Purification of Glucomannan of Porang (*Amorphophallus oncophyllus*) Flour using Combination of Isopropyl Alcohol and Ultrasound-Assisted Extraction

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Abstract

Porang tuber is rich of glucomannan content but contained irritable compounds to be consumed. Ultrasound-assisted extraction (UAE) using isopropyl alcohol (IPA) was developed as a purification method for glucomannan. This study aimed to determine the effects of UAE conditions (IPA concentrations, ratios of solvent-solid, and number of extraction stages) on the characteristics of purified glucomannan (PG) from porang flour (PF). Single-stage UAE using 80% IPA concentration and ratio of solvent-solid of 8:1 (ml/g) for 10 min produced the highest glucomannan content (76.10%). At this condition, the PG viscosity and yield were 12,800 cP and 96.10%, respectively. Furthermore, the three-stage UAE using 80% IPA concentration in each stage improved the glucomannan content to 83.26% with 15,960 cP and 90.02% of yield. The morphology showed that the purified glucomannan powders had smoother surface and less crump after the purification. Both carbonyl and acetyl groups were observed on PF and PG in different intensities.

Keywords: *glucomannan, isopropyl alcohol, Amorphophallus onophyllus, ultrasound-assisted extraction*

INTRODUCTION

Glucomannan, composed of β-1,4 linked D-mannose and D-glucose monomers, has ben used in various applications including food additives, pharmaceuticals, and fine chemical industries (Zhang *et al.,* 2005). The ratio of mannose-glucose of glucomannan is varied depending on its original source (Alonso-Sande *et al.,* 2009). Porang or iles-iles (*Amorphophallus oncophyllus*) tuber contains up to 60% glucomannan (Rahayu *et al.,* 2013). The presence of calcium oxalate in the tuber makes it less preferable for direct consumption.

Some methods had been developed to extract and purify the glucomannan, either by mechanical (dry processing) or chemical (wet processing) approach. Extraction using ethanol is the most common method to purify glucomannan due to its simplicity and high efficiency process, however, it requires relatively long duration to extract the glucomannan (Chua *et al.,*

2012). In this method, ethanol acts as an anti-solvent for glucomannan while removing its contaminants.

Ultrasound-assisted extraction (UAE) has been recognized as one of the efficient and eco-friendly method which produces high extraction yield using low energy and short extraction time (He *et al.,* 2016; Zhang *et al.,* 2016). Kimura *et al.* (2001) reported that higher dietary fibre and less trimethylamine and dimethylamine were obtained from 30% white konnyaku powder which was treated for 15 min under ultrasound exposure. Moreover, higher glucomannan content was reported using multiple stages of UEA. Widjanarko *et al.* (2011) found that three-stage UEA using ethanol for 25 min for each stage improved the glucomannan content to 87.83%, while the viscosity was increased from 4,900 to 8,200 cP.

Other than ethanol, isopropyl alcohol (IPA) was also reported as glucomannan coagulant (Ohashi *et al.,* 2000). Dielectric constant and dipole moment of IPA are comparable to those of ethanol with cheaper price. IPA is also safe to be consumed and widely used in food processing applications. Application of combination between IPA and UAE in glucomannan purification has not deeply explored yet. Hence, this work aims to study the suitable condition of UAE using IPA to produce high quality glucomannan purified from porang flour.

MATERIALS AND METHODS
Materials

Porang flour (PF, 80 mesh) as the main ingredient of this study was obtained from Ngawi, East Java-Indonesia with the composition shown on Table 1. IPA, 3.5-dinitrosalicylic acid (DNS), NaOH, $NaHSO_3$, H_2SO_4, glucose, potassium sodium tartrate, phenol crystals, formic acid, and other chemicals were in analytical grade.

Table 1. Characteristics of PF, the highest purity of PG and the commercial glucomannan (CG).

