Characterization of Glucomannan Extracted from Fresh Porang Tubers Using Ethanol Technical Grade

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ABSTRACT. Glucomannan is a polysaccharide consisting of β-1.4-linked D-mannose and D-glucose monomers, which have many benefits especially in the food and pharmaceutical industry. It has been widely reported that one of the main sources of glucomannan is porang tuber (*Amorphophallus muelleri* Blume). Generally, glucomannan extracted or purified from porang flour. However, the drying process causes other compounds than glucomannan stick strongly, resulting low levels of glucomannan. This study was to obtain glucomannan extract in an easy, effective, and inexpensive method, by direct extraction from fresh porang tubers using ethanol technical grade. We performed two extraction methods. The first is a fixed concentration method, the sample was repeatedly extracted using 50% ethanol (FC50) and 96% ethanol (FC96) 3 times, respectively. The second is a multilevel concentration method, the sample was repeatedly extracted using ethanol 60% (first step), 80% (second step), and 96% (third step), one replication each step. The highest glucomannan content (66.56%) was obtained by a multilevel concentration method. Moisture, lipid, protein, crude fiber, calcium oxalate level significantly reduce to 13.58%, 0.07%, 4.03%, 4.95%, 0.56% respectively. FTIR spectra confirmed the presence of functional groups (O-H, C=O, C-O, C-H), that compose the glucomannan compound. SEM image showed that the granules form of glucomannan were round and oval, began to change its phase from amorphous to crystalline, related to XRD data. The results showed that the direct extraction from fresh porang tuber using ethanol technical grade with a multilevel concentration method was an effective method to extract the glucomannan.

Keywords: Porang, *Amorphophallus muelleri* Blume, glucomannan, ethanol technical grade, multilevel concentration

INTRODUCTION

Glucomannan is a polysaccharide of the mannan family, very abundant in nature, specifically in softwoods, roots, tubers, and many plant bulbs (Nguyen, Do, Nguyen, Pham, & Nguyen, 2011). In Indonesia, the main sources of glucomannan are porang or iles-iles. One of the types of iles-iles which has high glucomannan content is yellow porang (*Amorphophallus oncophyllus* Prain or *Amorphophallus muelleri* Blume), about 55 % (dry basis) (Koswara, 2013). The benefits of glucomannan have been widely studied. In the food industry, glucomannan was used as a thickener of the syrup, jelly, edible film, noodle, and binder of sausage (Singh, Singh, & Arya, 2018). In the pharmaceutical industry, glucomannan was used in the preparation of hydrogel as a DNA-controlled release matriks (Tester & Al-Ghazzewi, 2017; Wang, Liu, Shuai, Cui, & Nie, 2014; Wang et al., 2014; Y. Yuan et al., 2018). As a functional food, glucomannan functions to reduce cardiovascular risk factors (Ho et al., 2017), reduce body weight and treatment of diabetes (Li et al., 2015).

The extraction process is one of the most important steps to produce glucomannan with high purity. Methods for the extraction and purification of glucomannan has been studied and developed. Glucomannan is extracted mechanically and chemically. The mechanical methods involve grinding and purified via wind-sifting (Parry, 2010; Takigami, 2000), grinding and purified via filtering, and polishing. The chemical methods have been carried out including centrifugation after flocculation using $Al_2(SO_4)_3$ (Tatirat & Charoenrein, 2011), the use of dimethyl sulfoxide (Ye et al., 2014), and ethanol (Chua et al., 2012; W. Xu et al., 2014) to extract glucomannan.

Generally, glucomannan extracted or purified from porang flour. Porang tubers were peeled, sliced, dried, and then made into flour. Problems encountered in the development of porang flour as food in Indonesia are the high content of calcium oxalate, which causes itching, brownish color, and relatively low glucomannan content around 47-48% (Sumarwoto, 2014). Those characteristics of porang flour cause a low selling price. To increase the economic value,

porang flour was extracted to obtain higher glucomannan content. But, the process of drying into flour, can make other components than glucomannan (impurities) stick strongly and reduce the purity of glucomannan. Therefore, we carry out the direct extraction of fresh porang tubers.

