinisation [210, 232], and size fractionation methods [233].

Patents on extraction, fractionation and isolation procedures to obtain *G. lucidum* proteins, peptidoglycans and glycoproteins are analysed in Table **10**. In addition to initial extractions with water or alkaline aqueous solutions, the processes contain preparative chromatographic techniques, such as gel filtration and ion exchange chromatographies [98, 102, 103, 234].

Based on the isolated glycoprotein fractions, some patents claimed specific immunostimulation methods (Table **11**), e.g. for increasing maturation of cells, production of immunoglobulins, or production and secretion of cytokines or chemokines [100, 224].

In some inventions, preparation methods of crude extracts with a variety of active components are described (Table **12**), such as using the supercritical fluid carbon dioxide ($SCF\text{-}CO_2$) extraction to obtain oleinaceous extracts with active substances from sporoderm-broken *G. lucidum* spores [235-236], and multi-step extractions with a sequence of solvents to obtain medicinally active extracts [237-239].

Table 6. Patent Documents on Pharmacologically Active Proteins Isolated from *G. lucidum*, Listed Chronologically.

Protein Product /Preparation	Effects/Activity	Testing	Applications	Patent, Year
Anti-angiogenic *G. lucidum* fraction from fruit body, mycelia, or spores, with optical absorbance 200 - 280 nm. Production: homogenization in phosphate buffered saline, filtering, precipitating supernatant with ammonium sulphate, dialyzing, purifying by gel filtration, ion-exchange column chromatography	Inhibition of angiogenesis: (1) inhibition of endothelial cells growth, (2) inhibition of pathological neovascularisation in a tissue	Testing on cell lines: Angiogenesis inhibition by endothelial cell culture assay. Anti-angiogenic activity determination on HEP-2 and calf pulmonary arterial endothelial (CPAE) cell lines. Cytolytic/cytotoxic assay on CPAE cells Matrix metalloproteinase assay. Endothelial cell tubule/cord formation assay. Endothelial cell migration assay. Testing on fertilized chicken eggs: inhibition of blood vessel development on chorioallantoic membrane	Pharmaceutical compositions for treating diseases associated with pathological neovascularisation: cancer, rheumatoid arthritis and osteoarthritis, neovascular-based dermatological conditions, diabetic retinopathy, Karposi's Sarcoma, age-related macular degeneration, restenosis, telangectasia, glaucoma, keloids, comeal graft rejection, wound granularization, angiofibroma, Osler-Webber Syndrome, myocardial angiogenesis, scleroderma	[224] 2003
LZ-8 protein, isolated from *G. lucidum* or *G. tsugae*, or prepared by recombinant protein technology in a yeast or bacterium system. LZ-8 protein-fused antigen, prepared by recombinant protein technology in a yeast or bacterium system, used as adjuvant in vaccine. LZ-8-treated dendritic cells	Enhancement of innate and adaptive immunity by activating dendritic cells and macrophages, to produce cytokines (TNF-α, IL-1β, IL-6, IL-10 and IL-12) or chemokines (monocyte chemoattractant protein , macrophage inflammatory proteins) Maturation of dendritic cells achieved through activation of mitogen-activated protein kinase pathway or NF-κB. T cells activation and proliferation to produce cytokines (IL-2, IL-4 and IFN-γ)	Testing on cell lines: LZ-8 stimulation of bone marrow-derived dendritic cells (BMDCs) to produce cytokines and chemokines. Promotion of BMDCs maturation. Activation of T cells. Activation of mitogen-activated protein kinase pathway and NF-κB . Activation of macrophages. Activation of human monocyte-derived dendritic cells	A method for enhancing innate and adaptive immunity by activating dendritic cells and macrophages by administering LZ-8 protein. A method for enhancing the immunogenicity of an antigen, by administering a LZ-8 protein-fused antigen	[225] 2009
Recombinant *G. lucidum* immunoregulatory protein (rLZ-8), with known nucleotide and amino acid sequence, and a particular spatial structure	Increasing the number of leukocytes. Prevention of systemic allergic reactions and immune rejection after organ transplantation	Testing on human leukaemia cells: growth inhibition, lethal effect, apoptosis induction *In vivo* animal tests: tumour growth inhibition in mice. Influence on normal tissue cells in rats and rabbits. Leukocyte-related treatments in mice. Prevention and treatment related to radiation in mice	Pharmaceutical compositions: (1) anticancer medicaments for lung, pancreatic, liver, intestinal, prostatic, uterus, bone and mammary cancers, lymphoma, leukaemia. (2) treatment of leucopoenia, neutropoenia and granulocytopoenia. (3) treatment of graft rejective reaction and reversion of immunosuppression resistance	[83] 2011
Vaccine composition for immunization, consisting of (1) an antigen – e.g. protein of a cancer cell or virus, or DNA; (2) LZ-8 protein as an adjuvant	Supporting immunization by immunomodulation and anticancer activity Elimination of pathogens and neoplastic cells	LZ-8 cloned and expressed in *Saccharomyces cerevisiae,* isolated and purified. LZ-8 testing on cell lines: TNF-α production in dendritic cells; T cell activation; citotoxicity on LN inguinal cells. *In vivo* testing on mice: tumor suppression efficacy; immunization / anititumor effect of DNA-LZ-8 vaccine	Pharmaceutical vaccine and a method for augmenting the immunogenicity of a mammal / human	[226] 2011

Table 7. Patent Documents on Pharmacologically Active Glycopolysaccharides and Peptidoglycans from *G. lucidum.*

Peptidoglycan Product / Preparation	Effects/Activity	Testing	Applications	Patent, Year
Proteoglycan G 009, produced from *G. lucidum* mycelia by submerged liquid cultivation, followed by aqueous NaOH extraction, dialysis, ethanol precipitation, purifications on DEAE-cellulose, Sephatex G-100, and Sephararose C1-4B chromatographies	Antitumor / anticancer effects Immunostimulating effects	*In vivo* testing on mice with transplanted sarcoma tumor cells: tumor growth inhibition; life prolongation; activation of macrophages, T-lymphocytes; effects on the complement system; immunostimulating action. G 009 toxicity test	Pharmaceutical preparations with antitumor and immunostimulating effects	[96] 1994
Fucose-containing glycoprotein fraction of *G. lucidum* extract, comprising fucose residues bound with α-1,2-fucosidic linkages. Production: 0.1 N NaOH alkaline extraction, neutralization, ethanol precipitation, gel filtration on a Sephacryl S-500 column	Immunomodulation associated with gene expression of inflammatory cytokine lnterleukin-1 (IL-1) and/or precursor protein pro-IL-1, and/or IL-1 converting enzyme in a mammalian cell. Activated pathway results in a transcriptional, posttranscriptional and posttranslational regulation of the IL-1 gene expression. *G. lucidum* extracts directly affect the connection between innate and adaptive immunity	Testing on cell lines: Proliferation activation of mouse splenocytes Cytokines expression in mouse spleen cells Cytokine activity determination by ELISA Testing on human macrophages Investigation of molecular mechanism of IL-1 expression and secretion Cytotoxicity assays on human erythroleukemia cell lines, enhancement of NK- cytotoxicity Effect on surface markers expression in human primary monocytes and dendritic cells Inhibition of macrophage expression of nitric oxides induced by LPS, Nitrite assay *in vitro*	A method to enhance cytotoxicity of NK cells against NK-sensitive tumor cells Mediation of immunomodulation associated with IL-1 gene expression in macrophages and spleen cells Stimulation of the inflammatory cytokine IL-1 expression Activation of multiple cytokine production Modulation of mononuclear cells differentiation Modulation of protein kinase pathways associated with inflammatory cytokine IL-1	[227] 2006
Fucose-containing glycoprotein fraction, produced from crude *G. lucidum* alkaline extract , and immunostimulatory compositions comprising a fucose-containing glycoprotein fraction from *G. lucidum* and an antigen, e.g. cancer antigen, bacterial or viral antigens, IgA protease, insulin peptide B	Increased maturation of dendritic cells. Increased immune response to pathogen antigens and cancer antigens. Increased production of cytokines and chemokines by dendritic cells Stimulation of a protective immune response in a host to a pathogen antigen	*In vitro* tests: Enzyme-linked immunosorbent assays - ELISAs Beadlyte Human-Cytokine Detection System Effects on differentiation in mouse primary splenic B cells Effects on primary human peripheral B cell activation	Pharmaceutical products for treating hypogammaglobulinemia, immunodeficiency or immunosuppression Pharmaceutical compositions for stimulating a protective immune response in a host to a pathogenic antigen, e.g. cancer, influenza, respiratory viruses, HIV, *Helicobacter pylori, Neisseria meningitidis pilins, N. gonorrhoeae pilins*, etc. Augmenting the immune response to a vaccine	[100] 2007
F3 Fucose-containing glycoprotein fraction, produced from crude *G. lucidum* alkaline extract. F3 fraction preparation: alkaline extraction with 0.1 N of NaOH, neutralization, ethanol precipitation, purification, fractionation on Sephacryl S-500 column, dialysis, lyophilisation	Anti-viral activity, particularly on H1N1 influenza virus infection	*In vivo* tests on mice: Treating H1N1 influenza virus infection with F3. Pretreatment with F3 prior to infection with H1N1 virus, with various dosages of F3, with different viral titres of influenza virus. Serum cytokine/chemokine profiles in response to F3. Activation of splenocytes. Effect of F3 on entry/replication of H1N1 virus *in vivo/in vitro*	Anti-viral pharmaceutical compositions, based on F3fucose-containing glycoprotein fraction. Formulations with different additives	[101] 2008

(Table 7) Contd….

Peptidoglycan Product / Preparation	Effects/Activity	Testing	Applications	Patent, Year
G. lucidum F3 subfractions F301 and F331 (polysaccharides or glycopeptides with terminal fucose residues), obtained by homogenizing *G. lucidum* tissue, extracting with 0.1 N NaOH aqueous alkaline solution; gel filtration chromatography on Sephacryl S-500 column, eluting with an aqueous Tris buffer solution, dialyzing, precipitating by ethanol, and resuspending in water, or evaporating the ethanol/aqueous solution	F3 fractions induce immunomodulatory, hematopoietic and tumor-inhibiting phenotypic changes in dendritic, epithelial, and monocyte cells, and cellular precursors, mediated through toll-like receptor (TLR) and other transmembrane receptors. Increased TLR mediated translation of mRNA to cytokines results in an increased production of cytokines (IL-6, IL-8, and TNF-α)	Testing by Reporter Gene Assay, RT-PCR or ELISA showed that F3 fractions increased toll-like receptor mediated transcription of messenger RNA coding for cytokines by at least 5 % F331 was capable of activating at least TLR-2 while F301 was capable of activating at least TLR-2, TLR-4, and TLR-5	Immunomodulatory, hematopoietic and tumor-inhibiting pharmaceutical applications	[202] 2009
G. lucidum purified alkaline extract, composed of polysaccharides / glycopeptides with terminal fucose residues (fraction F3), prepared by extraction of fruit bodies with NaOH solution, neutralization, HPLC purification, microfiltration and lyophilization, to obrain F3 fraction powder. Administration of F3 fraction alone or in conjunction with chemoterapeutic anticancer drugs	Anticancer and antimetastatic effects, achieved by: (1) reducing migration and invasion of cancer cells by immuno-modulation; (2) modulation of epithelial-mesenchymal transition (EMT) by reducing loss of epithelial cell markers, cell polarity and cell-junction proteins; conversion of fibroblastic to epithelial morphology; up-regulation of epithelial cell markers expression; down-regulation of mesenchymal cell markers expression	Testing on cell lines (human lung adenocarcinoma): analysis of protein expressions, Western blot analysis, *in vitro* cell migration, invasion and soft-agar colony formation assays *In vivo* testing on mice: metastasis assay - lung tumor number and volume determination	Pharmaceutical anticancer preparations for treatment and prevention of solid tumors, such as non-small cell lung cancer (NSCLC), pancreatic cancer, colorectal cancer, and hepatocellular cancer. Reducion of migration and invasion of lung cancer cells, neuroblastoma, melanoma, non- Hodgkin's lymphoma, Epstein-Barr related lymphoma, Hodgkin's lymphoma, retinoblastoma, small cell lung cancer, brain tumors, leukemia; epidermoid, transitional cell and renal cell carcinoma; cancer of prostate, breast, ovaries, lung, colon, liver, stomach, and other gastrointestinal cancers	[228] 2011

Table 8. Examples of Patents Disclosing Isolation Methods of *G. lucidum* Triterpenes.

Method / Process	Process Description, Main Stages, Process Parameters	Product Properties / Results	Patent, Year
Extraction of oxygenated triterpenes from pulverized *Ganoderma* fruit bodies with methanol or a combination of ethanol and water	(1) Extraction with methanol or ethanol and water at an elevated temperature to obtain a crude extract (2) Solvent partitioning or reverse phase chromatography to obtain the oxygenated triterpenes in an enriched form (3) Elution of oxygenated triterpenes using Cosmosil 5C18, 250x8 mm HPLC column with methanol:water:acetic acid=70:30:0.5 (V:V:V) at 2.0 mL min^{-1}, fraction collected between 4 and 22 minutes	Triterpene nutraceutical composition for treating patients with liver cancer. Application after sensitisation of cancer cells by a mevalonate-depleting compound, such as lovastatin or another 3-hydroxy-3-methyl-glutaryl-CoA reductase inhibitor	[229] 2002
A method for producing ganoderic acid extracts from *G. lucidum* by liquid carbon dioxide extraction	(1) Extraction of *G. lucidum* with liquid carbon dioxide - alone or with a polar cosolvent, e.g. ethanol (5 to 20 % by weight), ratio solvent biomass 6 mL to 1 g, pressure 100 to 310 bars, temperature 20 to 35°C (2) Carbon dioxide evaporation from the extract	*G. lucidum* extract. Contains significant levels of anti-inflammatory ganoderic acids. Identified. ganoderic acid B and C	[230] 2002

6. ANTICANCER COMPOSITIONS

Numerous anticancer formulations have been developed, patented and used as nutraceuticals and pharmaceuticals, mainly with *G. lucidum* fruit bodies, spores, and water, ethanol or SCF-CO_2 extracts, rarely with purified active compounds. Products became available commercially on the market, formulated as capsules, tablets, extracts, syrups, tea, bolus, or as food and feed additives [2, 240-244]. Examples of patents, revealing the composition, activities and testing of herbal and fungal anticancer preparations with *G. lucidum,* are presented in Table **13**, and sorted chronologically [245-254].

In a recent patent by Soeda *et al*. [255], a mixture of hot water extracts of *G. lucidum* and pine cones was proposed as a neovascularization inhibition agent, anticancer agent or cancer prophylactic agent, applied according to circadian rhythm. Kong [256] claimed *G. lucidum* coffee composition to strengthen health and increase the energy level, comprising 8 - 10 % (wt) of coffee and 15-20 % (wt) of Chinese medicines *G. lucidum*, cortex Eucommiae, rhizoma Polygonati, radix Codonopsis and radix Achyranthis bidentatae. A recent patent by Feng *et al*. [257] disclosed a medicinal anticancer composition prepared from 150 to 200 parts of *G. lucidum* spore oil, 75 to 100 parts of *Agaricus blazei* extract, and 75 to 100 parts of *Mortierella sp.*

7. MEDICAL EXPERIENCE AND CLINICAL TRIALS

Although *G. lucidum* has been used as a remedy in traditional Chinese and Japanese medicine for millennia, evidence on systematic medical research and clinical trials remains relatively scarce. The reports (Table **14**) predominantly come from Asian countries, where *G. lucidum* preparations have been used within the system of a traditional medicine, or as a natural supplement to other anticancer therapies. Although, the results may seem to be promising, a justified criticism remains that the methodologies in the described cases were often not sufficiently scientifically rigorous, and that the results were not statistically relevant. In addition, the experimental settings varied a lot, from scattered model case studies with either one or a few patients, to larger clinical observations of patients in hospitals, and systematically designed double-blind placebo-controlled randomized trials [258-269].

