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EXTRACTION AND PHYSICO-CHEMICAL CHARACTERIZATION OF WATER-SOLUBLE POLYSACCHARIDES FROM *OPUNTIA DILLENII* (KER GAWL.) HAW. CLADODES[#]

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Abstract. *Opuntia dillenii* (Ker Gawl.) Haw., a well-known member of the *Cactaceae* family, plays an important role in agriculture and medicine. In this study, the extraction conditions and the physico-chemical properties of water-soluble polysaccharides (OMP) from *Opuntia dillenii* mucilage were investigated. The cactus cladodes used in the study were collected from Binh Thuan province, Viet Nam. The optimum extraction conditions of water extraction included the conventional heating at temperature 70 ^{o}C for 120 min. The crude and deproteinized polysaccharides yield were 17.7 % and 7.9 %, respectively. By analysis of gel permeation chromatography (GPC), the study showed that the polysaccharides possessed a low average molecular weight (129,681 Da). In addition, the characterization of polysaccharide was determined by Fourier-Transform Infrared Spectroscopy (FTIR), and High-Performance Liquid Chromatography (HPLC). The results indicated that the types of sugars in the water-soluble polysaccharides extracted consisted of arabinose, galactose, rhamnose, xylose, and glucose.

Keywords: Opuntia dillenii (Ker Gawl.) Haw., *Cactaceae*, mucilage, polysaccharide, water-soluble polysaccharide.

Classification numbers: 1.1.1, 1.1.6.

1. INTRODUCTION

Opuntia dillenii Haw. is a cactus belonging to the *Cactaceae* family, widely grown in tropical countries [1]. In Viet Nam, it can live on all types of soil, especially, deserts and semi-desert regions as Ninh Thuan and Binh Thuan provinces.The *Opuntia dillenii* has capability of

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heat-resistant up to 49 ^{o}C in a dry season. Especially, when grown in the Northern provinces of Viet Nam, it can survive the winter weather, during the temperature decreasing to below 10^{o}. The cladodes were reported to have several health-promoting activities. They are considered edible, safe, and non-toxic, generally utilized as a fresh or processed vegetable for human consumption [2]. The cladodes are used as folk medicines for the treatment of diabetes, gastric ulcer, and several other diseases.

The mucilage that produced by the cladodes of *Opuntia dillenii* Haw. is a complex heteropolysaccharide and has many pharmacological activities, such as immunostimulatory, antidiabetic, antihyperlipidemic, antioxidant effects and promotion of wound healing [3 - 5]. Kalegowda *et al.* [6] found neutral sugars in mucilage such as: arabinose, galactose, rhamnose, xylose, glucose and uronic acid.

Cactus polysaccharides have paid many attentions because of their multiple important pharmacological actions. The hot water extraction technology is still the classical and main extraction method used to obtain the polysaccharides due to its safety, convenience, low cost, high activity and environment-friendly method [7]. However, the existential extraction methods have the disadvantage of producing a low extraction yield. In this report, the *Opuntia dillenii* Haw. was used as the raw material for the first time to improve the extraction yield and to analyze the physico-chemical properties of OMP in cactus, which was harvested in Binh Thuan province, Viet Nam.

2. MATERIALS AND METHODS

2.1. Materials

The fresh cladodes that identified by Dr. Van-Son Dang – Institute of Tropical Biology, Vietnam Academy of Science and Technology, were harvested from Binh Thuan province on March 2020. They were washed to remove their impurities and spines, then cut into small pieces (2×2 cm^2). After that, they were dried in an oven with a flow of air at 60 °C for 24 h. The dried pieces were grounded and stored at room temperature. All used chemicals and solvents were of analytical grade, such as: acetone, ethanol, n-hexane, chloroform, n-butanol, hydrochloric acid, sulfuric acid, copper (II) sulfate, potassium sulfate, sodium hydroxide, sodium carbonate, citric acid, sodium thiosulfate, potassium iodide.

