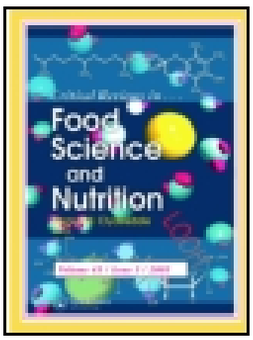


Taylor & Francis
Taylor & Francis Group

Critical Reviews in Food Science and Nutrition

ISSN: 1040-8398 (Print) 1549-7852 (Online) Journal homepage: http://www.tandfonline.com/loi/bfsn20

Advances in Antitumor Polysaccharides from Phellinus sensu lato: Production, Isolation, Structure, Antitumor Activity, and Mechanisms

Dr Jing-Kun Yan, Dr Juan-Juan Pei, Hai-Le Ma , Dr Zhen-Bin Wang & Dr Yuan-Shuai Liu

To cite this article: Dr Jing-Kun Yan, Dr Juan-Juan Pei, Hai-Le Ma , Dr Zhen-Bin Wang & Dr Yuan-Shuai Liu (2015): Advances in Antitumor Polysaccharides from Phellinus sensu lato: Production, Isolation, Structure, Antitumor Activity, and Mechanisms, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2014.984802

To link to this article: http://dx.doi.org/10.1080/10408398.2014.984802

Accepted author version posted online: 27 Oct 2015.

Submitted to: *Critical Reviews in Food Science and Nutrition*

(Original Research MS)

Advances in Antitumor Polysaccharides from *Phellinus* sensu lato: Production, Isolation, Structure, Antitumor Activity, and Mechanisms

Dr Jing-Kun Yan (Corresponding Author)

Email: jkyan_27@163.com

Jiangsu University, Zhenjiang, China

Dr Juan-Juan Pei

Email: weini_pjj@163.com

Jiangsu University, Zhenjiang, China

Professor Hai-Le Ma

Email: mhl@mail.ujs.edu.cn

Jiangsu University, Zhenjiang, China

Dr Zhen-Bin Wang

Email: wzhb@mail.ujs.edu.cn

Jiangsu University, Zhenjiang, China

Dr Yuan-Shuai Liu

Email: keysliu@ust.hk

The Hong Kong University of Science and Technology, Kowloon, Hong Kong, Hong Kong

Abstract

Edible and medicinal fungi (mushrooms) are widely applied to functional foods and nutraceutical products because of their proven nutritive and medicinal properties. *Phellinus* sensu lato is a well-known medicinal mushroom that has long been used in preventing ailments, including gastroenteric dysfunction, diarrhea, hemorrhage, and cancers, in oriental countries, particularly in China, Japan, and Korea. Polysaccharides represent a major class of bioactive molecules in *Phellinus* s. l., which have notable antitumor, immunomodulatory, and medicinal properties. Polysaccharides that were isolated from fruiting bodies, cultured mycelia, and filtrates of *Phellinus* s. l. have not only activated different immune responses of the host organism but have also directly suppressed tumor growth and metastasis. Studies suggest that polysaccharides from *Phellinus* s. l. are promising alternative anticancer agents or synergizers for existing antitumor drugs. This review summarizes the recent development of polysaccharides from *Phellinus* s. l., including polysaccharide production, extraction and isolation methods, chemical structure, antitumor activities, and mechanisms of action.

Keywords *Phellinus* sensu lato, Polysaccharides, Structure, Antitumor, Mechanism

INTRODUCTION

Nowadays, cancer is globally the second most life-threatening disease whose mortality follows immediately after that of cardiovascular disease. Excessive exposure to carcinogens, including tobacco, alcohol, chemicals, infectious agents, and radiation, results in different genetic (mutations), epigenetic (loss of heterozygosity), and global transcriptome changes (via inflammation pathways) and is closely related with increased cancer risk (Lieu et al., 2010). In 2014, the World Health Organization reported that approximately 14 million people were diagnosed with cancer globally and around 8.2 million people died of this disease in 2012. More than 24 million new cancer cases are also expected by 2035, of which, 70% would be contributed from developing countries, such as China (World Health Organization, 2014). Up to date, chemotherapy remains the mainstream therapeutic method for cancer. However, this treatment is often associated with severe side effects that could lead to opportunistic infections and even death (Mushiake et al., 2005). Therefore, new anticancer compounds should be studied and developed, particularly compounds derived from natural resources, to prevent the harmful side effects of chemotherapy without decreasing the efficacy against tumors (Efferth et al., 2007).

Polysaccharides represent a major class of bioactive molecules derived from renewable sources, including plants, animals, and microorganisms. Such biomolecules are extensively used in various biomedical applications and functional foods because of their notable and excellent bioactivities, such as immunostimulatory, antitumor, and antioxidant activities, as well as other

health benefits (Ooi & Liu, 2000; Moradali et al., 2007; Ren et al., 2013; Zong et al., 2012). Given their strong antitumor and immunomodulatory activities, various purified β-glucans as well as polysaccharide–protein and polysaccharide–peptide complexes (PSPs) from mushrooms (fungi) have been used in clinical applications for immunotherapy and cancer treatment (Giavasis, 2014; Stachowiak & Reguła, 2012; Zhang et al., 2007), such as lentinans from *Lentinus edodes* (Xu et al., 2014), polysaccharide D-fraction and MD-fraction from *Grifola frondosa* (Boh & Berovic, 2007), and PSP from *Coriolus versicolor* (Cui, 2003). These fungal polysaccharides and their derivatives have been proven useful against various cancers, particularly in the stomach, prostate, and lungs (Wasser, 2002; Zhang et al., 2007). Such polysaccharides and derivatives have been marketed as anticancer pharmaceutical agents in many oriental countries. Compared with many antitumor drugs, polysaccharides that were isolated from medicinal mushrooms show high efficiency and low toxicity for cancer. These mushrooms had also been consumed since antiquity (Lull et al., 2005). Therefore, in-depth investigation of highly antitumor polysaccharides from medicinal (or edible) mushrooms, particularly in developing new antitumor auxiliary drugs, is important.

In recent years, numerous studies have demonstrated that polysaccharides isolated from *Phellinus* sensu lato, including *P. linteus*, *P. igniarius*, and *P. baumii* Pilát, have a broad spectrum of biological activities, such as antitumor, immunomodulatory, anti-inflammatory, anti-allergic, anti-angiogenic, and anti-oxidant effects (Hsieh et al., 2013; Sliva, 2010; Zhu et al.,

2008). *Phellinus* s. l. is a species of medicinal mushrooms belonging to Hymenochaetaceae Basidiomycetes that is valuable in traditional Chinese medicine and is widely used in East Asia, particularly China, Japan, and Korea (Dai et al., 2010; Hsieh et al., 2013; Zhu et al., 2008). More than 40 years ago, an original study in Japan demonstrated that *P. linteus* had the strongest antitumor effects among other known medicinal mushrooms (Ikekawa et al., 1968). As previously reported, polysaccharides isolated from *Phellinus* s. l. with strong antitumor efficiency were not only immune stimulators or immune regulators that can stimulate the cell-mediated and innate immunity, activating T lymphocytes, B lymphocytes, native killer cells, dendritic cells (DCs), and macrophages, but also had direct cytotoxic activities against a wide spectrum of murine and human neoplasms, including prostate cancer, lung cancer, colon cancer, epidermoid, hepatocellular carcinoma, fibrosarcoma, neuroblastoma, and melanoma (Sliva, 2010; Zhu et al., 2008). The physicochemical properties and chemical structures of *Phellinus* polysaccharides should be elucidated to understand their antitumor and other medicinal properties.

Therefore, this review mainly focuses on the antitumor polysaccharides derived from *Phellinus* s. l. and presents a comprehensive overview of their production, extraction, isolation, and chemical structures, as well as antitumor activity and mechanisms of action. This review enhances the understanding of *Phellinus* polysaccharides and facilitates further studies on their distinct structure, properties, bioactivities, and potential applications in functional and health

foods.

MEDICINAL SPECIES AND MYCELIAL FERMENTATION

Phellinus s. l. (Japanese "meshimakobu", Chinese "song gen", Korean "sanghwang", English "meshima", American English "black hoof mushroom") is one of the largest medicinal mushrooms belonging to Hymenochaetaceae Basidiomycetes, which are mainly indigenous to tropic America, Africa, and East Asia (Dai et al., 2010; Sun et al., 2006; Zhu et al., 2008). Such mushrooms have been widely used for centuries in traditional Chinese medicine in East Asia, particularly in China, Japan, and Korea, to prevent ailments as diverse as gastroenteric dysfunction, diarrhea, hemorrhage, and cancers (Zhu et al., 2008). Around 220 species, including *P. linteus*, *P. igniarius*, *P. gilvus*, *P. ribis*, *P. baumii* Pilát, and *P. nigricans*, are presently recognized worldwide, and approximately 26 species found in China are recognized as medicinal mushrooms according to previous reports (Dai et al., 2009; Larsen & Cobb-Poulle, 1990). Among these species, *P. linteus* and *P. igniarius* are the most popular main species for their medicinal functions according to the practice of traditional Chinese medicine and recent scientific publications. These species have been used in traditional pharmacopoeias since ancient times and have elicited increasing attention worldwide. Some commercial health products have also been prepared from *Phellinus* s. l. (specifically *P. linteus* and *P. igniarius*) fruiting body or fungal mycelia in capsules and various other formulations in recent years (Fig. 1).

Given the limited and unstable supply of wild *Phellinus* s. l., cultivated fungal mycelia or

mushrooms (in fruit form) by solid and submerged fermentation has been the major source of fungal materials. In general, cultivation of the fruiting body of *Phellinus* s. l. requires approximately five to six months, and the product quality is difficult to control when *Phellinus* s. l. is traditionally cultivated in solid culture. The product composition also varies from batch to batch. In particular, liquid or submerged fermentation has potential advantages for higher mycelial and polysaccharide production in a more compact space over a shorter incubation time and availability of convenient control with less chance of contamination (Seviour et al., 2011; Zhong & Tang, 2004). Therefore, submerged fermentation has become a promising alternative for efficient production of mycelial biomass and bioactive compounds, including polysaccharides. Up to date, some *Phellinus* s. l. species, such as *P. linteus*, *P. igniarius*, *P. baumii* Pilát, and *P. nigricans*, have been used to produce mycelial polysaccharides and exopolysaccharides via submerged fermentation technology. Simultaneously, to achieve higher yield of polysaccharides, an optimal production medium and a set of optimal process operating conditions have been designed and applied in the submerged culture (Hwang et al., 2003; Kim et al., 2002; Lee et al., 1995; Luo et al., 2010; Wang, Quan et al., 2014; Zhu et al., 2011). More recently, our group developed a novel flat-plate ultrasound technology to stimulate polysaccharide production from *P. igniarius* mycelial fermentation. The results suggested that the yield of polysaccharides under ultrasonic treatment was much higher than that of the control, and the polysaccharides showed stronger radical scavenging capacities and antioxidant activities

(Zhang et al., 2014). Therefore, development of novel highly efficient strategies for polysaccharide production via submerged fungal culture remains a subject of research interest.

