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The Research Progress of Armillaria Mellea Polysaccharide

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Abstract: Armillaria mellea is also known as honey mushroom ring, wheel Ye Ji mushrooms or hazel mushroom, is one of the traditional medicine combination food fungi, its polysaccharide has many kinds of biological activity, such as antioxidation, anti-inflammatory, anti-tumor and so on.With the deepening of the research, more and more honey fungus polysaccharide was found, and the study of its biological activity has also made significant progress.This paper mainly discusses the armillaria polysaccharide extraction process and the optimization, structure, and physical and chemical properties, biological activity and so on, so as to provide reference for the development and utilization of the further research of the armillaria.

1. Introduction

Armillaria mellea is a parthenogenic parasitic fungus of the family Tricholomataceae in the order Hymenomycetes [1], and is also a hemiphyte of the famous Chinese medicinal herbs asparagus and porcupine [2], which is widely distributed in temperate regions of the Northern Hemisphere and is often found on the roots of coniferous or broad-leaved tree trunks in summer and autumn. It has a variety of bioactive components, including polysaccharides, sterols, sphingolipids, fatty acids, sesquiterpenes, non-hallucinogenic indole compounds, peptides, enzymes, adenosine derivatives and more than 110 others, among which polysaccharides are proven to play a key role, A. mellea polysaccharides, which can be extracted and taken out from the mycelium, ascospores, mycelium and fermentation broth of A. mellea [3-6]. With the development of biotechnology and in-depth research on the biological activity of A. mellea polysaccharides, more and more studies have shown that A.mellea polysaccharides have a wide range of biological activity and application value. Therefore, a comprehensive introduction and discussion of the physicochemical properties, extraction methods and their biological activities of A.mellea polysaccharides is of great significance to promote the in-depth research and application of A. mellea polysaccharides.

2. Extraction Process and Optimization of Polysaccharide from A.Mellea

Polysaccharide extraction can use different extraction methods according to the characteristics of raw materials and extraction sites and other factors. A variety of methods have been developed to extract polysaccharide components, mainly including acid-base extraction, hot water extraction, ultrasound-assisted extraction, compound enzyme-assisted extraction, microwave-assisted extraction, ultrasound-microwave-assisted extraction, pressurized extraction, supercritical extraction, etc. [7,8].

Acid-base extraction method, for some polysaccharides with low water solubility, the extraction rate of hot water extraction method is low, and the extraction rate can be increased by adding acid or alkali solution. The acid-base extraction method is influenced by the pH, temperature and extraction time of the solution, and the solution is too acidic or too alkaline to degrade the polysaccharides. Sun Yujiao [9] investigated different extraction methods to extract porcupine tea polysaccharides and found that the alkali extraction of porcupine tea polysaccharides yielded higher than water and acid extraction, and the highest glyoxalate content of porcupine tea polysaccharides was obtained by the alkali extraction method, which was more favorable to extract polysaccharides rich in glyoxalate; Sun Wei Xuan [10] used acid, alkali and enzymatic methods to extract potato pomace pectin polysaccharides and found that the alkali extraction method yielded the highest polysaccharides (23.1%).

Hot water extraction method, i.e. water extraction and alcoholic sedimentation method, is the traditional method of polysaccharide extraction, the required reagents and equipment are simple and easy to obtain, easy to operate, can avoid the destruction of polysaccharide by acid extraction method and alkali extraction method, and the polysaccharide activity of honeysuckle extracted by this method is high. The raw material is destroyed by the continuous action of hot water, which makes the intracellular polysaccharide flow out. The extraction rate of polysaccharide was affected by temperature, material-liquid ratio and extraction time.Du Guofeng [11] et al. used hot water extraction method to extract Astragalus polysaccharides, and compared the temperature, extraction time, and feed-liquid ratio, and found that the polysaccharide yield was proportional to temperature, extraction time, and feed-liquid ratio within a certain range. The optimum extraction conditions were 90 ℃, 2.5 h extraction time, and 1:8 g/mL ratio; when the conditions continued to increase, the polysaccharide yield decreased;By investigating the effect of different methods on the extraction rate of polysaccharides from A.mellea, Lu Qi [12] found that hot water extraction method gave higher extraction rate than ultrasonic extraction and homogeneous extraction, and the polysaccharide yield was less affected by the particle size of raw materials.

