

Mycotherapy of Cancer: An Update on Cytotoxic and Antitumor Activities of Mushrooms, Bioactive Principles and Molecular Mechanisms of their Action

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Abstract: Mycotherapy is defined as the study of the use of extracts and compounds obtained from mushrooms as medicines or health-promoting agents. The present review updates the recent findings on anticancer/antitumor agents derived from mushroom extracts and their metabolites. The increasing number of studies in the past few years revealed mushroom extracts as potent antitumor agents. Also, numerous studies were conducted on bioactive compounds isolated from mushrooms reporting the heteropolysaccharides, β -glucans, α -glucans, proteins, complexes of polysaccharides with proteins, fatty acids, nucleoside antagonists, terpenoids, sesquiterpenes, lanostanoids, sterols and phenolic acids as promising antitumor agents. Also, molecular mechanisms of cytotoxicity against different cancer cell lines are discussed in this review. Findings with *Antrodia camphorata* and *Ganoderma lucidum* extracts and isolated compounds are presented, as being the most deeply studied previously.

Keywords: Antitumor properties, compounds, extracts, molecular mechanisms, mushrooms.

INTRODUCTION

We defined the mycotherapy as the study of the use of extracts and compounds obtained from mushrooms as medicines or health-promoting agents. Mycotherapy of cancer is a relatively novel and promising scientific field, which deals with anticancerogenic agents derived from mushrooms. The term "mushroom" will be used for a medicinal fruiting body belonging to higher fungi.

Mushrooms are important dietary components in some cultures, some of them being traditionally used for the treatment of various conditions including cancer [1]. Identification of active principles in extracts, i.e. isolation of new antitumor substances from mushrooms became a matter of great importance, taking into account the complexity and distribution of various cancer types in population worldwide [2]. A great variety of compounds and complex fractions were isolated and/or purified from medicinal as well as from some edible mushrooms, with special importance regarding anticancer and cancer preventive activity [1]. Amongst the broad spectrum of constituents in medicinal and edible mushrooms, these activities are mainly attributed to polysaccharides (3-6), various polysaccharide-protein/peptide complexes [2], lectins [4, 7] terpenoids [8, 9], sterols [10, 11], etc. Special interest is devoted to polysaccharides from the fungal cell walls because of their immunomodulatory activity, being

biological response modifiers (BRM) that prevent carcinogenesis, but they also show direct anticancer effects and prevent tumor metastasis [12].

MUSHROOM EXTRACTS

Mushroom extracts are increasingly consumed as dietary supplements because of their properties, including the enhancement of immune function and antitumor activity [13]. It is well established that mushroom extracts contain a wide variety of compounds such as polysaccharides, protein, fiber, lectins and polyphenols, each of which may have pharmacological effects. More than 30 species of medicinal mushrooms are currently identified as sources of biologically active metabolites with potential anti-cancer properties [14]. The properties and mechanisms of mushroom extracts that have been recently evaluated are outlined in Table 1.

Ganoderma lucidum, commonly known as Lingzhi or Reishi has been traditionally administered throughout Asia for centuries as a cancer treatment [15]. The pharmacological activities of *G. lucidum*, particularly its intrinsic immunomodulating and antitumor properties, have been well documented. Several studies have demonstrated that various *G. lucidum* extracts interfere with cell cycle progression, induce apoptosis and suppress angiogenesis in human cancer cells and thus act as anticancer agents [16-18]. Recently, Suarez-Arrayo *et al.*, [19] evaluated the antitumor effect of a commercially available extract consisting of Reishi fruiting body and cracked spores and elucidated its potential mechanism *in vivo*. Mice injected with Inflammatory Breast Cancer (IBC) cells treated with Reishi for 13 weeks show tumor growth

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Table 1. Antitumor Activity of Mushroom Extracts⁰

Mushroom species	Active compounds/ extracts/fractions	Cell type	Activity/results	Mechanism of action	Reference
<i>Amauroderma rude</i>	hot water extract	human breast cancer cell lines MT-1, MDA-MB231, 4T1, MDA-MB468, MCF7		induction of apoptosis	[32]
<i>Antrodia camphorata</i>	cold water extract	human breast cancer cell lines MDA-MB-453 and BT-474	IC ₅₀ values 220 and 240 µg/ml for MDA-MB-453 and BT-474 cells respectively	inhibition of cell growth and induction of apoptosis through the induction of ROS, depletion of HER-2/neu, and disruption of the PI3K/Akt signaling pathway	[23]
	fermentation culture	human ovarian carcinoma (SKOV-3) cells	at 240 µg/mL colony formation was reduced by over 90% compared to the untreated control cells	modulation of HER-2/neu signaling pathway	[24]
<i>Antrodia cinnamomea</i>	ethanolic extract	murine leukemia WEHI-3 cells		inhibition of the proliferation and migration of WEHI-3 cells, MMP-9 protein expression reduction	[20]
<i>Clitocybe alexandri</i>	ethanolic extract	human non-small lung cancer NCI-H460		S-phase cell cycle arrest	[30]
<i>Ganoderma lucidum</i>	commercially available extract Reishi-Max GLP™	mice injected with IBC cells	reduction of tumor growth and weight by 45%	reduction in expression at both the gene and protein level of important molecules in the PI3K/Akt/mTOR and MAPK signaling pathways	[19]
<i>Hericium erinaceus</i>	50% ethanol extract	CT-26 mouse colon carcinoma cell	42% inhibition at 1 mg/mL	suppression of ERK and JNK activation, inhibition of lung metastasis <i>in vivo</i>	[31]
<i>Lentinula edodes</i>	aqueous extract	human tumor cell lines laryngeal carcinoma (Hep-2), cervical adenocarcinoma (HeLa)	IC _{50%} =0.46-1.03	apoptosis induction	[13]
	ethanol extract	HepG2 human hepatocellular carcinoma		apoptosis induction through caspase-3 and -8 death receptor pathway	[22]
<i>Lignosus rhinocereus</i>	cold water extract	human breast carcinoma MCF-7	IC ₅₀ =96.7 µg/mL		[23]
		human lung carcinoma A549	IC ₅₀ =466.7 µg/mL		
<i>Pleurotus pulmonarius</i>	hot water extract	human liver cancer cell lines Huh7, Hep 3B, SMMC-7721 and HepG2		inhibition of VEGF-mediated autocrine regulation of PI3K/AKT	[1]
<i>Pleurotus sajor-caju</i>	aqueous extract	human tumor cell lines laryngeal carcinoma (Hep-2) and cervical adenocarcinoma (HeLa)	IC _{50(%)} =0.25-0.78	apoptosis induction	[13]
<i>Ramaria flava</i>	ethanol extract	BGC-803, NCI-H520, MDA-MB-231	IC ₅₀ ranged from 66.54 to 743.99 µg/ml for the MDA-MB-231 and BGC-803 cell lines respectively		[20]
<i>Suillus collinitus</i>	methanolic extract	MCF-7 human breast cancer cell line		increases p53 expression and causes apoptosis	[28]

(Table 1) contd....

Mushroom species	Active compounds/ extracts/fractions	Cell type	Activity/results	Mechanism of action	Reference
<i>Suillus luteus</i>	methanolic extract	MCF-7 human breast cancer cell line, NCI-H460 human non-small cell lung cancer, AGS human gastric cancer, HCT-15 human colon cancer	GI ₅₀ values ranged from 17.75 to 32.25 µg/ml for the HCT-15 and MCF-7 cells, respectively	increases p53 expression and causes apoptosis	[29]
<i>Tricholoma giganteum</i>	80% ethanol extract	Ehrlich ascites carcinoma		apoptosis induction	[27]

and weight reduction by 45%. Furthermore, reduced expression of E-cadherin, mammalian target of rapamycin (mTOR), human eukaryotic translation initiation factor 4G (eIF4G), p70 ribosomal protein S6 kinase (p70S6K) and activity of extracellular regulated kinase (ERK 1/2) were observed. These results confirmed that Reishi is a potential natural therapeutic for breast and other cancers that selectively affects gene and protein expression and therefore, activity of molecules involved in cancer cell function [19].

Lentinula edodes, the shitake mushroom, has been used for traditional foods and medicine in Asia for over 2000 years and today it is also popular in many western countries [20]. It is the second most popular edible mushroom in the global market, and its importance being attributed to both nutritional value and medical application [21]. *L. edodes* has been extensively investigated for their medicinal benefits, most notably antitumor properties [21]. The low temperature (<50°C) aqueous extract of this mushroom significantly decreased proliferation of the human tumor cell lines laryngeal carcinoma (HEp-2) and cervical adenocarcinoma (HeLa), with IC₅₀% values ranging from 0.46% to 1.03% [13]. Yukawa *et al.*, [22] examined the antiproliferative effect of *L. edodes* mycelia extract on HepG2 human hepatocellular carcinoma cells. The extract efficaciously induced apoptosis of tested type of cells through the caspase -3 and -8 death receptor pathways [22].