2.2. Extraction and deproteinization of polysaccharides from cladodes

2.2.1. Extraction and deproteinization

The dried cladodes were milled to obtain the powder that soaked with n-hexane at 40 °C for 4 hours (repeat this process for 2 times) to remove fat in the sample. After that, the residue was soaked in ethanol to isolate the polarizer organic compounds. In order to extract mucilage, the powder was dispersed with distilled water at a ratio of 1/5 to 1/30 (w/v) between 30 to 90 °C in a flask and the mixture gently stirred at 600 rpm for 0.5 to 4 hours. The mucilage was separated by centrifugation at 4000 rpm for 20 min. The combined aqueous extracts were concentrated in a rotary evaporator under reduced pressure at 80°C. The concentrate was precipitated with ethanol 96^{o} (1/4, v/v), followed by centrifugation at 4000 rpm for 10 min to obtain the precipitate. Finally, the precipitate was washed with ethanol and acetone, respectively, before drying them in

a drying oven at a temperature of 45 °C to bring out the crude polysaccharide. The extract rate (%) is performed as follows:

$$\textbf{\textit{Extract rate}}\ (\%) = \frac{\textbf{\textit{polysaccharide weight}}}{\textbf{\textit{raw material weight}}} \times \mathbf{100}$$

The deproteinized polysaccharides were obtained by the Sevag method and described in [7]. The precipitate was dissolved in pure water, filtered out of the insoluble part. Next, the obtained solution was deproteinized with chloroform : n-butanol = 4:1 (v/v) at the ratio of 1/5 (v/v). The mixture was separated by centrifugation at 4000 rpm for 5 min. The supernatant was precipitated with ethanol 96^o and this process of deproteinization was repeated three times to collect the final solution of no protein.

2.2.2. Acid hydrolysis of deproteinized polysaccharides

150 mg deproteinized polysaccharide was added to 20 mL HCl 4N and hydrolyzed for 9 hours at 100 °C in a flask. At the end of the hydrolysis, the solution was neutralized by NaOH 30 %, filtered and stored in vials for determining total sugars content and HPLC analysis.

2.3. Physico-chemical properties of polysaccharides

2.3.1. pH and solubility

The pH of the 1 % w/v aqueous mucilage solution was measured with a calibrated pH meter (PH - 62K, APEL - Viet Nam).

Different solvents including as cold water, hot water, NaOH 0.1 N, HCl 0.1 N, acetone, chloroform and ethanol were used to estimate the solubility of mucilage.

2.3.2. Determination of total sugars

The hydrolyzed sample was used to determine the reducing sugar content. It was performed according to Luff-Schoorl method described in [8].

2.3.3. Sugar composition

After sample hydrolysis, the analysis of sugar composition was performed on a Agilent 1200 Series HPLC System, using the conditions as follows: quaternary pump, manual injection kit with 20 µL loop, wavelength of UV detector of 245 nm, column temperature of 30 °C, flow rate of 1.0 mL/min, and pH buffered ammonium acetate of 8.5. Mobile phase composed 82 % of ammonia buffer pH 8.5 and 18 % of acetonitrile.

2.3.4. Average molecular weight analysis by GPC

Gel Permeation chromatography (GPC) is a well-accepted method for determining the size and molecular weight. The GPC was performed on Agilent 1100 Series GPC-SEC Analysis System. The eluent was acetate buffer pH = 5, the flow rate was at 0.1 mL/min and the injection volume was 20 µL.

2.3.5. Swelling evaluation

0.01 g polysaccharide powder was immersed in 5 mL aqueous water and kept for 2 hours at room temperature. After the mucilage settled, it was removed off the excess water and weighed for the amount of the mucilage. The swelling of the mucilage in the water was calculated by comparison with the initial volume of mucilage.

$$Swelling\ evaluation = \frac{w_2 - w_1}{w_1} \quad [9]$$

where w_1 was the initial weight of the dried mucilage and w_2 was the weight of the mucilage in the swollen state.

