Chapter V

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

5.A. Introduction and review on earlier works

5.A.1. Polysaccharides from mushrooms

Wild edible mushrooms and their polysaccharides are useful for nutritional supplements as well as pharmaceuticals [130-132]. *Lentinus sajor-caju*, family polyporaceae, formerly known *Pleurotus sajor-caju* returned to the genus *Lentinus* by Pegler in 1975 [133]. It is distributed extending from southern regions of Africa to south-east Asia and down to the north-east region of Australia [134]. It grows abundantly on dead branches and woods. At the young stage the fruit bodies are consumed as nutritious food in Vietnam [135] and also in Malaysia as the vegetable [136]. Two water soluble polysaccharides from the cultivated edible mushroom previously named *Pleurotus sajor-caju* have been isolated, characterized and reported [98,137] from our 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.

5.A.2. 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 [134]. A reference specimen was deposited at the Calcutta University herbarium (Accession no: CUH AM352) adopting the method of Pradhan et al. [138].

5.B. Present work on PS-I

5.B.1. Isolation and purification

Crude polysaccharide (800 mg) was isolated from aqueous extraction of mushroom (600 g) by alcohol precipitation. Sepharose 6B gel filtration (**Fig. 1**) of crude polysaccharide (25 mg) yielded two fractions (fraction I; 14 mg and fraction II; 9 mg).

5.B.2. Physical characterization of PS-I

The specific rotation and average molecular weight (**Fig. 2**) of PS-I were shown in **Table 1**.

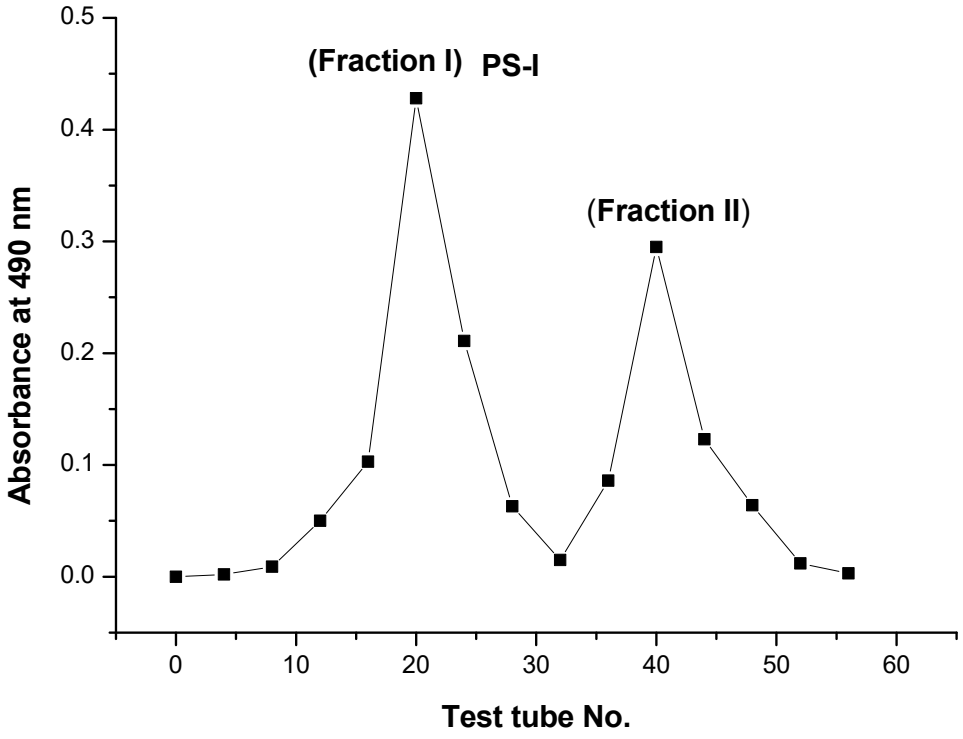


Figure 1: Gel permeation chromatogram of crude polysaccharide isolated from an edible mushroom *L. sajor-caju* using Sepharose 6B column.

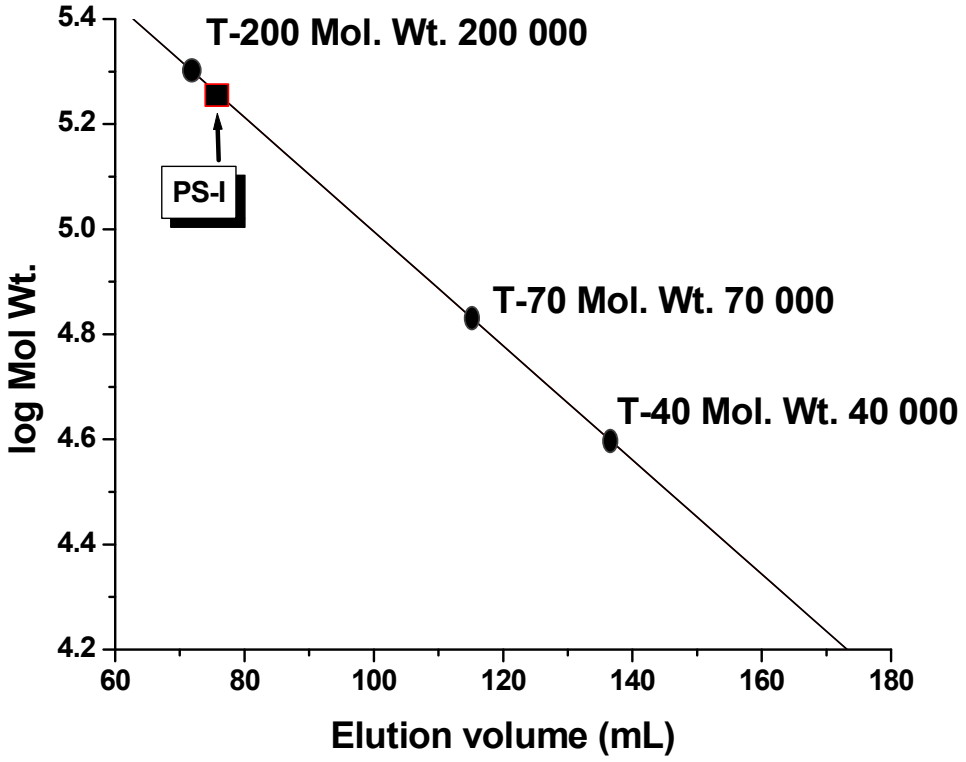


Figure 2: Determination of molecular weight of PS-I by gel permeation chromatography in Sepharose 6B column.

