Isolation and Chemical Characterization of D-Glucans from *Piptoporus betulinus* Mushroom

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Piptoporus betulinus is a polypore species mushroom and grows on birch trees of genus *Betula*. D-Glucans are the most common polysaccharides found in fungi, especially in basidomycetes, and present a variety of chemical structures. The aim of this study was to isolate and chemically characterize D-glucans present in the fruiting bodies of *Piptoporus betulinus*. The dried fungus was treated with chloroform and methanol (2:1; v/v). The residue sequentially was extracted with hot water and 5% KOH/$NaBH_4$ solution. The polysaccharides were recovered by ethanol precipitation (3:1; v/v). The D-glucans from hot aqueous extract (R1M) and alkaline extract (RK5) were isolated employing freeze-thawing, dialysis and ultrafiltration processes. The monosaccharide composition was determined by GC-MS. R1M and RK5 were analyzed by NMR. Per-*O*-methylation of each sample was carried out using NaOH–Me_2SO–MeI and analyzed by GC–MS. The RK5 molar mass distribution was performed by HPSEC-MALLS. The R1M and RK5 present 100% glucose. The main signals present in the R1M HSQC spectrum indicated β-configuration of C1 and substitutions at C3 and C6 (δ 102.8; 84.9 and 68.9 ppm, respectively). The RK5 ^{13}C-NMR spectrum showed similar profile (δ 102.7, 84.9 and 68.4 ppm), indicating that a (1→3),(1→6) β-D-glucan is present in both samples **.** Analyses by GC–MS of their partially *O*-methylated alditol acetates confirm this evidence, due to the presence of 2,3,4,6-Me_4-Glc*p*, 2,4,6-Me_3-Glc*p* and 2,4-Me_2-Glc*p* per-*O*-methylated derivatives. RK5 has a higher branching degree (2,4-Me_2-Glc*p*: 41%) than R1M (2,4-Me_2-Glc*p*: 16%). RK5 exhibits a homogeneous profile through HPSEC-MALLS and its M_w was 4.0×10^4 g/mol (d*n*/d*c* = 0.224). The present work reported the isolation and characterization of two (1→3),(1→6) β-D-glucans from aqueous and alkaline extractions and each of them has notably different size and branching degrees. The influence of such different chemical structure β-D-glucans in biological tests will be further investigated.

Key words: *Piptoporus betulinus,* D-glucans, chemical characterization
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