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Extraction, structure and bioactivities of the polysaccharides from *Pleurotus eryngii*: A review

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ABSTRACT

Pleurotus eryngii (also known as king trumpet mushroom or king oyster mushroom) is an edible mushroom cultivated widely in many regions of the world. Polysaccharides from *P. eryngii* have a variety of biological activities, including anti-oxidant, anti-hyperlipidemic, anti-tumor, immunoregulatory and bacteriostatic. This paper reviews the extraction/purification, structural analysis and pharmacological activities of polysaccharides from this mushroom and provides updated research progress in areas important for the processing and product development of *P. eryngii* derived agents.

Contents

1. Introduction

Pleurotus eryngii, also known as king trumpet mushroom or king oyster mushroom, is an edible mushroom [1,2]. It was originally grown in northern Italy and Switzerland where it was locally known as cardoncello [3,4]. It is commonly cultivated in Europe, the Middle East and North America as well as in many parts of Asia [5]. The cap of the mature *P. eryngii* has a shallow concave, fan-shaped or round shape, and the stipe is rod-shaped or bulbous, with a smooth surface and nearly white [6]. The fruiting bodies of *P. eryngii* are easy to cultivate [7] with high yield and the products have large market due to their good taste and an ability to be cooked directly.

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P. eryngii is reportedly rich in protein, carbohydrates, unsaturated fatty acids, vitamins and other nutrients and is low in fat, making it a high-quality, low-calorie food. In addition, *P. eryngii* is also a rich source of the disaccharide, trehalose [8]. Researchers found that this edible fungus has a variety of pharmacological activities and nutritional value, and its polysaccharides are important active substances in this edible fungus [9,10]. It has become an active research topic in recent years to extract polysaccharides from the fruiting bodies, mycelium and fermentation broth of edible fungi to explore their structure and physiological activities [11–13]. The polysaccharides of *P. eryngii* have become increasingly attractive due to their anti-oxidant, anti-tumor, immunoregulatory, antibacterial, anti-hyperlipidemic, and other activities.

Reviewing the available literature, there are many research reports on the polysaccharides of *P. eryngii*, but there are few reviews in the area. The purpose of this paper is to review the extraction, structure and bioactivities of *P. eryngii* polysaccharides, and to provide a scientific/theoretical basis for further processing and the product development of *P. eryngii* polysaccharides.

2. Extraction and purification

Over the past ten years, most of the research reports on the polysaccharides of *P. eryngii* have relied on its fruiting bodies as raw materials, and a few have adopted the use of the mycelium and fermentation broth of *P. eryngii* [14]. In addition, in recent years, polysaccharides have been isolated from the *P. eryngii* residue [15]. Most of the extraction of *P. eryngii* polysaccharides has been carried out by a hot water method. However, conventional extraction methods have shortcomings such as long extraction times and low extraction yields [16]. Therefore, new technologies have been developed in recent years, such as supercritical fluid extraction [17] and enzymatic extraction [18]. In the general procedure for extraction a sample is first treated with 95% ethanol to remove the lipophilic compounds, leaching requires a period of time at a certain ratio of material to liquid and a specific temperature. On using the selected extraction method, the supernatant is then collected by centrifugation or filtration, de-proteinized by Sevage method [19] or through an enzymatic method [20], and precipitated with 4-volumes of ethanol at 4 °C for 12 h. After centrifugation, the precipitate is washed successively with ethanol, dialyzed against deionized water, and freeze-dried to obtain crude *P. eryngii* polysaccharide.

The extraction process needs to be continuously optimized to obtain high yields of extracted polysaccharide. Therefore, for each extraction process, an optimal extraction process is obtained by comparing the extraction yields of polysaccharides under various significant variables such as extraction temperature, water-to-feed ratio and extraction times. For example, a response surface method can be used to study the effects of ultrasonic power, ultrasonic time and ratio of material to liquid on the extraction yields of *P. eryngii* polysaccharides [21]. The optimal extraction process identified in this study was: extraction time 39 min, ultrasonic power 517 W, ratio of material to liquid 19 mL/g, to obtain a yield of *P. eryngii* polysaccharides reaching 34% [21]. The purification of crude polysaccharides generally employs ion-exchange chromatography and gel filtration [22,23].

