

## A REVIEW ON ANTITUMOR ACTIONS OF POLYSACCHARIDE ISOLATED FROM MEDICINAL MUSHROOMS

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**ABSTRACT** Mushrooms have long been attracting a great deal of interest in many areas of foods and biopharmaceuticals. They are well known for their nutritional and medicinal values. Mushrooms comprise a vast and yet largely untapped source of powerful new pharmaceutical products. In particular, and most importantly for modern medicine, they represent an unlimited source of polysaccharides with antitumor and immunostimulating properties. Recently, basidiomycete fungi have been used for the treatment of cancer. Many, if not all, Basidiomycetes mushrooms contain biologically active polysaccharides in fruit bodies, cultured mycelium, culture broth. Polysaccharides and polysaccharide-protein complexes from medicinal mushrooms may enhance innate immune responses, resulting in antitumor activities. In this review, in the search for the development of new anticancer drugs, the effects of polysaccharides isolated from medicinal mushrooms on tumor were studied.

**Keywords:** Antitumor activity; polysaccharide; medicinal mushrooms

### INTRODUCTION

Cancer is the largest single cause of death in both men and women, claiming over 6 million lives each year worldwide. Cancer chemotherapy drugs such as 5-fluorouracil derivatives, cisplatin, mitomycin, adriamycin, taxol, etc., have been used extensively for the treatment of certain types of cancer. However, with these treatments, severe gastrointestinal toxicity, with diarrhea and mucositis and hematological toxicity, with leucopenia and immune suppression, appear to be dose-limiting factors. After the removal of a malignant tumor by surgical operation, radiation therapy and/or adjuvant therapy with cancer chemotherapy drugs may be curative. However, the removal of certain cancers, for example, breast carcinoma, colon carcinoma and osteogenic sarcoma, may be followed by the rapid growth of distant metastases to lung, liver etc. Therefore, it is necessary to develop new anticancer agents with antitumor and antimetastatic activities but without adverse reactions such as gastrointestinal toxicity, myelotoxicity and immune suppression caused by cancer chemotherapeutic drugs [1]. Recently, basidiomycete fungi have been used for the treatment of cancer. They comprise a vast and yet largely untapped source of powerful new pharmaceutical products. In particular, and most importantly for modern medicine, they represent an unlimited source of polysaccharides with antitumor and immunostimulating properties. Many, if not all, Basidiomycetes mushrooms contain biologically active polysaccharides in fruit bodies, cultured mycelium, culture broth. Polysaccharides and polysaccharide-protein complexes from medicinal mushrooms may enhance innate immune responses, resulting in antitumor activities. In this review, the effect of polysaccharides isolated from various medicinal mushrooms on tumor, was described.

#### *Dictyophora indusiata*

*Dictyophora indusiata* (Vent. Ex Pers.) Fish Phallaceae (Chinese name Zhu Sun, meaning the bamboo mushroom), synonymously called *Phallus indusiatus*, is frequently used under the name Veiled lady mushroom. The folkloric consumption of *D. indusiata* in ancient China began around 618 A.D., which pointed mainly to the nutritional bioactivities, like benefits to eyes and tonics to cardiovascular systems; and partially to the medicinal effect like antitumor, [2,3]. A review by Lindquist indicated that many edible

mushrooms exhibit a broad spectrum of bioactivities that are beneficially acting as a complementary alternative medicine (CAM) [4].

The polysaccharides contained in *D.indusiata* has revealed to be effective as anti-tumor, anti-agglutinating, anti-inflammatory, immune-enhancer, and anti-hyperglycemic. Furthermore, the short skirt Zhu Sun is a good superoxide anion scavenger; it protects the cellular membrane lipoproteins from peroxidation, a mechanism interpreting the anti-cancer and immune-enhancing effect [5]. The radiation protective effect of *D.indusiata* was studied by Guo et al. [6]. The thymus and pancreatic index, CD4<sup>+</sup>, CD16, CD57 and interleukin 2 were all improved, while CD8<sup>+</sup> was decreased [5]. Ke and Lin obtained a glycoprotein DIGP-2, having the molar ratio d-galactose : d-glucose : d-mannose = 0.78 : 2.12 : 1.00, which was shown to inhibit 36.2% of Sarcoma 180 cell-line viability [7]. Moreover, the extract of *Dictyophora* was shown to be effective antimutagenics [8]. As is well known, the antioxidative capability may be relevantly related with its immune-enhancing, anti-inflammatory and anticancer bioactivities. Recently, Yaw-Bee Ker et al Found that the soluble polysaccharides (SP) present in *D.indusiata* and their monosaccharide profiles can act as an important role affecting the antioxidative capability, which in turn would influence the biological activity involving anti-inflammatory, immune enhancing and anticancer (Figure 1). They obtained six SP fractions and designated them as D1, a galactoglucan; D2, a galactan; D3, the isoelectrically precipitated riboglucan from 2% NaOH; D4, a myoinositol; D5 and D6, the mannogalactans. The total SP accounted for 37.44% w/w, their molecular weight (MW) ranged within 801–4656 kDa [9].

