ISOLATION, CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF β-GLUCAN ISOLATED FROM EDIBLE MUSHROOM *AGROCYBE CYLINDRACEA*

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ABSTRACT

Objectives: Mushroom β-glucan has the ability to affect many cellular functions, including cellular glucose uptake. Although cumulative evidence in literature suggests a connection between β-glucan and reduction of blood glucose concentration, a mechanism of how β-glucan affects cellular glucose uptake has not been demonstrated. **Methods:** A set of spectroscopic, chemical and separation methods are used for this purpose. Among them, NMR spectroscopy is known as a powerful tool in structural analysis of glucans both in solution and in solid state. **Results:** In this study, β glucan isoalated from *Agrocybe cylindracea*, characterized by ^{1}H NMR, ^{13}C NMR spectroscopy and the *invitro* antidiabetic effect of β-glucan by the α-amylase inhibitory activity also analyzed. **Conclusions:** The highest inhibitory activity (75.82%) was detected at 2.0 mg/mL. This result indicated that β-glucan possessed higher inhibitory activitiy against α-Amylase.

Key words: β-glucan, *Agrocybe cylindracea* , *invitro* antidiabetic, antimicrobial

INTRODUCTION

Mushrooms have been valued throughout the world as both food and medicine for thousands of years. The main compounds commonly observed were various types of polysaccharides. Polysaccharides are large complex branched chain-like molecules built from many single units of monosaccharides. Mushroom is attributed by many medicinal properties, due to presence of bioactive compounds in fruiting bodies and cultured mycelium. Most excellent and thereuptically potent mushroom derived metabolite is a β-glucans a versatile, broad spectrum polysaccharide with anti-tumor and immunomodulating properties is best known for their biological activities. β-glucans are polysaccharides found in the cell wall of fungi, plants and some bacteria [1]. They consist of glucose molecules that link through β(1→3), β(1→4) and β(1→6) glycosidic bonds. β-glucan was shown to reduce total and LDL cholesterol level of hypercholesterolemic in adult individuals [2] and blood glucose in both animals and humans [3]. In addition, it also has the

ability to prevent occurrence of glucose intolerance in mice high-fat diet [4]. Thus, β-glucan possesses several activities, which depend on structure, size, solubility, and the degree of branching [5] For example, highly branched β-glucan was shown to be a better immune stimulator than one with less frequent branches [6]. In our recent study, we have extracted polysaccharides from two mushroom species, Pleurotus florida and Agrocybe cylindracea. The antioxidant abilities of polysaccharides were then analyzed by invitro systems including ferrous ion chelating activity and hydroxyl radical scavenging assay. The results suggest that extracted polysaccharides could be a promising source of natural antioxidant and be contributor to the health benefits of *P. florida* and *A. cylindracea* [7]. Here we report the evaluation of the antidiabetic activity of an isolated polysaccharide fraction of *Agrocybe cylindracea.* The isolated polysaccharide was characterized structurally by NMR spectroscopy.

MATERIALS AND METHODS

The fruiting bodies of *Agrocybe cylindracea* were collected from Ooty, Tamil Nadu and dried soon after harvest in a convection dryer. Subsequently, caps were separated from stems and ground in a mill to obtain fine powder. Isolation of crude β glucan was conducted according to Wasterlund *et al.,* [8] with slight modifications. The flow chart of β glucan isolation is shown in **Figure 1**. The isolated β glucan was then characterized by ^{1}H NMR and ^{13}C NMR spectroscopy.

