Analysis of glucomannan molecule in Porang (*Ammorphophallus Muelleri Blume*) flour using nuclear magnetic resonance

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Abstract: The porang tuber (*Ammorphophallus Muelleri Blume*) is a bulb plant belonging to the *Araceae* family, which contains the polysaccharide substance of glucomannan where the glucomannan polysaccharides consist of glucose and mannose molecular structures. The purpose of our study was to identify changes in the structure of glucomannan content consisting of glucose and mannose, where the molecular structure of glucose and mannose in the molecular structure of glucomannan experiences changes in the number of structures between mannose and glucose due to the purification process. In this study, the porang flour has been purified from tubers by a centrifugation method. To study the content of compounds and types of molecules that make up glucomannan, characterization was carried out using ^{1}H and ^{13}C nuclear magnetic resonance (NMR). The analysis on chemical shift data from both NMR spectra can be concluded that glucomannan in porang is more dominantly constructed by glucose molecules rather than mannose. If the concentration of the glucose molecular structure is compared to mannose, the sample can be directly absorbed into the body, especially for medical biomaterial applications. From these results, it is also known that the toxic compound Ca-oxalate which is part of the porang compound has been reduced during the centrifugation method.

Keywords: Porang flour; glucomannan; centrifugation; purification; NMR

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I. INTRODUCTION

The porang tuber plant (*Amorphophallus Mueller Blume*) belongs to the Araceae family and has a high Glucomannan content (15-64% dry weight) [1]. The porang tuber plant is also extensively distributed throughout Indonesia, especially in East Java Province. Porang plants are in great demand, particularly for foods [2], pharmaceuticals [3], and medical industries [4] because the porang plant has a high glucomannan content [5]. Glucomannan is a neutral polysaccharide derived from tuber plants like the konjac (*Ammorphophallus konjac* Koch) and porang (*Ammorphophallus Muelleri* Blume) [6].

The porang tuber plant, however, cannot be taken directly or used for medicinal or pharmaceutical purposes since it contains calcium oxalate, which has a negative impact on our living systems and impacts itching and irritation when consumed [2]. Consuming foods containing calcium oxalate can cause crystallization in the kidneys and other health problems. This is certainly a difficulty in the processing and synthesis of the porang tuber plant. So, a further treatment of reducing calcium oxalate content would be crucially needed and carried out optimally. Purification and evaporation are parts of approach for reducing oxalate compounds.

The molecular structure of glucomannan polysaccharides found in porang tuber plants consisting of glucose and mannose molecular structures with β-D-glucose and β-D-mannose molecular structures can be seen in Fig. 1. However, it should be noted that the molecular structure of glucose and mannose found in porang flour has various types of glucose and mannose molecular structures, including α-D-glucose, α-D-mannose, β-D-glucose and β-D-mannose which can be seen in Fig. 2. One may observe the picture of the molecular structure of mannose and glucose to differ from one to another. The difference between them is in the difference of OH and H positions at the C-6; where the position of OH is above and H below in mannose, while the opposite in glucose. Furthermore, the difference in and β is in the position of C-5 where the position of OH is below and H above in , and the

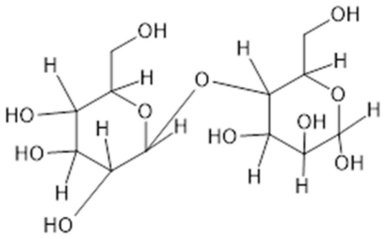


FIG. 1: The glucomannan consisting of two molecular structural units of β-D-glucose and β-D-mannose.

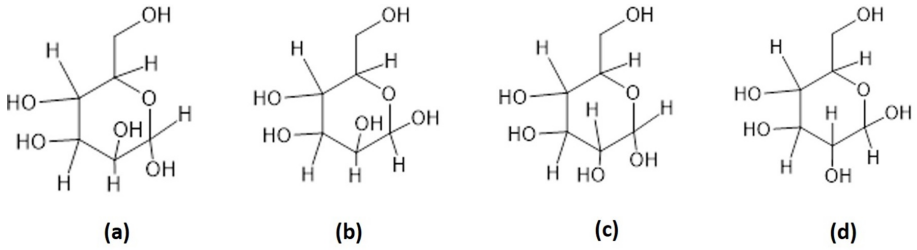


FIG. 2: Chemical structures: (a) α-D-Mannose (b) β-D-Mannose (c) α-D-Glucose and (d) β-D-Glucose.

opposite in β.The purification process can change the type of glucomannan molecular structure found in porang flour, therefore NMR spectroscopy characterization is needed to identify changes in the molecular structure of glucomannan found in porang flour after purification [7]. Identification of the molecular structure glucomannan and calcium oxalate can be done using Nuclei Magnetic Resonance (NMR) spectroscopy characteristics such as Proton Nuclei Magnetic Resonance (^{1}H-NMR) and Carbon Nuclei Magnetic Resonance (^{13}C-NMR).

