EFFECTS OF EXTRACTION CONDITIONS ON POLYSACCHARIDES OF CANTHIUM LEAVES (*CANTHIUM HORRIDUM* BLUME)

● DANG THI NGOC DUNG* - NGUYEN NGOC CHAU

ABSTRACT:

This study is to examine the factors affecting the extraction of polysaccharide from *Canthium horridum* Blume leaf. The extraction of polysaccharide from *Canthium horridum* Blume leaf was conducted under different conditions, including the ratio of water and raw materials (20, 25, 30, 35, 40, 45 and 50 mL/g), extraction temperature (50, 60, 70, 80, 90 and 95^{0}C) and extraction time (30, 60, 90, 120 and 150 minutes). The highest polysaccharide extraction yield was 22,022% (compared to the mass of raw materials) when the extraction was conducted at 90^{0}C for 60 minutes with the ratio of water/material of 40 mL/g. The FTIR spectrum of the *Canthium* polysaccharide sample showed the similarity of the *Tiliacora triandra* polysaccharide.

Keywords: Canthium leaf, polysaccharides, extraction, yield.

1. Introduction

Canthium horridum Blume is present in the countries such as India, Thailand, China, Vietnam, Singapore, Philippines; polysaccharides of *Canthium* leaves can produce jelly in water and drinks. *Canthium* leaves are often used by people in Vietnam's northern provinces (Cao Bang and Lang Son, etc), processed into cooling dishes such as jelly, sweet soup, etc. So studies of *Canthium* trees and the gel-forming properties of the *Canthium* leaves have not yet been researched popular. Polysaccharides belong to the carbohydrate group, which has a molecular mass that can reach tens of thousands or even millions of Daltons [18].

Polysaccharides are raw materials in the food industry because of their ability to change and control the functional properties of food systems. The most important characteristics of polysaccharides are their viscosity (including the ability to dense, gel) and bond to water [11]. Other important functions include stabilizing the emulsion system, preventing the re-crystallization of ice molecules, and improving sensory properties. Polysaccharide extracts from plants are interesting additives for industries, especially for the food industry, because consumers prefer the natural ingredients used in food products [12]. The extraction of polysaccharides in the *Canthium* leaves depends on many factors such as solvent, extraction temperature, extraction time, material and solvent ratio, extraction method, etc. To extract the polysaccharide content for high performance and safety in food and pharmaceuticals, water is considered the best because the water is not toxic, not flammable, cheap.

The goal of the study was to find conditions for extracting polysaccharides from high-*Canthium* polysaccharide yield, as a database for research and development of polysaccharide production technology from glove leaves on an industrial scale.

2. Materials and methods

2.1. Raw materials

Figure 1. Dry Canthium leaves before and after treatment

Dried *Canthium* leaves were purchased as households in Chi Linh town, Hai Duong province between March and May 2020. After buying, dry *Canthium* leaves are ground into powder, not filtered through sieves.

Dry powdery *Canthium* leaves are kept in PE bags. They are sealed and stored at room temperature.

2.2 Research methods

2.2.1. Survey on the effect of extraction temperature and the ratio of water/material to polysaccharides yield

Weigh 2 g of *Canthium* leaf powder into Erlen 250mL. Add distilled water with the ratio of water/material (v/w) of 20, 25, 30, 35, 40, 45 and 50 (mL/g), respectively.

After that, mix the *Canthium* leaf powder well with the water. Next, put Erlen in the water bath at temperatures of 50, 60, 70, 80, 90, 95oC and regularly shake Erlen with an extraction time of 60 minutes. After the extraction process is finished, Erlen in the water bath was taken and filtered out to collect the extract. The extract after filtration will be centrifugal at 3000 rpm for 10 minutes. After centrifugal, take the upper extract and the extract was dispersed in an alcohol 96% (96% alcohol is supplemented so that the alcohol content in the extract reaches 70% and soaking for 30 minutes). The product was then dried at 50^0C in an air oven to constant weight.

2.2.2. Survey on the effect of extraction time on polysaccharides yield

Weigh 2 g of *Canthium* leaf powder into Erlen 250mL. The extraction temperature and the ratio of water/material leaves (v/w) are carried out according to the results of the extraction performance survey in section 2.2.1. Next, samples were surveyed at different extraction times: 30, 60, 90, 120, and 150 minutes.