Antrodia (camphor tree mushroom) is a genus of mushrooms in the family Fomitopsidaceae. These mushrooms are highly valued in Taiwan [15]. The fermented culture broth of *A. camphorata* has been shown to promote growth inhibition and apoptotic induction of HER-2/neu-overexpressing human breast cancer cells MDA-MB-453 and BT-474 cells with IC₅₀ values of 220 and 240 µg/ml, respectively [23]. Among positive regulators of proliferation, HER-2/neu was found to be a complement protooncogene that regulates tumor progression in a variety of human cancers, including breast cancer. Anti-HER-2/neu receptor therapy has been increasingly recognized as a potential treatment of HER-2/neu-overexpressing breast and ovarian cancer patients, as supported by recent advances in this direction [24]. *A. camphorata* extract significantly down-regulated the expression of cyclin D1, cyclin E and cyclin-dependent kinase (CDK) followed by the suppression of PI3K/Akt, and their downstream effectors GSK-3β and β-catenin in tested cancer cell lines. Additionally, *A. camphorata* extract efficiently inhibited human ovarian carcinoma cell (SKOV-3) proliferation

(IC₅₀=196 µg/ml) as a result from ROS (reactive oxygen species) generation, loss of HER-2/neu activation and suppression of its downstream signaling, including the PI3K/Akt cascade [24]. *A. camphorata* induced apoptosis in SKOV-3 cells which was associated with internucleosomal DNA fragmentation and caspase-3 and caspase-9 activation, down-regulation of Bcl2 expression and up-regulation of Bax expression [24].

Ethanol extract of *A. cinnamomea* significantly inhibited the proliferation and migration of murine leukemia cell line WEHI-3 through the reduction of p-ERK1/2, p-Akt and MMP-9 protein expression and the upregulation of p21 and p27 protein expression [20]. Using an allograft tumor model in which BALB/c mice were injected with WEHI-3 leukemia cells, Liu *et al.*, [20] also found that this extract exhibited antitumor activity by significantly decreasing the average weights of liver, spleens and tumor.

Cultivation of the *Pleurotus* (oyster mushroom) species has increased greatly throughout the world during the last few decades. Today they represent the 3rd largest cultivated mushroom in the world [25]. Xu *et al.*, [1] reported that exposure of liver cancer cells to hot water extract of *Pleurotus pulmonarius* not only significantly reduced the *in vitro* and *in vivo* cancer cell proliferation and invasion, but also enhanced the drug-sensitivity to the chemotherapeutic drug Cisplatin [26]. The exhibited effect was mediated by the inhibition of autocrine vascular endothelial growth factor (VEGF)-induced PI3K/AKT signaling pathway. Aqueous extract of *P. sajor-caju* demonstrated inhibitory activity against the proliferation of Hep-2 and HeLa cell lines with IC₅₀% values ranged from 0.23% to 1.21% depending on the temperature used for extraction [13].

Tricholoma giganteum of the family Tricholomataceae, a wild edible mushroom, is most conspicuous in the tropical region during rainy season [27]. The 80% ethanol extract of *T. giganteum* exhibited significant potency against Ehrlich's ascites carcinoma (EAC) through induction of apoptogenic signal. It was observed that 80% ethanol extract enhanced the levels of pro-apoptotic proteins p53 and p21. Additionally, pro-apoptotic gene Bax was up-regulated, while no significant change in the expression of Bcl-2 was observed ensuing in decrease of the Bax/Bcl-2 ratio [27].

Suillus collinitus is an edible mycorrhizal mushroom found in European pine forests, belonging to the genus *Suillus* in the Suillaceae family. Vaz *et al.*, [28] studied the effect

of *S. collinitus* methanolic, ethanolic and boiled water extracts on the growth of four human tumor cell lines: MCF-7 (breast), NCI-H460 (non-small cell lung cancer), AGS (gastric) and HCT-15 (colon). The methanolic extract was the most potent in tested cell lines with concentrations that caused 50% of cell growth inhibition (GI_{50}) ranging from 25.2 to 103.2 $\mu\text{g/ml}$ for the MCF-7 and HCT-15 cells, respectively. The boiled water extract did not show any effect on the tested cell lines at the tested concentrations (up to 400 $\mu\text{g/ml}$). The results of flow cytometry showed that the methanolic extract induced G1 arrest in MCF-7 cells, with a concomitant decrease in the percentage of cells in the S phase. The apoptotic machinery was associated with strong increase in the levels of p53 and p21, decrease in the levels of XIAP and Bcl-2 and a concentration dependent increase in the levels of cleaved poly (ADP-ribose) polymerase (PARP). Additionally, methanolic, ethanolic and boiled water extracts of *S. luteus* were subjected to antitumor evaluation in the same human tumor cell lines (NCI-H460, MCF-7, HCT-15 and AGS). The methanolic extract was the most potent with GI_{50} values ranging from 17.75 to 32.25 $\mu\text{g/ml}$ for HCT-15 and MCF-7 cells, respectively. In HCT-15 cells, an increase in the levels of p53 was detected, but no alterations in some of the proteins transactivated by p53 (p21 or Bax) were found. Also, methanolic extract caused an increase in the cellular levels of p-H2A.X, which suggests DNA damage [29].

Lignosius rhinocerus, the tiger milk mushroom, belongs to the Polyporaceae family and is one of the most important medicinal mushrooms, used by natives in Southeast Asia and southern China. The cold water extract of *L. rhinocerus* exhibited significant antiproliferative activity against the breast cancer cell line MCF-7 and lung cancer cell line A549 [23]. The high-molecular-weight fraction of the extract was the one responsible for the observed activity against the two cancer cell lines tested, while the low-molecular-weight fraction was devoided of antiproliferative activity. The mechanism of growth inhibition was apoptosis induction [23].

Clitocybe alexandri is an edible saprophytic Basidiomycota mushroom belonging to the family of Tricholomataceae [15]. Ethanolic extract of *C. alexandri* has been demonstrated to possess cytotoxic and anti-proliferative activity towards a lung human tumor cell line (NCI-H460 cells). Flow cytometric analysis showed that the extract induced an S-phase cell cycle arrest and increased the percentage of apoptotic cells. Furthermore, an increase in the levels of (PARP) cleavage, caspase-3 cleavage and p53 were observed in the NCI-H460 cells treated with this extract [30].

The edible medicinal mushroom *Hericium erinaceus* commonly known as Lion's Mane has attracted great attention owing to its antitumor and immunomodulatory effects [15]. Kim *et al.*, [31] found that hot water and microwaved 50% ethanol extracts of *H. erinaceus* effectively inhibited the proliferation and invasion of CT-26 colon carcinoma cells, as well as the metastasis and invasion of CT-26 cells to the lungs by 66 and 69%, respectively. Their anti-invasive and antimetastasis activities might be ascribed to the suppression of extracellular matrix (ECM) degrading protease expression such as that of matrix metalloproteinases MMP-2 and MMP-9 and urokinase-type plasminogen activator (u-

PA) via down-regulating the upstream MAPK signaling pathway. Dietary administration of *H. erinaceus* extracts in BALB/c mice transplanted with CT-26 cancer cells reduced the formation of tumor nodules in the lung by 50 and 55% respectively, and prevented increase in lung weight caused by cancer cell metastasis [31].

Amauroderma rude, or bloody mushroom, of the family Ganodermataceae, is newly described and poorly studied fungus. Jiao *et al.*, [32] studied the effect of hot water extract of *A. rude* on invasive and metastatic breast cancer cell lines MT-1, MDA-MB231 and 4T1, less invasive breast cancer cell line MDA-MB468 and benign breast cell line MCF7. No cancer cells could survive after treatment with 600 $\mu\text{g/ml}$ of *A. rude* extract. Also, low concentration of this extract (50 $\mu\text{g/ml}$) exerted a significant activity in inducing cell apoptosis as compared with the control (29% vs. 1.8%). Furthermore, the antitumor effect of *A. rude* extract was assessed *in vivo* in regular mice injected with 4T1 cells. Locally administration of extract significantly inhibited the growth of tumor mass. Suppression of c-myc expression appeared to be associated with these effects [32].