EXTRACTION AND ISOLATION METHODS

In general, polysaccharides showing antitumor activity have been isolated from fruiting bodies, cultured mycelia, and filtrates of *Phellinus* s. l. These polysaccharides can be classified into two main types based on their locations in the fungal cells, that is, intracellular polysaccharides are extracted from the fruiting bodies and cultured mycelia of *Phellinus* s. l., whereas extracellular polysaccharides (exopolysaccharides) are extracted from the cultured filtrates of *Phellinus* s. l. Many extraction methods have been developed for antitumor polysaccharides from *Phellinus* s. l. Among these methods, hot water extraction is the most classical and popular approach for extraction of polysaccharides from mushrooms (or fungi) in industrial applications because of its simplicity, safety, and environmentally friendly compatibility (Wasser, 2002). However, hot water extraction is always associated with long duration and high temperature, leading to waste of energy and excessive degradation of polysaccharides. To improve the yield of polysaccharides, acidic solution, dilute alkali solution, and some supplementary methods, such as freeze/thaw method, ultrasound, microwave, ultrahigh pressure, and enzymatic method, have also been used to extract mushroom polysaccharides (Jin et al., 2012; Nie et al., 2013; Wasser, 2002; Xu et al., 2014; Zhang et al., 2007; Zhang et al., 2011). Similarly, these methods can be used to extract polysaccharides from *Phellinus* s. l. More

specifically, Park et al. developed a novel process for nanoparticle extraction of β-(1→3)-D-glucan from *P. linteus* using insoluble tungsten carbide as a model for nanoknife technology. This nanoknife method could be used to produce β-D-glucan for food, cosmetic, and pharmaceutical industries (Park et al., 2009). Some researchers have recently adopted a response surface methodology to optimize the extraction conditions for polysaccharides from *Phellinus* s. l. (Guo et al., 2010; He et al., 2012; Ma et al., 2014; Wang, Wang et al., 2014).

To avoid accumulation of biological wastes and loss of bioactive polysaccharides, Mizuno et al. developed reliable procedures for the extraction, fractionation, and purification of polysaccharides from fruiting bodies or cultured mycelia of medicinal mushrooms or fungi. In general, extraction of polysaccharides involves elimination of low molecular substances from mushroom material with 80% ethanol, followed by three successive extract media with water (100 °C, 3 h), 2% ammonium oxalate (100 °C, 6 h), and 5% sodium hydroxide (80 °C, 6 h) (Mizuno, 1999; Wasser, 2002). Based on this procedure, our group prepared three water-soluble polysaccharides (PL-W, PL-A, and PL-N) from *P. linteus* mycelia using three different extraction media, namely, hot water, 1% ammonium oxalate, and 1.25 M sodium hydroxide/0.05% sodium borohydride solutions in sequence (Fig. 2) (Wang, Pei et al., 2014). The results suggested that all of the three polysaccharides exhibited strong scavenging capacity and antioxidant activities. These polysaccharides could also be developed as potential natural antioxidants for applications in food additives and biomedical industries. Nonetheless, the

extraction method developed by Mizuno et al. can be varied according to structure and water solubility of polysaccharides from mushrooms.

The aqueous extracts or cultured filtrates of *Phellinus* s. l. have been subjected to ethanol precipitation (~80% v/v ethanol), fractional precipitation, and acidic precipitation with acetic acid to yield crude polysaccharides mixed with proteins and other substances, such as pigments. The extracted crude polysaccharides can be further purified using a series of combination technologies, including chemical/enzymatic treatment (to remove proteins), decolorization by active carbon or H_2O_2 (to remove pigments), and dialysis (to remove small molecules), followed by different methods of column chromatography, such as ion-exchange chromatography, gel-filtration chromatography, or affinity chromatography, eluting with an appropriate running buffer, collecting, concentrating, dialyzing, and lyophilizing. Finally, pure and homogeneous polysaccharides from *Phellinus* s. l. can be obtained (Ge et al., 2013; He et al., 2012; Kim et al., 2004; Kim, Oh et al., 2003; Kim, Park, Nam et al., 2003; Nakamura et al., 2004; Park et al., 2003). For example, Kim et al. isolated a 150 kDa acidic proteoglycan from *P. linteus* by hot water extraction, filtration, solvent precipitation, dialysis, and freeze drying, followed by anion-exchange column chromatography; the obtained proteoglycan exhibited both immunoregulatory and antitumor effects (Kim Park Nam et al., 2003). Various homogeneous antitumor polysaccharides have been obtained from *Phellinus* s. l. using different extraction methods and purification processes (Table 1).

STRUCTURAL AND PHYSICOCHEMICAL PROPERTIES

Polysaccharides derived from mushrooms with strong antitumor activities significantly differ in chemical structures and physical properties. The physicochemical and structural characterizations of polysaccharides are commonly defined by molecular weight, monosaccharide composition, configuration and position of glycosidic linkages, type and polymerization degree of branches, monosaccharide sequence, chain conformation, solubility, particle size, and rheological properties (Cui, 2005; Zhang et al., 2007). Various antitumor polysaccharides with different chemical structures have been identified from *Phellinus* s. l. The primary structural features including molecular weight, monosaccharide composition, and glycosidic linkages, as well as fungi species, polysaccharide source, extraction and isolation methods, and corresponding references are summarized in Table 1.

Different polysaccharides isolated from *Phellinus* s. l. vary with the extraction methods and origins of raw material. Furthermore, polysaccharides isolated from different strains of *Phellinus* s. l. with same extraction method and purification process shows different in chemical compositions, molecular weights, and chemical structures. In addition, polysaccharides belong to a structurally diverse class of macromolecules. The monosaccharide units in polysaccharides can interconnect at several points to produce various branched or linear structures (Sharon & Lis, 1993). In the literature, heteropolysaccharides or polysaccharide–protein complexes are the most reported antitumor polysaccharides that were isolated from *Phellinus* s. l. Kim Park Nam et al.

(2003) purified an acidic proteo-heteroglycan, which comprises 72.7% polysaccharide and 22.3% protein, from the fruiting body of *P. linteus* by hot water extraction. This polysaccharide–protein complex is composed of mannose, galactose, and glucose in a molar ratio of 2:1:1, and the molecular weight is approximately 150 kDa. The acidic proteo-heteroglycan was a novel biomolecule composed of both α- and β-linkages and a (1→6) branched type (1→3) glycan. Baker et al. (2008) isolated a water-soluble polysaccharide from the fruiting body of *P. linteus* by hot water extraction, ammonium sulfate precipitation, dialysis/concentration, and anion-exchange chromatographic steps. This polysaccharide is mainly composed of glucose and mannose with average molecular weight of ~25 kDa, and the primary structure was proposed as a main chain of β-(1→3)-glucan and side chains of β-(1→3)-mannose linked through a β-(1→6)-linkage (Fig. 3a).

As another important *Phellinus* s. l. species, *P. igniarius* is mainly found in China and is a titbit in traditional Chinese medicine for many years. Over the past decades, some bioactive polysaccharides with different chemical structures have been isolated from *P. igniarius*. For example, Wu et al. (2006) isolated a homogeneous polysaccharide, PIP_1, from the cultured mycelium of *P. igniarius*. PIP_1 has a molecular weight of 17 kDa and comprises glucose, galactose, and mannose in a molar ratio of 3.7:4.1:1.0. The main chain of PIP_1 is composed of glucose (1→3) and mannose (1→4) with side chains of glucose (1→3) and galactose (1→6). Two chains are linked via 6-O of glucose (1→3,6) and mannose (1→4,6). Yang et al. (2007)

isolated a novel heteropolysaccharide, PIP60-1, from the fruiting bodies of *P. igniarius*. PIP60-1 has a molecular weight of 17.1 kDa and is composed of L-fucose, D-glucose, D-mannose, D-galactose, and 3-O-Me-D-galactose in a ratio of 1:1:1:2:1, which also established a repeating unit. Yang et al. (2009) also isolated a heteropolysaccharide, PISP1 (~22 kDa), from the fruiting bodies of *P. igniarius* by hot water extraction, which was purified by DEAE-Sepharose anion-exchange and gel-filtration chromatography. PIPS1 is composed of fucose, galactose, mannose, and 3-O-Me-galactose in a ratio of 1:2:1:2. The chemical structure of PIPS1 has been elucidated (Fig.3b).

Recently, several other polysaccharides have been discovered in *P. baumii* Pilát, *P. ribis*, and *P. nigricans*. For instance, Ge et al. (2009) isolated a novel water-soluble heteropolysaccharide, PBF2, from the fruiting bodies of *P. baumii* Pilát. PBF2 (~2000 kDa) comprises L-fucose, D-mannose, and D-glucose. PBF2 mainly contains a β-(1→6)-D-glucopyranose backbone with a fucosyl unit on O-3 of the 3,6-di-O-substituted-D-glucosyl units, as well as a minor (1→3,6)-β-D-mannose residue and terminal glucose residues. Ge et al. (2013) purified another water-soluble polysaccharide (PBF3) from the fruiting bodies of *P. baumii* Pilát. PBF3 (~230 kDa) is a complex β-D-glucan. PBF3 has a backbone of (1→4)-β-D- and (1→3)-β-D-glucopyranosyl units, in which one single unit β-D-glucopyranosyl branch is substituted at O-6 of the 3,6-di-O-substituted-D-glucosyl units (Fig. 3c). Liu & Wang (2007) isolated a water-soluble polysaccharide, PRP, from the fruiting

bodies of *P. ribis* by hot water extraction, DEAE-cellulose, and Superdex 30 column chromatography. PRP is a β-D-glucan containing a (1→4), (1→6)-linked backbone with a single β-D-glucose at the C-3 position of (1→6)-linked glucosyl residue at every eight residues along the main chain. The glucan has an average molecular weight of approximately 8.59 kDa (Fig. 3d). Li et al. (2008) also isolated two proteoglycans, PNW1 and PNM1, from the mycelium of *P. nigricans* via submerged fermentation and culture medium. PNW1 and PNM1 with similar average molecular weight (33 and 29 kDa) are composed of glucose, galactose, mannose, arabinose, and fucose in the molar ratios of 3.3:8.8:6.4:1.4 and 20.1:8.7:6.9:1.0:0.8, respectively. The repeating units of PNW1 and PNM1 were also established.