Parameter	PF	PG	CG
Glucomannan (%)	64.28	83.26	97.81
Water (%)	8.46	8.48	0.63
Ash (%)	5.69	1.20	0.34
Protein (%)	4.28	0.28	0.20
Fat (%)	0.00	0.00	0.10
Starch (%)	11.20	3.02	0.33
Fibre (%)	5.13	2.71	0.15
Ca-oxalate (%)	1.98	0.17	0.03
Viscosity (cP)	8,600	15,960	25,900

Purification

The glucomanann purification was conducted based on the method of Widjanarko *et al.* (2011). PF were ultrasound-assisted extracted (UAE) (Krisbow Ultrasound Cleaner KW1801032) at 20 kHz using IPA (80%, 80 ml) as a solvent for 10 min. Concentrations of IPA, solvent-solid ratio, and number of purification stages were varied. The suspension was filtrated, and

the purified glucomannan (PG) cake was oven-dried at 40°C for 24 h prior the analysis.

Glucomannan content

Glucomannan content was determined using DNS method of Chua *et al.* (2012). Sample (0.2 g) was stirred in 50 ml of formic acid-NaOH buffer (0.1 M) for 4 h. After diluted to 100 ml using formic acid-NaOH buffer, the mixture was centrifuged at 4000 rpm for 20 min. Five mililiters of the glucomannan solution was hydrolyzed with sulphuric acid (3 M, 2.5 ml) in a boiling waterbath for 90 min. After the solution reached room temperature, NaOH solution (6 M, 2.5 ml) was added and brought to 25 ml using distilled water. Both of the glucomannan solution and hydrolyzed glucomannan solution were measured for the absorbance using UV-Vis spectrophotometer at 550 nm and compared with D-glucose standard curve. The glucomannan content of PF and PG per mass of the sample (m) was calculated using Equation (1).

$$GM content\ (\%) = \frac{5000f(5T - T_0)}{m} \qquad (1)$$

where f was the correction factor (0.9), T and T_0 were glucose content of hydrolyzed and initial glucomannan solution, respectively.

Calcium oxalate

Calcium oxalate was determined based on the method of Adeninyi *et al.* (2009). Sample (2 g) was digested with HCl (10 ml, 6M) for 1 h and brought to 250 ml in a volumetric flask. pH of the solution was adjusted using NH_4OH solution until the color changed from pink to faint yellow. $CaCl_2$ (10 ml, 5%) was added to precipitate the insoluble oxalate prior centrifuge at 4000 rpm for 10 min. The precipitate was dissolved with H_2SO_4 (10 ml, 20%) and brought to 300 ml. This solution (125 ml) was boiled and then titrated with 0.05 M $KMnO_4$ to give a faint pink color. Based on the the overall redox reaction below, the oxalate content was calculated using Equation (2):

$$2MnO_4^- + 5C_2O_4^{2-} + 16H^+ \rightarrow 2Mn^{2+} + 8H_2O + 10CO_2$$
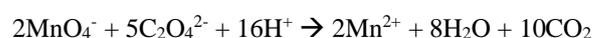
$$Oxalate\ (\%) = \frac{5 \times (M \times V)_{KMnO_4} \times MW_{CaC_2O_4}}{2 \times W_{sample}} \qquad (2)$$

Viscosity analysis

Viscosity of sample solution (1%, 30°C) was measured using Brookfield RVDV 2 Pro viscometer equipped with no 5 spindles at 150 rpm.

Proximate analysis

The proximate analyses followed the methods of AOAC (2005). To measure the water content, the sample (2 g) was oven-dried at 105°C until constant weight was obtained. The water content was calculated using Equation (3).

$$Water\ (\%) = \frac{W_{sample} - W_{dried}}{W_{sample}} \times 100\% \qquad (3)$$

The ash content was calculated by Equation (4) by heated 2 g of the sample in a furnace at 600ºC to produce the greyish ash with constant weight.

$$Ash\ (\%) = \frac{W_{ash}}{W_{sample}} \times 100\% \qquad (4)$$

The extraction of fat was conducted for 5 h using petroleum ether. The extracted fat was oven-dried at 105ºC for 30 min and weighed after cooled down. The fat content was calculated using Equation (5).

$$Fat\ (\%) = \frac{W_{fat}}{W_{sample}} \times 100\% \qquad (5)$$

Starch content was determined by dissolving the sample (5 g) in 50 ml of distilled water. The suspension was filtered and washed using 250 ml of distilled water. The remained residue was washed 5 times using 10 ml of ether. After evaporation, the residue was washed using ethanol (10%, 150 ml). Subsequently, the residue was washed using 200 ml distilled water before added with HCl solution (25%, 20 ml). The solution was heated in a waterbath for 150 min. After cooled down, the solution was neutralized using 45% NaOH solution, diluted to 500 ml, and filtrated. The filtrate (10 ml) was added with 10 ml of fehling solution and 5 ml of standard dextrose for Fehling titration method. The glucose content was calculated using Equation (6) and multiplied with 0.9 to obtained the starch content.