Ultrasound-assisted extraction method is to destroy the cell wall structure by high-frequency vibration of ultrasound in the liquid to promote the penetration of solvent and the release and diffusion of active ingredients, which has the advantages of simpler operation, less reagent dosage, less time consuming, lower temperature and significantly higher extraction efficiency compared with the hot water extraction method, but requires higher equipment. Ultrasound can effectively destroy the cell wall, so that the particle size of the extract can be reduced, the transfer of the cell contents can be enhanced, and the extraction rate of honeysuckle polysaccharide increases [13]. Wang Jiachu [14] et al. used ultrasound-assisted hot water extraction process to extract polysaccharides from A.mellea, and promoted the rapid dissolution of polysaccharides by ultrasound on the basis of simple and environmentally friendly operation. Although this method can enhance the polysaccharide extraction rate, it suffers from the problem that the heat production effect is not strong, which easily leads to insufficient extraction temperature.

Enzymatic extraction is a method to extract intracellular polysaccharides by disrupting the cell wall structure of raw materials under the action of enzymes, which can save time and obtain stable products. Enzymatic extraction is usually carried out in synergy with ultrasound, microwave or using complex enzymes, which can further enhance the extraction efficiency. With the synergistic effect of complex enzymes, the hydrolysis of raw materials is more complete and the polysaccharide extraction rate is improved. The mild reaction of compound enzyme method can better preserve the polysaccharide activity, which is a mild and efficient extraction method, but the enzyme price is high, and the extraction cost is large and affected by the enzyme addition, enzyme ratio, pH, temperature, etc. The optimal extraction conditions for the extraction of crude polysaccharides from M. gaultheriae using a complex enzyme method by Peiwen Su [15] were: particle size 80 mesh, enzyme addition (mass ratio) 4%, extraction time 2 h, pH=5, temperature 45 °C, enzyme ratio (papain: cellulase: pectinase) 2:2:1, material-liquid ratio 1:50 g/mL, and crude polysaccharide extraction rate of 18.86±0.04%.

Microwave-assisted extraction is the extraction of polysaccharides by microwave thermal effect, the principle is to use the combination of microwave and traditional hot water extraction method. The microwave power and extraction time will affect the polysaccharide extraction rate. Microwave power, extraction time, etc. will affect the polysaccharide extraction rate, polysaccharide yield and microwave power and extraction time are positively correlated, but too much power and too long time tend to make polysaccharide degradation. Liyuan Feng [16] extracted pectin polysaccharides from broccoli stems by microwave-assisted acid method with pH and microwave power as highly significant factors, and the polysaccharide yield was 4.8%. Jiaolong Fu [17] used microwave-assisted extraction and hot water extraction to extract pumpkin polysaccharides and found that the extraction rate of microwave-assisted extraction method was 5.25 % higher than that of hot water extraction method.

Ultrasonic-microwave-assisted extraction is the extraction of polysaccharides by using ultrasonic cavitation effect and microwave thermal effect synergistically, which can improve the extraction rate. Supercritical extraction method is an extraction method that uses supercritical fluid with similar penetration to gas and similar solubility to liquid for extraction and separation, which is suitable for separating heat-sensitive substances. Although this method has high extraction efficiency, the equipment is complicated and costly, so it cannot be widely used. The pressurized solvent method can accelerate the extraction of polysaccharide components by heating up the solvent under pressure and strengthening the penetration and diffusion ability of the solvent to the cells [18]. This method can accelerate the extraction of polysaccharide components.

The response surface analysis method can determine the optimal process conditions for the extraction of polysaccharides from A.mellea, thus enhancing the yield of polysaccharides obtained. It was found that the optimal conditions of each extraction method could be analyzed and screened by using factors such as extraction temperature, extraction time and liquid to material ratio as experimental factors and the extraction rate of honeysuckle polysaccharide as response values. Different extraction methods can also add experimental factors to participate in the analysis appropriately, such as the ultrasonic power for the ultrasound method, the amount of enzyme added for the enzyme method, and the pressure for the pressurization method.

3. Structure and Physicochemical Properties of A.mellea Polysaccharide

3.1. Composition Structure of A.mellea Polysaccharide

Polysaccharide is the main active component of A.mellea, which mainly includes intracellular polysaccharide and extracellular polysaccharide. The polysaccharide content of A.mellea varies in different developmental stages, and the experimental study showed that the polysaccharide content of A.mellea was 9% in mycelium, 2.27% in zygote, 1.12% in corpuscle and 0.87 g/100 m L in fermentation broth, indicating that the polysaccharide content of mycelium was the highest [19]. The fermentation broth contained 0.87 g/100 m L, indicating that the mycelium contained the most polysaccharides. The composition of the polysaccharides obtained from the fermentation broth also differed greatly depending on the extraction method of A.mellea.

