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Characterization of glucomannan from *Amorphophallus oncophyllus* and its prebiotic activity *in vivo*

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ABSTRACT

Porang (*Amorphophallus oncophyllus*) is local perennial plant rich in glucomannan. The aim of this study was to extract and characterize glucomannan from porang tuber and to evaluate its potency as prebiotic *in vivo*. The research consisted of the following steps, i.e. extraction of glucomannan, evaluation of its physico-chemical properties, and *in vivo* study. Extraction was done by immersing porang fluor with water at 55 °C followed by coagulating glucomannan using ethanol. Solubility, water holding capacity, viscosity, degree of acetylation, degree of polymerization (DP), and purity of the glucomannan were evaluated. *In vivo* study was done using thirty-two Wistar rats which were divided into four groups. Each group was treated for 14 days with standard AIN 93 (standard), porang glucomannan, commercial konjac glucomannan, and inulin diet as source of fiber. Bacterial population and chemical properties of digesta were analyzed after intervention. The results of the study indicated that the yield of glucomannan from porang flour was 18.05% with 92.69% purity. Compared to commercial glucomannan, porang glucomannan showed higher solubility (86.4%) and degree of acetylation (13.7%), but lower viscosity (5400 cps), WHC (34.5 g/g), and DP (9.4). Diet supplemented with porang glucomannan inhibited the growth of *Escherichia coli*, enhanced the production of total SCFA, and reduced pH value of cecal content. The study indicated that glucomannan from porang may be used as functional food.

1. Introduction

Porang (*Amorphophallus oncophyllus*) is a local perennial plant that commonly grown in Indonesian forest. Its tuber is rich in glucomannan that may offer many benefits as food ingredient as well as functional food. Farmer in Indonesia has exported porang to China and Japan in large quantity, but in low value since it is sold in chips form. So, it is important to explore the properties or the beneficial effect of porang glucomannan in order to enhance the value.

Konjac glucomannan extracted from *Amorphophallus konjac* is a linear random copolymer of (1 → 4) linked β-D-mannose and β-D-glucose. It is composed of mannose and glucose units at molar ratio of 1.6:1 with a low degree of acetyl groups (approximately 1 acetyl group per 17 residues) at the C-6 position (Dave & McCarthy, 1997). Gelation and high viscosity are the prominent properties of konjac glucomannan that can be developed for food industry, such as thickener of the syrup, jelly, edible film, noodle, and binder of sausage (Akesowan, 2002).

The characteristics of konjac glucomannan have been studied elsewhere. Those researches supported its role as health food, such as weight loss food by filling the stomach and making a person feel full (Li, Xia, Wang, & Xie, 2005), manage hyperglycemia and hypercholesterolemia (Chen et al., 2003; Vuksan et al., 2000 in Chen, Fan, Chen, & Chan, 2005), and increased fecal stool bulk (Chen, Cheng, Liu, & Wu, 2006).

Fermentation of glucomannan as dietary fiber in the lower gut can produce short chain fatty acid (SCFA). Previous studies proved that there were increased of acetic acid, propionic acid, and butyric acid when konjac glucomannan (*A. konjac*) was added, both *in vitro* and *in vivo* (Chen et al., 2006; Chen, Cheng, Wu, Liu, & Liu, 2008; Connolly, Lovegrove, & Tuohy, 2010). These also contribute to the health as antihypercholesterolemia and hyperglycemia, as well as antimicrobial agent.