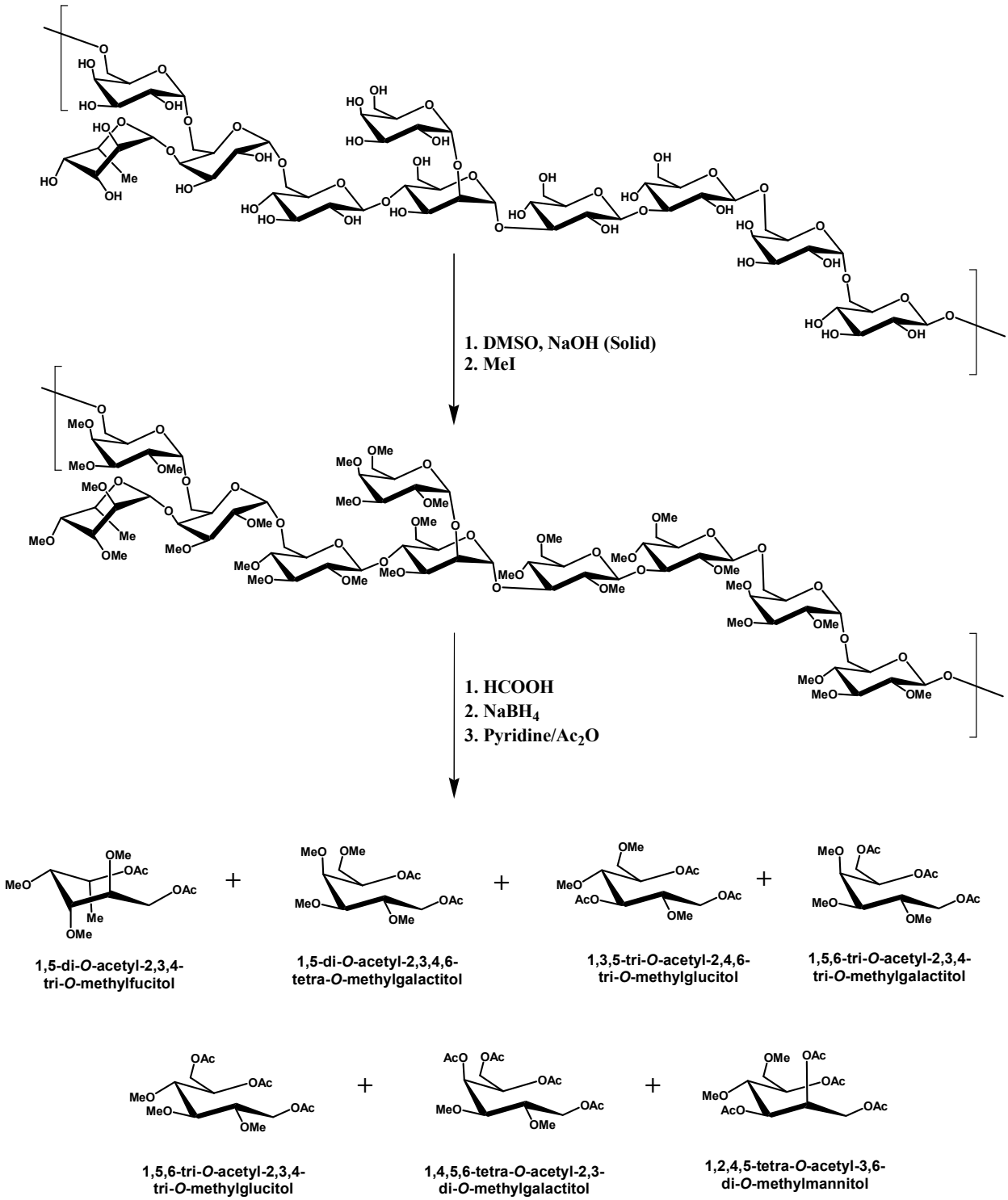
Table 1. Components of monosaccharide and properties of PS-I isolated from the mushroom *L. sajor-caju*

Properties	**Data**			
Monosaccharide component	Glucose	Galactose	mannose	fucose
Molar ratio	4	4	1	1
Absolute configuration	D	D	D	L
Specific rotation	$[\alpha]_D^{24.8}$ +13.6 (*c* 0.15, H_2O)			
Molecular weight	~1.79 ×10^5 Da			

5.B.3. Structural analysis of PS-I

5.B.3.1. Chemical analysis

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** (**Fig. 9**). 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. Methylation analysis of the PS-I is presented in the following diagram:

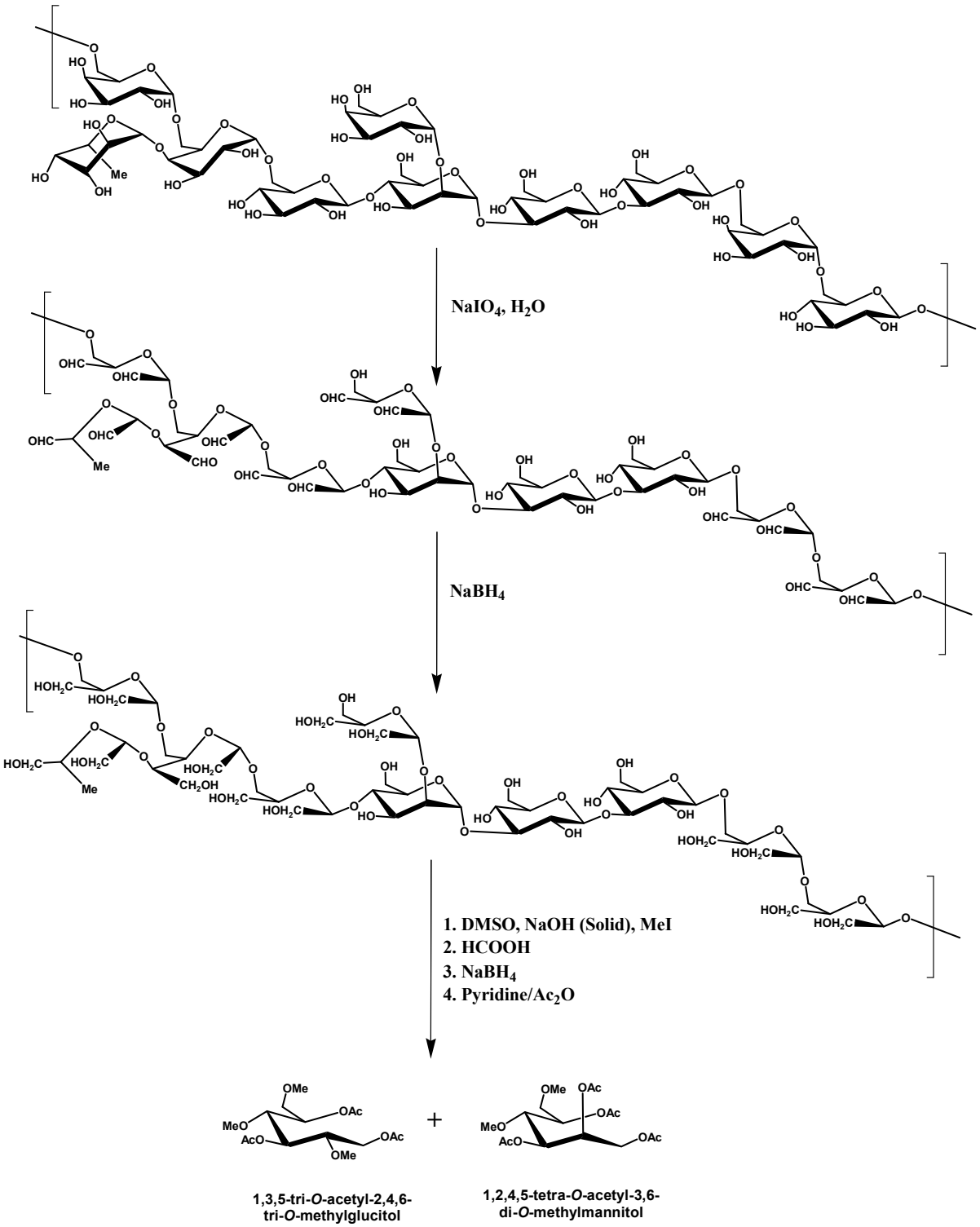


❖ Schematic representation of methylation analysis of PS-I.

Table 2. GLC-MS results of PS-I isolated from the mushroom *L. sajor-caju*

Methylated sugars	Molar ratio	Linkage type	Major Mass Fragments (m/z)
2,3,4-Me_3-Fuc	1	Fuc*p*-(1→	43,72,89,101, 115,117,131,161,175
2,3,4,6-Me_4-Gal	1	Gal*p*-(1→	43,45,59,71,87, 101,117,129,145,161,205
2,4,6-Me_3-Glc	2	→3)-Glc*p*-(1→	43,45,58,71,87,99,101, 117,129,143,161,173,217,233
2,3,4-Me_3-Gal	2	→6)-Gal*p*-(1→	43,45,58,71,87,99, 101,117,129,143,161,173,189,233
2,3,4-Me_3-Glc	2	→6)-Glc*p*-(1→	43,45,58,71,87,99, 101,117,129,143,161,173,189,233
2,3-Me_2-Gal	1	→4,6)-Gal*p*-(1→	43,58,85,87,99, 101,117,127,161,201,261
3,6-Me_2-Man	1	→2,4)-Man*p*(1→	43,59,74,87,99, 129,143,173,189,203,233

GLC analysis of periodate oxidized reduced material showed the presence of glucose and mannose in a molar ratio of nearly 2:1. GLC-MS analysis of alditol acetates of periodate oxidized reduced methylated product showed that (1→3)-Glc*p* and (1→2,4)-Man*p* moieties (**Table 3**) were unaffected during oxidation while other residues were consumed, which further confirmed the mode of linkages present in the polysaccharide. Periodate oxidation of the PS-I is presented in the following diagram:



❖ Schematic representation of periodate oxidation reaction of PS-I.