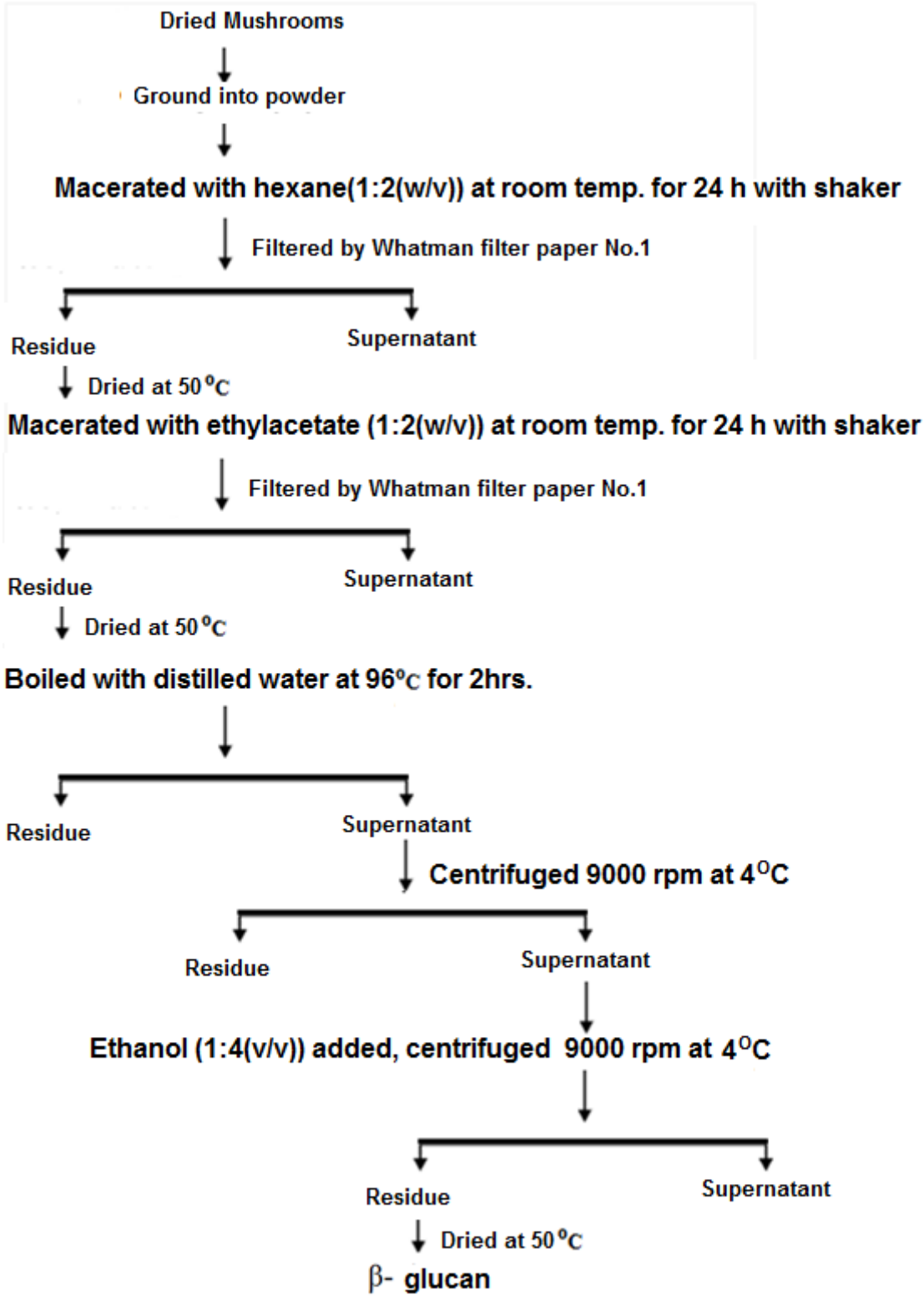

Figure 1: Flowchart of the isolation β-glucan

Bruker AVANCE III 500 MHz (AV 500) multi nuclei solution NMR spectrometer has been used for NMR studies.

α-Amylase Inhibition

The α -amylase inhibitory activity was performed by the method as described by Tadera *et al*. [9] with minor modification. Two hundred µL of varying concentration of extracts (0.125–2.0 mg/mL) were prepared in 20 mM, pH 6.9 phosphate buffer and then mixed with 200 µL of porcine pancreatic α -amylase (0.5 mg/mL) and incubated at 25°C for 10 min, and then 200 µL of starch solution (1%) was added and kept at 25°C for 30 min. The reaction was stopped by adding 1.0 mL of dinitrosalicylic acidreagent (1.0 g of 3.5-dinitrosalicylic acid in 20mL of 2 M NaOH + 50 mL distilled water + 30 g potassium sodium tartrate tetrahydrate). Then, the mixture was dissolved in distilled water to make a tota volume of 100 mL, and incubated in a water bath (100°C) for 5 min and cooled to room temperature. The reaction mixture was measured at 540 nm with a UV-Vis spectrophotometer. The α -amylase inhibitory activity was calculated using the following formula:

$$\text{Percent Inhibition} = [(Ac - As)/ Ac] \times 100$$

where Ac is the absorbance of the control reaction (containing all reagents except the test compound) and As is the absorbance of the test compound. Acarbose was used for the standard reference.