ANTITUMOR ACTIVITY AND MECHANISMS OF ACTION

Polysaccharides derived from fruiting bodies, cultured mycelia, and filtrates of mushrooms (or fungi) have exhibited strong antitumor activities. The antitumor effects of polysaccharides extracted from mushrooms were first published by Chihara in the 1960s (Chihara, 1969). Since then, researchers have isolated numerous structural diversified polysaccharides with strong antitumor activities from mushrooms. These antitumor activities, as well as their mechanisms of action, have been extensively studied (Ren et al., 2012; Wasser, 2002; Zhang et al., 2007; Zong et al., 2012). In contrast to traditional antitumor drugs, polysaccharides obtained from mushrooms could exhibit strong antitumor activities primarily based on the diversity of their mechanisms of action. The current understanding of antitumor effects of mushroom

polysaccharides includes: (1) prevention of oncogenesis by oral consumption of mushrooms or their preparations (cancer-preventing activity); (2) enhancement of immunity against bearing tumors (immune-enhancing activity); and (3) direct antitumor activity by inducing apoptosis of tumor cells and inhibiting tumor metastasis (direct tumor inhibition activity) (Wasser, 2002; Zhang et al., 2007). Numerous studies have demonstrated that polysaccharides isolated from *Phellinus* s. l. could inhibit tumor growth resulted from two basic mechanisms, that is, immune modulation and direct tumor inhibition activities (Table 2, Fig.4).

Immune modulation activity

Polysaccharides from *Phellinus* s. l. exert antitumor activities mainly via stimulation of host defense mechanisms involving activation of different immune cells, such as T and B lymphocytes, macrophages, DCs, and natural killer (NK) cells, which act as immunomodulators or biological response modifiers (BRMs) (Zhu et al., 2008). Purified polysaccharides from the mycelial culture of *P. linteus* stimulate the proliferation of T lymphocytes and the humoral immune function including acting as a polyclonal activator on B cells and inhibiting tumor growth and metastasis (Han et al., 1999; Kim et al., 1996). Kim Park Lee et al. (2003) demonstrated that an acidic proteoglycan (APG) from the fruiting body of *P. linteus* is a BRM that selectively stimulates proliferation and expression of co-stimulatory molecules in B cells but not T cells, probably by regulating protein tyrosine kinase (PTK) and protein kinase C (PKC) signaling pathways. Further study suggested that APG-treated murine peritoneal macrophages

(PM) *in vitro* and *in vivo* significantly showed enhanced production of NO for tumoricidal activity through PTK and PKC signaling pathways (Kim, Oh et al., 2003). Subsequently, the tumoricidal activity of PM cultured with APG against B16 melanoma and Yac-1 cells via upregulation of NO and TNF-α production has enhanced the expression of CD80, CD86, and MCF II in PM. These results suggested that the isolated polysaccharides from *P. linteus* acted as effective immunomodulators and enhanced the antitumor activities of PM (Kim, Choi et al., 2004). Kim et al. (2006) also demonstrated that a novel polysaccharide–protein complex extracted from *P. linteus* markedly increased B-cell proliferation, production of cytokines and nitric oxide from macrophages, and NK cell-mediated killing of Yac-1 lymphoma cells *in vitro*. Moreover, when HT-29 colon cancer cell-bearing mice were treated *in vivo* with proteoglycans extracted from *P. linteus*, a relative increase in spleen and thymus weights were noted. Significant changes in plasma biochemical parameters showed that polysaccharides from *P. linteus* acted as immunopotentiators partly through protecting T cells and enhancing mucosal IgA responses (Li et al., 2011).

DCs are potent antigen-presenting cells capable of capturing antigens, as well as processing and presenting antigenic peptide fragments. Mature DCs can migrate to lymphoid organs and can enhance prime T cells (Banchereau & Steinman, 1998). Given these properties, DCs are promising in cancer immunotherapy. An acidic polysaccharide isolated from *P. linteus* directly induced the maturation of BM-derived murine DC via PTK and PKC signal pathways with the

interaction of CD 11b and/or CD18 and enhanced the phenotypic and functional maturation of DC (Park et al., 2003). In another study, Kim Han et al. (2004) indicated that a proteoglycan (PG) isolated from *P. linteus* induced the phenotypic and functional maturation of murine DCs via toll-like receptors 2- and 4-mediated-NF-Kb, ERK, and p38 MARK signaling pathways. DCs do not only induce activation of T cells but are also associated with polarization of T cells. Kim Oh et al. (2004) found that the administration of PG-induced antitumor and immunomodulating activities through a mechanism leading to a Th-1 dominant immune state and activation of CD11c+CD8+ DC in MCA-102 tumor-bearing mice. These results suggested that the polysaccharides isolated from *P. linteus* augmented the antitumor effects of DC-based immunotherapy.

Some polysaccharides isolated from other *Phellinus* s. l. species, such as *P. igniarius* and *P. nigricans*, have also exerted strong antitumor effects by immunomodulation. For example, an endo-polysaccharide (PIE) extracted from the submerged fermentation product of *P. igniarius* suppressed the proliferation of tumor cells S180 and H22 in implanted mice through enhancement of cell-mediated immunity (Chen et al., 2011). Two proteoglycans (PNW1 and PNM1) isolated from the cultured mycelium of *P. nigricans* exhibited antitumor activity against mice-transplanted Sarcoma 180 *in vivo* by stimulating lymphocytes proliferation and increasing production of NO and TNF-α in macrophages (Li et al., 2008).

Direct tumor inhibition activity

Many studies have demonstrated that polysaccharides from *Phellinus* s. l. species did not only stimulate T lymphocytes and immune function via immunomodulation activity but also have direct tumor inhibition activity through diverse mechanisms, including cell-cycle arrest, induction of tumor cell death by apoptosis and secondary necrosis, inhibition of metastasis, and inhibition of angiogenesis according to *in vitro* and *in vivo* assays.

Induction of apoptosis

The death of tumor cells undergoing antitumor therapy can be through apoptosis and/or necrosis. Apoptosis is a form of cell death in which a programmed sequence of events results in the ingestion of cell remains by surrounding cells without releasing harmful substances (Harhaji et al., 2008). Apoptosis is tightly controlled by a number of gene products that either promote or block cell death at different stages of the cell cycle (Zhang et al., 2006). Deregulation of apoptosis is related to cancer progression. Several polysaccharides from *Phellinus* s. l. species exhibited direct inhibitory effects on cancer cell growth by modulating cell-cycle progression and inducing apoptosis.

Li et al. (2004) found that a protein-bound polysaccharide (PBP) from *P. linteus* had an antiproliferative effect for SW480 colon cancer cells, and the growth inhibition was mediated by induction of apoptosis and G2/M cell-cycle arrest, which were associated with a decrease in Bcl-2, increased release of cytochrome c, and reduced expression of cyclin B1. PBP could also cause a significant reduction in β-catenin protein levels and downregulation of certain

downstream genes in the Wnt/β-catenin pathway in SW480 colon cancer cells *in vitro* and *in vivo*. PBP could also significantly reduce invasiveness of SW480 cells through a direct effect on the activity of cellular MMPs, motility, and angiogenesis, which were strongly associated with Wnt/β-catenin signaling (Song et al., 2011). Recently, Li et al. (2011) reported that a novel proteoglycan (P1) purified from the fruiting body of *P. linteus* inhibited colorectal carcinoma by increasing the immune responses of T cells and IgA and by disrupting the Reg IV/EGFR/Akt signaling pathway. These results suggested that P1 did not only possess immunomodulatory activity but could also directly act on cancer cells. To investigate the underlying mechanism of P1 on colorectal cancer, cell-cycle alternation, and DNA damage, the effects of P1 on cell proliferation *in vitro* and *in vivo*, cell-cycle distribution, apoptosis, autophagy, and expression of several cell-cycle interrelated proteins in HT-29 cells were examined. The results showed that P1 inhibited cell proliferation of HT-29 cells by S-phase arrest through activation of the P27kip 1-cyclin D1/E-CDK2 pathway. Thus, P1 could be explored as a potential candidate for colorectal cancer treatment (Zhong et al., 2013). P1 also exhibited significant antiproliferative effects on HepG2 cells. The inhibition of HepG2 cell growth induced by P1 was mediated through the induction of S-phase arrest by suppressing CRT expression and activating the p27Kip1-cyclin A/D1/E-CDK2 pathway (Li et al., 2013). Zhu et al. (2007) also demonstrated that high doses of polysaccharides from *P. linteus* did not only activate the androgen receptor-dependent pathway via caspase 2, which is a specific intracellular switch for regulating susceptibility of prostate

cancer LNCaP cells, but also induced apoptosis in prostate cancer LNCaP, and PC3 cells activated the androgen receptor-independent pathway. Therefore, polysaccharides isolated from *P. linteus* induce cell-cycle arrest and apoptosis in different cancer cells, including human colon carcinoma, human hepatocellular carcinoma, melanoma, epidermoid, lung cancer, and prostate cancer cells by regulating signaling pathways and direct tumor inhibition activities.

In addition to polysaccharides from *P. linteus*, several polysaccharides isolated from other *Phellinus* s. l. species, such as *P. baumii* and *P. gilvus*, also exhibit antitumor effects via induction of apoptosis. For example, A 17-kDa water-soluble polysaccharide (PB) isolated from the cultured mycelium of *P. baumii* markedly inhibited the proliferation of HepG2 human liver cancer cells *in vitro* by inducing cell-cycle arrest at the S phase leading to apoptosis (Xue et al., 2011). Polysaccharides isolated from the fruiting body of *P. gilvus* does not only significantly inhibit B16F10 melanoma growth in mice by decreasing cell proliferation and increasing cell apoptosis but also inhibits BaP-induced forestomach carcinogenesis in mice and induces cancer cell apoptosis by down regulating mutant p53 expression (Bae et al., 2005a, b).

Inhibition of metastasis

Chemotherapy is an important therapeutic modality for managing various cancers. However, chemotherapy frequently fails to achieve a satisfactory therapeutic outcome, such as complete remission or prevention of distant metastasis without major chemotherapy-related side effects (Salgaller & Lodge, 1998). Cancer metastasis, in particular, is a major medicinal problem in

treating cancer. Cancer metastasis consists of several processes including abnormal cell proliferation, invasion, migration, and adhesion. The potential application of polysaccharides from *Phellinus* s. l. as an immunotherapeutic agent, specifically for cancer metastasis, has been actively investigated. For example, Han et al. (1999) demonstrated that an acidic polysaccharide (PL) isolated from the cultured mycelium of *P. linteus* alone significantly prolongs the survival rate of B16F10 cell-implanted mice, inhibits tumor growth in NCI-H23 cell-implanted nude, and reduces the frequency of pulmonary metastasis of B16F10 cell melanomas compared with adriamycin, which significantly inhibits tumor growth but only slightly inhibits metastasis. PL might be applied in immunochemotherapy of cancer because of its effective activities against tumor growth and metastasis via immunopotentiation of patients without toxicity. Further study also found that PL markedly inhibits cancer cell adhesion and invasion through interaction with cell-to-extracellular matrix *in vitro* and *in vivo* tests in B16F10 melanoma cells and in mice, respectively. However, PL has no direct effect on cancer cell growth. PL also increases NO production by macrophages (Han et al., 2006).