Table 3. GLC-MS results of periodate oxidized methylated PS-I isolated from the mushroom *L. sajor-caju*

Methylated sugars	Molar ratio	Linkage type	Major Mass Fragments (m/z)
2,4,6-Me_3-Glc	2	→3)-Glc*p*-(1→	43,45,58,71,87,99,101, 117,129,143,161,173,217,233
3,6-Me_2-Man	1	→2,4)-Man*p*(1→	43,59,74,87,99,129, 143,173,189,203,233

5.B.3.2. 1D and 2D NMR analysis

The proton NMR spectrum (500 MHz, in D_2O at 30 °C) of PS-I is shown in **Fig. 3** (**Table 4**). The anomeric region (δ_H 5.25 - 4.51) contained ten signals. These ten signals in decreasing order at δ_H 5.25, 5.11, 5.09, 5.05, 5.01, 4.99, 4.78, 4.75, 4.53, and 4.51 in PS-I were arbitrarily labeled as **A**, **B**, **C**, **D**, **E**, **F**, **G**, **H**, **I**, and **J** respectively. In ^{13}C NMR spectrum (125 MHz, in D_2O at 30 °C) the anomeric region (δ_C 102.8 - 97.6) contained nine signals (**Fig. 4a**). The anomeric signals of the residues **J** (δ_C 102.8), **I** (δ_C 102.6), **G** (δ_C 102.4), **H** (δ_C 102.4), **B** (δ_C 102.0), **C** (δ_C 101.3), **A** (δ_C 100.9), **E** (δ_C 98.3), **D** (δ_C 98.0), and **F** (δ_C 97.6) were correlated to the signals **J** (δ_H 4.51), **I** (δ_H 4.53), **G** (δ_H 4.78), **H** (δ_H 4.75), **B** (δ_H δ 5.11), **C** (δ_H 5.09), **A** (δ_H 5.25), **E** (δ_H 5.01), **D** (δ_H 5.05), and **F** (δ_H 4.99) respectively from HSQC spectrum (**Fig. 5a**; **Table 4**). All the ^1H and ^{13}C NMR signals (**Table 4**) were assigned from DQF-COSY, TOCSY, and HSQC experiments. Proton coupling constants were measured from DQF-COSY experiment.

Residue **A** was established as a terminal α-L-fucopyranosyl moiety. The H-1 (δ 5.25) and C-1 (δ 100.9) signals confirmed by HSQC experiment indicated that **A** residue is α-L-

Fuc*p*, which was strongly supported by the appearance of proton signal at δ 1.24, and a carbon signal at δ 15.7 for a exocyclic-CH_3 [58,128] group with respect to the standard methyl glycosides [66,97]. The rest of carbon values in ^{13}C corresponded to the standard values of methyl glycosides and considering the methylation analysis it indicated that residue **A** was glycosidically linked terminal α-L-fucopyranosyl moiety.

The anomeric chemical shift for residue **B** at H-1 (δ 5.11) and C-1 (δ 102.0) with coupling constant values ($J_{H\text{-}1,H\text{-}2}$~1.6 Hz, $J_{H\text{-}2,H\text{-}3}$~3.5 Hz, $J_{C\text{-}1,H\text{-}1}$ ~170 Hz) indicated that the residue **B** was present in α-configuration. The manno configuration of residue **B** was supported from large coupling constants ($J_{H\text{-}3,H\text{-}4}$ ~ 7.5 Hz, $J_{H\text{-}4,H\text{-}5}$ ~ 9.5 Hz). With respect to standard methyl glycoside values [66,97] the downfield shifts of C-2 (δ 77.6) and C-4 (δ 74.9) indicated that the moiety **B** was (1→2,4)-linked unit. Hence, these observations confirmed that residue **B** was (1→2,4)-α-D-mannopyranosyl moiety.

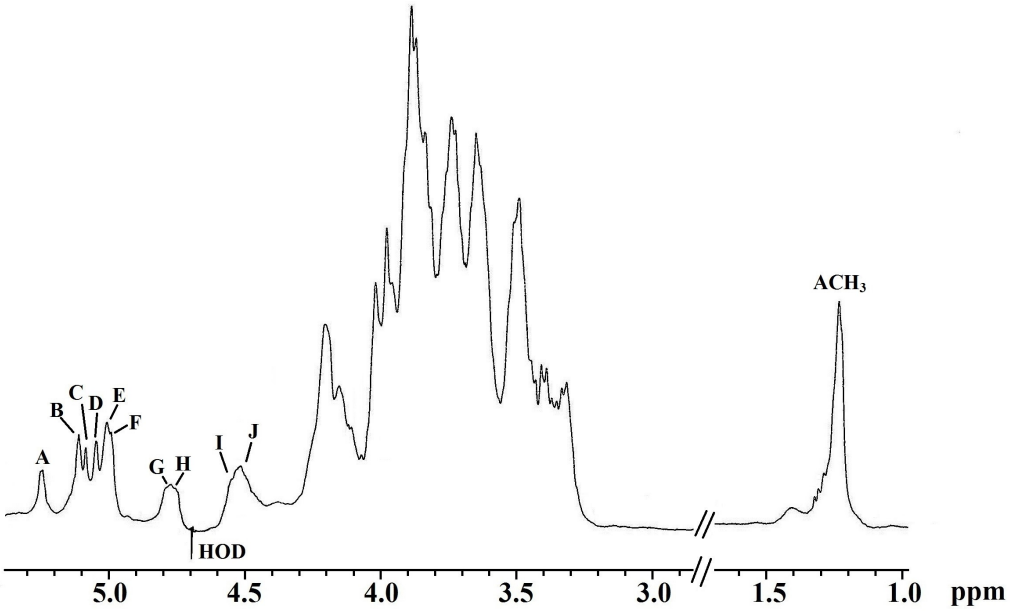


Figure 3: ^{1}H NMR spectrum (500 MHz, D_2O, 30 ºC) of PS-I, isolated from an edible mushroom *L. sajor-caju.*

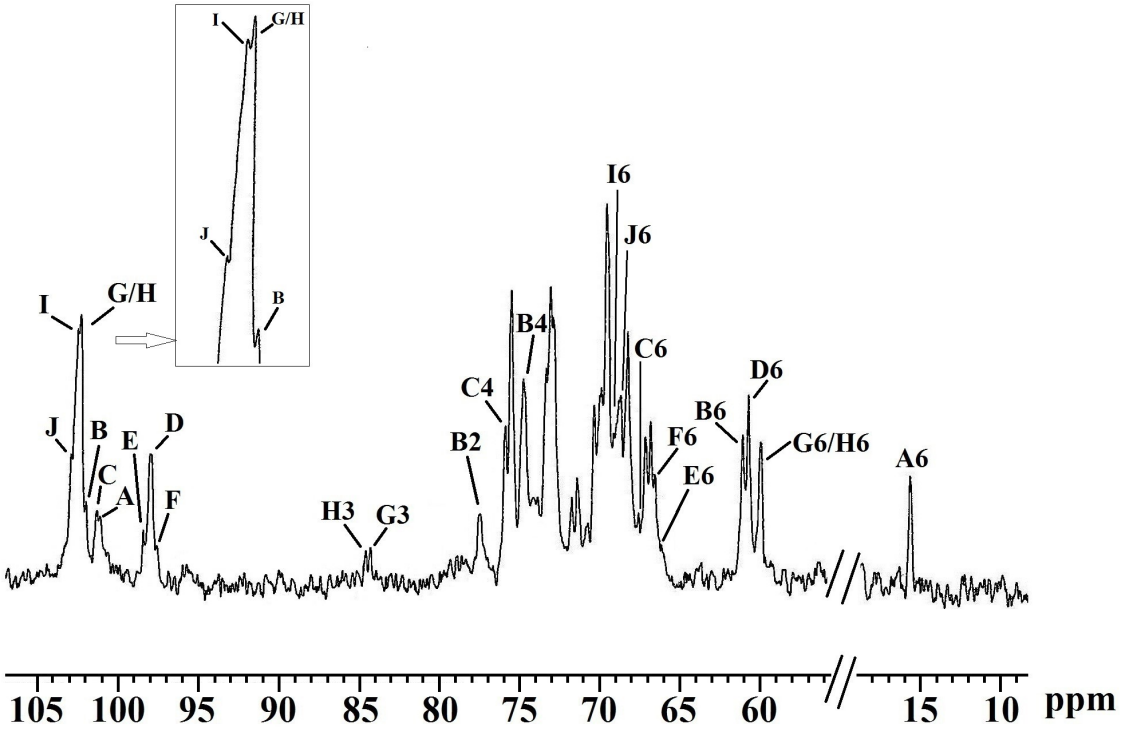


Figure 4(a): ^{13}C NMR spectrum (125 MHz, D_2O, 30 °C) of the PS-I, isolated from an edible mushroom *L. sajor-caju*.