$$Glucose\ (\%) = \frac{(V_{standard} - V_{sample}) \times C \times n \times 100\%}{W_{sample}} \qquad (6)$$

where $V_{standard}$ and V_{sample} are the volumes of titrant solution for dextrose and sample solution, respectively, C is the concentration of the dextrose, n is dilution factor, and W_{sample} is the weight of the sample.

The sample (1.5 g) was added with K_2SO_4 (1.9 g), HgO (40 mg), and H_2SO_4 (16 ml) in a Kjeldahl flask for protein determination. After boilling for 90 min, distilled water was added for washing before the addition of 5 ml of H_3BO_3, 2-4 drop of mixed indicator (methyl red and methyl blue in 2:1 ratio), and 10 ml of NaOH-$Na_2S_2O_3$ solution. The mixture was distilled to obtain ±15 ml of distillate. The distillate was titrated by HCl (0.02 N) until the color changed into grey. The protein calculation followed the Equation (7), with V is the acid volume used in titration, N is the normality of the acid, and W is the sample weight.

$$Protein\ (\%) = \frac{1.4V \times N}{W} \times 100\% \qquad (7)$$

The fibre content measurement was conducted by mixing the sample (2 g) with asbestos (1 g), H_2SO_4 (0.255 N, 200 ml), and a drop of antifoam in an Erlenmeyer flask equipped with a condensor. The mixture was boiled for 30 min and filtered. The residue was washed using boiled water until the filtrate was neutralized. The residue was then added by NaOH solution (0.313 N, 200 ml) in an Erlenmeyer flask equipped with a condensor and boiled for 30 min. A weighed filter paper was used to filter the mixture. The residue was washed using 10% K_2SO_4, boiled distilled water, and 15 ml of 95% ethanol in sequence. The residue in the filter paper was then oven-dried at 105ºC for 2 h and weighed after cooled down. The fibre content was calculated using Equation (8).

$$Fibre\ (\%) = \frac{W_{residue} - W_{asbestos}}{W_{sample}} \times 100\% \qquad (8)$$

Yield

The yield of PG from PF was calculated using Equation (9).

$$Yield\ (\%) = \frac{PG\ (g)}{PF\ (g)} x100\% \qquad (9)$$

Morphology and functional groups

The morphology of the samples was observed using FEI Inspect S50 Scanning Electron Microscope (SEM) at accelerating voltage of 10 kV and 2000× magnification. While the functional groups of the sample were analyzed using IR-Prestige-21 Shimadzu FT-IR spectrophotometer.

RESULTS AND DISCUSSION

In this research, UAE using IPA as a solvent was used for purified glucomannan of PF. Effect of IPA concentrations, ratios of solvent-flour, and extraction stages weres studied.

IPA concentrations

Effect of IPA concentrations (40, 60 80, and 99%) was studied in constant ratio (8:1) of solvent: PF, while the variation of the solvent-PF ratios (4:1, 8:1, and 12:1) were conducted at 80% IPA concentration.

Increasing the IPA concentration as a solvent for UAE removed higher amount of the impurites, thus produced porang flour with a higher glucomannan content. Figure 1 shows 80% IPA resulted in the highest glucomannan content (76.10%) and highest viscosity (12,800 cP). IPA, which is less polar than water, could dissolved non-polar compounds of glucomannan impurities. Table 1 showed that the PF contained various impurities such as calcium oxalate, ash, soluble protein, and starch. The presence of small amount of water helped to increase the purification capacity of solvent to dissolve more polar impurities by improving the solvent polarity (Razak *et al.,* 2012). Higher concentration than 80% had less impact on the purification, suggesting the presence of various polarities in PF impurities. However, lower than 80% of IPA was also not recommended for UAE as the glucomannan was soluble in water. More glucomannan was dissolved in higher water content of solvent. Figure 1 also shows linear correlation between glucomannan content and viscosity. It was reported that 0.5% and 1.5% konjac glucomannan

solution has ~800 and ~14,000 cP viscosity, respectively (Akesowan, 2002).