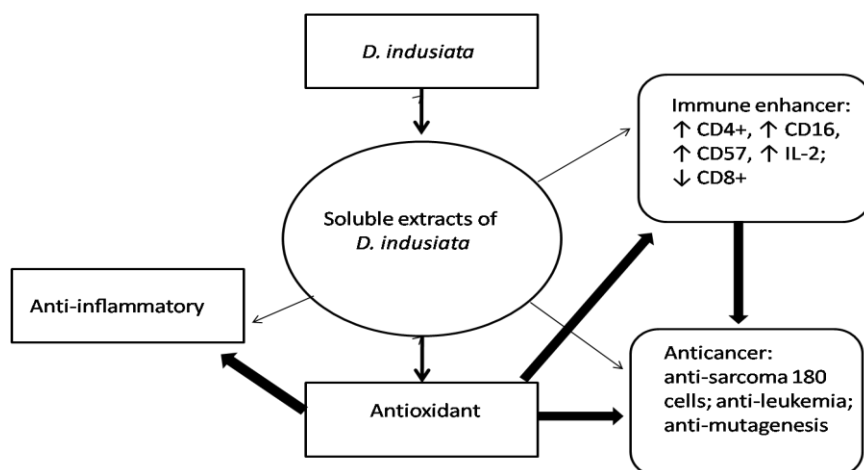


Fig. 1. The scheme showing the hypothesis confirmed by Yaw-Bee Ker et al.

### Proximate Composition

The fruiting bodies of *D.indusiata* contained huge amount of carbohydrates and crude fibers reaching 46.89 and 28.65%, respectively. In contrast, it had only 6.07% of crude protein (Table 1). The SP yielded 37.5%, accounting for ~80% ( $37.50/46.89 \approx 0.8$ ) of the total polysaccharides.

**Table 1.** proximate composition of the fruiting bodies of *D.indusiata*

Composition	Content (% w/w)
Moisture	10.55 ± 0.02
Crude ash	6.58 ± 0.01
Crude fat	1.26 ± 0.02
Crude fiber	28.65 ± 0.05
Crude protein	6.07 ± 0.03
Carbohydrate	46.89 ± 0.04

### Monosaccharide Composition

Ten kinds of monosaccharides were found in the SP of *D.indusiata*. Obeying the conventional naming rule, fraction D 1 was designated glucogalactan; D 3 , theriboglucan, and both D5 and D6, the mannogalactans. Interestingly, huge amount of myoinositol was simultaneously found in fractions D4, D2, and D 1( Table 2 ).

**Table 2.** Pattern of soluble polysaccharides isolated from the fruiting bodies of *D. indusiata* .

Sugar (mole%)	D1	D2	D3	D4	D5	D6
Rhamnose	1.0 ± 0.1	0.2 ± 0.0	13.6 ± 0.1	0.3 ± 0.0	1.8 ± 0.1	1.1 ± 0.0
Fucose	1.3 ± 0.1	0.3 ± 0.0	3.7 ± 0.0	0.3 ± 0.0	2.0 ± 0.1	2.3 ± 0.0
Ribose	1.4 ± 0.1	0.1 ± 0.0	24.3 ± 0.1	0.4 ± 0.0	ud	ud
Arabinose	1.6 ± 0.2	0.7 ± 0.1	3.3 ± 0.1	0.8 ± 0.1	ud	1.8 ± 0.0
Xylose	ud	1.4 ± 0.1	ud	ud	ud	ud
Allose	ud	ud	ud	0.3 ± 0.0b	ud	2.2 ± 0.0
Talose	ud	ud	ud	ud	ud	ud
Mannose	5.9 ± 0.3	0.4 ± 0.1	6.9 ± 0.1	2.4 ± 0.2	24.7 ± 0.3	28.0 ± 0.2
Galactose	14.2 ± 0.3	19.7 ± 0.3	2.9 ± 0.0	2.0 ± 0.1	66.2 ± 0.5	62.0 ± 0.3
Glucose	6.9 ± 0.3	2.0 ± 0.1	35.9 ± 0.2	1.1 ± 0.0	ud	2.7 ± 0.1
Myo-inositol	67.9 ± 0.3	76.5 ± 0.3	9.4 ± 0.1	92.5 ± 0.4	5.3 ± 0.2	ud