Inhibition of angiogenesis

Angiogenesis, the formation of new vessels from preexisting microvascular networks, plays a crucial role in tumor progression (Hanahan & Folkman, 1996). In cancers, new vessel formation contributes to the progressive growth and metastasis of solid tumors (Bergers & Benjamin, 2003). Thus, inhibition of angiogenesis can be a potential strategy to suppress tumor

growth and metastasis for cancer therapy. Recently, Liu et al. (2009) demonstrated that sulfated derivatives of a polysaccharide (PRP) obtained from *P. ribis* showed significant inhibition effects on the intersegmental vessel formation of zebrafish. Further study also indicated that the two sulfated derivatives (PRP-S1 and PRP-S2) could block formation of new vessels in zebrafish and inhibit the proliferation of HUVECs. They exhibited remarkably high antitumor activities *in vivo* (in BALB/c mice inoculated with H22 cells) and *in vitro* (against SKOV-3 cells), without producing any overt signs of general toxicity. In addition, PRP-S1 and PRP-S2 significantly reduced the average number of MVD and inhibited the expression of VEGF in tumor. The pronounced antitumor effects of PRP-S1 and PRP-S2 are mediated via their anti-angiogenic properties (Liu et al., 2014). These results demonstrated that sulfated polysaccharides obtained from *P. ribis* could be alternative therapeutic agents for angiogenesis-mediated tumor treatment.

RELATIONSHIP BETWEEN STRUCTURE AND ANTITUMOR ACTIVITY

It is well known that the antitumor activities of the polysaccharide are strongly related to its molecular structure. Polysaccharides with different monosaccharide composition, configuration of glycosidic linkages, backbone chains and branched structures, molecular weights, substituent groups, as well as their chain conformations have an important influence on their biological activities (Wasser, 2002; Zhang et al., 2007). Moreover, the effects of these factors on biological activities are also interrelated. Therefore, the development of structure-antitumor activity relationship will be beneficial for revealing the chemical basis of antitumor polysaccharides.

Meanwhile, the study on relationship between structure and antitumor activity will also provide important guidance for purpose screening, molecular modifications, and chemical synthesis of antitumor polysaccharides. Although it is difficult to clarify the correlation of the structure and antitumor activities of such complex macromolecules, some structure-antitumor activity relationship of *Phellinus* polysaccharides have been inferred as follows.

Previous studies have indicated that structural features such as β-(1→3) linkages in the main chain of the glucan and additional β-(1→6) branches were important for the antitumor activity by increasing immune-competent cell activity (Wasser, 2002). However, the antitumor polysaccharides isolated from *Phellinus* s. l. with different chemical structures were reported, such as hetero-β-glucans (Ge et al., 2013; Liu & Wang, 2007), heteroglycan (Baker et al., 2008), acidic proteo-heteroglycan (Kim Park Nam et al., 2003), acidic proteoglycan (Park et al., 2003), proteoglycan (Kim Han et al., 2004) and protein-bound polysaccharide (Li et al., 2004). More specifically, it has been postulated that fungal polysaccharides containing glucose and mannose may have some antitumor action because a polysaccharide receptor has been found on human macrophages, which has demonstrated high specificity for glucose and mannose (Lombard, 1994). For example, some polysaccharides isolated from *Phellinus* s. l. were found to be mainly composed of glucose and mannose possessing antitumor activities (Kim Park Nam et al., 2003; Kim et al., 2006; Barker et al., 2008).

In general, the greater the molecular weight and the higher the water solubility of the

polysaccharide, the higher the antitumor activity. A high molecular weight is necessary for extensively enhancing immunological and antitumor activities of the polysaccharide. It has been reported that the medicinal properties of β-(1→3)-glucans were strongly dependent on high molecular weight, ranging from 500 to 2000 kDa (Mizuno et al., 1996). However, some heteropolysaccharides and proteoglycan isolated from *Phellinus* s. l. being reported to show antitumor activities have the molecular weights ranging from 15 to 150 kDa (Table 2). In addition, Nakamura et al. (2004) isolated a protein-α-1,3-glucan complex from *P. linteus* mycelia by precipitating the 24% NaOH extract at pH 6.0, it had a molecular weight of 1000-2000 kDa and showed high anti-tumor activity toward solid tumors planted in mice.

The improvement of the biological activity of polysaccharides that show antitumor activity can be achieved by chemical modifications. Various sulfated, carboxymethylated, hydroxylated, formylmethylated, and aminethylated products have been designed in the literature (Ren et al., 2012; Wasser, 2002). For instance, Liu et al. (2009) reported that four sulfated derivatives (PRP-SI-IV) of the polysaccharide from *P. ribis* with variable degrees of substitution were prepared by the chlorosulfonic acid method. The sulfated derivatives except for PRP-SI showed significant inhibition effects on HepG2 cells comparison with the native non-sulfated polysaccharide (PRP). Furthermore, the polysaccharide isolated from *P. linteus* contained (1→3)-β-gulcans with a (1→6)-linkage was chemically modified by carboxymethylation, and the carboxymethylation stimulated *in vitro* cytotoxic activity against the HT1080 cell line. The

result indicated that the derivative exhibited the enhanced activity of immune systems, which would be explained by the improved water solubility and structural changes by carboxymethylation (Shin et al., 2007). Therefore, these investigations give the direction that the improvement of the antitumor activities of polysaccharides may be effectively approach by chemical modifications (Ooi & Liu, 2000).

CONCLUSION AND PERSPECTIVES

Phellinus s. l. is one of the most popular medicinal mushrooms in traditional Chinese medicine that has been widely used for centuries in China, Japan, and Korea to prevent or treat gastroenteric dysfunction, diarrhea, hemorrhage, allergy, and cancer. Polysaccharides are among the major components responsible for several biological activities, particularly antitumor activity, which have garnered increasing attention from both scientific researchers and common people. Some bioactive polysaccharides and/or polysaccharide–protein complexes have been isolated from fruiting bodies, cultured mycelia, and filtrates of *Phellinus* s. l. species, such as *P. linteus*, *P. igniarius*, and *P. baumii* Pilát. The structural characterizations of these biomolecules, including monosaccharide composition, molecular weight, configuration, and position of glycosidic linkages have been elucidated. The antitumor activities and mechanisms of these polysaccharides have been studied for many years, and the results suggest that polysaccharides from *Phellinus* s. l. exhibit strong antitumor effects mainly through several preliminary antitumor mechanisms, such as stimulation of macrophages, cell-cycle arrest, induction of tumor cell death by apoptosis and

secondary necrosis, inhibition of metastasis, and anti-angiogenesis. Therefore, polysaccharides from *Phellinus* s. l. have significant potential for further development in therapy or adjuvant therapy for cancers.

However, some important issues need urgent attention. First, the current procedure for isolation of polysaccharides is very tedious and intricate and is only useful for analytical or preparative purposes in the laboratory, but unsuitable for large-scale processing. New, facile, and efficient methods, such as ionic liquid-based aqueous two-phase system and nanoknife technology, for extracting and isolating polysaccharides from *Phellinus* s. l. should be developed. Second, elucidating the chemical structures and chain conformations of polysaccharides from *Phellinus* s. l. are very important to understand their biological activities. However, the precise structures (high-order structure) of these bioactive polysaccharides, as well as the relationship between the structure and bioactivities, are still not well established. Further structural characterization and evaluation of the bioactivities are important for their application in food and medicinal fields. Finally, although antitumor effects and mechanisms of polysaccharides from *Phellinus* s. l. have been frequently reported, more scientific studies are necessary to clarify the biochemical mechanisms and build upon the theories and to characterize the responsible structural parameters via chemical routes and biological molecular techniques.

ACKNOWLEDGEMENTS

This work was supported financially by the Natural Science Foundation of Jiangsu Province (BK20140542), China Postdoctoral Science Foundation funded project (2013M531292), the

Priority Academic Program Development (PAPD) of Jiangsu Higher Education Institutions and the Science and Technology Support Plan (Industry) of Zhenjiang (GY2013006).

REFERENCES

Bae, J.S., Jang, K., Yim, H., & Jin, H. (2005a). Polysaccharides isolated from *Phellinus gilvus* inhibit melanoma growth in mice. *Cancer Lett*. **218**: 43-52.

Bae, J.S., Jang, K.H., Yim, H., Park, S.C., & Jin, H.K. (2005b). Inhibitory effects of polysaccharides isolated from *Phellinus gilvus* on benzo(a)pyrene-induced forestomach carcinogenesis in mice. *W. J. Gastroentero.* **11**: 577-579.

Baker, J.R., Kim, J.S., & Park, S.Y. (2008). Composition and proposed structure of a water-soluble glycan from the Keumsa Sangwhang mushroom (*Phellinus linteus*). *Fitoterapia*. **79**: 345-350.

Banchereau, J., & Steinman, R.M. (1998). Dendritic cells and the control of immunity. *Nature*. **392**: 245-252.

Bergers, G., & Benjamin, L.E. (2003). Tumorigenesis and the angiogenesis switch. *Nat. Rev. Cancer*. **3**: 401-410.

Boh, B., & Berovic, M. (2007). *Grifola frondosa* (Dicks.: Fr.) SF Gray (Maitake mushroom): Medicinal properties, active compounds, and biotechnological cultivation. *Int. J. Med. Mushrooms*. **9**: 89-108.

Chen, L., Pan, J., Li, X., Zhou, Y., Meng, Q., &Wang, Q. (2011). Endo-polysaccharide of *Phellinus igniarius* exhibited anti-tumor effect through enhancement of cell mediated immunity. *Int. Immunopharmacol.* **11**: 255-259.

Chihara, G. (1969). The antitumor polysaccharide lentinan: an overview. In T. Aoki, et al. (Eds.), *Manipulation of host defense mechanism*. *Nature*. **222**: 687-694.

Cui, J. (2003). Polysaccharopeptides of *Coriolus versicolor*: physiological activity, uses, and production. *Biotechnol. Adv.* **21**: 109-122.

Cui, S.W. (2005). Structural analysis of polysaccharides. In Steve W. Cui (Ed.), *Food carbohydrates*: *Chemistry*, *physical properties and applications* (1 edition). Boca Raton, FL: CRC Press.

