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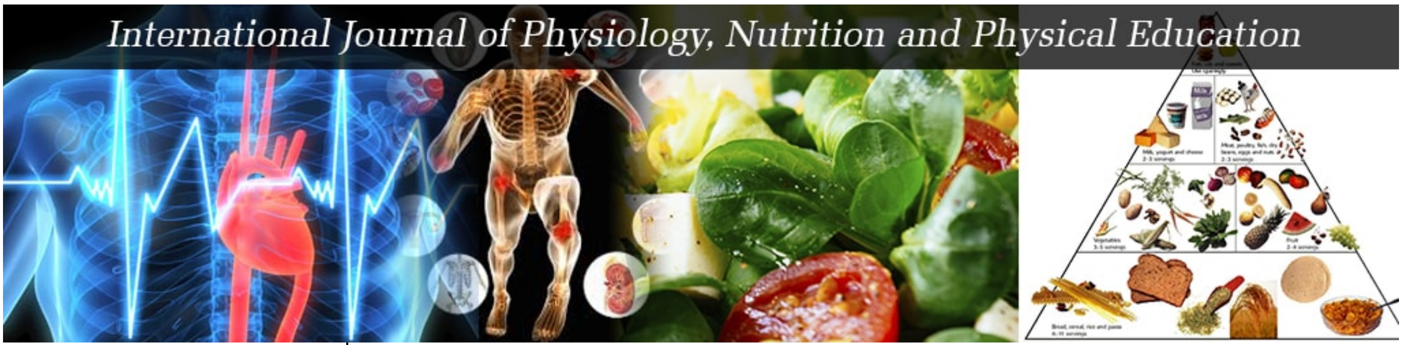

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A review on structural feature, extraction and bioactive properties of polysaccharides of *Pleurotus ostreatus*

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Abstract
The biodiversity of mushrooms is exciting due to its variability and diversity related to the composition of chemical structures such as polysaccharides, glycoproteins and secondary metabolites such as alkaloids, flavonoids etc. Recent studies of polysaccharides and their structural characterization have provided to direct research and development of technologies related to pharmacological action, production of bioactive foods and application of new and more sophisticated extraction tools. *Pleurotus ostreatus* is a valuable mushroom of dietary importance. It is rich in primary and secondary metabolites and chemical elements of physiological significance. In this review article the extraction, purification, structural analysis and biological activities of polysaccharides from this mushroom and provides updated research progress in areas important for the processing and product development of *Pleurotus ostreatus* derived agents.

Keywords: *Pleurotus ostreatus*, polysaccharide, isolation, structure, bioactivity.

1. Introduction
Mushrooms comprise a vast 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 basidiomycetes mushrooms contain biologically active polysaccharides in fruit bodies, cultured mycelium, and culture broth. These polysaccharides have (1→3)-β-linkages in the main chain of the glucan with additional β- (1→6) branch points. High molecular weight glucans appear to be more effective than those of low molecular weight. Chemical modification is often carried out to improve the antitumor activity of polysaccharides. *Pleurotus* species are commonly called oyster mushrooms. There are about 40 species of this mushroom [1] such as *Pleurotus ostreatus*, *Pleurotus sajor-caju, Pleurotus citrinopileatus*, *Pleurotus cornucopiae*, *Pleurotus tuber-regium*, *Pleurotus abalones, P. sapidus, P. corticatus, P. columbinus, P. spodoleucus, P. ferulae, P. nebrodensis, P. eryngii, P. pulmonarius, P. tuber-regium*, *P. cystiodisus, P. djamor, P. salmoneostramineus*, and *Pleurotus Florida* etc. They enjoy worldwide distribution, both in temperate and tropical parts of the world. Oyster mushrooms now rank second among the important cultivated mushrooms in the world [1].
The binomial name is *Pleurotus ostreatus* (Jacq.ex.fr) *P. Kumm*. The genus *Pleurotus* comprises about 40 species and they are commonly referred to as “oyster mushroom”, grow widely in tropical and subtropical areas and easily artificially cultivated. In recent decades a great increase in the international cultivation of *P. ostreatus* has been noted due to its significant tolerance of varied agro climatic conditions [2, 3].
Pleurotus ostreatus is a valuable mushroom of dietary importance. It is rich in primary and secondary metabolites and chemical elements of biological significance. Fresh fruiting body contains 15% of the recommended daily intake of vitamin C, 40% of niacin, riboflavin, and thiamin, and 0.5 mg of vitamin B12 of 100 g of fruit bodies. This species is also characterized by a high content of oleic acid (40%), linolenic acid (55%), and substances responsible for decreasing serum cholesterol levels [4]. High contents of lovastatin, an approved hypolipidemic drug, and pleuran, an immunomodulating polysaccharide, have been found in fruiting bodies of this species [5-7]. It exhibits anti atherosclerotic, hypoglycemic, antioxidant, anticancer and immunomodulatory properties [8-11].