3. Structure and Chemical modification characteristics

In the past ten years, the structural analysis of polysaccharides isolated from fungi has focused on polysaccharide molecular weight, monosaccharide composition, glycosidic bond type, and backbone structure. The analytical methods applied mainly include high performance liquid chromatography (HPLC), nuclear magnetic resonance spectroscopy (NMR), gas chromatography-mass spectrometry (GC-MS), infrared spectroscopy (IR), methylation analysis, and Smith degradation analysis [24–27]. Herein, we list reports on multiple types of *P. eryngii* polysaccharides, including their monosaccharide composition, molecular weight, structural characteristics and biological activities (Table 1).

The biological activities of polysaccharides are closely related to their structures. Therefore, elucidating the structural characteristics and biological activities of polysaccharides plays an important role in exploring the structure-activity relationship between polysaccharide structure and biological activity [28–31]. Ren and colleagues reported two heteropolysaccharides (PEP-1 and PEP-2) from *P. eryngii* fruiting bodies. Compared to PEP-1, the higher molecular weight PEP-2 had stronger anti-tumor activity on human hepatoblastoma HepG-2 cells [32]. Zhang and colleagues reported on polysaccharide IPS, extracted from *P. eryngii* SI-04 strain, and its two purified fractions (IPS-1 and IPS-2). In an *in vitro* antioxidant activity assay, IPS-2 showed the best antioxidant activity as well as a hepatoprotective effect on mice with an acute liver injury induced by CCl_4. The structures of these polysaccharides were analyzed, and it was found that only IPS-2 had special structural characteristics of C—O—Se, and it was concluded that the specific monosaccharide composition, the C—O—Se bond type, was responsible for the physiological activity of IPS-2 [33]. It has been demonstrated that the molecular weight and monosaccharide composition of natural polysaccharides are two important factors affecting their anticancer activity [34]. Ma and colleagues reported the characterization and antitumor activity of three polysaccharides (PEPE-1, PEPE-2 and PEPE-3) from *P. eryngii* residue. PEPE-2 and PEPE-3 were composed of mannose, glucose and galactose. PEPE-1 was composed of mannose, glucose, galactose and xylose. The molecular weight of PEPE-3 was large, reaching 4×10^5 Da, while the molecular weight of PEPE2 was the smallest. *In vitro*, the inhibitory effect on HepG-2 cells and cytotoxicity of different polysaccharides increased in the order, PEPE-3 > PEPE-2 > PEPE-1, the same as their uronic acid content, which suggests that the antitumor activities of these three polysaccharides was related to their molecular weight, monosaccharide composition, sulfate and uronic acid content [15].

Chemical modification can improve the biological activity of polysaccharides or create new functional properties, so chemically modified polysaccharides are receiving more and more attention [35]. Recent studies have shown that common methods of polysaccharide modification include sulfation, phosphorylation, methylation, carboxymethylation, acetylation, hydroxy-propylation, selenylation and etherification [36]. Sulfated polysaccharides from marine algae exhibit a variety of biological activities that can be beneficial to human health such as anticoagulant, antiviral, antioxidant and antitumor activities [37]. Li and colleagues compared the *in vitro* anti-inflammatory and anti-proliferative activities of natural polysaccharide (PEPS) from *P. eryngii* and sulfated polysaccharide (S.PEPS). The results showed that S.PEPS inhibited the release of reactive oxygen species (ROS) and nitric oxide (NO) from macrophages more strongly than PEPS and the anti-proliferative activity of S.PEPS against Caco-2 and HepG2 cells was also significantly stronger than that of PEPS [38]. Jung and colleagues succeeded in the preparation of *P. eryngii* polysaccharides with different degrees of sulfation. With an increase of sulfation degree, the water solubility of *P. eryngii* polysaccharide increased, as did its growth inhibitory affect on A549 human lung and H4IIE rat liver cancer cells and its DPPH free radical scavenging ability. In addition, compared with natural polysaccharides, sulfated polysaccharides had stronger inhibitory activity on growth of A549 human lung cancer cells [39].