Dai, Y.C., Yang, Z.L., Cui, B.K., Yu, C.J., & Zhou, L.W. (2009). Species diversity and utilization of medicinal mushrooms and fungi in China (review). *Int. J. Med. Mushrooms*. **11**: 287-302.

Dai, Y.C., Zhou, L.W., Cui, B.K., Chen, Y.Q., & Decock, C. (2010). Current advances in *Phellinus* sensu lato: medicinal species, functions, metabolites and mechanisms. *Appl. Microbiol. Biot.* **87**: 1587-1593.

Dong, W., Ning, L., Lu, W., Li, C., Chen, R., Jia, X., Wang, L., & Guo, L. (2009). Tumor-inhibitory and liver-protective effects of *Phellinus igniarius* extracellular polysaccharides. *World J. Microb. Biot.* **25**: 633-638.

Efferth, T., Fu, Y.J., Zu, Y.G., Schwarz, G., Konkimalla, V.S.B., &Wink, M. (2007). Molecular target-guided tumor therapy with natural products derived from traditional Chinese medicine. *Curr. Med. Chem.* **14**: 2024-2032.

Ge, Q., Mao, J., Zhang, A., Wang, Y., & Sun, P. (2013). Purification, chemical characterization, and antioxidant activity of a polysaccharide from the fruiting bodies of Sanghuang mushroom (*Phellinus Baumii Pilát*). *Food Sci. Biotechnol.* **22**: 301-307.

Ge, Q., Zhang, A., & Sun, P. (2009). Structural investigation of a novel water-soluble heteropolysaccharide from the fruiting bodies of *Phellinus Baumii Pilát*. *Food Chem.* **114**: 391-395.

Giavasis, I. (2014). Bioactive fungal polysaccharides as potential functional ingredients in food and nutraceuticals. *Curr. Opin. Biotechnol.* **26**: 162-173.

Guo, X., Zou, X., & Sun, M. (2010). Optimization of extraction process by response surface methodology and preliminary characterization of polysaccharides from *Phellinus igniarius*. *Carbohydr. Polym.* **80**: 344-349.

Han, S.B., Lee, C.W., Jeon, Y.J., Hong, N.D., Yoo, I.D., Yang, K.H., & Kim, H.M. (1999). The inhibitory effect of polysaccharides isolated from *Phellinus linteus* on tumor growth and metastasis. *Immunopharmacology*. **41**: 157-164.

Han, S.B., Lee, C.W., Kang, J.S., Yoon, Y.D., Lee, K.H., Lee, K., Park, S.K., & Kim, H.M. (2006). Acidic polysaccharide from *Phellinus linteus* inhibits melanoma cell metastasis by blocking cell adhesion and invasion. *Int. Immunopharmacol.* **6**: 697-702.

Hanahan, D., & Folkman, J. (1996). Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell*. **86**: 353-364.

Harhajia, Lj., Mijatovića, S., Maksimović-Ivanića, D., Stojanovića, I., Momčilovića, M., Maksimovićb, V., Tufegdžićb, S., Marjanovićb, Ž., Mostarica-Stojkovićc, M., Vučinićb, Ž., Stošić-Grujičić, S. (2008). Anti-tumor effect of *Coriolus versicolor* methanol extract against mouse B16 melanoma cells: *In vitro* and *in vivo* study. *Food Chem. Toxicol.* **46**: 1825-1833.

He, P., Geng, L., Wang, J., Wang, Z., Mao, D., & Xu. C. (2012). Purification, characterization and bioactivity of an extracellular polysaccharide produced from *Phellinus igniarius*. *Ann. Microbiol.* **62**: 1697-1707.

Hsieh, P.W., Wu, J.B., & Wu, Y.C. (2013). Chemistry and biology of *Phellinus linteus*. *BioMedicine*. **3**: 106-113.

Hwang, H.J., Kim, S.W., Choi, J.W., Yun, J.W. (2003). Production and characterization of exopolysaccharides from submerged culture of *Phellinus linteus* KCTC6190. *Enzyme Microb. Tech.* **33**: 309-319.

Ikekawa, T., Nakanishi, M., Uehara, N., Chihara, G., & Fukuoka, F. (1968). Antitumor action of some Basidiomycetes, especially *Phellinus linteus*. *Gann*. **59**: 155-157.

Jin, M., Zhao, K., Huang, Q., Xu, C., & Shang, P. (2012). Isolation, structure and bioactivities of the polysaccharides from *Angelica sinensis* (Oliv.) Diels: A review. *Carbohydr. Polym.* **89**: 713-722.

Kim, H.M., Han, S.B., Oh, G.T., Kim, Y.H., Hong, D.H., Hong, N.D., & Yoo, I.D. (1996). Stimulation of humoral and cell mediated immunity by polysaccharide from mushroom

Phellinus linteus. *Int. J. Immunopharmacol.* **18**: 295-303.

Kim, S.W., Hwang, H.J., Park, J.P., Cho, Y.J., Song, C.H., & Yun, J.W. (2002). Mycelial growth and exo-biopolymer production by submerged culture of various edible mushrooms under different media. *Lett. Appl. Microbiol.* **34**: 56-61.

Kim, G.Y., Oh, Y.H., & Park, Y.M. (2003). Acidic polysaccharide isolated from *Phellinus linteus* induces nitric oxide-mediated tumoricidal activity of macrophages through protein tyrosine kinase and protein kinase C. *Biochem. Biophys. Res. Commun.* **309**: 399-407.

Kim, G.Y., Park, H.S., Nam, B.H., Lee, S.J., & Lee, J.D. (2003). Purification and characterization of acidic proteo-heteroglycan from the fruiting body of *Phellinus linteus* (Berk., & M.A. Curtis) Teng. *Bioresource Technol.* **89**: 81-87.

Kim, G.Y., Park, S.W., Lee, M.K., Lee, S.H., Oh, Y.H., Kwak, J.Y., Yoon, S., Lee, J.D., & Park, Y.M. (2003). Proteoglycan isolated from *Phellinus linteus* activities murine B lymphocytes via protein kinase C and protein tyrosine kinase. *Int. Immunopharmacol.* **3**: 1281-1292.

Kim, G.Y., Choi, G.S., Lee, S.H., & Park, Y.M. (2004). Acidic polysaccharide isolated from *Phellinus linteus* enhances through the up-regulation of nitric oxide and tumor necrosis factor-α from peritoneal macrophages. *J. Ethnopharmacol.* **95**: 69-76.

Kim, G.Y., Han, M.G., Song, Y.S., Shin, B.C., Shin, Y.I., Lee, H.J., Moon, D.O., Lee, C.M., Kwak, J.Y., Bae, Y.S., Lee, J.D., & Park, Y.M. (2004). Proteoglycan isolated from *Phellinus linteus* induced Toll-like receptors 2- and 4-mediated maturation of murine dendritic cells via

activation of ERK, p38, and NF-κB. *Biol. Pharm. Bull.* **27**: 1656-1662.

Kim, G.Y., Oh, W.K., Shin, B.C., Shin, Y.I., Park, Y.C., Ahn, S.C., Lee, J.D., Bae, Y.S., Kwak, J.Y., & Park, Y.M. (2004). Proteoglycan isolated from *Phellinus linteus* inhibits tumor growth through mechanisms leading to an activation of CD11c+CD8+ DC and type I helper T cell-dominant immune state. *FEBS Lett.* **576**: 391-400.

Kim, G.Y., Lee, J.Y., Lee, J.O., Ryu, C.H., Choi, B.T., Jeong, Y.K., Lee, K.W., Jeong, S.C., & Choi, Y.H. (2006). Partial characterization and immunostimulatory effect of a novel polysaccharide-protein complex extracted from *Phellinus linteus*. *Biosci. Biotech. Biochem.* **70**: 1218-1226.

Larsen, M.J., & Cobb-Poulle, L.A. (1990). *Phellinus* (Hymenochaetaceae). A survey of the world taxa. *Synopsis Fungorum*. **3**: 1-206.

Lee, J.H., Cho, S.M., Ko, K.S., & Yoo, I.D. (1995). Effect of cultural conditions on polysaccharide production and its monosaccharide composition in *Phellinus linteus* L13202. *Korean J. Mycol.* **23**: 325-331.

Lee, J.H., Cho, S.M., Song, K.S., Hong, N.D., & Yoo, I.D. (1996). Characterization of carbohydrate-peptide linkage of acidic heteroglycopeptide with immune-stimulating activity from mycelium of *Phellinus linteus*. *Chem. Pharm. Bull.* (Japan). **44**: 1093-1095.

Li, Y.G., Ji, D.F., Zhong, S., Liu, P.G., Lv, Z.Q., Zhu, J.X., Chen, J.E., & Chen, H.P. (2013). Polysaccharide from *Phellinus linteus* induces S-phase arrest in HepG2 cells by decreasing

calreticulin expression and activating the P27kip1-cyclin A/D1/E-CDK2 pathway. *J. Ethnopharmacol.* **150**: 187-195.

Li, Y.G., Ji, D.F., Zhong, S., Zhu, J.X., Chen, S., & Hu, G.Y. (2011). Anti-tumor effects of proteoglycan from *Phellinus linteus* by immunomodulating and inhibiting Reg IV/ EGFR/ Akt signaling pathway in colorectal carcinoma. *Int. J. Biol. Macromol.* **48**: 511-517.

Li, X., Jiao, L.L., Zhang, X., Tian, W.M., Chen, S., & Zhang, L.P. (2008). Anti-tumor and immunomodulating activities of proteoglycans from mycelium of *Phellinus nigricans* and culture medium. *Int. Immunopharmacol.* **8**: 909-915.

Li, G., Kim, D.H., Kim, T.D., Park, B.J., Park, H.D., Park, J.I., Na, M.K., Kim, H.C., Hong, N.D., Lim, K., Hwang, B.D., & Yoon, W.H. (2004). Protein-bound polysaccharide from *Phellinus linteus* induces G2/M phase arrest and apoptosis in SW480 human colon cancer cells. *Cancer Lett.* **216**: 175-181.

Lieu, C.H., William, W.N., & Lippman, S.M. (2010). Cancer chemoprevention, in: M. Hidalgo, S.G. Eckhardt, E. Garrett-Mayer, N.J. Clendeninn (Eds.), *Principles of Anticancer Drug Development*, Springer Science, New York, pp. 463-482.

Liu, Y., Liu, C., Tan, H., Zhao, T., Cao, J., & Wang, F. (2009). Sulfation of a polysaccharide obtained from *Phellinus ribis* and potential biological activities of the sulfated derivatives. *Carbohydr. Polym.* **77**: 370-375.

Liu, Y., Liu, Y., Jiang, H., Xu, L., Cheng, Y., Wang, P.G., & Wang, F. (2014). Preparation,

antiangiogenic and antitumoral activities of the chemically sulfated glucan from *Phellinus ribis*. *Carbohydr. Polym.* **106**: 42-48.