Assay of Antibacterial Activity

Disc Preparation: The 6mm (diameter) discs were prepared from Whatmann No. 1 filter paper. The discs were sterilized by autoclave at 121°C. After the sterilization the moisture discs were dried on hot air oven at 50°C.Then discs were mixed with chemical compounds separately and control discs were prepared.

Collection of test microorganisms: The Bacterial strains of *Staphylococcus aureus , Klebsiella pneumonia* and *Escherichia coli* , obtained from Microbial Type culture Collection Centre (MTCC), Chandigarh.

The dried β-glucan (20 mg) was dissolved in 1ml of 20% DMSO (Dimethyl sulphoxide). From this stock solution 10 µl of was added to the disc (0.2 mg/disc) individually and aseptically. Each disc contained 0.2 mg of β-glucan. Then the discs were allowed for drying at room temperature. After drying they were used for screening the antibacterial activity.

Antibacterial activity test was carried out following the modification of the method originally described by Bauer *et al.*,[10] Muller Hinton agar was prepared and autoclaved at 15 lbs pressure for 20 minutes and cooled to 45 ºC. The cooled media was poured on to sterile petri plates and allowed for solidification. The plates with media were seeded with the respective microbial suspension using sterile swab. The various discs were placed individually on the each petri plates and also placed control and standard discs. Gentamicin antibiotic discs were used as standard. The plates were incubated at 37 ºC for 24 hrs. After incubation period, the diameter of the zone formed around the paper disc were measured and expressed in mm.

Statistical Analysis

All data were expressed as mean ± standard deviations (SD). One-way analysis of variance followed by Tukey multiple comparisons were used to compare means between groups. Differences between means at the 5% ($p \leq 0.05$) level were considered statistically significant.

RESULTS AND DISCUSSION

The ^{l3}C NMR spectrum showed peaks at 103.401, 69.457, 77.279, 76.771, 77.025 and 60.463 ppm could be easily assigned to C-l, C-4, C-3, C-2 and C-5 and C-6 respectively (**Figure 2**). The ^{13}C NMR spectrum showed one glycosidic carbon signal at 103.401 ppm due to β-linkage. The ^{1}H NMR (**Figure 3**) showed the glycosidic proton signals clearly differentiated in the lowest field. The signal at δ 4.627 ppm should be assigned to the overlapped resonance of β - (1-6) linkage, since the glycosidic signal of the corresponding glucobioses is very close each other. The spectra from 4.847 to 4.627 ppm represent the multiple repeating points in the resonances for the anomeric proton, H1 side chanin, and one of the methylene protons of the H6 side chain, of the (1→6)-β-linkage of the side chain respectively. Comparitive ^{13}C and ^{1}H NMR data for isolated polysaccharide shown in Table 1.