Due to its wide spectrum of biological activities, *P. ostreatus* is considered a medicinal mushroom. Fruiting bodies and extracts of *P. ostreatus* have found applications in the treatment of civilization – related diseases, especially diabetes [12, 13], arteriosclerosis and cancer [10, 11]. It is also a potential source of active ingredients in cosmetics and topically applied preparations.

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 [10, 11]. 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 [14, 15]. The polysaccharides of *P. ostreatus* have become increasingly attractive due to their antioxidant, antitumor, immunoregulatory, and other activities. The purpose of this paper is to review the extraction, structure and bioactivities of *P. ostreatus* polysaccharides, and to provide a scientific basis for further processing and the product development of *P. ostreatus* polysaccharides.

2. Extraction and purification

The polysaccharides of mushroom can be obtained from fruiting bodies, mycelial biomass or directly from liquid culture broth, such as exopolysaccharides released into the extracellular medium [16]. There are currently several polysaccharide extraction techniques, such as hot water extraction [17], microwave assisted extraction [19], pulse extraction, and ultrasound assisted extraction, enzyme assisted extraction [20], among others, each with its own peculiarity, advantages and disadvantages.

Most of the extraction of *P. ostreatus* polysaccharides has been carried out by a hot water method. The isolation of polysaccharides is initiated with hot water extraction, followed by precipitation in EtOH and centrifugation [19-22]. It is then dissolved in minimum volume of water and exhaustive dialysis is carried out to remove small carbohydrate molecules using DEAE cellulose bag. The high molecular weight polysaccharides remain inside the bag and the solution is then freeze-dried. Size exclusion chromatography (SEC) has been used to separate the mixture of polysaccharides. SEC also known as gel permeation chromatography (GPC) is a technique for characterizing polymer. It separates molecules on the basis of their size relative to the pores in the packing particles. For this purpose DEAE–cellulose column (3 x 30 cm) was used. The packing material was equilibrated with 1% NaCl solution. The elutes were detected with phenol-sulfuric acid reagent [23] at 490 nm using UV-vis spectrophotometer. This method [23] was also used to measure total carbohydrate content of the polysaccharide with reference to a standard carbohydrate. The apparent molecular weight [24] of each fraction was estimated using standard Dextrans.

The polysaccharides were prepared with distilled water using an ultrasonic-assisted cellulose method under optimized conditions (enzymatic hydrolysis temperature). Crude polysaccharides extract was further deproteinized with Savage reagent and dialyzed against distilled water, and then lyophilized [25].

Microwave-assisted extraction and pressurized liquid extraction (PLE) were compared asadvanced technologies to obtain polysaccharides from P. *ostreatus* fruiting bodies. These approaches are considered as environmentally friendly technologies due to their higher efficiency and consequently lower energy consumption, apart from lower emission of CO_2 as well as reduced use of pollutant solvents [26].

3. Structural analysis of polysaccharides

Structural analysis of polysaccharides requires the determination of molecular mass, chain composition, configuration and conformational isomers, sequence of monosaccharide residues, presence and position of branches and functional groups, and presence of inter glycosidic linkages. In recent time, there is a wide range of characterization techniques available to obtain structural details of polysaccharides. Chemical methods involve the use of reactions to get more insight into the chemical structure and arrangement. In contrast, analytical techniques are gaining more importance since sample preparation is straightforward and they give precise information. The analytical methods applied mainly include high performance liquid chromatography (HPLC), nuclear magnetic resonance spectroscopy (1D and 2D NMR), gas chromatography-mass spectrometry (GC-MS), infrared spectroscopy (IR), methylation analysis, and Smith degradation analysis [27-30]. 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 [31, 32].

The polysaccharide, pleuran, isolated from *Pleurotus ostreatus* consists of a backbone of (1→3)-linked D-glucan every fourth residue, being substituted at *O*-6 with single D-glucopyranosyl groups [33]. The linear polysaccharides α-(1→3)-linked D-glucan [34] and a β-(1→3), (1→6)-linked glucan [35] have been isolated from *P. ostreatus.* A glucan [36] was isolated from the *P. ostreatus var.* consists of a linear chain β-(1→3), (1→3, 6), (1→6)-linked D-glucopyranosyl and branching at C-3 of (1→3, 6)-D-glucopyranosyl by α-D-glucopyranosyl unit. A heteroglycan [37] was isolated from the mycelia of *P. ostreatus* was found to consist of the repeating unit had a branched backbone composed of (1→6)-linked α and β D-glucose and (1→2)–linked α-L-fucose. Branching occurred at C-4 position of (1→6)-linked α-D-glucopyranosyl residue with terminal β-D-mannose and C-3 position of (1→6)-linked β-D-glucopyranosyl residue with (1→6)-a-D-mannopyranosyl moiety terminated by a-D-glucose.

