ISOLATION OF POLYSACCHARIDES FROM THE BASIDIOCARP OF GANODERMA LUCIDUM AND STUDY OF THEIR MOLECULAR WEIGHT AND MONOSACCHARIDE COMPOSITION

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Abstract: In this article, polysaccharides from the basidiocarp of *Ganoderma lucidum* were isolated using aqueous and alkaline fractionation methods. The total sugar content of the polysaccharides was determined using the phenol-sulfuric acid method. The monosaccharide composition of the polysaccharides was analyzed using paper chromatography.

Keywords: fungus, monosaccharide, polysaccharide, fraction, chromatography, Sevag method.

Аннотация: В данной статье полисахариды, содержащиеся в базидиальном грибе *Ganoderma lucidum*, были выделены с использованием водной и щелочной фракционирующей методик. Общее содержание сахаров в составе полисахаридов определялось методом фенол-серной кислоты. Моносахаридный состав полисахаридов был определён методом бумажной хроматографии.

Ключевые слова: гриб, моносахарид, полисахарид, фракция, хроматография, метод Севиджа

Annotatsiya: Ushbu maqolada *Ganoderma lucidum* bazidial zamburug‘i tarkibidagi polisaxaridlar suvli va ishqoriy fraksiyalash usullari yordamida ajratib olindi. Polisaxarid tarkibidagi umumiy qand miqdori fenol-sulfat kislotali usulda aniqlandi. Polisaxaridlarning monosaxarid tarkibi qog’oz xromotografiyasi usuli bilan aniqlanadi.

Kalit so‘zlar: zamburug‘, monosaxarid, polisaxarid, fraksiya, xromotografiya, Sevage usuli.

Introduction

Ganoderma lucidum (Reishi) is a medicinal mushroom that has been widely used in traditional Asian medicine for centuries. Its richness in biologically active compounds has led to extensive scientific research in the fields of modern pharmaceuticals and biotechnology. One of the most important bioactive components of this mushroom is its water-soluble polysaccharides. These typically exist in the form of β-glucans and other complex polysaccharides, and they are known for enhancing the immune system, stimulating cell regeneration, reducing

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inflammation, and combating oxidative stress. The biological activity of these polysaccharides largely depends on their molecular weight, monosaccharide composition, and chain structure. In particular, optimizing the extraction process of water-soluble polysaccharides and conducting in-depth analysis of their composition is of great importance in contemporary phytopharmacology and biomedical science. This article investigates the extraction technology of water-soluble polysaccharides from *Ganoderma lucidum*, analyzes the structural composition of the extracted substances, and studies their biological activity. Additionally, it evaluates the possibilities of increasing polysaccharide yield and obtaining pharmacologically effective forms by selecting optimal extraction conditions.

The results of this study may serve as a scientific foundation for the development of pharmaceutical preparations and the creation of natural immunomodulatory agents in the future.

Literature Review

Currently, biologically active substances derived from natural compounds are widely used in medicine and pharmaceuticals. Water-soluble polysaccharides extracted from *Ganoderma lucidum* (*GLP - Ganoderma lucidum polysaccharides*) are known for their high biological activity. These compounds are recognized for enhancing the activity of macrophages and T-cells, suppressing inflammatory processes, and fighting oxidative stress. The polysaccharides generally have complex structures such as β-(1→3)-glucan and β-(1→6)-glucan, and their structure and molecular weight directly affect their biological activity [1].

The *Ganoderma lucidum* basidiomycete has a bitter and woody taste, which is attributed to the presence of 119 types of triterpenoids and aromatic compounds with anti-inflammatory, antitumor, and antiviral properties. More than 200 polysaccharides have been isolated from the fruiting bodies, spores, and mycelia of this mushroom species. The monosaccharide composition of these polysaccharides includes D-glucose, D-galactose, D-fructose, D-mannose, D-xylose, L-fucose, L-rhamnose, and L-arabinose. The polysaccharide content of this mushroom ranges from 10% to 25% [2].

Yew-Keong Choong and colleagues isolated two peptidoglycans, named Ganoderan B and C, from the fruiting body of *Ganoderma lucidum*. Their chemical and physicochemical properties revealed that Ganoderan B contains a main chain of β-1,3-D-glucopyranosyl with 1,6-linked side chains. Ganoderan C contains a main chain of β-1,3-D-glucopyranose with 1,6-linked D-galactopyranose side chains [3].

