Culinary-Medicinal Higher Basidiomycete Mushrooms as a Prominent Source of Dietary Supplements and Drugs for the 21st Century

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Abstract: The number of mushrooms on Earth is estimated at 140,000, yet perhaps only 10% (approximately 14,000 named species) are known. They make up a vast and yet largely untapped source of powerful new pharmaceutical products. Many, if not all, Basidiomycetes mushrooms contain biologically active substances in fruit bodies, cultured mycelium, and culture broth. These biologically active substances are of different chemical composition and mode of action. For instance, numerous bioactive polysaccharides or polysaccharide-protein complexes from medicinal mushrooms enhance innate and cell-mediated immune responses and exhibit antitumour activities in animals and humans. Stimulation of host immune defense systems by bioactive polymers from medicinal mushrooms has significant effects on the maturation, differentiation, and proliferation of many kinds of immune cells in the host. Many of these mushroom polymers were reported previously to have immunotherapeutic properties by facilitating growth inhibition and destruction of tumour cells. Whilst the mechanism of their antitumor actions is still not completely understood, stimulation and modulation of key host immune responses by these mushroom polymers appears central. Several of the Basidiomycetes compounds (primarily polysaccharides) have proceeded through Phase I, II, and III clinical trials, and are used in Asia to treat various cancers and other diseases. However, most of the mushroom-derived biologically active susbtaces are used as dietary supplements. The present review analyzes the pecularities of biologically active substances derived from Basiodiomycetes and their application as drugs and dietary supplements.

Key words: Medicinal mushrooms, dietary supplements, polysaccharides, polysaccharide-protein complexes, immunomodulator activity

1 Introduction

For millennia, mushrooms have been valued as edible and medical provisions for humankind. Currently, mushroom-derived substances with antitumor and immunomodulating properties are used as dietary supplements (DSs) or drugs.[1-8] Historically, hot-water-soluble fractions (decoctions and essences) from medicinal mushrooms, i.e., mostly polysaccharides, were used as medicine in the Far East, where knowledge and practice of mushroom use primarily originated.[9] Such mushrooms as *Ganoderma lucidum* (W. Curt. : Fr.) P. Karst. (Ling Zhi, Reishi), *Lentinus edodes* (Berk.) Singer (Shiitake), *Inonotus obliquus* (Ach. ex Pers.) Plilát (Chaga), and many others have been collected and used for centuries in Korea, China, Japan, and eastern Russia. Those practices still form the basis of modern scientific studies of fungal medical activities. It is remarkable how reliable are the facts collected by traditional eastern medicine in the area of medicinal mushrooms.[8-10]
Soon after the first reports on antitumor activity of polysaccharides from *L. edodes* and some other Polyporaceae (Aphyllophoromycetideae) species, the first three major drugs were developed from medicinal mushrooms. All three were polysaccharides, specifically β-glucans: Krestin from cultured mycelial biomass of *Trametes versicolor* (L. : Fr.) Lloyd (Turkey Tail), Lentinan from fruit bodies of *L. edodes,* and Schizophyllan from a liquid medium broth product of *Schizophyllum commune* Fr. : Fr. In the 40 years since then, medicinal mushrooms

have been intensively investigated for medicinal effects in *in vivo* and *in vitro* model systems, and many new antitumor and immunomodulating susbstances, primarily polysaccharides, have been identified and put to practical use.[2, 3, 6-8, 11]
In the present paper, antitumor and immunomodulating polysaccharides from higher Basidiomycetes mushrooms are analyzed. More attention is given to their common features than to specific peculiarities. The review summarizes a general state of knowledge in the area of biodiversity of mushrooms and their polysaccharides; the chemical structure of polysaccharides and its connection with their antitumor activity, including possible ways of chemical modification; results of experimental testing and clinical use of antitumor or immunostimulating polysaccharides; possible mechanisms of their biological action; and, finally, the difference in polysaccharide fraction composition in fruit bodies and pure culture mycelia in selected examples of the studied medicinal mushrooms.

2 The Diversity of Mushrooms with Antitumor and Immunomodulatory Compounds

Out of the total number of described fungi, mushrooms constitute 14,000 species, calculated from the *Dictionary of the Fungi,*[12] or go as high as 22,000.[13] However, the actual number of such species on Earth is undoubtedly much higher. We generally take the definition of the term "mushroom" from Chang & Miles:[14] "a macrofungus with a distinctive fruit body, which can be either hypogeous or epigeous, large enough to be seen with the naked eye and to be picked by hand". Two main reasons for the actual number being higher are (1) the great number of as yet undescribed species and (2) the fact that many morphologically defined mushroom 'species' prove to be assemblages of several biological[13] or phylogenetic[15] species. Studies of compatibility and molecular sequences between fungi previously considered to be the same species on morphological grounds revealed 'cryptic species', i.e., populations functioning as separate biological species but covered by a single scientific name. A single morphologically defined species may consist of 20 or more biological species[13] and a varying number of phylogentic species.[15]
Considering all this, it is reasonable to estimate the number of mushrooms on Earth at 140,000. Thus, 14,000 of known mushroom species represent perhaps only 10% of those that exist in nature. Meanwhile, of the approximately 14,000 species that we know today, about 50% are considered to possess varying degrees of edibility, more than 2,000 are safe, and about 700 species are known to possess significant pharmacological properties.[5, 8]
Clearly, mushrooms represent an important source of powerful new pharmaceutical products. Most of the main taxonomic groups of Higher Basidiomycetes possess such compounds. Biologically active substances (BAS) are contained in naturally collected or artificially grown fruit bodies, pure culture mycelia, and culture filtrates (culture broth). Polysaccharides represent the most numerous group of mushroom-derived BASs. For instance, antitumor or immunostimulating polysaccharides are known for 651 species and seven infraspecific taxa representing 182 genera of Hetero- and Homobasidiomycetes mushrooms.[5]

3 Structural Compositions of Antitumor and Immunomodulatory Polysaccharides in Higher Basidiomycetes

Polysaccharides belong to a structurally diverse class of macromolecules, polymers of monosaccharide residues joined to each other by glycosidic linkages. Compared with other biopolymers, such as proteins and nucleic acids, polysaccharides offer the highest capacity for carrying biological information because they have the greatest potential for structural variability.[16] This enormous potential variability in polysaccharide structure gives the necessary flexibility to the precise regulatory mechanisms of various cell-cell interactions in higher organisms.
Mushroom polysaccharides are mostly present as glucans with different types of glycosidic linkages such as (1→3), (1→6)-β-glucans and (1→3)-α-glucans, but some are true heteroglycans. The others mostly bind to

protein residues as PSP complexes (PSPC).[17] The main source of antitumor polysaccharides appears to be fungal cell walls.[3] Active polysaccharides are characterized with complex tertiary structures. For instance, active β-D-glucan of Schizophyllan has a triple-strand right-winding structure.[18] In addition to the well known antitumor β-(1→3)-glucans, a wide range of biologically active glucans and glycans with other structures are known (Table 1).