In order to make fermentation in colon and give the beneficial effect to the host, fiber must reach colon without digestion in the upper gut and selectively stimulate growth and/or activity of one or limited number of microbial in the gut microbiota that confer health benefits to the host. Those are related with the prebiotic activity. Chen et al. (2006, 2008) and Connolly et al. (2010) have studied the ability of glucomannan in stimulating growth of

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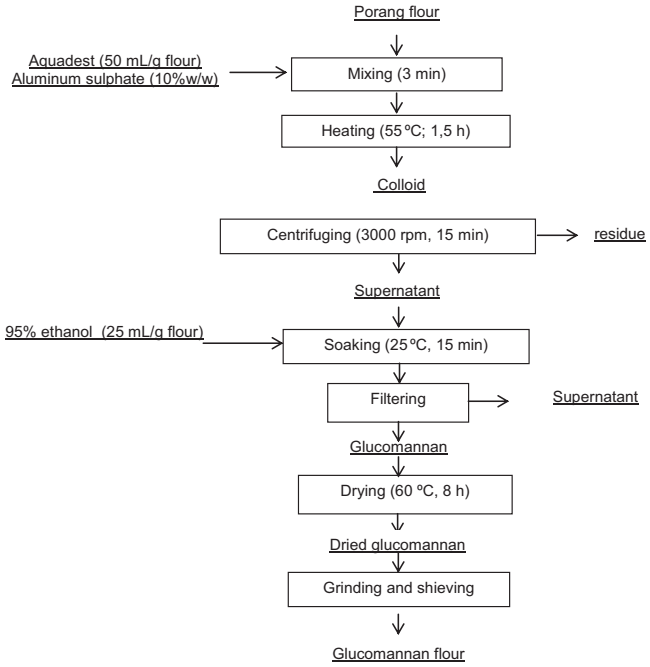

Fig. 1. Ethanol extraction of glucomannan from porang flour (Amanah, 1992).

bifidobacteria and *lactobacillus*, and suppressing *Escherichia coli* and *Clostridium perfiringens*.

Different species may produce different yield and characteristics of glucomannan. So far, there is limited information on the characteristics of porang glucomannan extracted from *A. oncophyllus* in Indonesia. Studies on the effect of porang glucomannan on selected bacterial population and chemical properties of cecal content of Wistar rats are also limited. The objectives of this research were to extract and characterize glucomannan from porang tuber and to evaluate its potency as prebiotic *in vivo*. In this study, characteristics of native glucomannan extracted from porang using method that usually done by local small scale industry (traditional) were evaluated. Findings of this study may enhance the added value of porang as functional food.

2. Materials and methods

2.1. Extraction of porang glucomannan

Porang was obtained from the low light intensity of young teak forest in Klangon, Saradan, East Java, Indonesia. Porang glucomannan was extracted from porang flour that was made by slicing the tuber, soaking it in Na-metabisulphite solution (1 g/L) for 5 min, dried the chips (60 °C), and then ground it. The extraction of glucomannan from porang flour was done by method of Amanah (1992) with slight modification as shown in Fig. 1.

2.2. Evaluation of porang glucomannan physico-chemical properties

The physico-chemical properties of glucomannan porang were evaluated for its solubility (Widowati, Sunarti, & Zaharani, 2005), viscosity at pH 6; 1% (w/v) (Akesowan, 2002), water holding capacity (WHC) (Umaru, Iya, Obidah, & Dahiru, 2010), degree of polymerization (DP) (Chen et al., 2005), and degree of acetylation (Pan, Meng, & Wang, 2011).

Table 1
Physico-chemical properties of porang glucomannan.

Character	Value
Solubility (%)	86.43 ± 1.32
Water holding capacity (g water/g glucomannan)	34.50 ± 2.32
Viscosity (cps)	5400 ± 40.82
Degree of polymerization	9.4
Degree of acetylation (%)	13.7
Purity (%)	92.69

2.3. In vivo study of porang glucomannan

In vivo study of porang glucomannan was approved by Medical and Health Research Ethics Committee, Ministry of National Education, Faculty of Medicine Gadjah Mada University (Ref: KE/FK/377/EC). Thirty-two of eight-week-old male Wistar rats were divided into 4 groups. Each group treated with standard AIN 93 diet (*American Institute of Nutrition*) (Reeves, Nielsen, & Fahey, 1993) as control and porang glucomannan, commercial konjac glucomannan (type konjac gum KJ-28 from Konson Konjac, China), and inulin (from Cosucra, Germany) diet as source of fiber for treated diet. Standard group used cellulose (CMC) as source fiber in the diet, while intervention groups were modified by replacing the same amount of cellulose with porang glucomannan, konjac glucomannan, and inulin.