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Today, polysaccharides extracted from basidiomycete fungi are known to exhibit immunomodulatory, antiviral, hepatoprotective, antiproliferative, and hypolipidemic activities. Numerous scientific studies and researchers - including Whistler, Zakany, Leung, Chihara, and Fachet - have successfully isolated polysaccharides from basidiomycetes [4].

These polysaccharides have demonstrated up to 90% inhibition of sarcoma S-180 and Ehrlich carcinoma growth, as well as significant antiviral activity against hepatitis B and herpes viruses [5].

Currently, the extraction of biologically active polysaccharides from mushrooms and their application in practice remains a highly relevant area of research.

Research Methodology

As the object of research, basidiomycete fungus *Ganoderma lucidum* collected from Bo‘stonliq district of Tashkent region was used. The fruiting body of *Ganoderma lucidum* was dried to a constant weight in a DC-80-01 SPU drying oven at a temperature of 30°C. The raw material was ground in a laboratory grinder to particle sizes ranging from 1 to 10 mm. To remove low molecular weight compounds from the ground raw material, a Soxhlet apparatus was used with a mixture of ethanol (96%) and chloroform in a 2:1 ratio at 90–95°C. The extraction continued until the solvent became completely colorless. The mixture was filtered, evaporated in a rotary evaporator at 50°C until a thick mass remained, and then dried at 100°C until the moisture content reached 5%.

To isolate water-soluble polysaccharides, the method used by A.P. Korzh was employed. This method examines the effects of raw material fineness, extraction time, and the ratio of extractant to raw material on the yield of extractive substances. For extraction, the raw material was extracted three times with distilled water at hydromodule ratios of 1:20, 1:15, and 1:10 for a total duration of 6 hours using a water bath. The resulting aqueous extracts were combined, concentrated in a vacuum evaporator (down to 1/5 of the total volume), and filtered. To remove polar solvent-soluble impurities, the concentrated sample was precipitated with ethanol at a ratio of 1:3 and kept at -5°C for 12 hours. The precipitate was washed three times with 96% ethanol, centrifuged, and dried in an oven at 40°C. To purify proteins from the water-extracted polysaccharide fractions, the Sevag method was used. Lyophilized polysaccharide fractions were dissolved in water and washed three times with a chloroform:n-butanol mixture (5:1 ratio). The solution was placed on a magnetic stirrer and mixed for 1 hour until an emulsion formed. The resulting emulsion was centrifuged at 10,000 rpm for 15 minutes. This process was

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repeated at least three times. After separating the aqueous phase, it was dialyzed through a dialysis membrane and then lyophilized.

The quantitative content of the polysaccharides was determined using the phenol-sulfuric acid method in accordance with the glucose calibration curve. For this, 0.5 ml of the polysaccharide solution was mixed with 0.5 ml of 5% phenol solution and 2.5 ml of concentrated sulfuric acid and incubated in a water bath for 10 minutes. The calibration curve was constructed using glucose solutions with concentrations ranging from 10 to 120 µg/ml. The analysis was carried out using a Shimadzu UV-VIS 1280 spectrophotometer (Shimadzu Europa GmbH, Germany) at a wavelength of 490 nm. The molecular weight distribution of the water-soluble polysaccharides was studied using gel chromatography. The research was conducted on a gel chromatograph equipped with an Agilent 1260 Infinity refractometric detector and a 7.5 x 300 mm PL aquagel OH Mixed chromatographic column (Waters, USA). The concentration and injection volume of the sample were 2 mg/ml and 10 µl, respectively. As the eluent, a 0.1 M sodium nitrate solution was used, with a flow rate of 0.8 ml/min. The columns were calibrated using dextran sulfate standards (Sigma-Aldrich Chemie GmbH, Germany).

The monosaccharide composition of the polysaccharides was determined using paper chromatography. The extracted polysaccharide samples were hydrolyzed in 1.0 N H_2SO_4 at 100°C for 8 hours, and the monosaccharide composition was determined using paper chromatography (PC). The separation of monosaccharides was carried out on Filtrak FN-12 (Germany) paper using a solvent system of butanol:pyridine:water (6:4:3 by volume). Aniline phthalate reagent was used for staining the monosaccharides.