In this paper, we use ^{1}H-NMR spectroscopy combined with ^{13}C-NMR to analyze and determine the molecular structure of purified glucomannan. NMR spectroscopy is generally used to identify the structure of organic molecules, proteins, and photosynthesis. It is additionally employed in physics to identify the magnetic moment of electrons, protons and their interactions with magnetic environments, according to the Zeeman and Stark effects [8]. Thus, this tool may clarify a sample with respect to its purity, chemical reactivity, and trigger other reactions [9]. It can also analyze molecular structure, kinetics and molecular dynamics, as well as the content of a biomaterial [10]. It has advantage of allowing us to investigate chemical structures without causing damage to the material. It is based on magnetic fields produced by the spins of electrically charged atomic nuclei [11].

The basic principle behind NMR is that some nuclei are in a certain state of nuclear spin when exposed to an external magnetic field ($B_\circ$). It observes transitions between states that are specific to a particular nucleus, as well as the chemical environment of that nucleus. However, this only applies to nuclei (I) whose spin, $I \neq 0$, so that nuclei with I = 0 are "not visible" in NMR spectroscopy. These properties mean that NMR can be used to identify molecular structures, monitor reactions, study metabolism in cells, and is used in medicine, biochemistry, physics, industry, and almost every branch of science [12].

The chemical change value (δ) is caused by the presence of electrons in a molecule which forms a shielding effect on the nuclear spin because it has a magnetic field direction opposite to the external magnetic field ($B_\circ$) so it has a low δ value (-CH_3 in ethanol). Atom that has a low δ value (near the TMS) is called protected (*high shielded field*) and conversely if the δ value is further away from the TMS it is called *deshield* (*low shielded field*) such as -CH_2OH in ethanol due to the industrial magnetic field effect. Electronegative oxygen atom (O) whose direction of circulation of the electron cloud is in the same direction as the external magnetic field ($B_\circ$) for precessions frequency, in Fig. 3, showing the effect of magnetic field induction [12].

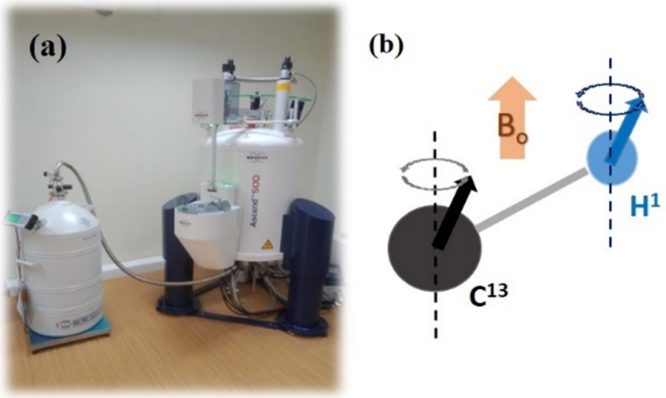


FIG. 3: (a) NMR's experimental set-up and (b) spin precession of 1H and 13C nuclei due to an external magnetic field ($B_\circ$) inside the set-up.

Research on purification methods has been conducted on glucomannan flour derived from corm fresh [13], konjac manan [14] and konjac flour [15]. However, these studies generally only determine the levels of glucomannan and calcium oxalate content in porang flour, and have not been specifically directed at determining changes and analysis of the molecular structure of glucose and mannose in the molecular structure of glucomannan contained in porang flour after the purification process, so this is an update of our research related to the identification and analysis of the molecular structure of glucose and mannose in the molecular structure of glucomannan and calcium oxalate from porang flour.

In this study, an analysis of the structure of glucomannan content was carried out in porang flour samples that were purified by the centrifugation method using ^{1}H- and ^{13}C-NMR spectroscopy. Glucomannan polysaccharides in porang flour experienced changes in molecular structure after purification by the centrifugation method; then there was a decrease in calcium oxalate levels based on the results of the identification of the molecular structure of calcium oxalate after the purification process of the porang flour sample using ^{1}H- and ^{13}C-NMR spectroscopy.

