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Optimized extraction conditions of polysaccharides from *Pseuderanthemum crenulatum* (Wall. ex Lindl.) Radlk.

Nghiên cứu các điều kiện thích hợp chiết xuất polysaccharide từ cây Xuân hoa răng Pseuderanthemum crenulatum (Wall. ex Lindl.) Radlk.

Research article

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Polysaccharide has attracted great attentions for its benefits to human health. Polysaccharide from natural sources have diverse anti-inflammatory, anticoagulant and wound healing activities. Polysaccharide is not only valuable in medicine, also widely used in foodstuffs such as gel thickening or emulsifying agents, emulsifiers, fillers. Recently there has been an increase in the demand for polysaccharides, so research into new sources of polysaccharide with plant-based bio-activity is essential. Pseuderanthemum crenulatum (Wall. ex Lindl.) Radlk belong to genus of Pseuderanthemum. Common names (Vietnamese): Xuân hoa răng. This species is native in the forests of Vietnam. The polysaccharide content in P. crenulatum leaves was (7.47 ± 0.6) % in dry weight. The appropriate polysaccharide extraction conditions were determined: material/ water ratio (1g/25ml), extracted temperature of 60°C, extraction time 12 hours. The polysaccharide composition was purified by TCA 10%, with a purity of (55.6 ± 1.19) %.

Trong những năm gần đây, polysaccharide là nhóm hợp chất rất được các nhà khoa học trên thế giới quan tâm do các tác dụng quan trọng của chúng về tăng cường miễn dịch, kháng viêm, làm lành vết thương, chống ung thư… Polysaccharide không những có giá trị trong Y học mà còn được sử dụng rộng rãi trong thực phẩm như các chất tạo độ đặc hay tạo gel, chất làm bền nhũ tương, chất độn… Hiện nay, nhu cầu sử dụng polysaccharide từ thực vật ngày càng gia tăng nên việc điều tra, khai thác nguồn polysaccharide mới có hoạt tính sinh học là rất cần thiết. Pseuderanthemum crenulatum (Wall. ex Lindl.) Radlk thuộc chi Pseuderanthemum sp, *tên thông thường là cây Xuân hoa răng, là cây mọc tự nhiên trong rừng Việt nam. Trong nghiên cứu này, chúng tôi đã tách chiết, xác định hàm lượng và tinh sạch sơ bộ polysaccharide từ lá cây Pseuderanthemum crenulatum. Hàm lượng polysaccharide trong lá cây Xuân hoa răng đạt (7.47 ± 0.6) % trọng lượng khô. Các điều kiện chiết rút polysaccharide thích hợp đã được xác định: nhiệt độ chiết rút 60°C, tỷ lệ nguyên liệu/nước (1g mẫu khô/25ml nước), thời gian chiết rút 12 giờ. Chế phẩm polysaccharide đã được tinh sạch bằng TCA 10%, có độ sạch đạt (55.6 ± 1.19)%.*

Keywords: Extraction, polysaccharide, Pseuderanthemum crenulatum

1. Introduction

Polysaccharides, a type of macromolecule carbohydrate polymer, are found in a host of herbal plants and fungus [5; 10; 12]. During past decades, more and more attentions were focused on it allowing for its multiple functional compounds and their bioactivities which will no doubt make a contribution to the potential and promising applications in the area of food, materials and pharmaceuticals. Polysaccharides have many general beneficial effects for human health and therefore been developed into potential cosmeceuticals and nutraceuticals. Polysaccharide from natural sources have diverse anti-inflammatory, anticoagulant and wound healing activities [13; 3]. The polysaccharides such as beta-glucans [14], pectin [7], group galactomannan [11] are more anti-inflammatory effective. Polysaccharides from several medicinal plants have been shown to stimulate prolifera-

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tion of keratinocytes and dermal fibroblasts [2]. Recently there has been an increase in the demand for polysaccharides, so research into new sources of polysaccharide with plant-based bio-activity is essential.