Increasing IPA concentration led to obtain more yield (Figure 1). Lower water content in the solvent drove less dissolved glucomannan. Interestingly, although higher glucomannan was found using 80% IPA, however, its yield was lower than sample using 99% IPA. In this case, the impurity removal was occured due to simultaneous work of UAE and the solvent dissolution. Hence, the simultaneous effect of the rupture of cavitation bubbles caused by ultrasonication and dissolution due to the high polarity solvent effectively erased the impurities was observed at 80% IPA. As a result, although the glucomannan increased but more impurities were removed, resulted in lower yield. Meanwhile, at 99% IPA the purification was less effective due to less water presence, which lowered the solvent polarity and dissolved less impurities (Bimakr *et al.*, 2017). Moreover, physical properties of water enhanced the cavitation in UAE which caused the water addition in UAE solvent was preferable (Natnoi & Pirak, 2019).

Ratios of solvent-solid

Extraction using a right volume of solvent was important to ensure all the solid was soaked but still ensure for conducting effective purification to reduce the cost (Yang *et al.*, 2013). In conventional extraction, higher amount of extract could be obtained when using higher volume of solvent (Elboughdiri, 2018). However, opposite performance was shown in UAE which due to the high amount of initial dissolved impurities in the solvent that influenced the further extraction (Pinchao-Pinchao *et al.*, 2019).

Increasing the ratio decreased the yield of PG. The highest glucomannan was shown using 8:1 (ml/g) of solvent-PF (Figure 1). Right amount of solvent was required to remove the impurities effectively. Nevertheless, more water was presence when high volume of solvent was applied which further could dissolved more glucomannan from PF and reduced the PG yield.
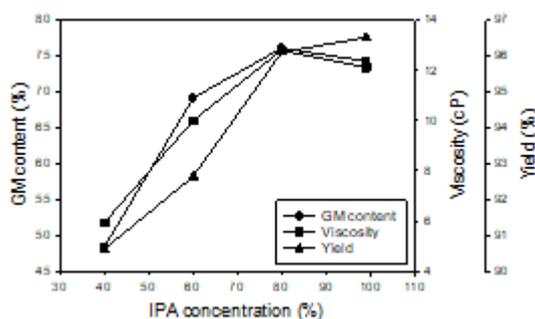
Figure 1. Effect of IPA concentration on glucomannan content, viscosity, and yield of PG.
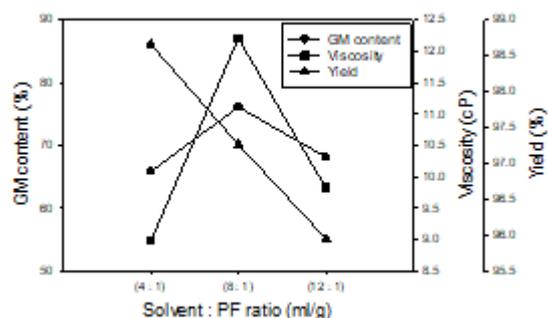
Figure 2. Effect of ratio solvent-PF on the glucomannan content, viscosity, and yield of PG.

Similar result was reported by Idris and Sulaiman (2017), which performed a better UAE on *Labisia Pumila Sp.* in lower solvent-solid ratio. Pinchao-Pinchao *et al.* (2019) also found the best ratio of solvent-solid for phenolic compound UAE was 40:1 (v/w), while increasing the ratio affected on decreasing phenolic content extracted.

The viscosity of glucomannan solution was related to its purity (Yanuriati, 2017). The impurities, especially starch, are less viscous than glucomannan and lowered the viscosity of PG (Xu *et al.*, 2014). The same correlation was applied on the result of this study, which shown as similar trend of Figure 1 and Figure 2. This result was also supported by Widjanarko *et al.* (2011).

Number of extraction stages

The PGs from single stage UAE were compared to the PGs produced using multiple stage of UAE in various IPA concentration on each stage. Multi-stage UAE was expected to increase the purity of PG by multi contacts between solids and the fresh solvent (Meullemiestre *et al.*, 2016). The use of fresh solvent in every stage lead to refresh the driving force of the purification and drive to obtain higher glucomannan content.