FIG. 4: Plant, tubers, and flour of Porang.

II. MATERIAL AND METHODS

The starting materials used in this study is porang flour from Nganjuk Regency in East Java, Indonesia, as Fig. 4. D_2O and water, including distilled water, were the solvents employed in this study. Porang flour was dissolved in 70% ethanol in the ratio (5g: 500 ml) and agitated for 90 minutes at a rotational speed of 300 rpm using a magnetic stirrer. Then centrifuged for 30 minutes at a rotation speed of 5000 rpm to separate the porang flour from the ethanol solution. This procedure is carried out to obtain pellets. The resulting pellet was dissolved in DI-Water in the ratio (5g: 1000 ml) agitated for 180 minutes at a rotational speed of 300 rpm using a magnetic stirrer, followed by centrifugation for 30 minutes at a rotational speed of 9000 rpm until the supernatant was obtained. The supernatant was evaporated to obtain dry glucomannan. Dry glucomannan was soaked in 96% ethanol for 24 hours at 4°C, then the results obtained were centrifuged again for 40 minutes at room temperature, this was done to separate the water content contained in the dry glucomannan sample. The resulting dry glucomannan pellets are purified with anhydrous ethanol (100%), then filtered and allowed to dry naturally until a glucomannan layer is obtained. Fig. 5 shows a schematic diagram of the centrifugation purification process of porang flour derived from porang tubers.

In this study, glucomannan from porang flour was carried out with a more complex compound of ^{1}H-NMR spectroscopic analysis using a Bruker-Avence Neo 500 Mhz NMR spectrometer at the ILRC University of Indonesia, as shown in Fig. 3(a). The ^{1}H-NMR and ^{13}C-NMR properties are used in NMR spectroscopic analysis. The glucomannan flour with a sample to solvent ratio of 10 mg: 0.5 ml D_2O, 8 scanning cycles, and an instrument frequency of 500 MHz are used for the ^{1}H-NMR characteristics. At the same time, 125 MHz is the frequency used for the ^{13}C-NMR characteristic measurements, and the Scan Number (NS) is 1024 Scan. Comparison of porang flour samples with a mass of 5 mg and 10 mg in relation to solvent.

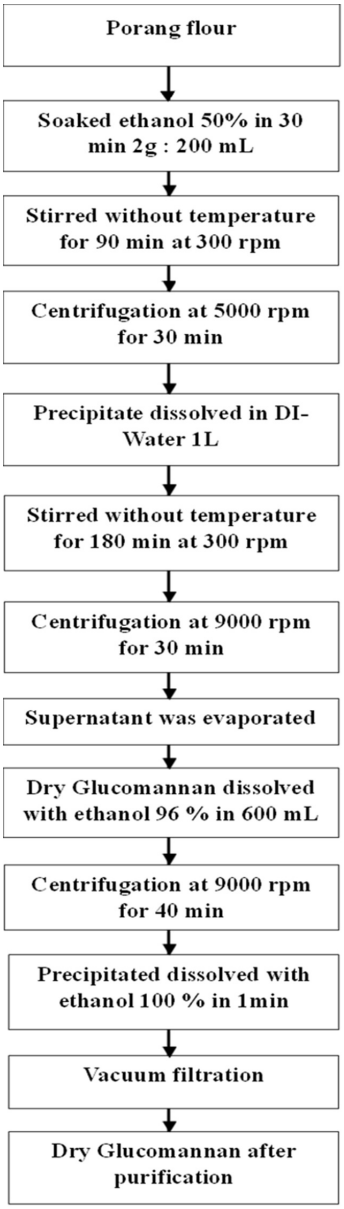
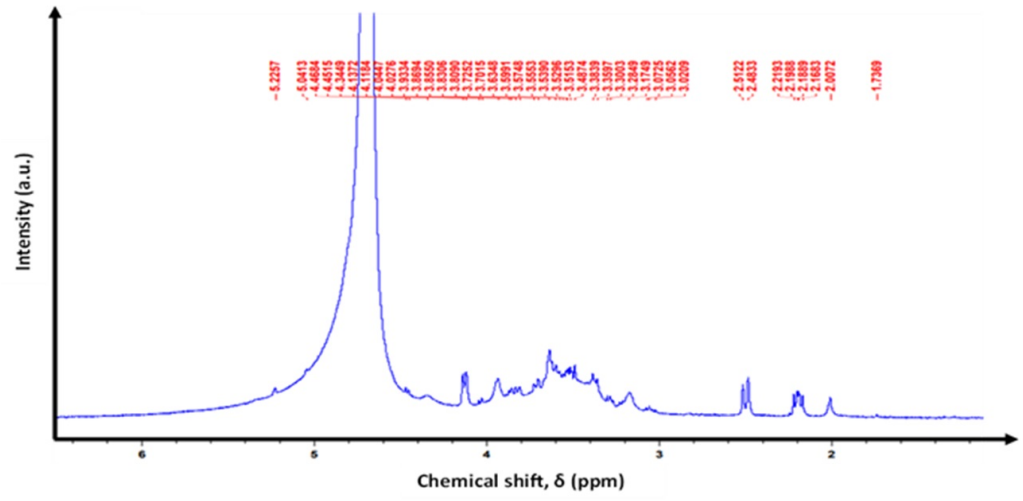