The reported results are mainly from *in vitro* studies and as a hint of the potential therapeutic value they mark the very first steps in preclinical screening. Often they are also used as advertising arguments for traditional medicines [14].

BIOACTIVE PRINCIPLES OF MUSHROOMS

Polysaccharides

Polysaccharides are biopolymers, consisted of monosaccharide units linked through glucoside bonds with high ability to carry biological information due to numerous structural variations. Many of them are shown to exert *in vitro* antiproliferative/cytotoxic as well as antitumor activity in animal models [2]. Polysaccharides are still mainly used as an adjuvant therapy in cancer treatment [3]. Several structural features are known to affect these biological activities, primarily specific structural features, molecular weight, backbone linkage, degree of branching, side-chain units, as well as monosaccharide composition [6].

Various mechanisms may determine the mode of action of polysaccharides on cancer cells, but these compounds mainly induce activation and modification of different immune responses in the host, rather than directly attacking cancer cells [3, 33]. These polysaccharides bind to serum specific proteins leading to activation of macrophages, T-helper, natural killer (NK) cells, and other effector cells and thereby increase the production of antibodies, interleukins such as IL-1 and IL-2, and interferon γ [34]. Such activities are strongly influenced by molecular mass, branching configuration, conformation and chemical modification of the polysaccharides [4]. Knowing that a specific pharmacological effect is dependent on structural features, apart from naturally occurring polysaccharides, many of the bioactive polysaccharides are produced semi-synthetically via chemical or enzymatic modification of the parent molecules [2]. Most often, such modifications are carried out in order to improve their water solubility by Smith degradation (oxydo-reducto-hydrolysis), formolysis and carboxymethylation [33].

Mushroom polysaccharides that exert antitumor activities have been isolated from fruiting bodies, cultured mycelia and culture filtrates of Basidiomycetes [4]. Traditional isolation of polysaccharides as active principles includes various techniques such as boiling water, alkali or acid extractions, alcohol precipitation, followed by fractionation on a sepharose and usually sephadex column chromatography [3, 35]. By various chemical modifications such as periodate oxidation, Smith degradation, partial acid hydrolyzation and methylation analysis of a polysaccharide in combination with spectroscopic methods such as Fourier Transform Infrared (FT-IR), and 1-D and 2-D Nuclear Magnetic Resonance (NMR) techniques, it is possible to determine backbone structure with specific configuration and/or linkage position [4, 34, 35].

Nowadays, considering backbone structure, it is known that glucose residues linked by β -(1 \rightarrow 3)-glycosidic bonds with attached β -(1 \rightarrow 6) branch points exhibit strong antitumor and immunostimulating properties [4]. In the following overview, besides well-known and commercially available products of a polysaccharide source, such as schizophyllan, lentinan and grifolan, a brief report on other polysaccharides that are currently investigated for their potential use in mycotherapy of cancer, will be given.

Heteropolysaccharides

Low molecular weight polysaccharide (LMW-ABP) isolated from the fruiting bodies of *Agaricus blazei* (syn. *A. brasiliensis*) inhibited tumor growth and angiogenesis *in vivo* by down-regulating VEGF. It was further shown that this polysaccharide inhibited tumor cell adhesion via depressing E-selectin protein expression and also NF- κ B protein expression, so it may be a promising therapeutic agent against E-selectin mediated neoplasm metastasis [36]. From the fruiting bodies of the same species, an heteropolysaccharide (MW 4.2×10^5 Da) consisting of glucose, mannose and galactose in a molar ratio 1:1:1 was purified, and cytotoxicity was tested in osteosarcoma HOC as well as in normal human osteoblast cells. This heteropolysaccharide showed significant inhibitory effect in HOS cell line by induction of apoptosis, whilst showing no or little toxicity in a normal cell line [37].

A heteroglucan polysaccharide isolated from *Astraeus hygrometricus* induced tumor regression in Dalton lymphoma bearing mice, and the possible mechanism, the elevation of macrophage and NK cells activation, with increase in Th1 cytokine production [38], was suggested.

Two heteropolysaccharides (MW 2 kDa and 40-70 kDa) consisted mainly of glucose, mannose, xylose, and fructose, were obtained by size-exclusion chromatography from medicinal mushroom *Agaricus bisporus*, and tested in MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide) assay for cytotoxic activity in four human cancer cell lines. Both polysaccharide fractions were active in human breast adenocarcinoma cell line MCF-7, while the activity in other tested cell lines was low [39]. In the same study, Jeong *et al.*, [39] exposed murine Sarcoma 180 cells to these polysaccharides, and implanted subcutaneously those cells into mice. A reduction in tumor growth compared to control group was observed.

Apart from β -glucans, from medicinal mushroom maitake (*Grifola frondosa*), a water-soluble heteropolysaccharide consisting of galactose, mannose, fucose and glucose in a molar ratio of 1.24:1:0.95:0.88 was purified. This polysaccharide inhibited colon-26 tumor-growth in BALB/cA mice, to a level achieved by the reference β -glucan, and the effect is thought to be associated with induced cell-mediated immunity [40].

An alkaline-soluble polysaccharide (MW 6.3 kDa) isolated and purified from *Inonotus obliquus* consisted of rhamnose, xylose, manose, galactose, glucose and galacturonic acid in a molar ratio of 3.09:1.61:2.06:4.45:19.7:1, showed excellent activity against solid tumor Sarcoma 180 formation in mice, and the exerted activity was associated with potent immunostimulating effect of this polysaccharide [41]. Another heteropolysaccharide (MW 93 kDa) was extracted and purified from *I. obliquus*, but was water-soluble and consisted of rhamnose, mannose and glucose in molar ratios of 1.0:2.3:1.7. For this polysaccharide, no significant *in vitro* cytotoxic effect was observed, but exerted significant anti-tumor effect in human gastric carcinoma SGC-7901-bearing nude mice. Similar to other polysaccharides, authors suggested possible mechanisms related to cancer-prevention, immuno-enhancement and direct tumor inhibition [42].

One of the polysaccharide fractions isolated from fruiting bodies of *Tricholoma matsutake*, unlike other purified fractions of this mushroom, was found to be consisted of glucose, galactose and mannose with a molar ratio 5.9:1.1:1.0. This fraction exerted strong antiproliferative activity on HepG2 and A549 cell lines in MTT test [43].

Several investigations revealed that water-solubility of heteropolysaccharides could be one of the key features for increased antitumor activity [4, 44].

Some previous investigation showed that carboxymethylated derivatives of linear α -(1 \rightarrow 3)-D-glucans isolated from *Agrocybe cylindracea* and *Amanita muscaria* exhibited high potentiating effect on peritoneal murine macrophages, playing an important role in tumor immunity [45]. Water insoluble, alkali soluble α -(1 \rightarrow 3)-D-glucans isolated from fruiting bodies of four fungal species: *Laetiporus sulphureus*, *Lentinus edodes*, *Pleurotus ostreatus* and *Piptoporus betulinus* were converted into water soluble fractions by carboxymethylation [5]. After carboxymethylation, the obtained derivatives were tested for their cytotoxic activities in MTT and neural red uptake (NR) assays, and all carboxymethylated α -(1 \rightarrow 3)-D-glucans exerted high cytotoxic activity on human cervical carcinoma (HeLa) and human acute T-cell leukemia cell line (IC₅₀ values in range 0.53-2.45 μ g/ml), with relatively low activity in cell line of normal human skin fibroblasts (IC₅₀ values >25 μ g/ml, or no activity at all) [5].

Water-insoluble, alkali-soluble polysaccharides that were identified as α -(1 \rightarrow 3)-D-glucans isolated from three fruiting bodies of the fungus *Ganoderma lucidum*, were carboxymethylated and tested for their cytotoxic activity in human cervical carcinoma HeLa cell line, as well as in two normal human cell lines (colon myofibroblasts CCD-18Co and epithelial cells CCD 841 CoTr). Tested carboxymethylated glucans decreased cell metabolism after 24 h incubation in both carcinoma and normal cell lines, and exerted inhibition of cell viability in normal cell lines while in HeLa

cell line did not induce cytotoxic effect [46]. Even though, carboxymethylation, leading to increased water-solubility, may be the suitable chemical modification for enhancing cytotoxic activity. Wiater *et al.*, [46] suggested that degree of substitution in carboxymethylated products strongly affects immunological parameters, whereas increased degree of substitution did not result in cytotoxic activity. Another study of intracellular polysaccharides from submerged fermentation of *Ganoderma lucidum* and their sulfated derivatives pointed out the importance of the structure of derivatives and their origin i.e. whether they are of intra- or extra-cellular origin and implication of these facts on their anticancer properties. Intracellular polysaccharides from mycelia of *G. lucidum* are believed to be more active against cancer cell lines than extracellular polysaccharides. Still, in the mentioned research, the intracellular polysaccharides of *G. lucidum* inhibited human hepatocarcinoma cell line HepG2 in the first 48 h but stimulated the cell growth after 72 h regardless the concentration applied, whilst for other human hepatocarcinoma cell lines, these polysaccharides showed dose- and time-dependent inhibition. Also, intracellular polysaccharides accelerated the growth of normal human liver cells. Sulfated extracellular polysaccharides performed high inhibition on HepG2 cell line, but also exerted certain toxicity in normal liver cell line. Supplementing sulfated extracellular polysaccharides with intracellular polysaccharides of *G. lucidum* reduced the harm to normal liver cells [47].

