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Studies on structure and antioxidant properties of a heteroglycan isolated from wild edible mushroom *Lentinus sajor-caju*

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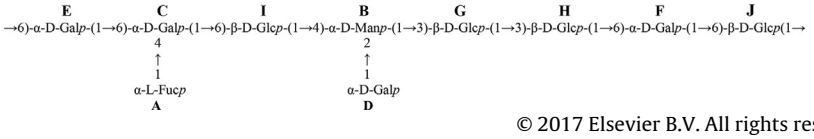
ABSTRACT

A water-soluble heteroglycan (PS-I) isolated from the aqueous extract of a wild edible mushroom *Lentinus sajor-caju* showed average molecular weight ~1.79×10^5 Da. The structure of the polysaccharide was determined using chemical and 1D/2D NMR experiments. Acid hydrolysis indicated the presence of D-glucose, D-galactose, D-mannose, and L-fucose in a molar ratio of nearly 4:4:1:1 respectively. The presence of terminal Fuc*p*, terminal Gal*p*, (1 → 3)-Glc*p*, (1 → 6)-Gal*p*, (1 → 6)-Glc*p*, (1 → 4,6)-Gal*p*, and (1 → 2,4)-Man*p* moieties were established from methylation analysis. The chemical and NMR analyses indicated that the PS-I was a heteroglycan composed of a repeating unit with backbone chain of three (1 → 6)-α-D-galactopyranosyl residues, two (1 → 6)-β-D-glucopyranosyl residues, one (1 → 4)-α-D-mannopyranosyl residue, and two (1 → 3)-β-D-glucopyranosyl residues where one (1 → 6)-α-D-galactopyranosyl residue was branched at *O*-4 position with terminal α-L-fucopyranosyl residue and (1 → 4)-α-D-mannopyranosyl residue was branched at *O*-2 position with terminal α-D-galactopyranosyl residue and the structure was proposed as;

The PS-I is a moderate antioxidant compound which showed DPPH radical scavenging activity, hydroxyl radical scavenging activity, ABTS radical scavenging property, reducing power, and ferrous ion chelating ability.



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1. Introduction

Wild edible mushrooms and their polysaccharides are useful for nutritional supplements as well as pharmaceuticals [1–3]. *Lentinus sajor-caju*, family polyporaceae, formerly known *Pleurotus sajor-caju* returned to the genus *Lentinus* by Pegler in 1975 [4]. It is distributed extending from southern regions of Africa to south-east Asia and down to the north-east region of Australia [5]. It grows abundantly on dead branches and woods. At the young stage the fruit bodies are consumed as nutritious food in Vietnam [6] and also in Malaysia as the vegetable [7]. Two water soluble polysaccharides from the cultivated edible mushroom previously named *Pleurotus sajor-caju* have been isolated, characterized and reported [8,9] by the present research group.

In the present investigation two water soluble polysaccharides have been isolated from the aqueous extract of the wild edible mushroom *Lentinus sajor-caju* through gel permeation chromatography. The first fraction was investigated as heteroglycan and found to contain glucose, galactose, mannose, and fucose. In the present study, attempts have been made to investigate the structure of only the first fraction which exhibits promising antioxidant activities as evidenced from its radical scavenging activity, chelating ability of ferrous ion, and high reducing power property. The detailed structural investigations and antioxidant properties of the polysaccharide were carried out and reported herein.

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2. Materials and methods

2.1. Collection and identification

Basidiocarps of *L. sajor-caju* were collected from Baruipur region adjacent to Kolkata, West Bengal, India during the month of July. The mushroom was collected growing on the dead woods and immediately transferred to the laboratory and identified by comparing with a voucher specimen according to the standard literature [5]. A reference specimen was deposited at the Calcutta University herbarium (Accession no: CUH AM352) adopting the method of Pradhan et al. [10].

2.2. General methods

L. sajor-caju (600 g) was the gently washed with H_2O, and then boiled at 100 °C with distilled H_2O for 10 h. The mixture was cooled, centrifuged, and the supernatant was precipitated in C_2H_5OH (1:5, v/v) to get crude polysaccharide (800 mg) and purified using the procedure as described in previous publication [11]. Two fractions of purified polysaccharide; fraction I (test tube no. 16–26) and fraction II (test tube no. 36–46) were collected [12] through Sepharose gel filtration, and freeze-dried. The purification process was repeated several times to get pure polysaccharide of fraction I (PS-I, 120 mg) and preserved for further reactions and analyses.

