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Research Article
Structural Characterization and Anti-Diabetic Activity of Polysaccharides from *Agaricus bisporus* Mushroom

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Abstract

Background and Objective: Mushroom polysaccharides have many health benefits. This study aimed to extract and purify polysaccharides from edible mushroom and to investigate its *in vitro* antidiabetic activity. **Materials and Methods:** *Agaricus bisporus* (*A. bisporus*) mushroom samples were collected at Thanjavur, Tamil Nadu. Extracted polysaccharides were characterized by ^{1}H NMR, ^{13}C NMR spectroscopy and the *in vitro* antidiabetic activity of the extracted polysaccharides was analyzed by α-amylase inhibitory activity. All data were expressed as mean standard deviations (SD) and SPSS version 16 was used for statistical analysis. **Results:** The highest inhibitory activity (78.85%) was detected at 2.0 mg mL^{-1}. This result indicated that polysaccharide possessed higher inhibitory activity against α-amylase. **Conclusion:** Hence, the present study showed that mushroom polysaccharides displayed antidiabetic activity. Mushroom polysaccharides are yet to be explored for a lot of various pharmaceuticals for applications in near future.

Key words: *Agaricus bisporus*, polysaccharide, α-amylase inhibitory activity, NMR, antidiabetic activity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Polysaccharides are polymeric carbohydrate molecules formed by many monosaccharide units linked by glycosidic bonds. They are widely distributed in the cell membranes of higher plants, algae, bacteria, fungi and animals[1]. Polysaccharides extracted from natural plants have been used as novel adjuvant with low toxicity, low side effects and stimulatory activities[2-5]. In recent years, studies have shown that polysaccharides display excellent immune-enhancing activity. It is well known that biological activities of polysaccharides depend on their structural characteristics, namely the glycosidic bond of the main chain sugar subunits[6-7]. Molecular modification of natural polysaccharides can significantly promote their immune-enhancing activity[8-10]. Diabetes mellitus (DM) is a chronic metabolic disease caused by the insufficient production of insulin from β-cells of the pancreas or reduced sensitivity of cells to insulin. To date, there is no satisfactory therapy available to cure type 2 DM[11]. Several drugs, α-glucosidase inhibitors such as acarbose, voglibose and miglitol are now available to treat the patients who suffer from post-prandial hyperglycemia. These type of drugs inhibited degradation of carbohydrates in the digestive system, thereby reducing the glucose absorption by the cells and decreasing the blood glucose level. However, these drugs are associated with side effects such as yellow eyes or skin and gastrointestinal disturbances including abdominal or stomach pain, diarrhea, passing of gas, thus searching for new natural anti-diabetic compounds is essential to overcome DM problems[12].

Agaricus bisporus is the most popular edible mushrooms, usually called as common mushroom, button mushroom or white mushroom. *A. bisporus* containing trace elements like sodium, potassium, phosphorus and common antioxidants vitamin C, phenol compounds and flavonoids. Ergosterol present in *A. bisporus* reduces the risk of breast cancer. In the present study, the *in vitro* antidiabetic activity of polysaccharides from *Agaricus bisporus* was assessed.

MATERIALS AND METHODS

Chemicals: All the analytical grade chemicals and solvents were purchased from Sigma Aldrich.

Mushroom: Fruiting bodies of *Agaricus bisporus* used in this experiment collected from the local area of Thanjavur district, India, during the month of May-July, 2017. The sample was washed with distilled water for several times and cleaved into small pieces. Fruit bodies were kept in freeze-drying flasks, -20°C and later subjected to freeze-drying. Freeze-dried mushroom was then ground using a dry grinder to obtain fine powdered sample. The freeze-dried samples were bottled and kept in dry container at room temperature before extraction. NMR: Bruker AVANCE III 500 MHz (AV 500) multi nuclei solution NMR spectrometer has been used for NMR studies.