2.3.6. FTIR spectrum analysis

The FTIR spectra were recorded using KBr pellets with an Equinox 55 IR spectrometer (Bruker, Germany) and the absorption bands were expressed in wavenumbers (cm^{-1}).

3. RESULTS AND DISCUSSION

3.1. Extraction of polysaccharides (OMP) from cladode

3.1.1. Effect of ratio of raw material and water on the yield of OMP

Ratio of water and raw material, as the remained important factor that affected on the extraction yield. In case of too small ratio, polysaccharides cannot be extracted up, completely. Oppositely, there is a dramatic increase in expenses. In this study, the effect of ratio of raw material to water on extraction yield of polysaccharides from *O. dillenii* was investigated, and the results were listed in Fig. 1A. The ratio of raw material to water was set at 1/5, 1/10, 1/15, 1/20, 1/25, 1/30 while other extraction variables were given as follows: extraction temperature of 80 °C and extraction time of 60 min. The results showed that the extraction yield of polysaccharides from the *O. dillenii* considerably grew up from 3.3 % to 15.0 %, with a rise in ratio of raw material to water from 1/5 to 1/15 (w/v). When the ratio exceeded 1/15 (w/v), the yield reached a peak.

3.1.2. Effect of extraction time on the yield of OMP

Extraction time plays an important role in the efficiency and energy cost of extraction of polysaccharides from natural products [10].

In this study, the effect of extraction time on extraction yield of polysaccharides from the *O. dillenii* was investigated and the result was shown in Fig. 1B. The extraction time was set at 0.5, 1, 2, 3 and 4 hours while other extraction variables were given as the followings: ratio of raw material and water of 1/15 (w/v) and extraction temperature of 80 °C. The result showed that the yield significantly increased from 11.3 % to 17.6 %, with the increasing of the extraction time from 0.5 to 2 hours, and then, there is a drop-off in the yield while the extraction time exceeded 3 hours. When extraction time rose from 3 to 4 hours (Fig. 1B), the extraction yield decreased, dramatically. The reason might be due to the partial nature in which the polysaccharides were hydrolyzed [11]. The maximum time was set for 2 hours in this study.

3.1.3. Effect of extraction temperature on the yield of OMP

The effect of various temperatures, of 30, 40, 50, 60, 70, 80 and 90°C, on the extraction efficiency of OMP was investigated in case of maintaining the other two factors as follows: extraction time and the ratio of raw material to water in 120 min and 1/15 (w/v), respectively. As shown in Fig. 1C, the OMP yield significantly increased from 6.3 % to 15.3 %, with the increasing of the extraction temperature from 30 to 70 °C and then decreased with increasing temperature from 80 to 90 °C. The yield was the highest at 70 °C.

At a higher temperature, the viscosity of the extracts decreased, leading to increasing the solubility of the OMP, which in turn accelerated the release and dissolution of these compounds [12]. The same fashion had been reported for polysaccharide extraction [13]. To prevent the yield loss and minimize the adverse effects of processing, temperature of 70 °C was set as the highest temperature in this study.