In 2012 Jin *et al*. in [270] reported on an evaluation of five randomised controlled trials that studied clinical effects of *G. lucidum* on long-term survival, tumour response, host immune functions and quality of life in cancer patients, with potential adverse events associated with *G. lucidum.* The critical review did not find sufficient statistically relevant evidence to justify the use of *G. lucidum* as a first-line treatment for cancer, and concluded that it remained uncertain whether and how much *G. lucidum* helped to prolong long-term cancer survival. However, based on results of the five trials, *G. lucidum* could be used as an alternative adjunct to conventional treatment, particularly due to the potential of enhancing tumour response and stimulating host immunity. The evaluation also reported that no major toxicity was observed across the analysed five randomised controlled trials. *G. lucidum* was generally well tolerated by patients, with some minor scattered adverse events. The report expressed a need for further clinical research on the effects of *G. lucidum* on long-term survival of cancer patients, and emphasized that the future studies should put more emphasis on the experimental settings and the improvement of methodological quality.

Although, traditional preparations of *G. lucidum* have been commonly used in Asia for millennia, there were, until recently, no published reports on the adverse side effects. However, in the last decade, two individual cases of hepatotoxicity have been reported with commercial *G. lucidum* products. In the first case, published in 2004 [271], a significant hepatotoxicity was observed in a 78-year-old Chinese female in Hong Kong, with liver biochemistry similar of acute cholangitis. The patient has had a regular intake of *G. lucidum* as a health supplement for at least 1 year. A month after she started to take a new powder formulation of *G. lucidum*, the symptoms of liver toxicity were evidenced, and were most likely due to the ingredients of the new *G. lucidum* powder formulation. In another case from 2007 [272], a 47-year-old female from Thailand took traditionally boiled *G. lucidum* for several years without any untoward effect. Two months after the patient changed to *G. lucidum* powder capsules (400mg of dry extract per day), the episode of jaundice and coma due to fulminant hepatitis occurred. The autopsy revealed a shrunken liver and extensive liver-cell necroses. The pathological features were interpreted as toxic or drug-induced hepatitis causing acute hepatic failure. Hepatotoxicity could be attributed to *G. lucidum* capsules and/or to a combination with psychotropic agents, such as perphenazine, administered to the patient. Contrary to the reports of potential hepatotoxicity, some research publications provided evidence on cell protective effects of *G. lucidum*, such as hepatoprotective effect in liver injury [71], antimutagenic and hepatoprotective activity in hepatic damage caused by mutagens [140], nephroprotective effect in a case of cisplatin induced nephrotoxicity [141], and a neuroprotective effect, observed as a reduction of neural inflammation in treatment of degenerative neurological disorders [207]. A recent study by Liu *et al*. [165] demonstrated that *G. lucidum* intracellular polysaccharides inhibited the human hepatocarcinoma cells, however, normal human liver cells were not inhibited but stimulated by polysaccharides in a positive dose- and time-dependent manner. Nevertheless, although the hepatotoxicity by traditionally boiled *G. lucidum* fruit bodies has never been reported in the scientific literature, the potential hepatotoxic role of various commercial products containing *G. lucidum* needs to be closely monitored.

8. CURRENT & FUTURE DEVELOPMENTS

Ganoderma lucidum is one of the most intensely studied wood degrading basidiomycete mushrooms with immunomodulatory and anticancer compounds. Promising medicinal results gave rise to the improvements in traditional cultivation methods and the development of bioreactor-based biotechnological processes in solid or liquid media. At present, the know-how of both production traditions seems to be developed and ready for the potential pharmaceutical industrial

Table 9. Patents Disclosing Isolation of *G. lucidum* Purified Polysaccharides.

Method / Process	Process Description, Main Stages, Process Parameters	Product Properties / Results	Patent, Year
Isolation of mucilaginous polysaccharides, e.g. *Ganoderma* 1,3- and 1,4-β-D-glucans, from aqueous solutions or extracts by complexation with hydrolysable tannins	(1) Complexation: aqueous solution of polysaccharides is treated with hydrolysable tannins (1 - 100 mg mL^{-1}) to form a complex with polysaccharide, which is separated from the solution (2) Decomplexation: the complex is treated with solvents (alcohols) or other substances (surfactants, proteins, resins, etc.) to remove tannins and release the polysaccharide	Polysaccharide products from plants and fungi (including *Ganoderma*). Applications in food, beverage, cosmetic and pharmaceutical compositions	[231] 2002
Production of deproteinised polysaccharide extracts from raw *Ganoderma* fruit bodies	(1) Pre-extraction of raw *Ganoderma* with alcohol (e.g. methanol) to remove non-polysaccharide ingredients (2) Extraction by water at an elevated temperature (e.g. boiling *Ganoderma* in water for 2 hours, 3x (3) Precipitation of polysaccharides by 50 % ethanol (4) Purification of polysaccharide extract with an agent to remove proteins (polysaccharide dissolution in water, adding equal volume of chloroform:butanol 3:1 mixture, partition 3x, then adding 50 % ethanol for precipitation to obtain deproteinised polysaccharide)	Polysaccharide extracts from *Ganoderma* for oral pharmaceutical compositions with immunopotentiating and antitumor effects	[210] 2003
Production of purified polysaccharides from *Ganoderma* spores	(1) Removal of impurities, breaking and shattering spores (2) CO_2 supercritical extraction of oil and fats at 35 - 65°C., extract pressure 20 - 35 MPa, 2-7 hours, CO_2 flow rate 0.5 - 1m^3 h^{-1} (3) Hot water extraction: per 1 g spore powder 10-20 g water, extraction 0.5-2.5 hours at 70-90°C., 1 - 3 times (4) Precipitation of condensed water extract by ethanol, to ethanol conc. 75-85 %. Separation, washing, drying of precipitated polysaccharides (5) Re-dissolving crude polysaccharides in water, pH 7.5-8.5, centrifugation, decolouration by H_2O_2 (6) Deproteinisation by Sevag's method. Dialysis (7) Precipitation by ethanol 70-90 %, separation, washing, drying	Pure polysaccharide preparations from *Ganoderma* spores. Anticancer preparations that statistically significantly inhibited growth of tumors in *in vivo* experiments on mice	[232] 2008
Isolation and fractionation of extracellular fungal polysaccharides (e.g. from *Lentinus, Ganoderma, Coriolus*) , produced by liquid state cultivation in a bioreactor	Isolation of polysaccharides from the liquid cultivation medium by size fractionation methods: ultracentrifugation, ultrafiltration, microfiltration or gel filtration. Applicable for the production of immunomodulatory polysaccharides, such as β-(1,3), β-(1,6) D-glucans, schizophyllan, grifolan, coriolan, glucuronoxylomannans; mannoglucans; glucomannans; galactoglucan and/or galactoglucomannans	Immunomodulatory polysaccharides of MW 30 - 3,000 kDa Galactose, mannose and glucose ratio 1:5 to 25:1 to 50 Immune stimulating pharmaceutical compositions, containing 10 to 1000 μg L^{-1} of soluble polypeptides	[233] 2009

Table 10. Patents on Isolation and Fractionation of *G. lucidum* Proteins, Peptidoglycans and Glycoproteins.

Method / Process	Process Description, Main Stages, Process Parameters	Product Properties / Results	Patent, Year
Isolation process of a glycoprotein from *G. lucidum* mycelia, produced by a liquid state cultivation	(1) Separation of mycelium from liquid cultivation medium by centrifugation at 13,000 ×g for 10 minutes (2) Resuspension in 10 mM tris-HCl buffer at pH 8.0, followed by grinding / extraction (3) Centrifugation (35,000 ×g, 20 minutes) to obtain extract as supernatant. (4) Chromatographic purification by gel filtration on Sephadex G-75, column chromatography on DEAE Sephadex A-25 (5) Dialysis, MW cut-off 3,500. (6) Lyophilisation by freeze drying	Immunosuppressive glycoprotein, MW 12,000-16,000. Saccharide content: 0.3-3.0 wt. %. Monosaccharides: galactose, mannose and hexosamine. Soluble in water, insoluble in ethanol. Absorption peaks at 210 nm and 276 nm. Potential therapeutic drug for allergy	[98] 1994

(Table 10) Contd….

Method / Process	Process Description, Main Stages, Process Parameters	Product Properties / Results	Patent, Year
Preparation of purified medicinally active extracts and fractions from *G. lucidum*, comprising polysaccharide or glycopeptide components having a terminal fucose residue with α1,2-fucosidic linkages	(1) Homogenization of *G. lucidum* tissue (2) Alkaline extraction with 0.1 N aqueous NaOH solution to obtain a crude extract (3) Gel filtration chromatography on Sephracryl S-500 to obtain fractions buffered at pH 7 (4) Partitioning of fractions with anion exchanger to form further subfractions	Extracts and fractions comprising polysaccharides or glycopeptides with terminal fucose residues with α1,2-fucosidic linkages. Effective in inhibition of tumor growth, modulation of immune response, and increase of hematopoietic activity	[103] 2009
Preparation process of *G. lucidum* polysaccharides and glycopeptides with terminal fucose residues, named F3 fractions: F301: total sugar content (%): 37, from which glucose 74, galactose 9, mannose 9, glucosamine 5, and fucose 4. F331 total sugar content (%) 19, from which glucose 59, glucosamine 18, mannose 13, galactose, fucose 3	(1) Homogenization of *G. lucidum* tissue (2) Alkaline extraction with 0.1 N NaOH aqueous solution (3) Gel filtration chromatography on Sephacryl S-500 column (4) Elution with an aqueous Tris buffer solution (5) Dialysis (6) Precipitation by ethanol (7) Resuspension in water	F3 fractions induce immunomodulatory, hematopoietic and tumor-inhibiting phenotypic changes in eukaryotic cells (dendritic, epithelial, and monocyte cells, and cellular precursors), mediated through toll-like receptor (TLR) and other transmembrane receptors	[102] 2009
Preparation of terminal fucose-containing fractions of *G. lucidum* extract: fractions F1, F2, F3, F4, F5, and further F3-F3G1, FRG2, F3 GH1, and F3 GH2	(1) Crude extract prepared by 0.1 N NaOH alkaline extraction, neutralization and ethanol precipitation) (2) Fractionation according to molecular weight: gel filtration chromatography on Sephacryl S-500 column with 0.1 N Tris buffer at pH 7.0 as the eluent (3) Spectrophotometric designation of F3 glycoprotein fraction with light absorbance peak at 625 nm. (4) Further fractionation of F3 on anion exchanger column Diaion-WA30 (Cal - form), eluted with 0.2 and 0.8 M NaCl, and with 2 M NaOH	Fucose-containing glycoprotein fractions. Included in pharmaceutical compositions for humans and other mammals	[234]

Table 11. Patents on Immunostimulation Methods, Based on the Isolated *G. lucidum* Glycoprotein Fractions.

Immunostimulation Method	Description	Applications / Results	Patent, Year
Immunostimulation methods for increasing: (1) maturation of dendritic cells, (2) immunoglobulin G and M production, (3) production of cytokines or chemokines by dendritic cells	*In vivo* or *ex vivo* applications of a fucose-containing glycoprotein fraction from *G. lucidum* to (1) immune cells (e.g. CD14 mononuclear cells, dendritic cells, human CD19 cells), optionally (2) transfer of cells to a subject in need of increased immunoprotection, or (3) administration to a subject	Improvement of immunodeficiencies, e.g. hypogammaglobulinemia, immunodeficiency associated with cancer, immunosuppression prior to the administration Augmenting the immune response to a vaccine Increased production of TNF-α, IL10, IL-1α, IL-1 β, IL 12, IL-6, IL-7, IL-8, IL-10, IFN-γ, IP-10, Eotaxin, MCP-1, MIP-1, RANTES	[100] 2007

(Table 11) Contd….

Immunostimulation Method	Description	Applications / Results	Patent, Year
Stimulation method to increase the secretion of IL-1 by F3 fucose-containing fractions of *G. lucidum*	IL-1 stimulation methods using effective amounts of terminal fucose-containing glycoprotein fraction F3 from *G. lucidum* , administered to: (1) lipopolysaccharide (LPS)-stimulated macrophage prior to or during LPS stimulation (2) a mammal prior to or during the contact with a lipopolysaccharide (3) a mammal to increase the serum level of IL-1 receptor antagonist (4) monocytes to increase the secretion of IL-1 (5) monocytes to increase the secretion of IL-1 receptor antagonist (6) macrophages to increase the secretion of IL-1 receptor antagonist by the macrophages	F3 increased macrophage-membrane surface expression of TLR4 and CD14; F3 enhanced macrophage phagocytosis of LPS; F3 promoted co-localization of internalized LPS; F3 increased IL-1 secretion within LPS-stimulated human primary macrophages and murine macrophage cells. Preinjection of F3 increased IL-1 and IL-1Ra secretion for LPS-injected mice *in vivo*	[234] 2010
Method for treating tumor progression and metastasis by modulating epithelial-mesenchymal transition (EMT). Method for reducing migration and invasion of cancer cells	Application of *G. lucidum* fraction F3 (purified polysaccharides / glycopeptides with terminal fucose residues)	EMT was modulated by EMT - related signaling pathways, driven via receptors. Effects: reduced loss of epithelial cell markers, reduced loss of cell polarity and cell-junction proteins by epithelial tumor cells; acquisition of protein mesenchymal-cell markers; conversion of fibroblastic to epithelial morphology; up-regulation of major epithelial cell markers expression; down-regulation of mesenchymal cell markers expression	[228] 2011

Table 12. Patents on Preparation Methods of Crude Extracts from *G. lucidum* with Complex Compositions.

Extraction Method	Process Description	Product Properties / Results	Patent, Year
Extraction of active substances from sporoderm-broken *G. lucidum* spores by supercritical fluid carbon dioxide (SCF-CO_2) extraction	(1) Induction of spore germination by incubation in a nutritional solution; culturing at constant temperature and humidity (2) Mechanically breaking spores to obtain sporoderm-broken spores (3) Extraction of oleaginous substances by SCF-CO_2, pressure 5 - 60 M Pa; temperature 32 - 85°C; flow capacity rate 5 - 80 kg h^{-1}; total extraction time 0.5 - 6 hours	Oleaginous, transparent extract with *Ganoderma* fragrance, with no deposits, solvent residues, or oxidized substances Extraction yield: 37% (wt.) of extracted substances from sporoderm-broken spores	[235] 2004
Preparation of transparent and odourless oleaginous substances from germination activation sporoderm-broken spores from *G. lucidum*, by SCF-CO_2 method	(1) Germination of spores in a soaking solution containing 5 % *Ganoderma* mycelia, 5 % *Corydeceps* mycelia, 5 % malt extract, and 5 % coconut juice in water, for 8 hours at 25°C (2) Germination of spores in a cultural box at 80 % relative humidity for 12 hours at 25°C (3) Extraction of oleaginous substances from sporoderm-broken *G. lucidum* spores by SCF-CO_2 extraction at 32° - 45°C	Therapeutic compositions: treatment of skin inflammation caused by skin injury, herpes zoster infection, psoriasis. Cosmetic compositions: anti- wrinkles, anti-aging, anti-inflammation, anti-pigmentation	[236] 2005

(Table 12) Contd….