Due to the different cultivation methods and extraction processes of A.mellea, the results obtained by different scholars are not the same. Hong Yi [20] et al. isolated the intracellular polysaccharide of A.mellea from its mycelium, and the polysaccharide fraction reacted pink with carbazole ethanol (Dische reaction), and the analysis proved that it contained glyoxalate, and the constituent monosaccharides were D-glucose, D-galactose, D-mannose, and D-xylose by paper chromatography and gas chromatography, and the constituent monomers were pyranose and did not contain protein.Chen-Chen Yu [21] et al. analyzed the structure of water-soluble polysaccharides in the fermentation broth of A.mellea using UV and IR spectroscopy and gas phase derivatization, and the results showed that the water-soluble polysaccharides were single in composition and all of them were pyranose type glucose. Xiaojie Liu [22] et al. obtained three homogeneous polysaccharides, AMFP-I, AMFP-II and AMFP-III, which were mainly composed of galacturonic acid, glucose, galactose and xylose, from the fermentation broth of A.mellea by dextran gel chromatography.

3.2. Physicochemical Properties of A.mellea Polysaccharide

Since the processes of extraction methods vary, there may be compositional differences in the A.mellea polysaccharides extracted under different conditions, so it is extremely necessary to analyze the physicochemical properties of A.mellea polysaccharides. The molecular mass, monosaccharide composition and glycosidic bond type of polysaccharides can be analyzed by hydrolysis, infrared spectroscopy, mass spectrometry, high performance liquid chromatography and gas chromatography in general. The analysis revealed that the chemical composition of Nectarobacterium polysaccharides is diverse in structure and has different biological activities. Two polysaccharide fractions with α-glucan structure were isolated from the substrates of A.mellea, which could reduce cholesterol and triglyceride concentrations, thus reducing the risk of cardiovascular diseases; the polysaccharide isolated from the sporophore of A.mellea had good antioxidant ability and its carbohydrate content was more than 58%; the polysaccharide isolated from the (1→6)-linked-α-d-glucan was more than 58%.) -linked-α-d-glucopyranosyl and (1→2,6)-linked-α-d, another A.mellea polysaccharide with immunomodulatory function [glycosyl and (1→2,6)-linked-α-d [23].

4. Biological Activity of A.mellea Polysaccharide

4.1. Antioxidant Activity

The significant antioxidant activity of A.mellea polysaccharide is determined by its special chemical structure and molecular weight. Its ability mainly comes from the various active groups contained in polysaccharide molecules, such as hydroxyl, methyl and aldehyde groups, which can react with free radicals, reduce their production, and thus play a protective role against free radical damage to cells and tissues. The results of traditional chemical antioxidant experiments on M. gaulis showed that Ag-1 and Ag-2 both have the ability to scavenge DPPH radicals, O2--radicals, ABTS radicals and reduce Fe3+, and it was found that both Ag-1 and Ag-2 have protective effects on HepG2, and Ag-1 is more effective, which proves that it has antioxidant activity [15] Fangjuan Zhao [24] et al. performed progressive high temperature tolerance domestication of three strains of A.mellea, and both the domesticated A.mellea and parental A.mellea showed different degrees of antioxidant capacity.Lianyu Zhou et al. studied the antioxidant activity and antibacterial activity of selenium polysaccharide of A.mellea yellow-green [25] showed that the scavenging ability of selenium polysaccharide on hydroxyl radical, DPPH radical and ABTS+ radical increased significantly ($P<0.05$) with the increase of its concentration, but the scavenging rate was less than that of ascorbic acid.

4.2. Antitumor Activity

A.mellea polysaccharide has a significant effect on the treatment of tumors. It can induce apoptosis of cancer cells through various pathways, thus exerting anti-tumor effects. It was found that polysaccharide extracted from A.mellea could inhibit the growth of H22 hepatocellular carcinoma cells and induce apoptosis of hepatocellular carcinoma cells by regulating the expression of Bax, Bcl-2, caspase-3 and other proteins. The polysaccharide from A.mellea could inhibit the proliferation of SMMC-7721 hepatocellular carcinoma cells in vitro, inhibit intracellular protein synthesis and inhibit Bcl-2 protein expression to promote apoptosis apoptosis [26].In lung cancer patients, A.mellea polysaccharide can improve SOD, GSH-Px, and GSH activity in the body, increase serum SOD, GSH-Px, and GSH levels in patients, reduce MDA levels, and decrease the damage caused by free radicals, which play an important role in the anti-tumor process [27]. In addition, it was found that A.mellea polysaccharide can also improve the activity of T lymphocytes and the killing effect of NK cells, thus enhancing human immunity and reducing the growth of cancer cells.