Analysis and Results

The initial weight of the mushroom prepared for extraction was 800 g, and it was dried to a constant weight in a drying oven at 30°C. The weight of the dried raw material was 650 g. The dried raw material was then ground using a laboratory grinder. The appearance of the ground raw material was brown in color with a wood-like texture.

First, the raw material was purified from low molecular weight compounds. The dried and ground *Ganoderma lucidum* basidiomycete extract was subjected to extraction using a Soxhlet apparatus with a mixture of ethanol and chloroform (in a 2:1 hydromodule ratio), until the solvent became completely colorless. The mixture was then filtered and evaporated in a rotary evaporator until a thick mass remained, and finally dried until the moisture content reached 5%. The extracted low molecular weight compounds included lipophilic substances, phenolic

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compounds, partial proteins, and pigments, which accounted for 3.6% of the total mass. Factors influencing the extraction process of water-soluble polysaccharides such as solvent ratio, degree of grinding of the raw material, and extraction time were studied. It was found that these factors significantly affect the yield of water-soluble polysaccharides.

Table 1

Effect of solvent (water) ratio and degree of raw material grinding on the yield of water-soluble polysaccharides

№	Particle size (mm)	Extraction time (h)	Temperature (°C)	Solvent ratio (1:x)	Yield (%)
1	4-6	2	100	1:15	8,5
2	8-10	2	100	1:15	7,4
3	1-3	2	100	1:10	8,5
4	1-3	2	100	1:15	10,5
5	1-3	2	100	1:20	12,1
6	1-3	2	100	1:20,1:15,1:10 (combined)	15,45

When the particle size was 8-10 mm, the yield of water-soluble polysaccharides was 7.4% and at 4-6 mm it was 8.5%. For raw material particles sized 1-3 mm, the yield of polysaccharides was 8.5% at a solvent ratio of 1:10, 10.5% at 1:15, and 12.1% at 1:20 (Table 1). The total yield of water-soluble polysaccharides obtained from the extraction process was 15.45%. The isolated water-soluble polysaccharides were purified from proteins using the Sevag method. UV spectra of the purified polysaccharides showed no absorption at 260–280 nm, which is characteristic for proteins. These results indicate the absence of proteins and peptides in the obtained polysaccharide samples. After purification, the total sugar content of the samples was determined by the phenol-sulfuric acid method. According to literature data, mushroom polysaccharides possess a β-glucan structure; therefore, glucose solutions in the range of 10–120 μg/ml were used to construct the standard calibration curve (Figure 1).

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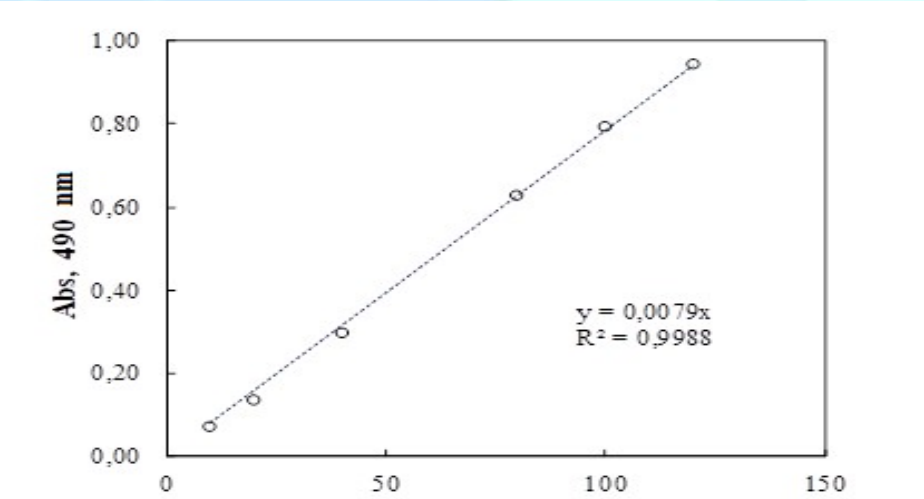


Figure 1. Calibration curve for determining the total sugar content in polysaccharides using the phenol-sulfuric acid method (glucose concentrations 10–120 µg/ml, λ = 490 nm).