They were housed individually for 3 days of adaptation periods and 14 days of intervention periods. All animals were allowed free access to water and food during the study. Food intake, body weight, and fecal weight were monitored during study. At the end of intervention periods, the rats were anaesthetized by ether and sacrificed. The digesta of caecum (cecal content) were removed immediately and were put into sterile container, taken and analyzed for certain number of cecal selected bacterial population, water content, pH, and SCFA.

Selective media used for microflora enumeration were Rogossa agar (Oxoid-England) for *lactobacilli*, Columbia agar (Merck-Germany) for *bifidobacteria*, and TBX agar (Oxoid-England) for *E. coli*. Fresh cecal content were collected directly from each rat, weighed, homogenized and serially diluted with 1% peptone water. From each of the dilutions, 0.1 ml was plated in triplicate onto selective media. TBX and rogosa agar plates were incubated aerobically at 37 °C for 24 h and Colombia agar were incubated anaerobically at 37 °C for 48–72 h.

Cecal content pH was determined directly by diluting cecal content in 1:10 distilled water using pH meter. SCFA were analyzed by centrifuging it at 3500 rpm for 20 min, the supernatant were then collected and analyzed by gas chromatography (Shimadzu GC-8A) using a capillary column (Φ180 cm × 4 mm).

Statistical analysis was performed using SPSS, version 16. Analysis of variance Duncan was used to determine the significance effect diet on bacterial group population, SCFA, pH value of cecal content. Differences were significant when $p < 0.05$.

3. Results and discussion

3.1. Extraction and physico-chemical properties of porang glucomannan

Extraction of glucomannan from porang flour yield 18.05% with 92.69% purity for cabinet drying and 93.84% for freeze drying. The other physico-chemical properties of glucomannan were shown in Table 1.

Glucomannan from porang had higher solubility (86.43%) than that from konjac (82.9%) (Du, Li, Chen, & Li, 2012). Solubility is partly attributed to the long side chains of the glucomannan (Hwan & Kokini, 1991; Zhau, 2008 in Samil, Abdelhameed, Ang, Morris, &

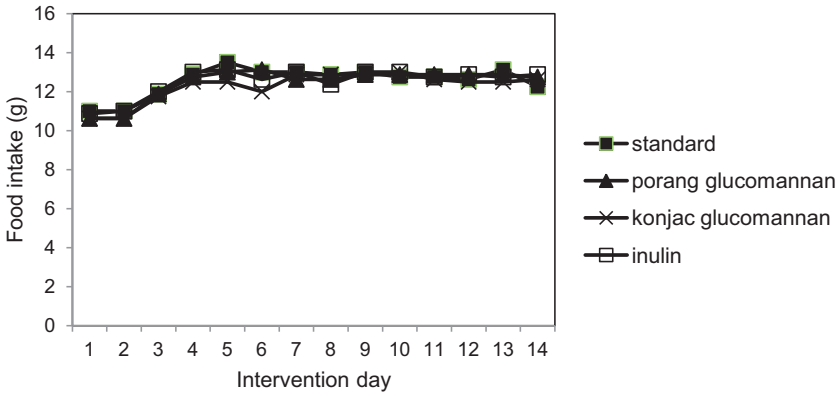

Fig. 2. Feed intake of Wistar rats during 14 days of intervention supplemented by various fibers.

Harding, 2009), the presence of acetyl units (Dave & McCarthy, 1997), glucuronic acid, and phosphoric acid groups (Nishinari, Takemasa, Zhang, & Takashi, 2007). In this research, the high solubility of porang glucomannan corresponding with its high degree of acetylation (13.7%) compared to konjac (5–10%) (Dea & Morrison, 1975). According to Du et al. (2012), water solubility was improved gradually by the introduction of acetyl groups. Solubility is very important for application of glucomannan in food because it can be used after solubilization in water.