Granules of konjac glucomannan are located in egg-shaped idioblasts within the parenchyma. Starch, cellulose, and nitrogen-containing materials are present in the parenchyma cells surrounding the idioblasts within the corm (Chua, Hocking, Chan, & Baldwin, 2013; Mekkerdchoo, Lei, & Jianrong, 2020). Other components (the impurities) are small and easily broken into fine particles than glucomannan, which is bigger and harder (Yanuriati, Marseno, Rochmadi, & Harmayani, 2017). Glucomannan is insoluble in ethanol (Li et al., 2014) but soluble in water. Previous research showed that glucomannan extraction directly from fresh tubers using ethanol absolute with multilevel concentration method succeeded in obtaining high purity glucomannan. However, for scale-up and mass production purposes, the use of ethanol absolute is highly cost. This study was to obtain glucomannan extract in an easy, effective, and inexpensive method, by direct extraction from fresh porang tubers using ethanol technical grade. The impurities such as starch, protein, lipid, crude fiber, calcium oxalate have different levels of polarity. So we assume that by varying the concentration of ethanol solution in the extraction process (multilevel concentration method), it can dissolve impurities more effectively, so the high purity glucomannan can be obtained, without further purification

EXPERIMENTAL SECTION
Extraction of Porang Glucomannan

The three years old Porang/Iles-iles Kuning (*A. morphophallus muelleri* Blume) tubers were obtained from the Bogor Agricultural University experimental garden, Bogor, Indonesia. Glucomannan was extracted from fresh porang tubers using ethanol technical grade. Porang tubers were peeled, washed, sliced to about 0,2-0,5 mm in thickness. Then it was soaked using Na-metabisulphite for 30 minutes. In the first method, the extraction was repeated using ethanol with a fixed concentration, that sliced porang milled in 50% technical grade ethanol (FC50) and 96% ethanol technical grade (FC96), filtered and pressed. The process was repeated three times from the start of the milling, filtering, and pressing process for each FC50 and FC96. The obtained glucomannan granules were dried in the oven at 50 ^{0}C.

In the second method, the extraction was repeated using ethanol with multilevel concentration (MC). After soaking with Na-metabisulphite, the sliced porang milled in 60% ethanol technical grade, filtered and pressed. Then the glucomannan granules are milled in 80% ethanol, filtered and pressed. The treatment repeated in 96% ethanol. The obtained glucomannan

granules were dried in the oven at 50 ^{0}C. The ratio of tubers (gram) and ethanol (ml) applied in both methods (fixed concentration and multilevel concentration) was 1:3.

The glucomannan yield (db) was calculated by the following formula:

$$Yield = \frac{m_1(1 - w_1)x\ 100\%}{m_2\ (1 - w_2)}$$

Where m_1 = mass of dried glucomannan, m_2 = mass of wet peeled porang tuber, w_1 = water content of dried glucomannan, and w_2 = water content of wet peeled porang tuber.

Chemical Properties

The tuber and glucomannan extract were determined for moisture, ash, fat, and protein, according to AOAC methods 967.03; 942.05; 920.39; 981.10 (Association of Official Analytical Chemists, 2007). The crude fiber content was measured by subtracting the sum of all the other components from 100 g. The starch was analyzed qualitatively by staining glucomannan granules using I_2-KI (Mekkerdchoo et al., 2020).

Glucomannan Content

Glucomannan content was analyzed using 3.5-Dinitro Salicylic Acid (3.5-DNS) colorimetric assay (Chua et al., 2012). The glucomannan sample solution was made by dissolving 0.2 g extracted glucomannan in buffer solution (formic acid-sodium hydroxide 0.1 mol/L 50 ml), then magnetically stirred for 4 hours at room temperature. The mixture was then diluted with formic acid–sodium hydroxide buffer to 100 mL in a volumetric flask, followed by centrifugation at 4000 rpm for 20 minutes, the supernatant was glucomannan sample solution. The next step was to made glucomannan hydrolysate, 5 mL of glucomannan sample solution was added to 25 mL volumetric flask followed by the addition of 2.5 mL of 3M sulfuric acid, heated in boiling water bath for 90 minutes. The solution allowed to cool to room temperature then added 2.5 mL of 6 M NaOH, then diluted with distilled water to 25 mL. Both the glucomannan sample solution and hydrolysate (2 mL) were added 3.5 DNS reagent (1.5 mL). Absorbance was then measured at 550 nm used a UV-VIS spectrophotometer. The 3.5-DNS reagent was made by mixing solution A (0.7 g phenol; 1.5 mL of 10% sodium hydroxide; 5 mL distilled water; and 0.7 g sodium bisulfite) with solution B (22.5 g potassium sodium tartrate; 30 mL of 10% sodium hydroxide; 88 mL dinitro salicylic acid 1%).