Liu, Y., & Wang, F. (2007). Structural characterization of an active polysaccharide from *Phellinus ribis*. *Carbohydr. Polym.* **70**: 386-392.

Lombard, Y.J. (1994). A new method for studying the binding and ingestion of zymosan particles by macrophages. *J. Immunol*. *Methods* **174**: 155-163.

Lull, C., Wichers, H.J., & Savelkoul, H.F.J. (2005). Anti-inflammatory and immunomodulating properties of fungal metabolities. *Mediat. Inflamm.* **2**: 63-80.

Luo, J., Liu, J., Sun, Y., Ye, H., Zhou, C., & Zeng, X. (2010). Medium optimization, preliminary characterization and antioxidant activity *in vivo* of ycelia polysaccharide from *Phellinus baumii Pilát*. *Carbohydr. Polym.* **81**: 533-540.

Ma, X., Zhang, H., Peterson, E.C., & Chen, L. (2014). Enhancing exopolysaccharide antioxidant formation and yield from *Phellinus* species through medium optimization studies. *Carbohydr. Polym.* **107**: 214-220.

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

Mizuno, T., Yeohlui, P., Kinoshita, T., Zhuang, C., Ito, H., & Mayuzumi, Y. (1996). Antitumor activity and chemical modification of polysaccharides from Niohshimeiji mushroom, Tricholima giganteum. *Biosci*. *Biotech*. *Bioch*. **60**: 30-33.

Moradali, M. F., Mostafavi, H., Ghods, S., & Hedjaroude, G. A. (2007). Immunonodulating and anticancer agents in the realm of macromycetes fungi (macrofungi). *Int. Immunopharmacol.* **7**: 701–724.

Mushiake, H., Tsunoda, T., Nukatsuka, M., Shimao, K., Fukushima, M., & Tahara, H. (2005). Dendritic cells might be one of key factors for eliciting antitumor effect by chemoimmunotherapy *in vivo*. *Cancer Immunol. Immun.* **54**: 120-128.

Nakamura, T., Matsugo, S., Uzuka, Y., Matsuo, S., & Kawagishi, H. (2004). Fractionation and anti-tumor activity of the mycelia of liquid-cultured *Phellinus linteus*. *Biosci. Biotech. Biochem.* **68**: 868-872.

Nie, S., Zhang, H., Li, W., & Xie, M. (2013). Current development of polysaccharides from *Ganoderma*: Isolation, structure and bioactivities. *Bioact. Carbohydr. Diet. Fibre*. **1**: 10-20.

Ooi, V.E.C., & Liu, F. (2000). Immunomodulation and anti-cancer activity of polysaccharide-protein complexes. *Curr. Med. Chem.* **7**: 715–729.

OuYang, F., Wang, G., Guo, W., Zhang, Y., Xiang, W., & Zhao, M. (2013). AKT signaling and mitochondrial pathways are involved in mushroom polysaccharide-induced apoptosis and G1 or S phase arrest in human hepatoma cells. *Food Chem.* **138**: 2130-2139.

Park, S.K., Kim, G.Y., Lim, J.Y., Kwak, J.Y., Bae, Y.S., Lee, J.D., Oh, Y.H., Ahn, S.C., & Park, Y.M. (2003). Acidic polysaccharides isolated from *Phellinus linteus* induce phenotypic and functional maturation of murine dendritic cells. *Biochem. Biophys. Res. Commun.* **312**:

449-458.

Park, H.G., Shim, Y.Y., Choi, S.O., & Park, W.M. (2009). New method development for nanoparticle extraction of water-soluble β-(1→3)-D-glucan from edible mushrooms, Sparassis crispa and *Phellinus linteus*. *J. Agric. Food Chem.* **57**: 2147-2154.

Ren, L., Perera, C., & Hemar, Y. (2012). Antitumor activity of mushroom polysaccharide: a review. *Food Funct.* **3**: 1118-1130.

Ren, L., Reynisson, J., Perera, C., & Hemar, Y. (2013). The physicochemical properties of a new class of anticancer fungal polysaccharides: Acomparative study. *Carbohydr. Polym.* **97**: 177-187.

Salgaller, M.L., & Lodge, P.A. (1998). Use of cellular and cytokine adjuvants in the immunotherapy of cancer. *J. Surg. Oncol.* **68**: 122-138.

Seviour, R., McNeil, B., Fazenda, M.L., & Harvey, L.M. (2011). Operating bioreactors for microbial exopolysaccharide production. *Crit. Rev. Biotechnol.* **31**: 170-185.

Sharon, N., & Lis, H. (1993). Carbohydrates in cell recognition: Telltale surface sugars enable cells to identify and interact with one another; new drugs aimed at those carbohydrates could stop infection and inflammation. *Sci. Am.* **268**: 74-81.

Shin, J.Y., Lee, S.Y., Bae, I.Y., Yoo, S.H., & Lee, H.G. (2007). Structural and biological study of carboxymethylated *Phellinus linteus* polysaccharides. *J. Agric. Food Chem.* **55**: 3368-3372.

Sliva, D. (2010). Medicinal mushroom *Phellinus lintues* as an alternative cancer therapy (review).

Exp. Ther. Med. **1**: 407-411.

Song, K.S., Cho, S.M., Lee, J.H., Kim, H.M., Han, S.B., Ko, K.S., et al. (1995). B-lymphocyte-stimulating polysaccharide from mushroom *Phellinus linteus*. *Chem. Pharm. Bull.* (Japan). **43**: 2105-2108.

Song, K.S., Li, G., Kim, J.S., Jing, K., Kim, T.D., Kim, J.P., Seo, S.B., Yoo, J.K., Park, H.D., Hwang, B.D., Lim, K.L., & Yoon, W.H. (2011). Protein-bound polysaccharide from *Phellinus linteus* inhibits tumor growth, invasion, and angiogenesis and alters Wnt/β-catenin in SW480 human colon cancer cells. *BMC Cancer*. **11**: 307.

Stachowiak, B., & Reguła, J. (2012). Health-promoting potential of edible macromycetes under special consideration of polysaccharides: a review. *Eur. Food Res. Technol.* **234**: 369-380.

Sun, P.L., Xu, S.Y., Yang, K., Zhang, J., & Song, L. (2006). Advance of researches on *Phellinus* spp. A rare and precious medicinal fungus. *Microbiology*. **33**: 119-123.

van Griensven, L.J.L.D., & Verhoeven, H.A. (2013). *Phellinus linteus* polysaccharide extracts increase the mitochondrial membrane potential and cause apoptosis death of THP-1 monocytes. *Chin. Med.* **8**: 25.

Wang, Z.B., Pei, J.J., Ma, H.L., Cai, P.F., & Yan, J.K. (2014). Effect od extraction media on preliminary characterizations and antioxidant activities of *Phellinus linteus* polysaccharides. *Carbohydr. Polym.* **109**: 49-55.

Wang, Z., Quan, Y., & Zhou, F. (2014). Optimization of medium composition for exopolysaccharide production by *Phellinus nigricans*. *Carbohydr. Polym.* **105**: 200-206.

Wang, Z., Wang, C., & Quan, Y. (2014). Extraction of polysaccharides from *Phellinus nigricans* mycelia and their antioxidant activities *in vitro*. *Carbohydr. Polym.* **99**: 110-115.

Wasser, S.P. (2002). Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. *Appl. Microbiol. Biot.* **60**: 258-274.

World Health Organization. (2014). Cancer. Retrieved from http://www.who.int/mediacentre/factsheets/fs297/en/.

Wu, M., Jiang, D., Liu, T., & Zhang, L. (2006). Structural analysis of water-soluble polysaccharide PIP1 extracted from the cultured mycelium of *Phellinus igniarius*. *Chem. Res. Chinese U.* **22**: 708-711.

Xu, X.F., Yan, H.D., Tang, J., Chen, J., & Zhang, X.W. (2014). Polysaccharides in *Lentinus edodes*: isolation, structure, immunomodulating activity and future prospective. *Crit. Rev. Food Sci. Nutr.* **54**: 474-487.

Xue, Q., Sun, J., Zhao, M., Zhang, K., & Lai, R. (2011). Immunostimulatory and anti-tumor activity of a water-soluble polysaccharide from *Phellinus baumii* mycelia. *World J. Microb. Biot.* **27**: 1017-1023.

Yang, Y., Ye, L.B., Zhang, J.S., Liu, Y.F., & Tang, Q.J. (2009). Structural analysis of a bioactive polysaccharide, PISP1, from the medicinal mushroom *Phellinus igniarius*. *Biosci. Biotech. Bioch.* **73**: 134-139.

Yang, Y., Zhang, J.S., Liu, Y.F., Tang, Q.J., Zhao, Z.G., & Xia, W.S. (2007). Structural elucidation of a 3-O-methyl-D-galactose-containg neutral polysaccharide from the fruiting

bodies of *Phellinus igniarius*. *Carbohydr. Res.* **342**: 1063-1070.

Zhang, M., Cheung, P.C.K., Chiu, L.C.M., Wong, E.Y.L., & Ooi, V.E.C. (2006). Cell-cycle arrest and apoptosis induction in human breast carcinoma MCF-7 cells by carboxymethylated β-glucan from the mushroom sclerotia of *Pleurolus tuber-regium*. *Carbohydr. Polym.* **66**: 455-462.

Zhang, M., Cui, S.W., Cheung, P.C.K., Wang, Q. (2007). Antitumor polysaccharides from mushrooms: a review on their isolation process, structural characteristics and antitumor activity. *Trends Food Sci. Tech.* **18**: 4-19.

Zhang, H., Ma, H., Liu, W., Pei, J.J., Wang, Z., Zhou, H., & Yan, J. (2014). Ultrasound enhanced production and antioxidant activity of polysaccharides from mycelia fermentation of *Phellinus igniarius*. *Carbohydr. Polym.* **113**: 380-387.

Zhang, Y., Li, S., Wang, X., Zhang, L., & Cheung, P.C.K. (2011). Advances in lentinan: Isolation, structure, chain conformation and bioactivities. *Food Hydrocolloid.* **25**: 196-206.

Zhong, S., Ji, D.F., Li, Y.G., Lin, T.B., Lv, Z.Q., & Chen, H.P. (2013). Activation of P27kip1-cyclin D1/E-CDK2 pathway by polysaccharide from *Phellinus linteus* leads to S-phase arrest in HT-29 cells. *Chem-Biol. Interact.* **206**: 222-229.

Zhong, J.J., & Tang, Y.J. (2004). Submerged cultivation of medicinal mushrooms for production of valuable bioactive metabolites. *Adv. Biochem. Eng./ Biot.* **87**: 25–59.