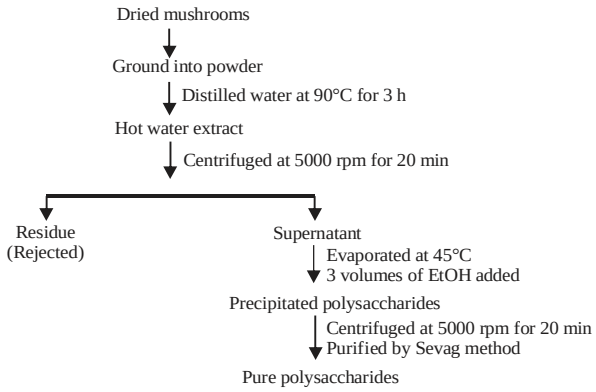

Fig. 1: Flow chart for the isolation and purification of polysaccharides

The extraction and purification of fruiting body polysaccharides were carried out according to the method of Lu *et al.*[7]. The precipitated materials were collected by centrifugation at 5000 rpm for 20 min and then purified using the classic Sevag method[8]. Extraction process was shown in flow chart (Fig. 1). Under these conditions, the maximal polysaccharide yield was 12.56 g/100 g.

α-amylase inhibition: The α-amylase inhibitory activity was performed by the method as described by Tadera *et al.*[13] with minor modification. About 200 µL of varying concentration of extracts (0.125-2.0 mg mL^{-1}) were prepared in 20 mM, pH maintained as 6.9 using phosphate buffer and then mixed with 200 µL of porcine pancreatic α-amylase (0.5 mg mL^{-1}) 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 acid reagent (1.0 g of 3.5-dinitrosalicylic acid in 20 mL 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 total 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:

$$\text{Percent inhibition} = \frac{Ac - As}{Ac} \times 100$$

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.

Statistical analysis: All data were expressed as mean±standard deviations (SD) and SPSS version 16 was used for statistical analysis. One-way analysis of variance ANOVA followed by Tukey's multiple comparisons were used to compare means between groups. Differences between means at the 5% ($p<0.05$) level were considered statistically significant.

RESULTS AND DISCUSSION

NMR spectroscopy is a powerful analytical technique that is often employed to study polysaccharides. The detailed structural information obtained from NMR is frequently not available through other analytical techniques. The ^{1}H-NMR spectra of the extracted polysaccharides is shown in Fig. 2. ^{1}H signal at δ4.62-4.84 ppm indicated that the glycosidic linkages of monosaccharides are both α and β configurations in the extracted polysaccharides[14]. The chemical shifts from 3.70-3.74 ppm were assigned to protons of C2-C5 of the glycosidic ring[15,16]. The ^{13}C-NMR spectra of the extracted polysaccharides is shown in Fig. 3.

SEM images of polysaccharides: Scanning electron micrographs of extracted polysaccharides are illustrated in Fig. 4. SEM images showed that extracted polysaccharides had a rough surface with many cavities. Though the preparation of polysaccharides might cause damage to the samples as some rigid fragments were appeared in the micrographs.

At low magnification, it was displayed irregular shape (Fig. 4a). The smooth surface indicated that polysaccharides had stronger intermolecular forces which could contribute to more stable structure (Fig. 4c and d). The SEM images of polysaccharides indicated that it had stronger intermolecular forces and interaction with other molecules which might profit from the functional groups in protein. The protein in polysaccharides highly affected its physicochemical properties and hence its bioactivities[17]. Also monosaccharide composition/combinations significantly affect polysaccharide bioactivity. Increasing research attention has been paid to regulation of synthesis of polysaccharide with stronger bioactivities. Variability in monosaccharide composition/combinations among mushroom polysaccharides may result from strain variations, developmental stage, culture method and conditions, medium composition, extraction method and even drying method[18-22].