ud: undetected. Different superscripts in each column denote significant difference ( $P < .05$ ) between fractions D1–D6. D1: the 3-fold ethanol precipitate from hot water extracts. D2: the 3-fold ethanol precipitate from 0.04 N HCl extracts. D3: the isoelectric precipitate from 2% NaOH extracts. D4: the 3-fold ethanol precipitate from 2% NaOH extracts. D5: the isoelectric precipitate from 10% KOH extracts; and D6: the 3-fold ethanol precipitate from 10% KOH extracts. Carbohydrate content (% w/w) was measured by the phenol-H<sub>2</sub>SO<sub>4</sub> method. Protein content (% w/w) was determined by Bradford protein assay.

### Antioxidative Capability

Fraction D3 exhibited the most potent DPPH radical scavenging capability, D6 and D2 were the next, respectively. The order in strength was D3 > D6 > D2 > D4 > D1. Regarding the scavenge for the hydroxyl radicals, fraction D3 also showed the most prominent bioactivity (reaching 50%) with the order D3 > D2 > D6 > D4 > D1. Data revealed that only fractions D1, D2, D4 and D6 were peptidoglycan in nature. As a contrast, fractions D3 and D5 were typically characteristic of glycoproteins. Moreover, the high total SP content (37.44%) may also implicate an alternate therapeutic use. By insight of monosaccharide profile, fraction D1 was designated a glucogalactan; D3, a riboglucan; D5 and D6, mannogalactans. Fraction D4, having myo-inositol content 92.5%, almost was approaching the pure myo-inositol. As well known, inositol epimerase governs the conversion of myo-inositol to chiro-inositol [10, 11], an insulin dependent process related with immunological activity. The actual role of such an unusually high content of myo-inositol in *D.indusiata* acting as immunomodulator remains to further investigation. D3 possessed the most potent DPPH radical scavenging capability prevailing over the mannogalactan (D6), implicating the complicate phytochemical-therapeutics relationship [12]. As well cited, the binding of glucans having (1 → 3)-β-d-glucosan main frame with β-d-(1 → 6) branches onto the cell surface of cytotoxic macrophages, helper T and NK cells will trigger immunopotential [12, 13]. The degree of branching (DB) between 0.20 and 0.33 and the triple helical structures are important for immunopotential [12]. Conversely, some insoluble aggregates also had been reported to be more stimulatory than the soluble polymers.

The fruiting bodies of *D.indusiata* contain huge amount of SP (37.5%) that exhibits MW ranging within 801–4656 kDa. Among which, the fraction D3 (the isoelectrically precipitated riboglucan from 2% NaOH) has the smallest MW 801 and is the most potent SP regarding the antioxidative capability. Furthermore, the huge amount of myo-inositol present can be relevantly associated with its additional immunobioactivity. Thus Yaw-Bee Ker et al confirmed their hypothesis that the bioactivity of *D.indusiata* is related in majority, if not entirely, to its soluble polysaccharides [9].

### Maitake (*Grifola frondosa*)

is the Japanese name for an edible fungus with a large fruiting body characterized by overlapping caps. It is a premier culinary as well as medicinal mushroom. Maitake is increasingly being recognized as a potent source of polysaccharide compounds with dramatic health-promoting potential. The most recent development is the MD-fraction, a proprietary maitake extract its Japanese inventors consider to be a notable advance upon the preceding D-fraction. The D-fraction, the MD-fraction, and other extracts, often in combination with whole maitake powder, have shown particular promise as immunomodulating agents, and as an adjunct to cancer and HIV therapy. They may also provide some benefit in the treatment of hyperlipidemia, hypertension, and hepatitis [14].

### Mushrooms' Unique and Active Compounds

Some 50 of the 38,000 species of mushrooms have been found to have medicinal properties, according to mushroom researcher Cun Zhuang. Three have been used as the source for extracts now employed clinically as anticancer drugs in Japan:

- Kawaratake (*Coriolus versicolor*) is the source for PSK (Krestin). Developed in the late 1970s, PSK was the first mushroom-based anticancer drug and is now one of the most popular anticancer drugs in Japan. It is taken orally for gastric and other cancers.
- Shiitake is the source for Lentinan, which has been approved since the mid-1980s to treat gastric cancer. Because of poor absorption when taken orally, this compound is best administered by injection.

