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## Isolation of $\beta$ -(1-3) Glucan Compound from the Water Extract of Indonesian Jamur Tanduk (*Termitomyces eurirrhizus* Berk)

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**Abstract:** Jamur Tanduk (*Termitomyces eurirrhizus* Berk) is one of uncultivated edible mushrooms from genus *Termitomyces* found in various regions in Indonesia. The purpose of this study is to analyze the chemical structure of its  $\beta$ -glucan compound that possesses bioactivity as lowering cholesterol levels. Isolation and identification of  $\beta$ -glucan compound from water extract of Jamur tanduk (local name), *Termitomyces eurirrhizus* Berk collected in Sumedang has been done.  $\beta$ -glucan was isolated with Wasterlund methods and obtained as white powder. Isolated compound was identified based on interpretation of spectroscopies data such as Ultra-Violet (UV), Infra-Red (IR) and nuclear magnetic resonance one dimensional such as <sup>1</sup>Hydrogen-Nuclear Magnetic Resonance (<sup>1</sup>H-NMR), <sup>13</sup>Carbon- <sup>13</sup>C-NMR) and Distortionless Enhancement by Polarization Transfer (DEPT) and two dimensional such as Correlation spectroscopy (COSY), Hetero Multiple Quantum Connectivity (HMQC) and Hetero Multiple Bond Connectivity (HMBC). The determination of molecule weight of sample based on viscosity measurements and obtained that  $\beta$ -glucan has 322 molecules of glucose.

**Key words:** *Termitomyces eurirrhizus* Berk,  $\beta$ -glucan compound, Indonesian natural, product, jamur, agaricaceae

### INTRODUCTION

Mushroom, possessing spores without chlorophyll, grows between living and dead creatures. This plant has been recognized to exhibit some living characteristics, such as heterotrophy, saprophyte, mutualistic and parasite (Hawksworth, 2001).

Some harmless mushrooms, called edible mushrooms, have been widely cultivated as foodstuff with high economical value. Many edible mushrooms, such as, *Auricularia* sp., *Flammulina velutipes*, *Ganoderma lucidum*, *Grifola frondosa*, *Hericium erinaceus*, *Hypsizygus marmoreus*, *Lentinula edodes*, *Morchella esculenta*, *Pleurotus* sp., *Pholiota nameko*, *Tremella fuciformis* dan *Volvariella* sp., are extensively developed. Some of which has been utilized for health improvement, since they exhibit many pharmacologically active compounds (Wasser, 2002).

One of the mushroom's special qualities is its capability to convert cellulose and lignin to polysaccharide and cholesterol free-proteins. With this interesting capability, the phyto-protein contents in

mushroom are almost equal to or greater than that of chlorophyll plants and its fat and calorie content are less than that of meats, making it very suitable as foodstuffs for diet purposes. Moreover, consuming certain mushrooms regularly is able to prevent hypercholesterolemia, thus, reducing the risk of hypertension. Considering those benefits, therefore, most people are accustomed to consuming various edible mushrooms as daily food (Domer, 1989).

The pharmacologically active constituents of mushrooms could be polysaccharide and other compounds such as fiber, lectin, triterpenoids, glycolipids and compounds those produced through shikimate pathway. Those compounds have been recognized as Biological Response Modifiers (BRM) which have capability to stimulate body immune system response against infections as well as abnormal metabolism (Waiser and Weis, 1999; Mizuno, 1999).

The BRM is naturally produced by human body as needed, however, these compounds are often required in greater quantity especially in the certain condition that causes insufficient biosynthetic BRM to combat diseases.

Either functional food or myconutraceutical can be used as additional source for BRM. Several polysaccharide compounds isolated from mushrooms can be served as BRM which can increase body immune system response (Wasser, 2002; Kim *et al.*, 2006).

Unlike proteins and nucleic acids, the chemical structure of BRM is polymer from monosaccharide residue which binds to one another via glycosidic bond, because the variability of the chemical structure of this polysaccharide might have huge capacity for carrying biological information.

Most mushroom polysaccharides are polymer from glucose with glycosidic bond such as glycosidic  $\beta$ -(1-3),(1-6) or  $\beta$ -(1-3),  $\beta$ -(1-4) with branch at  $\beta$ -(1-6). This polymer form is distinctive for interconnection mechanism with either amino acids in proteins or nucleotides in nucleic acids which can only interconnect in one way, whereas, polysaccharide in some positions, making it is able to bring different biological information (Sharon and Lis, 1993; Wasser, 2002).