Table 1
Composition of monosaccharides, molecular weight, linkage type and bioactivities of polysaccharides isolated from *P. eryngii*.

NO.	Name	Monosaccharide composition	Mw (Da)	Linkage type	Bioactivities	Ref.
1	EP-1	D-Glc, D-Gal, D-Man, molar ratio: 96.39:2.26:1.35	3.67×10^4	β-1,3-D-Glucan with α-D-Glc*p* side chain	Inhibit lipid accumulation in foam cells	[2]
2	PEPE-1	D-Man, D-Glc, D-Gal, D-Xyl, molar ratio: 8.01:74.82:11.14:1.24	2.08×10^5	Unknown	Antitumor activity	[15]
3	PEPE-2	D-Man, D-Glc, D-Gal, molar ratio: 5.23: 86.74: 5.12	1.20×10^4	Unknown	Antitumor activity	[15]
4	PEPE-3	D-Man, D-Glc, D-Gal, molar ratio: 4.08:90.93:2.89	4.13×10^5	Unknown	Antitumor activity	[15]
5	WPEP-N-b	D-Gal, D-Man, 3-*O*-Me-D-Gal, D-Glc, molar ratio: 43.8:39.3: 11.7:9.20	2.14×10^4	α-1,6-D-Gal*p* and 3-*O*-Me-D-Gal*p*	Immunomodulatory activities	[19]
6	PEP-1	D-Man, D-Glc, D-Gal, molar ratio: 23.02:49.21:27.77	2.54×10^4	1,3-D-Glc*p*, 1,6-D-Man*p* and 1,2,6-Gal*p*	Antitumor activity	[32]
7	PEP-2	D-Man, D-Glc, D-Gal, molar ratio: 10.90:78.60:10.48	4.63×10^5	1,3- D-Glc*p*, 1,6-D-Glc*p*, T-D-Glc*p*, 1,3-D-Man*p*, 1,2,3-D-Man*p*, 1,2,6-D-Man*p* and 1,6-D-Gal*p*	Antitumor activity	[32]
8	IEPW-PE	D-Glc,D-Gal, molar ratio: 99:0.5	Unknown	β-1,3-linked-D-Glc*p*	Unknown	[40]
9	RFP-PE	D-Gal, 3-O-Me-D-Gal, molar ratio: 3.0:1.0	1.79×10^5	partially 3-O-methylated, α-1,6-D-Galactans containing Gal and 3-Me-Gal*p*	Unknown	[41]
10	PEPS1	D-Man,D-Gal,3-O-Me-Gal, molar ratio:1:0.94:1.14	1.88×10^4	α-1,6-D-Gal*p* and α-1,6–3-*O*-Me-D-Gal*p*	Unknown	[42]
11	PEPw	L-Ara, D-Man, D-Gal molar ratio: 1.2:2.3:6.2	2.50×10^4	1,6-D-Gal*p*, 1,2,6-D-Gal*p* and 1,4-D-Man*p*	Antitumor activity	[43]
12	PEPE-A1	D-Glc, D-Gal, D-Man, molar ratio:92.26:2.32:1.42	3.75×10^4	β-1,3-D-Glucan with branched α-D-Glc*p*	Reduction of lipid content	[44]
13	PEPE-A2	D-Glc, D-Gal, D-Man, molar ratio:95.54:3.28:1.18	3.00×10^4	β-1,3-D-Glucan	Reduction of lipid content	[44]
14	KOMAP	D-Glc, D-Man, L-Ara, molar ratio:6.20:2.10: 2.00	2.10×10^4	β-1,4-D-Glc*p* and β-1,3,6-D-Man*p*	Immunoregulatory and anti-tumor activities	[45]
15	EPA-1	D-Man, D-Glc, D-Gal, molar ratio: 2.20:1.00:3.20	9.97×10^4	1,6-linked Gal*p*	Immunomodulatory activities	[46]
16	MG-Pe	D-Man, 3-O-Me-D-Gal, D-Gal, molar ratio: 32.9:15:52.10	2.09×10^4	α-1,6-D-Gal*p* and α-1,6–3-*O*-Me-D-Gal*p*	Anti-melanoma	[47]
17	EPS1	L-Ara, D-Man, D-Gal, D-Glc, molar ratio: 1.00:1.70:1.00:4.30	2.33×10^3	Unknown	Antioxidant, anti-hyperglycemic, and renoprotective effects	[48]
18	EPS2	D-Man, D-Glc, molar ratio: 1.00:1.36	1.02×10^3	Unknown	Antioxidant, anti-hyperglycemic, and renoprotective effects	[48]