- Suehirotake (*Schizophyllum commune*) is used to derive Shizophyllan, which is used to treat cervical cancer (it also is injected).

These anticancer medications, as well as many additional medicinal mushrooms such as reishi, hiratake or oyster (*Pleurotus ostreatus*), and enokitake (*Flammulina velutipes*), contain various compounds with diverse biological and therapeutic effects. The content and bioactivity of these compounds depend on how the mushroom is prepared and consumed [15]. Among the most important constituents are certain polysaccharides, known as beta-glucans, which are bound to proteins. PSK, Lentinan, and Shizophyllan are all forms of beta-glucan. Maitake's prominent immune-boosting effects are thought to be due predominantly to these polysaccharides.

Polysaccharides such as beta-glucans found in a number of medicinal mushrooms are increasingly being recognized for their non-specific immunomodulatory effects. These so-called biological response modifiers can be potent antiviral and antitumor agents, not by killing viruses or cancer cells directly but by stimulating the body's innate ability to marshal cellular defenses. Augmenting what Japanese cancer researchers have termed "intrinsic host defense mechanisms" is particularly promising because it is a property generally lacking in conventional anticancer drugs.

### **The Immunopotentiating Maitake Fractions**

The polysaccharides in maitake have a unique structure and were among the most powerful to be studied to date, demonstrating more pronounced antitumor activity in animal tests than other mushroom extracts [16]. Maitake also demonstrated the most promise as an orally effective immunomodulator. This made it potentially much easier to use compared to, for example, shiitake extracts that worked optimally only when administered by injection. In the early 1980s, a Japanese mycologist Hiroaki Nanba decided to focus exclusively on maitake, and he and a number of other Japanese scientists began to extract various polysaccharides from maitake and test them for antitumor and immunomodulating potential.

In 1984 Nanba identified a fraction found in both the mycelia and the fruit body of maitake with the ability to stimulate macrophages. The resulting D-fraction is a standardized form of isolated beta-glucan polysaccharide compounds (beta-1,6 glucan and beta-1,3 glucan) and protein with a molecular weight of about 1,000,000. In the same year a patent was issued in Japan to Nanba and others [17]. While other medicinal mushrooms have been shown to have bioactive beta-glucan constituents, Nanba notes that various beta-glucans differ and that the beta-glucans found in the maitake D-fraction have a unique and complex structure, containing both a 1,6 main chain having a greater degree of 1,3 branches, and a 1,3 main chain having 1,6 branches. Most other mushroom-derived beta-glucans have a 1,3 main chain with 1,6 branches only.

The D-fraction's high molecular weight may also be a factor in its immunomodulating effects, according to research into antitumor activity and glucose consumption by macrophages. One investigation concluded, "These results suggest that an anti-tumor glucan is not always a multiple enhancer of host defense mechanisms and that a large molecular weight is required to augment multiple immunological activities [18]. A subsequent study by some of the same researchers suggested that "the branching ratio and molecular weight of (1→3)-beta-D-glucans are important factors for the production of cytokines from macrophages [19].

A recent review of the existing data on the mechanism of whole mushrooms and isolated mushroom compounds, particularly certain beta-glucans, concluded the antitumor mechanisms of several species, including maitake, are mediated largely by T-cells and macrophages. According to the researchers, "Despite the structural and functional similarities of these glucans, they differ in their effectiveness against specific tumors and in their ability to elicit various cellular responses, particularly cytokine expression and production [20].

In 1990s Nanba and colleague Keiko Kubo continued to study maitake, trying to improve upon the antitumor and immunopotentiating activity of the D-fraction. Further purification of the D-fraction yielded the MD-fraction. In appearance the MD-fraction is a hygroscopic powder in shades of brown, is neutral to weakly acidic, and has a molecular weight distributed around 1,000,000.

The MD-fraction provided superior results over the D-fraction in an antitumor test described in the patent. The researchers found the group given the MD-fraction experienced a significantly stronger inhibitory effect on tumor growth than that of the group given the D-fraction. The researchers also compared the substances' effects on macrophage and killer T-cell activity five days after each test substance was given. They determined that the MD-fraction exhibited stronger antitumor activity and immunopotentiating activity than the D-fraction [21]. Both the D- and MD-fractions are considered to have low toxicity and high safety.

Maitake is among the most promising natural sources of immunotherapeutic products. Standardized beta-glucan extracts such as the D- and MD-fraction show particular potential as carcinostatic agents that can be used in conjunction with conventional medical treatments to treat cancer. Most of the published studies to date have been animal studies; additional human studies and clinical trials in particular are needed.

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