D-glucose and D-mannose standard curves were built up by d-Glucose stock solution (1 mg/mL) were placed (0.40; 0.80; 1.20; 1.60 and 2.00 mL) into 25 ml volumetric flasks, respectively (using DI water as a blank). DI water was then added to the volume of 2.00 mL, followed by the addition of 3.5-DNS (1.50 mL) to each flask. Each mixture was heated for 5 min in a boiling water bath and cooled to room temperature

before being diluted to 25 mL with DI water in a volumetric flask. Absorbance was then measured at 550 nm, and a plot of the measured absorbance against the glucose content (mg) constructed. A d-mannose standard curve was constructed using the procedure as described for glucose. The glucomannan content was calculated by the following equation:

$$GM content(\%) = \frac{5000 \, f \, (5T - To)}{m \, (1 - w)}$$

Where f = correction factor, T = glucose content of glucomannan hydrolysate (mg), To = glucose content of glucomannan sample solution (mg), m = mass of extracted glucomannan (200 mg) and w = water content of glucomannan

Statistical Analysis

Statistical significance of the effect of the extraction methods based on the obtained of glucomannan and the chemical properties of extracted glucomannan was assessed with a one-way analysis of variance (ANOVA).

Calcium Oxalate Content

Calcium oxalate was analyzed by spectrophotometry (Ardhian, Dhike & Indriyani, 2013). The sample weighed 5 g. Then the furnace was treated with a temperature of 600-800 ^{0}C for 30 minutes and cooled for 3 hours. The residue added 10 mL of HNO_3:aquadest (1:1) solution. Then heated to 5 mL. The mixture is filtered to a 25 mL volumetric flask so that the filtrate is obtained and then treated with an aquadest. Samples were analyzed using AAS. The calcium content of the AAS results is converted to the oxalate content with the following formula (Association of Official Analytical Chemists, 2007):

$$Oxalate \; level = \frac{Ca \; conc. \left(\mu \frac{g}{mL}\right) x \; MW \; Ca \; oxalate \; x \; SV \; (mL)}{sample \; weight \; (g) x \; MW \; Ca}$$

Where: MW Ca-oxalate = 128
MW Ca = 40
Solvent volume = 25 mL

Characterization of Glucomannan

The obtained glucomannan (FC50, FC96, MC, and commercial glucomannan) was determined its functional groups of glucose and mannose using Fourier Transform-Infra Red (FTIR) Thermo scientific iD5 ATR. XRD pattern of glucomannan was analyzed using XRD Maxima 7000 Shimadzu. The diffraction angle ranged from $2\theta=10°$ -2θ =$80°$. The XRD analysis was conducted to determine the phase of glucomannan granule and crystallinity of extracted

glucomannan, then compared with the commercial glucomannan.

The SEM photomicrographs were recorded by using SEM-EDX Carl Zeiss Bruker type EVO MA10To study the morphology or the surface characteristic of extracted glucomannan.

RESULTS AND DISCUSSION
Chemical Properties of Raw Material

In this study, glucomannan extracted from fresh tubers of porang/iles-iles kuning (*Amorphophallus muelleri* Blume). The chemical composition of the porang tubers was presented in **Table 1**. The highest content in porang tubers was moisture content. High moisture content in raw food can cause some damage, including microbial growth, browning reactions, and fat hydrolysis. Therefore the process of handling post-harvest of porang tubers must be done quickly and correctly. Generally, porang farmers immediately process the harvested tubers into dried chips and sell them directly to collectors, but some people process them further into porang flour. This process conducted to reduce the moisture content of the material to inhibit the growth of microorganisms and the activity of enzymes.

One of the chemical methods to isolate glucomannan is extraction or repeated leaching using ethanol. Ethanol solution will dissolve the components (impurities) such as ash, starch, fat, protein, and crude fiber to obtain high level of glucomannan extract.