Abbreviations: Mw, molecular weight; *p*, pyranose; Glc, glucose; Gal, galactose; Man, mannose; Ara, arabinose; Xyl, xylose; Rha, rhamnose.

4. Biological activities

4.1. Antioxidant activity

ROS, mainly composed of superoxide anions, hydrogen peroxide and hydroxyl radicals [49], are continuously produced at low levels during the process of human energy production to maintain normal physiological functions, and organisms have an endogenous antioxidant defense system such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) to avoid oxidative damage [50]. High concentrations of ROS or a defense system imbalance can lead to nucleic acid destruction, protein oxidation and lipid peroxidation, which can impact many cellular functions, leading to health problems such as cancer, inflammation and cardiovascular disease [51]. Therefore, it is often necessary to reduce the oxidative damage of the human body by supplementing antioxidants.

A large body of literature has shown that *P. eryngii* polysaccharide exhibits good antioxidant activity. Zhang and colleagues extracted crude polysaccharides (PEPS30, PEPS60 and PEPS80) from the fruiting body of *P. eryngii* by ultrasonic extraction. *In vitro*, the concentration of these crude polysaccharides is positively correlated with DPPH free radical scavenging rate and superoxide anion radical scavenging ability, and PEPS80 demonstrates the best antioxidant activity [21]. Li and colleagues confirmed that the crude polysaccharides and sulfated polysaccharides of *P. eryngii* had the ability to scavenge ABTS radical scavenging activity and directly inhibit the antioxidant capacity of intracellular ROS and MDA, and also regulate the activity of antioxidant enzymes (SOD, GPx and CAT) in the defense system of organisms, reducing oxidative damage [52]. The intracellular polysaccharide (IPS-2) from *P. eryngii* SI-04 improved the levels of alanine aminotransferase activity (ALT) and aspartate aminotransferase activity (AST) in CCl_4-induced liver injured mice and alleviated the impact of CCl_4 on the levels of bilirubin (BIL), albumin (ALB) and triacylglycerol (TG) in the serum. At the same time, IPS-2 significantly prevented the increase of malondialdehyde (MDA) and lipid peroxide (LPO) levels in the liver. These results suggested that this polysaccharide could effectively prevent liver injury by mediating antioxidant and free radical scavenging activities [33].