Table 1. Chemical structure of antitumor and immunostimulating polysaccharides of higher Basidiomycetes [5]

Polysaccharide	Species
Glucans	
α-(1→3)-glucan	*Armillariella tabescens*
Linear α-(1→3)-glucan	*Amanita muscaria*
	Agrocybe aegerita
α-(1→4)-; β-(1→6)-glucan	*Agaricus brasiliensis S. Wasser et al. (= A. blazei ss. Heinem.)*
α-(1→6)-; α-(1→4)- glucan	*A. brasiliensis*
β-(1→6)-glucan	*Lyophyllum decastes*
	A. tabescens
β-(1→6)-; β-(1→3)-glucan	*A. brasiliensis*
	Grifola frondosa
β-(1→6)-; α-(1→3)-glucan	*A. brasiliensis*
β-(1→3)-glucuronoglucan	*Ganoderma lucidum*
Mannoxyloglucan	*G. frondosa*
Galactoxyloglucan	*Hericium erinaceus*
Xyloglucan	*G. frondosa*
	Polyporus confluens
	Pleurotus pulmonarius (= P. sajorcaju)
Xylogalactoglucan	*Inonotus obliquus*
Mannogalactoglucan	*P. pulmonarius*
	Pleurotus cornucopiae
	G. lucidum
	A. brasiliensis
Galactomannoglucan	*Flammulina velutipes*
	Hohenbuehelia serotina
	Leucopaxillus giganteus
Arabinoglucan	*Ganoderma tsugae*
Riboglucan	*A. brasiliensis*
Glycans	
Arabinogalactan	*Pleurotus citrinopileatus*
Glucogalactan	*G. tsugae*
Fucogalactan	*Sarcodon aspratus*
α-(1→6)-mannofucogalactan	*Fomitella fraxinea*
Fucomannogalactan	*Dictyophora indusiata*
Mannogalactan	*P. pulmonarius*
Mannogalactofucan	*G. frondosa*
Xylan	*H. erinaceus*
Glucoxylan	*H. erinaceus*
	P. pulmonarius
Mannoglucoxylan	*H. erinaceus*
α-(1→3)-mannan	*D. indusiata*
Glucomannan	*A. brasiliensis*
β-(1→2)-; β-(1→3)-glucomannan	*A. brasiliensis*
Galactoglucomannan	*Lentinus edodes*

The number of antitumor active fractions in fruit bodies and cultured mycelium of mushrooms is remarkably high. In particular, 20 polysaccharide fractions out of the 29 obtained from *G. frondosa* fruit bodies demonstrated different levels of antitumor activity, and 24 polysaccharide fractions out of the 28 obtained from cultured mycelium of this mushroom showed antitumor activity.[19] Another example can be seen in an analysis of polysaccharides of fruit bodies of *Pleurotus pulmonarius (= P. sajorcaju):* 16 polysaccharide fractions from 21 extractions demonstrated different levels of antitumor activity.[20] The number of polysaccharides extracted from the fruit bodies or the cultured mycelium of the same species is strongly dependent on the methods of fractionation used, but analyzed literature data indicate that, in general, the total amount of polysaccharides is higher in the fruit bodies.

4 Correlation of Structure and Antitumor Activities of Mushroom Polysaccharides

Polysaccharides with antitumor action differ greatly in their chemical composition and configuration as well as in their physical properties. Antitumor activity is exhibited by a wide range of glucans and glycans extending from homopolymers to highly complex heteropolymers.[4] Differences in activity can be correlated with solubility in water, size of the molecules, branching rate, and form. Although it is difficult to correlate the structure and antitumor activity of complex polysaccharides, some relationships can be inferred.

Structural features such as β-(1→3) linkages in the main chain of the glucan and additional β-(1→6) branch points are needed for antitumor action. β-Glucans containing mainly (1→6) linkages have less activity. High-molecular weight glucans appear to be more effective than those of low-molecular weight.[2, 3] However, distinct variations in antitumor polysaccharides have also been noted. Antitumor polysaccharides may have other chemical structures, such as hetero-β-glucans, β-glucan-protein, α-manno-β-glucan, α-glucan-protein,[21] and heteroglycan-protein complexes.[20, 21]

A triple-helical tertiary conformation of medicinal mushroom β-(1→3)-glucans is known to be important for their immune-stimulating activities, such as macrophage nitrogen oxide synthesis and limulus factor G activation. Denaturation of Lentinan with dimethyl sulfoxide, urea, or sodium hydroxide affected the tertiary structure, while the primary structure stayed intact. These conformation changes following progressive denaturation lowered tumor inhibition properties.[18] The same results, which confirmed the correlation between antitumor activity and triple helix structure, were obtained upon investigation of Schizophyllan,[18] indicating that some of the immuno-stimulating activities are dependent on the triple-helix conformation. However, other activities are independent of it, e.g., synthesis of interferon-γ and colony stimulating factor;[22] this indicates that the β-(1→3)-mannan backbone structure is of more importance than the tertiary structure of the molecule.

Unlike β-(1→3)-glucans (ranging from 500 to 2000 Da) with medicinal properties that are heavily dependent on high-molecular weight,[2] α-(1→3)-glucuronoxylomannans, which are characteristic of Jelly mushrooms, are not heavily dependent on molecular weight. Gao et al.[23] reported that acidic hydrolysate fractions of *Tremella fuciformis* fruit bodies' glucuronoxylomannans with molecular weights from 53 to 1 kDa induced human monocytes to produce interleukin-6 as efficiently as non-hydrolyzed heteropolysaccharides.

An important aspect concerning biologically active polysaccharides is the possibility of improvement of their antitumor activity by chemical modification. Different approaches to improving antitumor activity of mushroom polysaccharides by chemical modification are known. The most successful schemes have been developed for *Ganoderma lucidum, Grifola frondosa and Leucopaxillus giganteus.* Main modification procedures include the Smith degradation (oxydo-reducto-hydrolysis); activation by the method of formolysis;[2, 3, 21] carboxymethylation;[24] and enzymatic reactions. Chemically modified active substances have been derived from *Pleurotus ostreatus* (Jacq.:Fr.) P. Kumm. (pleuran)[25] and S. *commune*[26, 27] (chemically sulfated Schizophyllans with different sulfur content). It was suggested that the sulfur content in Schizophyllan is more important in inhibiting growth of human immunodeficiency virus (HIV) than the molecular weight or the nature of the sugar component.[9, 27] Medical tests indicate that sulfated Schizophyllan with a sulfur content of

5% can be useful as an anti-HIV agent for treatment of HIV-infected hemophiliacs.[9, 26] Another example of successful modification is a new class of biologically active polysaccharides developed in Japan. Active hexose correlated compounds (AHCC) is a proprietary compound produced by cultivation and enzymatic modification of mushroom mycelia of several species. In contrast to other anticancer glucans, the glucans of AHCC are low molecular weight, α-1,3 structures.[7, 28] AHCC's most notable observed effects have been in enhancing or boosting the immune system to allow other cancer treatments such as radiation or chemotherapy, to work successfully.[7, 28] According to industry analysts in Japan, currently, over 700 hospitals and medical clinics recommend AHCC to patients as part of an immune enhancement maintance regimen.[29]
Chemical modification is necessary in many cases to improve not only the antitumor activity of mushroom polysaccharides, but also their clinical qualities, most importantly, water solubility and the ability to permeate stomach walls after oral ingestion.