FIG. 5: Schematic diagram purification of Porang flour.

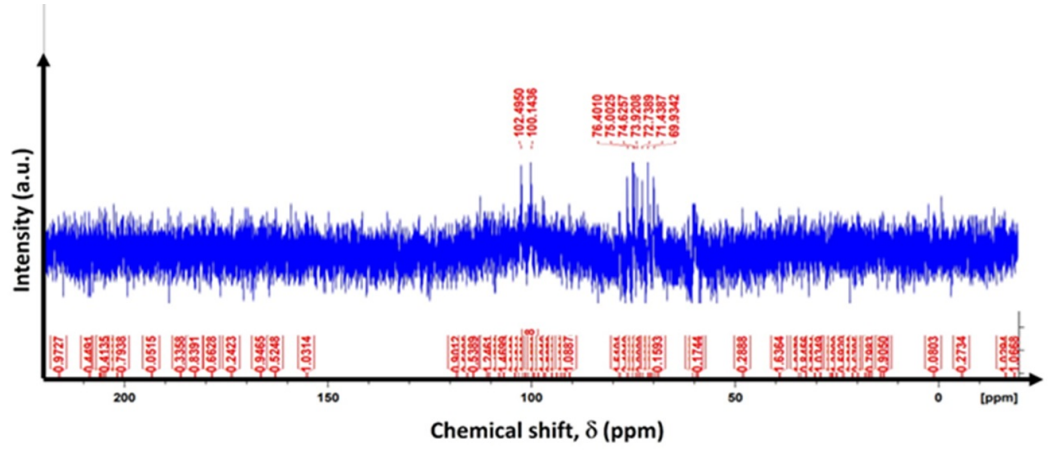
III. RESULT AND DISCUSSION

The ^{1}H-NMR spectroscopy is an analytical method used to determine the structure of a compound based on the type of proton or hydrogen spin [16]. The proton resonance occurs at specific frequency along the frequency sweep [17]. Each proton is in the different chemical environment characterized by its chemical shift (δ). The chemical shift value of an atom will have a low value (δ) if it approaches TMS (tetra methyl silane, $\delta = 0$, as a standard) where it can be said to be *shielded*; while if an atom has a high chemical shift (δ) relatively to TMS, it can be said to be *deshielded* [9]. Fig. 6 shows the molecular structure of alpha and beta mannose and glucose found in the glucomannan, where the structure of mannose and glucose is obtained from the identification of the ^{1}H-NMR and ^{13}C-NMR spectra.

The peak of NMR spectrum for the molecular structure of glucose and mannose is divided into 6 groups, namely H-1, H-2, H-3, H-4, H-5, H-6 and C-1, C-2, C-3, C-4, C-5, C-6. Representing the Hydrogen and Carbon element found in the



(a)



(b)

FIG. 6: (a) ^{1}H-NMR and (b) ^{13}C-NMR spectra from glucomannan in porang flour.

TABLE I: ^{1}H-NMR data and related molecule types.

Glucomannan Str	H-1	H-2	H-3	H-4	H-5	H-6
α-D-Mannose	-	3.9334	-	-	-	-
β-D-Mannose	-	-	-	-	3.3839	-
α-D-Glucose	5.2257	3.2849	3.7252	-	3.8306	3.7252
β-D-Glucose	-	-	3.5153	-	-	-

TABLE II: ^{13}C-NMR data and related molecule types.