The potential ability of polysaccharide as immunoceutical has not been relatively investigated yet, however, there are some studies that revealed immunoceutical ability of several mushrooms, such as, *Termitomyces robustus*, *T. striatus* and *T. clypeatus*.

The immunostimulation mechanism of polysaccharide involved the immunoreactivity upsurge in macrofag, cytotoxic T-cells and natural killer cells (Mizuno, 1999; Ikekawa, 2000; Feng *et al.*, 2001; Lindequist *et al.*, 2005; Oyetayo, 2008).

Jamur tanduk or jamur bulan, one of species from *Termitomyces* genus, is an edible mushroom which is found mostly in many regions and relatively undeveloped. In the initial rain season, it is often seen in most farms and used by local residents as a food ingredients. Even in Sumedang, it is sold in the morning immediately after picking from its natural habitat.

Interested in jamur tanduk especially that found in Sumedang region, the research to analyze its chemical constituents was carried out, including the  $\beta$ -glucan and other compounds which have been successfully elucidated.

**$\beta$ -glucan in mushroom:** The mushroom's cell-wall is composed of carbohydrates with  $\beta$ -glucan bond, proteins and lipids, however, its structure is different than that of in higher species, including in mammals (Kapteyn *et al.*, 1995; Sanjuan *et al.*, 1995).

Glucane is polymer from  $\beta$ -D-glucose anhidrate Glucan-anhydroglucose repeat units (AGRUs)-with glycosidic bond between C<sub>1</sub>/C<sub>3</sub> position and backbone bond position (Fig. 1a). Some glucane polysaccharides

show chain branching at C<sub>6</sub> position in AGRU connecting structure backbone (Fig. 1b, c) (Kim *et al.*, 2000). Water soluble glucane presents in either single or triple helix. Single helix glucane is known as semi-flexible coil, whereas, triple helix glucane is composed of three combinations of single helix glucane that are connected through hydrogen bond at hydroxyl group from C<sub>2</sub> position. Within cell-wall, glucan is composed of three dimensional tissue from (1-3- and 1-6)- $\beta$ -D linked AGRU connected with carbohydrates, proteins and other lipids (Stone and Clarke, 1992).

Jamur Tanduk (*Termitomyces eurhizus* Berk.) which is found mostly in many regions and relatively undeveloped (Fig. 2). In the initial rain season, it is often seen in most farms and used by local residents as food ingredients.

## MATERIALS AND METHODS

**Materials:** For this study, Jamur Tanduk whose botanical name is *Termitomyces eurhizus* Berk was used is taken from Sumedang, West Java. The sample was collected during rainy season between March and April 2004. Solvents for extraction were petroleum ether, isopropanol, aquadest, asetonitril, asetone, n-buthanol, methanol, chloroform, raw papaya latex (proteinase enzyme), ammonium sulphate and ethanol. Solvents for structure elucidation were DMSO, D<sub>2</sub>O and CDCl<sub>3</sub>.

**Extraction, isolation and purity examination:** The plant material (545, 6 g) was percolated at room temperature using petroleum ether as solvent (11,5 L), followed by filtration. Filtrate (Ftr-1) was then separated and the residue was dried in open air, followed by repercolation using isopropanol (9 L) and filtration. Filtrate (Ftr-2) was then separated and the residue was air dried. The residue was macerated with aquadest (5 L) at 96°C for 2 h, followed by filtration. Filtrate in water (Ftr-3) would be used for subsequent experiments to isolate active compounds (Wasterlund *et al.*, 1993).

**Isolation of active compounds from water extract:** Dried raw papaya latex (2 g) was added to water filtrate (Ftr-3), followed by heating at 40°C for 3 h and cooling. Subsequently, ethanol was added up to approximately 60% of concentration. The filtrate was then stored at 4°C overnight, followed by centrifugation at 4000 rpm. The sediment was dissolved in H<sub>2</sub>O at 80°C. Subsequently, the  $\beta$ -glucane compound was precipitated with ammonium sulphate 30% and stored at 4°C for 72 h. Centrifugation, ethanol recrystallization and freeze drying were employed respectively in order to yield compound A as powder (12,3371 g, 2,2612%) (Wasterlund *et al.*, 1993).