Apart from carboxymethylation, it has been shown that *O*-sulfonated derivatives of native water-insoluble (1→3)- α -D-glucans, isolated from fruiting bodies of *Lentinus edodes*, exert inhibition of growth of solid tumor Sarcoma 180 implanted in mice. Also, cytotoxic activity of *O*-sulfonated glucans exerted cytotoxic activity in MTT assay on the same cell line. *O*-Sulfonation increased antitumor and cytotoxic activities of naturally occurring glucans, in both *in vitro* and *in vivo* tests [48].

Polysaccharides isolated from submerged fermentation broth of *G. lucidum* SB1997 were sulfated and the effect of this modification on the antitumor and cytotoxic properties was estimated. The sulfated polysaccharides exhibited high antiproliferative activity in MTT test on four human and one rat carcinoma cell lines in concentration-dependent manner and also remarkable but not dose-dependent antitumor activity in Heps hepatoma in mice. In comparison, naturally occurring extracellular water-soluble polysaccharide isolated from *G. lucidum* showed to lack antiproliferative activity in these cell lines, but reduced Heps in rat models also in non-dose-dependent manner [49].

Similarly, a sulfated polysaccharide from *Grifola frondosa* was tested for antiproliferative activity in Hep2 cells. This derivative concentration-dependently inhibited proliferation of Hep2 cells and it was related to a potential mechanism involving apoptosis through cell cycle arrest in S phase [50].

β -GLUCANS

β -Glucans represent fundamental building blocks in fungi, since their cell walls are composed of two polymers: chitin and β -glucan that are interlinked by covalent bonds and hydrogen bridges, which makes strong foundation for

chitin fibers network incorporated in glucan matrix. β -Glucans are polysaccharides where glucose is a sole monomer unit, from tens to thousands of kilodaltons, more or less soluble in water, which increases with temperature of the solvent. Glucans that are isolated from mushrooms are mainly β -1,3-D-glucan or β -1,6-D-glucans [44].

Even though chemical structure of β -glucans of cell walls of fungi has not been examined fully, it is known that immunomodulating activity is mainly dependent on single helix glucan structure capable to interact and/or link to immunoglobulins present in blood serum. Several structural features contribute to these effects such as higher degree of substitution, presence of hydrophilic groups on the helix surface and higher molecular weight. In the human body, glucans are intensively oxidized, and formed metabolites are temporarily and less effective than β -glucans themselves [44]. Basically, underlying mechanisms for antitumor activities of β -glucans such as lentinan, schizophyllan and grifolan, include stimulation of hematopoietic stem cells, activation of the alternative complement pathway, and activation of immune cells such as lymphocytes, macrophages, DC, NK cells, Th cells, Tc cells, and B cells [5].

In order to define specific features that contribute to antitumor activity, a series of tests were conducted on glucans obtained from *Grifola frondosa*. The most active glucan appeared to be branched β -(1 → 3)-glucan, known as grifolan, that exerted antitumor activity in Sarcoma 180 mice in a single dose 20 μ g/mice (daily dose 20 μ g/mice three times a day). Using specific solvents in extraction, may favour the enriching of extracts of *G. frondosa* in grifolan, and for this purpose the predominantly used solvent is sodium hydroxide [32]. A soluble β -(1 → 3)-(1 → 6)-D-glucan, purified also from *G. frondosa*, named maitake D-fraction, was proven to exert antitumor activity after intraperitoneal injection, by activating host immune system. The same fraction after oral administration significantly inhibited tumor growth in murine tumor models [40]. A soluble homogeneous β -glucan (MW 300 kDa) was purified from the fraction of the fruit bodies of *G. frondosa*. Its structure was determined to be a β -(1 → 3)-D linked glucan backbone with a single β -(1 → 6)-D linked glucopyranosyl residue branched at C-6 on every third residue. This glucan inhibited Sarcoma-180 growth allografted in ICR (Imprinting Control Region) mice but not in immunodeficient BALB/c nu/nu mice and the effect was partially associated with the activation of macrophages, which suggested the molecule as promising biological response modifier [51].

β -(1 → 6)-D-glucan, major polysaccharide component obtained from fruit bodies of *Agaricus blazei* (*A. brasiliensis*), a mushroom used for cancer prevention, exerted significant antitumor activity in Sarcoma 180 mice, as demonstrated in several tests [10].

Lentinan (MW 400-800 \times 10³ Da), the main β -glucan of *L. edodes* fruit bodies, is a right handed triple helix, with five β -(1 → 3)-glycose residues in a linear linkage and two β -(1 → 6)-glucopyranoside branches in side chains. Due to the specific conformation, it exerts specific immunomodulatory and anti-cancer effects in tumor models, which are attributed, not directly to inhibition of cell growth *in vitro*, but to the activation of T-cell- or peritonealexudate-cell-mediated

immune responses [44, 52]. Lentinan is considered relatively safe in doses used by *i.v.* administration (0.001-30 mg/kg) for 5-6 weeks, but longer term use is not recommended due to its toxicity. Also, taken orally, lentinan exerts little activity and may cause different gastro-intestinal disturbances and allergic reactions [53].

Schizophyllan (MW 450×10^3 Da) is also β -(1 \rightarrow 3)-glycan, with a side chain of β -(1 \rightarrow 6)-glucopyranosyl group and at each third glucose of main chain [44].

β -(1 \rightarrow 3)-D-Glucan with linked branches of primarily single β (1 \rightarrow 6)-glucopyranosyl groups, two for every seven residues in the main chain, was isolated from the fruiting bodies of *Amanita muscaria*. For this β -glucan, it was demonstrated a high antitumor activity in Sarcoma 180 in mice, and a mixture of this compound with mytomycin C, reference compound, increased the antitumor effect of mitomycin [54].

α -GLUCANS

Apart from β -glucans, immunostimulatory and potential anticancer activity of α -glucans was also shown. A branched α -(1 \rightarrow 4)-glucan (L10) purified from *Lentinula edodes* induced a significant reduction of viability (66% to 37%) of irradiated human lung adenocarcinoma A549 cells cocultured with monocytes (THP-1) by Toll-like receptor 4 mediated induction of THP-1 [6]. Lo *et al.*, [6] stated that L10-monocytes have the potential to enhance the antitumor immune response and antitumor effect of radiotherapy.

A low molecular complex of glucans (20 kDa) derived from *Agaricus blazei* consisted of α -(1 \rightarrow 4)-glucan and β -(1 \rightarrow 6)-glucan, and it showed *in vitro* selective cytotoxicity in MethA tumor cells, without affecting normal cells [55].

Proteins

Mushrooms contain high amounts of proteins, which account 10-40% of dry body weight [3]. Normally, a protein or peptide is not regarded as a drug candidate due to intensive metabolism in alimentary tract and serum, and development of corresponding antibodies by the immune system. However, there are few studies that support cytotoxic and antitumor properties of proteins isolated from mushrooms [3, 56-58]. Lectins are proteins of non-immune origin that reversibly bind to mono- and oligosaccharides with high specificity; through these processes they may be involved in biological activities such as cell-to-cell interactions and innate immunity [59]. Antitumor and cytotoxic properties of several lectins isolated from mushrooms, have been demonstrated [7, 57, 59].