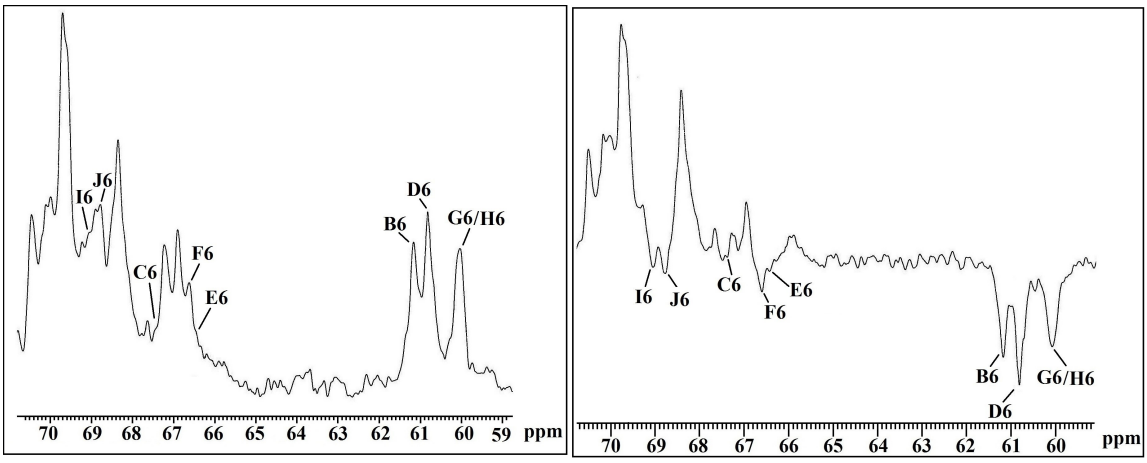


Figure 4(b): Part of ^{13}C NMR and DEPT-135 spectrum (D_2O, 30 °C) of the PS-I.

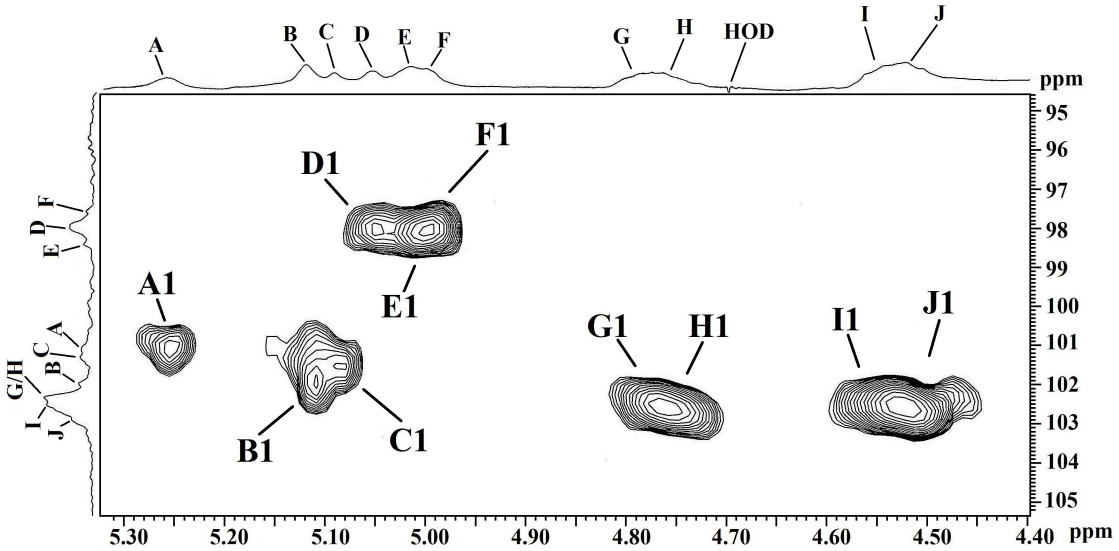


Figure 5(a): HSQC spectrum (D_2O, 30 °C) of anomeric part of PS-I isolated from an edible mushroom *L. sajor-caju.*

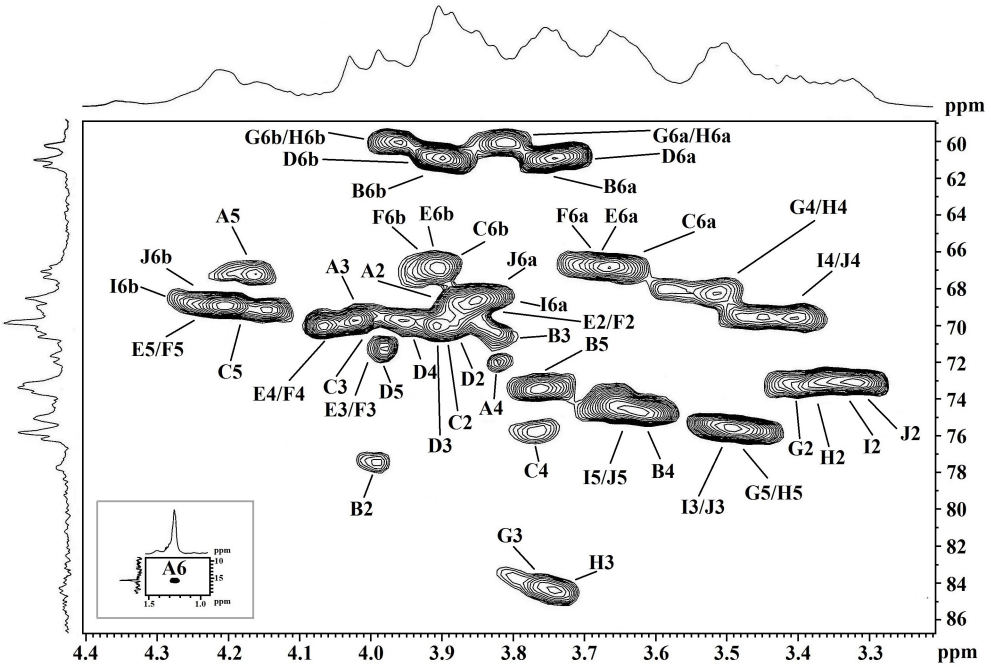


Figure 5(b): HSQC spectrum (D_2O, 30 °C) of other than anomeric part (Inset: C-6/H-6 correlation of α-L-Fuc*p* moiety) of PS-I isolated from an edible mushroom *L. sajor-caju.*

Residues **C**, **D**, **E**, and **F** were established as D-galactopyranosyl moieties, as revealed from coupling constant values ($J_{H-2,H-3}$ ~9 Hz, $J_{H-3,H-4}$ ~3.5 Hz). The α-configuration of **C**, **D**, **E**, and **F** residues were assigned from anomeirc ^{1}H and ^{13}C values. The coupling constant values $J_{H-1,H-2}$ ~3.1 Hz and $J_{C-1,H-1}$ ~171 Hz also confirmed the α-configuration. In case of residue **C** the downfield shifts of C-4 (δ 76.0) and C-6 (δ 67.4) with respect to standard [66,97] indicated that it was (1→4,6)-linked α-D-galactopyranosyl. The downfield shifts of C-6 of residues **E** (δ 66.4) and **F** (δ 66.6) indicated that residues **E** and **F** were (1→6)-linked α-D-galactopyranosyl moieties [58]. Hence, these observations confirmed that the residue **C** was (1→4,6)-α-D-galactopyranosyl unit and residues **E** and **F** were (1→6)-α-D-galactopyranosyl units. All the proton and carbon signals of residue **D** almost matched corresponding to the standard values of methyl glycosides. Thus the residue **D** was terminal α-D-galactopyranosyl moiety. Linkage at C-6 positions were established from DEPT-135 spectrum (**Fig. 4b**).