Extraction Method	Process Description	Product Properties / Results	Patent, Year
Three-step extraction of ground *Ganoderma* sp. material by SCF-CO_2 , ethanol and water. Production of (1) essential oil fraction, (2) triterpene fraction, and (3) polysaccharide fraction	(1) Extraction by SCF-CO_2 extraction (6,000 - 80,000 kPa, 35 - 90°C, 30 min - 2.5 hours) to yield an essential oil fraction (2) Extraction of the first residue by ethanol (30°C - 100°C, 1-10 hours), and purification by liquid-liquid extraction in chloroform - saturated $NaHCO_3$ aqueous solution, to yield the triterpene fraction (consisting of ganoderic acid, lucidenic acid, ganolucidic acid, ganoderiol, lucidone, lucidumol, ganodermenonol, ganodermadiol, ganodermatriol, ganodermanondiol, ganodermanontriol) (3) Extraction of the second residue by water (70°C - 90°C, 1 - 5 hours) and precipitation of polysaccharides with ethanol to yield the polysaccharide fraction (monomer structure glucose, arabinose, galactose, rhamnose, xylose uronic acid)	Products: (1) essential oil fraction, (2) triterpene/ polyphenol fraction, (3) polysaccharides, (4) formulations of combined fractions Pharmaceutical products for immune enhancement, cancer prevention and therapy, treatment of diabetes mellitus, thrombosis, cardiovascular and cerebrovascular diseases, atherosclerosis, hypercholesterolemia, hypertension, inflammations, allergies, arthritis, rheumatism, other auto immune diseases, viral infections (cold, influenza, HIV, Herpes simplex, Herpes zoster, and hepatitis B), bacterial infections	[237] 2008
Production of crude and purified extracts *from G. lucidum or G. tsugae.* Methods of Ganoderma-mediated enhancement of human tissue progenitor cell adhesion and differentiation	I. Crude water extract: (1) Homogenization of tissue. (2) Extraction in water, stirring 24 hours, T = 40°C. (3) Removal of insoluble materials by centrifugation. (4) Concentration by evaporating a portion of water at T ≥ 35°C. (5) Lyophilisation of the concentrate. (6) Resuspension of lyophilized crude extract in a liquid phase II. Crude alkaline extract: (1) Alkaline extraction with 0.1 N NaOH. (2) Neutralization. (3) Precipitation with ethanol. III. Purified extract: (1) Resuspension of crude extract in 0.1 N Tris buffer. (2) Removal of insoluble materials by centrifugation. (3) Purification by gel filtration on Sephacryl S-500, eluent 0.1 N Tris buffer, pH 7.0. (4) Dialysis to remove excessive salt. (5) Lyophilisation. (6) Optional further fractionation by anion exchange chromatography on Diaion-W A30 anion exchanger, Cl-form, eluted with 0.2 and 0.8 M NaCl	Medicinally active extracts for enhancement and modulation of immune response and hematopoietic activity. Achieved increase of eukaryotic cell adhesion, differentiation of eukaryotic cells to produce increased numbers of B cells, dendritic cells and chondrocytes, and maintain undifferentiated hematopoietic cells	[238] 2008
Preparation of extracts for anticancer compositions: *G. lucidum* (10-20 %) Radix Ginseng, *Cordyceps sinensis*, *Codonopsis pilosula*, *Lycium barbarum, Ligustrum lucidum, Glycyrrhiza uralensis, Hedyotis diffusa, Agastache rugosa* and *Prunella vulgaris*	Extraction of active substances: (1) Extraction of dry plant/mushroom material in aqueous solution of an organic acid, e.g. acetic acid (2) Extraction from acetic acid solution with an organic solvent. (3) Extraction with alcohol, water and an organic aprotic solvent (4) Concentration under vacuum. (5) Extraction of the extract with petroleum ether to remove fatty components, and extraction with ethanol (6) Filtration of the extract and concentration under vacuum	Herbal pharmaceutical compositions, food or beverage with anticancer activity, recommended for treatment of breast cancer or lung cancer in combination with radiation therapy, chemotherapy, surgery, immunotherapy and photodynamic therapy	[239] 2010

Table 13. Selected Patents on Anticancer Pharmaceutical Formulations, Containing *G. lucidum,* Listed Chronologically.

Composition of the Formulation	Effects/Activity	Testing	Applications	Patent, Year
A mixture of dry alcohol extracts *of Panax pseudo-ginseng, Isatis Indigotica, Ganoderma lucidum, Dendranthema morifolium, Glycyrrhiza glabra, Scutellaria baicalensis, Rabdosia rubescens, Serenoa repens*	Anticancer treatment of prostate carcinoma. Decrease in PSA, immune system stimulation, improvement in appetite and well-being	(1) Tests on prostate cancer cells (decrease in % of S and G1 phases, accumulation of cells in G2M phase, increased cancer cell apoptosis) (2) Clinical study: 72 years old male with prostate carcinoma with metastases, PSA value 182. Hormone therapy and herbal composition 1,2 g per day for 3 month. PSA decrease to 0.84	Pharmaceutical composition for treating prostate carcinoma, administered orally or by suppository May be administered in combination with hormones, antibiotics antimetabolites or cytotoxic agents Can also be used as a dietary supplement	[245] 2000
Immune system stimulator composition comprising extracts of 5-80 % (wt.) *Ganoderma lucidum, Dioscorea opposita, Chrysanthemum morifolium, radix Astragali*, and *folium Isatidis*	Enhanced production of IL-1-β without causing an increase in the production of IL-4	Tests on human blood cells: lymphocyte assay for IL-1β and IL-4; peripheral blood mononuclear cells for IL-1β and IFN-γ	Pharmaceutical immune system stimulating compositions (pills, capsules, gels, 100 mg per dose)	[246] 2002
Phytoestrogenic compositon with an anticancer agent and/or an immune stimulant. The anticancer component is *G. lucidum* or *Coriolus versicolor* extract. The phytoestrogen is selected from wogonin, isoliquiritigenin, coumestrol, their salts, esters, substituted analogs, or combinations thereof	Simultaneous anticancer and phytoestrogenic activity, particularly useful in treatment of hormone-related cancers	(1) Anticancer tests on cell lines: taxol-resistant ovarian cancer cells, hormone-sensitive prostate cancer cells, androgen-dependent and androgen-independent prostate cells, breast cancer cells (2) Trials with two elderly volunteer patients diagnosed with prostate cancer, capsules 6 times a day	Treating or preventing cancer and/or estrogen-related disorders, particularly hormone-related cancers (prostate, breast, endometrial, colon, lung, bladder, testicular, ovarian, thyroid, or bone cancer)	[247] 2004
Hot water extract compositions of *Ganoderma lucidum* and/or *Coriolus versicolor* fruit bodies, and *Panax ginseng* and/or *Panax japonicus* root	Hypoglycaemic effects and antitumor activity	(1) Measurement of the oxidation-reduction potential of compositions (2) Antitumor activity and proliferation inhibition in human leukocyte cancer cells (3) Clinical administration to cancer patients (26 patients with severe cancer, doses 150 mL, 3 times a day for 35 days) (4) Clinical administration to diabetic patients	Pharmaceutical compositions: (1) Antitumor agents (aprox. 0.18 g mushroom and 0.09 g, ginseng root extract per 1 kg body weight per day) (2) Treatment of diabetes (aprox. 0.08 g mushroom and 0.04 g, ginseng root extract per 1 kg body weight per day)	[248] 2004
Extract of *G. lucidum* or *G. atrum* fruit bodies, containing matrix metalloproteinase (MMP) inhibitor. Extraction with water, lower alcohols, or liquid polyhydric alcohols. Preferably hot water extract	Inhibition of MMP. Suppression of MMP-related disorders and diseases, such as cancer metastasis	(1) MMP inhibition test against gelatinase produced by mouse melanoma cells (concentration dependent inhibition) (2) Cancer metastasis suppression, as reduction of metastasized cell nests in mice lungs	(1) Pharmaceutical products for treating MMP-caused disorders and diseases (2) Cosmetic applications: suppression skin aging (3) Food and drink products	[249] 2004

(Table 13) Contd….

Composition of the Formulation	Effects/Activity	Testing	Applications	Patent, Year
Anticancer and anti-metastatic compositions, comprising *Ganoderma* (spores and/or a fruit body extract) and a reovirus (human, animal mammalian and/or avian reovirus). Reovirus inhibits the synthesis of drug transporter proteins and enables the drug to accumulate in the cell	Destruction of neoplastic cells by: (1) administering of reovirus for infection of neoplastic cells; and (2) administering of *G. lucidum* extract (aqueous extract, precipitated by ethanol)	Patent description provides no data on testing	Pharmaceutical products for treatment of metastatic cancer cells in humans and other mammals, Reovirus is administered prior or concurrently to the administration of *Ganoderma* extract	[250] 2005
Anticancer compositions containing extracts of *G. lucidum, Salvia miltiorrhiza, Scutellaria barbata.* Optionally with *Hippophae rhamnoides, Camellia sinensis*, and a chemotherapeutic agent (e.g. gemcitabine, methotrexate, paclitaxel, docetaxel, etoposide, vincristine, vinblastine, vinorelbine, cyclophosphamide, 4-hydroperoxycyclophosphamide, thiotepa, taxol, doxorubicin, daunorubicin, neocarzinostain)	Cytostatic effects by: (1) boosting the immune system, (2) reducing oxidative damage, (3) reducing inflammation, (4) arresting proliferation of cells, (5) anti-oxidant activity, and (6) anti-mutagenic effects	Testing of extracts: (1) anti-proliferative effect on human lung cancer cells in tissue cultures (2) Inhibition of cyclooxygenase activity (3) Anti-oxidant properties (4) Release of TNF-α (5) Proliferation of lymphocytes (6) Mutagenicity test Animal testing on mice: (7) Maximum tolerable dose determination (8) Human tissue xenograft / mouse models: human cancer cells of lung, prostate, cervical, breast and colon cancer cells	Botanical extracts for cancer prevention and therapy. Advised for individuals at risk of developing cancer, and patients with early stages of lung, breast, cervical or prostate cancer Optionally in conjunction with chemotherapeutic agents, radiation therapy, surgery, immunotherapy, photodynamic therapy, to achieve a synergistic action	[251-252] 2005
Mushroom extracts with anticancer activity, e.g. *G. adspersum, G. applanatum, G. resinaceum* (extraction of dry mycelium from submerged liquid cultivation with organic solvents: methanol, ethanol, acetonitrile, ethyl acetate, chloroform, hexane, cyclohexane, isooctane and dichloromethane)	Selective antiproliferative activity, and/or selective apoptosis-inducing activity	Tests on cell lines: proliferation inhibition of human chronic myelogenous leukemia blast cells; human T lymphoblasts; human colon adenocarcinoma cells; cytostatic and cytotoxic effects; mediation of apoptosis; induction of leukemic blast cell differentiation, erythroid differentiation; cell growth inhibition; expression of proteins correlated with carcinogenesis. Selective inhibition of the growth of prostate cancer cells	Pharmaceutical anticancer preparations: treatment of chronic myelogenous leukemia, acute lymphoblastic leukemia, prostate cancer; and treatment of β-globin disorders. Food and beverage compositions	[253] 2006
Composition of organic solvent extracts from *G. lucidum* (10-20 %), *radix Ginseng, Cordyceps sinensis, Codonopsis pilosula, Lycium barbarum, Ligustrum lucidum, Glycyrrhiza uralensis, Hedyotis diffusa, Agastache rugosa* and *Prunella vulgaris*	Anticancer activity, recommended for treatment of breast cancer or lung cancer	(1) Tests on HeLa cells: viability, antiproliferative activity, cell morphology, apoptosis (2) Animal studies: antitumor effect on mice (0.04, 0.1, 0.2 mL per day per mouse; concentration 30mg mL^{-1}): inhibition of the breast adenocarcinoma cancer cells, reduced tumor weight	(1) Pharmaceutical compositions for anticancer treatment, in combination with radiation therapy, chemotherapy, surgery, immunotherapy and photodynamic therapy. (2) Health food or beverages	[254] 2010

Table 14. Examples of Published Medical Investigations with *G. lucidum* Preparations: Case Studies and Clinical Trials, Listed Chronologically.

Description of the Investigation	Evidence of Effects / Results	Reference, Year
A patient diagnosed with hepatoma, located at the portal vein region of the liver. High dose of *Ganoderma* spores treatment from May to August 1999, no other medicines taken	X-Ray investigation: tumor size 5.1 x 6.6 x 7.7 cm in May 1999, reduced to 3.5 x 3.4 x 3 cm in August, 1999	[112] 2001
Two month clinical investigation of Green Valley Lingzhi capsule; 130 patients suffering from type 2 diabetes mellitus	Synergistic effect in hypoglycaemic action combined with regular hypoglycaemic drugs: significant decrease of clinical symptoms, compared to a control group treated only with regular hypoglycaemic drugs	[258] 2002
Clinical observation assessment of a Chinese *G. lucidum* Essence with 547 medium and late phase cancer patients	Significantly lower death rate of patients in the long-term treatment. A continuous 2-3 month active treatment with a daily dosage of 4-6g of *G. lucidum* essence was proposed, with further dosage of 2 g per day continuously after the third month of therapy. Short-term treatments were less successful	[259] 2002
Clinical study of Ganopoly, composed of polysaccharide fractions from *G. lucidum*. 30 advanced-stage cancer patients, treated orally with 1800 mg Ganopoly, three times daily, before meals, for 12 weeks	Ganopoly enhanced the immune responses in patients with advanced-stage cancer. Significantly increased mean plasma concentrations of IL-2, IL-6, and IFN-γ. Levels of IL-1 and TNF-α significantly decreased. Variability among patients observed in the numbers of each lymphocyte subset at baseline. PHA responses enhanced in most patients. A significant increase in the mean NK activity observed, compared to baselines	[260] 2003
8 Clinical examples of individual patients with advanced cancers (prostate, lung, breast, stomach, rectum, liver and lung). Oral composition of *G. boninense* mycelia, 100 mg - 2,000 mg kg^{-1} body weight per day	Levels of IL-12 increased, various tumor markers decreased due to potentiation of the immunity	[209] 2003
37 Cancer patients, treated with a therapeutic agent comprising a polysaccharide with α-1,3-glucoside linkage from mycelia of *Shizophyllum commune, Coriolus versicolor, Lentinula edodes* - Shiitake, and *Ganoderma lucidum,* dosage 6 g paer day per body for 3 months, alone or in combination with OK432 (Picibanil). Cancer types: breast, uterine, ovarian, prostatic, liver, gastric, pancreatic, colon, rectal, lung, oesophagus, hypopharynx, multiple metastasis	Measurement of activation of NKT and increase in number of NKT cells in blood. Measurement of IL-12, IL-10, IFN-γ, Vα24+/Vβ11+, CD3+/CD161+, TH1/Th2. Studying the correlations between cytokines, and of the clinical effects with cytokines; e.g. a strong positive correlation observed between Th1/Th2 ratio and IL-12; Th1/Th2 ratio and IFN-γ; IFN-γ and IL-12; IL-12 and the ratio of CD3*CD161 (NKR-P1)-positive cells (CD3+/CD161+); and IFN-γ and the ratio of CD3*CD161 (NKR-P1)-positive cells. Of 37 cases, 6 patients showed complete recovery, 14 partial recoveries, in 14 cases there was no progress of cancer, in 3 cases the treatment was ineffective	[208] 2003
26 Patients with severe cancer (stages 3 or 4). Hot water extract of *G. lucidum, Coriolus versicolor Panax ginseng,* at doses of 150 mL each, 3 times a day for 35 days	In all cases reported alleviation of complications and body pain, increased appetite, reduced stress, improvement in sleep and body functions. With 5 patients ≥ 50 % regression of carcinoma observed (cervical, lung, ovaries, breast and cutaneous carcinoma), and regression of metastasis of cutaneous and breast cancer	[248] 2004
25 Patients with diabetes mellitus (age 16 to 74 years). Hot water extract of *G. lucidum, Coriolus versicolor* and *Panax ginseng,* at doses of 150 mL each, 2 times a day, for 25 days	Decrease in the blood glucose level in all patients (24.1 % to 72.8 % lower values). Average improvement 52.2 % with herbal preparation only, 45.9 % in combination with insulin treatment. No adverse effects observed	[248] 2004
4 Patients with cancer stage 4. Hot water extract of *G. lucidum, Coriolus versicolor* and *Panax ginseng* at doses of 150 mL each, 3 times a day for 35 days. Patient 1: breast cancer, no other medications. Patient 2: lung cancer, in addition to chemotherapy. Patient 3: sarcoma, in addition to radiotherapy, Patient 4 (control): intestine cancer, not administered any treatment	Blood tests and immunological tests performed for each patient before and after the administration. Improvement of blood and immunological conditions of Patients 1, 2 and 3. Alleviation of body pain, increased appetite, improved sleep and body functions, with alleviation of complications and better recovery. Improved immunological conditions of patients 2 and 3, who were subjected to chemotherapy or radiotherapy	[248] 2004

(Table 14) Contd….