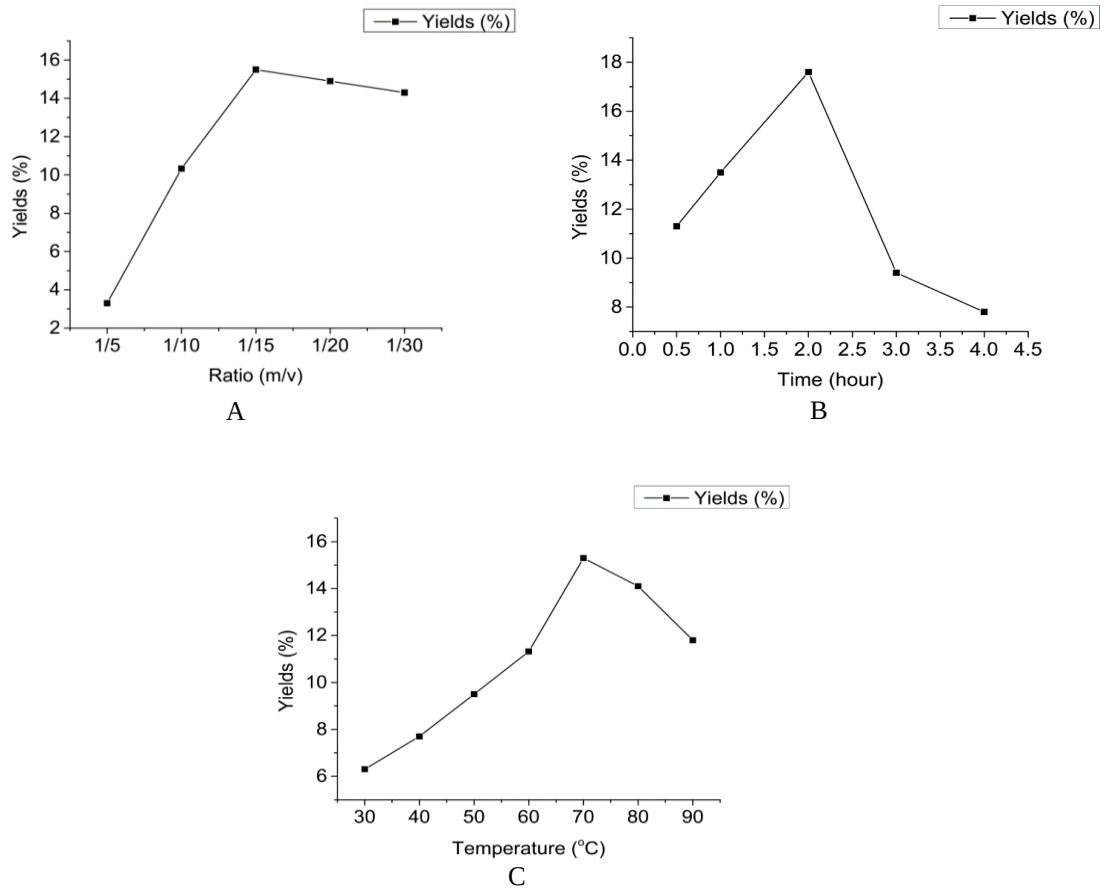

Figure 1. Effects of different raw material-to-water ratio (A), times (B), and temperature (C) on extraction yield of OMP.

After investigating the time, temperature, and raw material/water ratio, we proposed the optimum conditions for extracting water-soluble polysaccharides at 70 °C for 2 hours and the ratio of raw materials to water of 1/15 (w/v). The yield of crude polysaccharides from the *Opuntia dillenii* reached 17.7 % in these conditions. Accordingly, the report of Kalegowda *et al.* showed that the extraction yield of mucilage in water was 6.2 % [6]. The difference of two studies can be explained so that the same raw materials were not utilized, we used dry cladodes

while fresh cladodes were provided by Kalegowda, P. *et al.*. Nevertheless, in comparison to the extraction of the *Opuntia cochenillifera* (L.) Miller and using the powder cladodes, the yield of mucilage extraction was lower than that of this study. That previous report showed the obtained product from dry cladodes was 21.3 % [14]. The reason can be explained by the diversity of the type of plant, climate, and the growing environment. Especially, the difference in the raw material state enormous affects the extraction results. Moreover, the amount of obtained mucilage was also depended on the extraction conditions, the age of the cactus and the harvest season.

After removing the protein, the content of obtained polysaccharide was only half of the crude polysaccharide and the value was 7.9 %. Because in the process of washing, polysaccharide components that possess small molecular weight will be eliminated from the final product.