4.3. Immunomodulatory Activity

A.mellea polysaccharide has immunomodulatory activity and can act by promoting the phagocytosis of macrophages, enhancing human immunity and regulating immune levels. A.mellea polysaccharide can stimulate the phagocytosis of macrophages, promote the activation of macrophages and secrete inflammatory cytokines.Hanzhen [28] isolated and purified AAMP-A70, a polysaccharide with a molecular weight of 5.6 KDa, from the cotyledons of A. honeysuckle, and showed that AAMP-A70 exerts immunomodulatory activity by activating NFKB and MAPK signaling pathways through binding to TLR-2 and activating macrophages. In addition, A.mellea polysaccharide can enhance the immune function of the body and improve the activity of T-lymphocytes and B-lymphocytes, thus strengthening the resistance of the body.In a study by Huiguo Wang [29] et al. found that A.mellea polysaccharide enhances the phagocytic function of phagocytes and the proliferation capacity of splenic T lymphocytes, and is an immunomodulator that can improve the immunity of the body. In addition, it has been suggested that A.mellea polysaccharide can enhance immune function by modulating the action of immune cells and immune molecules [30].

4.4. Anti-inflammatory Activity

The anti-inflammatory activity of A.mellea polysaccharides is one of the hot topics of research in recent years. Anti-inflammatory effect refers to the inhibition of cellular and molecular activities in the inflammatory process, thus reducing the inflammatory response and tissue damage. Several studies have shown that A.mellea polysaccharides are able to exert their anti-inflammatory effects by inhibiting the activity of inflammatory cells, modulating the immune system and scavenging free radicals in various ways [6]. A.mellea polysaccharide reduced the inflammatory response of periodontitis in mice by regulating the gene expression of pro-inflammatory factors IL-1β, TNF-α and anti-inflammatory factor IL-10 in periodontal tissues [31]. In laboratory studies, the anti-inflammatory effects of A.mellea polysaccharides have been initially demonstrated. Through in vivo and in vitro experiments, researchers found that A.mellea polysaccharide was able to reduce the inflammatory response in the liver of mice and lower the level of inflammatory indicators, as well as enhance the function of the immune system of mice. The anti-inflammatory effect of A.mellea polysaccharide is exerted by the synergistic effect of its multiple bioactive components [32]. However, further clinical studies are needed to evaluate the mechanism of action and the prospect of clinical application.

4.5. Modulation of Glycolipid Metabolic Activity

A.mellea polysaccharide can regulate glucolipid metabolism. A.mellea polysaccharide can improve insulin resistance through the regulation of lipid metabolism. The alkali-extracted polysaccharide AAMP significantly reduced fasting blood glucose levels and improved glucose intolerance in type 2 diabetic rats, and increased the expression levels of lipoprotein lipase (LPL), adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) by regulating the transcription factor SREBP-1c, and decreased the expression levels of serum triglycerides (TG) and free fatty acids (FFA). Expression levels, thereby inhibiting adipogenesis [31]. In addition, it was shown that the total sugar AMP and neutral sugar AMP-N of A.mellea had significant oral hypoglycemic activity, and the hypoglycemic effect of AMP-N was superior to that of AMP. The mechanism of hypoglycemic effect of AMP-N, on the one hand, increased the level of autophagy in the liver of diabetic mice, reduced fat accumulation, and improved insulin sensitivity; on the other hand, protected the pancreatic islets of diabetic mice and improved the damaged function [33]. On the other hand, it protected the islets of diabetic mice and improved the damaged function.

4.6. Other Roles

In addition to the above effects, many studies have confirmed that A.mellea polysaccharides have biological activities such as anti-Alzheimer's disease [34], anti-aging, neuroprotective, hepatoprotective, and antibacterial [35].

5. Summary and Outlook

A.mellea is one of the famous traditional Chinese herbal medicines for tonicity and disease prevention in China, which has a long history of application in tonicity and disease prevention. In recent years, the biological activity of fungal polysaccharides has attracted more and more attention, and the extraction, structure identification, pharmacological activity and mechanism of action of A.mellea polysaccharides have also become hot spots for research. At present, there are many experimental studies on the pharmacological effects of A.mellea polysaccharides, but most of them are on the whole animal level, and the studies on the mechanism of action at the cellular and molecular levels still need to be explored in depth.

In recent years, a lot of research has been done at home and abroad on the polysaccharide of A.mellea and its medicinal value, and good research results have been achieved, and nutritional functional health food such as A.mellea tablets, A.mellea punch and A.mellea syrup with health functions have been developed. More in-depth research and development on A.mellea has been going on, and as the research on A.mellea polysaccharide continues, more preparations will definitely be developed to better serve human health.

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