Optical rotation was measured at 24.8 °C by using Jasco Polarimeter, model P-1020. Average molecular weight of PS-I was determined adopting the procedure as described earlier [13–15]. By using the method of Gerwig et al. [16] the absolute configuration of the polysaccharide was determined. PS-I (3 mg) was hydrolyzed according to the method as applied in earlier publications [14,15] for finding the sugar units present in the polysaccharide. The PS-I (4 mg) was methylated by Ciucanu and Kerek method [17]. Methylation experiment was performed for identifying the linkage pattern of the sugar residues present in the PS-I adopting the procedure as described in previous publication [14,15]. Periodate oxidation of PS-I (9 mg) was performed to confirm the linkages obtained from the results methylation studies. The periodate-oxidized material [18,19] was divided into two parts. One portion was hydrolyzed for GLC analysis to identify the sugar residues and other part was methylated for linkage confirmation using GLC–MS. Structure characterizations of the molecule were performed applying ^1H, ^{13}C, TOCSY, DQF-COSY, NOESY, ROESY, and HSQC NMR experiments [20] as reported in previous publication [21–23]. The Smith degradation reaction of the IO_4^- oxidized material was performed [24,25] and ^{13}C NMR studies of the degraded material was carried out adopting the procedure as described in earlier publication [11,14].

2.3. Antioxidant activities

DPPH radical scavenging assay of PS-I was evaluated by the procedure as described previously [26,27]. Ascorbic acid was used as standard for comparison. Hydroxyl radical scavenging activity [11,28], reducing power [11,29], and Chelating ability of ferrous ions [30] assays were determined by adopting the procedures as described earlier. Butylated hydroxyltoluene (BHT), Ascorbic acid, and EDTA were used as positive control, respectively. The scavenging activity of PS-I against ABTS [2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] radicals was determined according to the method as applied in earlier publications [31].

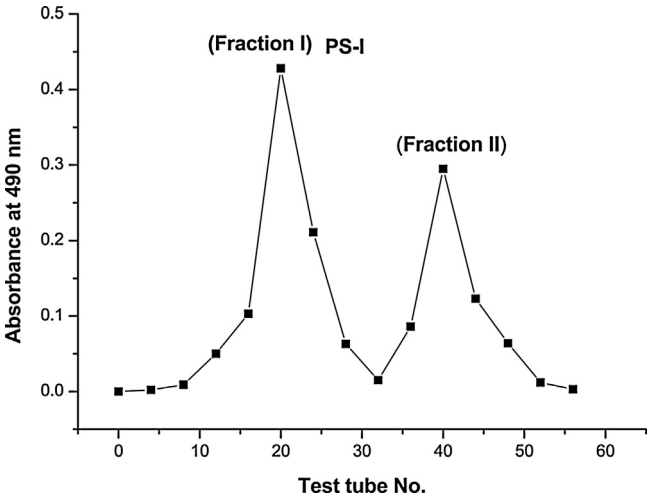

Fig. 1. Gel permeation chromatogram of crude polysaccharide isolated from an edible mushroom *L. sajor-caju* using Sepharose 6B column.

2.4. Statistical analysis

Values of antioxidant activities were expressed as means ± standard deviation of five separate experiments.

3. Results and discussion

3.1. Isolation, purification, and chemical analysis of PS-I

Crude polysaccharide (800 mg) was isolated from aqueous extraction of mushroom (600 g) by alcohol precipitation. Sepharose 6 B gel filtration (Fig. 1) of crude polysaccharide (25 mg) yielded two fractions (fraction I; 14 mg and fraction II; 9 mg). The specific rotation and average molecular weight (Fig. 2) of PS-I were shown in Table 1. GLC analysis of the hydrolyzed product of PS-I revealed the presence of monosaccharides as shown in Table 1 along with their corresponding molar ratio and absolute configuration. GLC–MS analysis of the methylated product showed the presence of seven components as presented in Table 2. These results indicated that the repeating unit of PS-I consisted of terminal Fuc*p*, terminal Gal*p*, (1 → 3)-Glc*p*, (1 → 6)-Gal*p*, (1 → 6)-Glc*p*, (1 → 4,6)-Gal*p*, and (1 → 2,4)-Man*p* moieties. GLC analysis of periodate oxidized

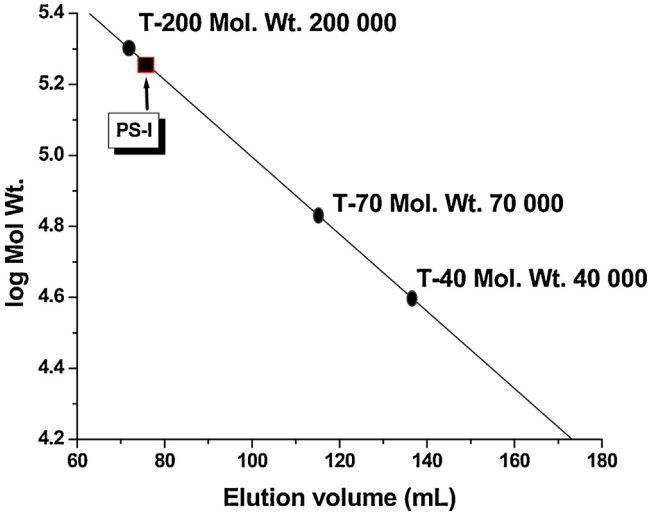

Fig. 2. Determination of molecular weight of PS-I by gel permeation chromatography in Sepharose 6B column.

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