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Structural features and antioxidant activity of a new galactoglucan from edible mushroom *Pleurotus djamor*

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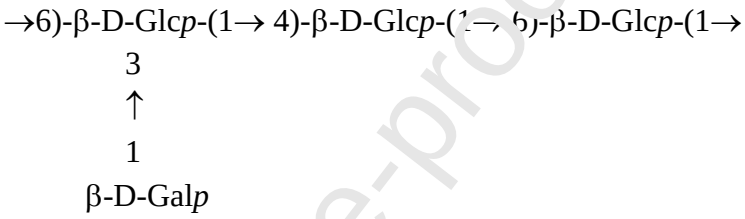
Abstract

A new water soluble galactoglucan with apparent molecular weight ~1.61×10^5 Da, was isolated from the edible mushroom *Pleurotus djamor* by hot water extraction followed by purification through dialysis tubing cellulose membrane and sepharose 6B column chromatography. The sugar analysis showed the presence of glucose and galactose in a molar ratio of nearly 3:1 respectively. The structure of the repeating unit in the polysaccharide was determined through chemical and NMR experiments as:



In vitro antioxidant studies showed that the PDPS exhibited hydroxyl radical scavenging activity (EC_{50}= 1.681 ± 0.034 mg/ml), DPPH radical scavenging activity (EC_{50}= 3.83 ± 0.427 mg/ml), reducing power (EC_{50}= 4.258 ± 0.095 mg/ml), and ABTS radical quenching activity (EC_{50}= 0.816 ± 0.077 mg/ml). So, PDPS should be explored as a natural antioxidant.

Keywords: *Pleurotus djamor*, Galactoglucan, Antioxidant activity

Abbreviations: AcOH= Acetic acid; ABTS=2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonate); BHA= Butylated hydroxyl anisole; CF_3COOH= Trifluoro acetic acid; DEPT= Distortionless enhancement by polarization transfer; DPPH= Diphenyl–picrylhydrazine; DMSO= Dimethyl sulfoxide; DQF-COSY= Double-quantum filtered correlation spectroscopy; GLC= Gas-liquid chromatography; GLC-MS= Gas-liquid chromatography mass spectrometry; HSQC= Heteronuclear single quantum coherence; NMR= Nuclear magnetic resonance; NOESY= Nuclear overhauser enhancement spectroscopy; PDPS= *Pleurotus djamor* polysaccharide (heteroglycan); P_2O_5= Phosphorus pentoxide; TOCSY= Total correlation spectroscopy.

1. Introduction

Edible mushrooms are very important foodstuff and folk medicine all over the world [1, 2]. From edible mushrooms prepared hot aqueous solutions, containing mostly polysaccharides, were considered as a medicine since ancient times [3]. Polysaccharides from edible mushrooms are considered as an antitumor agent [4-8]. Specifically some edible mushrooms, *Lentinus, Hericium, Grifola, Flammulina, Pleurotus,* and *Tremella* have highly medicinal and functional properties [9]. *Pleurotus* genus (Class Basidiomycetes) is among the most popular and edible macrofungi which are commonly regarded as oyster mushrooms. This species is widely cultivated in several countries especially due to its flavour, texture and health benefits [10]. Generally, Pleurotus *spp.* is grown in the woody forests of the earth [11, 12]. These mushrooms contain mainly carbohydrates, proteins, dietary fiber, minerals, some essential amino acids, and vitamins [13]. *Pleurotus* spp. has been demonstrated in many previous studies to possess valuable medicinal attributes such as antioxidant, immunomodulatory, hypocholesterolemic, anti-inflammatory, anti-hyperglycaemic, anti-genotoxic, anti-tumour, anti-viral, anti-Human

Immunodeficiency Virus (HIV), hepatoprotective, anti-mutagenic, anti-allergic and anti-ageing effects [14]. The genus encompasses about 50 species of which nearly 26 taxa have been included from India. One such common oyster mushroom is *Pleurotus djamor* that is appreciated worldwide as food encouraging artificial cultivation and economic benefit. In earlier experimental studies, *P. djamor* has been reported to possess strong anti-inflammatory, analgesic, antioxidant, antimicrobial, antiplatelet and antipyretic activity [15]. They have been reported to modulate the immune system, and to inhibit tumor growth [11, 16]. The cultivation of the genus *Pleurotus* such as *Pleurotus sajorcaju, Pleurotus citrinopileatus, Pleurotus ostreatus, Pleurotus florida and Pleurotus djamor* has been increasing at a rapid rate [17]. Previously a non-toxic water insoluble β-glucan was isolated from the alkaline extract of the fruit body of *Pleurotus djamor*, and the detailed structural investigation and study of cytotoxic effect were carried out and reported [18]. A new water soluble polysaccharide (PDPS) has been isolated from the aqueous extract of the edible mushroom *Pleurotus djamor* through gel permeation chromatography. The present study was carried out to evaluate the detailed structural investigation and antioxidant activities of PDPS.

2. Materials and methods

2.1. Isolation and purification of the polysaccharide

Dry fruiting body of edible mushroom *Pleurotus djamor* (500 g) were extracted by distilled water at 100 ˚C for 7 h. The mixture was kept overnight and filtered through fresh linen cloth. The supernatant was collected after centrifugation at 8000 rpm at 4◦C for 1 h and then precipitated by adding 5 times volumes of ethanol. The precipitate was dialyzed through tubing cellulose membrane against distilled water for 2 days. The dialyzed material was freeze-dried to

obtain crude polysaccharide (800 mg). During purification process, a single homogeneous fraction (test tube 26-37) was collected and freeze-dried [19, 20]. The same route was repeated several lots to get 120 mg of PDPS for further analysis.