Glucomannan was also popular for its extraordinary high WHC, highly viscous solutions when dissolved in water. Study showed that 1 g of konjac glucomannan could absorb up to 200 ml of water (Maeda et al., 1980 in Samil et al., 2009). In this study, WHC of porang glucomannan was 34.5 ± 2.32 g water/g glucomannan which lower than that of konjac glucomannan. The WHC may be influenced by thermal processing of polysaccharides that construct stronger interchain interaction due to extensive hydrogen bonding (Dave & McCarthy, 1997), especially drying process in high temperature for extended period (Zhao, Zhang, Srzednicki, Kanlayanarat, & Borompichaichartkul, 2010). In this study, after extraction, glucomannan was dried using cabinet drying. This process may reduce WHC of glucomannan. WHC of glucomannan may be improved by freeze drying. However, in this study we intended to evaluate native glucomannan obtained from porang that commonly processed by small scale industry (traditional). Further, we have analyzed the WHC of porang glucomannan dried by freezed dryer and the result was not significantly different ($p < 0.05$; data not shown).

Viscosity of porang glucomannan was 5400 cps. This value was in agreement with study done by Akesowan (2002) who analyzed the viscosity of konjac glucomannan. When we compared to commercial glucomannan, it becomes lower (19,679.33 cps). The high viscosity means high beneficial potency of fiber in health, such as disrupts the absorption of cholesterol in the small intestine, thereby decreasing rate of lipid absorption, slow digestion rate and absorption of carbohydrate affecting insulin activity, and increasing production of SCFA in the colon. This could inhibit the growth of harm bacteria (Truswell, 1990; Johnson, 1990 in Umaru et al., 2010) and may be used as functional food.

Glucomannan DP from porang was 9.4 with cabinet drying and 12 with freeze drying. The result was in agreement with konjac glucomannan (Chen et al., 2005) which found that the degree of polymerization was 12. The low DP of cabinet drying glucomannan may be caused by higher and longer heating process.

3.2. *In vivo study of porang glucomannan*

3.2.1. *Food intake and body weight of rat*

Supplementation of porang glucomannan to diet was also studied for its influence on food intake during 14 days (Fig. 2).

Generally, food intake of rats increased and the slopes were similar. The slopes of food intake of standard diet, porang glucomannan, konjac glucomannan, and inulin diets were 0.091; 0.133; 0.130; 0.112, respectively. Therefore, the feed palatability of rats supplemented with porang glucomannan was not significantly different with standard ($p > 0.05$).

Supplementation porang glucomannan was also studied for the body weight of the rats (Fig. 3). Standard diets had the least body weight gain among others and it was significantly different ($p < 0.05$). Cellulose is insoluble food fiber and the others are soluble fibers. Insoluble fiber was more difficult to fermentate than soluble fiber (inulin, konjac glucomannan, porang glucomannan). It made cellulose more undigestible and excreted out of the body and did not influence the body weight.

3.2.2. *Bacterial population (lactobacilli, bifidobacteria, and E. coli) of cecal content*

Prebiotic activity of glucomannan was determined *in vivo* by enumerating the bacterial population (*lactobacilli*, *bifidobacteria*, and *E. coli)* in cecal content (Table 2).