Description of the Investigation	Evidence of Effects / Results	Reference, Year
Open-label clinical study of Ganopoly (polysaccharide fractions from *G. lucidum,* Encore International Corp). 30 advanced-stage lung cancer patients, treated orally with Ganopoly 5.4 g per day, for 12 weeks	Treatment with Ganopoly did not statistically significantly alter the mean mitogenic reactivity to phytohemagglutinin, mean counts of CD3, CD4, CD8, and CD56, mean plasma concentrations of IL-2, IL-6, and IFN-γ, or NK activity, but the results were significantly variable. Some cancer patients demonstrated markedly modulated immune functions. IL-1 changes were correlated with IL-6, IFN-gamma, CD3, CD8, and NK activity, and IL-2 changes were correlated with IL-6, CD8, and NK activity. The results suggested that subgroups of cancer patients might be responsive to Ganopoly in combination with chemotherapy or radiotherapy	[261] 2005
Phase I study of a methanol extract of *G. lucidum;* male volunteers of age >50, with mild symptoms of bladder outlet obstruction	Statistically significant reductions in International Prostate Symptoms Score (I-PSS) versus placebo, observed at the 6 mg and 60 mg dose. The extract of *G lucidum* was well tolerated, a significant improvement in I-PSS was observed, and a 6 mg dose of the extract was recommended	[262] 2005
Clinical studies of *G. lucidum* preparations used in conjunction with radiation and chemotherapy	G. *lucidum* preparations exerted synergistic therapeutic effect when used in conjunction with radiation and chemotherapy by enhancing tolerance for radiation and chemotherapy, potentiating the therapeutic efficacy, ameliorating adverse toxicity of radiation and chemotherapy, and reducing the side effects, such as leucopoenia, thrombocytopoenia, anemia, nausea, vomiting, appetite loss, anti-infection deficiency and immunosuppression	[263] 2005
47 Patients with advanced colorectal cancer. A study of *G. lucidum* polysaccharides, oral dose 5.4 g per day, for 12 weeks	Treatment tended to increase mitogenic reactivity to phytohemagglutinin, counts of CD3, CD4, CD8 and CD56 lymphocytes, plasma concentrations of IL-2, IL-6 and IFN-γ, and NK activity. Plasma concentrations of IL-1 and TNF-α were decreased. For these parameters no statistical significance was observed when a comparison was conducted between baseline and values after a 12-week treatment. The changes of IL-1 were correlated with those for IL-6, IFN-γ, CD3, CD4, CD8 and NK activity, and IL-2 changes were correlated with those for IL-6, CD8 and NK activity. The results indicated that *G. lucidum* may have potential immuno-modulating effect in patients with advanced colorectal cancer	[264] 2006
Double-blind and placebo-controlled randomized trial with 88 men with lower urinary tract symptoms, over the age 49, for 12 weeks, daily dosage 6 mg *G. lucidum* extract once a day	*G. lucidum* extract was well tolerated, effective and significantly superior to placebo. Overall administration was well tolerated with no severe adverse effects	[265] 2007
A double-blind, randomized, placebo-controlled pilot trial on safety and efficacy of *G. lucidum* and San miao San supplementation in patients with rheumatoid arthritis. 32 patients with active rheumatoid arthritis despite disease-modifying antirheumatic drugs received *G. lucidum* (4 g) and San Miao San (2.4 g), and 33 patients a placebo in addition to their current medications, for 24 weeks	Pain and patient's global scores improved significantly. However, no significant antioxidant, anti-inflammatory, or immunomodulating effects were demonstrated (unchanged CD4+/CD8+/natural killer/B lymphocytes ratio, CD3, CD4, and CD8 lymphocyte counts and markers of inflammation, including plasma IL-18, IFN-γ-inducible protein 10, monocyte chemoattractant protein 1, monokine induced by IFN-γ, and RANTES). Conclusion: *G. lucidum* and San Miao San preparation may have analgesic effects for patients with active rheumatoid arthritis, and were generally safe and well tolerated. However, no significant antioxidant, anti-inflammatory, or immunomodulating effects could be demonstrated	[266] 2007

(Table 14) Contd….

Description of the Investigation	Evidence of Effects / Results	Reference, Year
Model case study of a stage IIIa breast cancer patient, taking a *G. lucidum* triterpene-enriched polysaccharide extract of a hybrid Reishi Gano 161, containing 12 % β-D-glucan and 6 % triterpenes, in conjunction with a standard regimen of breast cancer treatments (pre-surgery chemotherapy, surgery, radiotherapy, and post-surgery chemoprevention)	Application of *G. lucidum* triterpene-enriched polysaccharide extract containing 12 % β-D-glucan and 6 % triterpenes was evidenced as beneficial to the patient: effective tumor shrinkage, minimal or transient side effects from chemotherapy and radiotherapy, and improvement of the quality of life, compared to the experience of patients who used only conventional cancer treatments. The molecular mechanism of the antitumor activity appeared to be based on the inhibition of the nuclear transcription factor NF-κB	[267] 2007
48 Breast cancer patients with cancer-related fatigue undergoing endocrine therapy, randomized into the experimental or control group. Effectiveness of spore powder of *G. lucidum* investigated. Data collected at baseline and 4 weeks after treatment	Concentrations of TNF-α, IL-6, and liver-kidney functions measured before and after intervention. The experimental group showed statistically significant improvements in physical well-being and fatigue. Patients reported less anxiety and depression, and better quality of life. Immune markers of CRF were significantly lower. No serious adverse effects occurred during the study. Conclusion: the spore powder of *G. lucidum* may have beneficial effects on cancer-related fatigue and quality of life in breast cancer patients undergoing endocrine therapy without any significant adverse effect	[268] 2012
Study to assess the cardiovascular, metabolic, antioxidant and immunomodulatory responses to therapy with *G. lucidum* in patients with borderline elevations of blood pressure and/or cholesterol. Randomised, double-blind, cross-over study with placebo-controlled run-in and cross-over periods. 26 patients received 1.44 g *G. lucidum* daily or matching placebo for 12 weeks	Measurements of body weight, blood pressure, metabolic parameters, urine catecholamines and cortisol, antioxidant status and lymphocyte subsets. Results indicated that *G. lucidum* might have mild antidiabetic effects and potentially improve the dyslipidaemia of diabetes	[269] 2012

applications. Although fruit bodies and spores remain the basic raw materials in *G. lucidum* commercial products in traditional Asian medicines, cultivation from spawn to cropping remains lengthy. Therefore, submerged liquid or solid state biotechnological processes in bioreactors under controlled conditions offer a faster option for the production and isolation of *G. lucidum* pharmaceutically active substances.

Downstream processes for the production of purified polysaccharides usually combine consecutive steps of aqueous extraction (neutral, acidic or basic), typical precipitation by ethanol, and additional purifications on chromatographic columns, followed by dialysis or ultrafiltration. Similarly, preparations of protein and peptidoglycan fractions begin with water extractions, followed by gel filtration and other chromatographies. Triterpene isolation methods typically begin by extractions with organic solvents, such as methanol or ethanol, or by supercritical fluid carbon dioxide**,** followed by liquid-liquid and column chromatographic separations. However, due to possible synergistic effects of individual compounds, current research does not strive towards the development of sophisticated industrial downstream processes for the isolation of pure substances. Instead, newer patents describe multi-step extractions for the production of mixed extracts.

Most research studies agree that water-soluble and water-insoluble fractions of *G. lucidum* play a promising role in anticancer treatment and prevention, and are able to both improve the anticancer immune response and minimize the collateral effects of chemotherapeutic treatments. At present, *G. lucidum* seems to be well accepted in Asian medical systems, with parallel attempts to elucidate the structure, activities and effects of active components by rigorous scientific research.

Based on reviews on mechanisms of the anticancer action of *G. lucidum,* published in the last years [1, 115, 151, 152, 273-276]**,** and on the results of numerous individual research articles and patents, it has become clear that *G. lucidum* preparations induce anticancer activity *in vitro* and *in vivo* on animal models, and the anticancer effects of *G. lucidum* are mainly due to polysaccharides /peptidoglycans and triterpenoids of the fungus. Reports on extracts and isolated compounds from *G. lucidum* are convincing. However, the specific mechanisms have not been understood yet in all details. At the present state of the research, the results show that it is not a single compound with a single mechanism responsible for the anticancer activity of *G. lucidum*. There seems to be a variety of *G. lucidum* active compounds, naturally present in a complex mixture in the mushroom that trigger different mechanisms, and lead to a number of responses, evidenced and described in *in vivo* and *in vitro* tests. Some compounds may contribute to synergistic effects, or may possess individually different activities that together have a balancing effect on the organism. In any case, the present review suggests that the anticancer activity of *G. lucidum* can be attributed mainly to the following five groups of mechanisms:

1) **Activation/modulation of the immune response of the host**. This has been accepted as the most evident mechanism, by which *G. lucidum* polysaccharides and triterpenoids prevent and/or treat cancer, mainly through activation of the immune effector cells, such

as lymphocytes, macrophages, monocytes, dendritic cells, neutrophils, and natural killer cells, and through modulation of cytokine levels, such as IL-1, IL-2, IL-3, IL-6, IL-8, IL-10, IL-12, TNF-α, and IFN-γ.

2) **Direct cytotoxicity to cancer cells.** In addition to immune functions that were initially accepted as the only explanation of anticancer activity, other research results provided evidence that *G. lucidum* preparations exhibit direct cytotoxicity to tumor cells lines, in absence of cells of the immune system. Explanations suggested a few possible mechanisms, targeted to cancer cells, such as an inhibition of DNA polymerase, inhibition of the nuclear transcription factor, DNA fragmentation, induction of cell cycle arrest, endoplasmic reticulum stress-mediated autophagic death of cancer cells, mitochondrial dysfunction, and other mechanisms leading to cancer cell apoptosis.
3) **Inhibition of tumor-induced angiogenesis.** Studies brought evidence that the antitumor activities of *G. lucidum* are also due to the inhibition of tumor-induced angiogenesis, caused either by the direct inhibition of vascular endothelial cell proliferation, or indirectly by decreasing vascular endothelial growth factor expression of tumor cells.
4) **Inhibition of cancer cells proliferation and invasive metastasis behaviour.** Observations of inhibition phenomena, induced by *G. lucidum* preparations in cultures of highly invasive cells, suggested a palette of potential explanations and mechanisms of inhibiting cancer cells proliferation and metastasizing, such as: inhibition of the nuclear transcription, inhibition of distinct signalling pathways in different cancer cells, cell apoptosis, induction of cell differentiation and maturation, and inhibition of metastatic cells motility.
5) **Carcinogens deactivation and protection of cells**. Studies also reported on protective effects of *G. lucidum,* e.g. on antioxidant activities, prevention of injury of cells induced by reactive oxygen species, and induction of metabolizing enzymes for the detoxification of carcinogenic and toxic electrophilic compounds, thus contributing to deactivation of carcinogens.

In addition to anticancer activities, the following other pharmacological effects of *G. lucidum* triterpenoid, polysaccharide and polypeptide preparations have been evidenced:

- Reduction of the immunosuppressive response, induced by anticancer drugs,
- Abatement of allergies and auto-immune diseases by decreasing levels of IgE and/or IgG2,
- Anti-inflammatory and anti-arthritic effects by reduced production of proinflammatory cytokines,
- Antioxidant activity and free radical scavenging, contributing to protection of living cells from oxidative injury or radiation damage,
- Hepatoprotective, nefroprotective and neuroprotective effects,
- Anti-viral and antibacterial activity,
- Hypoglycaemic and antidiabetic effects,
- Dermatocosmetic beneficial effects.

The largest portion of scientific articles and patents on *G. lucidum* originate from research laboratories of Asian countries, such as Japan, China, and Korea. The Western biochemical and biotechnological sciences have been increasingly involved to catch-up with *G. lucidum* research, while the pharmaceutical and medical communities seem to remain restrained and cautious about potential *G. lucidum* anticancer applications. However, *G. lucidum* is becoming accepted as a natural adjuvant supplement in combination with anticancer therapies, such as radiotherapy or chemotherapy, to enhance the curative effects by supporting the immune system, and to reduce side effects, such as immune system suppression and fatigue of cancer patients.

The major obstacle for the acceptance of natural products, such as *G. lucidum*, in the doctrines of Western pharmaceutical and medical systems, is the complexity and variability of preparations from natural sources. However, complex mixtures, if of a standardised high quality, can bring significant advantages due to synergistic effects. For example, *G. lucidum* triterpenes could directly suppress growth and invasive behaviour of cancer cells, whereas *G. lucidum* polysaccharides could synergistically stimulate the immune functions, resulting in the activation of anticancer activities of immune cells, and production of cytokines.

Although the data from recent *in vitro* and *in vivo* studies demonstrate promising anticancer effects of *G. lucidum,* a need remains to reach a deeper scientific understanding of *G. lucidum* anticancer mechanisms, and their interrelationships. To evaluate and justify potential acceptance of *G. lucidum* in Western pharmacy and medicine, future research directions and prospects should continue to address the following areas:

(1) Isolation and purification of individual compounds, to enable further systematic studies of anticancer effects and mechanisms of single compounds on the molecular level.
(2) Systematic studies of combinations of active compounds, to understand whether the anticancer compounds in *G. lucidum* act synergistically or independently, and to elucidate potential synergistic effects.
(3) Well designed *in vivo* tests and randomized controlled clinical studies with *G. lucidum,* to provide statistically significant results and better validation of *G. lucidum* preparations in treatment and prevention of cancer.
(4) New scientific studies to unveil the molecular mechanisms of the antitumor and immuno-supportive activities, including the genome-wide analysis, research on gene expression, and deeper understanding of enzymes and regulatory molecules.
(5) Standardisation and quality control for *G. lucidum* strains, cultivation processes, extracts and commercial formulations, to enable further acceptance of *G. lucidum* as a natural product for potential use in the prevention and treatment of cancer.

CONFLICT OF INTEREST

The author has no financial interest in any of the companies or patents reviewed in this article.

ACKNOWLEDGEMENTS

Many thanks to Prof. Shu-Ting Chang, a prominent mycologist and medicinal mushrooms researcher. More than two decades ago, by his knowledge, experience and enthusiasm, Prof. Chang inspired the *Ganoderma* research at the University of Ljubljana. I would also like to thank my colleagues Prof. Margareta Vrtačnik, Dr. David Heath and Jakob Boh for valuable comments on the draft manuscript.