A water soluble component from *Agrocybe aegerita* (Yt), consisting of 60% of proteins (two lectins, serine proteinase and several unknown proteins), 40% of low molecular weight substances and trace carbohydrates, was demonstrated to induce significant cytotoxicity in six tumor cell lines in concentration 50 μ g/ml, while fibroblast cell line NIH3T3 was not affected by the presence of Yt. Furthermore, this protein complex induced tumor rejection in hepatocellular carcinoma H22 and sarcoma S180 tumor bearing BALB/c mice in a low concentration (2.5 mg/kg) without significant cytotoxicity. In a nude mouse H22 tumor model,

Yt did not produce significant changes, but prolonged the lifespan. The contribution of a protein part of the molecule to exerted activities was proven by weakening of tumor rejection properties when proteins were degraded by proteinase K. Using the same isolation procedure from water extracts of fungi *Cordyceps militaris*, *Ganoderma lucidum* and *Lentinus edodes*, similar protein-low molecular weight compound complexes were extracted and purified, and anti-cancer activity was examined in H22 hepatocellular carcinoma bearing mice, and those tumor rejection effects were similar to those of Yt complex, pointing out that protein components, at least in part, contribute to antitumor properties of these mushrooms [3].

Agaricus bisporus lectin (ABL) has the remarkable property of binding selectively and with high affinity to Thomas-Friedereich antigen or T-antigen, a disaccharide hidden in healthy cells but exposed in high percentage in human carcinomas [60]. *In vitro* tests of ABL showed that it causes concentration-dependent inhibition on proliferation of HT29 human colorectal carcinoma, Caco-2 human colorectal cancer cells, human breast cancer MCF-7 cells, as well as rat mammary fibroblast Rama-27 cells via specific oligosaccharide binding sites (TF antigens) on cell membranes [7]. Also, from *Boletus edulus*, another lectin with similar structure to ABL was isolated, and it was found that it selectively and dose-dependently inhibits the growth of three cell lines, with inhibition of colon cancer cell line HT29 being the most pronounced [59]. A novel lectin marked as a BEL β -trefoil, for its structure of homodimer and each promoter folds as β -trefoil domain, was isolated and characterized from fruiting bodies of the same *Boletus* species; furthermore, it was shown to exert concentration-dependent cytotoxic effect in four human cell lines by MTT assay [57].

An immunomodulatory recombinant protein Lz-8, that was previously isolated and purified from the mycelia of *Ganoderma lucidum*, induced endoplasmic reticulum stress-mediated autophagic cell death in SGC-7901 human gastric cancer cells. Described mechanism is neither a caspase dependent cell death nor apoptosis, and may be a novel strategy for cancer treatment [58].

Alkaline protease (MW 15 kDa) that was isolated from fruiting bodies of *Amanita farinosa* was shown to inhibit proliferation of HepG2 cells concentration-dependently (IC₅₀ of about 25 μ M) using MTT assay [56].

Complexes of Polysaccharides with Proteins

Biological effects of mushroom polysaccharides may be promoted by the presence of a peptide or protein part complexed to them [44, 52, 61]. In previous investigations it was shown that highly active of *Ganoderma lucidum* are immunostimulating glycoproteins called fungal immunomodulatory proteins (FIMs) and *Ganoderma* polysaccharides peptide (GPP). Proteoglucans polysaccharide peptide (PSP) and polysaccharide-krestin (PSK) that are present in *Trametes versicolor* or *Schizophyllum commune* are also known to possess antitumor properties [61].

Krestin, polysaccharide protein complex purified from *Coriolus versicolor*, is a β -(1 \rightarrow 4)-glycan with a β -(1 \rightarrow 3)- and β -(1 \rightarrow 3)-glucosidic branches, containing about 25% of protein that exert antitumor effects in various animal tumor

models and has been given orally to cancer patients. The killer T cell activity was increased in tumor-bearing mice by intraperitoneal or oral administration of krestin [52].

A specific heteroglycan-protein conjugate (LEM), known to possess antitumor properties is extracted from *L. endodes mycelia* before the cap and the stem grow and contains 24.6% of protein and 44.0% of sugars, comprising mostly glucose, but also galactose, mannose and fructose. In much lower quantity, it contains nucleic acid derivatives, vitamin B complex, ergosterol and water-soluble lignins [53].

Soluble proteoglycan isolated from the fruit bodies of *Agaricus blazei* Murill consists of (1→3)- α -D-glucan and (1→6)- β -D-glucan in ratio 2:1 (MW 170 kDa), and small amounts of proteins. This complex was shown to be able to selectively suppress tumor growth by both apoptotic processing and host immune responses in solid tumor glioblastoma cells, as well as by NK cells-mediated immune response and related tumoricidal activity in Balb/c mice [62].

Furthermore, a complex RNA-glucoprotein complex named FA-2-b- β -fraction, containing adenine, aminopurine, chloropurine and other modified bases as nucleic acid base components, 15.7% of protein and D-ribose as the major constitutive sugar, was isolated from *A. blazei* and exerted cytotoxic effect in HL-60 cells measured by MTT assay, causing induction of apoptosis by combined effect of down-regulation of telomerase activity and up-regulation of mRNA expression of caspase-3 gene [63].

A 21-kDa heteropolysaccharide, coded as GFPS1b was isolated from the cultured mycelia of *Grifola frondosa* GF9801, is an acidic polysaccharide with approximately 16.60% protein and 4.3% uronic acid. Monosaccharide units were found to be D-glucose, D-galactose, and L-arabinose in a molar ratio of 4:2:1, forming a backbone consisting of α -(1 → 4)-linked D-galacopyranosyl and α -(1 → 3)-linked D-glucopyranosyl residues substituted at O-6 with glycosyl residues composed of α -L-arabinose-(1 → 4)- α -D-glucose (1 → 6) linked residues. This polysaccharide was shown to induce strong antiproliferative effect in MCF-7 human breast adenocarcinoma cells [35].

Ganoderan, a β -glucan isolated from *G. lucidum*, is consisted of glucose and 4% of protein, and induced antitumor immunity in tumor-bearing mice [4].

Sixteen peptide polysaccharide complexes were isolated from the mycelium of *Ganoderma tsuaga* and tested for anti-tumor properties on Sarcoma 180 mice. Amongst the complexes tested, the most active were heteropolysaccharides consisted of glucose, xylose and mannose with 9.3% of proteins and two more glucan-protein complexes: one containing 25.8% of protein and other having glycan (protein ratio of 42:58 w/w with the polysaccharide part consisting mainly of glucose, associated with arabinose, mannose, xylose and galactose). The three polysaccharide protein complexes exerted the highest tumor inhibition, and the most prolonged life span [64].

A protein bound polysaccharide composed mainly of mannose, galactose and glucose in a molar ratio of 1:1.28:4.91 (MW 1013 kDa) was isolated from fruiting bodies of *Ganoderma atrum*, a medicinal mushroom with a long history of use in Asia, and shown to inhibit proliferation of

mouse colon cancer cell line (CT26) via activation of peritoneal macrophages. Furthermore, this polysaccharide-protein complex showed significant antitumor activity in CT26 tumor bearing mice model, as well as inhibition of sarcoma 180 cells proliferation via macrophage activation and anti-tumor activity in Sarcoma 180 bearing mouse model [65-66].

A polysaccharide peptide complex purified from the fruiting bodies of the edible mushroom *Plurotus abalonus*, that contains glucose, rhamnose, glucuronic acid and galactose (molar ratio 22.4:1:1.7:1.6) demonstrated *in vitro* anti-proliferative activity in hepatoma HepG2 and breast adenocarcinoma MCF 7 cell lines [67].

FATTY ACIDS

Ethanol extracts of spores of *Ganoderma lucidum* inhibited tumor cell proliferation and induced apoptosis of HL-60 cells; the active constituents appeared to be long chain fatty acids, particularly carbon-19 fatty acids. Nonadecanoic acid and *cis*-9-nonadecanoic acid were pointed out as the active components [68].

NUCLEOSIDE ANTAGONISTS

Amongst nucleoside antagonists, cordycepin i.e. 3'-deoxyadenosine isolated originally from *Cordyceps militaris*, is known to exert antitumor activity, mostly by interfering with RNA synthesis [69]. Also its cytotoxic activity was shown in several cancer cell lines, where it suppresses NF- κ B signaling pathway [70]. It was shown that cordycepin induced apoptosis and persistent cell cycle arrest in human breast cancer cell lines, highly de-differentiated cell lines being more affected by this compound than less aggressive cell lines or non-malignant breast epithelial cells [69].