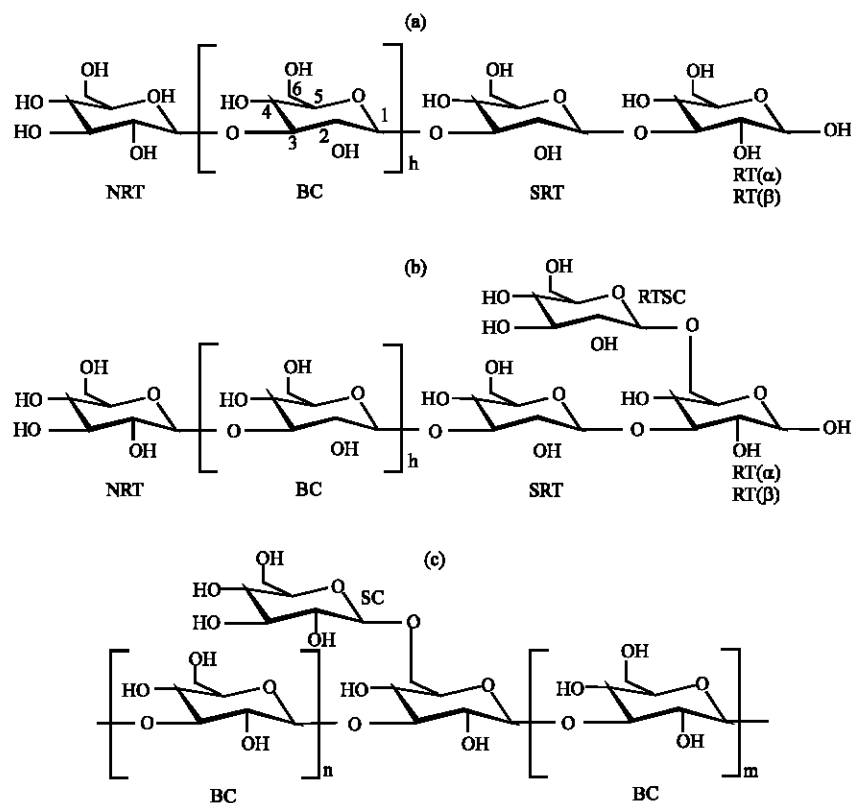


Fig. 1: (a) Chemical structure and type of  $\beta$ -glucan bond. Glucan is a polymer composed of "backbone chain (BC)" with the bond of (1 $\rightarrow$ 3)- $\beta$ -D-anhidroglikosa repeat unit (AGRU). (b and c) Branch chains occur at the position (1-6). The polymer of glucan possess a reduction group (RT) and nonreduction group (NRT) as well as one secondary reduction functional group (SRT). RT is present as  $\alpha$  dan  $\beta$  anomers. Some glucane polysaccharides show chain branching at C<sub>6</sub> position in AGRU connecting structure backbone



Fig. 2: Jamur Tanduk (*Termitomyces eurhizus* Berk.)

## RESULTS AND DISCUSSION

**Spectroscopical analysis:** The interpretation of IR spectra of compound A gave wave number ( $\nu$ ) at 3203-3263  $\text{cm}^{-1}$  (widening) which is very distinctive for some of the

hydroxyl group (OH) from saccharide compounds (sugar). While the presence of bond between the -CO- appeared at the wave numbers 1101  $\text{cm}^{-1}$ . The UV spectrum gave no absorption at wavelengths above 200 nm.

The proton magnetic resonance spectrum of compound A gave protons within chemical shifts ( $\delta\text{H}$ ) 3.34 ~ 4.41 ppm, indicating that the compound A possess CH and CH<sub>2</sub> functional groups that resonate with the oxygen atom of the hydroxyl group. Three anomeric protons at  $\delta\text{H}$  4.31; 4.38 and 4.41 ppm showed that the compound A is a sugar compound (saccharide). This result was supported by NMR carbon data and DEPT (Distortionless Enhancement by Polarization Transfer) which exhibited the existence of 18 carbon atoms. Three carbons indicated that glycosidic carbon contained in the chemical shift  $\delta\text{C}$  102.30; 102.45 and 103.34 ppm; three methylene carbon (CH<sub>2</sub>) at  $\delta\text{C}$  60.26; 60.27 and 60.70 ppm. Whereas the other 12 carbons came from CH methine group on chemical shifts  $\delta\text{C}$  68.22; 72.10; 72.90; 73.41; 74.26; 74.56; 74.75; 74.86; 76.15 ; 79.77; 80.01 and 86.87 ppm.

