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ISOLATION, CHARACTERIZATION AND *INVITRO* ANTIDIABETIC ACTIVITY OF β-GLUCAN ISOLATED FROM EDIBLE MUSHROOM *PLEUROTUS FLORIDA*.

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ABSTRACT

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. Isolation of glucans from raw sources needs removal of ballast compounds including proteins, lipids, polyphenols and other polysaccharides. Purity control of glucan fractions is necessary to evaluate the isolation and purification steps; more rigorous structural analyses of purified polysaccharides are required to clarify their structure. 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. In this study, β glucan isoalated from *Pleurotus florida* ,characterized by ^{1}H NMR, ^{13}C NMR spectroscopy and the *invitro* antidiabetic effect of β-glucan by β-glucosidase inhibitor activity also analyzed. The highest inhibitory activity (76.68%) was detected at 2.0 mg/mL. This result indicated that β-glucan possessed higher inhibitory activitiy against α-glucosidase.

KEYWORDS: β-glucan, *Pleurotus florida*, *invitro* antidiabetic, β-glucosidase inhibitor activity.

INTRODUCTION

Mushroom is attributed by many medicinal properties, due to presence of bioactive compounds in fruiting bodies and cultured mycelium. Fruiting bodies as well as active mycelia of *Pleurotus* species also possesses a number of therapeutic properties to combated cancer, high cholesterol level, high blood pressure, blood sugar etc, in humans.[1] 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.[2] 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[3] and blood glucose in both animals and humans.[4] In addition, it also has the ability to prevent occurrence of glucose intolerance in mice high-fat diet.[5] Thus, β-glucan possesses several activities, which depend on structure, size, solubility, and the degree of branching.[6] For example, highly branched β-glucan was shown to be a better immune stimulator than one with less frequent branches.[7]

One of the best sources for β(1→3) with β(1→6) glucan is mushroom. β-glucan isolated from mushrooms has been subjects for many intense research investigations. One of the mushrooms: the white oyster mushroom (*Pleurotus florida*), which is easily found in India, has been used for β-glucan isolation. It was found that β-glucan from this mushroom was highly branched, it contained a single β(1→6) glucose side chains on every second and third glucose residues.[8] This research was aimed to isolate and characterize crude β glucan from *Pleurotus florida* and its β-glucosidase inhibitor activity also analyzed.

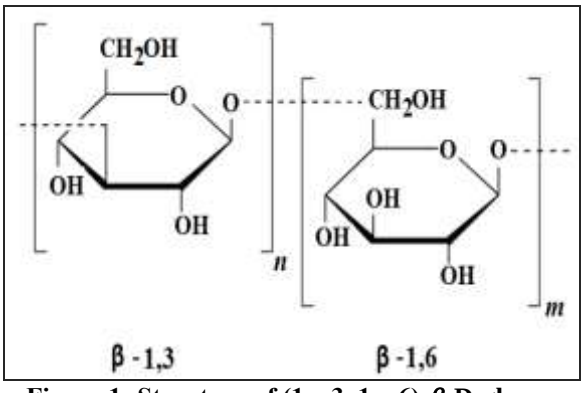

Figure 1: Structure of (1→3, 1→6)-*β*-D-glucan

MATERIALS AND METHODS

The fruiting bodies of *Pleurotus florida* 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.*,[9] with slight modifications. The flow chart of β glucan isolation is shown in **Figure 2**.

To remove impurities successive extraction were done for 24 hrs.by hexane followed by ethyl acetate, finally hot water extraction was done for 2 hrs at 100 ^{0}C for getting water soluble β glucan. 1:4(v/v) ethanol was used to precipitate β glucan. The isolated β glucan was then characterized by ^{1}H NMR and ^{13}C NMR spectroscopy.