All of intervention groups had similar population of *lactobacilli* ($p > 0.05$), but population of *bifidobacteria* was similar among porang glucomannan, konjac glucomannan, and standard. It means that *lactobacilli* and *bifidobacteria* could not be stimulated by porang glucomannan. This result was different with previous study that konjac glucomannan had bifidogenic effect in Balb mice (Chen et al., 2005). Theoretically, glucomannan might be used by *lactobacilli* and *bifidobacteria* as there were enzymes secreted by other colon microbiota. It could hydrolyzed glucomannan to produce oligosaccharide or monosaccharide (Pokusaeva, Fitzgerald, & Sinderen, 2011), such as mannopentaose, mannotetraose, mannotriose, mannobiose, and mannose (Katrolia et al., 2012). The enzyme were β-glucanase dan 1,4 β-D-mannanase secreted by bacilli and clostridium groups (Jiangyang et al., 2008; Matsuura, 1991 in Nakajima, Ishihara, & Matsuura, 2002). Hydrolyzed product may be fermented easily by cecal *bifidobacteria* through bifid

Table 2
Bacterial population in cecal content of Wistar rat fed with diets containing cellulose (standard), porang glucomannan, konjac glucomannan, and inulin.

Intervention group	Number of bacteria (log10CFU/g)		
	Bifidobacteria	*Lactobacilli*	*E. coli*
Standard	8.22 ± 0.34[a]	7.48 ± 0.45[a]	8.28 ± 0.31[a]
Porang glucomannan	8.09 ± 0.63[ab]	7.46 ± 0.61[a]	6.93 ± 0.77[b]
Konjac glucomannan	7.81 ± 0.31[ab]	7.18 ± 0.54[a]	7.14 ± 0.59[b]
Inulin	7.54 ± 0.71[b]	7.39 ± 0.44[a]	6.60 ± 0.50[b]

Values in the same column with different superscripts are significantly different ($p < 0.05$) using one-way ANOVA and multiple comparison Duncan's test.

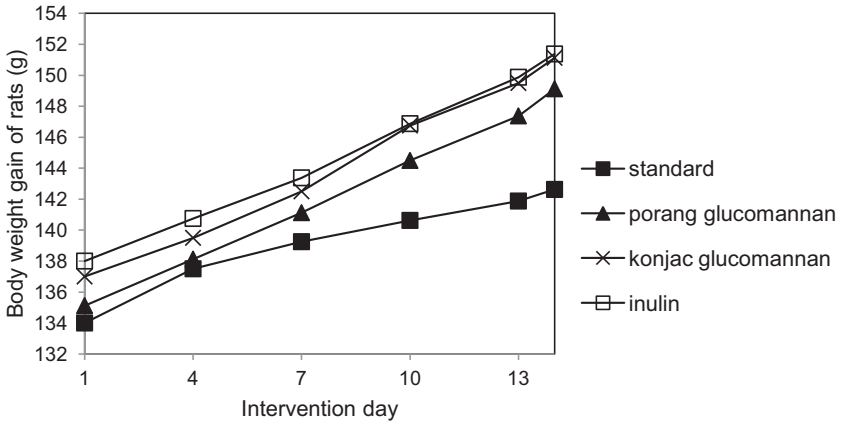

Fig. 3. Body weight gain of Wistar rats during 14 days of intervention supplemented by various fibers.

shunt (Vries & Stouthamer, 1967 in Pokusaeva et al., 2011) and EMP (Embden Meyerhoff Parnas) pathway. In addition, bifidobacteria had the ability of secreting glycoside hydrolase that was very active in degrading plant polysaccharide (Van den Broek & Voragen, 2008). In this study, glucomannan may be more difficult to hydrolyze because of strong interchain interaction from extensive hydrogen bonding influenced by thermal processing (Dave & McCarthy, 1997), especially in high temperature and extended period (Zhao et al., 2010).

Prebiotic potency of porang glucomannan showed greatly suppressing the growth of *E. coli*. It was agreed with the observation of Chen et al. (2005) that reported decreasing of *E. coli* in cecal content of rat fed with diet containing konjac glucomannan. The mechanisms by which bifidobacteria exerted these effects include the increase in acidic fermentation that inhibit the growth of *E. coli.*

It is clearly showed that porang glucomannan had potential prebiotic property to balance the microbiota toward better host health.

3.2.3. Characters of cecal content

Physical and chemical properties of cecal contents of rats were shown in Table 3.