5 Mechanisms of Antitumor and Immunodulating Action by Mushroom Polysaccharides

Mushroom polysaccharides exert their antitumor action mostly via activization of the immune response of the host organism and are regarded as biological response modifiers (BRMs).[8, 11]
The immunomodulating action of mushroom polysaccharides is especially valuable as a prophylactic, a mild and non-invasive form, and in the prevention of metastatic tumors, etc. Polysaccharides from mushrooms mostly do not attack cancer cells directly, but produce their antitumor effects by activating different immune responses in the host. Such results suggest that the antitumor action of polysaccharides requires an intact T-cell component and that the activity is mediated through a thymus-dependent immune mechanism.[4] Also, their antitumor activity is inhibited by pretreatment with antimacrophage agents (such as carrageenan). Thus, the various effects of polysaccharides are thought to be due to potentiation of the response of precursor T cells and macrophages to cytokines produced by lymphocytes after specific recognition of tumor cells. In addition, the induction of a marked increase in the amounts of CSF, IL-1, and IL-3 by polysaccharides results in maturation, differentiation, and proliferation of the immunocompetent cells for host defense mechanisms.[4] Mushroom polysaccharides are known to stimulate natural killer cells, T-cells, B-cells, and macrophage-dependent immune system responses.

6 Antitumor and Immunomodulating Activity of Mushroom BRMs/Biologically Active Substances

We would like to emphasize the principal points of antitumor and immunomodulating effects of mushroom biologically active substances the most significant of which are polysaccharides. Most important among them are: (1) prevention of oncogenesis through oral consumption of mushrooms or their preparations; (2) direct antitumor activity against various allogeneic and syngeneic tumors; (3) immunopotentiation activity against tumors in combination with chemotherapy; (4) preventive effect on tumor metastasis.

6.1 *Lentinus edodes*

An immense amount of literature deals the anticancer effects of Lentinan on animals and humans, and only the more relevant and recent medical studies will be presented here. The purified polysaccharide has been shown in numerous xenographs to cause tumor regression and in some cases even a complete response (for extensive review of animal studies, see Hobbs,[9] Wasser & Weis,[8] and Yap & Ng[30]). The cytostatic effect of Lentinan is due to the activation of the host's immune system. Also, pre-clinical and clinical toxicity with Lentinan is rarely noted. Accumulated information on the antitumor activity, the prevention of metastasis, and the suppression of chemical and viral oncogenesis in animal models by Lentinan is summarized in Wasser & Weis.[8]

While Lentinan is a pure polysaccharide composed only of atoms of carbon, oxygen, and hydrogen, LEM (= glycoprotein from *L. edodes* mycelia) and LAP (= glycoprotein from *L. edodes* culture media) have also demonstrated antitumor activity in xenograft models and clinical trials. Both LEM and LAP activate the host immune system.[31] In Japan, Lentinan is presently classified as a medicine whereas LEM and LAP are considered food supplements (nutriceuticals).
There have been numerous clinical trials of Lentinan in Japan, although none have been placebo-controlled and double-blinded. However, Lentinan has been approved for clinical use in Japan for many years, and is manufactured by several pharmaceutical companies. Intraperitoneal Lentinan is widely used as an adjuvant treatment for certain cancers in Japan and China. Lentinan has proved successful in prolonging the overall survival of cancer patients, especially those with gastric and colorectal carcinoma. In patients with inoperable or recurrent gastric cancer, tumor responses and prolonged median survival were also noted.[32] It is also important to note that very few adverse reactions to Lentinan have been noted. Furthermore, it is able to greatly reduce the debilitating effects of chemotherapy, e.g. nausea, pain, hair loss, and lowered immune status.[32]
Apart from the polysaccharides, *L. edodes* has been shown recently to harbor substances with medicinal properties other than polysaccharides, for instance the protein Lentin. The protein exerts antifungal activity and inhibitory activity on HIV-1 reverse transcriptase and proliferation of leukemia cells.[33]

6.2 *Trametes versicolor*

T. versicolor is not an edible mushroom but, from ancient times, its extracts have been used in traditional Chinese medicine for therapeutic effects, including the treatment of cancer. Today, two compounds, PSK (polysaccharide-K, commercial name 'Krestin') and PSP (polysaccharide-peptide) are purified from this fungus by deep tank fermentation of the mycelium using a variety of strains. PSK was first isolated in Japan in the late 1960s while PSP was isolated in 1983 in China. Each compound has shown remarkable anticancer properties with few side effects. Remarkably, by 1987 PSK accounted for more than 25% of total national expenditure for anticancer agents in Japan.[31]
PSK has remarkable immune-enhancing activity and a broad anti-neoplastic scope. It acts directly on tumor cells, as well as indirectly in the host to boost cellular immunity.[9] It has been shown to be effective against many types of cancer,[7] but seldom has satisfactory results when administered alone. An intriguing feature of this compound is that injection of PSK at one tumor site has been shown to inhibit tumor growth at other sites, thus helping to prevent metastasis. PSK has been used both orally and intravenously in clinical medicine.
While PSK has been almost exclusively developed and tested in Japan, PSP is a product of China and continues to be assessed for efficacy safety by China's scientists and oncologists. There is a close similarity between PSK and PSP polypeptides although PSP lacks fucose and instead contains arabinose and rhamnose. Since the first development of PSP in 1983, there has been rapid progress through human clinical trials. Phase I clinical trials of PSP [34] showed that an oral dose of up to 6g/day was well tolerated and devoid of side-effects. Patients showed improvement in appetite and general condition, together with a stabilization of haematopoietic parameters. The Phase II and Phase III trials conducted in Shanghai hospitals showed that PSP improved disease-free survival of gastric, oesophageal, and non-small-cell lung cancers substantially reducing the usual unpleasant side effects of conventional treatments.[35, 36] Such a protective effect on the immunological functions of conventionally treated patients demonstrates that PSP can be classified as a clinical biological response modifier (BRM). Other BRMs such as LAK cells, IL-2, α y IFN or TNF-(are also being used in the treatment of advanced cancer cases.[36, 37] However, these drugs in effective doses often produce quite severe side effects such as fevers, chills, rashes, arthralgia, hypotension, pulmonary oedema, congestive heart failure, and CNS toxicities. A further observation noted that PSP in combination with radiotherapy induced a significant increase in the percentage of apoptotic cells at 24 h compared with radiation alone, and it has been surmised that the antitumor mechanism of PSP action may also involve the induction of DNA damage by apoptosis in the target

cancer cells.[38] Another beneficial side of PSP application is a strong amelioration of haematopoietic toxicity,[36, 37] a common adverse reaction of radiotherapy and chemotherapy. Moreover, PSP produced no teratogenic effects in mice or rats and exerted analgesic action in mice.[39]

Mutagenicity testing can now be viewed against an impressive background of basic scientific knowledge of genetic mechanisms and also the development of a wide range of experimental procedures that can be used as test systems. Recently, Zhong *et al.*[40] carried out an extensive series of experiments on possible genetic toxicity of the PSP polysaccharopeptide. No evidence of mutagenic activity has been revealed in: (1) mutagenicity tests using a special strain of *Salmonella typhimurium;* (2) cytotoxicity tests of PSP with V_{79} Chinese hamster cells *in vitro;* (3) *in vivo* micronucleus tests for the cytogenotoxicity on mammalian somatic cells, and; (4) chromosome observation tests, metaphase analysis of bone marrow cells in mice.

The corpus of laboratory and clinical evidence that PSP offers considerable benefits to patients suffering from cancers of the stomach, oesophagus, and lung led to the Chinese Ministry of Public Health granting it a regulatory license. However, despite the use of PSK and PSP in humans for many years, bioavailability and pharmacokinetics have received little detailed study. More work in this area, as well as blind RCT's, are required.