From several combination of multi-stage UAE, the best condition for glucomanann's purification was using three-stages UAE with 80% IPA concentration on each stage (Table 2). From this purification condition, PG with the highest purity of 83.26% had the viscosity of 15,960 cP but low yield (90.02%).

Table 2. Effect of number of stages on glucomannan (GM) content, viscosity and yield of PG at 20 kHz and room temperature for 10 min.

Number of stages	IPA concentration (%)	GM content (%)	Viscosity (cP)	Yield (%)
1	80	76.10	12,800	96.10
1	99	74.29	11,760	97.32
2	80; 80	80.07	14,200	94.59
2	80; 99	76.84	13,020	95.08
3	80; 80; 80	83.26	15,960	90.02
3	80; 90; 99	77.78	13,980	92.99

This result confirmed previous result in which a higher polarity of the mixture of IPA-water improved the UAE performance and produced high purity of PG. However, the use of lower IPA concentration was not recommended as more water facilitated the dissolution of glucomannan content.

Morphology and functional groups
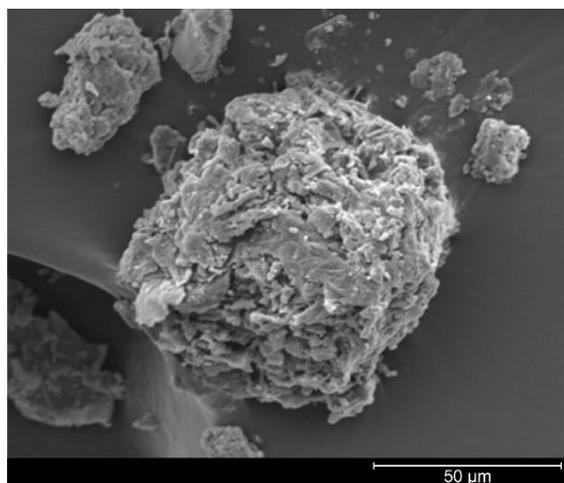
(a)
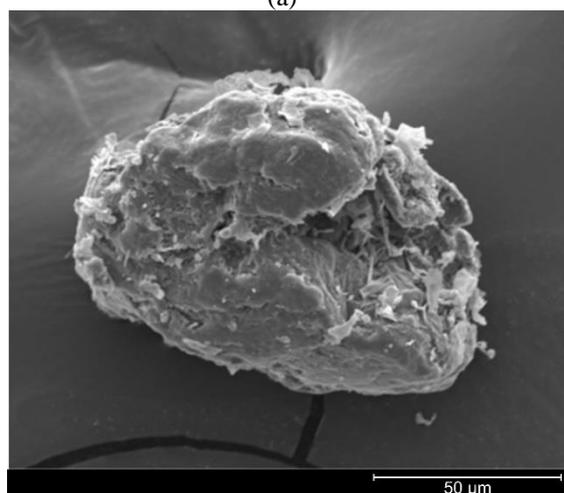
(b)
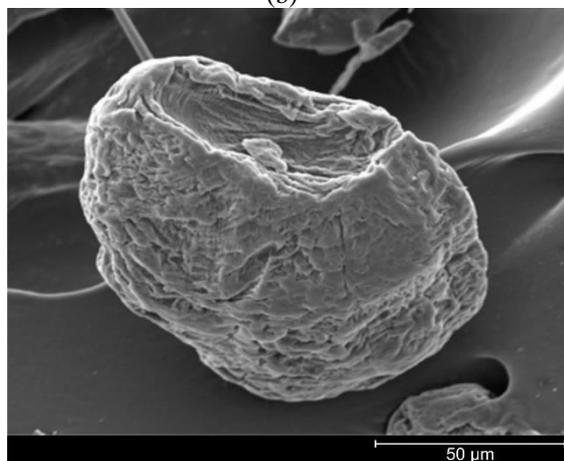
(c)