REFERENCES

[1] Boh B, Berovic M. *Ganoderma lucidum* Production of Pharmaceuticals. In: Liong MT, Ed. Bioprocess Sciences and Technology: Biochemistry Research Trends. Hauppauge, Nova Science Publishers, 2011, pp. 233-68.

[2] Zhou XW, Su KQ, Zhang YM. Applied modern biotechnology for cultivation of *Ganoderma* and development of their products. Appl Microbiol Biotechnol 2012; 93: 941-63.

[3] Web of Science: Advanced search. Available at: http://apps.webofknowledge.com/ (Accessed on: July 30, 2012).

[4] Espacenet: Advanced search. Available at: http://worldwide.espacenet.com/advancedSearch?locale=en_EP (Accessed on: July 30, 2012).

[5] Stamets P. *Ganoderma lucidum* (Wm. Curtis: Fries) Karsten. In: *Growing Gourmet and Medicinal* Mushrooms. Berkeley, Ten Speed Press, 2000, pp. 352-66.

[6] Shigeru, Y. Cultivation of Reishi. JP3083521 (**1991**).

[7] Hitoshi, N. Mycelial extract of *Ganoderma lucidum* and production thereof. JP60149369 (**1985**).

[8] Li, R., Chen, Y., Suo, J. Method for production of *Ganoderma lucidum* mycelium. CN1099217 (**1995**).

[9] Toshio, M. Production of high purity mycelium of *Ganoderma lucidum*. JP10066535 (**1998**).

[10] Habijanic J, Berovic M. The relevance of solid-state substrate moisturing on *Ganoderma lucidum* biomass cultivation. Food Technol Biotechnol 2000; 38: 225-8.

[11] Habjanic, J., Berovic, M. Process of cultivation of fungus *Ganoderma lucidum* on a solid cultivation substrate. SI20923 (**2002**).

[12] Li, P.J., Shen, C.G. Method for propagating fungi using solid state fermentation. US6558943 (**2003**).

[13] Kohlmünzer S, Wegiel J, Sitarz J. Polysaccharides in mycelial culture of *Ganoderma applanatum* (Pers.). Pat Herba Hung 1989; 28, 87-94.

[14] Fang QH, Zhong JJ. Effect of initial pH on production of ganoderic acid and polysaccharide by submerged fermentation of *Ganoderma lucidum*. Process Biochem 2002; 37: 769-74.

[15] Fang QH, Zhong JJ. Submerged fermentation of higher fungus *Ganoderma lucidum* for production of valuable bioactive metabolites - ganoderic acid and polysaccharide, Biochem Eng J 2002; 10: 61-5.

[16] Tang YJ, Zhong JJ. Fed-batch fermentation of *Ganoderma lucidum* for hyperproduction of polysaccharide and ganoderic acid. Enzyme Microb Technol 2002; 31: 20-8.

[17] Tang YJ, Zhong JJ. Role of oxygen supply in submerged fermentation of *Ganoderma lucidum* for production of *Ganoderma* polysaccharide and ganoderic acid. Enzyme Microb Technol 2003; 32: 478-84.

[18] Xu P, Ding ZY, Qian Z, Zhao CX, Zhang KC. Improved production of mycelial biomass and ganoderic acid bysubmerged culture of *Ganoderma lucidum* SB97 using complex media. Enzyme Microb Technol 2008; 42: 325-31.

[19] Tang YJ, Zhang W Zhong JJ. Performance analyses of a pH-shift and DOT-shift integrated fed-batch fermentation process for the production of ganoderic acid and Ganoderma polysaccharides by medicinal mushroom *Ganoderma lucidum*. Bioresour Technol 2009; 100: 1852-9.

[20] Tang YJ, Zhang W, Zhu LW. Hyperproduction of *Ganoderma* polysaccharides and ganoderic acid during fermentation of medicinal mushroom *Ganoderma lucidum* by multi-stage control and induced culture. J Biosci Bioeng 2009; 108: S114-S34.

[21] Zhang WX, Zhong JJ. Effect of oxygen concentration in gas phase on sporulation and individual ganoderic acids accumulation in liquid static culture of *Ganoderma lucidum*. J Biosci Bioeng 2010; 109: 37-40.

[22] Papinutti L. Effects of nutrients, pH and water potential on exopolysaccharides production by a fungal strain belonging to *Ganoderma lucidum* complex. Bioresour Technol 2010; 101: 1941-6.

[23] Zhao W, Xu JW, Zhong JJ. Enhanced production of ganoderic acids in static liquid culture of *Ganoderma lucidum* under nitrogen-limiting conditions. Bioresour Technol 2011; 102: 8185-90.

[24] Misaki, A., Sone, Y., Yoshida, M., Takeuchi, K. Beta-glucan, its production and use. JP60188402 (**1985**).

[25] Yamazaki, A. Method of cultivating mycelium of basidiomycetes. JP61234776 (**1986**).

[26] Tsunoo, H., Kino, K., Yamasita, A. Novel glycoprotein, production process thereof and immunosuppressive agent containing same as effective ingredient. EP0288959 (**1988**).

[27] Murata, T., Ishigami, Y., Ishikawa, H. Mucilaginous polysaccharide substance and production thereof. JP1121302 (**1989**).

[28] Tsujikura, Y., Higuchi, T., Miyamoto, Y., Sato, S. Production of ganoderic acids. JP4304890 (**1992**).

[29] Zore, I., Berovic, M., Boh, B., Hodzar, D., Pohleven, F. Procedure for preparation of inoculum for growing of fungus *Ganoderma lucidum* by submersion fermentation. SI9700014 (**1998**).

[30] Li, B.J., Li, Q.S., Li, G. Ganoderma lucidum 10 ton tank fermentation technology. CN1188801 (**1998**).

[31] Kosuna, K.-I., Yuan, L., Miura, T., Sun, B. Novel substance originating in basidiomycete culture, process for producing the same and use thereof. EP1154022 (**2001**).

[32] Song, C.H., Yang, B.G. Method for preparing exo-polymers from mycelial culture of *Ganoderma lucidum* (KCTC 10241BP) and the use thereof. KR20030097062 (**2003**).

[33] Yao, Q., Gao, X., Gong, Z., Ren, P., Han, J., Ren, H., Liu, Y., Zhao, J., Qu, L., Li, J.Liquid fermentation method for improving yield of *Ganoderma lucidum* triterpenoid by using rare earth element. CN101892282 (**2010**).

[34] Yao, Q., Gao, X., Gong, Z., Ren, P., Ren, H. Han, J., Liu, Y., Li, J., Qu, L., Zhao, J.Liquid fermentation method capable of improving yield of *Ganoderma lucidum* polysaccharide. CN101880700 (**2010**).

[35] Mikhajlovna, K.L., Vitalevna, A.A., Ilinichna, L.E.M., Petrovich, T.V., Vladimirovna, S.T., Mikhajlovich, B.V., Mikhajlovna, T.E. Method For Producing Agent Showing Anti-Cancer Activity, And Agent Produced By This Method. RU2416418 (**2011**).

[36] Petre, M.,Teodorescu, A. Process for cultivating macromycetes of the species *Ganoderma lucidum* and nourishing fungal biomass. RO126278 (2011).

[37] Mizuno T, Wang G, Zhang J, Kawagishi H, Nishitoba T, Li J. Reishi, *Ganoderma lucidum* and *Ganoderma tsugae*: Bioactive substance and medicinal effects. Food Rev Int 1995; 11: 151-66.

[38] Miyahara R, Yoshimoto T, Asawa K. Chemical structures and changes of extracts during growth of reishi (*Ganoderma lucidum*). Mokuzai Gakkaishi 1987; 33: 416-22.

[39] Chan K. Linking chemical and biological characteristics in assuring the quality of Chinese medicinal materials and OTC products. Ganoderma: Genetics, chemistry, pharmacology and therapeutics, *Proceedings of International symposium on Ganoderma research*. Shanghai, China: October 21-23, 2002.

[40] Chen XL, Liu XC, Sheng DP, Huang DK, Li WZ, Wang X. Distinction of broken cellular wall *Ganoderma lucidum* spores and *G. lucidum* spores using FTIR microspectroscopy. Spectrochim Acta - A Mol Miomol Spectrosc 2012; 97: 667-72.

[41] Chen Y, Xie MY, Zhang H, Wang YX, Nie SP, Li C. Quantification of total polysaccharides and triterpenoids in *Ganoderma lucidum* and *Ganoderma atrum* by near infrared spectroscopy and chemometrics. Food Chem 2012; 135: 268-75.

[42] Wubshet SG, Johansen KT, Nyberg NT, Jaroszewski JW. Direct ^{13}C NMR detection in HPLC hyphenation mode: Analysis of *Ganoderma lucidum* terpenoids. J Nat Prod 2012; 75: 876-82.

[43] Hirotani M, Ino C, Furuya T, Shiro M. Ganoderic acids T, S, and R, new triterpenoids from the cultured media of *Ganoderma lucidum.* Chem Pharm Bull 1986; 34: 2282-5.

[44] Kikuchi T, Kanomi S, Murai Y, Kadota S, Tsubono K, Ogita Z. Constituents of the fungus *Ganoderma lucidum* (Fr.) Karst. I. Structures of ganoderic acids C2, E, I, and K, lucidenic acid F and related compounds. Chem Pharm Bull 1986; 34: 3695-712.

[45] Morigiwa A, Kitabatake K, Fujimoto Y, Ikekawa N. Angiotensin converting enzyme inhibitory triterpenes from *Ganoderma lucidum*. Chem Pharm Bull 1986; 34: 3025-8.

[46] Nishitoba T, Sato H, Sakamura S. Triterpenoids from the fungus *Ganoderma lucidum*. Phytochemistry 1987; 26: 1777-84.

[47] Nishitoba T, Goto S, Sato H, Sakamura S. Bitter triterpenoids from the fungus *Ganoderma applanatum*. Phytochemistry 1989; 28: 193-7.

[48] Lin LJ, Shiao MS. Seven new triterpenes from *Ganoderma lucidum*. J Nat Prod 1988; 51: 918-24.

[49] Lin LJ, Shiao MS, Yeh SF. Triterpenes from *Ganoderma lucidum*. Phytochemistry 1988; 27: 2269-71.

[50] Shiao MS, Lin LJ, Yeh SF, Chou CS. Two new triterpenes of the fungus *Ganoderma lucidum*. J Nat Prod 1987; 50: 886-90.

[51] Chairul, Tokuyama T, Hayashi Y, Nishizawa M, Tokuda H, Chairul SM, Hayashi Y. Applanoxidic acids A, B, C and D, biologically active tetracyclic triterpenes from *Ganoderma applanatum*. Phytochemistry 1991; 30: 4105-9.

[52] Chairul, Hayashi Y, Chairul SM. Lanostanoid triterpenes from *Ganoderma applanatum*. Phytochemistry 1994; 35: 1305-8.

[53] Boh B, Berovic M, Wraber B, Hodzar D, Habijanic J, Pohleven F, *et al*. *Ganoderma lucidum* (W.Curt.:Fr.) Lloyd and *G. applanatum* (Pers.) Pat. (Aphyllophoromycetideae) from Slovenian habitats: Cultivation, isolation, and testing of active compounds. Int J Med Mushrooms 2004; 6: 15-32.

[54] Yang M, Wang X, Guan S, Xia J, Sun J, Guo H, Guo D. Analysis of triterpenoids in *Ganoderma lucidum* using liquid chromatography coupled with electrospray ionization mass spectrometry. J Am Soc Mass Spectrom 2007; 18: 927-39.

[55] Toth JO, Luu B, Ourisson G. Les acides ganoderiques T a Z: triterpenes cytotoxiques de *Ganoderma lucidum* (Polyporaceae). Tetrahedron Lett 1983; 24: 1081-4.

[56] Gao JJ, Min BS, Ahn EM, Nakamura N, Lee HK, Hattori M. New triterpene aldehydes, lucialdehydes A-C, from *Ganoderma lucidum* and their cytotoxicity against murine and human tumor cells. Chem Pharm Bull 2002; 50: 837-40.

[57] Lin SB, Li CH, Lee SS, Kan LS. Triterpene-enriched extracts from *Ganoderma lucidum* inhibit growth of hepatoma cells via suppressing protein kinase C, activating mitogen-activatedprotein kinases and G2-phase cell cycle arrest. Life Sci 2003; 72: 2381-90.

[58] Wang JL, Li YB, Liu RM, Zhong JJ. A new ganoderic acid from *Ganoderma lucidum* mycelia. J Asian Nat Prod Res 2010; 12: 727-30.

[59] Cheng CR , Li YF, Xu PP, Feng RH, Yang M, Guan SH, Guo DA. Preparative isolation of triterpenoids from *Ganoderma lucidum* by counter-current chromatography combined with pH-zone-refining. Food Chem 2012; 130: 1010-6.

[60] Hattori, Y., Ri, G., Hatta, A. Extraction method of *Ganoderma lucidum* extract. JP2003235502 (**2003**).

[61] Yang, X.H. Method for extracting antineoplastic components in *Ganoderma* triterpene. CN1546519 (**2004**).

[62] Xu, Z.X. Technique for extracting triterpene substane of mycelium of *Ganoderma lucidum*. CN1563408 (**2005**).

[63] Minto, R., Kennedy, E. Process for preparing delta-7,9(11) steroids from *Ganoderma lucidum* and analogs thereof. WO2012044817 (**2012**).

[64] Bao XF, Wang XS, Dong Q, Fang JN, Li XY. Structural features of immunologically active polysaccharides from *Ganoderma lucidum*. Phytochemistry 2002; 59: 175-81.

[65] Wasser SP. Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. Appl Microbiol Biotechnol 2002; 60: 258-74.

[66] Chen JZ, Seviour R. Medicinal importance of fungal ß-(1-3), (1-6)-glucans. Mycol Res 2007; 111: 635-52.

[67] Moradali MF, Mostafavi H, Ghods S, Hedjaroude GA. Immunomodulating and anticancer agents in the realm of macromycetes fungi (macrofungi). Int Immunopharmacol 2007; 7: 701-24.

[68] Guo L, Xie J, Ruan Y, Zhou L, Zhu H, Yun X, *et al.* Characterization and immunostimulatory activity of a polysaccharide from the spores of *Ganoderma lucidum*. Int Immunopharmacol 2009; 9: 1175-82.

[69] Wang J, Zhang L. Structure and chain conformation of five water-soluble derivatives of a β-D-glucan isolated from *Ganoderma lucidum*. Carbohydr Res 2009; 344: 105-12.

[70] Dong Q, Wang Y, Shi L, Yao J, Li J, Ma FL, *et al*. A novel water-soluble beta-D-glucan isolated from the spores of *Ganoderma lucidum*. Cabohydr Res 2012; 353: 100-5.

[71] Li YQ, Fang L, Zhang KC. Structure and bioactivities of a galactose rich extracellular polysaccharide from submergedly cultured *Ganoderma lucidum*. Carbohydr Polym 2007; 68: 323-8.

[72] Ye LB, Zhang JS, Yang Y, Zhou S, Liu YF, Tang QJ, *et al.* Structural characterisation of a heteropolysaccharide by NMR spectra. Food Chem 2009; 112: 962-6.

[73] Wang J, Ma Z, Zhang L, Fang Y, Jiang F, Phillips GO. Structure and chain conformation of water-soluble heteropolysaccharides from *Ganoderma lucidum*. Carbohydr Polym 2009; 86: 844-51.

[74] Pan D, Wang LQ, Chen CH, Teng BS, Wang CD, Xu ZX, *et al*. Structure characterization of a novel neutral polysaccharide isolated from *Ganoderma lucidum* fruiting bodies. Food Chem 2012; 135: 1097-103.