6.3 *Schizophyllum commune*

The polysaccharide derived from this mushroom is a β-(1,3)-D-glucan with β-(1,6)-D-glucan side-chains and is called Schizophyllan (or Sonifilan, Sizofiran, Sizofilan). As with all glucan preparations, they are never homologous in terms of molecular weight but consist of molecules with a wide range of molecular weights. In the case of Schizophyllan, the molecules are large and are normally administered in the clinical setting via the intramuscular or intraperitoneal route.

Various clinical trials have been carried out in Japan. Schizophyllan has been approved for clinical use in Japan. Early clinical studies with Schizophyllan in combination with conventional chemotherapy (tegafur or mitomycin C and 5-fluorouracil) in a randomized controlled study of 367 patients with recurrent and inoperable gastric cancer resulted in a significant increase in median survival.[9] Recently, Schizophyllan has also been shown to increase the overall survival of patients with head and neck cancers.[41]

In a randomized controlled study of Schizophyllan in combination with radiotherapy, Schizophyllan significantly prolonged the overall survival of Stage II cervical cancer patients, but not for those patients in Stage III.[18] In a prospective, randomized clinical trial involving 312 patients treated with surgery, radiotherapy, chemotherapy (fluorouracil), and Schizophyllan in various combinations, patients treated with Schizophyllan had a better overall survival than patients who had not received the polysaccharide.[18] The variety of treatment regimes significantly reduced the value of these results. However, separate analyses of patients with 10% or more activated $CD4^+$ cells out of their total $CD4^+$ population and with more than 25% activated $CD8^+$ cells before the beginning of treatment showed that in this group the Schizophyllan-induced increase in survival was highly significant. Furthermore, when Schizophyllan was injected intratumorally to cervical cancers there was a significant infiltration of Langerhans cells and T-cells.[42] Several Japanese pharmaceutical companies currently produce Schizophyllan commercially.

6.4 *Ganoderma lucidum*

Studies indicate that the active constituents of *G. lucidum* possess a variety of therapeutic effects. Numerous polysaccharides demonstrate antitumor and immunostimulating activities. For example, β-D-glucan (GL-1) from the fruit body and as a medium product has a potent effect against Sarcoma 180 in animals. This substance appears to act as a new type of a carcinostatic agent because its effect is based on enhancement of the host's immune system. Unlike general carcinostatic agents (chemotherapy), it appears to be nontoxic. Additionally, polysaccharides of *G. lucidum* enhance DNA synthesis of spleen cells in mixed lymphocytes culture, stimulate

both RNA and DNA synthesis in the bone marrow of mice.[43]

Furthermore, *G. lucidum* contains other substances that may enhance various aspects of homeostasis and physis such as the reduction of blood pressure and blood sugar level, elimination of cholesterol, antithrombotic reaction, hepatitis healing, etc.: (1) triterpenes and related compounds (cytotoxic to tumors, anti-HIV, anti-hyperlipidemic, hypotensive, anti-platelet aggregation, hepatoprotective, analgesic, immunomodulatory), LZ-8 (anti-hypersensitivity, anti-autoimmune diabetes, anti-hepatitis B, immunomodulatory activities), adenosine and derivatives (inhibit platelet aggregation), organic germanium (anti-Lewis-lung carcinoma, promotes blood circulation/0_2 utilization), oleic acids and cyclooctasulphur (inhibit histamine release), RNA (anti-viral, immunomodulatory activity).[10, 43]

The sterols in *G. lucidum* are reported to act as hormone precursors, while adenosine (a component of RNA) has been found to inhibit platelet aggregation. Ganoderal A and B glycans of the fruit bodies significantly reduce plasma sugar levels in hyperglycemic mice.

Triterpenes of *G. lucidum* have adaptogenic, anti-hypertensive, and anti-allergic potency. Ganoderic acid C appears to be the most active of the anti-allergic constituents followed in activity by ganoderic acids A and D. Also, ganoderic acids B and D are reported to act as strong anti-hypertensive agents. Ganoderic acids T through Z exhibit antitumor activity against hepatoma cells. Ganoderic acids A through D have been shown to inhibit histamine release.

Over the last ten years, there have been numerous reports of pre-clinical antitumor activity of *G. lucidum* extracts in a variety of tumors.[44, 45] Such extracts effectively inhibited metastasis in animal (mouse) models and increased survival when administered as monotherapy or in combination with conventional chemotherapy.[44, 45] Some pre-clinical studies have suggested that the antitumor action of *G. lucidum* polysaccharides could be the result of its biological response modifying effects. Ganopoly (an aqueous extract of *G. lucidum*) has been shown in *in vitro* systems and in xenographs to have immunomodulating effects, through the activation of macrophages, T-lymphocytes, and natural killer cells.[46] An extensive open, non-randomized clinical trial has recently been carried out on patients with advanced cancers using Ganopoly.[45, 47] This compound is marketed as an over-the-counter product in Hong Kong, New Zealand, and Australia.

Within the realms of traditional herbal medicine in China and in several Asian countries, many cancer patients use *G. lucidum* proprietary extracts as an adjunct to conventional treatment or as the sole therapy. However, no clinical trials with *G. lucidum* extracts against various human cancers have been published in English in peer-reviewed journals.[46]

6.5 *Grifola frondosa*

Grifola frondosa (maitake) is one of the most popular medicinal mushrooms. Fruit bodies of this mushroom contain β-glucans with different chain conformation, heteroglucans, or glucoproteins.[19, 48]

In contrast to fruit body polysaccharide composition, no β-glucan has been detected among antitumor active fractions obtained from culture mycelium (grown on Whatman filter paper soaked in liquid nutrient medium) that was collected before initiation of fruit bodies.[49] Polysaccharides from *G. frondosa* cultured mycelium are heteromannans, heterofucans, and heteroxylans, or their complexes with protein, i.e., types of polysaccharides that were not found in fruit bodies of this mushroom.

Several studies have shown that β-D-glucan and glycoprotein complexes derived from this mushroom have strong antitumor activity in xenographs. More recently, a highly purified extract, β-glucan (β-1,6 glucan branched with a β-1,3-linkage) (Grifon-D©=GD) has become available. GD has considerable immunomodulating and antitumor activities in animal models, and is orally bioavailable.[19] Maitake D-fraction and crude maitake powder have demonstrated remarkable inhibition of metastasis in an immuno-competent mouse model, especially in the prevention of hepatic metastases, which in one series of experiments was reduced by 81 % (maitake powder) to 91 % (D-fraction).[50] GD has been shown to have a cytotoxic effect on human prostate cancer cells