α-amylase inhibitory activity: α-amylase is a prominent enzyme found in the pancreatic juice and saliva which breaks

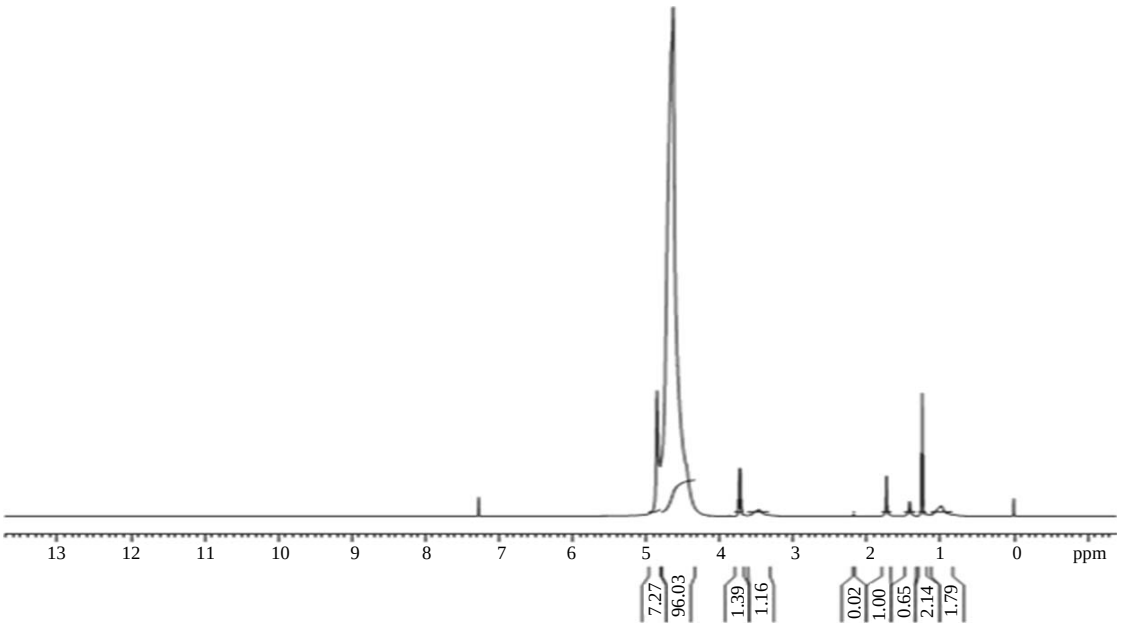

Fig. 2: ^{1}H NMR spectrum extracted polysaccharide

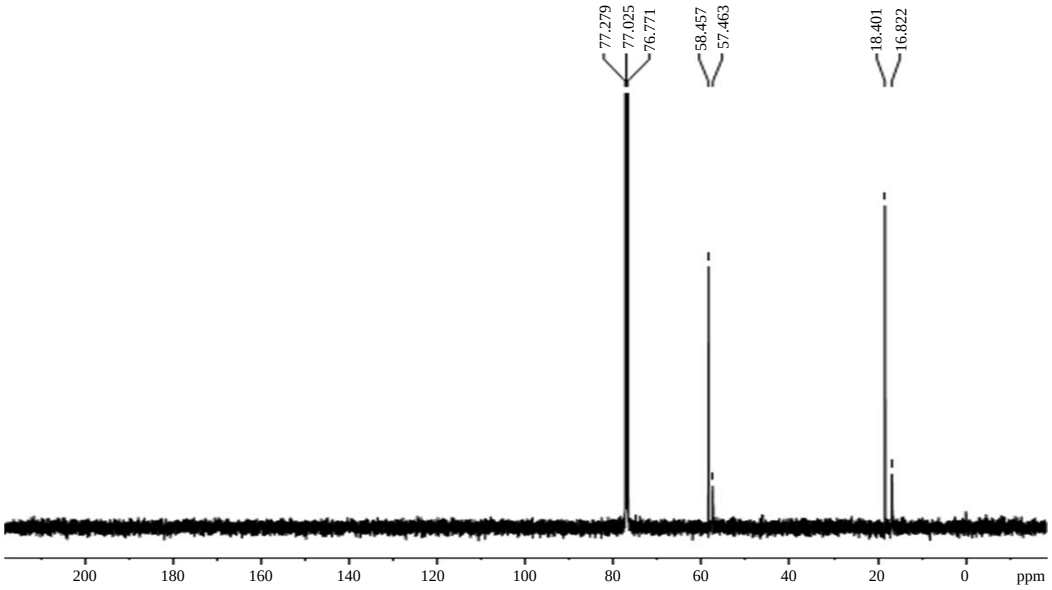