4.2. Antitumor and immunomodulatory activities

According to the World Health Organization (WHO), 13% of people who die were suffering from cancer [53]. Chemotherapy is one of the most commonly used treatments for cancer, but most of the anticancer drugs, currently used in chemotherapy, in addition to causing nausea and vomiting, and acute cholinergic gastrointestinal effects, also exhibit cytotoxic effects on normal mitotic cells, leading to alopecia, anemia and leukopenia [54]. Therefore, finding new alternative anticancer drugs and overcoming the shortcomings of conventional chemotherapy drugs has become is increasingly important in cancer therapy [55]. Numerous studies have reported that natural polysaccharides exhibit good anticancer activity in both *in vitro* studies and *in vivo* animal studies, and have minimal toxic side effects, providing a new direction in cancer treatment [56]. Ren and colleagues reported two heteropolysaccharides (PEP-1 and PEP-2) from *P. eryngii* fruiting bodies. Compared to PEP-1, the higher molecular weight PEP-2 induced apoptosis by regulating the activity of caspase-3 and caspase-9 and ROS-dependent mechanisms, and showed stronger antitumor activity *in vitro* experiments with human hepatoblastoma HepG-2 cells. PEP-2 could also prevent the S phase of the cell cycle, thereby inhibiting the growth of HepG-2 cells [32]. *In vitro*, the cell viability, cell proliferation and cell morphology of B16-F10 murine melanoma cells treated with *P. eryngii* polysaccharide (MG-Pe) were normal, confirming that MG-Pe was not cytotoxic and that MG-Pe could also reduce the invasive ability of B16-F10 cells. In further *in vivo* experiments in mice, MG-Pe was shown to be a novel anticancer molecule as the tumor volume in mice injected with 10d MG-Pe was 60% smaller than that of the control mice, and no changes were observed in mouse physiological parameters [47].

Chemotherapy alone still has not achieved satisfactory results. In 1957, Byerrum and colleagues first reported the antineoplastic effect of mushroom polysaccharides [57]. Since then, a large number of edible fungi have been reported to have antitumor activity, and the mechanism of action is thought to be that the polysaccharide exerts its antitumor activity indirectly through the host's immune system, rather than through direct cytotoxicity [58,59]. Macrophages play a vital role in the immune regulation of the body, secreting and producing tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6 and other cytokines, and NO and ROS [60]. Liu and colleagues reported that KOMAP, a polysaccharide from *P. eryngii*, significantly inhibited the growth of tumors in Renca-bearing mice and enhanced the immune function of these mice. The data showed that KOMAP increased immune organ index in tumor mice, LPS and ConA-induced splenocyte proliferation, serum levels of cytokines IL-2, TNF-α and IFN-γ, and activity of NK and CTL cells, but did not adversely affect liver and kidney function of Renca-bearing mice [45]. Similarly, the water-soluble polysaccharide PEPw from the fruiting body of *P. eryngii* also promoted the proliferation of spleen cells stimulated by ConA and LPS in Renca-bearing mice, enhancing the killing activity of NK cells and CTLs of spleen cells and increase the level of IL-2 and TNF-α in the serum of Renca-bearing mice, which indirectly exerts antitumor activity [43].

4.3. Regulation of lipid metabolism

Atherosclerosis is a complex chronic disease that can be caused by hyperglycemia and hyperlipidemia [61]. It is known that high levels of total cholesterol (TC), total triglycerides (TG), low-density lipoprotein cholesterol (LDL-C) and low levels of high-density lipoprotein cholesterol (HDL-C) in the serum can cause hyperlipidemia [62]. In the mouse model of type 2 diabetes, *P. eryngii* polysaccharide (PEP) effectively reduced TG, TC and LDL-C levels in mice, increased serum HDL-C levels, showing hypolipidemic activity [63]. The extracellular polysaccharides, EPS1 and EPS2, extracted from *P. eryngii* SI-04 showed inhibitory effects on the activities of α-amylase and α-glucosidase *in vitro*, indicating that they had potential hypoglycemic effects. The elevated levels of TC, TG, LDL-C and very low-density lipoprotein (VLDL-C) as well as the decreased levels of HDL-C in STZ-induced diabetic mice were significantly ameliorated after feeding EPS1 and EPS2 [48]. Meanwhile, the results in the mouse model showed that *P. eryngii* polysaccharide (PEPE) effectively prevented hyperlipidemia in mice with high-fat diet. In another group of mice with hyperlipidemia, after feeding PEPE, serum levels of TC, TG and LDL-C were significantly decreased, HDL-C levels were significantly increased, and lipids in liver tissues were reduced. This indicates that PEPE not only has the potential to prevent hyperlipidemia, but also can be used as a drug candidate for the treatment of hyperlipidemia [64].

