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# CHARACTERIZATION OF GLUCOMANNAN FROM AMORPHOPHALLUS PANOMENSIS IN VIETNAM

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## ABSTRACT

A glucomannan has been isolated from the tuber of Amorphophallus panomensis by a simple method without using toxic chemicals. The glucomannan content of the tuber was 4% (w/w). The structure of the glucomannan was investigated by IR and NMR spectroscopy revealing that it consists of mannose and glucose units with a mannose/glucose ratio of about 1.00/0.13. The degree of crystallization was investigated by XRD diffraction revealing that the glucomannan was amorphous. The molecular weight was determined by viscosimetry to be of 1.2×10<sup>6</sup> Da.

Keywords: Amorphophallus panomensis, glucomannan, konjac.

## 1. INTRODUCTION

Amorphophallus sp. is a perennial herbaceous herb. It grows in mountain or hilly areas in subtropical regions mainly in the South East of Asia. It has been used as food and food additives in China and Japan for more than 1000 years. Glucomannan (GM) is a polysaccharide of the mannan family, very abundant in nature, specifically in softwoods (hemicellulose), roots, tubers and many plants bulbs. Despite the variety of sources, the most commonly used type of GM is named Konjac glucomannan (KGM), which is sextracted from tubers of Amorphophallus plants. Irrespective of its origin, GM is composed of  $\beta$ -1,4-linked D-mannose and D-glucose monomers. However, the mannose/glucose monomer ratio may vary depending on the original source of GM. For example, it has been reported that Konjac GM has a molar ratio of around 16:1, whereas GMs extracted from Scotch pine and orchid tubers have ratios of 2.1:1 and 3.6:1, respectively. These values should be regarded cautiously given the variability observed depending on the studies and, in particular, on the analytical procedures [1, 2].

Studies on glucomannan showed that it has many applications in many fields. Due to its biggradability and gel-forming ability, konjac glucomannan can be widely used for drug delivery such as capsule for chronic stomach disease. Wang et al. reported that a kind of alginate-konjac glucomannan-chitosan beads could be used as controlled release matrix [3].

Glucomannan has very good film-forming ability so several kinds of transparent blend films of konjac glucomannan with polyacrylamide, gelatin, sodium carboxymethylcellulose, polyvinyl pyrrolidone, chitosan, sodium alginate and cellulose, were invented [4, 5]. The occurrence of intra- and intermolecular interaction of the pure components, as well as the intermolecular interactions between KGM and these substance come from hydrogen bond formation. Li et al also reported a kind of edible membrane material made from the mixture of konjac glucomannan and xanthan, which showed better strength and water-resistance than konjac glucomannan itself [6]. In the patent of Yang et al coating materials containing konjac glucomannan were invented for preserving fresh produce (e.g. apples). The coating composition was effective to control respiratory exchange, i.e. the passage of gases, particularly oxygen, ethylene, carbon dioxide and water vapor, into and out of the produce, thereby controlling maturation and ripening of the produce [7].

In the field of cosmetics, Omura et al invented a hair composition containing glucomannan and/or keratose quaternary ammonium derivatives which provided excellent conditioning effect and moisture-retaining effect without causing stickiness. Some kinds of hair-styling preparations containing glucomannan with less stickness and giving natural gloss and smoothness to hair. Some kinds of cosmetic oil-in-water emulsions containing konjac glucomannan were also invented [8]. Takada invented a kind of water-insoluble glucomannan gel particles as mild scrubbing agents for cosmetics. The dried gel particles did not damage the skin and tooth surface. Therefore they were effective as scrubbing agents [9].

Besides the applications mentioned above, konjac glucomannan and its derivatives were also used in many other areas: they can be used as biodegradable resin compositions, soil modifier, soil amendment [9]. In addition, konjac glucomannan powder can be mixed with powdery or granular charcoal and water and press-molded to charcoal shaped products having excellent properties in dehumidification, deodorization, sound absorption, and electromagnetic shielding and are suitable for water treatment, health products (such as using glucomannan to lower blood cholesterol and sugar level, help weight loss, promote intestinal activity and immune function etc) [10].

Being a member of the Araceae (arum) family, Amorphophallus panomensis was expected to contain polysaccharide, usually named glucomannan, with mannose and glucose units. Therefore, the aim of this work was to perform isolation and characterization (using FTIR, NMR, X-Ray) of polysaccharide from Amorphophallus panomensis. The polysaccharide obtained is intended to be used in food, as film-forming material, for packaging or encapsulating of pharmaceutical and biomedical products, and for designing new drug carriers.