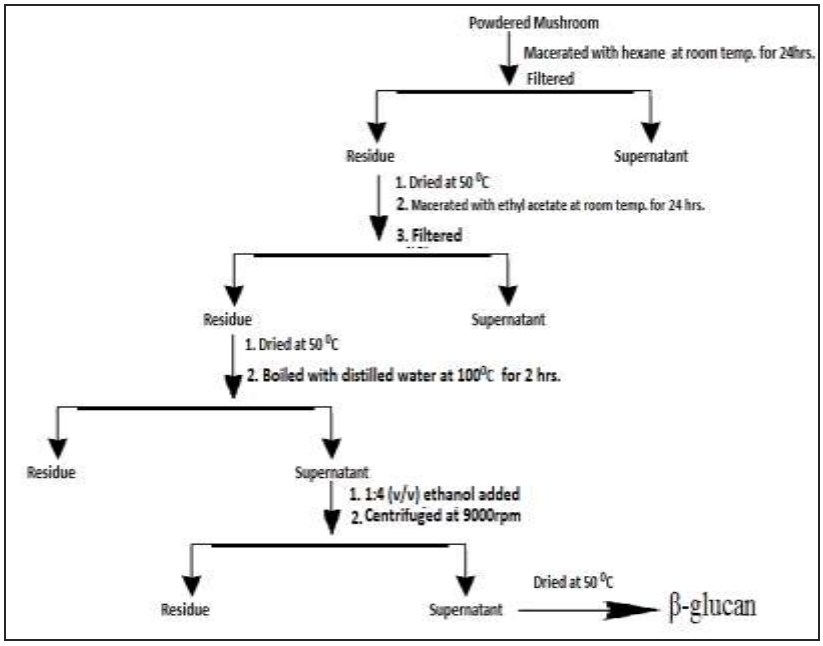

Figure 2: Flowchart of the isolation β-glucan

^{1}H NMR spectra were recorded on 400 MHz Brucker Spectrometer (NM-3410) using DMSO-d_6 (H: 2.50 ppm) as the standard, chemical shifts are expressed in ppm. ^{13}C NMR spectra were recorded on 100 MHz using an internal deuterium lock. The following internal reference was used: DMSO-d_6 (C: 39.5 ppm) at SASTRA University, Thanjavur, Tamil Nadu.

α -Glucosidase Inhibition

100 µL aliquots of varying extract concentrations (0.125, 0.25, 0.5, 1.0 and 2.0 mg/mL) were prepared in 100 mM phosphate buffer (pH 6.9) and 100 µL of 1.0 U/mL α-glucosidase enzyme solution were mixed and kept at 37^{0}C for 10 min. Then, 100 µL of 5 mM p-nitrophenyl- α -D-glucopyranoside solution was added and the reaction mixture was incubated at 37^{0}C for 10 min. Then, 20µL of the mixture was diluted into 1.0 mL of deionized distilled water. The absorbance was measured at 405 nm by a UV-Vis spectrophotometer.[10] The α-glucosidase inhibitory activity was calculated using the formula:

% Inhibition = [(Ac- As)/ Ac] X 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). 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 ^{13}C NMR spectrum showed peaks at 103.6, 69.4, 76.2, 73.2, 75.8 and 60.9 ppm could be easily assigned to C-l, C-4, C-3, C-2 and C-5 and C-6 respectively (**Figure 3**). The ^{13}C NMR spectrum showed one glycosidic carbon signal at 103.6 ppm due to β-linkage.

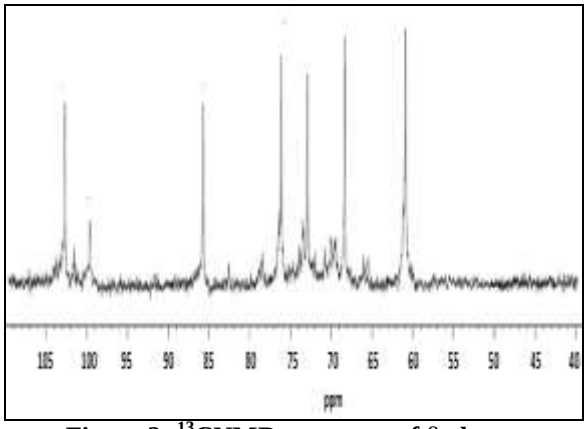

Figure 3: ^{13}CNMR spectrum of β glucan

The ^{1}H NMR (**Figure 4**) showed the glycosidic proton signals clearly differentiated in the lowest field. The signal at δ 4.54 ppm should be assigned to the overlapped resonance of β -(1-3) and β -(1-6) linkages, since the glycosidic signal of the corresponding glucobioses is very close each other. The signal at δ 4.73 should also be assigned to the glycosidic proton of β -(1-3) linkage.

The expanded region of the spectra from 4.32 to 4.73 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.