Extraction of Porang Glucomannan

In this study, the result showed the glucomannan granules extracted using ethanol with multilevel concentrations (MC) had the glucomannan content: 66.56%, and yield: 51.35% (**Table 2**). It was higher than the glucomannan obtained by a fixed concentration method with 50% ethanol (FC50) and 96% ethanol (FC96). The impurities such as starch, lipids, protein, crude fiber have different polarity levels. Therefore it was more effective if extracted using ethanol with different concentrations. A one-way ANOVA was conducted to examine the effect of the extraction methods based on the obtained of glucomannan (**Table 2**). There were statistically significant deferences between the extraction methods using ethanol with fixed concentration FC50 and multilevel concentration (MC), but there were insignificant differences between the extraction methods FC50 and FC96; FC96 and MC.

Table 1. Chemical Composition of Porang Tuber

Chemical Composition	Analysis Results (%)
Moisture	84.01
Ash	0.83
Starch	+
Fat	0.83
Protein	9.50
Crude Fiber	9.70

+= dark blue colour (positive/indicated high starch content)

Table 2. Glucomannan Content

	Fixed Concentration		Multilevel Concentration
	FC50 (%)	FC96 (%)	MC (%)
Glucomannan	54.20±0.62[a]	60.20±4.86[a,b]	66.56±0.63[b]
Yield	49.99[a]	51.99[c]	51.35[b]

Different superscripts among mean values in each row indicated a significant difference (p ≤ 0.05)

Table 3. Chemical Composition of Glucomannan Extract

Chemical Composition	Fixed Concentration		Multilevel Concentration
	FC50 (%)	FC96 (%)	MC (%)
Moisture	14.07[c]	13.13[a]	13.58[b]
Ash	1.46[a]	1.71[b]	1.66[b]
Starch	-	-	-
Lipid	0.13[a,b]	0.18[b]	0.07[a]
Protein	5.14[c]	3.9[a]	4.03[b]
Crude Fiber	9.60[c]	7.25[b]	4.95[a]
Calcium oxalate	0.56[a]	0.76[b]	0.75[b]

Different superscripts among mean values in each row indicated a significant difference (p ≤ 0.05)
- = no color (negative/indicated no starch content)

In the multilevel concentration method, at the first stage, the porang tuber was extracted using 60% ethanol. The high moisture content of porang tubers is 84.01% (Table 1) adding polarity to the 60% ethanol solution used as a solvent in the extraction process, so at this stage, polar impurities such as protein and sugar more easily dissolved (Xu et al., 2014) In the second stage, we use 80% ethanol. The high moisture content of the tuber was reduced the ethanol concentration to moderate polarity, caused the starch to dissolve. Whereas in the third stage, low polarity (96% ethanol) caused dissolved fat, crude fiber, and calcium oxalate.

Decreasing of lipids, protein, and crude fiber content in glucomannan extract (Table 3) compared to the porang tubers before extracting, indicated that the components were dissolved and washed out by ethanol solution. While, the ash content in glucomannan extract was higher than in porang tubers, due to the moisture content in porang tubers very high compared to glucomannan extracts. That affects the amount of ash content. Ash content indicates the mineral content of the material (Koswara, 2013). Ash content contained in the obtained glucomannan still meets the requirements of the standard by The Ministry of Agriculture of the People's Republic of China (2002).

The glucomannan content in this study was lower compared to our previous study using ethanol absolute with the same method: multilevel concentration method (Nurlela, Ariesta, Santosa, & Muhandri, 2019). Due to the higher purity of ethanol absolute compared to technical grade ethanol, which has an impact on the effectiveness of extraction in dissolving impurities.

For the scale-up purposes and mass production of glucomannan flour, the use of technical grade ethanol was certainly more economical. In this study, the glucomannan obtained by the multilevel concentration method of ethanol technical grade, considered as common konjac flour (the glucomannan level is ≥ 60% on dry basis) according to the standard by The Ministry of Agriculture of the People's Republic of China (2002). In Indonesia, the refined porang flour with 60% glucomannan content is sold at Rp. 250,000/Kg, while porang chips are sold at Rp. 27,000/Kg (Saputro, Lefiyanti, & Olim, 2014).