2.2. Determination of molecular weight

The apparent molecular weight of the PDPS was determined by gel-permeation chromatography [19]. Standard dextrans T-200, T-70, and T-40 were passed through a Sepharose 6B column, and then the elution volumes were plotted against the logarithms of their respective molecular weights. The elution volume of PDPS was plotted in the same graph and the average molecular weight of PDPS was measured.

2.3. Absolute configuration of monosaccharide

According to the method Gerwig, Kamerling, and Vliegenthart, the D and L notation of a sugar was evaluated [19, 21]. PDPS (1.0 mg) was hydrolyzed with CF_3COOH, and then the acid was removed by co-distillation with water. 250 µl of 0.625 M HCl solution treated with R- (+)-2-butanol was added to the hydrolyzed product and heated at 80 °C for 16 h. Then the reactants were evaporated and TMS-derivatives were prepared with N,O-bis (trimethylsilyl) trifluroacetamide (BSTFA). The products were analyzed by GLC using a capillary column SPB-1 (30 m × 0.26 mm) with a temperature program (3 °C/min) from 150 to 210 °C. The resulting 2,3,4,6-tetra-*O*-TMS-(+)-2-butylglycosides were identified by comparison with those prepared from the D and L enantiomers of different monosaccharides.

2.4. Optical rotation

Optical rotation was measured through a Jasco Polarimeter model P-1020 at 31 °C.

2.5. Monosaccharide analysis

The neutral monosaccharide composition of the PDPS was determined by gas liquid chromatography (GLC) analysis. PDPS (3.0 mg) was hydrolyzed with 2 M CF_3COOH (2 mL) at 100 °C in a round-bottom flask for 18 h in a boiling water bath. The excess acid was completely removed by co-distillation with water. Then, the hydrolyzed product was reduced with $NaBH_4$ (8 mg), followed by acidification with dilute CH_3COOH, and then co-distilled with pure CH_3OH to remove excess boric acid. The reduced sugars were acetylated with 1:1 pyridine–acetic anhydride in a boiling water bath for 1 h to give the alditol acetates, which were analyzed by GLC [18, 19].

2.6. Methylation analysis

The PDPS (4.0 mg) was methylated according to the method of Ciucanu and Kerek method where distilled DMSO and finely ground NaOH were used. The methylated product was then hydrolyzed with 90 % formic acid (1 ml) at 100 °C for 1 h and excess formic acid was evaporated by co-distillation with distilled water. The hydrolyzed product was then reduced with sodium borohydride and acetylated with pyridine-acetic anhydride (1:1). The alditol acetates of methylated sugars were analyzed by GLC-MS [18, 19].

3.7. Periodate oxidation

PDPS (10 mg) was added to 2 mL 0.1 M sodium metaperiodate solution and the mixture was kept in the dark for 72 h at room temperature. The excess periodate was destroyed by adding 1,2-ethanediol and the solution was dialyzed against distilled water for 2 h. The volume of the dialyzed material was concentrated to 2–3 ml. This material was reduced with $NaBH_4$, 12 h, neutralized with 50% AcOH, and dialyzed with distilled water and finally freeze dried. The periodate-oxidized material was divided into two parts. One portion was hydrolyzed with 2 M CF_3COOH for 18 h and this hydrolyzed material was used for alditol acetates preparation as

usual for GLC analysis. Another portion was methylated, followed by preparation of alditol acetates which were analyzed by GLC-MS [18, 19].

2.8. NMR analysis

PDPS was dried over P_2O_5 in vacuum for several days and then exchanged with deuterium followed by lyophilizing with D_2O (99.96 % atom ^2H, Aldrich) for four times. The ^1H and ^{13}C NMR experiments were carried out at 500 MHz and 125 MHz, respectively with a Bruker Avance DPX-500 spectrometer. The ^1H, ^{13}C, TOCSY, DQF-COSY, NOESY, and HSQC NMR spectra were recorded in D_2O at 30 ºC. The ^1H NMR spectrum was recorded by suppressing the HOD signal (fixed at δ 4.70) using the WEFT pulse sequence using acetone as internal standard fixing methyl proton signal at δ 2.19. Acetone was used as an internal standard (δ 31.05 ppm) for ^{13}C spectrum. The 2D-DQF-COSY experiment was performed using standard BRUKER software. The TOCSY experiment was recorded at mixing time of 150 ms, and complete assignment required several TOCSY experiments having mixing times ranging from 60 to 300 ms. The NOESY mixing delay was 300 ms.

2.9. Antioxidant properties

2.9.1. Hydroxyl radical scavenging assay

For the assay of hydroxyl radical scavenging activity, a 1 ml reaction mixture was prepared consisting of KH_2PO_4–KOH buffer (20 mM, pH 7.4), 2-deoxy-D-ribose (2.8 mM), variable concentration (0.5–2.5 mg/ml) of PDPS, $FeCl_3$(100 mM), EDTA (100 mM), ascorbate (100 mM) and H_2O_2 (1 mM) [22]. It was incubated at 37 ℃ for 1 h. Then the reaction mixture was added to 2 ml thiobarbituric acid (TBA) and trichloroacetic acid (TCA) solution (3.75% (w/v) TBA, 0.15% (w/v) TCA and 0.25 N HCl) and the whole mixture were incubated in a

boiling water bath for 15 min. A pink colour was developed. After cooling, absorbance was measured at 535 nm. BHA was used as standard. The scavenging activity was calculated using the following equations:

$$\text{Hydroxyl radical Scavenging rate (\%)} = \{(A_0-A_1)/ A_0\} \times 100$$

Where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of sample.