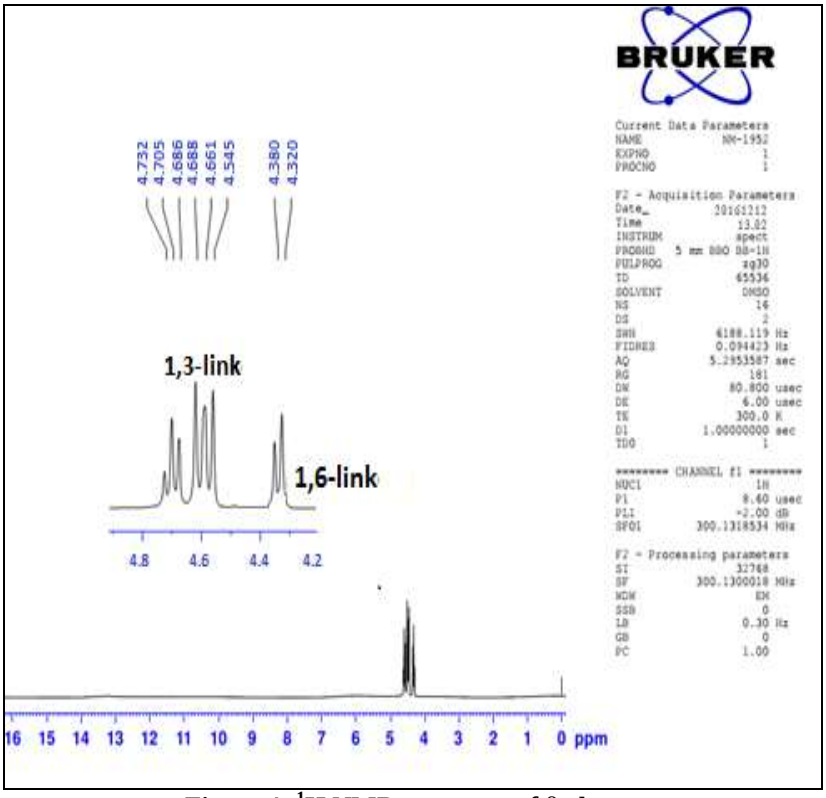

Figure 4: ^{1}H NMR spectrum of β glucan

α-Glucosidase Inhibitory Activity

The control of the early stage of Diabetes mellitus (DM) is crucial for preventing chronic DM development. Several synthetic drugs are now available to treat DM, including α-glucosidase inhibitors (AGIs). AGIs work in the gastrointestinal tract by inhibiting digestion of starch, thereby increase the glycemic control and postprandial hyperglycemia modulation.[11] In this experiment, α-glucosidase inhibitory effects of β-glucan extracts increased gradually with the increasing extract concentration.

The highest inhibitory activity (76.68%) 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 against α-glucosidase. The α-glucosidase inhibitory activity of β-glucan showed in **Figure 5.**

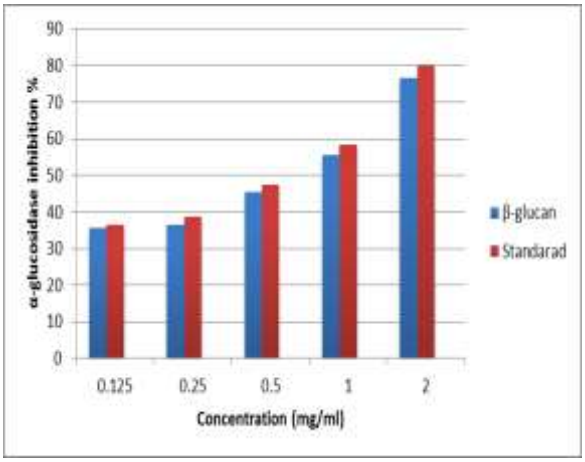

Figure 5: The α-glucosidase inhibitory activity of β-glucan and standarad drug Acarbose. Values are means ± S.D (n=3)

CONCLUSION

We analysed the *in vitro* anti-diabetic activity of β glucan which is isolated from *Pleurotus florida*. As a result, we found that the β glucan have inhibitory activity against α--glucosidase. These results support the evidence that the

anti-diabetic activity exhibited by *Pleurotus florida* polysaccharides is mainly related to the presence of *β*-glucans. Further studies on the isolation and characterization of active compounds from *Pleurotus florida* extracts are needed using various techniques. The effect of purified mushroom β-glucan requires additional analyses, and mechanistically how the mushroom β-glucan affects blood glucose concentration becomes our future goal.

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