2.3. Polysaccharides content determination method

The content of polysaccharides is determined by the Long method [8]:

$$\% \text{ Gel} = \frac{m_1 - m_2}{m_{material}} \times 100\,(\%) \qquad (1)$$

m_1, m_2, $m_{material}$ (g): the mass of polysaccharides and petri dish before drying, the mass of polysaccharides and petri dish after drying; the mass of leaf powder.

2.4. Surface structure analysis

Fourier transform infrared spectra (FTIR) of samples were collected using FTIR-47000 (Jasco, Japan) with wavelengths ranging from 400 - 4000 cm^{-1}.

2.5. Statistical analysis

The experiments were conducted in three replicates. The values were expressed as the mean ± standard deviation. Statistical analysis was performed using the Microsoft Excel 2010 data analysis software and R software (version 3.1.1, 32-bit) for Windows. The confidence limits used in this study were based on 95% ($P< 0.05$).

3. Results and discussion

3.1. Chemical components in Canthium leaves

Table 1. Chemical components in Canthium leaves

Parameter	Unit	Content (% dry basic)
Moisture	%	9,8199 ± 0,0514
Ash	%	6,6351 ± 0,0247
Protein	%	10,9670 ± 0,0452
Lipid	%	2,6674 ± 0,0392
Total sugars	%	4,3746 ± 0,2053

As shown in Table 1, the chemical components in *Canthium* leaves include: moisture, ash, protein, lipids, total sugar content is 9,8199; 6,6351; 10,9670; 2,6674 and 4,3746%.

When comparing the chemical content of *Canthium* leaves with *Tiliacora triandra* leaves and Platostoma palustre leaves, the composition of these leaves also has similarities. Specifically, in the report of Singthong, J. et al. (2009), moist, ash, protein, and lipid components in dry *Platostoma palustre* leaves were 7,63; 8,46; 6,59 and 1,26%. Moreover, in the research result of Trinh Khanh Son et al. (2018), the moist, ash, protein, lipid in dry *Platostoma palustre* leaves are 9,12; 26,46; 9,11 and 2,03%.

3.2. Survey results of factors affecting the extraction of polysaccharides from Canthium leaves

3.2.1. Survey results of factors affecting the extraction temperature and the ratio of water/material to polysaccharides yield

Temperature as an independent variable increases the solubility of compounds and reduces the viscosity of the solvent, which allows the solvent to better penetrate into the solid substrate [10].

At the ratio of water/material = 20 (mL/g), polysaccharide extraction yield increased from 13,221 to 19,170% when the extraction temperature increased from 50 to 95^{0}C. This result is consistent with the report of Samavati, V., (2013) in extracting polysaccharides from okra and Yin, G., et al. (2008) on a polysaccharide extract from Lycium barbarum. Polysaccharide extraction yield increases when extraction temperature increases (Samavati, V., (2013) and Yin, G., et al. (2008)). It explains the above result as the polysaccharide extraction yield increases when the extraction temperature rises due to increased solubility of polysaccharides in solvents[2]. On the other hand, the extraction process at high temperatures leads to quicker and easier extraction of polysaccharides from the cell wall into the water [1]. As the extraction temperature rises, the proportion of raw materials and water increases, the yield of extracting polysaccharides into the water increase [15]. Li, Cui, & Kakuda (2006) reported that the rise of the polysaccharide diffusion coefficient and the solubility of polysaccharides in solvents when extracted at high temperatures, resulted in an increase in polysaccharide content coming out of the material into the extract solution.

Table 2. Survey on the effect of extraction temperature and the ratio of water/material to polysaccharide yield