(PC9) *in vitro,* possibly acting through oxidative stress, and causing 95 % cell death by apoptosis.[51]
The addition of vitamin C reduced the effective level of GD required. This potentiation of GD action by vitamin C and the chemosensitizing effect of GD on carmustine may well have significant clinical implications. Data against using prostate cancer cells *in vitro* have shown that the cytotoxic effects of the anticancer drug was significantly potentiated or enhanced with GD, possibly mediated through the inactivation of glyoxalase I, a vital detoxifying enzyme responsible for detoxification of cytotoxic metabolites/substances. This study suggests that GD may be useful with some anticancer drugs to improve the efficacy of ongoing clinical chemotherapy.
The maitake D-fraction is a relatively new compound and there are a number of clinical trials involving breast, prostate, lung, liver and gastric cancers underway in the US and Japan. Most of these are at an early clinical stage (Phase I/II).[48]
Early pilot studies from China published in abstract form involving 63 cancer patients reported a response rate (partial and complete) against solid tumors at 95% and for leukaemia (type not specified) 90%.[50] A recent Japanese non-randomised clinical study using the D-fraction has been carried out in a variety of advanced cancer patients (n=165). Patients took either oral D-fraction plus crude maitake powdered tablets, or D-fraction plus placebo tablets in addition to chemotherapy.[52] Tumor regression or significant symptomatic improvement was observed in 11 out of 15 advanced hepatocellular carcinomas with D-fraction plus maitake. When D-fraction plus maitake was combined with chemotherapy, the overall response rates rose by 12-28% when results from all cancer types were combined. As the authors of this study observed, chemotherapy itself could also significantly lower the immune system of patients. They reported that many of the patients recovered from the severe side effects caused by chemotherapy when D-fraction was given. In a similar manner to Lentinan, there are now increasing examples of synergism between maitake D-fraction and crude maitake powder and conventional chemotherapy.
The US Food and Drug Administration has approved GD for trial under an Investigational New Drug Application (IND) for patients with advanced cancer, and some US-based clinical trials are currently underway at various institutions.[52] No details are yet available. In conclusion, GD has few side effects and anecdotal clinical reports appear to suggest that it might alleviate some of the side effects of chemotherapy. The apparent success of crude maitake powder by oral administration in cancer therapy and immune stimulation would also support its suitability as a nutriceutical.

6.6 *Phellinus linteus*

Phellinus linteus (Berk. et Curt.) Teng has long been used in traditional Chinese medicine in the form of hot water extracts from fruit bodies.[53] In the last decade, the effects of these extracts for improving symptoms of digestive system cancers such as oesophageal, duodenal, colorectal, as well as hepatocellular, have been reported by practitioners of TCM. As with most of these mushroom polysaccharide extracts, tumor responses and/or symptomatic improvement (enhanced quality of life) have mainly been reported in combination with conventional chemotherapy in an adjuvant or neo-adjuvant setting.[53] In Korea, there has been a major national project involving industry, government, and academic laboratories using fermenter-cultivated mycelium from several *Ph. linteus* strains.[54] The major polysaccharide product has been approved as a medicine and has been manufactured by the Korean New Pharmaceutical Co. since 1997. Similar studies are also taking place in Japan by the Applied Microbiology Laboratory, Obiken Co. Ltd. Meshima, the hot water extracted polysaccharide product now manufactured by the Korean company, has become available in Japan for sale as a functional food (an immunity activation substance).
Water-soluble fractions from the fruit bodies and the mycelium of Ph. linteus have immune stimulating activity specifically enhancing B-lymphocytes.[55] Ikekawa[11] noted that water fractions of this mushroom did not inhibit the growth of implanted, solid-type Sarcoma tumors in mice. Conversely, it was found that this mushroom had the highest rate of inhibition against implanted Sarcoma 180 tumors in mice, resulting in 96.7 % inhibition.

[56] Mizuno[53] reported clinical studies at Seoul University. In post chemotherapy with 45 stomach cancer patients, *Ph. linteus* significantly enhanced NK activity resulting in recovery of T-3 and T-4 lymphocytes to near-normal conditions. Research by Song et al.[55] and Kim et al.[57] reported that the water extract from the mycelium induced B-lymphocytes and enhanced cellular immunity. Han *et al.* [58] found that *Ph. linteus* polysaccharides, when combined with the chemotherapeutic agent, adriamycin, increased effectiveness against tumor growth and metastasis, while the polysaccharides by themselves did not influence the growth of pulmonary cancers in mice. Apart from polysaccharides, a novel β-glycosidase inhibitor, called cyclophellitol, has been isolated from this species.[59]
Although there have been only few Phase II trials, tumor responses to the combination of *Ph. linteus* with conventional chemotherapy have been reported. Further trials with the *Ph. linteus* polysaccharide product (oral formulation) are ongoing.

6.7 *Agaricus brasiliensis* [1]

Agaricus brasiliensis (= *A. blazei* Murrill ss. Heinem.)[62] (the Royal Sun *Agaricus,* ABM, Himematsutake, Cogmelo de Deus), is one of the newly discovered medicinal mushrooms. During the 1980s and 1990s, it was demonstrated to be an immune system stimulant, promoting the body's natural defense mechanisms to fight a variety of infectious agents including cancers. Immunostimulating, antitumor and antimutagenic activities of *A. brasiliensis* extracts were investigated on different laboratory models.[60, 61, 63]

6.7.1 Anti-tumor polysaccharide fractions

A. brasiliensis is used by approximately 300,000-500,000 persons for the prevention of cancer and/or as an adjuvant with cancer chemotherapy drugs after the removal of a malignant tumor.[60, 61] The most intensively studied and thus ubiquitous group of antitumor active substances is comprised of polysaccharides, obtained mostly by extraction from fruit bodies. Fruit bodies contain water-soluble (1→3)-β-D-glucan with (1→6)-β-branches ($F1_0$-a-β),[60, 61] AB-P (glucan-protein complex with a Glc:protein ratio 34:30), protein-bound polysaccharide-proteoglucans AB-I, AB-II-b, AB-III-b, acidic heteroglucans, immunostimulating heteroglucans AG-2, AG-3, AG-6, and (1→6)-β-galactoglucans. All these fractions, with the exception of AB-I, AB-II-b, AB-III-b possess pronounced antitumor activity against Sarcoma 180.[60, 61]
The acid-treated fraction (ATF) of fruit bodies induces infiltration of the distant tumor with marked tumoricidal activity, as well as directly inhibiting tumor cell growth *in vitro* by inducing apoptotic processing. The ATF has no effect on normal splenic mononuclear mouse cells, indicating that it is selectively cytotoxic for the tumor cells. The ATF is composed of antitumor-active high-molecular (HM) and low-molecular (LM) weight fractions. The most active HM fraction, HM3-G, consists of primarily (1→4)-α-D-glucan with (1→6)-β branching.[60, 61] ATF constituents have a unique mode of action in that they have both a direct cytotoxic action on tumor cells and an indirect immunopotentiating action on tumor-bearing mice. The mechanism of action has not yet been elucidated. It is speculated that the inhibition of the distant tumor might be due to the increased migration of granulocytes, enhanced by the effect of extract injections on the primary tumor side. Another important trait of ATF is that it retained its full tumoricidal activity even when administrated orally.[60, 61]
In addition to LM fractions of ATF, other active low-molecular weight substances have been isolated from *A. brasiliensis*,[60, 61] namely, ABMK-22 and HACCP, which are able to activate the manifestation of the cytokine gene of the macrophages; they are suggested to possess a preventive effect on cancer.
Apart from water-soluble antitumor active polysaccharides, *A. brasliensis* fruit bodies contain water-insoluble polysaccharides and polysaccharide-protein complexes: FIV-2b (xyloglucan containing 9% of protein and 4%

[1] Full lists of literature sources are given in Didukh et al.[60, 61]

of uronic acid) and FIII-2b fractions (glucan-protein complex consisting of 43.3% of protein and 50.2% of carbohydrate).[60, 61] Interestingly, the data received on partial formolysis of FIII-2b led to the conclusion that the protein component is essential for antitumor activity.[60, 61]

The liquid cultured mycelium is also a source of biologically active substances, mainly polysaccharides. The antitumor active polysaccharide-protein complex, ATOM, inhibits Sarcoma 180, Ehrlich ascites carcinoma, Shionogi carcinoma 42, and Meth-A fibrosarcoma in mice on i.p. or p.o. administration. The tumor growth-inhibitory effect of ATOM is apparently due to immunological host-mediated mechanisms.[60, 61] The hot-water soluble fraction of the mycelia contains glucomannan with a main chain of (1→2)-β-D-mannopyranosyl and side chains of (1→3)-β-D-glucopyranosyl. The polysaccharide inhibits Sarcoma 180, and is completely different from the antitumor polysaccharide from *A. brasiliensis* fruit bodies.[60, 61]