Residues **G**, **H**, **I**, and **J** were established as β-configuration from the coupling constant values ($J_{H-1,H-2}$~8.0 Hz, $J_{C-1,H-1}$~160Hz) as well as from the corresponding H-1 and C-1 values. Large $J_{H-2,H-3}$ and $J_{H-3,H-4}$ values (~10.0 Hz) of residues **G**, **H**, **I**, and **J** confirmed their D-glucopyranosyl configuration. The downfield shifts of C-3 of residues **G** (δ 84.2) and **H** (δ 84.5) with respect to standard [66,97] indicated that residues **G** and **H** were (1→3)-linked β-D-glucopyranosyl. The downfield shifts of C-6 of residues **I** (δ 69.0) and **J** (δ 68.8) with respect to standard indicated that it was (1→6)-linked β-D-glucopyranosyl. The *O*-6 substitution of both residues **I** and **J** were confirmed from the respective reverse peak in the DEPT-135 spectrum (**Fig. 4b**). Hence, these observations

confirmed that the residues **G** and **H** were (1→3)-β-D-glucopyranosyl and **I** and **J** were (1→6)-β-D-glucopyranosyl moieties.

Table 4. The ^{1}H NMR[a] and ^{13}C NMR[b] chemical shifts for the PS-I isolated from the mushroom *L. sajor-caju* in D_2O at 30 ^{o}C

Glycosyl residue	H-1/ C-1	H-2/ C-2	H-3/ C-3	H-4/ C-4	H-5/ C-5	H-6a, H-6b/ C-6
α-L-Fuc*p*-(1→	5.25	3.89	4.01	3.82	4.16	1.24
A	100.9	68.3	69.4	71.8	67.2	15.7
→2,4)-α-D-Man*p*-(1→	5.11	3.98	3.83	3.62	3.75	3.75[c], 3.90[d]
B	102.0	77.6	70.4	74.9	73.1	61.2
→4,6)-α-D-Gal*p*-(1→	5.09	3.90	3.98	3.78	4.16	3.63[c], 3.89[d]
C	101.3	70.0	69.9	76.0	68.3	67.4
α-D-Gal*p*-(1→	5.05	3.88	3.90	3.96	3.98	3.73[c], 3.90[d]
D	98.0	69.7	70.4	69.9	71.4	60.8
→6)-α-D-Gal*p*-(1→	5.01	3.85	3.98	4.02	4.21	3.66[c], 3.90[d]
E	98.3	69.6	70.7	70.0	69.1	66.4
→6)-α-D-Gal*p*-(1→	4.99	3.85	3.98	4.02	4.21	3.66[c], 3.90[d]
F	97.6	69.6	70.7	70.0	69.1	66.6
→3)-β-D-Glc*p*-(1→	4.78	3.41	3.75	3.51	3.46	3.79[c], 3.95[d]
G	102.4	72.9	84.2	68.3	76.0	60.1
→3)-β-D-Glc*p*-(1→	4.75	3.38	3.73	3.51	3.46	3.79[c], 3.95[d]
H	102.4	72.9	84.5	68.3	76.0	60.1
→6)-β-D-Glc*p*-(1→	4.53	3.34	3.51	3.41	3.62	3.83[c], 4.21[d]
I	102.6	73.1	75.6	69.7	75.0	69.0
→6)-β-D-Glc*p*-(1→	4.51	3.32	3.51	3.41	3.62	3.83[c], 4.21[d]
J	102.8	73.1	75.6	69.7	75.0	68.8

[a] The values of chemical shifts were recorded keeping HOD signal fixed at δ 4.70 at 30 ^{o}C.

[b] The values of chemical shifts were recorded with reference to acetone as internal standard and fixed at δ 31.05 at 30 °C.

[c,d] Interchangeable.

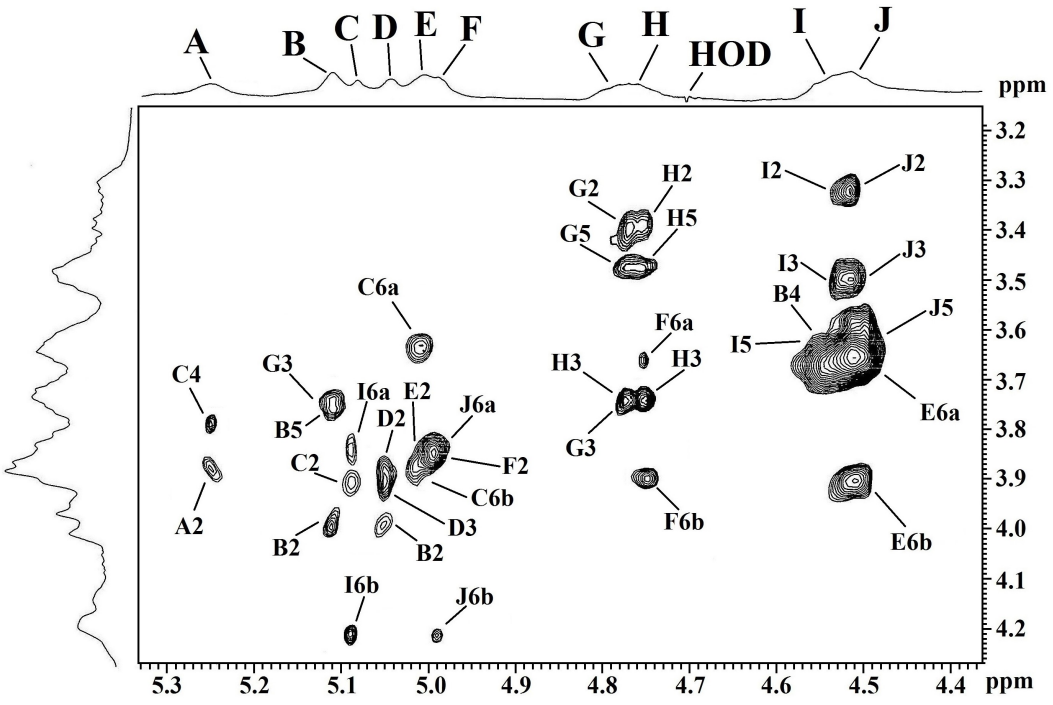


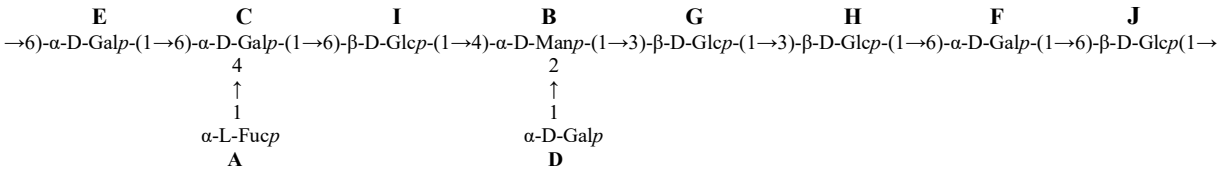
Figure 6: Part of ROESY spectrum of PS-I from an edible mushroom *L. sajor-caju*. The ROESY mixing time was 300 ms.

From ROESY (**Fig. 6**; **Table 5**) and NOESY (not shown) experiments the sequences of glycosyl residues were determined. The ROESY experiment showed the inter-residual contacts: **E**H-1/ **C**H-6a, 6b; **C**H-1/ **I**H-6a, 6b; **I**H-1/ **B**H-4; **B**H-1/ **G**H-3; **G**H-1/ **H**H-3; **H**H-1/ **F**H-6a, 6b; **F**H-1/ **J**H-6a, 6b; **J**H-1/ **E**H-6a, 6b; **A**H-1/ **C**H-4; **D**H-1/ **B**H-2 along with other intra-residual contacts (**Fig. 6**).