[75] Habijanic J, Svagelj M, Berovic M, Boh B, Wraber B. Submerged and solid-state cultivation of bioactive extra- and intracellular polysaccharides of medicinal mushrooms *Ganoderma lucidum* (W. Curt.: Fr.) P. Karst. and *Grifola frondosa* (Dicks.: Fr.) S. F. Gray (Aphyllophoromycetideae). Int J Med Mushrooms 2009; 11: 409-18.

[76] Berovic M, Habijanic J, Zore I, Wraber B, Hodzar D, Boh B, *et al*. Submerged cultivation of *Ganoderma lucidum* biomass and immunostimulatory effects of fungal polysaccharides. J Biotechnol 2003; 103: 77-86.

[77] Kino K, Yamashita A, Yamaoka K, Watanabe J, Tanaka S, Ko K, *et al.* Isolation and characterization of a new immunomodulatory protein, ling zhi-8 (LZ-8), from *Ganoderma lucidium*. J Biol Chem 1989; 264: 472-8.

[78] Tanaka S, Ko K, Kino K, Tsuchiya K, Yamashita A, Murasugi A, *et al.* Complete amino acid sequence of an immunomodulatory protein, ling zhi-8 (LZ-8). J Biol Chem 1989; 264: 16372-7.

[79] Kino K, Sone T, Watanabe J, Yamashita TH, Miyajima H, Tsunoo H. Immunomodulator, LZ-8, prevents antibody-production in mice. Int J Immunopharmacol 1991; 13: 1109-15.

[80] Van Der Hem LG, Van Der Vliet JA, Bocken CFM, Kino K, Hoitsma AJ, Tax WJM. Ling zhi-8 studies of a new immunomodulating agent. Transplantation 1995; 60: 438-43.

[81] Yeh CH, Chen HC, Yang JJ, Chuang WI, Sheu F. Polysaccharides PS-G and protein LZ-8 from Reishi (*Ganoderma lucidum*) exhibit diverse functions in regulating murine macrophages and T lymphocytes. J Agric Food Chem 2010; 58: 8535-44.

[82] Sun, F., Liang, C., Zhang, X. Use of rLZ-8 for the prevention or treatment of thrombocytopenia and a pharmaceutical composition comprising rLZ-8. WO2010135854 (**2010**).

[83] Sun, F., Zhang, X. Recombinant *Ganoderma lucidium* immunomodulatory protein (rLZ-8) and uses thereof. US2011009597 (**2011**).

[84] Zhou, X., Wang, X., Li, Q., Su, K., Cong, Y. Recombinant fungal immunomodulatory protein gene in *Ganoderma lucidum*, protein coded whereby and application thereof. CN102199201 (**2011**).

[85] Wang HX, Ng TB, Chiu SW. A distinctive ribonuclease from fresh fruiting bodies of the medicinal mushroom *Ganoderma lucidum*. Biochem Biophys Res Commun 2004; 314: 519-22.

[86] Tian YP, Zhang KC. Purification and characterization of a novel proteinase A inhibitor from *Ganoderma lucidum* by submerged fermentation. Enzyme Microb Technol 2005; 36: 357-61.

[87] Wang HX, Ng TB. Ganodermin, an antifungal protein from fruiting bodies of the medicinal mushroom *Ganoderma lucidum*. Peptides 2006; 27: 27-30.

[88] Zhang JS, Tang QJ, Zimmerman-Kordmann M, Reuter W, Fan H. Activation of B lymphocytes by GLIS, a bioactive proteoglycan from *Ganoderma lucidum*. Life Sci 2002; 71: 623-38.

[89] Wang YY, Khoo KH, Chen ST, Lin CC, Wong CH, Lin CH. Studies on the immuno-modulating and antitumor activities of *Ganoderma lucidum* (Reishi) polysaccharides: Functional and proteomic

analyses of a fucose-containing glycoprotein fraction responsible for the activities. Bioorg Med Chem 2002; 10: 1057-62.

[90] Liu J, Yang F, Ye LB, Yang XJ, Timani KA, Zheng Y, *et al*. Possible mode of action of antiherpetic activities of a proteoglycan isolated from the mycelia of *Ganoderma lucidum in vitro*. J Ethnopharmacol 2004; 95: 256-72.

[91] Thakur A, Rana M, Lakhanpal TN, Ahmad A, Khan MI. Purification and characterization of lectin from fruiting body of *Ganoderma lucidum*. Biochim Biophys Acta 2007; 1770: 1404-12.

[92] Ye LB, Zhang JS, Ye XY, Tang QJ, Liu YF, Gong CY, *et al.* Structural elucidation of the polysaccharide moiety of a glycopeptide (GLPCW-II) from *Ganoderma lucidum* fruiting bodies. Carbohydr Res 2008; 343: 746-52.

[93] Shi Y, Sun J, He H, Guo H, Zhang S. Hepatoprotective effects of *Ganoderma lucidum* peptides against D-galactosamine-induced liver injury in mice. J Ethnopharmacol 2008; 117: 415-9.

[94] Ye L, Li J, Zhang J, Pan Y. NMR characterization for polysaccharide moiety of a glycopeptides. Fitoterapia 2010: 81: 93-6.

[95] Lee, K.H., Chung, H., Lee, C.W., Chung, C.H. *Ganoderma lucidium* IY009 which produces proteoglycan (G009) having effect of enhancing antitumor immunity. EP0563383 (**1993**).

[96] Lee, K.H., Chung, H., Lee, C.W., Chung, C.H. Proteoglycan (G 009) effective in enhancing antitumor immunity. EP0610499 (**1994**).

[97] Tsunoo, H., Kino, K., Yamashita, A. Novel glycoprotein, production process thereof and immunosuppressive agent containing same as effective ingredient. EP0288959 (**1988**).

[98] Tsunoo, H., Kino, K., Yamashita, A. Glycoprotein isolated from *Ganoderma* having immunosuppressive activity. US5334704 (**1994**).

[99] Lin, Z.B., Lin, S.Q., Wang S.Z. *Ganoderma lucidum* polysaccharide protein and its preparing method and use. CN1629186 (**2005**).

[100] Yu, A.,Yu, J., Lin, K.I., Yang, W.B., Wong, C.H. Fungal immunostimulatory compositions. US2007231339 (**2007**).

[101] Yu, A., Wong, C.H., Jan, J.T., Cheng, S.E.Y. Anti-viral effect of an extract of *Ganoderma lucidum*. US20080214442 (**2008**).

[102] Liang, S.M., Chen, Y.P., Yang, W.B., Wong, C.H. Reishi F3 subfraction polysaccharides and methods of using same. US20090263897 (**2009**).

[103] Wang, Y.Y., Khoo, K.H., Chen, S.T., Lin, C.C., Wong, C.H., Lin, C.H., Chen, H.-s., Tsai, Y.-f. Immuno-modulating antitumor activities of *Ganoderma lucidum* (Reishi) polysaccharides. US7560114 (**2009**).

[104] Russell R, Paterson M. *Ganoderma* - A therapeutic fungal biofactory. Phytochemistry 2006; 67: 1985-2001.

[105] Ziegenbein FC, Hanssen HP, Konig WA. Secondary metabolites from *Ganoderma lucidum* and *Spongiporus leucomallellus*. Phytochemistry 2006; 67: 202-11.

[106] Gao P, Hirano T, Chen Z, Yasuhara T, Nakata Y, Sugimoto A. Isolation and identification of C-19 fattyacids with anti-tumor activity from the spores of *Ganoderma lucidum* (Reishi mushroom). Fitoterapia 2012; 83: 490-9.

[107] Liu X, Wang JH, Yuan JP. Pharmacological and anti-tumor activities of *Ganoderma* spores processed by top-down approaches. J Nanosci Nanotechnol 2005; 5: 2001-2013.

[108] Xie YZ, Li SZ, Yee A, La Pierre DP, Deng ZQ, Lee DY, *et al. Ganoderma lucidum* inhibits tumour cell proliferation and induces tumour cell death. Enzyme Microb Technol 2006; 40: 177-85.

[109] Fukuzawa M, Yamaguchi R, Hide I, Chen ZQ, HiraiY, Sugimoto A, *et al*. Possible involvement of long chain fatty acids in the spores of *Ganoderma lucidum* (Reishi Houshi) to its anti-tumor activity. Biol Pharm Bull 2008; 31: 1933-7.

[110] Zhang JP, Zheng LM, Wang JH, Magnusson KE, Liu X. Lipid extract from completely sporoderm-broken germinating *Ganoderma sinensis* spores elicits potent antitumor immune responses in human macrophages. Phytother Res 2009; 23: 844-50.

[111] Zhao H, Zhang QY, Zhao L, Huang X, Wang JC, Kang XM. Spore powder of *Ganoderma lucidum* improves cancer-related fatigue in breast cancer patients undergoing endocrine therapy: A pilot clinical trial. Evid Based Complement Alternat Med 2012; Art. No. 809614. doi:10.1155/2012/809614.

[112] Liu, X., Chung, C.K. Germination-activated red *Ganoderma lucidum* spores and method for producing the same. EP1092765 (**2001**).

[113] Chung, C.K., Tong, S.K. *Ganoderma lucidum* spores for treatment of autoimmune diseases. US20030143246 (**2003**).

[114] Kosuna, K.C., Yuan, L., Miura, T., Sun, B. Substance derived from basidiomycetes culture, method for producing it and its use. US7883708 (**2011**).

[115] Boh B, Berovic M, Zhang JS, Lin ZB. *Ganoderma lucidum* and its pharmaceutically active compounds. Biotechnol Annu Rev 2007; 13: 265-301.

[116] Min BS, Gao JJ, Nakamura N, Hattori M. Triterpenes from the spores of *Ganoderma lucidum* and their cytotoxicity against meth-A and LLC tumor cells. Chem Pharm Bull 2000; 48: 1026-33.

[117] Gao JJ, Min BS, Ahn EM, Nakamura N, Lee HK, Hattori M. New triterpene aldehydes, lucialdehydes A-C, from *Ganoderma lucidum* and their cytotoxicity against murine and human tumor cells. Chem Pharm Bull 2002; 50: 837-40.

[118] Lin SB, Li CH, Chen YR, Kan LS, Lee SS. Triterpene extract from *Ganoderma lucidum* inhibits growth of hepatoma Huh7 cells: Involvement of oxidative stress induction. Ganoderma: Genetics, chemistry, pharmacology and therapeutics. *Proceedings of International symposium on Ganoderma research*. Shanghai, China: October 21-23, 2002.

[119] Kimura Y, Taniguchi M, Baba K. Antitumor and antimetastatic effects on liver of triterpenoid fractions of *Ganoderma lucidum*: Mechanism of action and isolation of an active substance. Anticancer Res 2002; 22: 3309-18.

[120] Lin SB, Li CH, Lee SS, Kan LS. Triterpene-enriched extracts from *Ganoderma lucidum* inhibit growth of hepatoma cells via suppressing protein kinase C, activating mitogen-activatedprotein kinases and G2-phase cell cycle arrest. Life Sci 2003; 72: 2381-90.

[121] Yu SP, Zhang JS, Tang QJ, Shi XM, Liu YF, Yan Y, *et al*. Correlation between intracellular triterpenes from mycelia of *Ganoderma lucidum* in different growth stages and inhibition effect on tumor cells. Mycosystema 2004; 23: 548-54.

[122] Liu GQ, Zhang KC. Mechanisms of the anti-cancer action of *Ganoderma lucidum* (Leyss. ex Fr.) Karst: A new understanding. J Integr Plant Biol 2005; 47: 129-35.

[123] Mueller CI, Kumagai T, Kelly JO, Seeram NP, Heber D, Koeffler HP. *Ganoderma lucidum* causes apoptosis in leukemia, lymphoma and multiple myeloma cells. Leuk Res 2006; 30: 841-8.

[124] Tang W, Liu HW, Zhao WM, Wei DZ, Zhong JJ. Ganoderic acid T from *Ganoderma lucidum* mycelia induces mitochondria mediated apoptosis in lung cancer cells. Life Sci 2006; 80: 205-11.

[125] Guan SH, Xia JM, Yang M, Wang XM, Liu X, Guo DA. Cytotoxic lanostanoid triterpenes from *Ganoderma lucidum*. J Asian Nat Prod Res 2008; 10: 695-700.

[126] Yuen JWM, Gohel MDJ. The dual roles of *Ganoderma* antioxidants on urothelial cell DNA under carcinogenic attack. J Ethnopharmacol 2008; 118: 324-30.

[127] Chen NH, Liu JW, Zhong JJ. Ganoderic acid Me inhibits tumor invasion through down-regulating matrix metalloproteinases 2/9 gene expression. J Pharmacol Sci 2008; 108: 212-6.

[128] Yue QX, Song XY, Ma C, Feng LX, Guan SH, Wu WY, *et al*. Effects of triterpenes from *Ganoderma lucidum* on protein expression profile of HeLa cells. Phytomedicine 2010; 17: 606-13.

[129] Thyagarajan A, Jedinak A, Nguyen H, Terry C, Baldridge LA, Jiang J, *et al*. Triterpenes from *Ganoderma lucidum* induce autophagy in colon cancer through the inhibition of p38 mitogen-activated kinase (p38 MAPK). Nutr Cancer 2010; 62: 630-40.

[130] Liu RM, Li YB, Zhong JJ. Ganoderic acid Mf and S induce mitochondria mediated apoptosis in human cervical carcinoma HeLa cells. Phytomedicine 2011; 18: 349-55.

[131] Liu RM, Li YB, Zhong JJ. Anti-proliferation and induced mitochondria-mediated apoptosis of ganoderic acid Mk from *Ganoderma lucidum* mycelia in cervical cancer HeLa cells. Lat Am J Pharm 2012; 31: 43-50.

[132] Wu GS, Lu JJ, Guo JJ, Li YB, Tan W, Dang YY, *et al*. Ganoderic acid DM, a natural triterpenoid, induces DNA damage, G1 cell cycle arrest and apoptosis in human breast cancer cells. Fitoterapia 2012; 83: 408-14.

[133] Akihisa T, NakamuraY, Tagata M, Tokuda H, Yasukawa K, Uchiyama E, *et al.* Anti-inflammatory and anti-tumor-promoting effects of triterpene acids and sterols from the fungus *Ganoderma lucidum*. Chem Biodiversity 2007; 4: 224-31.

[134] Ko HH, Hung CF, Wang JP, Lin CN. Antiinflammatory triterpenoids and steroids from *Ganoderma lucidum* and *G. tsugae*. Phytochemistry 2008; 69: 234-9.

[135] Dudhgaonkar S, Thyagarajan A, Sliva D. Suppression of the inflammatory response by triterpenes isolated from the mushroom *Ganoderma lucidum*. Int Immunopharmacol 2009; 9: 1272-80.

[136] Kohda H, Tokumoto W, Sakamoto K, Fujii M, Hirai Y, Yamasaki K, *et al.* The biologically active constituents of *Ganoderma lucidum* (Fr.) Karst histamine release-inhibitory triterpenes. Chem Pharm Bull 1985; 33: 1367-74.

[137] El-Mekkawy S, Meselhy MR, Nakamura N, Tezuka Y, Hattori M, Kakiuchi N, *et al.* Anti-HIV-1 and anti-HIV-1 protease substances from *Ganoderma lucidum*. Phytochemistry 1998; 49: 1651-7.

[138] Min BS, Nakamura N, Miyashiro H, Bae KW, Hattori M. Triterpenes from the spores of *Ganoderma lucidum* and their inhibitory activity against HIV-1 protease. Chem Pharm Bull 1998; 46: 1607-12.

[139] Iwatsuki K, Akihisa T, Tokuda H, Ukiya M, Oshikubo M, KimuraY, *et al.* Lucidenic acids P and Q, methyl lucidenate P, and other triterpenoids from the fungus *Ganoderma lucidum* and their inhibitory effects on Epstein-Barr virus activation. J Nat Prod 2003; 66: 1582-5.