Calcium Oxalate

Oxalic acid in plants is stored in two forms, water-soluble oxalate and water-insoluble oxalate (Ardhian et al., 2013). Insoluble oxalate compound in porang is calcium oxalate. The benefits of the presence of calcium oxalate are oxalate poison binding, regulating calcium, and as self-defense against herbivores (Chairiyah, Harijati, & Mastuti, 2013). But, high concentrations of calcium oxalate can cause irritation and itching. Problems encountered in the development of porang flour as food in Indonesia are the high content of calcium oxalate, which causes itching, brownish color, and relatively low glucomannan content around 47-48% (Sumarwoto, 2014). Those characteristics of porang flour cause a low selling price.

Glucomannan extracted by FC50, FC96, and MC still contain calcium oxalate, but in a low percentage 0.56 - 0.76% (Table 2). Maximum calcium oxalate consumption in adults ranges from 0.6 grams to 1.25 grams per day for six consecutive weeks (Jiang et al., 2011). Foods with low oxalate levels don't need to be removed because calcium oxalate in food is not efficiently absorbed by the body, so consumption of foods with low oxalate levels will not have a significant effect on health. The body can also synthesize oxalates from several precursors such as ascorbic acid.
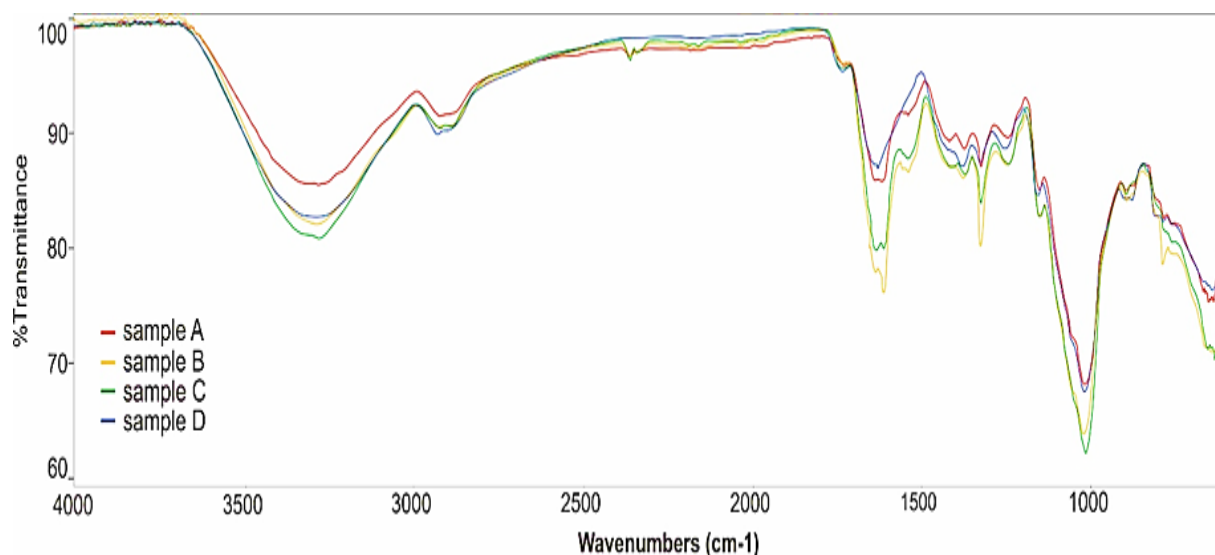
Figure 1. FTIR Spectrum of Glucomannan Granules Extracted by FC50% (**sample A**), FC96% (**sample B**), MC (**sample C**), and Commercial Glucomannan (**sample D**)

Fourier Transform Infra-Red (FTIR) Analysis of Glucomannan

Figure 1 showed the FTIR spectra of the glucomannan in the wavelength range of 4000-400 cm^{-1}. The FTIR spectra of the glucomannan obtained by fixed concentration 50% ethanol (sample A), fixed concentration 96% ethanol (sample B), multilevel concentration (sample C) and compared to the commercial glucomannan (sample D) had almost the same absorption band pattern but with different intensities. The broadband was observed at 3000-3600 cm^{-1} represented to the O-H stretching of the glucose and mannose of glucomannan. Absorption bands in the range of 2850-2925 cm^{-1} shown stretching asymmetry and symmetry vibration of the alkane group. The strong peak at 1600-1700 cm^{-1} indicated the presence of the carbonyl group (C=O). Absorption bands at $\pm$ 1444 cm^{-1} and 1383 cm^{-1} showed asymmetric bending vibrations and symmetries of CH, CH$_2$, and CH$_3$. The C-O group on the alcohol bond could be seen in the uptake of the 1027 cm^{-1} band. The peaks at 875 cm−1 and 805 cm−1 belonged to the characteristic peak of the mannose (Da Silva et al., 2020; Wang, Lee, Yuan, Wang, & Lu, 2020; Wang, Zhou, Wang, & Li, 2015).