Table 5. ROESY data for the PS-I isolated from an edible mushroom *L. sajor-caju*

Glycosyl residue	Anomeric proton	ROE contact proton		
	δ	δ	residue	atom
α-L-Fuc*p*-(1→	5.25	3.78	**C**	H-4
A		3.89	**A**	H-2
→2,4)-α-D-Man*p*-(1→	5.11	3.75	**G**	H-3
B		3.98	**B**	H-2
		3.75	**B**	H-5
→4,6)-α-D-Gal*p*-(1→	5.09	3.83	**I**	H-6a
C		4.21	**I**	H-6b
		3.90	**C**	H-2
α-D-Gal*p*-(1→	5.05	3.98	**B**	H-2
D		3.88	**D**	H-2
		3.90	**D**	H-3
→6)-α-D-Gal*p*-(1→	5.01	3.63	**C**	H-6a
E		3.89	**C**	H-6b
		3.85	**E**	H-2
→6)-α-D-Gal*p*-(1→	4.99	3.83	**J**	H-6a
F		4.21	**J**	H6b
		3.85	**F**	H-2
→3)-β-D-Glc*p*-(1→	4.78	3.73	**H**	H-3
G		3.41	**G**	H-2
		3.75	**G**	H-3
		3.46	**G**	H-5
→3)-β-D-Glc*p*-(1→	4.75	3.66	**F**	H-6a
H		3.90	**F**	H-6b
		3.38	**H**	H-2
		3.73	**H**	H-3
		3.46	**H**	H-5
→6)-β-D-Glc*p*-(1→	4.53	3.62	**B**	H-4
I		3.34	**I**	H-2
		3.51	**I**	H-3
		3.62	**I**	H-5
→6)-β-D-Glc*p*-(1→	4.51	3.66	**E**	H-6a
J		3.90	**E**	H-6b
		3.32	**J**	H-2
		3.51	**J**	H-3
		3.62	**J**	H-5

Thus, the ROESY connectivity established the sequences as, **E** (1→6) **C**; **C**(1→6) **I**; **I** (1→4) **B**; **B** (1→3) **G**; **G** (1→3) **H**; **H** (1→6) **F**; **F** (1→6) **J**; **J** (1→6) **E**; **A** (1→4) **C**; **D** (1→2) **B**. Hence, from all these chemical and NMR studies the repeating unit motif of PS-I was proposed as:



Finally, the structure of main chain of the heteroglycan was identified by Smith degradation, which produced an oligomeric product (SDPS) that was analyzed by ^{13}C NMR. The ^{13}C NMR (125 Hz; at 30 ^{o}C) spectrum (**Fig. 7**; **Table 6**) of SDPS showed three anomeric signals at δ 103.2, 103.1, and 102.2.

The anomeric carbon signals at δ 103.2 and 103.1 resembled both to the (1→3)-β-D-glucopyranosyl residues and arbitrarily labeled as **K** and **L** respectively. Whereas, the carbon signal at δ 102.2 corresponded to the terminal α-D-mannopyranosyl (**M**) residue. The carbon signals of the glycerol moiety (Gro) [128,129] were assigned as C-1 (δ 66.5), C-2 (δ 72.2), and C-3 (δ 62.5).

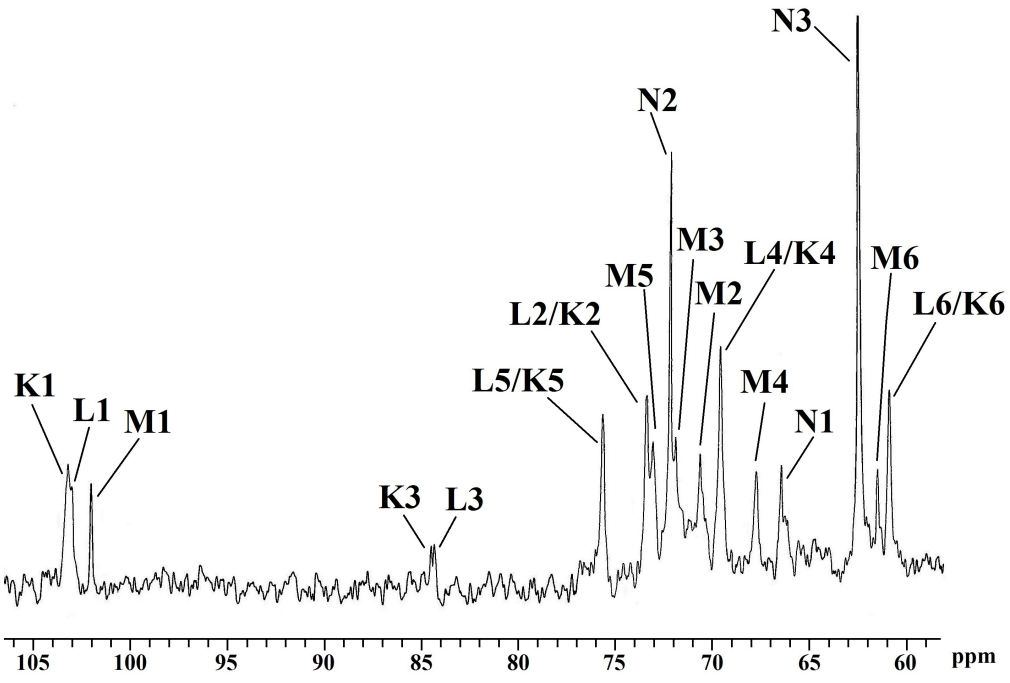


Figure 7: ^{13}C NMR spectrum (125 MHz, D_2O, 30 ^{o}C) of the Smith-degraded glycerol containing trisaccharide of PS-I isolated from an edible mushroom *L. sajor-caju*.

Table 6. The ^{13}C NMR^{n} chemical shifts of Smith-degraded glycerol-containing trisaccharide (SDPS) of PS-I isolated from an edible mushroom *L. sajor-caju* in D_2O at 30 ^{o}C

Sugar residue	C-1	C-2	C-3	C-4	C-5	C-6
→3)-β-D-Glc*p*-(1→ **K**	103.2	73.2	84.6	69.6	75.6	60.9
→3)-β-D-Glc*p*-(1→ **L**	103.1	73.2	84.4	69.6	75.6	60.9
α-D-Man*p*-(1→ **M**	102.2	70.5	72.0	67.8	73.0	61.4
→3)-Gro **N**	66.5	72.2	62.5			

[n] The values of chemical shifts were recorded with reference to acetone as internal standard and fixed at δ 31.05 at 30 °C.

The terminal α-D-mannopyranosyl (**M**) residue of SDPS was generated from the (1→2,4)-α-D-mannopyranosyl moiety (**B**) of parent PS-I during Smith degradation. The downfield shifts of C-3 of residues **K** (δ 84.6) and **L** (δ 84.4) of SDPS indicated that residues **K** and **L** were (1→3)-linked β-D-glucopyranosyl. The (1→3)-β-D-Glc*p* residues of SDPS (**K** and **L**) were retained during oxidation previously denoted as **H** and **G** respectively of the parent PS-I. During Smith degradation, the glycerol moiety **N** was generated from (1→6)-α-D-Gal*p* residue (**F**). Thus, the residue **N** was attached to **K**. Therefore, a glycerol containing trisaccharide was formed from the parent PS-I through Smith degradation. Hence, the structure of SDPS was proposed as:

M **L** **K** **N**
α-D-Man*p*-(1→3)-β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→3)Gro

The above results further confirmed the repeating sugar unit in PS-I.

Two similar kinds of polysaccharides, one from *Termitomyces striatus* [93] and another from *Termitomyces clypeatus* [128] were reported earlier which contain same sugar composition but in different ratios. The present polysaccharide (PS-I) contain D-glucose, D-galactose, D-mannose, and L-fucose in a molar ratio of nearly 4:4:1:1 whereas, for *T. striatus* and *T. clypeatus* the ratios are 4:2:2:2 and 4:2:1:1 respectively. In *T. clypeatus* α-D-Man*p* and β-D-Glc*p* remain in the backbone chain terminated with α-L-Fuc*p* and α-D-Glc*p*. In *T. Striatus* α-D-Gal*p* and α-D-Glc*p* remain in the skeleton chain and terminated with α-D-Glc*p* and β-D-Glc*p* respectively whereas, in the PS-I α-D-Gal*p*

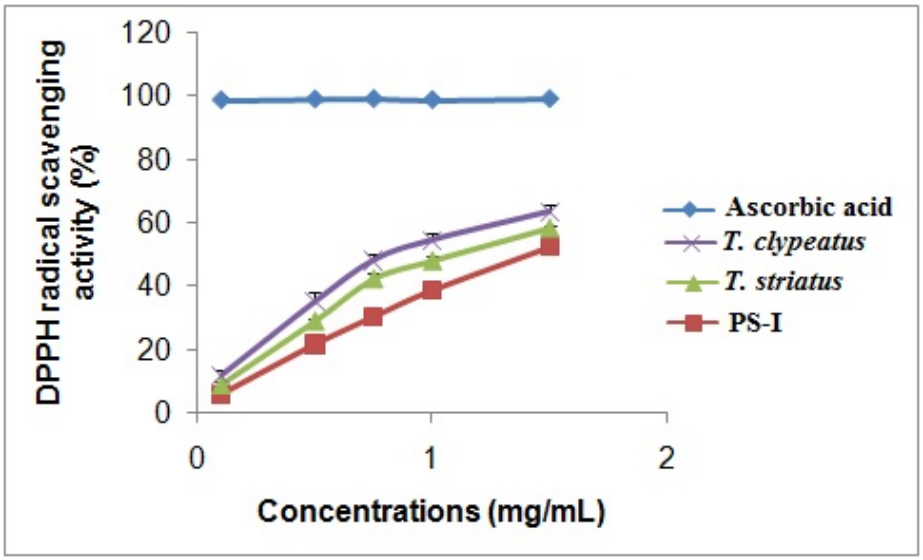
and α-D-Man*p* remain in the backbone chain terminated with α-L-Fuc*p* and α-D-Gal*p* correspondingly. The linkages and ^{1}H, ^{13}C signals are also different in three polysaccharides.