Pseuderanthemum crenulatum (Wall. ex Lindl.) Radlk belong to *Pseuderanthemum*. Common names in Vietnamese is Xuân hoa răng. The species is native to China, India, Laos, Malaysia, Thailand and Vietnam where it lives in the forests. This species usually does not surpass the 1-3m of height. The body is dark brown, fluffy. Petiole 1-4 cm long, with fluffy. Leaf-oval to ovate margin, size 5-15 x 3-5,5 cm, top face light green and feathery along the veins, with less feathering surface. Cluster of towers size 3-10 cm, carrying thick flowers. racemose terminal inflorescences, up to 12cm long, with a crowd of bilabiate flowers, of about 2,5 cm of diameter, pale blue to lilac with a white spot at the centre of the lower lip, that open gradually [8].

Pseuderanthemum genus belongs to the Acanthacea family have 9 species in Vietnam, but only 2 species (*P. palatiferum* and *P. carruthersii* var. *atropurpureum*) are used to treat and prevent many diseases, especially the anti-inflammatory and wound healing effect. However, the research on effective constituents from *Pseuderanthemum* genus has mainly been focused on small-molecular compounds. Leaves of *P. crenulatum* has a very high viscosity. Therefore, the investigation and exploitation of polysaccharide source from this plant will provide new scientific information on the source of medicinal herbs in Vietnam.

Very limited work has been done on polysaccharide of *Pseuderanthemum* sp which calls for a more detailed research. The aim of this paper was to determine the optimal extract conditions using water extract by considering some factors: temperature, heating time and sample to water ratio.

2. Materials and methods

2.1. Materials

Pseuderanthemum crenulatum (Wall. ex Lindl.) Radlk were collected in Hien Chung commune, Quan Hoa district, Thanh Hoa province on the 6th October 2017. Samples were kept in the Herbarium, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, Ha Noi, Vietnam. Sample (code DVH 171) was identified by Dr. Do Van Hai.

The standard glucose and phenol (Sigma-Aldrich Chemical Co., USA), sulfuric acid and trichloroacetic acid (TCA) (Merck); UV/VIS Spectrophotometer **(**Shimazu, Japan) were used in analysis.

2.2. Methods

***Extraction of polysaccharides preparation*:** 1.00 g of dried and shattered *P. crenulatum* was weighed to dip into water at certain ratios ranging (1/15; 1/20; 1/25; 1/30 g/ml (sample/water).

Five groups of the mixture were heated at different temperature (50, 60, 70, 80 and 90ºC) with constant stirring for set times (3, 6, 9, 12, 15 h). To obtain the crude polysaccharide, ethanol was applied for precipitation with ratio of 3/1 (ethanol/sample). The depurated sediments were dried at the temperature of 60ºC and the polysaccharides were obtained for further analysis.

***UV spectrometer detection preparation*:** phenol-sunlfuric acid method was used to determine the polysaccharide content [4]. It is necessary to make sure that the detection with UV spectrometer can finish in a set time after polysaccharides extraction, for the color would change with time lasting.

***Standard curve*:** Glucose was used to obtain a standard curve. The glucose solutions at concentrations of 0, 25, 50,75, 100 µg/ml were prepared to test by the sulfuric acid -phenol method.

***Deproteination by TCA method* [9]:** Deproteination of crude polysaccharides by trichloroacetic acid method: removed protein by TCA 5%, 10%. The mixture was kept at 4ºC for 4 h, then centrifuged to remove the precipitate.

Polysaccharide Spectrophotometry: Polysaccharide extraction was performed on a spectrophotometer using the method of Liang et al [6]. Polysaccharide after extraction is diluted with distilled water at a concentration of 100 µg/ ml and then scanned with a UV-vis spectrophotometer (Shimazu, Japan) at a wavelength of 200 to 700 nm.

Statistical analysis

All extractions were performed in triplicate. The apparent content of polysaccharide obtained at different conditions were analysed and expressed as mean ± standard deviation. T test were used to determine the differences amongst the means. *p*-values< 0.05 were considered to be significantly different.