Zhu, T., Guo, J., Collins, L., Kelly, J., Xiao, Z.J., Kim, S.H., & Chen, C.Y. (2007). *Phellinus*

linteus activate different pathways to induce apoptosis in prostate cancer cells. *Brit. J. Cancer*. **96**: 583-590.

Zhu, T., Kim, S.H., & Chen, C.Y. (2008). A medicinal mushroom: *Phellinus Linteus*. *Curr. Med. Chem.* **15**: 1330-1335.

Zhu, H., Sun, S., & Zhang, S. (2011). Enhanced production of total flavones and exopolysaccharides *via Vitreoscilla* hemoglobin biosynthesis in *Phellinus igniarius*. *Bioresource Technol.* **102**: 1747-1751.

Zong, A., Cao, H., & Wang, F. (2012). Anticancer polysaccharides from natural resources: A review of recent research. *Carbohydr. Polym.* **90**: 1395-1410.

Table 1 The antitumor polysaccharides isolated from *Phellinus* s. l. species.

Species	Source	Extraction and Isolation Methods	Molecular weight (Da)	Monosaccharide composition	Structures	Reference
P. linteus	the fruiting body	hot water extraction, DEAE-Cellulose anion exchange and Sepharose CL-4B gel filtration chromatographies	150	mannose, galactose, glucose =2.0:1.0:1.0	both α- and β-linkages, and a (1→6) branched type (1→3) glycan	Kim, Park, Nam et al., 2003
P. linteus	The cultured mycelium	Precipitating the 24% NaOH extract at pH 6.0	1000-2000	fructose, xylose, mannose, glucose, galactose =1.1:3.6:3.4:87.3:4.7	a protein-α-1,3-glucan complex	Nakamura et al., 2004
P. linteus	the fruiting body	hot water extraction, DEAE-Cellulose anion exchange and Sepharose CL-4B gel filtration chromatog	73	Glucose, mannose=3:2	polysaccharide-protein complex,PPC	Kim et al., 2006

		raphies				
P. linteus	the fruiting body	hot water extraction, ethanol precipitation	860	glucose	β-(1→3)-glucans with a (1→6) linkage	Shin et al., 2007
P. linteus	the fruiting body	hot water extraction, ammonium sulfate precipitation, anion exchange chromatography	25	glucose, mannose	β-(1→3)-glucan via β-(1→6) links with β-(1→3)-mannose chains	Baker et al., 2008
P. linteus	the fruiting body	hot water extraction, HPLC purification	18.8	fucose, rhamanose, galactoseglucose, xylose, mannose, 3-O-Me-galactose=1.0:3.1:33.5:2.0:4.0:1.1:2.9	P1, proteoglycan	Li et al., 2011
P. ignia rius	the cultured mycelium	hot water extraction, ethanol precipitation, Sepharose CL-6B gel filtration chromatography	17	glucose, galactose, mannose=3.7:4.1:1.0	(1→3)-linked glucose in main chain, side chains at 6-O	Wu et al., 2006
P. ignia rius	the fruiting body	hot water extraction, DEAE-Sepharose Fast Flow column chromatography	17.1	fucose, glucose, mannose, galactose, 3-O-Me-galactose = 1:1:1:2:1	PIP60-1, 3-O-Me-galactoe residues in the main chain	Yang et al., 2007

P. ignia rius	the fruiti ng body	hot aqueous extraction, DEAE-Se pharose anion –exchange and gel filtration chromatog raphy	22	fucose, galactose, mannose, 3-O-Me-galactose = 1:2:1:2	PISP1	Yang et al., 2009
P. ignia rius	the cultur ed mycel ium	hot water extraction, Sepharose G-100 gel filtration chromatog raphy	12	Xylose, mannose, fucose, glucose, galactose =2.3:1.0: 22.1:19.8	PIE	Chen et al., 2011
P. baum ii Pilát	the fruiti ng body	hot water extraction, Sephacryl S-1000 gel-permeatio n chromatog raphy	2000	glucose, mannose, fucose	β-(1→6)-D-gluco pyranose as the backbone	Ge et al., 2009
P. baum ii Pilát	the fruiti ng body	hot water extraction, DEAE-Se pharose fast flow anion exchange and Sephacryl S-1000 gel-flitrati on	230	glucose	(1→3)-β-D-, (1→4)-β-D-, branched (1→3,6)-β-D-glu copyranosyl resiues	Ge et al., 2013

		chromatography				
P. ribis	the fruiting body	hot water extraction, DEAE-cellulose and Superdex 30 column chromatography	8.59	glucose	(1→4), (1→6)-linked glucose backbone, β-D-glucan	Liu & Wang, 2007
P. nigricans	the cultured mycelium	hot water extraction, DEAE-Cellulose anion exchange and Sepharose CL-4B gel filtration chromatographies	33	glucose, galactose, mannose, arabinose, fucose = 20.1:8.7:6.9:1.0:0.8	proteoglycan, PNW1	Li et al., 2008
P. nigricans	the mycelium	hot water extraction, DEAE-Cellulose anion exchange and Sepharose CL-4B gel filtration chromatographies	29	glucose, galactose, mannose, arabinose, fucose = 3.3:8.8:6.4:1.0:1.4	proteoglycan, PNM1	Li et al., 2008

Table 2 Antitumor effects and mechanisms of polysaccharides from *Phellinus* s. l. species

Species	Polysaccharide source	Compound name	Cell/animal models	Antitumor effects and mechanisms	Reference
P. linteus	the mycelial cutlure	polysaccharide	normal splenocytes, mice	stimulate the proliferation of T lymphocytes and the humoral immune function including acting as a polyclonal activator on B cells	Kim Han et al., 1996
P. linteus	the fruiting body	acidic proteoglycan	MSLs, mice	Stimulate proliferation and expression of co-stimulatory molecules in B cells by regulating PTK and PKC signaling pathways.	Kim, Park, Lee et al., 2003
P. linteus	the fruiting body	acidic proteoglycan	BM-derived murine DC, mice	induce phenotypic and functional maturation of murine dendritic cells	Park et al., 2003
P. linteus	the fruiting body	acidic proteoglycan	B16 cells, mice	induce nitric oxide-mediated tumoricidal	Kim, Oh et al., 2003

				activity of macrophages through protein tyrosine kinase and protein kinase C	
P. linteus	the fruiting body	acidic proteoglycan	B16 and Yac-1 cells, mice	enhance through the up-regulation of NO and TNF-α from peritoneal macrophages	Kim, Choi et al., 2004
P. linteus	the fruiting body	proteoglycan	BM-derived murine DC, mice	induce Toll-like receptors 2- and 4-mediated of murine dendritic cells via activation of ERK, p38 and NF-κB signal pathways	Kim Han et al., 2004
P. linteus	the fruiting body	proteoglycan	BM-derived murine DC, MCA-102-bearing mice	Inhibit the tumor growth through a mechanism leading to a Th-1 dominant immune state and the activation of CD11c+CD8+ DC	Kim Oh et al., 2004
P. igniarius	the cultured mycelium	PIE	S180- and H22-bearing mice	exhibit antitumor effect through enhancement of cell mediated	Chen et al., 2011

				immunity	
P. nigricans	the cultured mycelium	PNW1,PNM1	S180-bearing mice	Stimulate lymphocytes proliferation, increase production of NO and TNF-α in macrophages	Li et al., 2008
P. linteus	the cultured mycelium	polysaccharide	B16F10 and NCI-H23 cells mice	inhibit tumor growth and metastasis as chemotherapy and immunotherapy	Han et al., 1999
P. linteus	the cultured mycelium	polysaccharide	B16F10 cells, mice	activate macrophage functions; inhibit cancer cell metastasis by blocking cell adhesion and invasion	Han et al., 2006
P. linteus	the fruiting body	Protein-bound polysaccharide	SW480 cells	induce apoptosis and G2/M cell cycle arrest by decreasing Bcl-2 and cyclin B1 expressions	Li et al., 2004
P. linteus	the fruiting body	polysaccharide	LNCaP and PC3 cells	activate different pathways to induce apoptosis in prostate cancer cells	Zhu et al., 2007
P.	the fruiting	P1	HT-29 cells,	inhibit	Li et al.,

linteus	body		HT-29-bearing mice	colorectal carcinoma by enhancing the immune response of T cells and IgA, and disrupting the Reg IV/EGFR/Akt signaling pathway	2011
P. linteus	the fruiting body	P1	HT-29 cells, HT-29-bearing mice	inhibit cell proliferation of HT-29 by S-phase arrest through activation of the P27kip1-cyclin D1/E-CDK2 pathway	Zhong et al., 2013
P. linteus	the fruiting body	P1	HepG2 cells, mice	induce S-phase arrest in HepG2 cells by decreasing calreticulin expression and activating the P27kip1-cyclin A/D1/E-CDK2 pathway	Li et al., 2013
P. linteus	the fruiting body	polysaccharide	HepG2 and Bel-7404 cells	inhibit cell proliferation, induce apoptosis and G1 or S phase arrest in HepG2 and Bel-7404 cells by AKT	OuYang et al., 2013

				signaling and mitochondrial pathways	
P. linteus	the fruiting body	polysaccharide	THP-1 cells	increase the mitochondrial membrane potential and cause apoptotic death of THP-1 monocytes	van Griensven & Verhoeven, 2013
P. linteus	the fruiting body	Protein-bound polysaccharide	SW480 cells, mice	suppress tumor growth, invasion and angiogenesis through the inhibition of Wnt/β-catenin signaling in certain colon cancer cells	Song et al., 2011
P. igniarius	the cultured filtrate	exopolysaccharide	S180- and H22-bearing mice	inhibit tumor without toxicity via immune mechanism	Dong et al., 2009
P. baumii	the cultured mycelium	PB	HepG2 cells	inhibit the proliferation of HepG2 cells by inducing cell cycle arrest at S phase, leading to apoptosis	Xue et al., 2011
P. gilvus	the fruiting body	polysaccharide	B16F10 cells, mice	inhibit melanoma growth in mice; decrease cell proliferation and increase cell apoptosis	Bae et al., 2005a

P. gilvus	the fruiting body	polysaccharide	BaP-induced mice	induce cancer cell apoptosis by down-regulating mutant *p53* mRNA expression	Bae et al., 2005b
P. ribis	the fruiting body	PRP-SII-IV	HepG2 cells, zebtafish	inhibit the growth of HepG2 cells; block new angiogenic vessel formation in zebrafish assay	Liu et al., 2009
P. ribis	the fruiting body	PRP-S1, PRP-S2	HUVECs, SKOV-3 cells zebrafish, H22-bearing mice	exhibit antiangiogenic and antitumoral properties, and the antitumoral effect was mediated via the antiangiogenic property.	Liu et al., 2014

Figure 1 The fruiting body (or fungal mycelium) of *Phellinus linteus* and Commercial *Phellinus* health food and cosmetic products.