4. Biological activities

Mushrooms have been traditionally employed as medicinal agents due to their proven healthy properties. Among the occurring bioactive compounds, polysaccharides are responsible for a wide range of biological activities, the modulation of the immune system being the most studied. Polysaccharides from mushrooms showing a β-linkage have demonstrated a boost in the human immune system and the modulation of the immunological response under certain circumstances, thus they are commonly termed biological response modifiers (BRM). As a result of the activation of the host's immune system, these polysaccharides show significant antitumor, antiviral and antimicrobial activity, among other effects

Antitumor and immunomodulatory activities

According to the World Health Organization (WHO), 13% of people who die were suffering from cancer [38]. 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. Therefore, finding new alternative anticancer drugs and overcoming the shortcomings of conventional chemotherapy drugs has become is increasingly important in cancer therapy [39]. 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 [40].

Tong *et al.* [19] suggested that a hetero polysaccharide (POPS-1) from *Pleurotus ostreatus* exhibited strong antitumor activities against Hela tumor cells *in vitro*. No antiproliferative effect of these polysaccharides on human embryo kidney 293T cells was observed, implying that they had no direct cytotoxicity to non-cancerous cells. The results suggest that POPS-1 could be considered as a potential candidate for antitumor agent. The novel polysaccharides [41, 42] isolated from the mycelia and fruit body of *Pleurotus ostreatus* exhibited immunological functions which include lymphocyte proliferation, macrophage activation (nitric oxide production, ROS generation, phagocytosis, TNF-α production) as well as macrophage and NK cell mediated cytotoxicity. *In vivo* studies with Dalton's Lymphoma mice tumor model considered the immune enhancing and tumor regression potential of the two glucan molecules. Highest tumor inhibition of about 75% and 71.4% were observed at 20 mg/kg of mycelia and fruit body glucan/glycan treatments. Both the polysaccharide molecules showed similar degree of immune response *in vitro*.

Kong *et al.* [43] reported the alkali extracted polysaccharide (WPOP-N1) from the fruiting bodies of *P. ostreatus* showed immunomodulating and antitumor activities effects *in vivo* and *in vitro*. In this publication they showed that WPOP-N1 could activate macrophages through NF- κB signalling pathway, and the antitumor effects of WPOP-N1 can be achieved by its immunostimulating property. Facchini *et al.* [44] studied the antitumor activity of *Pleurotus ostreatus* polysaccharide fractions (FS, FI, FII, FIII-1, FIII-2) on Ehrlich tumor and Sarcoma-180. FS, FI and FII fractions promoted the highest rates of inhibition of the development of Ehrlich tumor of 60.6, 76.5 and 73.6%, respectively. FII and FIII-2 fractions exhibited the highest rates of inhibition of Sarcoma-180 of 85.6 and 93.6%. The fraction FII was effective against both tumors and in a concentration of 30 mg/kg, showed no toxic effect.

Maity *et al.* [38] was isolated a pure glucan from aqueous extract of an edible mushroom, *Pleurotus ostreatus*, cultivar showed the immunomodulating properties through macrophage, splenocyte, and thymocyte activations.

5. Antioxidant Activity

Oxidative damage is related to the prevalence of a great number of common diseases. Therefore, substances capable of acting as antioxidant agents usually prevent the development of certain diseases. Polysaccharides from different mushrooms showed free radical scavenging activity [45], superoxide radical scavenging activity, reducing properties, lipid peroxidation inhibition, suppression of proliferation and oxidative stress etc. [46]. The activity not only depended on the fungal species but also on the chemical structure and arrangement of the active polysaccharide [47-50]. Similarly, mixed carbohydrates, such as polysaccharide–peptide complexes, have also shown a potent antioxidant activity [51].

Patra S. *et al.* studied the antioxidant activity of a heteroglycan [42] was isolated from the mycelia of *P. ostreatus.* The scavenging activity of hydroxyl free radicals whose EC_{50} value of heteroglycan at 943 µg/mL was determined. Further, EC_{50} value of the heteroglycan at 53 µg/mL was observed for scavenging activity of superoxide free radicals. Chelating effects (54.82%) of ferrous ions were observed at 1 mg/mL of the polysaccharide.

6. Conclusions

Polysaccharides of mushroom from the genus *Pleurotus spp*, are important biological structures, responsible for several bioactivities with scientific relevance and profound impacts on society, economy, income generation, and technology development. In recent times, the research reports on polysaccharides of *P. ostreatus* have gradually increased, mainly focused on the extraction, separation and purification, structural characterization and biological activities. These polysaccharides have been considered as biological response modifiers due to their ability to enhance the immune system and, therefore, prevent and treat several common diseases and promote health. A large body of literature has confirmed that *P. ostreatus* polysaccharides have pharmacological activities such as anti-oxidant and antitumor, immuno regulatory activities. *P. ostreatus* polysaccharide can indirectly exert antitumor activity through activating the body's own immune system, and it has little or no cytotoxicity, which makes it a potential new type of anticancer drug.

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