Scanning Electron Microscope (SEM) Analysis

Figure 2 showed the morphologies of glucomannan granules extracted by fixed concentration 50% ethanol (A), fixed concentration 96% ethanol (B), multilevel concentration (C) with 200 X magnification showed that the granules form of glucomannan were round and oval. The surface of the glucomannan granules was still covered by impurities, but the surface of glucomannan granules extracted by the multilevel concentration of ethanol

(C1) more clear than FC50 and FC96. The native structure of konjac glucomannan was primarily composed of lamella structure units (Manab, Purnomo, Widjanarko, & Radiati, 2016), as seen in **Figure 2.C1**.

From the micrographs with 3000X magnification, the shape of particles from glucomannan obtained by FC96 (B2) and MC (C2) begins to transition from the amorphous phase to the crystalline phase. The transition was shown by the formation of irregular cubes, whereas it was not found in FC50 (A2). There are needle-shape crystals in **figures B2** and **C2**, which show the presence of calcium oxalate crystals. The shape of calcium oxalate crystals is like a needle (Koswara, 2013). As seen in **Figure 2**, needle-shape crystals are more visible in **figures B2** (FC96) and **C2** (MC) than in figure **A2** (FC50). This was consistent with the results of the calcium oxalate data in **Table 2** that the calcium oxalate content in glucomannan obtained by FC96 and MC were higher (0.76% and 0,75%) than in glucomannan obtained by FC50 (0,56%).