3. Results and discussion

3.1. Effect of temperature on the extracted amount

Based on the published plant polysaccharide extraction studies [1; 15], polysaccharide from *P. crenulatum* was extracted by distilled water. The extraction ratio increased with increasing temperature to 70ºC and decreased with further increases in temperature (Fig.1). At the temperatures between 50-70ºC, the concentration of polysaccharide extracted increased at a relative stable speed. This suggests that the higher the temperature is, the greater the level of destruction of the cell wall. There were no significant differences when extracted at 60°C and 70°C ($p>0.05$).

Therefore, 60ºC was fixed as a constant parameter for the subsequent experiments. This temperature also suitable for high temperature studies for extracting polysaccharides. However, increasing the extraction temperature to

90ºC can lead to evaporation of solvents, energy costs, and more contaminants.

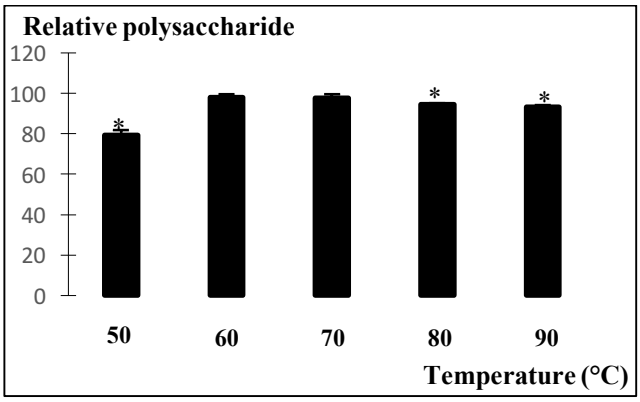

Figure 1. Effect of the extraction temperature on the polysaccharide content. (sample/water ratio=1/20; (g/ml), time=6 h) * = $p < 0.05$ vs 60°C

3.2. Effect of extraction time on extraction of polysaccharide

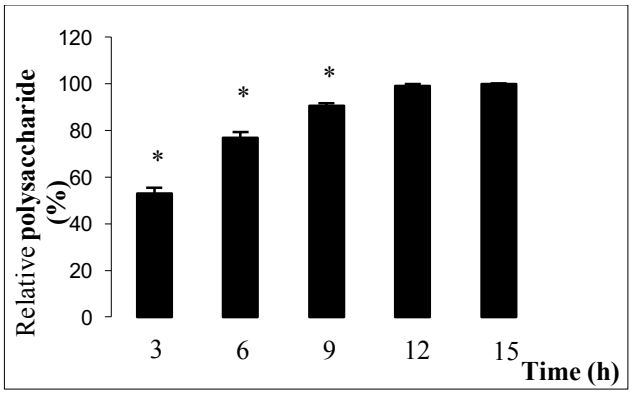

Figure 2. Effect of the extraction time on the polysaccharide content. (sample/water ratio=1/20 (g/ml), temp. = 60 ºC) * = $p < 0.05$ vs 12h

The heating time plays a major role in the polysaccharides extraction efficiency. In this study, the polysaccharides distributing in the cell of plants require time to dissolve in water. Therefore, the heating (60°C) time was examined over the range of 3-15 h (Fig. 2). The highest peak was observed after heating for 12 h. There were no significant differences when extracted at 12h and 15h ($p>0.05$). Therefore, 12 h was selected for the optimal heating progressing.

3.3. Effect of sample/water ratio on extracted amount

The extraction rate was also related to sample/water ratio. Large volumes of solvent are not only uneconomical but can also influence the extract efficiency. Higher solvent extraction rates will make the extraction process faster, low density and viscosity will facilitate the release of polysaccharide molecules in water, the amount of polysaccharide dissolved in higher extracts. A series of extractions were carried out at different sample/water ratios (1/15, 1/20, 1/25, 1/30) to evaluate its effect (Figure 3).