The ratio of water/ powder (/g)	Polysaccharides yield (%)					
	50^{0}C	60^{0}C	70^{0}C	80^{0}C	90^{0}C	95^{0}C
20/1	13,221 ± 0,344^{bC}	15,218 ± 0,741^{cB}	15,371 ± 0,522^{cB}	15,995 ± 0,308^{cB}	18,188 ± 0,483^{dA}	19,170 ± 0,229^{aA}
25/1	13,750 ± 0,778^{bD}	15,622 ± 0,357^{cbC}	15,763 ± 0,240^{cCB}	16,975 ± 0,560^{cbB}	19,522 ± 0,290^{cA}	19,435 ± 0,288^{aA}
30/1	16,623 ± 0,513^{aB}	16,690 ± 0,414^{baB}	16,970 ± 0,212^{bB}	17,510 ± 0,135^{bB}	20,160 ± 0,203^{cbA}	19,705 ± 0,723^{aA}
35/1	16,615 ± 0,354^{aD}	16,717 ± 0,195^{baD}	18,080 ± 0,360^{aC}	18,712 ± 0,454^{aCB}	20,612 ± 0,358^{bA}	19,805 ± 0,624^{aB}
40/1	17,110 ± 0,761^{aE}	17,190 ± 0,324^{baED}	18,167 ± 0,175^{aDC}	18,763 ± 0,103^{aC}	22,022 ± 0,147^{aA}	20,040 ± 0,241^{aB}
45/1	17,060 ± 0,291^{aD}	17,201 ± 0,498^{aD}	18,140 ± 0,324^{aC}	18,778 ± 0,321^{aC}	22,103 ± 0,123^{aA}	20,110 ± 0,253^{aB}
50/1	17,135 ± 0,320^{aD}	17,223 ± 0,098^{aD}	18,217 ± 0,357^{aC}	18,710 ± 0,634^{aC}	22,112 ± 0,154^{aA}	20,210 ± 0,297^{aB}

*Data represents the mean ± SD (n = 3). Statistically significant: *p = 0,001.*
Survey on the effect of extraction temperature on polysaccharide yield

At the proportion of water/materials since 20 (mL/g) to 50 (mL/g), showing that the polysaccharide content increased with rising heat from 50÷90^{0}C (Tab. 2). The extraction polysaccharide yield ranged from 16,615 to 20,612% (water/material = 35mL/g) and from 17,110 to 22,022% (water/material = 40mL/g) (Table 2).

Besides, the result shows that the polysaccharide extraction yield increase from 13,221 to 19,170% (water/material=20mL/g) with inreasing heat from 50÷90^{0}C. Contrast, the polysaccharide extraction yield decreased with increasing heat from 90 to 95^{0}C, the extraction performing decreases from 22,022 to 20,040% (water/material = 40 mL/g) and declined from 22,103 to 20,110% (water/raw material = 45 mL/g).

This result is explained when the extraction temperature is raised too high, polysaccharides in *Canthium* leaves are altered properties leading to a decrease in extraction performance [6]). Survey on the effect of the ratio of water/material to polysaccharide yield

The Govender, S report that the ratio of water to raw materials will influence the extraction yield[5]. Polysaccharides in *Canthium* powder cannot be extracted well when the water/material scale is too small. But process costs are high when the water/material proportion is too large [17]. The effects of ratio water and material on the polysaccharide extraction yield are shown in Table. 3.

The analysis result, extraction heat increased from 50 to 95^{0}C for 60 mins, performing extracting polysaccharides from *Canthium* leaves increased, as the ratio of water to material increased. Specifically, the extraction yield gained from 13,221 to 16,623% in 50^{0}C (water/material = 20 mL/g; and extended from 15,218 to 16,690% at 60^{0}C (water/raw = 30 mL/g). The water/material ratio continues to increase from 40 to 50 mL/g, the extraction yield varies is not significant (from 17,19 to 17,223% at 60^{0}C).

Polysaccharide extraction yield grows faster, 15,371 ± 0,522% (water/material = 20 (mL/g), 18,167 ± 0,175% (water/material = 40(mL/g) with extraction temperature at 70oC); 15,995 ± 0,308% (water/material = 20 (mL/g), 18,778 ± 0,321% (water/material = 45(mL/g), with extraction temperature at 80^{0}C); but they are reduced when increasing the ratio of water/ powder from 45 to 50(mL/g), such as 18,778 ± 0,321% (water/material = 45(mL/g), 18,710 ± 0,634% (water/material = 50(mL/g), with extraction temperature at 80^{0}C). The trend to reduce polysaccharide extraction yield of *Canthium* leaves is again received at the extraction condition of 90 and 95^{0}C with a water/material proportion of 40 mL/g. This result is explained by an expansion of water/material properties that can increase the diffusion capacity of solvents into the cell and enhance the release of polysaccharides from the cell [10].

Some studies are according with the results; Yin, G, (2018) extracted polysaccharides from Lycium barbarum fruit; XuJie, H., and Wei, C. (2008) extracted polysaccharides from Bachu mushroom (extraction efficiency raised from 1.5% to 8.8% as the water/material scale increased from 1 to 6 mL/g).

3.1.2. The result of the effect of the extraction time on polysaccharide yield.

The effect of the extraction time: 30, 60, 90, 120, 150 minutes (with water/material = 40 mL/g, at the extraction condition of 50^{0}C) to performing receiving polysaccharide leaves are shown in Table 3.