TERPENOIDS

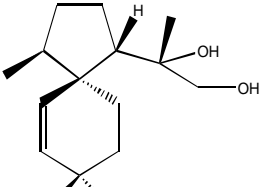
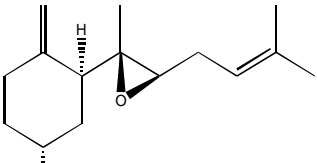
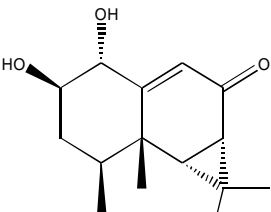
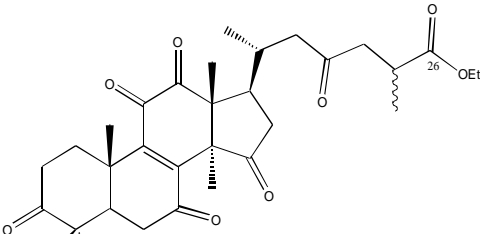
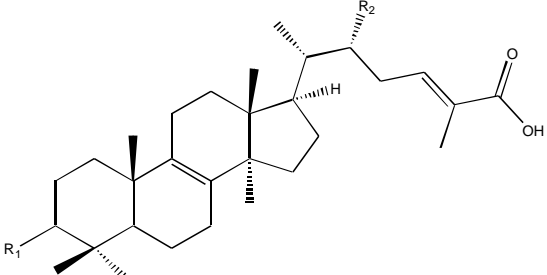
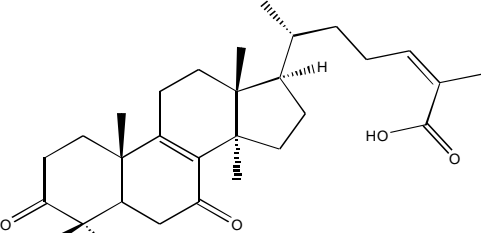
Certain classes of terpenoid compounds were isolated from some mushroom species; their structure was completely elucidated. The most important class is lanostane triterpenes, isolated from species such as *Ganoderma lucidum*, *Poria cocos*, *Laetiporus sulphureus*, *Inonotus obliquus* and *Anthrodia camphorata* that were investigated for their cytotoxic or apoptotic effects [71]. In the following chapter, a brief overview of terpenoid compounds is given and some of the structures are provided in Table 2.

SESQUITERPENES

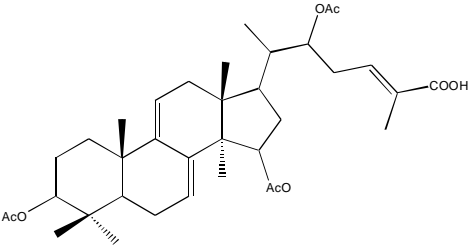
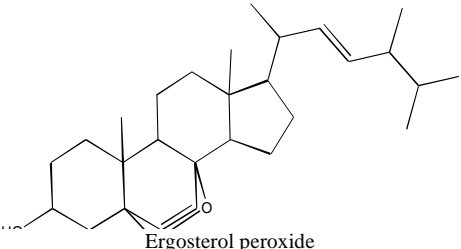
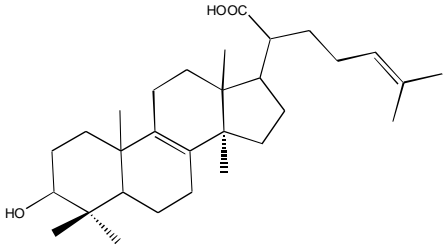
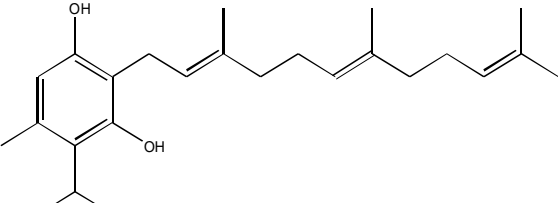
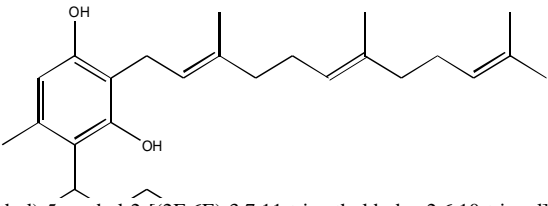
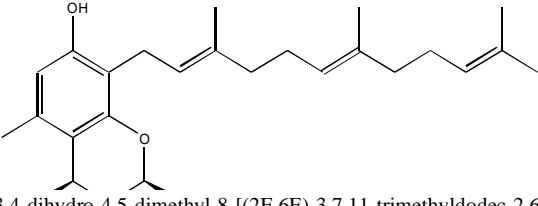
Three unusual sesquiterpenes namely cordycepol A-C of spiro[4.5]decane type and one fumagillol analogue cordycol, were isolated from the cultured mycelia of *Cordiceps ophioglossoides* and all the compounds were tested for their cytotoxic properties in HeLa, A549, HepG2 and MCF-7 cell lines using MTT assay. Cordycepol C and cordycol (Table 2) concentration- and time-dependently reduced the number of carcinoma cells, with relatively low effect on normal liver LO₂ cell line. Results imply that these two molecules may be promising lead compounds in treating human hepatic carcinoma [72].

Sesquiterpene nambinone C (Table 2) and dimer sesquiterpenes aurisin A and aurisin K, isolated from

Table 2. Some of the Compounds Isolated from Medicinal Mushrooms that Exert Cytotoxic or Apoptotic Effects⁰

Compound	Exerted activity	Reference
 <p>Cordycepol C</p>	Cytotoxic effects in HeLa, A549, HepG2 and MCF 7 cell lines	[72]
 <p>Cordycol</p>		
 <p>Nambinone C</p>	Cytotoxic effect in NCI/H187 cells	[73]
 <p>Ethyl 3,7,11,12,15,23-hexaoxo-5α-lanost-8-en-26-oate</p>	Cytotoxic effect in B16F1, B16F10, Huh-7, MCF 7 and A 2058	[74]
 <p>R₁=O; R₂=OAc Astraodoric acid A R₁=O; R₂=OH Astraodoric acid B R₁=α-OH; R₂=OAc Astraodoric acid D</p>	Cytotoxic effect in KB, NCI-H187 and MCF 7 cell line	[79]
 <p>Ganoderic acid DM</p>	Anti-prostate cancer activity and cytotoxic effect in MCF 7 cell line	[8]

(Table 2) contd....

Compound	Exerted activity	Reference
 <p data-bbox="493 516 647 541">Ganoderic acid T</p>	Three human carcinoma cell lines	[9]
 <p data-bbox="483 789 659 814">Ergosterol peroxide</p>	Induction of cell death of the miR-378-transfected cells Cytotoxic effects in human breast and prostate cell lines	[11] [81]
 <p data-bbox="490 1077 649 1102">Trametenolic acid</p>	Cytotoxic effects in human breast and prostate cell lines	[81]
 <p data-bbox="354 1335 787 1381">4-(1-methoxyethyl)-5-methyl-2-[(2E,6E)-3,7,11-trimethyldodec-2,6,10-trienyl]benzene-1,3-diol</p>	Cytotoxic effect in human lung carcinoma and human and mouse melanoma cell lines	[82]
 <p data-bbox="185 1608 950 1654">4-(1-ethoxyethyl)-5-methyl-2-[(2E,6E)-3,7,11-trimethyldodec-2,6,10-trienyl]benzene-1,3-diol</p>	Cytotoxic effect in human lung carcinoma and human and mouse melanoma cell lines	[82]
 <p data-bbox="196 1881 943 1919">(2R*,4R*)-3,4-dihydro-4,5-dimethyl-8-[(2E,6E)-3,7,11-trimethyldodec-2,6,10-trienyl]-2H-[1]benzopyran-2,7-diol</p>	Cytotoxic effect in human lung carcinoma and human and mouse melanoma cell lines	[82]

luminescent mushroom *Neonothopanus nambi*, exerted cytotoxic activity. All three compounds induced cytotoxicity in human small cell lung cancer cells (NCI-H187), and aurisins A and K additionally exerted cytotoxic activity in chloran-giocarcinoma cell lines [73].

LANOSTANOIDS

Lanostanoids are a type of tetracyclic triterpenoids derived from lanosterol that are being intensively investigated for their anticancer effects. Various mechanisms are believed to support apoptotic effects of these compounds: modification of transcriptional activities via nuclear factors or genes and the activation or inhibition of pro- and antiapoptotic proteins. Some data on cytotoxicity were reported, but mechanisms of their action were not fully elucidated [71].

From fruiting bodies and mycelia of solid cultures of *Antrodia camphorata*, a parasitic fungus on heartwood inner wall of endemic and endangered *Cinamomum kanehirai* Hay, six compounds: three lanostane triterpenes, two sterols and a steroid were isolated and elucidated. Cytotoxic activity of these compounds was tested in seven cell lines: human squamous cell carcinoma (HSC-3), murine melanoma (B16F1 and B16F10), hepatocarcinoma Huh-7, ovarian carcinoma (SKOV3), human breast adenocarcinoma (MCF-7) and human melanoma (A 2058). Even all the compounds exerted moderate activity, there were differences in observed effects; the presence of ethylester at position C-26, as in ethyl 3,7,11,12,15,23-hexaoxo-5 α -lanost-8-en-26-oate (Table 2), seemed to play a key role in mediating cytotoxicity [74].