Figure 3. SEM images of (a) PF, (b) PG, and (c) commercial glucomannan in 2,000× magnification
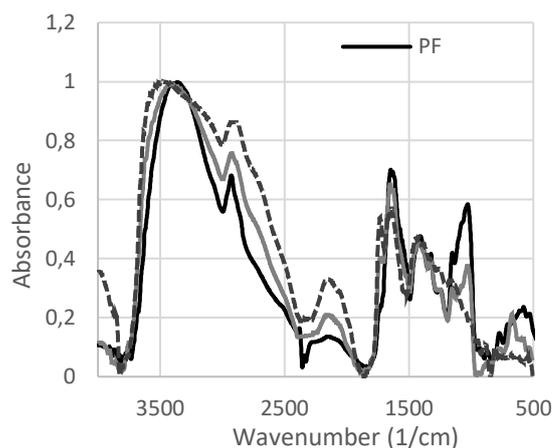
Figure 4. Functional groups of PF, PG and the commercial glucomannan

The morphology of PF, PG, and commercial glucomannan was observed using SEM and the images were shown at Figure 3. PF had rough surfaces, which became smoother after UAE process. The ultrasonication mediated the impurities removal in particles as described by Nguyen *et al.* (2017) who studied the purification of paracetamol crytals. Liu *et al.* (2018) also reported that ultrasonication formed a smoother surface on potato-starch based film. Commercial glucomannan, which had the highest purity, had the smoothest surface of all granules. From these results, the purity of glucomannan could be indicated by the morphology of PF granules. Similar result was also reported by Wardhani *et al.* (2019a).

The Infrared (IR) spectra of PF, PG, and commercial glucomannan were shown at Figure 4, which had similar peaks but in different intensities.

A wide band of 3000-3700 cm^{-1} was attributed to O-H stretching vibration, while the peak of ~2900 cm^{-1} was indicated to –CH$_2$ – stretching vibration. The carbonyl groups (C=O) of β-1,4 linked glucose and mannose were identified from the peak at ~1650 cm^{-1} (Wardhani *et al.,* 2019b). The acetyl groups of glucomannan were shown by the peak at ~1726 cm^{-1} (Nguyen *et al.,* 2011). The peak at 900 – 800 cm^{-1} of PG had a lower intensity than PF, which indicated that some β-mannosidic linkages were broken during the purification process as described by Chua *et al.* (2012).

Chemical composition

Chemical composition of PF, the highest glucomannan content of PG, and commercial glucomannan were shown at Table 1. Although the glucomannan content of PG was lower than the commercial one, UAE using IPA solvent increased the glucomannan purity from 64.2 to 83.26%. The impurities of these flours were quantitatively represented by proximate (water, ash, protein, starch and fiber) and calcium oxalate content. After UAE using IPA, these impurities decreased along with increasing glucomannan content and its viscosity. The commercial glucomannan has superior purity of

glucomannan than PG. Chinese standard set the maximum moisture content and ash for glucomannan flour are 10 and 3%, respectively (Peiying *et al.,* 2002). Meanwhile, WHO assigned that the maximum moisture content and ash of konjac flour were 15 and 5%, respectively (Dannan & Ekelman, 2005). Therefore, the produced PG using UAE using IPA solvent met these both standards.

Calcium oxalate was the impurity that had to be removed from edible glucomannan as this compound gives allergic effect on human body (Wardani & Handrianto, 2020). UAE using IPA solvent reduced the calcium oxalate content from 1.98% to 0.17% after three-stage purification. Widjanarko *et al.* (2011) reported that calcium oxalate was soluble in water and leached out in extraction using ethanol. However, the amount of calcium oxalate in CG was still far lower than PG. The different compositions of raw material, either porang tuber or PF, might differ the characteristics of the purified flour (Anam, 2008). The process conditions including the ultrasound instrument, solvent type, frequency and temperature also influenced the extraction performance (Capello-Martinez, 2009).

CONCLUSION

The UAE using IPA as solvent successfully purified glucomannan from porang flour. Single stage purification using 80% IPA in 8:1 ratio of solvent-flour resulted 76.10% glucomannan content with 12,800 cP viscosity and 96.1% yield. The glucomannant content increased to 83.26% in three-stage UAE purification using 80% IPA with 10 min contact in each stage. The purified glucomannan showed cleaner particle surface than the crude flour. The purified glucomannan had higher intensity of carbonyl and acetyl groups than the flour. These results provided the alternative method in glucomannan purification by combining UAE and IPA.