Table 3. The result of the effect of the extraction time on polysaccharide yield.

Extraction time (mins)	The extraction yield (%)
30	16,520 ± 0,304^a
60	17,210 ± 0,774^a
90	17,230 ± 0,667^a
120	17,312 ± 0,0,271^a
150	17,322 ± 0,202^a

Mean value from triplicate means ± standard deviation. Values with different superscripts in the same column are significantly different (p< 0.05).

The extraction time is one factor that affects the polysaccharide extraction yield in raw materials. The longer the extraction point will also affect polysaccharide content is extracted[16]. The performance of extracting polysaccharides from *Canthium* leaves increased from 16,520 to 17,210%

when the extraction time extended from 30 mins to 60 mins. The time needed for polysaccharide contact with the extracted environment where the liquid penetrates the material dissolves polysaccharides and then diffuses out of *Canthium* leaves[10]. Next, the polysaccharide extraction yields do not change significantly when the extraction time increases, 17,210% (60 minutes) to 17,322% (150 minutes). The above research is also consistent with Samavatis analysis results (2013) on the effect of time on the okra polysaccharide extraction yield, 8,8% (0,5h) to 15,7% (6,5h); The extraction performance does not change if increase the time (over 150 mins). Some studies are according to the results; XuJie, H., et al, C. (2008) extracted polysaccharide from wild BaChu mushroom; the output of extraction rise greatest when extraction time extends from 2 to 8 hours, after 8 hours, sugar yield doesnt increase the value.

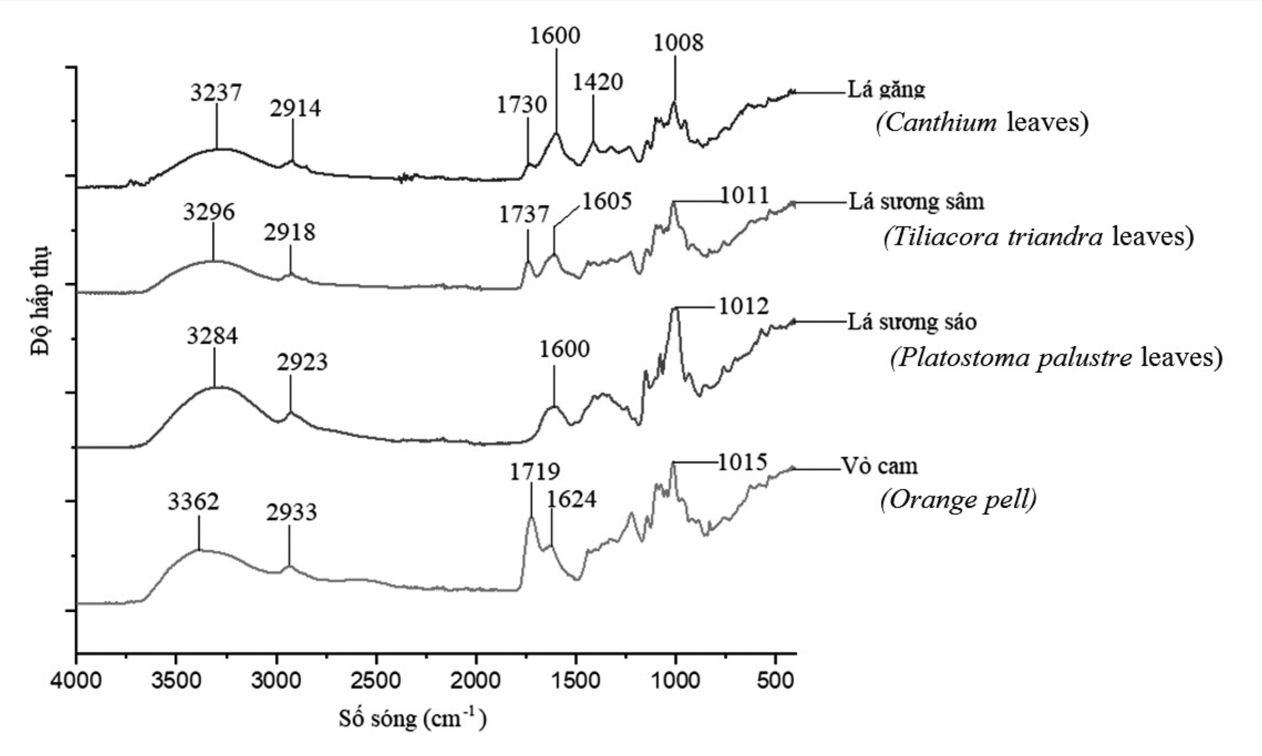
3.3. FTIR analysis

The FTIR spectrum of polysaccharide *Canthium* leaves, Tiliacora triandra leaves, Platostoma palustre and orange peel are shown in Figure 1.