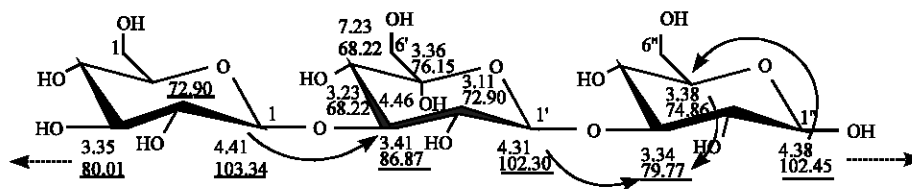


Fig. 3: Chemical structure of compound A based on 1D (Proton and carbon NMR) and 2D-NMR (HMBC, COSY and HMQC)

Table 1: The <sup>13</sup>C-NMR data for one Glucose of Compound A Compared to glucose isolated by Ensley *et al.* (1994) and Hisamitsu *et al.* (1983)

Compound A	(1-3)-β-glucan (Ensley)	(1-2)-β-glucan (Hisamitsu)
103.34	102.70	102.79
72.90	72.59	76.28
86.87	85.98	82.64
68.22	68.23	69.71
76.15	76.14	77.18
60.70	60.71	61.50

Table 2: The value of viscosity reduction ( $\eta_{red}$ ) and specific viscosity ( $\eta_{sp}/c$ ) of compound A

Concentration (g dL <sup>-1</sup> )	Average time (detik)	Specific $\eta$	Reduction $\eta$
0.2616	13.64	0.1212	0.4632
0.3904	15.06	0.2165	0.5546
0.5992	16.64	0.3441	0.5743
0.8188	18.38	0.4846	0.5919
0.9912	20.24	0.6349	0.6405

**Analysis of two-dimensional NMR spectra (HMQC, COSY and HMBC):** The relationship between protons in compound A can be observed in COSY (Correlation Spectroscopy) as  $\delta$ H 3.23 (H4) associated with protons at  $\delta$ H 3.11 (H5). HMBC spectra (Hetero Multiple Bond Connectivity) showed the existence of protons and carbon connectivity that possess distance of more than one bond. In the HMBC spectra of compounds A showed that the proton signal at  $\delta$ H 4.41 ppm (H-1) has a correlation with carbon at position C-3' ( $\delta$ C 86.87 ppm),  $\delta$ H 4.31 ppm (H-1') connected with the spot at the  $\delta$ C 79.77 (C-3''). The combination of HMQC, COSY and HMBC spectra and comparing chemical shift with β-glucan isolated by Ensley *et al.* (1994) (Table 1), indicating that the polymer is 1-3 linked polyglucose (Fig. 3).

**The determination of molecular weight of compound A:** The determination of compound polymer A molecular weight is based on viscosity measurements by using Huvink Mark-Sakurada equation:  $[\eta] = K \cdot Mv^a$ . Intrinsic viscosity value  $[\eta]$  and viscosity reduction data are shown in Table 2.

Based on the  $\eta_{red}$  regression curve against concentration, we obtained value  $[\eta] = 0.4369$ , then inserted into the Mark-Sakurada Huvink equation with

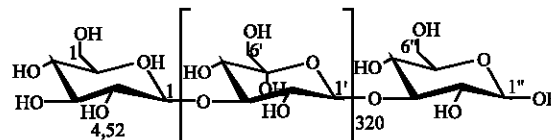


Fig. 4: Chemical structure of compound A which has been isolated in this study

the K value =  $3.5 \times 10^4$  and a value = 0.65 which belongs to the Curdland (1.3) -β-glucan compound) taken from (Futatsuyama *et al.*, 1999) so that the molecular weight for compounds A is 58,021 g mol<sup>-1</sup>. (1,3)-β-glucan is a polymer from carbohydrate with the repetition of glucose which has a molecular weight 180 g mol<sup>-1</sup>, therefore, it can be concluded that compound A has 322 molecules of glucose (Fig. 4).

From the results obtained in this research, we indicated that (1). β-glucan compound isolated from a fungus that grows in Indonesia exhibits a cholesterol-lowering activity as, to be published in another journal. (2). There is a possibility that the structure of β-glucan obtained and analyzed in this research is different in terms of the numbers of glucose molecules as compared with the results of other studies.

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