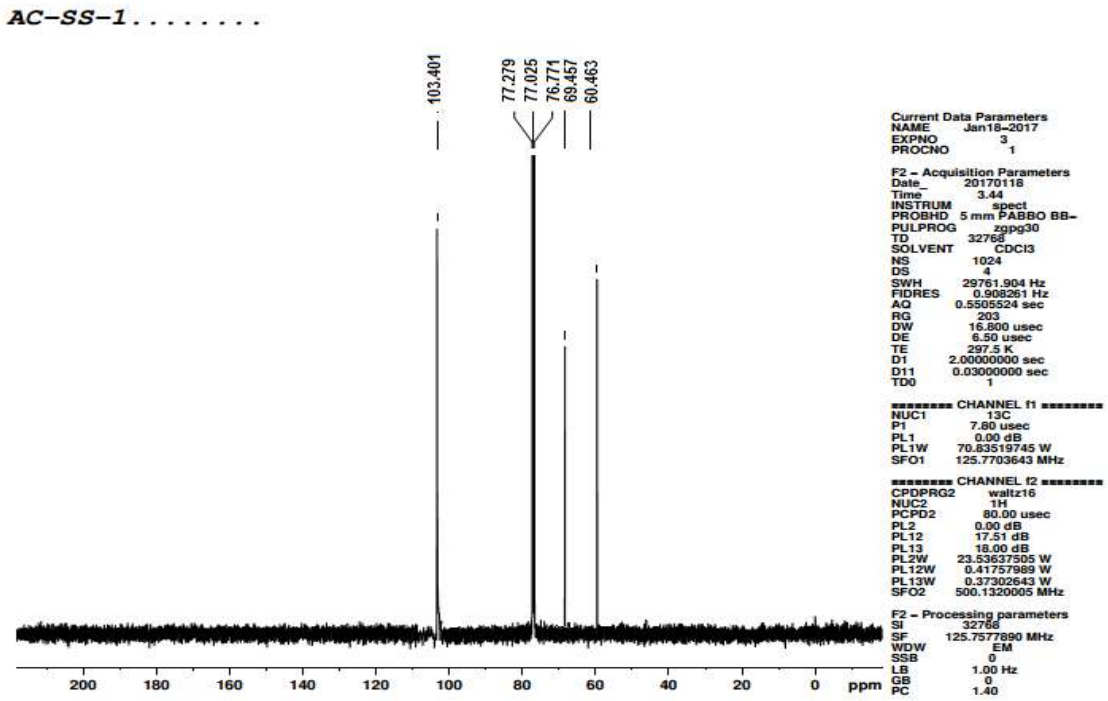

Fig 2. ^{13}C NMR spectrum of isolated polysaccharide

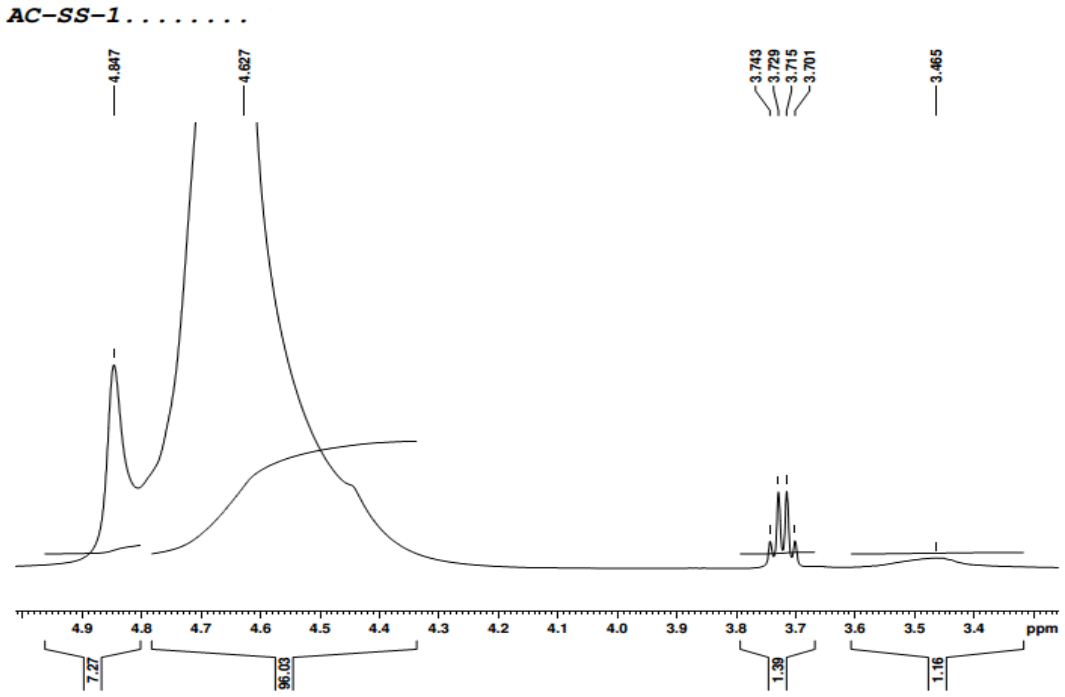

Fig 3. ^{1}H NMR spectrum of isolated polysaccharide

Table 1. Comparitive ^{13}C and ^{1}H NMR data for isolated polysaccharide

	C1	C2	C3	C4	C5	C6
^{13}C NMR	103.401	76.771	77.279	69.457	77.025	60.463
	H-1	H-2	H-3	H-4	H-5	H-6,6'
^{13}H NMR	4.847	3.715	3.743	3.701	3.729	3.465 4.627

The possible structure of β-D-(1→6) glucan shown in Figure 4.