5.B.4. Antioxidant activities of PS-I

5.B.4.1. DPPH radical scavenging activity

The antioxidant activity of PS-I was determined as their scavenging capability against free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH). Antioxidant compounds have the ability to donate electron or hydrogen atom. DPPH is a stable free radical which accepts an electron/hydrogen to gain stability. When the stable DPPH radical accepts an electron from the antioxidant compound, the violet color of the DPPH radical was reduced to yellow colored diphenylpicrylhydrazine radical which was measured colorimetrically. The degree of discoloration demonstrates it ability to scavenge free radicals. In the present study, the PS-I was found as a notable scavenger of DPPH radicals. Results showed that the radical scavenging activity of the PS-I increased in concentration dependent manner. **Fig. 8a** revealed that 0.1, 0.5, 0.75, 1.0 and 1.5 mg/mL concentrations of polysaccharide exhibited radical scavenging activity at the rate of 6.14%, 21.88%, 30.56 %, 38.89 % and 52.5 %, whereas the standard ascorbic acid showed the activity at 98.6%, 98.9%, 99%, 98.7% and 99% respectively. In DPPH assay, the radical scavenging activity, from strongest to weakest, was as follows: *T. clypeatus* (EC_{50} = 0.80 ± 0.23 mg/mL), *T. striatus* (EC_{50} = 1.25 ± 0.23 mg/mL), and PS-I (EC_{50} = 1.375 ± 0.02 mg/mL). Thus PS-I shows moderate DPPH radical scavenging activity.

Fig. 8(a)

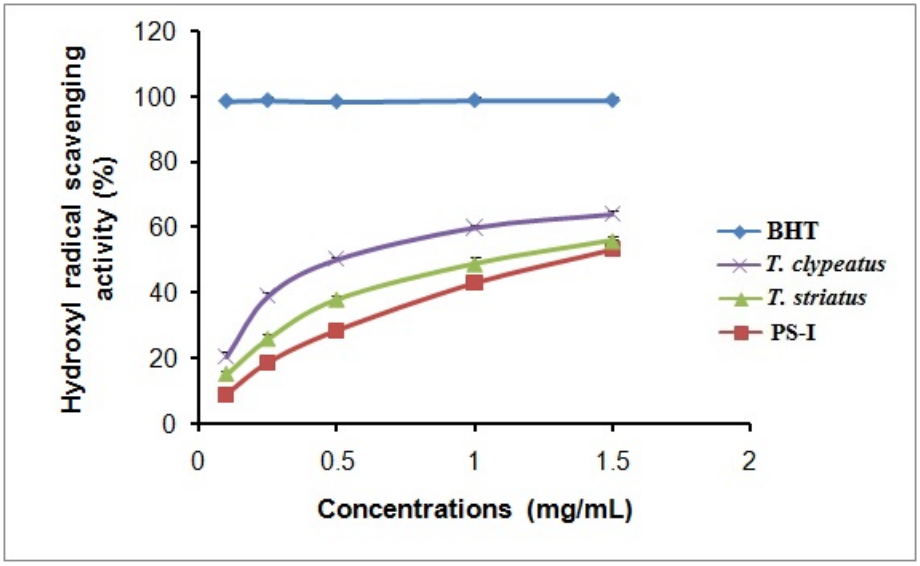


5.B.4.2. Hydroxyl radical scavenging activity

Hydroxyl radical is the most reactive oxygen centered species and can be generated in biological cells through the Fenton reaction. It can damage mostly the major biomolecule [139] DNA by attacking purine, pyrimidine and deoxyribose. When test sample (PS-I) was added to the reaction mixture, hydroxyl radicals are removed and prevented sugar degradation. The PS-I showed potent hydroxyl radical scavenging activity which gradually increases with increasing concentration (**Fig. 8b**). The hydroxyl radical scavenging rate of PS-I at 0.25, 0.50, 0.75, 1.0, and 1.5 mg/mL were found as 18.7%, 28.5%, 36.5%, 43.06%, and 53.3% respectively. But BHT showed hydroxyl radical scavenging rate to that of corresponding concentrations at 98.7%, 99%, 98.6%, 99%, and 99%. The EC_{50} value of the PS-I was found to be 1.31 ± 0.048 mg/mL whereas, the polysaccharides from *T. clypeatus* and *T. striatus* show EC_{50} at 0.50 ± 0.25 mg/mL

and 1.10 ± 0.18 mg/mL respectively. These results indicated that PS-I and the polysaccharide from *T. striatus* have almost equal hydroxyl radical scavenging ability but the polysaccharide from *T. clypeatus* is better among these three.

Fig. 8(b)



5.B.4.3. Reducing power

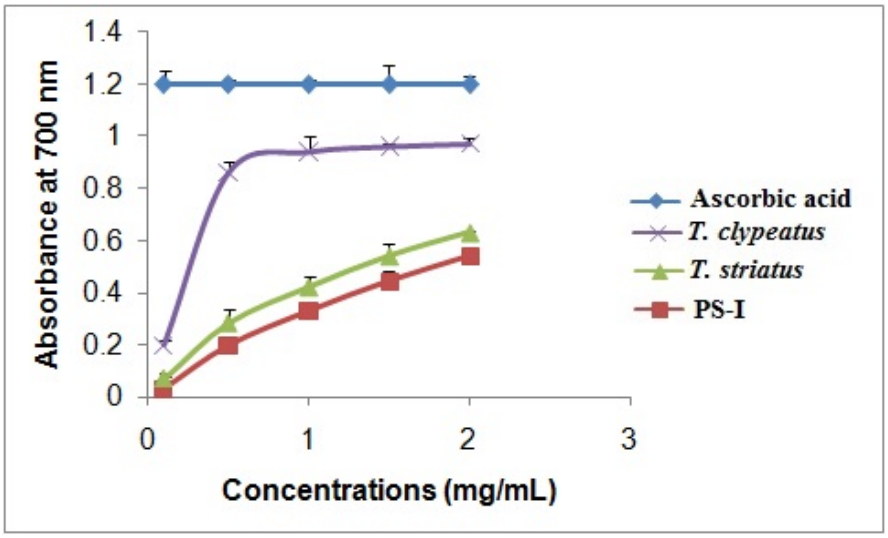
The antioxidant principles present in the PS-I caused the reduction of Fe^{3+}/ferricyanide complex to the ferrous form and thus proved its reducing power ability. In the present study, the conversion of Fe^{3+}–Fe^{2+} was investigated in the presence of the mushroom polysaccharide, PS-I. Literature studies revealed that the reducing properties are generally associated with the presence of reductones, which break free radical chain by donating a hydrogen atom thereby indicating its antioxidant potentiality [140]. The PS-I showed good reducing power ability in a dose dependent manner which was comparable to that of ascorbic acid, used as standard. Higher absorbance of the reaction

mixture indicated greater reducing power. At concentration of 0.1 mg/mL, the absorbance value of PS-I was 0.03 but in case of ascorbic acid the value was 1.2. The PS-I showed the absorbance value 0.196 at 0.5 mg/mL, whereas the ascorbic acid showed the value of 1.19 at the same concentration. Reducing power of the PS-I increases with the increase in concentration. At concentrations of 1.0, 1.5, and 2.0 mg/mL the absorbance value of ascorbic acid was fixed at 1.2 whereas the value of PS-I increases to 0.328, 0.443 and 0.539 respectively. Herein, PS-I could reduce Fe^{3+} to Fe^{2+} with 50 % inhibition capacity at a concentration of 1.75 ± 0.035 mg/mL (**Fig. 8c**). These results suggest that PS-I is a moderate electron donor and may terminate the radical chain reaction by converting free radicals to more stable product. The polysaccharides isolated from *T. striatus* (EC_{50} = 1.21 ± 0.12 mg/mL) and *T. clypeatus* (EC_{50} = 0.30 ± 0.05 mg/mL) shows better reducing power compare to that of PS-I.