2.9.2. DPPH radical scavenging capacity

A methanol DPPH-solution (0.2 mL) would be added to 1.0 mL of sample solution, and 2.0 mL of water was added. After 30 min the absorbance will be measured at 517 nm using a spectrophotometer [23, 24]. BHA was used as positive control. The DPPH radical scavenging activity was calculated by the following formula:

$$\text{Scavenging activity (\%)} = \{(A_0-A_1)/ A_0\} \times 100$$

Where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of sample.

2.9.3. ABTS radical *scavenging* activity

ABTS radicals were generated freshly by adding 2.45 mM of potassium persulfate in 7 mM ABTS solution and the mixture was incubated overnight. After 12–16 h, $ABTS^{\bullet+}$ solution was diluted to an absorbance of 0.7 ± 0.02 at 750 nm. Further, the radicals were allowed to react with extracts at variable doses in 200 µl reaction mixture in 96 well plates. The plate was shaken at medium speed for 10 seconds and absorbance was perceived at 750 nm [23, 24]. BHA was used as a positive control. Scavenging degree was deliberated by adopting the equation as mentioned below:

$$\text{Quenching effect (\%)} = \{(A_0–A_1)/ A_0\} \times 100$$

Where A_0 and A_1 were the absorbance of negative control and experimental sets respectively.

2.9.4. Determination of reducing power

The reducing power of was determined according to the method described by Oyaizu [31]. Various concentrations of PDPS (1-6 mg/mL) were mixed with 2.5 mL sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1 %). The mixture was incubated for 20 min and then 2.5 mL of trichloroacetic acid (10 %) was added. 2.5 mL of solution mixture was mixed with 2.5 mL distilled water and 0.5 mL $FeCl_3$ (0.1 %) and incubated for 15 min. The absorbance was measured at 700 nm against buffer [23, 24]. BHA was used as standard. EC_{50} value is the effective concentration at which the absorbance was 0.5 for reducing power.

Fig. 1

Table 1a

3. Results and discussion

3.1. Isolation, purification and chemical analysis of PDPS

800 mg of crude polysaccharide was isolated from hot water extraction of fruit bodies of *Pleurotus djamor*. 18 mg of pure PDPS was found from 25 mg crude polysaccharide through sepharose 6B column (Fig. 1). The same method was repeated several times to get 120 mg of PDPS. The PDPS showed specific rotation $[\alpha]_D^{31}$ - 3.47 (*c* 0.10, H_2O). The average molecular weight of PDPS was estimated as ~1.61×10^5 Da. Chemical composition analysis by the phenol-sulphuric acid method [20], shows the total carbohydrate content of PDPS was up to 98.8 ± 1.2%. GLC analyses of the alditol acetates of this polysaccharide revealed the presence of glucose and galactose in a molar ratio nearly 3:1. The absolute configuration of monosaccharides were determined by the method of Gerwig et al. (1978) and found that all the monomeric sugar units had the D configuration. The molecular weight, sugar composition, absolute configuration,

and specific rotation of the PDPS were shown in Table 1a. GLC–MS study of the methylated product showed the presence of four components as presented in Table 1b. This result indicated that (1→3,6)-linked glucopyranosyl, (1→4) linked glucopyranosyl, (1→6)-linked glucopyranosyl and terminal galactopyranosyl moieties were present in the PDPS. GLC study showed that only glucose was present in periodate oxidized reduced material of PDPS. GLC–MS study of alditol acetates of periodate oxidized reduced methylated product showed that (1 → 3,6)-Glc*p* was unaffected during oxidation while other residues were destroyed during oxidation.

Table 1b

Fig. 2a

Fig. 2b

Table 2a

3.2. NMR and structural analysis of PDPS

The ^{1}H NMR spectrum (500 MHz, 30 °C, Fig. 2a) of the PDPS showed four anomeric signals at 4.65, 4.64, 4.44 and 4.43 ppm. They were chosen as **A**, **B, C, D** according to their falling anomeric proton chemical shifts (Table 2a). In ^{13}C NMR spectrum (125 MHz, Fig. 2b) at 30 °C, four signals were found in the anomeric region at 103.0, 102.7, 102.5 and 102.3 ppm.

All the ^{1}H and ^{13}C signals (Table 2a) were assigned using DQF-COSY, HSQC (Fig. 2c), and TOCSY (Fig. 2d) spectrum. Continue with the attribution of signals but correlate H and C chemical shifts (Fig. 2c, Table 2a) to a given unit: δ 4.65/103.0 (**A**), 4.64/102.7 (**B**), 4.44/102.5 (**C**) and 4.43/102.3 (**D**).