#### 2. EXPERIMENTAL

## 2.1. Materials

Tubers of Amorphophallus panomensus were collected from hilly area of Lang Son province of Vietnam. All other chemicals and reagents used were of analytical grade.

# 2.2. Isolation and purification of polysaccharide from Amorphophallus tuber

Polysaccharide from the tuber of Amorphophallus panomensis was extracted and purified as follows: Amorphophallus tubers were sliced to about 8 - 10 mm in thickness, then pulverized by a mill. The crude flour was dispersed in distilled water and laid for 3 h, the white powder

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s Dae te ed for de e s kind d ex [3] (glucomannan) at the bottom was separated and then washed many times with water. Then the powder was dried at 50 °C and used for further characterization. The glucomannan content (GF) was calculated using the following formular:  $GF^{\circ} = (m_1/m_2) \times 100$  % with  $m_1$  and  $m_2$  were weight of final white powder and original Amorphophallus tubers, respectively.

## 2.3. Characterization of polysaccharide

FTIR spectrum of the glucomannan was recorded on the FTIR-Impact 410 spectrometer in the range between 4000 and 400 cm<sup>-1</sup>. <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra were recorded on the 500 MHz Bruker Avance spectrometer, the sample concentrations being about 5 g/l and 20 g/l, respectively, in 0.5N NaOH/D<sub>2</sub>O at 353 °K.

The intrinsic viscosity of glucomannan was measured by Ubbelohde viscometer according to n =  $5.96 \times 10^{2} \times M_{\odot}^{0.317}$  following the n =  $5.96 \times 10^{2} \times M_{\odot}^{0.317}$  following to n =  $5.96 \times 10^{2} \times M_{\odot}^{0.317}$  following to n =  $5.96 \times 10^{2} \times M_{\odot}^{0.317}$  following the new formula of the new formul

X-ray diffraction pattern was analyzed using a Siemens D5000 (Japan) diffractometer equipped with a CuK $_{\rm cc}$  target at 40 kV and 30 mA with a scan rate of 4°/min. The diffraction angle ranged from 20 = 5° to 20 = 65°.

#### 3. RESULTS AND DISCUSSION

#### 3.1. Isolation of polysaccharide from Amorphophallus tuber

In recent years, there were many studies on glucomannan and its applications. The isolation and purification of glucomannan from Amorphophallus plants was one of the most important part of these studies. According to traditional methods, the isolation and purification process were often carried out with dried Amorphophallus tubers [5]. However, in our process, glucomannan could be directly obtained by dispersion of the crude flour in water because of its low solubility and high density. In this process, the fat and protein were dissolved by water. The glucomannan content was about 4 % of original tuber. Thus, this glucomannan could be easily isolated from Amorphophallus tubers without using toxic chemicals. The molecular weight of glucomannan measured according to method of Li et al. was about 1.2 × 10° Da.

## 3.2. FTIR analysis

The FTIR spectrum of glucomannan in the wavelength range of  $4000 + 400 \text{ cm}^{-1}$  is shown in Fig.1.

In the spectrum, the wide band observed at 3000 - 3700cm<sup>-1</sup> could be attributed to O-H stretching. The band at 2926 cm<sup>-1</sup> was attributed to the asymmetric stretching of C-H, while the band at 1645 cm<sup>-1</sup> was ascribed to adsorbed water and the bands at 1426 and at 1372 cm<sup>-1</sup> to the angular deformation of C-H. The C-O ether bond shows stretching at 1159 cm<sup>-1</sup> while the C-O alcohol bond shows stretching at 1082 and 1014 cm<sup>-1</sup>. Peaks from 768 to 927 cm<sup>-1</sup> were typical for C-H linkage of  $\beta$ -pyranose ring (glucose and mannose). Those results were in agreement with those of Liu et al and Jia et al. [11, 12].

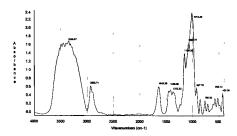


Figure 1. FTIR spectrum of panomensis glucomannan.

# 3.3. NMR spectroscopy

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The <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra of the glucomannan was shown in Fig. 2.

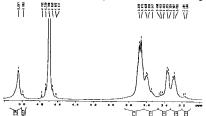


Figure 2. <sup>1</sup>H-NMR spectra of panomensis glucomannan in D<sub>2</sub>O at 25°C.

The peaks were assigned by comparison with chemical shift data reported in literature [1]. Signals were assigned as follows: H-1 of mannose (δ 5.047 ppm); H-1 of glucose (4.993); H-2 of both mannose and glucose (3.423 - 3.440, overlapped); H-3 of mannose (3.292) and that of glucose (3.307); H-4 of mannose (3.359) and H-4 of glucose (3.375); H-5 of mannose (3.671), H-5 of glucose (3.690); H-6a and H-6b of mannose and glucose are (3.608), (3.442), respectively.