A liquid medium filtrate separated after submerged cultivation of *A. brasiliensis* also contains an antitumor active mannan-protein complex, AB-FP.[60, 61]

Antitumor polysaccharides from fruit bodies, cultured mycelia, or produced extracellularly in a culture medium have different chemical structures. Polysaccharides from fruit bodies represent glucans with different types of glucose unit connections or heteroglucans; cultured mycelia contain glucomannans, and the mannan-protein complex is produced in a culture medium under submerged cultivation. Polysaccharides or proteoglucans, isolated from *A. brasiliensis* have shown marked tumoricidal activity in different experimental models (allogeneic, syngeneic mouse tumor models, double grafted tumor system). These models provide screening of anti-metastatic drugs and facilitate examination of the anti-metastatic effects and the mechanism of action of these biological response modifiers. Although it has been postulated that the inhibitory action of polysaccharides or proteoglucans results from enhancement of host immunity against tumor cell growth, no direct evidence has yet been obtained.

6.7.2 Anti-tumor lipid fractions

Antitumor active lipid fractions are not as numerous as the polysaccharide fractions. Ergosterol (a precursor of ergocalciferol) contained in fruit bodies has been shown to be highly effective against Sarcoma 180 and highly metastatic, drug-resistant mouse Lewis lung carcinoma (LLC) cells via oral or intraperitoneal administration.[60, 61] Besides pronounced antitumor activity, administration of ergosterol (both i.p. and p.o.) was devoid of side effects that are usually caused by cancer chemotherapy drugs (e.g., myelotoxicity, immunotoxcity, reduction in body weight). The antitumor activity of ergosterol may be due to the direct inhibition of angiogenesis induced by solid tumors. Besides ergosterol, six steroids were isolated from acetone extract of *A. brasiliensis* fruit bodies. Three of them effectively inhibit cell proliferation of cervical cancer cells (HeLa cells).[60, 61]

To date, a wide range of biological response modifiers derived from *A. brasiliensis* have been revealed. A clear understanding of their mechanisms of action at different levels is needed in order to apply them successfully to the treatment of human diseases. Besides substances with a relatively known mode of action, *A. brasiliensis* harbors biologically active compounds of unknown or poorly known structure possessing immunomodulatory, antiviral, antigenotoxic, antimutagenic, and antioxidant activities (see reviews by Didukh et al.[60, 61]). Thus, *A. brasiliensis* fruit bodies, cultured mycelia, and culture broth possess a wide range of biologically active compounds. However, the chemical nature of a number of these substances remains unknown, as well as the mechanisms of action of both known and unknown active fractions.

7 Dietary Supplements from Medicinal Mushrooms

As can be seen, culinary-medicinal higher Basidiomycetes contain numerous BASs, which can be successfully applied either for disease prevention and treatment. However, only a few mushroom-derived substances are classified as 'drugs' (e.g., Krestin, Lentinan, and Schizophyllan). They are used mostly as DSs because their

mechanims of action are poorly studied.

In the second half of the 20th century, mushroom-producing technologies grew enormously. In 2003, the value of world mushroom production was estimated at about $20 billion USD, which is almost the same as the value of coffee production,[64] Such increase in production of mushrooms is due to the fact that they present a unique combination of wholesome food, BRMs, and a source of DSs.

Although the variety of mushroom-derived DSs is great, they can be divided into several major classes: (1) naturally growing dried mushroom fruit bodies in the form of capsules or tablets; (2) artificially cultivated fruit body powders, hot water or alcohol extracts of these, or the same extract concentrates and their mixtures; (3) dried and pulverized preparations of the combined substrate, mycelium, and mushroom primordia after inoculation of edible semisolid medium (usually grains), and; (4) biomass or extracts from mycelium harvested from submerged liquid culture grown in a fermentation tank. When considering DSs, it should be noted that there are several debatable issues concerning DS regulations, safety control, etc.

8 Conclusions

Higher Basidiomycetes mushrooms are still far from thoroughly studied; even the inventory of their known species is incomplete. Only an insignificant percentage of the currently known species have been screened for biologically active substances and for cultivation. Nevertheless, the species studied so far represent a vast source of biologically active substances.

Out of the huge variety of activities, the most frequently sought, hence, the best known for the majority of screened species is anticarcinogenic activity. Mushroom-derived carcinogenic agents are of a varied chemical nature and have different modes of action. In fact, anticarcinogenic action can hardly be separated from immunomodulatory activity. These data indicate that mushroom-derived products might yield a wealth of commercially available anticarcinogenic agents.

Practical application of Basidiomycetes mushroom BASs is dependent not only on their unique properties but also on biotechnological availability. Isolation and purification of BAS from mushroom material is relatively simple and straightforward, and can be done with minimal effort.[2, 3] Nowadays, along with the cultivation of fruit bodies of edible and medicinal mushrooms, tank culture of mycelia of both edible and nonedible medicinal mushrooms can be used to derive active principles. Mycelia formed by growing pure cultures in submerged conditions are of constant composition, and submerged culture is the best technique for obtaining consistent and safe mushroom products.[5, 62]

However, the potential activity of a species should be evaluated carefully. For instance, in a test on antimutagenic activity of *A. Brasiliensis,* extracts derived from a single strain were not active, while those derived from a mixture of strains exhibited activity.[65] In other studies of antimutagenic/antigenotoxic activities, single strain-derived extracts were active.[9, 30] These conflicting results have not yet been explained. It is suggested that the antimutagenic component is not equally distributed between different lineages and/or that it is not equally present in the mushrooms at different periods of the year.[65] In tests on antimicrobial activity, small or large differences were commonly observed with respect to the ability of strains of the same species to produce antimicrobial compounds. The differences may reflect genetic divergence at the intraspecific level, but they may also be attributable, in part, to experimental error and flask-to-flask variation.[66] This supports the idea already emphasized by Mizuno[31] that higher Basidiomycetes properties should be considered together with crop management, stock, and processing, as these factors interfere with the components present in the mushrooms.[60, 61]

Another anticipated trait of Basidiomycetes BASs is the possibility of improvement of their qualities by chemical modification.

A large body of experimental and clinical evidence demonstrates benefits of Basidiomycetes DSs and drugs of mushroom polysaccharides for the following purposes: (1) prevention of oncogenesis by oral consumption of mushrooms or their preparations; (2) direct antitumor activity against various allogeneic and syngeneic tumors;

(3) immunopotentiation activity against tumors in conjuction with chemotherapy, and; (4) preventive effects on tumor metastasis. Most of the clinical evidence comes from the commercial polysaccharides Lentinan, PSK (Krestin), and Schizophyllan, but there are also impressive new data for polysaccharides from *Phellinus linteus, Flammulina velutipes, Hypsizygus marmoreus, Agaricus brasiliensis,* and others.

The biochemical mechanisms for mediating the biological activity of mushroom BASs are still not clearly understood. Existing data primarily concern polysaccharides. Polysaccharides from mushrooms do not attack cancer cells directly, but produce their antitumor effects by activating different immune responses in the host. The antitumor action of polysaccharides requires an intact T-cell component; their activity is mediated through a thymus-dependent immune mechanism.[1] Mushroom polysaccharides are known to stimulate natural killer cells, T-cells, B-cells, and macrophage-dependent immune system responses. The immunomodulating action of mushroom polysaccharides is especially valuable as a means of prophylaxis, a mild and non-invasive form of treatment, prevention of metastatic tumors, and as a co-treatment with chemotherapy.