Fig. 2c

Fig. 2d

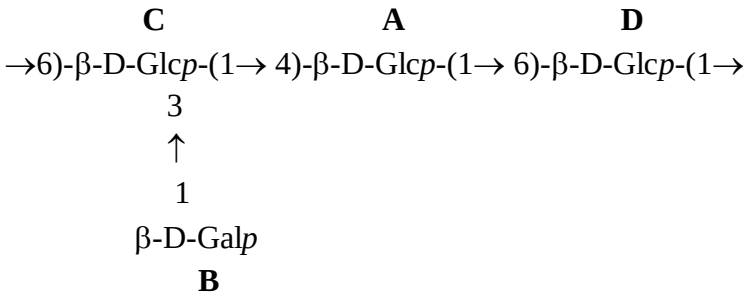
The β-configuration of the residues (**A**-**D**) was confirmed by low resonance frequency of H-1 (4.43-4.65 ppm) and high resonance frequency of C-1 (102.3-103.0 ppm) signals [22, 24-26]. The downfield shifts of C-4 (δ 78.7) with respect to standard value of methyl glycoside indicated that residue **A** was (1 → 4)-linked β-D-Glc*p*. In residue **B**, all the proton and carbon chemical shifts values were found nearly to the standard value of methylgalactosides. Thus, residue **B** was terminal β-D-Gal*p*. The downfield shifts at C-3 (δ 84.4) and C-6 (δ 68.6) with respect to standard value of methyl glycoside indicated that residue **C** was (1 → 3,6)-β-D-Glc*p*. Since the downfield shifts of C-6 (δ 68.7) with respect to standard value of methyl glycoside indicated that residue **D** was (1 → 6)-linked β-D-Glc*p* [27, 28]. The *O*-6 substitution of residues **C** and **D** were confirmed from the respective reverse peak in the DEPT-135 spectrum (Fig. 2b).

Table 2b

Fig. 3

The sequence of glycosyl residues (**A** to **D**) was determined from the NOESY experiment (Table 2b, Fig. 3). The NOESY experiment showed the inter-residual contacts: **A**-H1/**D**-H6a, **D**-H6b; **B**-H1/**C**-H3; **C**-H1/**A**-H4, **D**-H1/**C**-H6a, **C**-H6b along with other intra-residual contacts (Fig. 3). The above NOESY connectivities established the following sequences: **A** (1 → 6) **D**; **B** (1 → 3) **C**; **C** (1 → 4) **A**; **D** (1 → 6) **C**.

Based on the chemical and NMR spectral evidences the probable structure of repeating unit in the PDPS was proposed as:



3.3 Evaluation of antioxidant activities

3.3.1 Hydroxyl radical scavenging activity

Firstly, hydroxyl radical (ȮH) scavenging potential was determined as the radical can damage biomolecules immediately after generation. This most reactive radical can be formed inside human body and can damage DNA by attacking purines, pyrimidines and deoxyribose resulting pathogenesis of several kinds of diseases [29]. Thus, direct scavenging of ȮH by antioxidants in a biological system is highly required to combat against oxidative stress. In this context, classic Fenton's reaction is generally followed to determine ȮH quenching effect of natural or synthetic product. The synthesized radical then can react with deoxyribose forming MDA that can be measured as a pink MDA–TBA chromogen [30]. When test sample is added to the reaction mixture, the reaction progression is inhibited resulting decolourization of solution. Following the method, effect of PDPS on ȮH was evaluated where extremely potent activity was found that increased gradually with rise of concentration (Fig 4a). Results showed that the polysaccharides inhibited 8.57%, 37.52% and 55.44% radicals at the level of 0.1, 1 and 2 mg/ml respectively. EC_{50} value of PDPS was found 1.681 ± 0.034 mg/ml. EC_{50} value is the effective concentration at which 50% of the ȮH were scavenged.

Fig 4a

3.3.2 DPPH radical scavenging activity

The DPPH radical scavenging method is an easy, sensitive, valid, accurate and economic mean to evaluate antioxidant potential of investigating drug(s). It is characterized by virtue of delocalisation of spare electron over the molecule giving rise to deep violet colour. After mixing the $DPPH^{\bullet}$ solution with a substance that can donate hydrogen atom or electron, the radical is transformed to yellow coloured reduced form i.e. diphenyl–picrylhydrazine [31]. Thus, magnitude of lowering in signal intensity of reactant solution is directly proportional to increased antioxidant activity. As presented in Fig 4b, PDPS possessed moderate $DPPH^{\bullet}$ scavenging abilities that increased in dose dependent manner. At the level of 2 and 4 mg/ml, the polysaccharides quenched radicals at the rate of 27.41% and 52.31% respectively. EC_{50} value of PDPS was found 3.83 ± 0.427 mg/ml.

Fig 4b

Fig 4c

3.3.3 ABTS radical quenching activity

ABTS salt can generate blue–green $ABTS^{\bullet+}$ by reacting with a strong oxidizing agent like potassium persulfate. However, the chromophore can quickly be converted back to its colourless neutral form in presence of hydrogen–donating antioxidant compounds [30]. Thus, the radical scavenging performance of antioxidative molecule can be determined by monitoring decrease in absorbance. According to the result, PDPS possessed strong radical quenching potential that amplified steadily with increase of concentration (Fig 4c). The polysaccharides quenched 17.4% and 35.08% $ABTS^{\bullet+}$ respectively at the level of 0.1 and 0.5 mg/ml and the effect was further

amplified to 59.64% at the concentration of 1 mg/ml. EC_{50} value of PDPS was found 0.816 ± 0.077 mg/ml.

