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A novel polysaccharide with antioxidant, HIV protease inhibiting and HIV integrase inhibiting activities from *Fomitiporia punctata* (P. karst.) murrill (Basidiomycota, hymenochaetales)

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ABSTRACT

A novel polysaccharide fraction (G_1) was obtained from the fungus *Fomitiporia punctata* (P. Karst.) Murrill. G_1 exhibited a molecular weight of approximately 151 kDa. The *FT-IR* results suggested that the monosaccharide components of G1 possessed furanoid rings and there were β-glycosidic bonds between the sugar units. *The* ^{1}H NMR results showed that G1 was composed of arabinose, fructose, galactose and glucose in the molar ratio of 1.6:3.8:19.7:19.7, as determined by gas chromatography-mass spectrometry (GC–MS) and high performance liquid chromatography (HPLC). G_1 produced significant antioxidant effects as evidenced by its potency in inhibiting erythrocyte hemolysis, and in scavenging hydroxyl radicals and superoxide radicals. The highest rates of inhibition achieved were 73.58%, 36.55% and 50.98% respectively. In addition, G_1 brought about 19.6% inhibition of HIV-1 protease activity at the concentration of 50 μg/mL. G1 displayed inhibitory activity toward HIV-1 integrase in the concentration range of 100–1000 μg/mL. The present study indicates that G_1 from *Fomitiporia punctate* (P. Karst.) Murrill is a novel natural antioxidant.

1. Introduction

Reactive oxygen species (ROS) include superoxide radicals ($O_2^{\bullet -}$), hydroxyl radicals(•OH), singlet oxygen($^1O_2$), hydroxyl peroxide(H_2O_2) and peroxides, which are generated in metabolism in the human body and other exogenous factors [1,2]. Normally, ROS levels fluctuate within a certain range [3] and play an important role in maintaining the stability of vital life activities. However, in some cases such as the introduction of physical and chemical factors, a cell cannot neutralize the increased amounts of ROS, which damage normal cells and tissues, and cause various diseases and aging. Antioxidants protect against the accumulation of ROS by scavenging them from the system [4].

Polysaccharides, an important type of biological macromolecules, are involved in many biological processes, such as cell–cell communication, embryonic growth, infection by bacteria and/or viruses, and humoral and cellular immunity [5]. Bioactive polysaccharides manifest various biological activities such as antioxidative, antitumor, antiviral, anti-inflammatory, immunomodulatory, anti-aging, and antidiabetic activities [5–12]. It has been shown that synthetic antioxidants have potential cytotoxicity, and hence the development of effective and safe natural antioxidants is one of the main pursuits in the field of research on antioxidants. In recent years, a variety of polysaccharides were obtained from plants and fungi, and their chemical structures and bioactivities were elucidated. The research findings

Abbreviations: AAPH, 2,2′-azobis- (2-amidinopropane) dihydrochloride; BHA, Butylated Hydroxyanisole; BHT, Butylated Hydroxytoluene; BRM, Biological Response Modifiers; BSA, Bovine Serum Albumin; CAT, Catalase; DBZ, N,N"—disalicylhydrazine; DPPH, 1,1-Diphenyl-2-Picryl-Hydrazyl; ELSD, Evaporative Light—Scattering Detector; FTIR, Fourier Transform Infrared spectroscopy; GC, Gas Chromatography; GSH, Glutathione; H_2O_2, Hydrogen peroxide; HIV-1, Human Immunodeficiency Virus-1; HPLC, High Performance Liquid Chromatography; IPTG, Isopropyl β-D-1-Thiogalactopyranoside; MDA, Malonaldehyde; MS, Mass Spectrometry; NADH, Reduced form of nicotinamide-adenine dinucleotide; NBT, NitroblueTetrazolium Chloride; NMR, Nuclear Magnetic Resonance; PMS, Phenazinemethosulfate; PPC, polysaccharide-protein complex; PS, Polysaccharide; RBC, Red Blood Cell; ROS, Reactive Oxygen Species; SOD, Super Oxide Dismutase; TBA, ThiobarbituricAcid; TCA, Trichloroacetic Acid.

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demonstrated that polysaccharides possessed potent antioxidant activity [2,5,6,13]. Polysaccharides produced by medicinal and edible mushrooms are good candidates for use as natural antioxidants [6].

Fomitiporiapunctata (P. Karst.) Murrill, as an important medicinal resource, has been used to treat coronary heart disease and angina pectoris. To date, reports on *Fomitiporia punctata* (P. Karst.) Murrill have mainly concentrated on the study of its fermentation broth and mycelial extracts with respect to their actions on coronary heart disease and angina pectoris. Research showed that the abovementioned diseases are related to ROS [4]. Hence, research on antioxidants in this mushroom species may reveal their mechanism of action. The present study aimed to purify and identify the polysaccharide from *Fomitiporia punctata* (P. Karst.) Murrill, and investigate its antioxidant and antiviral activities.