Fig. 1.
Yan et al.

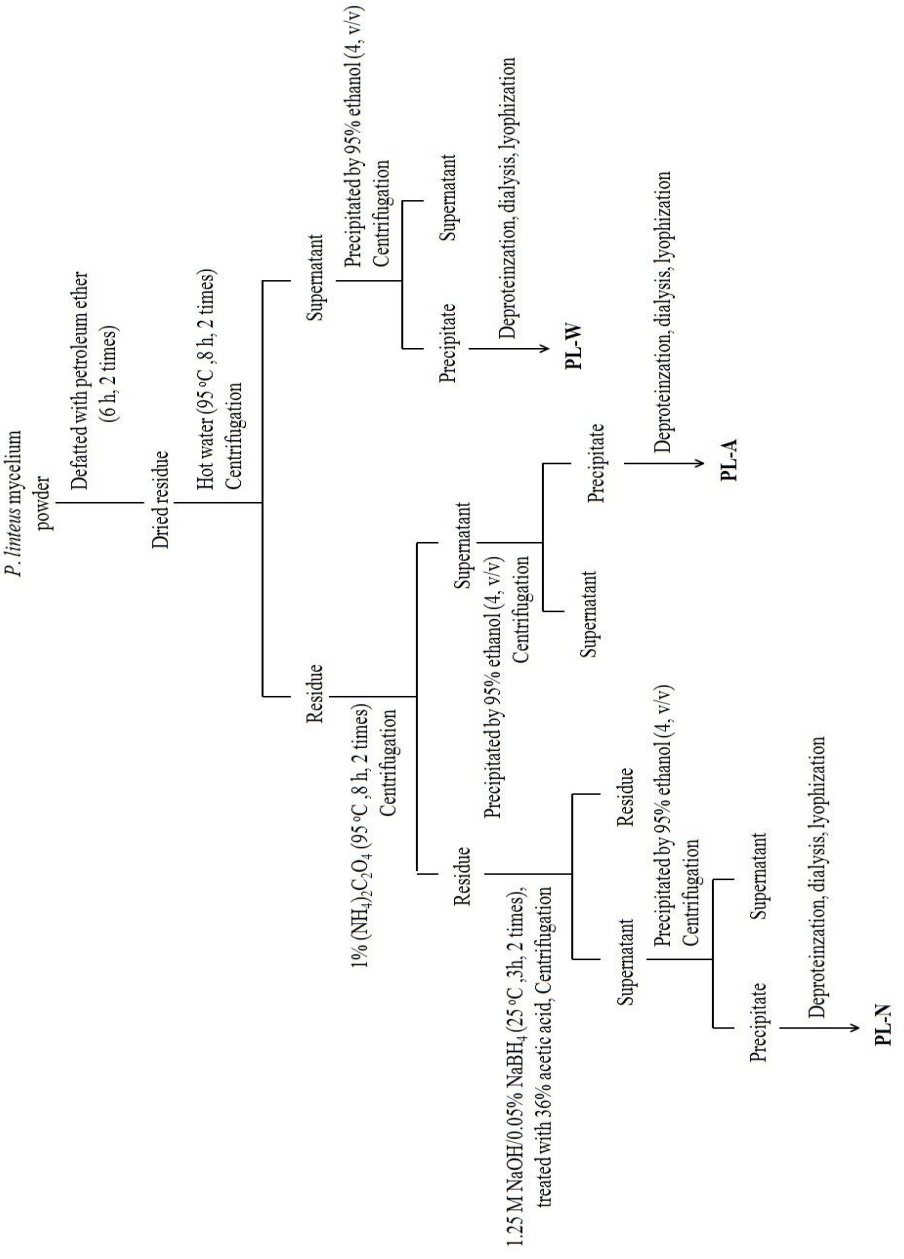
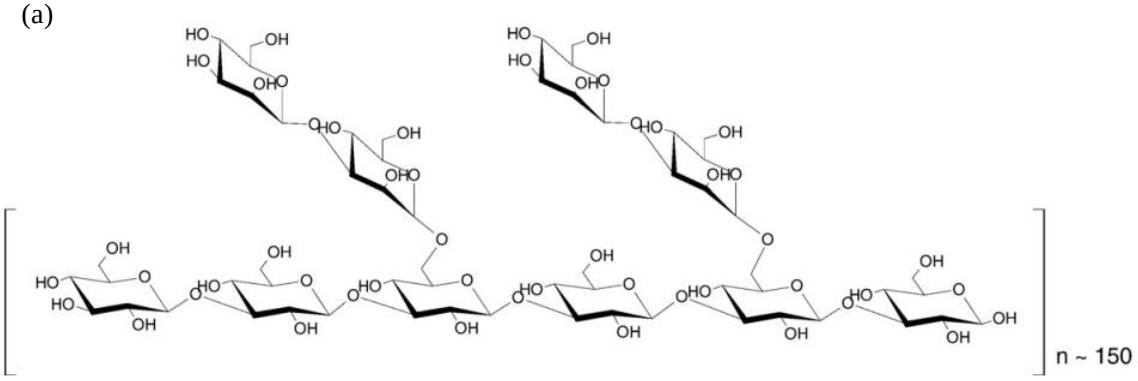


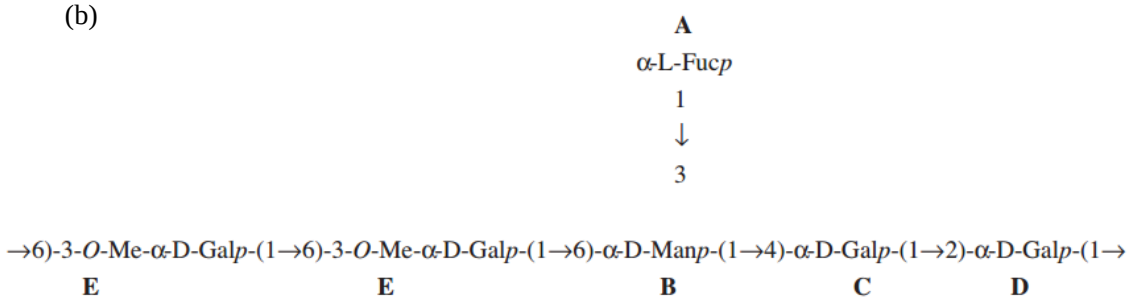
Fig. 2. Yan et al.

Figure 2 Scheme for extraction and isolation of polysaccharides from *P. linteus* mycelia by different extraction media (Wang et al., 2014).

(a)



(b)



(c)

(d)

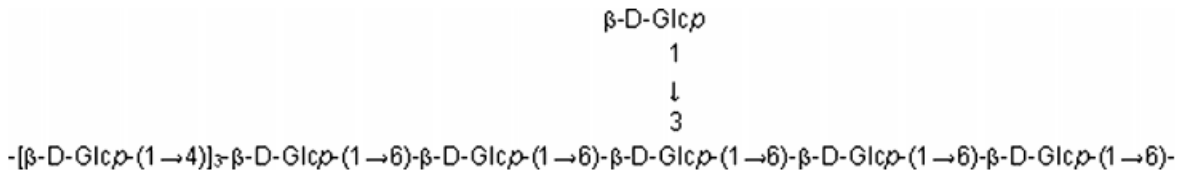


Figure 3 Chemical structures of antitumor polysaccharides from *Phellinus* s. l. species. Take from (a) *P. linteus* (Baker et al., 2008); (b) *P. igniarius* (Yang et al., 2009); (c) *P. baumii Pilát* (Ge et al., 2013); (d) *P. ribis* (Liu & Wang, 2007).

Fig. 3.
Yan et al.

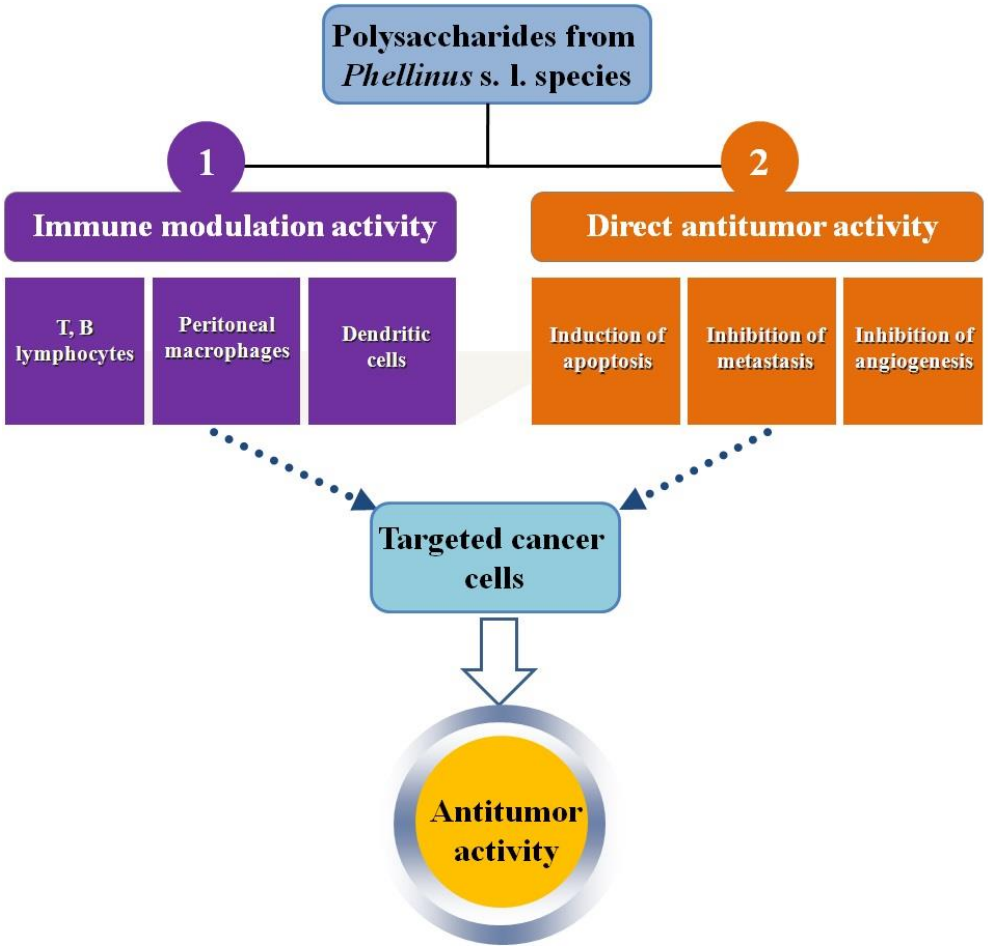


Figure 4 Summary of mechanisms of antitumor activity of polysaccharides from *Phellinus* s. l. species.