It could be seen from Fig. 2 that the signals attributed to hydrogens linked to C2 to C6 of both glucose and mannose units were not well separated. This was due to the complex nature of the spectra of polysaccharides. Meanwhile, the signals attributed to H-1 of both glucose unit (4.993 ppm) and mannose unit (5.047 ppm) were well separated. Therefore, the mannose/glucose ratio in the glucomannan molecule could be calculated using the H-1 integrals. According to this method, the mannose/glucose ratio in the glucomannan molecule was 1.00/0.13.

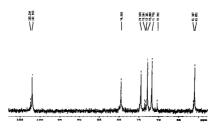


Figure 3. 13C-NMR spectra of panomensis glucomannan in D2O at 25°C.

The <sup>13</sup>C-NMR spectrum (Fig. 3) showed characteristic anomeric signals at 102.39 ppm for β-D-glucose, and at 101.99 ppm for D-mannose residues. The C-4 chemical shifts of the glucosyl and mannosyl units involved in glycosidic linkages were not separated and appeared at 79.56 ppm. The signals at 74.55, 72.90 and 71.77 ppm were assigned to C-5, C-3 and C-2 of mannose residues, respectively. The characteristic resonances of C-5, C-3 and C-2 of β-1,4 linked glucose residues were observed at 74.55 (overlapped with the C-5 of mannose unit), 73.54 and 73.32 ppm, respectively. The signals in the high magnetic field at 61.33 and 61.07 ppm were generated by the resonances of nonsubstituted C-6 of glucosyl and mannosyl residues, respectively. Besides, a low intensity signal at 70.38ppm could be assigned to substituted C-6 of glucosyl or mannosyl residues. Thus, the results confirmed a linear structure of glucomannan composed of 1,4-linked D-mannosyl and D-glucosyl units with a mole ratio of 1/0.1 and the β configuration of glycosidic bond in the main chain and the presence of short side chains at C-6.

## 3.4. X-ray diffraction

The X-Ray curve of panomensis glucomannan is shown in Fig. 4. As observed, the pattern of polysaccharide had both sharp peaks at  $2\theta = 15^\circ$ ;  $22^\circ$ ;  $24^\circ$ ;  $26^\circ$  (quite low intensity) and a very broad peak around  $2\theta = 25 \div 60^\circ$ . This results showed that the panomensis glucomannan had both amorphous and crystallized structure.

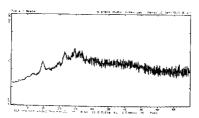


Figure 4. X-ray diffraction of panomensis glucomannan.

## 4. CONCLUSIONS

In this study, a glucomannan was isolated in 4 % yield (w/w) from tubers of the Vietnamese Amorphophallus panomensis plant by a simple method without using toxic chemicals. IR and NMR spectra indicated that the obtained panomensis glucomannan consists of  $\beta$ -1,4-linked D-mannosyl and D-glucosyl units with a mole ratio of 1.00/0.13. The presence of short side chains at C-6 was also observed. The molecular weight was determined by viscosimetry to be about 1.2× 10<sup>6</sup> Da. X-ray diffraction showed that this glucomannan has both amorphous and crystallized structure.

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## TÓM TẮT

## ĐẶC TRƯNG CỦA GLUCOMANNAN TỪ CÂY NƯA THÁI (AMORPHOPHALLUS PANOMENSIS) Ở VIỆT NAM

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Bài báo này trình bày phương pháp tách glucomannan từ củ cây nưa thái (Amorphophallus panomensis) bằng phương pháp đơn giản không sử dụng các hoá chất độc hại. Hàm lượng phán trăm, cấu trúc hoá học (xác định bằng phương pháp phổ hồng ngoại và phổ cộng hưởng từ hạt nhân), mức độ kết tinh (xác định bằng phương pháp đo nhiều xạ tia X) và khối lượng phân từ (xác định bằng phương pháp đo độ nhớt) của glucomannan đã được xác định. Kết quả cho thấy, hàm lượng glucomannan trong củ nưa là 4 %, khối lượng phân từ đạt khoảng 1,2.10° Da, có cấu trúc vở định hình và được cấu tạo từ các đơn vị cấu trúc là mannozo và glucozo với tỉ lệ tương ứng là 1,00/0,13.

Từ khoá: Nưa thái (Amorphophallus panomensis), glucomannan, konjac.