3.3.4 Reducing power

Ferric reducing antioxidant power assay is a widely used, simple, sensitive, precise and inexpensive method that uses antioxidants as reductants in a redox-linked colorimetric reaction. The method is based on reduction of ferric iron (Fe^{3+}) by electron-donating antioxidants under investigation to its ferrous form (Fe^{2+}). Consequently, the iron colorimetric probe complex develops a dark blue colour product which can be measured at 540-600 nm [32]. Thus, increase in absorbance denotes an increment in reducing power as well as antioxidant effects. At concentration of 1.0 mg/ml, the absorbance value of PDPS was 0.142 but in case of BHA, the value was 1.2. The PDPS showed the absorbance value 0.587 at 5.0 mg/ml, whereas the BHA showed the value of 1.2 at the same concentration. Reducing power of the PDPS increases with the increase in concentration. At concentrations of 2.0, 3.0, and 4.0 mg/mL the absorbance value of BHA was fixed at 1.2 whereas the value of PDPS increases to 0.25, 0.354 and 0.471 respectively. EC_{50} value of PDPS was found 4.258 ± 0.095 mg/ml (Fig. 4d). This result suggests that PDPS has potency to donate electron to reactive free radicals, converting them into more stable non reactive species and terminating the free radical chain reaction.

Fig 4d

4. Conclusion

A water soluble galactoglucan (PDPS) was isolated from the aqueous extract of an edible mushroom *Pleurotus djamor* and characterized using chemical and 1D/2D NMR experiments. PDPS had an average molecular weight of 1.61×10^5 Da and was composed of glucose and

galactose. The repeating unit was composed of a backbone containing two (1→6)-β-D-glucopyranosyl, and one (1→4)-β-D-glucopyranosyl residues. One of (1→6)-linked glucose units was branched at *O*-3 position with terminal β-D-galactopyranosyl residue. The PDPS showed hydroxide radical scavenging activity (EC_{50}=1.681 ± 0.034 mg/ml), DPPH radical scavenging activity (EC_{50}=3.83 ± 0.427 mg/ml), ABTS radical scavenging activity (EC_{50}=0.816 ± 0.077 mg/ml), and reducing power (EC_{50}= 4.258 ± 0.095 mg/ml). So PDPS could be used as a natural antioxidant and also employed as an ingredient in dietary health or functional products due to its antioxidant advantages.

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Conflict of Interest

The authors declare no Conflict of Interest.

Author Contribution Statement

Gajendra Nath Maity: Methodology, analysis and Writing

Prasenjit Maity: Conceptualization, Writing and Editing

Somanjana Khatua: Analysis and Writing

Krishnendu Acharya: Supervision

Sudipta Dalai: Supervision

Soumitra Mondal: Editing and Supervision

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Figure caption

Fig. 1. Gel permeation chromatogram of crude polysaccharide isolated from an edible mushroom *Pleurotus djamor* using Sepharose 6B column.

Fig. 2 a) ^{1}H NMR spectrum (500 MHz, D_2O, 30 °C) of the PDPS isolated from the fruit body of *Pleurotus djamor*. **b)** ^{13}C NMR spectrum (125 MHz, D_2O, 30 ºC) of a PDPS isolated from an edible mushroom *Pleurotus djamor*. Part of DEPT-135 spectrum (D_2O, 30 ºC) of a PDPS isolated from an edible mushroom *Pleurotus djamor* (inset).

Fig. 2 c) The HSQC spectrum (D_2O, 30 °C) of the PDPS isolated from the fruit body of *Pleurotus djamor*. **d)** TOCSY spectrum of the PDPS, isolated from an edible mushroom *Pleurotus djamor*.

Fig. 3. Part of NOESY spectrum of a PDPS isolated from an edible mushroom *Pleurotus djamor*. The NOESY mixing time was 300 ms.

Fig. 4. (a) Hydroxyl radical scavenging activity of the PDPS. Results are the mean ± SD of five separate experiments, each in triplicate. (b) DPPH radical scavenging activity of the PDPS. Results are the mean ± SD of four separate experiments, each in triplicate. (c) ABTS radical scavenging activity of the PDPS. Results are the mean ± SD of five separate experiments, each in triplicate. (d) Determination of reducing power of the PDPS. Results are the mean ± SD of five separate experiments, each in triplicate.

Table 1a. Components of monosaccharide and properties of PDPS from *Pleurotus djamor*

Properties	Data
$[\alpha]_D^{31}$	- 3.47 (*c* 0.10, H_2O)
Molecular weight	~1.61×10^5 Da
Monosaccharide component	Glucose, Galactose
Molar ratio of sugar unit	Glucose (3) : Galactose (1)
absolute configuration	D

Table 1b

GLC-MS analysis of methylated polysaccharide (PDPS) isolated from mushroom *Pleurotus djamor*.

Methylated sugars	Molar ratio	Linkage type	Major Mass Fragments (m/z)
2,3,6-Me_3-Glc	1	→4)-Glc*p*-(1→	43,45,71,87,99,101,113,117,129,131,161,173,233
2,4-Me_2-Glc	1	→3,6)-Glc*p*-(1→	43,58, 87,101,117,129,139,159,189,201,233
2,3,4-Me_3-Glc	1	→6)-Glc*p*-(1→	43, 45, 58, 71, 87, 99, 101, 117, 129, 161, 173, 189, 233
2,3,4,6-Me_4-Gal	1	Gal*p*-(1→	43, 45, 59, 71, 87, 101, 117, 129, 161, 205

Table 2a

The $^{1}H^{a}$ and $^{13}C^{b}$ NMR chemical shifts for the polysaccharide (PDPS) isolated from mushroom *Pleurotus djamor* D_2O at 30 ^{o}C.