[140] Lakshmi B, Ajith TA, Jose N, Janardhanan KK. Antimutagenic activity of methanolic extract of *Ganoderma lucidum* and its effect on hepatic damage caused by benzo[a]pyrene. J Ethnopharmacol 2006; 107: 297-303.

[141] Pillai TG, John M, Thomas GS. Prevention of cisplatin induced nephrotoxicity by terpenes isolated from *Ganoderma lucidum* occurring in Southern parts of India. Exp Toxicol Pathol 2011; 63: 157-60.

[142] Hajjaj H, Mace C, Roberts M, Niederberger P, Fay LB. Effect of 26-oxygenosterols from *Ganoderma lucidum* and their activity as cholesterol synthesis inhibitors. Appl Environ Microbiol 2005; 71: 3653-8.

[143] Kim SD. Isolation and structure determination of a cholesterol esterase inhibitor from from *Ganoderma lucidum*. J Microbiol Biotechnol 2010; 20: 1521-3.

[144] Wang CN, Chen JC, Shiao MS, Wang CT. The aggregation of human platelet induced by ganodermic acid S. Biochim Biophys Acta 1989; 986: 151-60.

[145] Su CY, Shiao MS, Wang CT. Predominant inhibition of ganodermic acid S on the thromboxane A2-dependent pathway in human platelets response to collagen. Biochim Biophys Acta 1999; 1437: 223-34.

[146] Su CY, Shiao MS, Wang CT. Potentiation of ganoderemic acid S on prostaglandin E(1)-induced cyclic AMP elevation in human platelets. Thrombosis Res 2000; 99: 135-45.

[147] Liu J, Shimizu K, Konishi F, Noda K, Kumamoto S, Kurashiki K, *et al*. Anti-androgenic activities of the triterpenoids fraction of *Ganoderma lucidum*. Food Chem 2007; 100: 1691-6.

[148] Liu J, Shimizu K, Konishi F, Kumamoto S, Kondo R. The anti-androgen effect of ganoderol B isolated from the fruiting body of *Ganoderma lucidum*. Bioorg Med Chem 2007; 15: 4966-72.

[149] Takashita, T., Yamashita, A., Ishihara, T. Neovascularization inhibitor. JP2004196761 (**2004**).

[150] Watson, S., Muir, K., Kumari, R., Fang, L.Y., Guo, D.A. Compositions comprising *Ganoderma lucidum* extracts and uses thereof. WO2009087368 (**2009**).

[151] Xu ZT, Chen XU, Zhong ZF, Chen LD, Wang YT. *Ganoderma lucidum* polysaccharides: Immunomodulation and potential anti-tumor activities. Am J Chin Med 2011; 39: 15-27.

[152] Camargo MR, Kaneno RR. Antitumor properties of *Ganoderma lucidum* polysaccharides and terpenoids. Annu Rev Biomed Sci 2011; 13: 1-8.

[153] Lee SS, Chen FD, Chang SC, Wei YH, Liu I, Chen C. *In vivo* anti-tumor effect of crude extracts from the mycelium of *Ganoderma lucidum*. J Chin Oncol Soc 1984; 5: 22-7.

[154] Hwang SF, Liu KJ, KuanYH, Tung KS, Su CH, Tung TC. The inhibitory effect on artificial pulmonary metastasis of murine S-180 Sarcoma cells by orally administered *Ganoderma lucidum*. J Chin Oncol Soc 1989; 5: 10-5.

[155] Furusawa E, Chou SC, Furasawa S, Hirazum A, Dang Y. Antitumor activity of *Ganoderma lucidum*, an edible mushroom, on intraperitoneally implanted Lewis lung carcinoma in synergeneic mice. Phytother Res 1992; 6: 300-4.

[156] Lu H, Kyo E, Uesaka T, Katoh O, Watanabe H. Prevention of development of N,N′-dimethylhydrazine-induced colon tumors by a water-soluble extract from cultured medium of *Ganoderma lucidum* (Rei-shi) mycelia in male ICR mice. Int J Mol Med 2002; 9: 113-7.

[157] Liu X, Yuan JP, Chuang CK, Chen XJ. Antitumor activity of the sporoderm-broken germinating spores of *Ganoderma lucidum*. Cancer Lett 2002; 182: 155-61.

[158] Lu H, Kyo E, Uesaka T, Katoh O, Watanabe H. A water-soluble extract from cultured medium of *Ganoderma lucidum* (Rei-shi) mycelia suppresses azoxymethane-induction of colon cancers in male F344 rats. Oncol Rep 2003; 10: 375-9.

[159] Gao YH, Gao H, Chan E, Tang WB, Xu AL, Yang HY, *et al.* Antitumor activity and underlying mechanisms of Ganopoly, the refined polysaccharides extracted from *Ganoderma lucidum*, in mice. Immunol Invest 2005; 34: 171-98.

[160] Pang X, Chen Z, Gao X, Liu W, Slavin M, Yao W, *et al*. Potential of a novel polysaccharide preparation (GLPP) from Anhui-grown *Ganoderma lucidum* in tumor treatment and immunostimulation. J Food Sci 2007; 72: S435-S42.

[161] Li N, Hu YL, He CX, Hu CJ, Zhou J, Tang GP, *et al*. Preparation, characterisation and anti-tumour activity of *Ganoderma lucidum* polysaccharide nanoparticles. J Pharm Pharmacol 2010; 62: 139-44.

[162] Zhang J, Tang Q, Zhou C, Jia W, Silva L, Nguyen LD, *et al.* GLIS, a bioactive proteoglycan fraction from *Ganoderma lucidum*, displays anti-tumour activity by increasing both humoral and cellular immune response. Life Sci 2010; 87: 628-37.

[163] Shang DJ, Li Y, Wang C, Wang XM, Yu Z, Fu X. A novel polysaccharide from Se-enriched *Ganoderma lucidum* induces apoptosis of human breast cancer cells. Oncol Rep 2011; 25: 267-72.

[164] Zhang J, Liu YJ, Park HS, Xia YM, Kim GS. Antitumor activity of sulfated extracellular polysaccharides of *Ganoderma lucidum* from the submerged fermentation broth. Carbohydr Polym 2012; 87: 1539-44.

[165] Liu YJ, Shen J, Xia YM, Zhang J, Park HS. The polysaccharides from *Ganoderma lucidum*: Are they always inhibitors on human hepatocarcinoma cells? Carbohydr Polym 2012; 90: 1210-5.

[166] Wang SY, Hsu ML, Hsu HC, Tzeng CH, Lee SS, Shiao MS, *et al*. The anti-tumor effect of *Ganoderma lucidum* is mediated by cytokines released from activated macrophages and T lymphocytes. Int J Cancer 1997; 70: 699-705.

[167] Hu YH, Lin ZB. Polysaccharides isolated from mycelia of *Ganoderma lucidum* induced HL-60 cell apoptosis by enhancing macrophage activity. Chin Pharmacol Bull 1999; 5: 27-30.

[168] Lei LS, Li MC, Sun LS. *Ganoderma lucidum* and its components on the function of macrophages. Ganoderma: Genetics, chemistry, pharmacology and therapeutics. *Proceedings of International Symposium on Ganoderma Research*. Shanghai, China: October 21-23, 2002.

[169] Cao LZ, Lin ZB. Comparison of the effects of polysaccharides from wood-cultured and bag-cultured *Ganoderma lucidum* on murine spleen lymphocyte proliferation *in vitro*. Acta Pharmacol Sin 2003; 38: 92-7.

[170] Wang YY, Khoo KH, Chen ST, Lin CC, Wong CH, Lin CH. Studies on the immuno-modulating and antitumor activities of *Ganoderma lucidum* (Reishi) polysaccharides: Functional and proteomic analyses of a fucose-containing glycoprotein fraction responsible for the activities. Bioorg Med Chem 2002; 10: 1057-62.

[171] Lee SS, Lee PL, Chen CF, Wang SY, Chen KY. Antitumor effects of *Ganoderma lucidum*. Ganoderma: Genetics, chemistry, pharmacology and therapeutics. *Proceedings of International Symposium on Ganoderma Research*. Shanghai, China: October 21-23, 2002.

[172] Cao LZ, Lin ZB. Regulation of *Ganoderma lucidum* polysaccharides on cytotoxic T lymphocytes induced by dendric cells *in vitro*. Ganoderma: Genetics, chemistry, pharmacology and therapeutics.

Proceedings of International Symposium on Ganoderma Research. Shanghai, China: October 21-23, 2002.

[173] Cao LZ, Lin ZB. Regulation on maturation and function of dendritic cells by *Ganoderma lucidum* polysaccharides. Immunol Lett 2002; 83: 163-9.

[174] Boh B, Berovic M, Wraber B, Hodzar D, Habijanic J, Pohleven F, *et al*. *Ganoderma lucidum* (W.Curt.:Fr.) Lloyd and *G. applanatum* (Pers.) Pat. (Aphyllophoromycetideae) from Slovenian habitats: Cultivation, isolation, and testing of active compounds. Int J Med Mushrooms 2004; 6: 15-32.

[175] Chen HS, Tsai YF, Lin S, Lin CC, Khoo KH, Lin CH, *et al*. Studies on the immuno-modulating and anti-tumor activities of *Ganoderma lucidum* (Reishi) polysaccharides. Bioorg Med Chem 2004; 12: 5595-601.

[176] Zhu XL, Lin ZB. Effects of *Ganoderma lucidum* polysaccharides on proliferation and cytotoxicity of cytokine-induced killer cells. Acta Pharmacol Sin 2005; 26: 1130-7.

[177] Zhu XL, Lin ZB. Modulation of cytokines production, granzyme B and perforin in murine CIK cells by *G. lucidum* polysaccharides. Carbohydr Polym 2006; 63: 188-97.

[178] Cheng KC, Huang HC, Chen JH, Hsu JW, Cheng HC, Ou CH, *et al. Ganoderma lucidum* polysaccharides in human monocytic leukemia cells: From gene expression to network construction. BMC Genomics 2007; 8: 411.

[179] Lai CY, Hung JT, Lin HH, Yu AL, Chen SH , Tsai YC, *et al*. Immunomodulatory and adjuvant activities of a polysaccharide extract of *Ganoderma lucidum in vivo* and *in vitro*. Vaccine 2010; 28: 4945-54.

[180] Meng JJ, Hu XF, Shan FP, Hua H, Lu CL, Wang EH, *et al*. Analysis of maturation of murine dendritic cells (DCs) induced by purified *Ganoderma lucidum* polysaccharides (GLPs). Int J Biol Macromol 2011; 49: 693-9.

[181] Cao QZ, Lin ZB. *Ganoderma lucidum* polysaccharides peptide inhibits the growth of vascular endothelial cell and the induction of VEGF in human lung cancer cell. Life Sci 2006; 78: 1457-63.

[182] Song YS, Kim SH, Sa JH, Jin C, Lim CL, Park EH. Anti-angiogenic and inhibitory activity on inducible nitric oxide production of the mushroom *Ganoderma lucidum.* J Ethnopharmacol 2004; 90: 17-20.

[183] Stanley G, Harvey K, Slivova V, Jiang J, Sliva D. *Ganoderma lucidum* suppresses angiogenesis through the inhibition of secretion of VEGF and TGF-β1 from prostate cancer cells. Biochem Biophys Res Commun 2005; 330: 46-52.

[184] Shi YL, James AE, Benzie IFF, Bushwell JA. Mushroom derived preparations in the prevention of H_2O_2-induced oxidative damage to cellular DNA. Teratog Carcinog Mutagen 2002; 22: 103-11.

[185] Pillai TG, Nair CKK, Janardhanan KK. Enhancement of repair of radiation induced DNA strand breaks in human cells by *Ganoderma* mushroom polysaccharides. Food Chem 2010; 119: 1040-3.

[186] You YH, Lin ZB. Protective effects of *Ganoderma lucidum* polysaccharides peptide on injury of macrophages induced by reactive oxygen species. Acta Pharmacol Sin 2002; 23: 787-91.

[187] Zhang HN, He JH, Yuan L, Lin ZB. *In vitro* and *in vivo* protective effect of *Ganoderma lucidum* polysaccharides on alloxan-induced pancreatic islets damage. Life Sci 2003; 73: 2307-19.

[188] Lai CSA, Yu MS, Yuen WH, So KF, Zee SY, Chang RCC. Antagonizing β-amyloid peptide neurotoxicity of the anti-aging fungus *Ganoderma lucidum*. Brain Res 2008; 1190: 215-24.

[189] Lei L, Lin Z, Chen Q, Li R, He Y. Antagonistic effect of *Ganoderma lucidum* polysaccharide on the immunosuppressive response induced by cyclosporin A, hydrocortisone and antitumor agents. Chin J Biotechnol Toxicol 1993; 7: 183-5.

[190] Zhu XL, Chen A F, Lin ZB. *Ganoderma lucidum* polysaccharides enhance the function of immunological effector cells in immunosuppressed mice. J Ethnopharmacol 2007; 111: 219-26.

[191] Li M, Lei L, Liang D. Effect of Ganderma polysaccharide on intracellular free calcium in murine peritoneal macrophages. Chin Pharm J 1999; 34: 805-7.

[192] Sadava D, Still DW, Mudry RR, Kane SE. Effect of *Ganoderma* on drug-sensitive and multidrug-resistant small-cell lung carcinoma cells. Cancer Lett 2009; 277: 182-9.

[193] Tu, T.R., Li, C.F., Su, S.H., Wong, C.H., Fan, E. Compositions and methods for treating allergies, auto-immune diseases, and improving skin condition by *Ganoderma lucidum* (Reishi) polysaccharides. US20090060939 (**2009**).

[194] Tu, T.R., Li, C.F., Su, S.H., Wong, C.H., Fan, E. Compositions and methods for treating psoriasis by *Ganoderma lucidum* (Reishi) polysaccharides. US20090060940 (**2009**).

[195] Nwanneka OL, Ogbe AO, Olufunke O. Effect of the mycelial culture of *Ganoderma lucidum* on human pathogenic bacteria. Int J Biol 2011; 3: 111-4.

[196] Fujiwara, H., Sawai, K. Antimicrobial agent for *Staphylococcus* comprising an extract of *Ganoderma lucidum*. EP0591603 (**1994**).

[197] Wachtel-Galor, S., Benzie, I.F.F., Boost, M.V. Methods of reducing minimal inhibitory concentration of antibiotics and compositions resulting therefrom. US20070059321 (**2007**).

[198] Kim HW, Shim J, Cho EC, Kim BK. Inhibition of cytopathic effect of human immunodeficiency virus-1 by water-soluble extract of *Ganoderma lucidum*. Arch Pharmacal Res 1997; 20: 425-31.

[199] Stamets, P. Antiviral activity from medicinal mushrooms. US20060171958 (**2006**).

[200] Lam FFY, Ko IWM, Ng ESK, Tam LS, Leung PC, Li EKM. Analgesic and anti-arthritic effects of Lingzhi and San Miao San supplementation in a rat model of arthritis induced by Freund's complete adjuvant. J Ethnopharmacol 2008; 120: 44-50.

[201] Jia J, Zhang JJ, Hu YS, Wu Y, Wang QZ, Li NN, *et al*. Evaluation of *in vivo* antioxidant activities of *Ganoderma lucidum* polysaccharides in STZ-diabetic rats. Food Chem 2009; 115: 32-6.

[202] Seto SW, Lam TY, Tam HL, Au ALS, Chan SW, Wu J, *et al.* Novel hypoglycemic effects of *Ganoderma lucidum* water-extract in obese/diabetic (+db/+db) mice. Phytomedicine 2009; 16: 426-36.