2. Materials and methods

2.1. Chemicals and materials

The fruiting bodies of *Fomitiporia punctata* (P. Karst.) Murrill were purchased from Yipin antler Co. Ltd(Jilin Province, China) of Changbaishan development zone. Male Kunming mice (18–22 g), were obtained from China Academy of Military Medical Science. The mice were housed under normal laboratory conditions (21 ± 2 °C, 12 h light-dark cycle) with free access to standard rodent chow and water. For determination of antioxidant activities use mouse brains and blood were used,in addition to 500 μg/mL butyl hydroxyanisd (BHA), 1% thiobarbituric acid (TBA), 20 mM 2,2′-azobis (2-methyl- propionamidine) dihydrochloride (AAPH), 0.1 mM1,1- diphenyl- 2-picrylhydrazyl (DPPH), and 12 μM cytochrome C. TheDEAE-cellulose 32 were purchased from Sigma-Aldrich Co., theSephadex G-75 was purchased from Pharmacia Co., and glucose dextran series was purchased from Dingguo Biotechnology Co.Ltd. Evaporative light-scattering detector was purchased from SofTA corporation.

2.2. Isolation and purification of polysaccharide

Fomitiporia punctata (P. Karst.) Murrill fruiting bodies were dried and shattered, and the powder (20 g) was added into 20 vol. of distilled water. The mixture was incubated in a water bath at 80 °C for 5 h. After centrifugation, the residue was extracted again, and the supernatants were pooled and concentrated by using a rotary vacuum evaporator. The solution was dialyzed and mixed with anhydrous ethanol and left overnight at 4 °C. The precipitate was obtained by centrifugation, and washed sequentially with anhydrous ethanol, acetone, and absolute ether. The precipitates were redissolved in distilled water, and proteins were removed with the Sevage method [14].

The supernatant was concentrated with a rotary evaporator and then lyophilized with a vacuum freezedrier to yield crude polysaccharide (P).

The crude polysaccharide (P) was dissolved in Tris–HCl buffer (pH 7.4) and passed through a DEAE-cellulose column (2.6 cm × 40 cm), and eluted with a linear gradient of 0–2 M NaCl. The carbohydrate content of the eluate collected in each tube was determined using the anthrone–sulphuric acid method [15] by measuring the absorbance at 620 nm after the color reaction. The eluates in each absorbance peak were pooled, dialyzed and then lyophilized to obtain the fractions P_1 and P_2. P_2 was further purified by using a Sephadex G-75 column (1.5 cm × 60 cm) which was eluted with distilled water, and as a result two fractions were obtained (G_1, G_2). The lead acetate reaction showed that G_2 was composed of tannins. Total sugar content of G_1 and its erythrocyte hemolysis inhibiting activity were determined. G_1 was used for further studies.

2.3. Molecular weight determination

Molecular weight of G1 was first analyzed using SDS-PAGE (5% stacking gel and 12% separating gel). After electrophoresis, the gel was treated with periodic acid-Schiff stain (PAS). Molecular weight and purity were estimated from electrophoretic mobilities and number of the resulting bands. In addition, the purity of the polysaccharide was determined by using an HPLC system equipped with a SUGAR KS-804 column (8.0 mm × 300 mm) and an evaporative light-scattering detector.

G1 sample was dried to constant weight at 105C, and then the elements were analyzed by using a vario EL cube element analyzer.

Molecular weight of the purified polysaccharide was identified by using a High Performance Liquid Chromatography-Evaporative Light Scattering Detector (HPLC-ELSD). The Dextran T-series of standards with different molecular weights (Dextran T-10, T-50, T-70, T-100, T-200) were used to plot the calibration curve. The sample concentration was 10 mg/mL and filtered using a 0.22-μm filter prior to injection (injection volume = 20 μL). The mobile phase was double distilled water.

2.4. Analysis using fourier transform infrared (FT-IR) spectroscopy and nuclear magnetic resonance (^{1}H NMR) spectroscopy

The purified polysaccharide (1 mg) was ground with KBr powder and then pressed into pellets for FT-IR measurement in the frequency range of 4000–500 cm^{-1} (Tensor 37, Bruker Co., Germany).

The purified polysaccharide was dissolved in D_2O containing a trace of tetramethylsilane (TMS) as internal standard. ^{1}H NMR spectra were recorded on a BrukerAvance AV400 nuclear magnetic resonance spectrometer.

2.5. Analysis of monosaccharide composition

Monosaccharide composition of the purified polysaccharide was analyzed by using a gas chromatography-mass spectrometer (7890A-5975C, Agilent Co., USA). The purified polysaccharide was dissolved in 2 M trifluoroacetic acid (TFA) and hydrolysed at 100 °C for 6 h. Then, the solution was concentrated by using a rotary vacuum evaporator and the residue was redissolved in methanol and evaporated again to dryness. Subsequently, hydroxylamine hydrochloride and pyridine were added followed by incubation at 90 °C for 30 min. After cooling down to room temperature, acetic anhydride was added. Acetylated products were analysed by GC–MS after return to room temperature. In addition, HPLC was used to identify the monosaccharide composition of the purified polysaccharide. Solutions of six standard monosaccharides (glucose, laevulose, galactose, arabinose, sorbitol, and mannose) were mixed and filtered using a 0.22-μm filter prior to HPLC-ELSD analysis. Each of the six standard monosaccharides was also analyzed. Different concentrations of derived standard monosaccharides were separated by HPLC-ELSD to prepare a standard curve. After acid hydrolysis of the purified polysaccharide, the sample was subjected to HPLC-ELSD. The composition and content of monosaccharides were determined by using a standard curve.

2.6. Antioxidant activity

2.6.1. Determination of erythrocyte hemolysis inhibiting activity

Erythrocyte hemolysis inhibiting activity of G_1 was determined according to the method of Ng et al. [16]. Blood was sampled from the eyes of male Kunming mice and placed in a centrifuge tube