3.2. Physico-chemical properties of mucilage polysaccharide

3.2.1. pH and solubility

Mucilage from the *Opuntia Dillenii* at 1 % w/v suspension was determined and it was found to be slightly acidic mucilage with pH of 6.53 ± 0.01. This result is different from the data reported by Kalegowda *et al.* research whereby the mucilage was neutral [6]. This acidic nature concerned the appearance of uronic acids in the structures of the mucilage [6].

The solubility of mucilage was evaluated in a range of solvents. The results showed that mucilage was insoluble in organic solvents such as acetone, chloroform, ethanol. It formed colloidal solution in cold water but dissolve in hot water, acid and base.

3.2.2. Determination of total sugars

After titration, the hydrolysis solution by the Luff-Schoorl method, as a result, the content of mucilage was 475.54 ± 4.72 (mg/g). In case of comparison to the extraction of the *Opuntia ficus-indica*, it is hard to differentiate the specific divergence [15]. The contrast in soil, species, climate growth, and taking care of plants, along with different analytical techniques lead to this difference.

3.2.3. Analysis of average molecular weight

The average molecular weight of the extracted mucilage was found to be 129,681 Da by GPC. This result showed that the average molecular weight was quite smaller than that by Kalegowda *et al.* which was 1.9×10^3 kDa [6]. On the other hand, when comparing to other cactus like the *Opuntia ficus-indica*, the average molecular weight in this study was in the range of molecular weight that published from 2.4×10^4 to 4.3×10^6 (Da) [16]. Polysaccharides are polymeric carbohydrate structures, formed of repeating monosaccharides, so that the average molecular weight depends on the number of monosaccharide units in a polysaccharide.

3.2.4. Swelling evaluation

The swelling capacity of mucilage in water reached 10.73 %. This value shows that the swelling ability of mucilage is not good and lower than that of Kalegowda *et al.* study which was 20 % [6]. Since the molecular weight of the polysaccharide chains in our report was low, so

it was readily dissolved in the water again. Consequently, the polysaccharide's ability of swell was not as expected.

3.2.5. HPLC analysis

The results of HPLC analysis was illustrated in the Table 1. The presence of galactose, arabinose, rhamnose, xylose, glucose and the different monomeric sugars in extracted mucilage was confirmed. However, this result is different from the one reported by Kalegowda *et al.* [6] about the percentage of those monosaccharides. The highest percentage in that study was galactose with 39.05, in contrast to our study, Kalegowda showed arabinose was the highest with 38.80 %. According to Table 1 the amount of glucose and galactose increased correspondingly from 5.1 % to 10.03 % and 33.00 % to 39.05 %; however, a proportion of xylose sugar was dropped slightly from 5.10 % to 3.08 %. Especially, it is clear that the percentage of arabinose in the present research was much lower than that of the Kalegowda *et al.* report. On the other hand, the sugar content of different species of the *Opuntia* depended on not only the conditions of extraction but also the environmental factors such as location, the age of the cladodes, climatic conditions, and soil where the cactus grow. The details of sugar content of the *Opuntia* species were showed in Table 1.

Table 1. Monosaccharide composition in mucilage of *Opuntia* cladodes.

Monosaccharide	Content (%)					
	Opuntia Dillenii (This study)	*Opuntia Dillenii* [6]	*Opuntia tomentosa* [17]	*Opuntia atropes* [17]	*Opuntia hyptiacantha* [17]	*Opuntia ficus-indica* [17]
Rhamnose	14.54	15.70	2.58	1.44	1.41	1.93
Glucose	10.03	5.10	16.21	9.05	6.03	5.18
Xylose	3.08	5.10	16.02	16.62	17.05	16.32
Galactose	39.05	33.00	21.59	26.75	30.83	27.26
Arabinose	14.75	38.80	33.50	34.36	32.82	35.36