Fig. 3: ^{13}C NMR spectrum of extracted polysaccharide

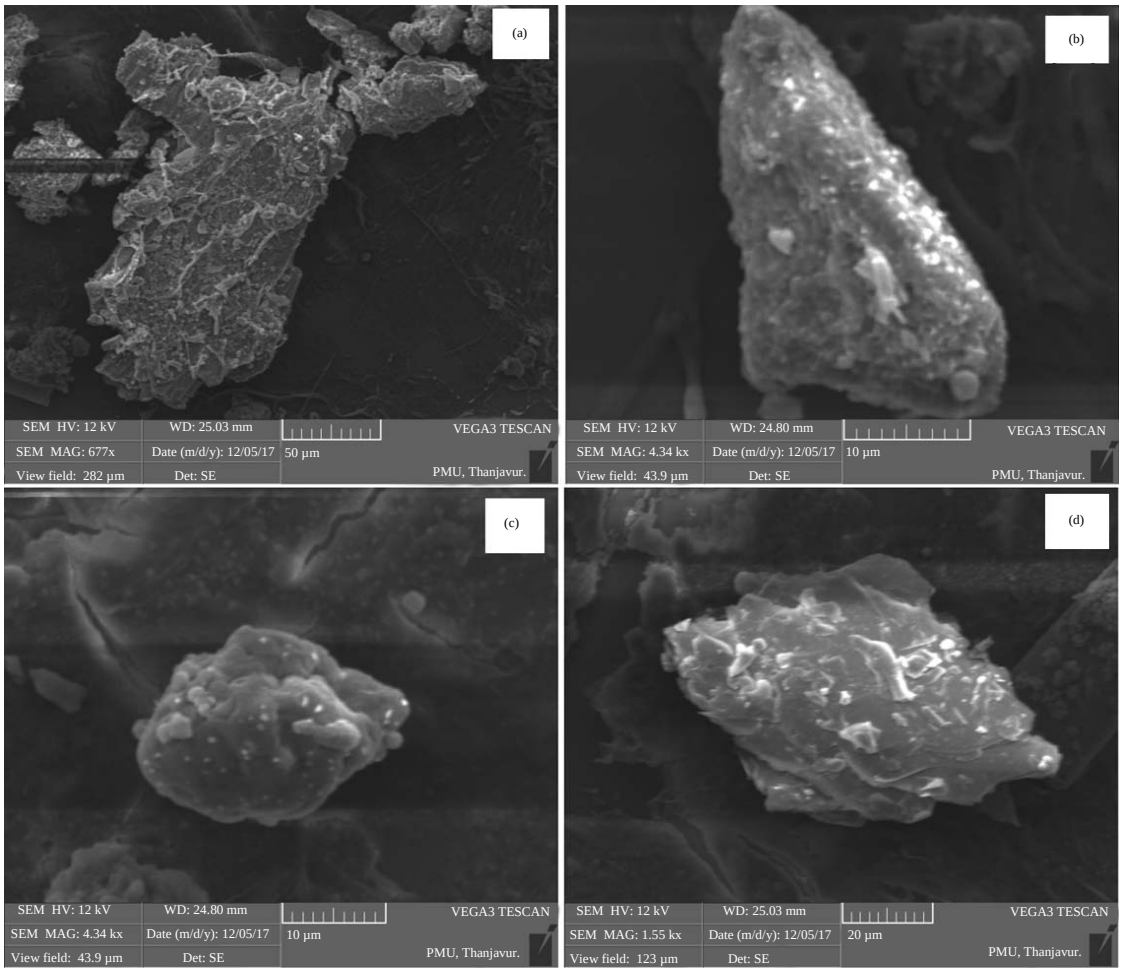

Fig. 4(a-d): SEM images of polysaccharides at different magnifications, (a) 677x (scale bar is 50 µm), (b) 4.34 kx (scale bar is 10 µm), (c) 3.54 kx (scale bar is 10 µm) and (d) 1.55 kx (scale bar is 20 µm)