The total carbohydrate content in the isolated polysaccharide fraction was found to be 55.74%. These results indicate the presence of melanins and other impurities in the extract in addition to polysaccharides. The molecular weight distribution of the isolated water-soluble polysaccharides was studied using gel chromatography. It is known that β-(1,3)-glucans in aqueous solutions are resistant to temperature changes and a wide range of pH values, and exhibit high viscosity. The molecular weight of the isolated polysaccharides was determined to be 9,800 Da, with a polydispersity index of 1.3. The relatively low polydispersity index indicates that the polysaccharides have a homogeneous nature.

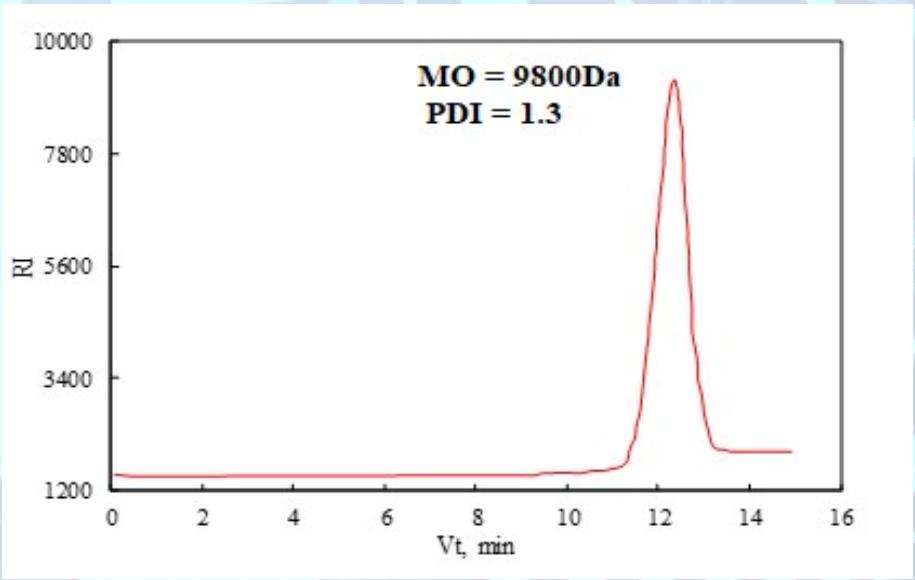


Figure 2. Gel chromatogram of water-soluble polysaccharides isolated from *Ganoderma lucidum* mushroom (eluent: 0.1 M sodium nitrate, flow rate: 0.8 ml/min, column standard: dextran sulfate).

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Solubility and Composition of Polysaccharides the water solubility of these polysaccharides is directly related to their molecular weight; as molecular weight increases, solubility decreases. This suggests that the polysaccharides may form complexes with other types of compounds. Additionally, the dark brown color of the isolated samples indicates the presence of other functional groups, since neutral polysaccharides are typically pale yellow to white.

Many studies have shown that basidiomycetes contain melanin pigments responsible for the brown coloration in addition to polysaccharides. Aniline phthalate and diphenylamine-aniline reagents were used for monosaccharide staining. The analysis showed that the monosaccharide composition of the isolated water-soluble polysaccharides mainly consisted of glucose residues. In addition to glucose, minor amounts of galactose, mannose, and rhamnose residues were also detected in the polysaccharides isolated from the mushroom.

These results indicate that the water-soluble polysaccharides isolated from *Ganoderma lucidum* predominantly possess a glucan structure.

Conclusions

In conclusion, water-soluble polysaccharides were successfully isolated by fractionation from *Ganoderma lucidum* basidial mushroom raw material, which was first purified from low molecular weight compounds (yield 3.6%). Factors affecting the extraction of water-soluble polysaccharides, such as the degree of grinding and extraction time, were studied. Optimal conditions were determined as extraction of raw material sized 1–3 mm with solvent ratios of 1:20, 1:15, and 1:10, performed three times at 100°C for 2 hours each, resulting in a high yield (15.45%) of water-soluble polysaccharides. The isolated polysaccharide fractions contained a total carbohydrate content of 55.74%, molecular weight of 9,800 Da, and a polydispersity index of 1.3. The monosaccharide composition mainly consisted of glucose residues, with minor amounts of galactose, mannose, and rhamnose. Further detailed studies on the composition of the water-soluble polysaccharides are ongoing using other physicochemical methods.

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