Glycosyl residue	H1/C1	H2/C2	H3/C3	H4/C4	H5/C5	H6b,H6a /C6
→4)-β-D-Glc*p*-(1→	4.65	3.30	3.52	3.50	3.43	3.87, 3.72
A	103.0	73.3	74.8	78.7	75.9	60.0
β-D-Gal*p*-(1→	4.64	3.31	3.56	3.43	3.41	3.83, 3.65
B	102.7	69.5	74.8	68.1	75.9	60.7
→3,6)-β-D-Glc*p*-(1→	4.44	3.44	3.70	3.56	3.67	4.12, 3.51
C	102.5	73.1	84.4	70.0	75.8	68.6
→6)-β-D-Glc*p*-(1→	4.43	3.27	3.56	3.41	3.66	3.80, 3.41
D	102.3	73.1	75.5	69.5	74.1	68.7

[a] Values of the ^{1}H chemical shifts were recorded with respect to the HOD signal fixed at δ 4.70 at 30 ^{o}C.

[b] Values of the ^{13}C chemical shifts were recorded with reference to acetone as the internal standard and fixed at δ 31.05 at 30 ^{o}C.

Table 2b

NOE effects of PDPS, observed in the NOESY spectrum recorded in D_2O at 30 ^oC.

Unit	NOE signals		
	From	Intraunit	Interunit
A	**A**-H1	**A**-H2, **A**-H3, **A**-H5	**D**-H6a, **D**-H6b
B	**B**-H1	**B**-H2, **B**-H3, **B**-H5	**C**-H3
C	**C**-H1	**C**-H2, **C**-H3, **C**-H5	**A**-H4
D	**D**-H1	**D**-H2, **D**-H3, **D**-H5	**C**-H6a, **C**-H6b

Author Statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Highlights

- A galactoglucan (PDPS) was isolated from edible mushroom *Pleurotus djamor*.
- Its structure was established by chemical and 1D/2D NMR analyses.
- It showed antioxidant activities.

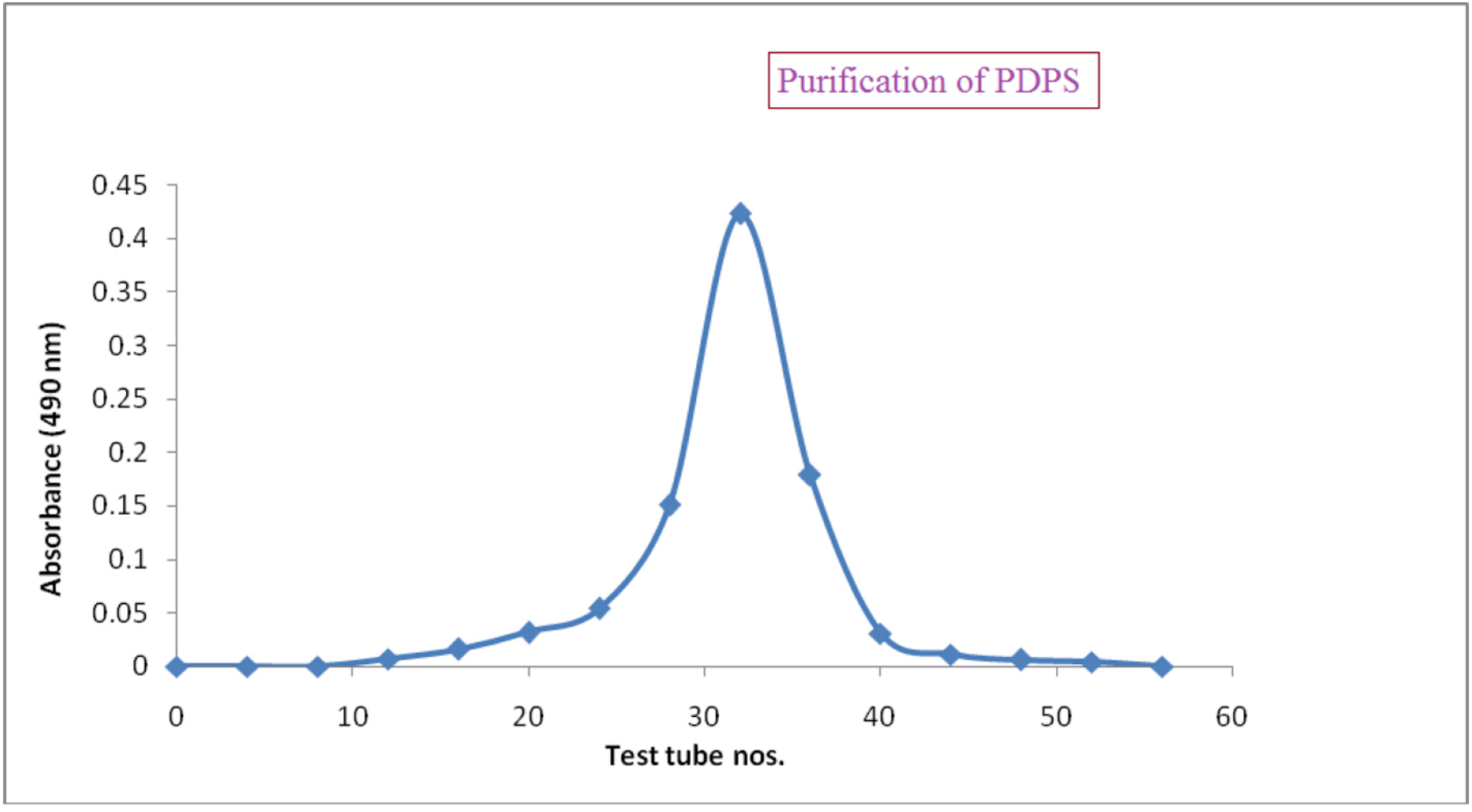
Purification of PDPS
Absorbance (490 nm)
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
10
20
30
40
50
60
Test tube nos.