At least 31 triterpenoids were identified in *Antrodia cinnamomea*, and some of them shown to possess anti-cancer activity [75]. Three ergostane type triterpenes: methyl antcininate B, zhankuic acid A and C isolated from fruiting bodies of this species, exerted cytotoxic activity in four human carcinoma cell lines [76]. Five triterpenoids, camphoratins B-F, that were also isolated from the fruiting bodies of *A. cinnamomea* showed moderate to potent cytotoxicity in KB and KBVIN human nasopharyngeal cancer cell lines [77]. Furthermore, anticin K, anticin C, zhankuic acid C and zhankuic acid A, isolated from the fruiting bodies of the same species were also shown to inhibit three human leukemia cell lines [78].

Four novel lanostane triterpenes: astraodoric acids A-D, and new 5-hydroxyhyphaporine, together with ergosterol, astraodorol (artabotryols A), nicotinic acid and hypaphorine were isolated and elucidated from *Astraeus odoratus*, a Thai edible mushroom, and were tested for their *in vitro* cytotoxic activity in three human carcinoma cell lines [79]. In the mentioned research, among the compounds tested, astraodoric acid A, B and D (Table 2) exerted the highest cytotoxic activity (IC₅₀ values of 34.69, 19.99 and 31.55 μ g/ml against human epidermoid carcinoma cell line and 18.57, 48.35 and 34.15 μ g/ml against human small cell lung cancer, respectively, and IC₅₀ value of astraodoric acid D against human breast adenocarcinoma MCF-7 was 40.15 μ g/ml).

A series of lanostane triterpenes has been isolated from *Ganoderma lucidum*, a species intensively used in Traditional Chinese Medicine [8]. Ganoderic acid DM (Table 2),

isolated from this species, exerted anti-prostate cancer activity via inhibiting 5 α -reductase activity. Furthermore, it inhibited cell proliferation and colony formation in MCF-7 human breast adenocarcinoma cell line via induction of G1 cell cycle arrest and apoptosis [8]. Ganoderic acid Mk, isolated from mycelia of *G. lucidum* dose-dependently inhibited proliferation of HeLa cells via induction of apoptosis [80]. Ganoderic acid S, isolated from the same mushroom, caused cell cycle arrest in S phase, whilst ganoderic acid Mf causes cell cycle arrest in G1 phase [9]. Some of the lanostane triterpenoids served as model compounds for the synthesis of derivatives with more pronounced cytotoxic and/or antitumor activities. Ganoderic acid T (Table 2), a promising anticancer agent, was modified to obtain more potent derivatives with cytotoxic and pro-apoptotic effects on three human carcinoma cell lines, with minimal effect on non-tumor cell line [9].

STEROLS

Various ergosterol derivatives have been isolated from mushrooms such as *Lentinus edodes*, *Polyporus umbellatus* and *Agaricus blazei*, mainly from the lipid fraction [10].

Oral administration of ergosterol (400 and 800 mg/kg during 20 days) to Sarcoma 180 bearing mice, significantly reduced tumor growth without side effects, such as decreases in body, epididymal adipose tissue, thymus, spleen weight and leukocyte numbers. Ergosterol did not induce cytotoxic effects in tumor cells, but acted as antiangiogenic substance in two *in vivo* models of tumor- and Matrigel-induced neurovascularization [10].

Ergosterol peroxide induced death of the miR-378-transfected cells; miR-378 are expressed in a number of cancer cell lines. This data point out that ergosterol peroxide may be a new reagent for overcoming the problem of drug-resistance in tumor cells [11].

Ergosterol peroxide and trametenolic acid (Table 2), isolated from *Inonotus obliquus* exerted cytotoxic activity in human prostate and breast carcinoma cell lines [81].

PHENOLIC COMPOUNDS

Three novel farnesyl phenols, grifolin derivatives (Table 2) isolated from the fresh wild mushroom *Boletus pseudocapellatus* showed high cytotoxic activity against two human and one mouse cancer cell lines, using SRB assay [82].

Protocatechuic acid (phenolic acid) and a related compound (cinnamic acid), detected as the main components in *Clitocybe alexandri* ethanol extract induced significant cell growth inhibition of human lung cancer cell line (NCI-H460), by SRB assay; the effect of cinnamic acid being the most pronounced. These compounds, at least partly contributed to cell cycle arrest and apoptosis in the lung cancer cell line induced by *C. alexandri* extract [30].

Molecular Mechanisms Of Action – *A. camphorata* and *G. lucidum*

Antrodia camphorata and *Ganoderma lucidum* were the most deeply studied mushrooms regarding their molecular mode of action on anticarcinogenesis. Therefore, we focused

further on explaining the data concerning molecular mechanisms of action of these mushrooms and their bioactive constituents.

DNA Damage and Apoptosis Biomarkers Activation

Studies on *Antrodia camphorata* (AC) extracts showed that they can promote immune responses by exhibiting antileukemic activity in WEHI-3 leukemia BALB/c mice [83] and can activate the immunomodulation of macrophages in a human hepatoma cell model [84].

In numerous studies it has been shown that AC demonstrates cell cycle inhibition and apoptotic cell death that are both contributing to its antitumor effect. Tu and his team [85] demonstrated that a purified compound from AC (4,7-dimethoxy-5-methyl-1,3-benzodioxole; SY-1) can be used as apoptosis inducer in COLO-25 colon cancer cells. SY-1 also induced cell cycle arrest in G0/G1 phase through the activation of p53-mediated cyclin-dependent kinase (CDK) inhibitor expression. These findings showed that AC can be used as adjuvant antitumor agent for T 29 human colon cancer cell xenograft tumors [86]. Tu *et al.*, [85] demonstrated that SY-1 isolated from dried fruiting bodies of AC inhibited the growth of COLO-25 cell xenograft tumors through the inhibition of p53-mediated cell cycle regulatory genes. SY-1 inhibited the cell growth in both COLON-205 and HepG2 cells that exhibits wild type p53 and cancer cell lines that have mutant p53 in dose and time-dependent manner. But it has to be pointed out that those cells with wild type p53 are more sensitive to SY-1. SY-1 has its effect through increased expression of p53, which leads to inhibition of cell cycle by inducing p27/Kip1. P27/Kip1 is an important step, which directly has effect on cell cycle that leads to cell growth cycle arrest.

Apoptosis Induction (Up-Regulation of p21Waf1/Cip1 and K-ras)

Antroquinonol is a compound which is an ubiquinone derivative isolated from AC. It has been shown that this compound exhibits anticancer activity against hepatocellular carcinoma (HCC) through activation of 5'adenosine-monophosphate-activated protein kinase (AMPK) and inhibition of mTOR pathway [87]. The aberrant induction of checkpoint arrest renders cells to apoptosis, which makes this compound good anticarcinogenic. Antroquinonol inhibited phosphorylation of mTOR at Ser²⁴⁴⁸ which is a site dependent on mTOR kinase activity [87], but in AsPC-1 cell line this is not the mechanism by which antroquinonol exhibits its effect. In this particular cell line, antroquinonol has effect indirectly on mTOR by first blocking PI3-kinase/Akt pathway by inhibiting the phosphorylation of Akt at Ser⁴⁷³. This pathway is important upstream regulator of mTOR. Antroquinonol also displays effect on other important proteins by inhibiting phosphorylation of p70S6K, eIF4E and 4E-BP1 thus blocking whole mTOR/p70S6K/4E-BP1 signaling pathway.

Antroquinonol also down-regulated expression of other cyclins that are important in cell cycle. This compound decreased expression of cyclins D1, E, A, B1 and CDK4 in a time dependent fashion. Antroquinonol also induced significant increase in K-ras expression and phosphorylation. This

protein belongs to Ras family that regulates a wide variety of biological effects including cell proliferation, survival and transformation [88]. This protein can also induce growth arrest, apoptosis, senescence and autophagy. Ras displays proapoptotic activity through a direct interaction with Bcl-2 or related family members. Bcl-2 regulates integrity of mitochondrial membrane and it is a well known fact that mitochondrion plays important role in apoptosis. So, increasing level of K-ras antroquinonol causes down-regulation of Bcl-2 family proteins (specifically Bcl-xL) that leads to loss of mitochondrial integrity and programmed cell death through apoptosis. Therefore, data reported by Yu *et al.*, [89] suggest that antroquinonol induces anticancer activity in human pancreatic cancer AsPC-1 cells through a sequential signaling cascade. It induces an inhibitory effect on PI3-kinase/Akt activities that in turn block mTOR/p70S6K/4E-BP1 signaling pathways, leading to the down-regulation of cyclin proteins and CDKs. The translational inhibition results in G1 arrest of the cell cycle and an ultimate mitochondria-dependent apoptosis. The upregulated K-ras may also contribute to apoptosis through the association with Bcl-xL. Moreover, autophagic cell death and accelerated senescence also explain, at least partly, the antroquinonol-mediated anticancer effect in AsPC-1 cells [89].