Glucomannan Str	C-1	C-2	C-3	C-4	C-5	C-6
α-D-Mannose	-	-	-	-	-	-
β-D-Mannose	-	-	74.6257	-	-	-
α-D-Glucose	-	72.7389	73.9208	-	-	-
β-D-Glucose	-	75.0042	76.4010	-	76.4010	-

chemical compound structure of glucose and mannose as the element reviewed in the ^{1}H-NMR and ^{13}C-NMR characterization [7, 18].

Different peaks in the spectrum can be seen to appear with different intensities which are related to the number of protons that give rise to the signal. Some proton resonances can interact with neighboring atoms. The absorption peaks that appear in the ^{1}H-NMR spectrum are measured by the difference in the nuclear resonance frequency relative to the standard in units of ppm (*part per million*) or chemical shift (δ). The value of chemical changes is influenced by several factors such as inductivity effects, bond anisotropy and hydrogen bond formation.

The peaks of ^{1}H-NMR spectrum explain the chemical shift as described in refs [7]. The emergence of peaks in massive chemical shifts, often referred to as de-shielded, occurs by the inductive impact of electronegative atoms, such as nitrogen and oxygen. This is possible because of the magnetic field induction effect produced by electronegative atoms like O, which have the electron cloud's circulating direction

aligned with the external magnetic field. Chemical shifts in compounds containing alkene (C=C), alkyne (CC), carbonyl (C=O), and aromatic (Ar) groups are also affected by chemical bond anisotropy, in addition to the induction effect of the presence of electronegative atoms like N and O [17].

Fig. 6(a) shows that peaks emerge at greater chemical shifts when the double bonds are present. The ^{1}H-NMR spectrum displays chemical shift, for instance, at 3.7252 and 3.5153 ppm, signifying the presence of α-D-Glucose and β-D-Glucose respectively. The whole chemical shift values for the remaining peaks are listed in Table I. [7]. Meanwhile, Fig. 6(b) exhibits the ^{13}C-NMR spectrum for the sample, presenting also the chemical shifts and giving analysis as listed in Table II, as reported in reference [7]. This result provides an explanation for the identification of the mannose and glucose constituting the glucomannan molecules in the porang flour sample. Further analysis for illustration of glucomannan structures is given in Fig. 6. It is finally known that the content of glucose in porang flour is more dominant than mannose. If the concentration of the glucose molecular structure is compared to mannose, the sample can be directly absorbed into the body, especially for medical biomaterial applications [19].

Porang flour that has been purified by centrifugation method experiences several changes in the component of sugar so that the other types of mannose and glucose cannot be detected, and/or changed their chemical structure. In the ^{1}H-NMR spectrum (Fig. 6(a)), it can be explained that the proton solvent resonance is at the sharpest peak of 4.75 ppm [20]. Meanwhile, the presence of Ca-Oxalate in porang flour should have peak of around 2-2.5 ppm, which was confirmed as an aromatic ketone group[21]. Several peaks (in Fig. 6(a)) have been confirmed around 3-4.5 ppm to be the chemical structure of aromatic rings [21]. The chemical shift in the ^{13}C-NMR spectrum, on the other hand, of oxalic acid should be at 176.2 and 179.3 ppm [21]. However, no chemical shift peaks of 176.2 and 179.3 ppm were detected in Fig. 4(b), indicating that the porang flour is free from calcium oxalate. If the oxalate content is reduced, it can be concluded that the sample is of good quality, since the oxalate content can have negative effects for the body.

IV. CONCLUSION

The purification process of porang flour prepared from tubers has been carried out using the centrifugation method. The molecular content of glucomannan was characterized using ^{1}H- and ^{13}C-NMR. The results showed that the glucomannan molecule was composed of glucose and mannose, where glucose content was more dominant, according to ^{1}H-NMR characteristics, the glucose molecular structure's peak chemical shift values are 5.2257, 3.2849, 3.7252, 3.8306, and 3.5153, but mannose's is just 3.9334. The ^{13}C-NMR characterization yielded chemical shift values of 72.7389, 73.9208, 75.0042, and 76.4010 for the glucose molecular structure, but only 74.6257 for the mannose molecular structure. Based on the NMR chemical shift data, it is proven that the studied porang flour has been free from the toxic compound of Ca-oxalate.

Acknowledgments

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