ACKNOWLEDGEMENT

REFERENCES

Akesowan, A. (2002). Viscosity and gel formation of a konjac flour from Amorphophallus oncophyllus. Faculty of Science, University of the Thai Chamber of Commerce, Bangkok, Thailand.

Alonso-Sande, M., Teijeiro-Osorio, D., Remuñán-López, C., & Alonso, M.J. (2009). Glucomannan, a promising polysaccharide for biopharmaceutical purposes. *European Journal of Pharmaceutics and Biopharmaceutics*, 72, pp.453–462.

Anam K., Rodiyati A., & Gustini E. (2008). The Comparison of Glucomannan and Calcium Oxalate Compounds in Several Variants of Porang (Amorphophallus muelleri blume.) From Klangon Village, Saradan Sub-district, Madiun Regency, East Java. Malang. Biology Departement of Brawijaya University. (in Indonesia).

Adeniyi, S A., Orjiekwe,, C L., & Ehiagbonare, J E. (2009). Determination of alkaloids and oxalates in some selected food samples in Nigeria. *African Journal of Biotechnology*, 8(1), pp. 110-112.

AOAC (2005). *Official of analysis of the Association of Official Analytical Chemistry*. Arlington. AOAC Inc.

Bimakr, M., Ganjloo, A., Zarringhalami, S., & Ansarian, E. (2017). Ultrasound-assisted extraction of bioactive compounds from Malva sylvestris leaves and its comparison with agitated bed extraction technique. *Food Science and Biotechnology*, 26(6), pp. 1481-1490.

Capello-Martinez, J.C. (2009). *Ultrasound in chemistry: analytical applications*. Weinheim-Germany: Wiley-VCH Verlag GmbH & Co. KGaA.

Chua, M., Chan, K., Hocking, T. J., Williamns, P. A., Perry, C. J. , & Baldwin, T. C. (2012) Methodologies for the extraction and analysis of konjac glucomannan from corms of *Amorphophallus konjac K. Koch*, *Carbohydrate Polymers*, 87, pp.2202-2210.

Dannan, G.A. & Ekelman, K.B. (2005) *Konjac flour*. WHO Food Additives Series 32. Food and Drug Administration, Washington, DC, USA. Retrieved from http://www.inchem.org/documents/jecfa/jecmono/v32je10.htm

Elboughdiri, N. (2018). Effect of time, solvent-solid ratio, ethanol concentration and temperature on extraction yield of phenolic compounds from olive leaves. *Engineering, Technology & Applied Science Research*, 8(2), pp. 2805-2808.

He, B., Zhang, L.-L., Yue, X.-Y., Liang, J., Jiang, J., Gao, X.-L., & Yue, P.-X. (2016). Optimization of Ultrasound-Assisted Extraction of phenolic compounds and anthocyanins from blueberry (*Vaccinium ashei*) wine pomace. *Food Chemistry*, 204, pp.70–76.

Idris, N. A. N., & Sulaiman, A. Z. (2017). Comparison between conventional extraction and ultrasound assisted extraction of labisia pumila Sp. in 25-L mobile extractor using water as solvent of extraction. *Chemical Engineering Transactions*, 56, pp. 781-786.

Kimura, T., Sugahara, T., Kameda, K., Sasaki, H., Fukuya, Y., & Goto, M. (2001). Effect of ultrasonication on the physicochemical properties and sensory characteristics of konnyaku. *Journal of Home Economics of Japan*, 52(3), pp.227-234.

Liu, P., Wang, R., Kang, X., Cui, B., & Yu, B. (2018). Effects of ultrasonic treatment on amylose-lipid complex formation and properties of sweet potato starch-based films. *Ultrasonics Sonochemistry*, *44*, pp. 215-222.

Meullemiestre, A., Petitcolas, E., Maache-Rezzoug, Z., Chemat, F., & Rezzoug, S. A. (2016). Impact of ultrasound on solid–liquid extraction of phenolic compounds from maritime pine sawdust waste: Kinetics, optimization and large scale experiments. *Ultrasonics Sonochemistry*, *28*, pp. 230-239.

Natnoi, S., & Pirak, T. (2019). Effect of ultrasonic-assisted extraction on the properties, antioxidant and inflammatory activities of carotenoids from gac (Momordica cochinchinensis) fruit pericarp. *Cogent Food & Agriculture*, *5*(1), pp.1696512.