Depletion of HER-2/neu, and Disruption of the PI3K/Akt Signaling Pathway

Antrodia camphorata can also be used against human breast cancer cell lines that exhibit high level of HER-2/*neu* [90]. It mediates growth inhibition and apoptotic induction through intercellular ROS generation, suppression of the HER-2/*neu* signaling cascade, and disruption of the PI3K/Akt-dependent pathway. Activation of the HER-2/*neu* network leads to autophosphorylation of the C-terminal tyrosine and the recruitment to these sites of cytoplasmic signal transducers that regulate cellular processes, such as proliferation, inhibition of apoptosis, and transformation [91]. Results showed that AC reduces the basal tyrosine kinase phosphorylation and constitutive activation of HER-2/*neu* receptors in HER-2/*neu*-overexpressing breast cancer cells. It seems that AC mechanism of induction doesn't influence the mRNA level in cells, in another words, it has no effect on post-transcriptional mechanism. Since it has no effect at post-transcriptional level, another way by which AC can exert effect is by proteolysis, which was the case of the results presented by Lee *et al.* [90]. These authors demonstrated that proteasomal activity was critically involved in AC-induced HER-2/*neu* degradation in human breast cancer MDA-MB-453 cells. Also incubation of cells with AC caused a significant increase in ROS accumulation that leads to cell death. Key mechanism by which HER-2/*neu*-overexpression stimulates tumor cell growth and renders cells chemoresistant involves the HER-2/*neu* receptor. This mechanism involves the PI3K/Akt signaling pathway, and human breast cancer cells with overexpression and amplification of HER-2/*neu*, have been shown to make increased use of the PI3K/Akt signaling pathway [90]. Results suggest that AC treatment significantly inhibits the expression of the Akt upstream kinase, PI3K, in MDA-MB-453 cells. AC causes a similar dose-dependent reduction in Akt phosphorylation in BT-474 cells, whereas the levels of total Akt re-

mains unaffected by AC under the same treatment conditions. These data established that AC induced HER-2/*neu* depletion and growth inhibition may be mediated by the inactivation of PI3K/Akt activity in HER-2/*neu*-overexpressing breast cancer cells.

When PI3K/Akt is active, a number of substrates are activated that involve apoptosis, cell-cycle regulation, and protein synthesis [91]. PI3K/Akt could potentially regulate cell cycle progression by phosphorylating and inactivating GSK-3 β , thereby stabilizing nuclear translocation of β -catenin and increasing cyclin D1 and Cdk4 transcription [92]. So, it was demonstrated that AC may inhibit cell proliferation and the induction of cell death by suppressing GSK-3 β and the β -catenin pathway in HER-2/*neu*-overexpressing breast cancer cells. In this study it was also shown that AC treatment causes dose dependent reduction of cyclin D1 and cyclin E expression in HER-2/*neu*-overexpressing MDA-MB-453 cells. Cyclin D1 is regulatory subunit of CDK4 and contributes to its stability. In addition, Akt may contribute to the induction of cell-cycle progression by regulating the CDK inhibitors p27KIP and p21CIP [93]. Previous studies have shown that the modulation of both p27KIP and p21CIP is required for oncogenic growth driven by HER-2 [94]. Both p27KIP and p21CIP protein levels increased dose-dependently in response to AC treatment. A similar pattern of results were also observed in BT-474 cells; AC down-regulates cyclin D1 and upregulates p21CIP expression in a dose dependent fashion.

AC significantly increased the release of cytochrome c from mitochondrion, which is evidence that AC causes membrane damage. It is a known fact that cytochrome c is involved in triggering apoptosis [95]. AC induces cell apoptosis through another mechanism by disregulating Bax/Bcl-2. So, induction of apoptosis could be a major mechanism of AC-induced growth inhibition in HER-2/*neu*-overexpressing breast cancer cells. One important feature of AC effect on HER-2/*neu*-overexpressing breast cancer cell lines is that it also suppresses their transformation ability.

Cell Cycle Arrest by *G. lucidum*

Among the active compounds in *Ganoderma lucidum* (GL), triterpenoids have been demonstrated as one of the main components responsible for the pharmacological activities including immunomodulation, anti-oxidative, anti-metastasis, and anti-tumor effects [16-18]. *In vitro* and *in vivo* assays have revealed that the mixtures of triterpenoids from GL exerted antiproliferation effects by inducing apoptosis and cell cycle arrest [96, 97]. Ganoderic acid DM (GADM) is a lanostane-type triterpenoid extracted from the GL that inhibits osteoclastogenesis by regulation of c-Fos and nuclear factor of activated T cells c1 [98] has been shown to exert anti-proliferation effects on both androgen-dependent and independent prostate cancer cell lines in a concentration dependent manner [8]. One of the underlying mechanisms is that GADM attenuates the conversion of testosterone to dihydrotestosterone by inhibition of the 5 α -reductase activity and blocks DHT binding to the androgen receptor by competitive inhibition in prostate cancer cells [99]. Human breast cancer is another hormone sensitive malignancy. Wu *et al.*, [8] used breast cancer cell lines MCF-7 (ER-positive) and MDA-MB-231 (ER-negative) to evaluate

anti-proliferative potential of GADM. GADM decreased the percentage of adherent cells in a concentration-dependent manner in MCF-7 cells. GADM also notably decreased the viability of MCF-7 cells as detected by MTT assay. Wu *et al.*, [8] found out that MCF-7 cells were much more sensitive to GADM compared with MDA-MB-231 cells. GADM effectively induced G1 cell cycle arrest and apoptosis in MCF-7 cells. Consistently the cells distributed in S phase were significantly reduced in affected cell line. Both CDK2 and CDK6 are catalytic subunits of the cyclin-dependent kinase complex are essential for the G1/S transition. Cyclin D is one of the major cyclins and cyclin D-CDKs complex partially phosphorylates Rb which is an important regulator of genes responsible for progression through G1 phase. The expression of CDK2, CDK6, cyclin D1 and p-Rb was down-regulated after GADM treatment. These proteins are all important for G1 cell cycle progression [100], and might partially be responsible for GADM-induced G1 cell cycle arrest. It has been demonstrated that c-Myc promotes cell proliferation and many investigators have uncovered the target genes that regulate the cell cycle such as CDKs and cyclins [101]. It's notable that the oncoprotein c-Myc, which is vital for cells progressing into S phase [101], was also reduced after GADM treatment. Thus, GADM mediated c-Myc down-regulation may also contribute to the G1 cell cycle arrest. These results indicate that GADM induced G1 cell cycle arrest in MCF-7 cells may be partially due to modulating of CDKs, cyclins and c-Myc. Results suggested that G1 cell cycle arrest and apoptosis induction both contributed to the anti-cancer activity of GADM. DNA damage is one of the molecular events associated with cell cycle arrest and apoptosis and many anti-cancer reagents induce DNA damage [102]. Incubation with GADM leads to internucleosomal DNA fragmentation in time dependent manner. After GADM treatment, apoptosis was observed by detecting a cleavage fragment of PARP, indicating GADM indeed induces apoptosis in MCF-7 cells. It has been known that all organisms have the ability to restore genomic integrity through DNA repair. If the repair is faulty or the cell is overwhelmed by damage, chances are that the cell will despair and be removed by apoptosis [103, 104]. Wu *et al.*, [8] found that GADM elicited DNA damage after 6-h treatment measured by the comet assay and the up-regulated γ -H2AX protein levels, so it can be supposed that the cell cycle arrest and apoptosis may be attributed to GADM-induced DNA damage.

CONCLUSION

Mycotherapy of cancer is promising discipline in current scientific and medical battle against serious, widespread and very frequent disease of nowadays. Studies to date have identified a number of compounds and elucidated underlying mechanisms. Further research focused on mycotherapy of cancer, especially clinical trials, are needed to validate the usefulness of mushrooms and their compounds, either alone or in combination with existing therapies.

CONFLICT OF INTEREST

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