5.B.4.4. ABTS radical scavenging assay

ABTS radicals possess blue green chromophore, formed by the reaction between ammonium persulphate and ABTS salt [141]. The reaction is based on the reduction of ABTS radicals which visibly reflects in discoloration of the sample solution due to hydrogen donating ability of the antioxidant compounds. The radical scavenging activity was compared with Trolox, commercially available water soluble vitamin E analog. In this assay, radical scavenging activity was evaluated and the results showed 501.3 ± 0.042 µM of Trolox equivalent/mg of polysaccharide antioxidant potentiality.

Fig. 8(c)



5.B.4.5. Chelating ability of ferrous ion

Chelating ability of any compounds may serve as a reflection of its antioxidant activity. In this assay, ferrous ion being a catalyst has the ability to generate free radicals by the Fenton and Haber-Weiss reaction. Ferrozine quantitatively forms complexes with Fe^{2+} and changes the solution color to violet. In presence of any chelating agent, the ferrozine- Fe^{2+} formation is disrupted with decrease in color of the complex. Resultant color reduction demonstrates ferrous ion chelating efficacy of the sample. In this assay, polysaccharide (PS-I) and standard antioxidant compound EDTA interfered with the formation of ferrous and ferrozine complex, suggesting that they have chelating activity. **Fig. 8d** reveals that PS-I exhibited a marked capacity for iron binding ability. At 0.5, 1, 1.5, 2 and 3 mg/mL concentrations the polysaccharide showed chelation at the rate of 25.03 %, 33.6%, 38.2 %, 43.7 %, and 52.5%, whereas the used standard EDTA also chelated 99% at the same concentrations. From the EC_{50} value of PS-I (2.67 ± 0.02

mg/mL), *T. striatus* (1.17 ± 0.14 mg/mL), and *T. clypeatus* (0.48 ± 0.08 mg/mL), it is revealed that *T. clypeatus* is strongest chelator of ferrous ions.

Fig. 8(d)

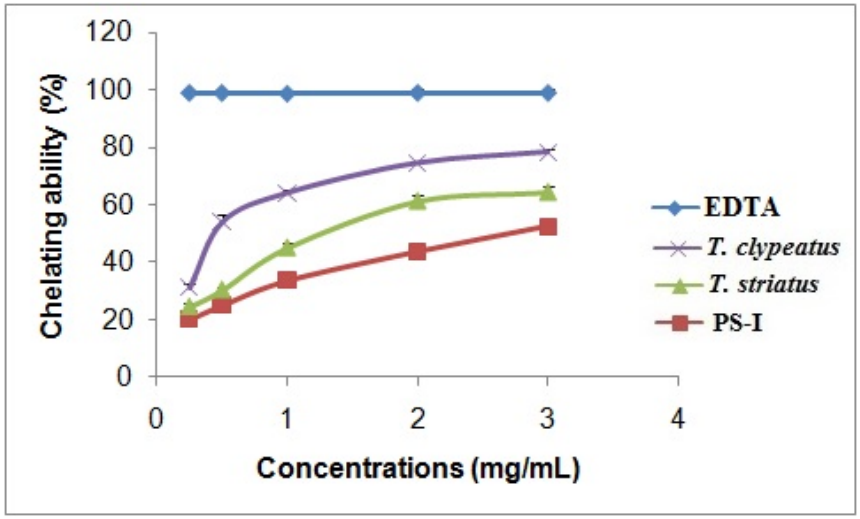
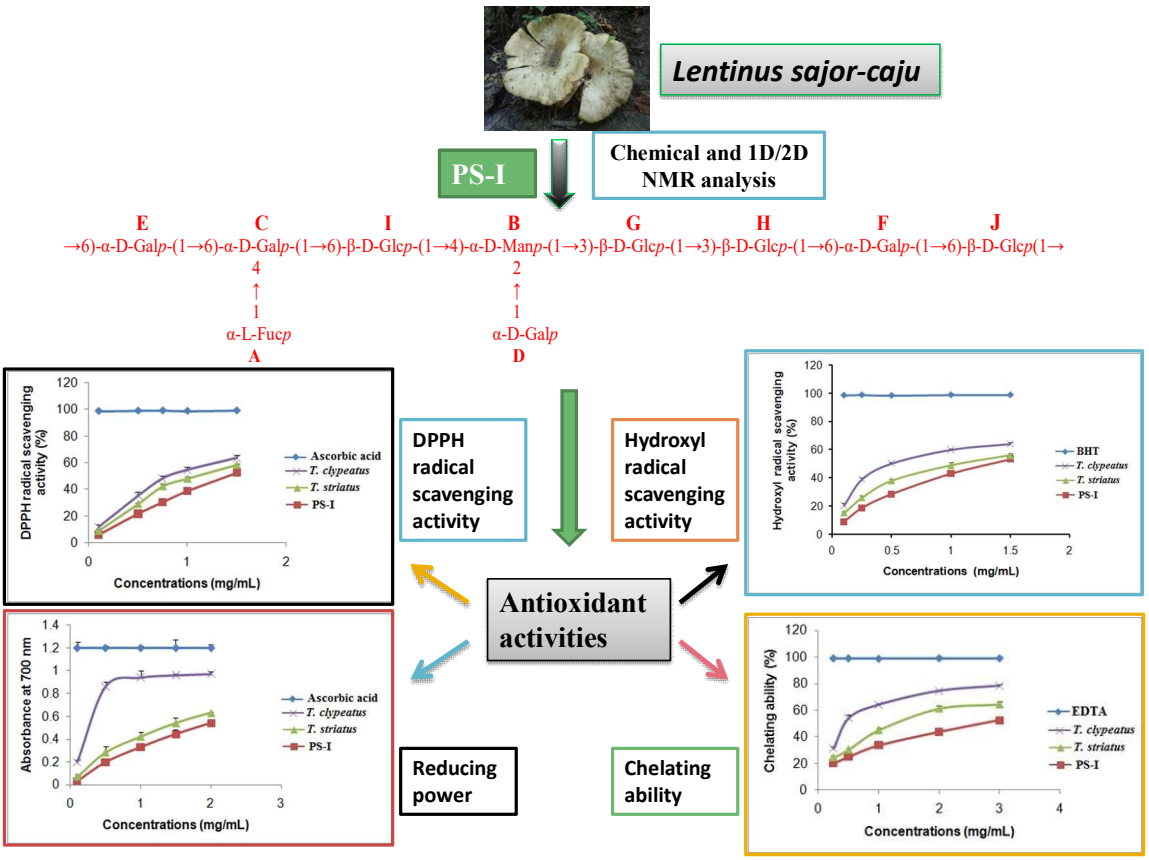


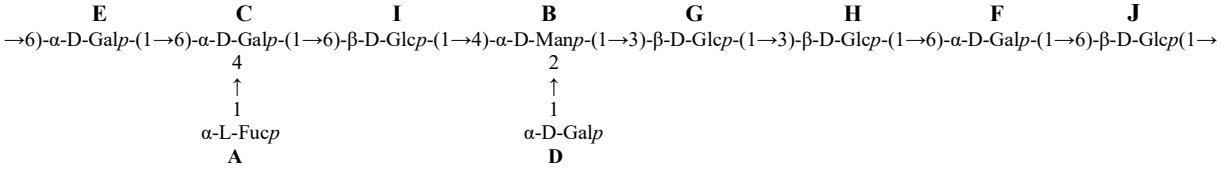
Figure 8: Antioxidant activities of polysaccharides isolated from *T. clypeatus*, *T. striatus*, and PS-I (*L. sajor-caju*) **(a)** DPPH radical scavenging activity, **(b)** Hydroxyl radical scavenging activity, **(c)** Reducing power, **(d)** Chelating ability. Results are the mean ± SD of five separate experiments, each in triplicate.

❖ **All the structural and antioxidant properties of a polysaccharide (PS-I) from an edible mushroom *Lentinus sajor-caju* are presented in the following diagram:**



5.C. Conclusion

A purified water soluble heteroglycan of an edible mushroom *L. sajor-caju* obtained by gel permeation chromatography showed specific rotation $[\alpha]_D^{24.8}$ +13.6 (*c* 0.15, H_2O) with average molecular weight ~1.79 $\times 10^5$ Da. 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 showed DPPH radical scavenging activity, hydroxyl radical scavenging activity, ABTS radical scavenging property, reducing power, and ferrous ion chelating ability.