[203] Ping CX, Yan C, Bing LS, Guo CY, Yun LJ, Ping LL. Free radical scavenging of *Ganoderma lucidum* polysaccharides and its effect on antioxidant enzymes and immunity activities in cervical carcinoma rats. Carbohydr Polym 2009; 77: 389-93.

[204] Guo CY, Ji SZ, Ping CX. Modulatory effect of *Ganoderma lucidum* polysaccharides on serum antioxidant enzymes activities in ovarian cancer rats. Carbohydr Polym 2009; 78: 258-62.

[205] Heleno SA, Barros L, Martins A, Queiroz MJRP, Santos-Buelga C, Ferreira ICFR. Fruiting body, spores and *in vitro* produced mycelium of *Ganoderma lucidum* from Northeast Portugal: A comparative study of the antioxidant potential of phenolic and polysaccharidic extracts. Food Res Int 2012; 46: 135-40.

[206] Fan LP, Li JW, Deng KQ, Ai LZ. Effects of drying methods on the antioxidant activities of polysaccharides extracted from *Ganoderma lucidum*. Carbohydr Polym 2012; 87: 1849-54.

[207] Chan, B.P., Zhang, R.P. Neuroprotective *Ganoderma* compositions and methods of use. US20100278855 (**2010**).

[208] Akikuni, Y. Therapeutic agent for a cancer and method of screening the same, and health-care auxillary food. US20030064076 (**2003**).

[209] Yagita, A.Y. Anti-cancer compositions. US20030118606 (**2003**).

[210] Wu, R.T. Polysaccharide-based extract from *Ganoderma*, pharmaceutical use thereof, and process for preparing the same. US6613754 (**2003**).

[211] Kosuna, K.C., Yuan, L., Miura, T., Sun, B. Substance derived from basidiomycetes culture, method for producing it and its use. US7883708 (**2011**).

[212] Vincent ECO, Fang L. Immunomodulation and anti-cancer activity of polysaccharide-protein complexes. Curr Med Chem 2000; 7: 715-29.

[213] Ye LB, Zheng XL, Zhang JS, Yang Y, Meng YC, Li JR, *et al.* Composition analysis and immunomodulatory capacity of peptidoglycan from Ling zhi or Reishi medicinal mushroom, *Ganoderma lucidum* (W. Curt.: Fr.) P. Karst. strain 119 (Aphyllophoromycetideae). Int J Med Mushrooms 2010; 2: 157-65.

[214] Tsai CC, Yang FL, Huang ZY, Chen CS, Yang YL, Hua KF, *et al.* Oligosaccharide and peptidoglycan of *Ganoderma lucidum* activate the immune response in human mononuclear cells. J Agric Food Chem 2012; 60: 2830-7.

[215] Cao QZ, Lin ZB. Antitumor and anti-angiogenic activity of *Ganoderma lucidum* polysaccharides peptide. Acta Pharmacol Sin 2004; 25: 833-8.

[216] Liang CY, Li HR, Zhou H, Zhang SQ, Liu ZY, Zhou QL, *et al*. Recombinant LZ-8 from *Ganoderma lucidum* induces endoplasmic

reticulum stress-mediated autophagic cell death in SGC-7901 human gastric cancer cells. Oncol Rep 2012; 27: 1079-89.

[217] Ho YW, Yeung JSL, Chin PKY, Tang WM, Lin ZB, Man RYK, *et al*. *Ganoderma lucidum* polysaccharide peptide reduced the production of proinflammatory cytokines in activated rheumatoid synovial fibroblast. Mol Cell Biochem 2007; 301: 173-9.

[218] Eo SK, Kim YS, Lee CK, Han SS. Possible mode of antiviral activity of acidic protein bound polysaccharide isolated from *Ganoderma lucidum* on herpes simplex viruses. J Ethnopharmacol 2000; 72: 475-81.

[219] He H, He JP, Sui YJ, Zhou SQ, Zhou SQ, Wang J. The hepatoprotective effects of *Ganoderma lucidum* peptides against carbon tetrachloride-inducedliver injury in mice. J Food Biochem 2008; 32: 628-41.

[220] Sun J, He H, Xie BJ. Novel antioxidant peptides from fermented mushroom *Ganoderma lucidum.* J Agric Food Chem 2004; 52: 6646-52.

[221] You YH, Lin ZB. *Ganoderma lucidum* polysaccharides peptide (GLPP) protects ECV304 cells from oxidantive injury. Acta Pharmacol Sin 2006; 27: 355-5.

[222] Yuan BJ, Zhang WZ, Yu ZQ, Zhang RJ. *In vitro* evaluation of antioxidant property of the exopolysaccharides peptides from *Ganoderma lucidum* CAU5501 in submerged culture. J Food Agric Environ 2012; 10: 97-101.

[223] Bishop, M., Bishop, E., Smith, W., Gillis, G. Anti-glycation hydrolysate of *G. lucidum.* US7524504 (**2009**).

[224] Wong, K.P., Wong, M.C. Compositions containing an active fraction isolated from *Ganoderma lucidum* and methods of use. US2003095981 (**2003**).

[225] Chu, C.L., Chen, T.C. Methods for enhancing innate and adaptive immunity and antigen immunogenicity. US20090285789 (**2009**).

[226] Chu, C.L., Chen, T.C. Method for augmenting the immunogenicity of an antigen. US20110280906 (**2011**).

[227] Wong, C.-H., Hsu, H.-Y., Hua, K.-F., Lin, C.-H., Hsu, J., Chen, S.-T., Lin, K.-I., Yang, W.-B., Yu, J., Yu, A. Methods and compositions associated with administration of an extract of *Ganoderma lucidum.* WO2006044616 (**2006**).

[228] Wong, C.-H., Fan, E., Hsu, H.-Y., Tu, T.-R., Lin, W.-C., Li, C.-F., Weng, S.-T., Wang, W.-T. Reishi polysaccharide-based compositions and methods for treatment of cancer. WO2011133983 (**2011**).

[229] Shiao, M.-S. Method for sensitizing cancer cells to cancer therapies with a mevalonate-reducing compound. US2002173538 (**2002**).

[230] Wheatley, G.W., Chapman, T.B. A method for producing an extract from *Ganoderma lucidum* containing ganoderic acid. EP0943006 (**2002**).

[231] Vittori, N. Method of isolating mucilaginous polysaccharides and uses thereof. US6482942 (**2002**).

[232] Feng, P., Feng, M., Huang, J.S., Qian, Y.F., Shen, J., Chen, L., Wang, L.A. Process for refining *Ganoderma* spore polysacchoride. US20080112967 (**2008**).

[233] Kristiansen, B. Immune modulating compounds from fungi. US7514085 (**2009**).

[234] Hua, K.F., Hsu, H.Y., Wong, C.H. Methods and compositions associated with administration of an extract of *Ganoderma lucidum*. US7687064 (**2010**).

[235] Liu, X., Huang, X.-N., Chung, P.C.-K. Method for extracting oleaginous substances from *Ganoderma lucidum* spores. EP1245235 (**2004**).

[236] Chung, C.K., Tong, S.K. External preparation for skin containing oleaginous substances extracted from *Ganoderma lucidum* and method of using the same. US200501800988 (**2005**).

[237] Gow, R.T., Li, D., Sypert, G.W., Alberte, R.S. Extracts and methods comprising *Ganoderma* species. US20080112966 (**2008**).

[238] Shih, D.T.B., Chen, W.Y., Wong, C.H. Reishi-mediated enhancement of human tissue progenitor cell adhesion and differentiation. US20080247989 (**2008**).

[239] Lu, E.S., Young J. Herbal composition for treating cancer. WO2010028075 (**2010**).

[240] Wasser SP, Weiss AL. Medicinal mushrooms - *Ganoderma lucidum*, Reishi mushroom. Pedeifus Publishing House: Haifa 1997.

[241] Chang ST, Buswell JA. Mushroom nutriceuticals. World J Microbiol Biotechnol 1996; 12: 473-6.

[242] Sanodiya BS, Thakur GS, Baghel RK, Prasad GB, Bisen PS. *Ganoderma lucidum*: A potent pharmacological macrofungus. Curr Pharm Biotechnol 2009; 10: 717-42.

[243] Wasser SP. Current findings, future trends, and unsolved problems in studies of medicinal mushrooms. Appl Microbiol Biotechnol 2010; 89: 1323-32.

[244] Wachtel-Galor S, Yuen J, Buswell JA, Benzie IFF. Chapter 9: *Ganoderma lucidum* (Lingzhi or Reishi). In : Benzie IFF, Wachtel-Galor S, (Eds). Herbal Medicine: Biomolecular and Clinical Aspects. 2nd ed. Boca Raton (FL): CRC Press; 2011.

[245] Chen, S., Wang, X. Herbal composition for treating prostate carcinoma. EP0977556 (**2000**).

[246] Lam, P.Y.S. Herbal immune system stimulant. US6464982 (**2002**).

[247] Chen, S. Botanical extract compositions with anti-cancer or phytoestrogenic activity comprising prenyl flavonoids. WO2004080474 (**2004**).

[248] Goino, T. Physiologically active compositions based upon active ingredients of basidiomycotina and araliaceae. WO0156589 (**2001**) & US 6746675 (**2004**).

[249] Hattori, K., Mizutani, H., Osumi, K. Matrix metalloprotease inhibitor. EP1449534 (**2004**).

[250] Cheung, D.C.L. Combined therapy with a herbal extract and a reovirus for killing neoplastic cells in a subject. WO2005112966 (**2005**).

[251] Dao, J., Dao, T.C.S., Tong, D.D., Wilson, L., Jordan, M.A., Gerwick, W. Compositions of botanical extracts for cancer therapy. US20050208070 (**2005**).

[252] Dao, J., Dao, J.J. Compositions of botanical extracts for treating malignancy-associated changes. US20050196409 (**2005**).

[253] Mahajna, J.A., Yassin, M., Wasser, S.P. Mushroom extracts having anti-cancer activity. US20060057157 (**2006**).

[254] Lu, E.S., Young J. Herbal composition for treating cancer. WO2010028075 (**2010**).

[255] Soeda, S., Kuramoto, Y., Hayashida, S., Kosako, T., Urano, K., Honda, S., Motonagare, R., Nakaji, K. New anticancer agent or cancer prophylactic agent having both neovascularization-inhibiting effect and immune control effect with preparation or mixture of basidiomycetes belonging to polyporaceae and component of pinaceae plant. JP2011098922 (**2011**)

[256] Kong, P. Ganoderma lucidum coffee and preparation method thereof. WO2011077295 (**2011**).

[257] Feng, M., Shen, J. *Ganoderma lucidum* spore oil-containing Chinese medicinal composition with efficient cancer resistance. CN102379905 (**2012**).

[258] Zhang CY, Li YM. Clinical investigation of Green Valley Lingzhi capsule on type 2 diabetes mellitus. Ganoderma: Genetics, chemistry, pharmacology and therapeutics, *Proceedings of International Symposium on Ganoderma Research*. Shanghai, China: October 21-23, 2002.

[259] Shi KG, Quing LH. The follow-up observation assessment of medium and late phases cancer treated by Chinese *Ganoderma lucidum* essence (CGLE). Ganoderma: Genetics, Chemistry, Pharmacology and Therapeutics, *Proceedings of International Symposium on Ganoderma Research*. Shanghai, China: October 21-23, 2002.

[260] Gao Y, Zhou S, Jiang W, Huang M, Dai X. Effects of ganopoly (a *Ganoderma lucidum* polysaccharide extract) on the immune functions in advanced-stage cancer patients. Immunol Invest 2003; 32: 201-15.

[261] Gao Y, Tang W, Dai X, Chen G, Ye J, Chen E, *et al.* Effects of water-soluble *Ganoderma lucidum* polysaccharides on the immune functions of patients with advanced lung cancer. J Med Food 2005; 8: 159-68.

[262] Noguchi M, Kakuma T, Tomiyasu K, Konishi F, Kumamoto S, Kondo R, *et al*. Phase I study of a methanol extract of *Ganoderma lucidum*, edible and medicinal mushroom, in men with mild symptoms of bladder outlet obstruction. Urology 2005; 66 (Suppl 3A): 21.

[263] Chan WK, Lam DT, Law HK, Wong WT, Leung KMW, Lau YL, *et al*. *Ganoderma lucidum* mycelium and spore extracts as natural adjuvants for immunotherapy. J Altern Complement Med 2005; 11: 1047-57.

[264] Chen X, Hu ZP, Yang XX, Huang M, Gao Y, Tang W, *et al.* Monitoring of immune responses to a herbal immuno-modulator in pa-

tients with advanced colorectal cancer. Int Immunopharmacol 2006; 6: 499-508.

[265] Noguchi M, Kakuma T, Tomiyasu K, Itoh K, Konishi F, Kumamoto S, *et al.* A randomized clinical trial of an ethanol extract of *Ganoderma lucidum* (edible and medicinal mushroom) in men with lower urinary tract symptoms. Urology 2007; 70 (Suppl 3A): 254.

[266] Li EK, Tam LS, Wong CK, Li WC, Lam CWK, Wachtel-Galor S, *et al.* Safety and efficacy of *Ganoderma lucidum* (Lingzhi) and San miao San supplementation in patients with rheumatoid arthritis: A double-blind, randomized, placebo-controlled pilot trial. Arthritis Rheum - Arthritis Care Res 2007; 57: 1143-50.

[267] Chen AW, Seleen J. Potential benefits of Ling Zhi or Reishi mushroom *Ganoderma lucidum* (W. curt.: Fr.) P. Karst. (Aphyllophoromycetideae) to breast cancer patients. Int J Med Mushrooms 2007; 9: 29-38.

[268] Zhao H, Zhang QY, Zhao L, Huang X, Wang JC, Kang XM. Spore powder of *Ganoderma lucidum* improves cancer-related fatigue in breast cancer patients undergoing endocrine therapy: a pilot clinical trial. Evid Based Complement Alternat Med 2012; Art. No. 809614. doi:10.1155/2012/809614.

[269] Chu TTW, Benzie IFF, Lam CWK, Fok BSP, Lee, KKC, Tomlinson B. Study of potential cardioprotective effects of *Ganderma lucidum* (Lingzhi): Results of a controlled human intervention trial. Br J Nutr 2012; 107: 1017-27.

[270] Jin X, Beguerie JR, Sze DMY, Chan GCF. *Ganoderma lucidum* (Reishi mushroom) for cancer treatment. Cochrane Database Syst Rev 2012; 6:CD007731. doi: 10.1002/14651858.CD007731.pub2.

[271] Yuen MF, Ip P, Ng WK, Lai CL. Hepatotoxicity due to a formulation of *Ganoderma lucidum* (lingzhi). J Hepatol 2004; 41: 685-90.

[272] Wanmuang H, Leopairut J, Kositchaiwat C, Wananukul W, Bunyaratvej S. Fatal fulminant hepatitis associated with *Ganoderma lucidum* (Lingzhi) mushroom powder. J Med Assoc Thai 2007; 90: 179-81.

[273] Lin ZB, Zhang HN. Anti-tumor and immunoregulatory activities of *Ganoderma lucidum* and its possible mechanisms. Acta Pharmacol Sin 2004; 25: 1387-95.

[274] Liu GQ, Zhang KC. Mechanisms of the anti-cancer action of *Ganoderma lucidum* (Leyss. ex Fr.) Karst.: A new understanding. J Integr Plant Biol 2005; 47: 129-35.

[275] Yuen JWM, Gohel MDI. Anti-cancer effects of *Ganoderma lucidum*: A review of scientific evidence. Nutr Cancer 2005; 53: 11-7.

[276] Sliva D. *Ganoderma lucidum* in cancer research. Leuk Res 2006; 30: 767-8.