Figure 1

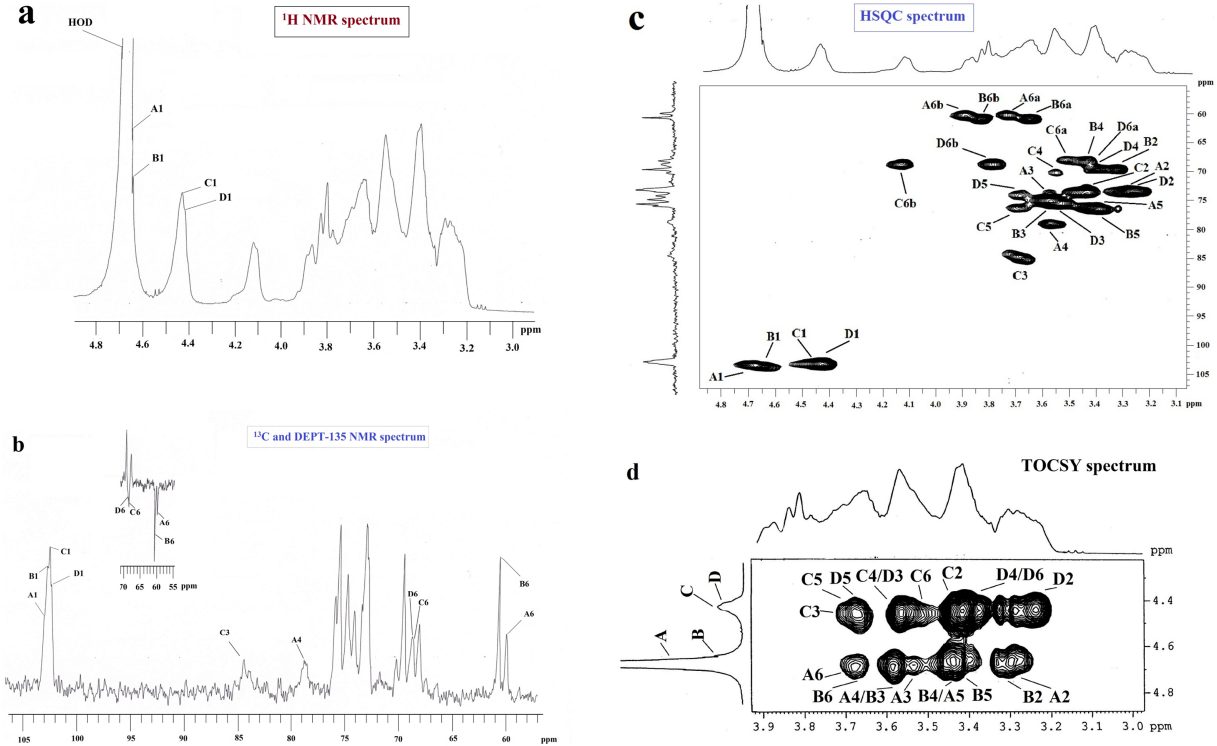
a
HOD
A1
B1
C1
D1
^{1}H NMR spectrum
4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 ppm
b
^{13}C and DEPT-135 NMR spectrum
D6 C6 A6 B6
70 65 60 55 ppm
B1 A1 C1 D1
C3 A4 D6 C6 B6 A6
105 100 95 90 85 80 75 70 65 60 ppm
c
HSQC spectrum
A6b B6b A6a B6a C6a B4 D6a B2 D4 C2 A2 D2 A5 B5 D3 B3 A4 C3 C4 A3 D5 C5 D6b C6b B1 C1 D1 A1
ppm
4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 ppm
d
TOCSY spectrum
A B C D
C5 D5 C4/D3 C6 C2 D4/D6 D2 C3
A6 B6 A4/B3 A3 B4/A5 B5 B2 A2
ppm
4.4 4.6 4.8
3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 ppm