3.2.6. FTIR analysis

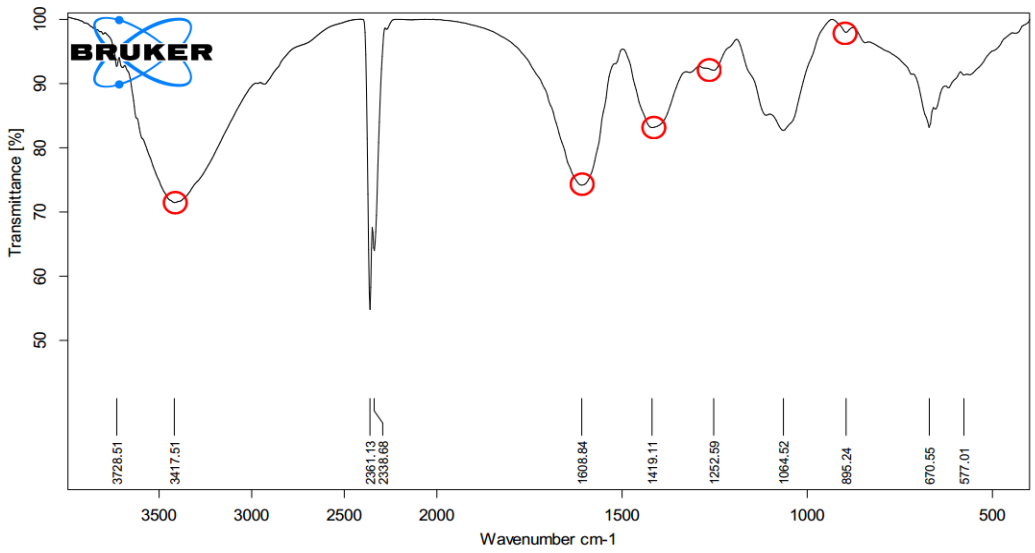

Figure 2. FT-IR spectra of mucilage.

The FTIR analysis of mucilage was reported in Fig. 2. The band at 3417 cm^{-1} is assigned for hydroxyl group. The band at 1608 cm^{-1} indicates the presence of O-H. The peak at 1419 cm^{-1} represents the methylene group CH_2-CO. The band at 1254 cm^{-1} reflects the existence of the C-O-C link. The peak at 895 cm^{-1} is attributed to the absorbance of β-glycoside.

4. CONCLUSIONS

This study reported the physico-chemical characterization of the *Opuntia dillenii* mucilage. The optimum conditions of extraction of mucilage were at 70^o for 2 hours and the ratio of the raw materials to water of 1/15 (w/v). By these extraction conditions, the yield of crude polysaccharide extracted from powder cladodes reached 17.7 % and after removing protein, the yield of deproteinized polysaccharide was 7.9 %. The total sugar content using titration method reached to 475.54 ± 4.72 (mg/g). In addition, mucilage extracted from cladodes had the average molecular weight of 129,681 Da, and swelling evaluation of 10.7 %. The HPLC spectral study revealed the presence of complex mixture of sugar such as galactose, arabinose, rhamnose, xylose, and glucose and the content of galactose was 39.05 % which is more than that of the others in the polysaccharide. The difference in the growing conditions, as well as the age of the plant and the harvest season, which would extremely affect the types and content of sugars in extracted polysaccharides. Simultaneously, the methods of extraction affect not only the sugar content but also the physico-chemical properties of polysaccharide. Combining structural analysis methods (FTIR) provides a certain reason for polysaccharide structure.

Authors contributions: THAN: conceptualization, methodology, resources, data curation, formal analysis, software, supervision, validation, visualization, writing-original draft, writing-review & editing; TKDH: conceptualization, methodology, resources, data curation, formal analysis, finance, investigation, supervision, validation, visualization, writing-original draft, writing-review & editing; TTHN: data curation, formal analysis, validation, visualization, writing-original draft, writing-review & editing; All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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