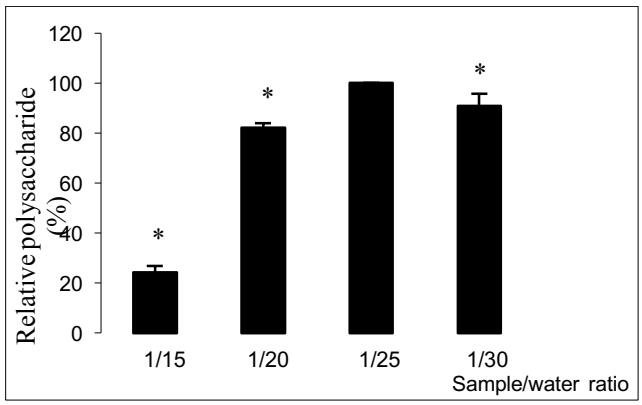

Figure 3. Effect of sample/water ratio on the polysaccharide content (temp. = 60 ºC, time = 12h), * = $p <$ 0.05 vs 1/25 (sample/water)

With increasing amount of water, the leaching-out rates is elevating until the sample/water ratio reaches to 1/25 g/mL and decreased with further increases in rate. Therefore, the ratio of 1/25 g/mL was considered as the optimal ratio choice for next testes.

Due to the multiple bioactivities and extended medical applications of polysaccharides extracting from different edible nature plants, the most efficient method and conditions for the extraction acting as the most important preparation for further separation and activities study were urgent to figure out. Based on the whole experiment, the optimal parameters were 60ºC, 1/25 of ratio of dried sample to water and heating time of 12h. According to the results, the polysaccharide content in *P. crenulatum* leaves was (7,47 ± 0,6) % in dry weight.

3.4. Deproteination

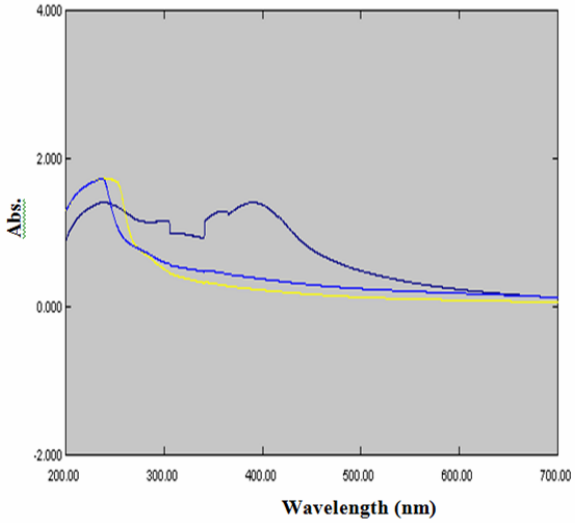

Figure 4. The UV absorbance spectra of polysaccharide before and after purification by TCA

: Crude polysaccharide
: Purified polysaccharide by TCA 10%
: Purified polysaccharide by TCA 5%

P. crenulatum was extracted with water (60ºC, 1/25 of ratio of dried sample to solution and heating time of 12h) and precipitated with ethanol (1V extracted polysaccha-

ride/3 V ethanol). To remove the proteins in crude extracts, TCA was added to the extract as described in the methods section. The purified polysaccharides after treatment with TCA were evaluated for purity by UV-vis spectrophotometer. Samples were scanned at a wavelength of 200–700 nm [9].

The UV-visible spectra (Figure4) showed that purified polysaccharides by TCA had an absorption peak at 210 nm only, which is the characteristic UV absorption peak for a polysaccharide. Purified polysaccharide by TCA 10% has higher purity than purified polysaccharide by TCA 5% (table 1).

Table 1. Deproteination of polysaccharides extracts from *P. crenulatum*

Characteristics	Polysaccharide were removed protein by TCA 5%	Polysaccharide were removed protein by TCA 10%
% yield (g/100 g dry weight)	1.33 ± 0,15 %	1.25 ± 0.13 %
Polysaccharides determination	47.4 ± 1,65 %	55,6 ± 1,19 %*

Note: * = $p < 0.05$ vs removed protein by TCA 5%.

4. Conclusion

In this study, we extracted and determined the polysaccharide content from the leaves of Pseuderanthemum crenulatum (Wall. ex Lindl.) Radlk was (7.47 ± 0.6) % in dry weight. The appropriate polysaccharide extraction conditions were determined as: material/water ratio (1g/25ml), extracted temperature of 60°C, extraction time 12 hours. The polysaccharide composition was purified by TCA 10%, with a purity of (55.6 ± 1.19) %.

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