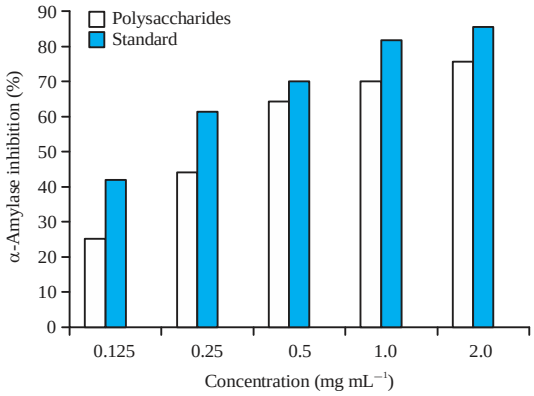

Fig. 5: α-Amylase inhibitory activity of polysaccharides and standard drug acarbose. Values are Means±S.D (n = 3)

down large insoluble starch molecules into absorbable molecules[23]. Inhibitors of α-amylase and α-glucosidase delay the breaking down of carbohydrates in the small intestine and diminish the postprandial blood glucose excursion[24]. Thus α-amylase inhibitor that offer a therapeutic approach in reducing the postprandial hyperglycemia. In order to ultimately slowing glucose breakdown from starch, α-amylase enzyme that cleaves at internal bonds of large polysaccharides is inhibited[25]. This α-amylase inhibitor inhibits the action of α-amylase enzyme leading to retard the liberation of maltose from starch hydrolysis which shows beneficial effects on glucose level control in diabetic patients[26]. In this experiment, α- amylase inhibitory effects of polysaccharides increased gradually with the increasing polysaccharides concentrations. The highest inhibitory activity (78.85%) was detected at 2.0 mg mL^{-1}. However, this inhibitory activity was lower than that of Acarbose. These results indicated that polysaccharides possessed higher inhibitory activity. The α- amylase inhibitory activity of polysaccharides are shown in Fig. 5.

α-amylase and α-glucosidase are key enzymes to digest starch in mammals[27]. Inhibition of starch digestive enzymes or glucose transporters can suppress postprandial hyperglycemia by reducing the rate of glucose release and absorption in the small intestine[28]. Chen *et al.*[29] reported that mushroom polysaccharides improved the impaired glucose tolerance from developing into DM through its inhibiting digestive enzymes. This result supports current findings. The biological activities of polysaccharides are correlated with their structural characterization. The type of monomer, linkage type and position, the number and position of branches occurring within the polymer chain strongly influence the three-dimensional arrangement and in addition to the molecular size, these factors determine polysaccharide behaviour[30]. Therefore, the structural elucidation of polysaccharides is very important for predicting their biological behavior. Realizing the combined chemical and pharmacological properties of mushroom polysaccharides will give information into their efficiency to prevent and treat chronic diseases. The current study described the structural characteristics of polysaccharides extracted *A. bisporus* and for the first time the present study was undertaken to evaluate *in vitro* antidiabetic activity by performing α-amylase inhibitory evaluation.

CONCLUSION

Based on the results obtained in this study, it was concluded that the extracted polysaccharides have significant antidiabetic activity. These results suggested that *A. bisporus* polysaccharides could be a promising source of natural antidiabetic and be contributor to the health benefits. Mushroom polysaccharides might simultaneously help multiple human disease syndromes associated with allergy, cancer, diabetes, infections and obesity with inflammatory.

SIGNIFICANT STATEMENT

Understanding of the overlapping chemical and pharmacological aspects of mushroom polysaccharides will provide valuable insights into their potential to prevent and treat chronic diseases. Here, the study aimed to extract and characterize polysaccharides from *Agaricus bisporus* and the *in vitro* antidiabetic activity of the extracted polysaccharides was analyzed first time by α-amylase inhibitory activity. The results from the study may open up a great field of disease management in near future.

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