Cecal content weight of all intervention groups were not significantly different ($p > 0.05$), but the water content of cecal in standard was significantly higher ($p < 0.05$) than that was in other interventions groups. It may be caused by imbalance of intestinal microbiota. Krehbiel et al. (2003), Ouwehand et al. (2002) in Mendoza, Heinrichs, and Jones (2011) reported that diarrhea can be caused by the increased of coliform and decreased of *lactobacilli* and *bifidobacteria*. The pH values of cecal contents of all samples were low, except in rat fed with konjac glucomannan. The lower pH value of cecal content in rats with porang glucomannan is beneficial to reduce the incidence of colon cancer.

3.2.4. SCFA

The concentration of short fatty acid (SCFA) in cecal contents could be seen in Table 4. Carbohydrate having prebiotic potency will be fermentate in small intestine that produce SCFA. Variety and amount of SCFA were influenced by microbiota in colon, type of substrate, and transit time in the gut (Cumming et al., 1979 in Cho & Finocchiaro, 2010).

Table 3
Characteristics of cecal contents.

Intervention	Weight (g)	pH	Water content (%)
Standard	1.80 ± 0.29[a]	6.94 ± 0.17[a]	82.57 ± 2.02[a]
Porang glucomannan	1.80 ± 0.70[a]	6.90 ± 0.29[a]	71.69 ± 5.14[b]
Konjac glucomannan	1.76 ± 0.77[a]	7.33 ± 0.43[b]	73.72 ± 2.87[b]
Inulin	1.66 ± 0.34[a]	6.94 ± 0.42[a]	74.10 ± 1.89[b]

Values in the same column with different superscripts are significantly different ($p < 0.05$) using one-way ANOVA and multiple comparison Duncan's test.

Table 4
SCFA total concentration and molar ratio of SCFA of cecal contents.

Intervention	SCFA total (%)	Acetic acid (%)	Propionic acid (%)	Butyric acid (%)
Standard	57.13	57.50	36.84	5.66
Porang glucomannan	70.40	60.78	33.17	6.05
Konjac glucomannan	50.82	57.56	36.50	5.94
Inulin	76.49	59.22	35.17	5.61

Porang glucomannan had higher total SCFA than standard and konjac glucomannan groups. It meant that porang glucomannan could increase capacity of fermentation. Molar ratios of acetic acid of all samples were higher than propionic and butyric acid. It is agreed with previous observation that supplementation of konjac glucomannan in the diet of Balb mice demonstrated increasing of acetic acid but decreasing butyric acid (Chen et al., 2005).

Cumming et al. (1979) in Cho and Finocchiaro (2010) reported that generally, molar ratio of SCFA (acetic acid > propionic acid > butyric acid) produced by nondigestible carbohydrate was 60:20:20. In this study, SCFA ratio of those in porang glucomannan sample was 61:33:6. It means that porang glucomannan tend to have higher propionic acid. Propionic acid is able to block cholesterol synthesis in a heart and has hypolipidemic effect (Cho & Finocchiaro, 2010). It also enhanced calcium absorption from rectal liquid (Gibson & Roberfroid, 2008). Thefore, it can be expected that porang glucomannan was potential to reduce cholesterol concentration. Futher reseach on this effect was needed.

4. Conclusion

Porang glucomannan had different characteristics to konjac glucomannan. It was relatively higher solubility (86.43 ± 1.32%) and degree of acetylation (13.7%), but lower WHC (34.50 ± 2.32 g water/g glucomannan), viscosity (5400 cps), and DP (9.4) as compared to available commercial glucomannan. Diet supplemented with porang glucomannan had good effect on inhibiting the growth of *E. coli* but it had little effect on stimulating the growth of *bifidobacteria* and *lactobacilli*. Diet with porang glucomannan enhanced the production of total SCFA and decreased pH value of cecal content in Wistar rats. The study indicated that glucomannan from porang may be improved as functional food.

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