Uncontrolled uptake of oxidized low-density lipoprotein (oxLDL) by macrophages induces foam cell formation, resulting in excessive lipid accumulation in the cell [65]. In a macrophage-derived foam cell model induced by oxLDL, PEPE-A1 purified from PEPE showed significant ability to inhibit lipid accumulation in foam cells [44]. Subsequently, further studies by Chen and colleagues confirmed that the excreted polysaccharide EP in the submersion culture of *P. eryngii* can cause the down-regulation of

scavenger receptor-CD36 at the transcriptional and protein levels to inhibit lipid accumulation in foam cells, thereby inhibiting the uptake of oxLDL [2].

4.4. Other activities

In addition to the above physiological activities, the polysaccharide of *P. eryngii* has other activities and is anti-inflammatory, antibacterial, hypoglycemic and hepatoprotective.

Glucan extracted from *P. eryngii* in an experimental *in vitro* model of the human cell line HL-60 and mouse experiments exhibits anti-inflammatory activity by modulating neutrophilic effects, and the results of the dextran sodium sulfate (DSS)-induced inflammatory bowel disease (IBD) mouse model showed that the glucan extracted from *P. eryngii* could effectively improve the clinical symptoms of IBD and maintain the percentage of activated monocytes in the circulating blood. The percentage of nuclear cells that inhibited the up-regulation of the pro-inflammatory cytokines TNF-α, CXCL1, significantly reduced the mRNA expression level of iNOS, indicating that dextran had an anti-inflammatory effect in IBD [66].

S.PEPS and PEPS significantly inhibited the secretion of IL-1β, IL-6, IL-10, TNF-α, and ROS and NO by RAW264.7 macrophages. At 800 μg/mL, the strongest anti-proliferative activities of S.PEPS and PEPS against Caco-2 cells were 81.9 ± 4.5 and 62.2 ± 9.7%, and those against HepG2 cells were 78.2 ± 2.7 and 57.5 ± 3.2%, respectively [38]. In the antibacterial activity test, the S.PEPS and PEPS showed antibacterial activity against *E. coli*, *S. aureus* and *L. monocytogenes*, and the antibacterial activity of S.PEPS was stronger than that of PEPS [67]. In the mouse model of type 2 diabetes, PEP effectively reduced serum insulin in mice, increased glycogen content in the liver, and showed hypoglycemic activity [63].

In mice with acute liver injury caused by injection of 1% CCl_4, the activity of SOD and GSH in liver tissue was significantly increased after feeding PEPE. At the same time, the accumulation of lipid droplets in liver tissue decreased and hepatocytes remained normal in high fat-loaded mice fed with PEPE. All the above results suggest that PEPE has a hepatoprotective effect [64].

5. Conclusions and future prospects

In recent years, the research reports on polysaccharides of *P. eryngii* have gradually increased, mainly focused on the extraction, separation and purification, structural analysis and pharmacological activities of *P. eryngii* polysaccharides. A large body of literature has confirmed that *P. eryngii* polysaccharides have pharmacological activities such as anti-oxidant, anti-hyperlipidemic, anti-tumor, immunoregulatory and liver protection activities. *P. eryngii* polysaccharide can indirectly exert anti-tumor activity through the body's own immune system, and it has little or no cytotoxicity, which makes it a potential new type of anti-cancer drug. However, most of the studies have only reported the *in vitro* pharmacological activity of polysaccharides extracted from *P. eryngii* and *in vivo* studies, and reports on the development of *P. eryngii* polysaccharide products are rare. In addition, research on the relationship between the structure and pharmacological activity of *P. eryngii* polysaccharides and its mechanism of action lacks depth. Therefore, further exploration of the structure-activity relationship of *P. eryngii* polysaccharides is required to provide a greater scientific and theoretical basis for the extensive processing and product development of *P. eryngii* polysaccharides.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijbiomac.2019.10.144.

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