Figure 2

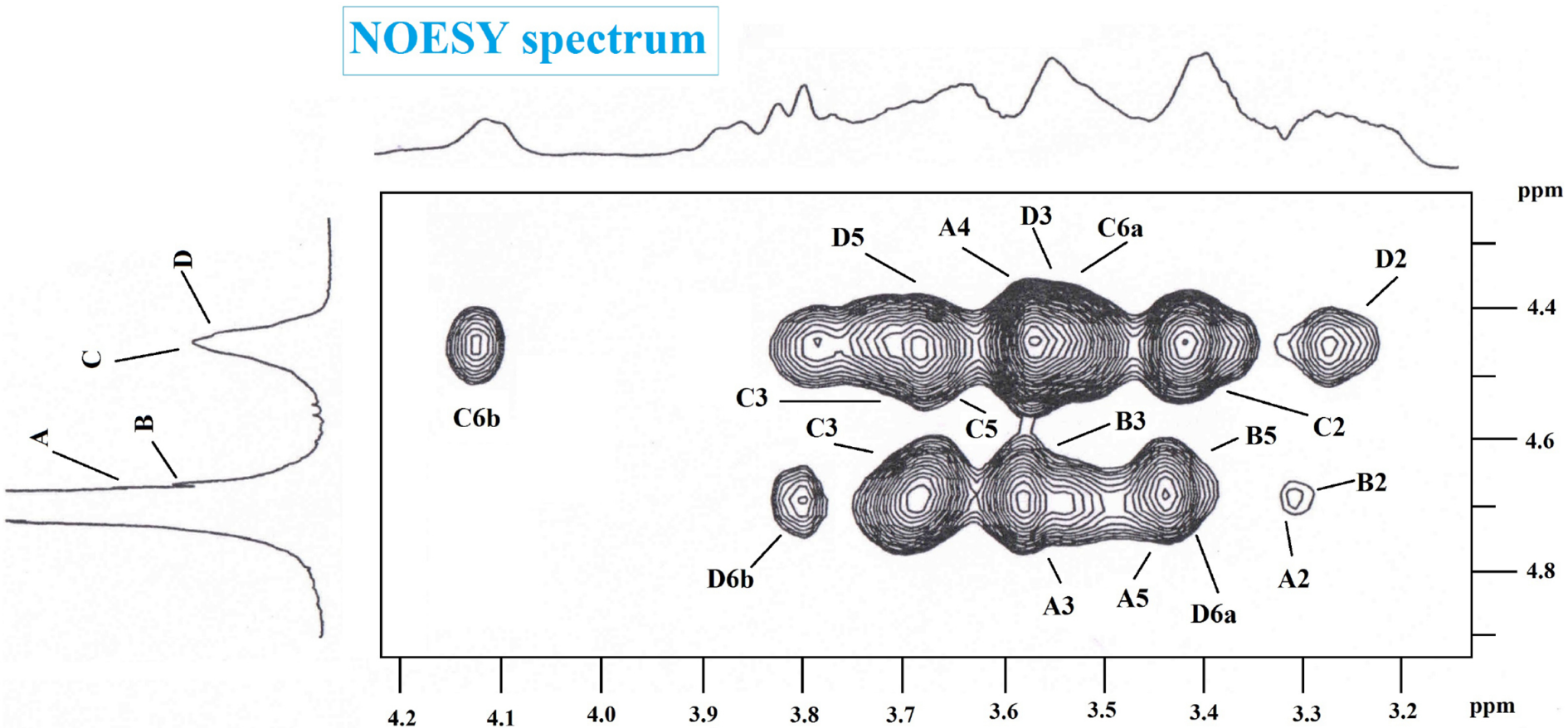
NOESY spectrum
A
B
C
D
C6b
D5
A4
D3
C6a
D2
C3
C5
B3
B5
C2
C3
B2
D6b
A3
A5
D6a
A2
ppm
4.4
4.6
4.8
4.2
4.1
4.0
3.9
3.8
3.7
3.6
3.5
3.4
3.3
3.2
ppm

Figure 3

Antioxidant activities

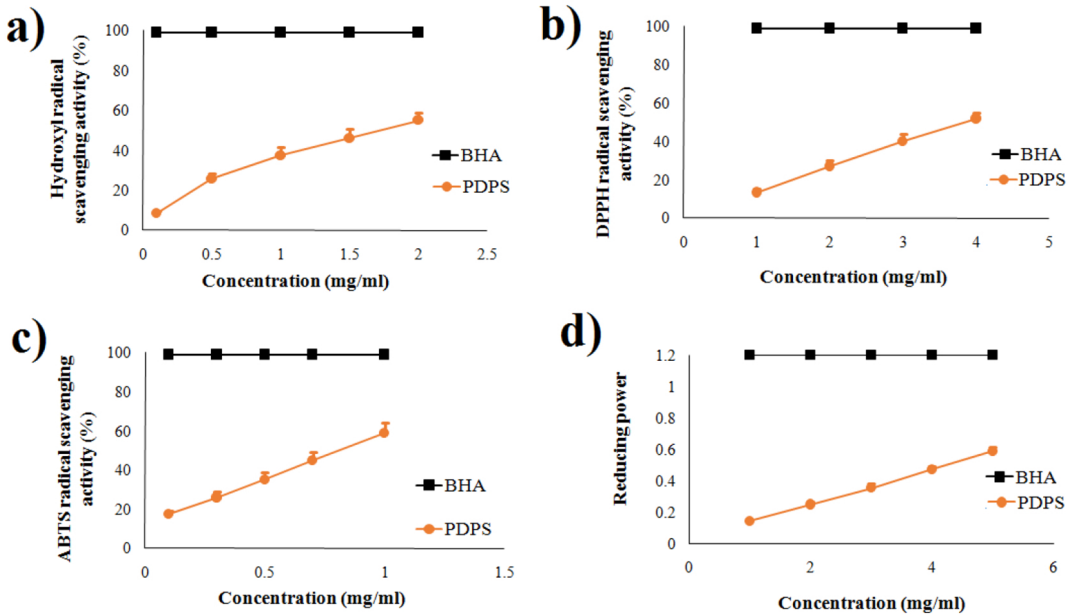

Figure 4