The FTIR spectra showed the functional groups and structural information of different extracted polysaccharides. The wavelength range of 950 and 1200 cm^{-1} are considered as the finger print region for carbohydrates as it allows to identify of the major chemical groups (ether R-O-R and cyclic C - C bond) in polysaccharides[3]. The FTIR spectrum of *Canthium* polysaccharides and other polysaccharides have similarities and non-similarities waves (Fig. 3).

The *Canthium* polysaccharides peaks with high absorption of the following wavenumbers: 3237cm^{-1}, 2914cm^{-1}, 1730cm^{-1}, 1600cm^{-1}, 1200÷950cm^{-1}; especially, concentrated in 1800÷950cm^{-1}. The carbonyl bands at 1730cm^{-1} show the presence of esterified carboxyl groups [4]. According to analytical results (Table 3), the FTIR spectrum of *Canthium* polysaccharides and *Platostoma palustre* polysaccharides are the similarities. The broadband, from 2,900 to 3,600cm-1, was due to absorbed moisture in the samples. The O=C-O structure at 1630 to 1420cm^{-1} [9], the absorption of the *Canthium* polysaccharide is similar to the *Platostoma palustre*

Figure 2. The FTIR spectrum of polysaccharide Canthium leaves, Tiliacora triandra leaves, Platostoma palustre and orange peel



polysaccharides. In addition, the absorption of the polysaccharide sample at 1730 cm^{-1} is lower than at 1600 cm^{-1} (Figure 1). Therefore, it can be concluded that the *Canthium* polysaccharide sample belongs to low methoxyl pectin [14, 4].

4. Conclusion

Cathium leaves are an origin to collect polysaccharides. The extraction conditions (such as temperature, water/material, time) influence the polysaccharides yield. The polysaccharides extraction yield reached 22,022% (extracted at 90^0C, water/powder = 40mL/g, 60 minutes. Besides, the FTIR spectrum of *Canthium* polysaccharides and *Platostoma palustre* polysaccharides are the similarities. The *Canthium* polysaccharide belongs to low methoxyl pectin ■

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Author information:

1. DANG THI NGOC DUNG[*1]
2. NGUYEN NGOC CHAU[1]
[1]**Ho Chi Minh city of University of Technology & Education**
** Corresponding Author: Dang Thi Ngoc Dung; Email: dzungdang@hcmute.edu.vn; 0908801818*

ẢNH HƯỞNG CỦA MỘT SỐ ĐIỀU KIỆN TRÍCH LY ĐẾN HÀM LƯỢNG POLYSACCHARIES TỪ LÁ THẠCH GĂNG *(CANTHIUM HORRIDUM BLUME)*

● **ĐẶNG THỊ NGỌC DUNG**
● **NGUYỄN NGỌC CHÂU**
Trường Đại học Sư phạm Kỹ thuật TP. Hồ Chí Minh

TÓM TẮT:

Nghiên cứu nhằm khảo sát các điều kiện trích ly polysaccharides từ lá găng (*Canthium horridum* Blume). Quá trình trích ly polysaccharides từ lá thạch găng đã được thực hiện với các điều kiện chiết xuất khác nhau: tỷ lệ nước và nguyên liệu lần lượt là: (20, 25, 30, 35, 40, 45 và 50 (ml/g)), nhiệt độ trích ly (50^0C, 60^0C, 70^0C, 80^0C, 90^0C và 95^0C) và thời gian trích ly (30, 60, 90, 120 và 150 phút). Hiệu suất thu nhận gel lá găng thô cao nhất là 22,022% (so với khối lượng nguyên liệu) khi trích ly ở nhiệt độ 90^0C, tỷ lệ nước/bột lá găng là 40 ml/g trong thời gian trích ly 60 phút. Phổ FTIR của mẫu bột gel lá găng cho thấy sự tương đồng của gel lá găng với gel lá sương sâm.

Từ khoá: lá găng, polysaccharires, trích ly, hiệu suất.