All in all, mushroom-derived compounds seem to be more effective in some applications (wound healing, immunotherapy, and biotherapy) than currently used chemical agents. In view of rapidly growing popularity of mushroom-based products, including numerous products of Agaricaceae species, further elucidation of active principles, mechanisms of action, and their possible adverse effects is crucial for implementing safety measures for public health.

References

[1] Borchers AT, Stern TS, Hackman RM, *et al.* Mushrooms, tumours and immunity. Proc. Soc. Exp. Biol. Med. 1999, 221:281-293

[2] Mizuno T. A development of antitumor polysaccharides from mushroom fungi. Foods Food Ingred. J. Jpn. 1996, 167:69-85.

[3] Mizuno T. The extraction and development of antitumor-active polysaccharides from medicinal mushrooms in Japan (Review). Int. J. Med. Mush. 1999, 1: 9-29.

[4] Ooi VEC, Liu F. A review of pharmacological activities of mushroom polysaccharides. Int. J. Med. Mush. 1999, 1: 195-206.

[5] Reshetnikov SV, Wasser SP, Tan KK.. Higher Basidiomycota as source of antitumor and immunostimulating polysaccharides. Int. J. Med. Mushr. 2001, 3: 361-394.

[6] Rowan NJ, Smith JE, Sullivan R. Immunomodulatory activities of mushroom glucans and polysaccharide-protein complexes in animals and humans (a review). Int. J. Med. Mush. 2003, 5: 95-110.

[7] Smith JE, Sullivan R, Rowan NJ. 2003.The role of polysaccharides derived from medicinal mushrooms in cancer treatment programs: Current perspectives (Review). Int. J. Med. Mush. 2003, 5: 217-234.

[8] Wasser SP, Weis A. Medicinal properties of substances occurring in higher Basidiomycetes mushrooms: current perspectives (Review). Int. J. Med. Mush. 1999, 1: 31-62.

[9] Hobbs ChR. Medicinal mushrooms: an exploration of tradition, healing and culture. 1995, Botanica Press, Santa Cruz, CA.

[10] Wasser SP, Weis A. Reishi mushroom [*Ganoderma lucidum* (Curt. : Fr.) P. Karst.]. - In: E. Nevo [ed.]. Medicinal mushrooms. 1997. Peledfus, Haifa, Israel.

[11] Ikekawa T. Beneficial effects of edible and medicinal mushrooms on health care. - Int. J. Med. Mush. 2001, 3: 291-298.

[12] Kirk PM, Cannon PF, David JC, *et al.* Ainsworth & Bisby's Dictionary of the Fungi. 9th edn. 2001, CAB International, Wallingford.

[13] Hawksworth D.L. Mushrooms: the extent of the unexplored potential. Int. J. Med. Mush. 2001, 3: 333-340.

[14] Chang ST, Miles PG. Mushroom biology - a new discipline. Mycologist. 1992, 6:64-65.

[15] Taylor JW, Geiser DM, Burt A, *et al.* The evolutionary biology and population genetics underlying strain-typing. Clin. Microbiol. Rev. 1999, 12: 126-146.

[16] Sharon N, Lis H. Carbohydrates in cell recognition. Sci Amer, Jan., 1993,74-81.

[17] Gorin PAJ, Barreto-Berger E. The chemistry of polysaccharides of fungi and lichens. - In: G.O. Aspinall [ed.]. The polysaccharides. Vol. 2. Pp. 365-409. 1983, Academic Press, Orlando, FL.

[18] Hobbs ChR. The chemistry, nutritional Value, immunopharmacology, and safety of the traditional food of medicinal split-gill fungus *Schizophyllum commune* Fr.:Fr. (Schizophyllaceae). A literature review. Int. J. Med. Mush. 2005, 1-2 [In press].

[19] Zhuang C, Wasser SP. Medicinal value of culinary-medicinal Maitake mushroom *Grifola frondosa* (Dicks.:Fr.) S.F. Gray (Aphyllophoromycetideae). Review. Int. J. Med. Mush. 2005, 6: 287-313.

[20] Zhuang C, Mizuno T, Shimada A, *et al.* Antitumor protein-containing polysaccharides from a Chinese mushroom Fengweigu or Houbitake, *Pleurotus sajorcaju* (Fr.) Sing. Biosci. Biotechnol. Biochem. 1993, 57: 901-906.

[21] Mizuno T, Yeohlui P, Kinoshita T, *et al.* Antitumor activity and chemical modification of polysaccharides from Niohshimeji mushroom, *Tricholoma giganteum.* Biosci. Biotechnol. Biochem. 1996, 60: 30-33.

[22] Yadomae T. Structure and biological activities of fungal β-1,3-glucans. Yakugaku Zasshi. 2000, 120: 413-431.

[23] Gao QP, Jiang RZ, Chen HQ, *et al.* Characterization and cytokine stimulating activities of heteroglycans from *Tremella fuciformis.* Pl. Med. 1996, 62: 297-302.

[24] Karácsonyi S, Kuniak L. Polysaccharides of *Pleurotus ostreatus:* Isolation and structure of pleuran, an alkali-insoluble β-D-glucan. Carbohydr. Polym. 1994, 24: 107-111.

[25] Paulik Š, Švrčec M, Mojžišóvá J, *et al.* The immunomodulatory effect of the soluble fungal glucan (*Pleurotus ostreatus*) on delayed hypersensitivity and phagocytic ability of blood leucocytes in mice. J. Vet. Med. B. 1996, 43: 129-135.

[26] Hirata A, Itoh W, Tabata K, *et al.* Anticoagulant activity of sulfated schizophyllan. - Biosci. Biotechn. Biochem. 1994, 58: 406-407.

[27] Itoh W, Sugawara I, Kimura S, *et al.* Immunopharmacological study of sulfated schizophyllan (SPG) I: Its action as a mitogen and anti-HIV agent. Int. J. Immunopharmacol. 1990, 12: 225-234.

[28] Kidd P. The use of mushroom glucans and proteoglycans in cancer treatment. - Altern. Med. Rev. 2000, 5: 4-27.

[29] Matsushita K, Kuramitsu Y, Ohiro, *et al.* Combination therapy of active hexose correlated compound plus UFT significantly reduces the metastasis of rat mammary adenocarcinoma. Anti Cancer Drugs. 1998 9: 343-350.

[30] Yap AT, Ng ML. Immuno-potentiating properties of lentinan (1→3)-β-D-glucan extracted from culinary-medicinal shiitake mushroom *Lentinus edodes* (Berk.) Singer (Agaricomycetideae). Int. J. Med. Mush. 2003, 5: 239-258.

[31] Mizuno T. Bioactive biomolecules of mushrooms: food functions and medicinal effects of mushroom fungi. Food. Rev. Int. 1995, 11: 7-21.

[32] Hobbs ChR. Medicinal value of *Lentinus edodes* (Berk.) Sing. (Agaricomycetideae). A literature review. Int. J. Med. Mush. 2000, 2: 287-302.

[33] Ngai PHK, Ng TB. Lentin, a novel and potent antifungal protein from shitake mushroom with inhibitory effects on activity of human immunodeficiency virus-1 reverse transcriptase and proliferation of leukaemia cells. Life Sci. 2003, 73: 3363-3374.