Nguyen, T.A., Do, T.T., Nguyen, T.D., Pham, L.D., & Nguyen, V.D. (2011) Isolation and characteristics of polysaccharide from *Amorphophallus corrugatus* in Vietnam*, Carbohydrate Polymers*, 84, pp.64-68.

Nguyen, T. T., Khan, A., Bruce, L. M., Forbes, C., O'Leary, R. L., & Price, C. J. (2017). The effect of ultrasound on the crystallisation of paracetamol in the presence of structurally similar impurities. *Crystals*, *7*(10), pp. 294.

Ohashi S. Shelso GJ. Moinaro AL. Drinkwater WL. (2000). Clarified Konjac Glucomannan. *US Patent no. 6,162,196.*

Peiying, L., Zhang, S., Zhu, G., Chen, Y., Quyang, H., Han, M., Wang, Z., Xiong, W., & Peng, H. (2002). Professional Standart of The Peoeple' Republic of China for Konjac Flour. NY/T : 494-2002.

Pinchao-Pinchao, Y. A., Ordoñez-Santos, L. E., & Osorio-Mora, O. (2019). Evaluation of the effect of different factors on the ultrasound assisted extraction of phenolic compounds of the pea pod. *DYNA*, *86*(210), pp.211-215.

Rahayu, L. H., Wardhani, D.H., & Abdullah. (2017) The effect of frequency and washing time on ultrasound-assisted extraction using isopropanol on glucomanann content and porang viscosity (*Amorphophallus oncophyllus*). *Metana*, 9(1), pp. 45-52 (in Indonesian)

Razak, M. F. B. A., Yong, P. K., Shah, Z. M., Abdullah, L. C., Yee, S. S., & Yaw, I. T. C. S. (2012). The effects of varying solvent polarity on extraction yield of *Orthosiphon stamineus* leaves. *Journal of Applied Sciences*, 12(11), pp.1207-1210.

Wardani, R. K., & Handrianto, P. (2020). The effect of soaking porang tubers in acid solution on decreasing calcium oxalate levels. *Annals of Biology*, 36(2), pp.173-176.

Wardhani, D. H., Vázquez, J. A., Ramdani, D. A., Lutfiati, A., Aryanti, N., & Cahyono, H. (2019a). Enzymatic purification of glucomannan from *Amorphophallus oncophyllus* using α-Amylase. *Bioscience Journal*, 35(1).

Wardhani, D. H., Kumoro, A. C., Hakiim, A., Aryanti, N., & Cahyono, H. (2019b). Kinetic Modeling Studies of Enzymatic Purification of Glucomannan. *Chemistry & Chemical Technology, 3 (13), 2019*, (3), pp. 384-390.

Widjanarko, S. B., Faridah, A., & Sutrisno, A. (2011). Effect of multi level ethanol leaching on physico-chemical properties on konjac flour (Amorphophallus Oncophyllus). *In The 12th Asean Food Conference.* pp. 16-18.

Xu, W., Wang, S., Ye, T., Jin, W., Liu, J., Lei, J., Li, B. and Wang, C. (2014). A simple and feasible approach to purify konjac glucomannan from konjac flour–Temperature effect. *Food Chemistry*, 158, pp.171-176.

Yang Y.C, Wei M.C, Huang T.C, Lee S.Z, and Lin S.S. (2013). Comparison of modified ultrasound-assisted and traditional extraction methods for the extraction of baicalin and baicalein from Radix Scutellariae. *Industrial Crops and Products,* 45, pp. 182– 190.

Yanuriati, A., Marseno, D. W., & Harmayani, E. (2017). Characteristics of glucomannan isolated from fresh tuber of Porang (*Amorphophallus muelleri Blume*). *Carbohydrate polymers*, 156, pp.56-63.

Zhang, W. N., Zhang, H. L., Lu, C. Q., Luo, J. P., & Zha, X. Q. (2016). A new kinetic model of ultrasound-assisted extraction of polysaccharides from Chinese chive. *Food Chemistry*, 212, pp.274-281.

Zhang, Y-q., Xie, B-j., & Gan, X. (2005). Advance in the applications of konjac glucomannan and its derivatives, *Carbohydrate Polymers*, 60, pp. 27-31.