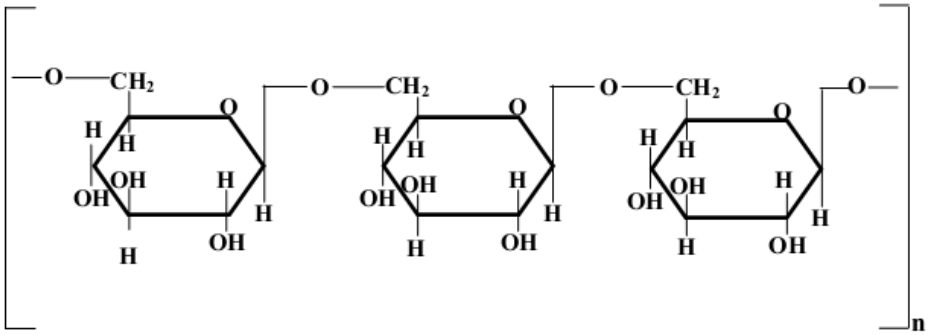

Fig. 4. Structure of β-D-(1→6) glucan isolated from *Agrocybe cylindracea*

α- Amylase Inhibitory Activity

In this experiment, α- Amylase inhibitory effects of β-glucan increased gradually with the increasing β-glucan concentrations. The highest inhibitory activity (75.82%) was detected at 2.0 mg/mL. However, this inhibitory activity was lower than that of Acarbose. These results indicated that β-glucan possessed higher inhibitory activitiy . The α- Amylase inhibitory activity of β-glucan showed in **Figure 5.**

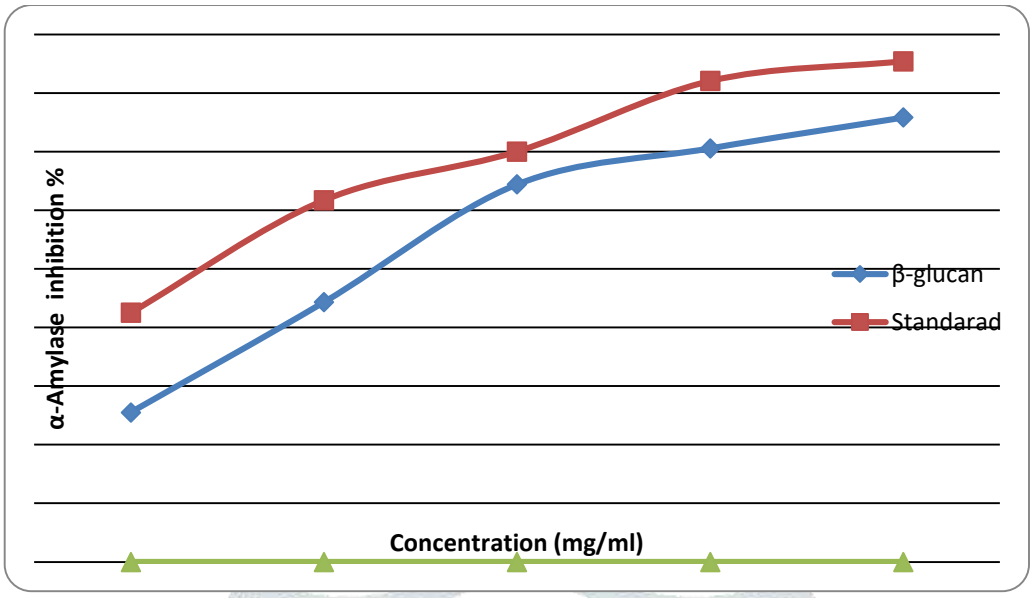

Figure 5: The α- Amylase inhibitory activity of β-glucan and standarad drug Acarbose. Values are means ± S.D (n=3)

Antibacterial Activity

Disk diffusion method allowed for preliminary evaluation of antibacterial activity of isolated β-glucan. The results of antibacterial tests are presented in **Fig.6** and **Table 2**. The isolated β-glucan was demonstrated to possess antibacterial activity against gram-positive bacterial strains *Staphylococcus aureus*; and gram-negative bacterial strains *Klebsiella pneumonia* and Escherichia coli. The β-glucan had greatest activity against gram-positive bacterial strain *Staphylococcus aureus* (20mm) .

Figure 6. Antibiogram of β-glucan against common pathogens

Table 2. Antimicrobial activity of isolated β-glucan againt common pathogens

S. No.	Bacteria	Zone of Inhibition (mm in diameter)		
		Control	Standarad*	Sample (β-glucan)
1	*Escherichia coli*	-	18	15
2	*Klebsiella pneumonia*	-	14	17
3	*Staphylococcus aureus*	-	15	20

CONCLUSION

The antibacterial activity of isolated β-glucan was evaluated against *Escherichia coli* , *Klebsiella pneumoniae* and *Staphylococcus aureus* . β-glucan showed good bactericidal activity. Also the findings indicated that the β-glucan isolated from *Agrocybe cylindracea* possess anti-diabetic properties . So, the edible mushrooms can be used to develop natural drugs which may be used in lieu of commonly used strong allopathic drugs which possess a number of harmful side effects.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest

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