X-Ray Diffraction (XRD) Analysis

XRD analysis was conducted to determine the crystallization of glucomannan. **Figure 3** showed that the XRD pattern of glucomannan obtained by FC96 and MC were similar, exhibited very broadband, indicated the amorphous phase begins to transition to the crystalline phase. It was related to the SEM image (**Figure 2.B2 and C2**). Both diffractograms had high peaks at 2θ = 20-25^0 (Wu et al., 2012). XRD pattern of FC 50 exhibited sharper peak and higher intensity than the others. It can be concluded that the glucomannan extracted by FC50, FC96, and MC contain more amorphous form than the crystalline form.
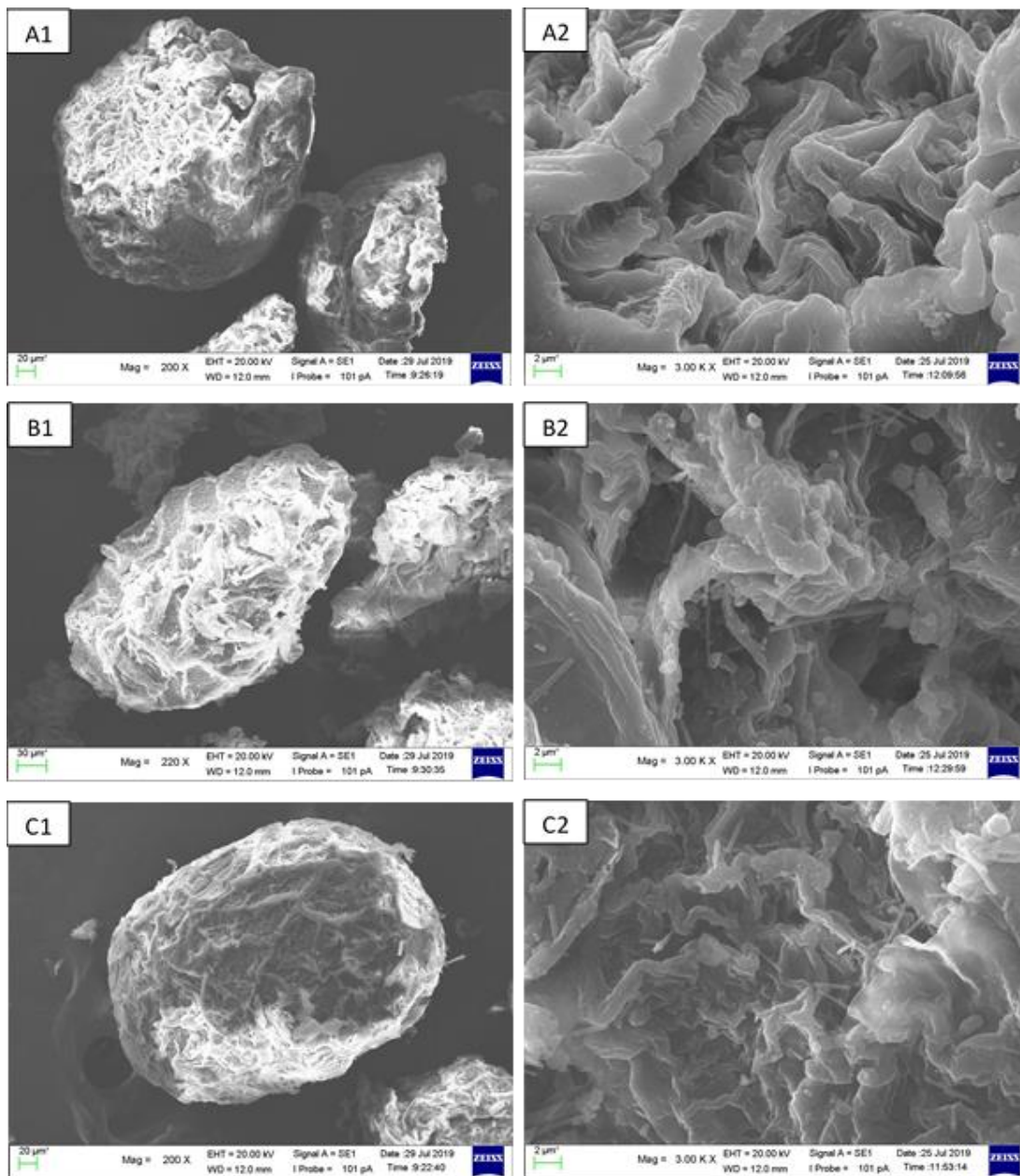
Figure 2. SEM Image of Glucomannan Granules Extracted by FC50 (**A**), FC96 (**B**), MC (**C**) with 200X (**1**), 3000X (**2**) Magnification
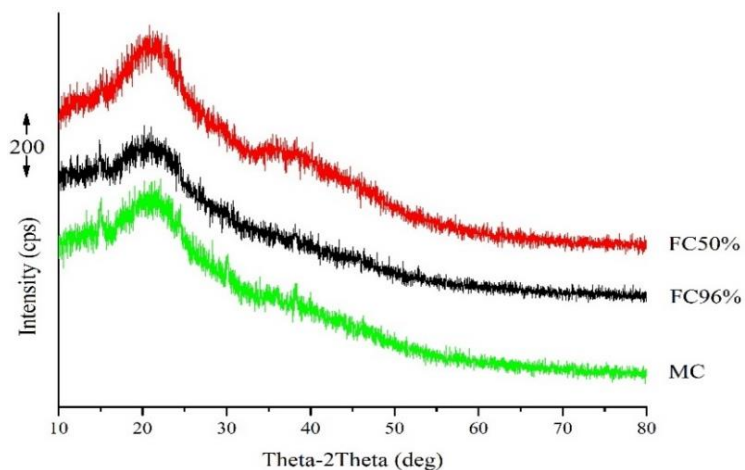

Figure 3. XRD Pattern of Glucomannan Granules Extracted by FC50 (**A**), FC96 (**B**), MC (**C**)

CONCLUSIONS

The highest glucomannan content (66.56%) was obtained by the multilevel concentration method (MC). Moisture, lipid, protein, crude fiber, calcium oxalate level significantly reduce to 13.58%, 0.07%, 4.03%, 4.95%, 0,56% respectively. FTIR spectra confirmed the presence of functional groups (O-H, C=O, C-O, C-H), that compose the glucomannan compound. SEM image showed that the granules form of glucomannan were round and oval, began to change its phase from amorphous to crystalline, related to XRD data. The results showed that the direct extraction from fresh porang tuber using ethanol technical grade with a multilevel concentration method was an effective method to extract the glucomannan.

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