[34] Xu GM. Phase I clinical reports of PSP capsules. - In: PSP International symposium, Anthology of theses and abstracts, pp. 179-182. 1993.

[35] Sun T, Zhu Y. The effect of PSP on immune function and living quality in patients receiving chemotherapy for gynaecological malignances. - In: Q. Yang [ed.]. Advanced Research in PSP, pp. 308-309. 1999, Hong Kong Association for Health Care Ltd., Hong Kong.

[36] Sun Z, Yang QY, Fei HL. The ameliorative effect of PSP on the toxic and side eactions of chemo- and radiotherapy of cancers. - In: Q. Yang [ed.]. Advanced Research in PSP, pp. 304-307. 1999, Hong Kong Association for Health Care Ltd., Hong Kong.

[37] Shiu WCT. A clinical study of PSP on peripheral blood counts during chemotherapy. Physiol. Res. 1992, 6: 217-218.

[38] Stephens LC, Ang KK, Schulthesis TE. Apoptosis in irradiated murine tumours. Rad. Res. 1991, 127: 308.

[39] Jin TY. Toxicological research on Yun Zhi polysaccharopeptide (PSP). - In: Q. Yang [ed.]. Advanced Research in PSP, pp. 76-79. 1999, Hong Kong Association for Health Care Ltd., Hong Kong.

[40] Zhong BZ, Zhon YG, Zhou LF, *et al.* Genetic toxicity test of Yun Zhi polysaccharide (PSP). - In: Q. Yang [ed.]. Advanced Research in PSP, pp. 285-294. 1999, Hong Kong Association for Health Care Ltd., Hong Kong.

[41] Kimura Y, Tojima H, Fukase S. Clinical evaluation of schizofilan as assistant immunotherapy in treatment of head and neck cancer. Acta Otolaryn. 1994, 511: 192-195.

[42] Nakano T, Oka K, Hanba K, *et al.* Intratumoral administration of sizofilan activates Langerhans cells and T-cell infiltration in cervical cancer. Clin. Immun. Immunopath. 1996,79: 79-86.

[43] Chen AW, Miles PG. Biomedical research and the application of mushrooms nutriceuticals from *Ganoderma lucidum.* - In: D.J. Royse [ed.]. Mushroom Biology and Mushroom Products, pp. 161-176. 1996, Pennsylvania State University, University Park.

[44] Lee SS, Wei YH, Chen CH, *et al.* Antitumour effects of *Ganoderma lucidum.* J. Chin. Med. 1995, 6: 1-12.

[45] Zhou Sh, Gao Y, Chen G, *et al.* A phase I/II study of a *Ganoderma lucidum* (Curt. : Fr.) P. Karst. (Ling Zhi, Reishi mushroom) extract in patients with chronic hepatitis B. Int. J. Med. Mush. 2002, 4: 321-328.

[46] Gao Y. The miracle herb scientific reports of *Ganoderma.* 2000, Yuangizai Publisher, Taipei.

[47] Gao Y, Zhou S, Chen G, *et al.* A Phase I/II study of a *Ganoderma lucidum* extract (Ganoply) in patients with advanced cancer. Int. J. Med. Mush. 2002, 4: 207-214.

[48] Stott K, Mohammed C. Cultivation of the edible and medicinal mushroom *Grifola frondosa* (Dicks. : Fr.) S.F. Gray (Maitake)-relevance of literature to production in Australia (review). Int. J. Med. Mush. 2003, 5: 199-216.

[49] Mizuno T, Zhuang C. Maitake, *Grifola frondosa:* pharmacological effects. Food Rev. Int. 1995, 11: 135-149.

[50] Nanba H. Activity of Maitake D-fraction to inhibit carcinogenesis and metastasis. Ann. New York Acad. Sci. 1995, 768: 243-245.

[51] Minato KI, Mizuno M, Kawakami S, *et al.* Changes in immunomodulating activities and contents of antitumor polysaccharides during the grows of two medicinal mushrooms, *Lentinus edodes* (Berk.) Sing. and *Grifola frondosa* (Dicks. : Fr.) S.F. Gray. Int. J. Med. Mush. 2001, 3: 1-7.

[52] Nanba H. Effect of Maitake D-fraction on cancer prevention. Ann. New York Acad. Sci. 1997, 833: 204-207.

[53] Mizuno M, Shiomi Y, Minato K, *et al.* Fucogalactan isolated from *Sarcodon asparatus* elicits release of tumor necrosis factor-alpha and nitric oxide from murine macrophages. - Immunopharmacol. 200, 46: 113-121.

[54] Aizawa K. Antitumour-effective mushroom meshimakoku *Phellinus linteus.* 1998, Gendai-Shorim,Tokyo.

[55] Song KS, Cho SM, Lee JH, *et al.* B-lymphocytestimulating polysaccharide from mushroom *Phellinus linteus.* Chem. Pharm. Bull. 1995, 43: 2105-2108.

[56] Mizuno T. Development of an antitumor biological response modifier from *Phellinus linteus* (Berk. et Curt.) Teng (Aphyllophoromycetideae) (Review). Int. J. Med. Mushr. 2000, 2: 21-33.

[57] Kim HM, Han SB, Oh GT, *et al.* Stimulation of humoral and cell mediated immunity by polysaccharides from mushroom *Phellinus linteus.* Int. J. Immunopharmacol. 1996, 18: 295-303.

[58] Han SB, Lee CW, Jeon YJ, *et al.* The inhibitory effect of polysaccharides isolated from *Phellinus linteus* on tumor growth and metastasis. Immunopharmacol. 1999, 41: 157-164.

[59] Atsumi S, Umezawa K, Iinuma H, *et al.* Production, isolation and structure determination of a novel beta-glucosidase inhibitor, cyclophellitol, from *Phellinus* sp. J. Antibiot. (Tokyo). 1990, 43: 49-53.

[60] Didukh MYa, Wasser SP, Nevo E. Medicinal value of species of the family Agaricaceae Cohn (higher Basidiomycetes): current stage of knowledge and future perspectives. Int. J. Med. Mush. 2003, 5: 133-152.
[61] Didukh M, Wasser SP, Nevo. E. Impact of the family Agaricaceae (Fr.) Cohn on nutrition and medicine. 2004, Rugggel A.R.G. Gantner Verlag K.-G.
[62] Wasser SP, Didukh MYa, Amazonas MALA, *et al.* Is widely cultivated culinary-medicinal Royal Sun Agaricus (the Himematsutake mushroom) indeed *Agaricus blazei* Murrill? Int. J. Med. Mush. 2002, 4: 267-290.
[63] Menoli RC, Mantovani MS, Ribeiro LR, *et al.* Antimutagenic effects of the mushroom *Agaricus blazei* Murrill extracts on v79 cells. Mutat. Res. 2001, 496: 5-13.
[64] Chang ST. A 40-year journey through bioconversion of lignocellulosic wastes to mushrooms and dietary supplements. Int. J. Med. Mush. 2001, 3:299-311.
[65] Delmanto RD, de Lima PLA, Sugui MM, *et al.* Antimutagenic effect of *Agaricus blazei* Murrill mushroom on the genotoxicity induced by cyclophosphamide. Mutat. Res. 2001, 496: 15-21.
[66] Suay I, Arenal F, Asensio FJ, *et al.* Screening of Basidiomycetes for antimicrobial activities. Antonie van Leewenhoek. 2000, 78: 129-139.