Botanical Characteristics and Pectin Properties of *Canthium parvifolium* Roxb.

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Abstract

Canthium parvifolium Roxb. is a native plant found in the northern region of Thailand. *Canthium parvifolium* Roxb. has been found to have pectin in the fruits. Accordingly, the botanical characteristics of *C. parvifolium* and characterization of its pectin were evaluated. It was found that the habit of this plant is shrub with paired straight supra axillary thorns. Leaves are simple and opposite in arrangement with interpetiolar stipule. Young branches and lower surface of leaves are covered with pubescence. The inflorescence is axillary cymose. Calyx is green and limb is very short undulating 5-lobed with subglobse ovary portion. Corolla is tubular with 5 lobes, green to white or yellow in color. There are 5 stamens inserted at corolla throat. The ovary is inferior, 2-loculate with only 1 ovule each. The edible fruits are ovoid in shape and the skin is green or yellow, depending on maturity. The fruits of *C. parvifolium* were used as plant materials for pectin properties. The extracted pectin was designated as pectin with high degree of esterification due to its low moisture of 10.00% and the approximately 76.92% degree of esterification (DE) with methoxyl content around 7.06%. In addition, *C. parvifolium* yielded pectin around 38.00%. The chemical structure of pectin was studied through Fourier Transform Infrared (FTIR) spectroscopy and ^{1}H NMR spectroscopy. On the other hand, the molecular vibration was confirmed by using Density Functional Theory (DFT).

Keywords: *Canthium parvifolium*, botanical characteristics, pectin, density functional theory

1. Introduction

Canthium parvifolium Roxb. or "Nam Ma Khet" in the Thai vernacular [1], is one of the dicotyledonous plants belonging to the family Rubiaceae, order Gentianales [2]. *C. parvifolium* is a local plant which is widely distributed in the northern region of Thailand, e.g. Chiang Mai and Chiang Rai. In Chiang Rai Province, *C. parvifolium* is found in Wiang Chiang Rung District and Chiang Rai Rajabhat University. The fruits of this plant are very interesting because it could serve as another source of pectin [3].

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Pectin is a carbohydrate polymer compound that is found in plants. Pectin is a substrate of the enzyme pectinase which is found in plant cells between fibers at the top of the first layer cell wall. Pectin serves to anchor cells together. Pectin is the key component of parenchyma tissue which contains arabinose, galactose, and galacturonic acid [4]. It is a high-molecular weight compound with pectinic acid that could be served as a gelling agent when mixing it with food and water. Also, it is used as thickener and stabilizer in many types of food products, such as jam and jellies [5]. The gelling properties of pectin are known to be closely related to the degree of methylation (DM) and distribution of ester groups. Both rupture strength and gel threshold were found to be partly proportional to the free energy of hydrophobic interaction between the CH_3-groups in the model systems [6]. However, there are many factors which could affect the functionality of pectin, e.g. their composition, DM, solubility, pH, temperature and presence of soluble solids. Typically, the objective of commercial extraction of pectin is to obtain certain water-soluble and high molecular weight pectin [7]. There are different approaches to elucidate the pectin structure reported in the literature [8]. Nuclear Magnetic Resonance (NMR) was extensively to study pectin in food carbohydrates at a structural level [9]. However, the application of NMR as a quantitative analytical tool in carbohydrate determination is still limited despite the NMR's great capability [10]. DFT was used to study biology of some methylxanthines. According to the previous theoretical study [11], B3LYP was employed to calculate the gap between highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO). The result from the gap is representative for pharmaceutical properties of pectin. The result of this important properties of pectin is supported by the experimental study [12].

Moreover, the studies of silk retardant through plasma treatment, computational simulation, and vibration give a good agreement with FTIR in the work of Chaiwong and co-worker [13].

Thus, the objective of this research was to study the botanical characteristics of *Canthium parvifolium* Roxb. In addition, the physical and chemical properties of pectin in *C. parvifolium* such as methoxyl content, degree of esterification, equivalent weight and moisture were also evaluated. Lastly, we preliminary investigated the structural properties of pectin in *C. parvifolium* using the DFT as a tool for calculation together with the experimental approach.

2. Materials and Methods

2.1 Plant and extraction methods

The intact *C. parvifolium* plants were collected from Chiang Rai Rajabhat University, Chiang Rai Province, Thailand. The fruits of *C. parvifolium* were used as plant materials for pectin extraction. Fresh fruits were cut into small pieces and dried in an oven at 105 °C. Pectin was extracted from the ground *C. parvifolium* fruits with water (Ratio 25:1w/w) mainly to remove pigments. The material was filtered and the solids were treated with a solution (6% w/w) of sodium hexameta phosphate (SHMP) at 80 °C for 90 min. A pH 3.0 was obtained by adding HCl (1 M). After extraction, the pectin solution was filtered through a white filter cloth. Then, 95% acidified Alcohol and Acetone were utilized to precipitate the filtrate. To explain this step clearly, there is the persuasive detail: at the first, acidified alcohol was employed to remove impurities, and then the residue was dried by acetone. This process continues literately. 5 iterations were required to produce colorless filtrate.

The formed gel was allowed to precipitate at 25 °C for 24 h. After that, the pectin was dried at 35 °C for 24 h. The dry pectin was ground to pass through 60 mesh sieves for further study. The functional groups of *C. parvifolium* pectin were investigated by FTIR method with

Nicolet 6700 FTIR spectrophotometer (Bruker, Germany). The spectra were collected by averaging 64 scans at a resolution of 4 cm^{-1} from 400 – 4,000 cm^{-1}. The ^{1}H NMR spectra were acquired on a Bruker 400 MHz spectrometer, operating at 400 MHz for proton. Spectra were collected at 298 K, with 32K complex points, using a 90 ° pulse length. Sixteen scans were acquired with a spectral width of 8278 Hz, an acquisition time of 3.95s.

2.2 Physio-chemical

C. parvifolium fruit was analyzed as dry matter content [14]. Crude protein, crude fat (ether extract), crude fiber, and ash content using the standard techniques [14]. All samples were analyzed in duplicate and the results were reported including the total energy (cal/g) with bomb calories.

2.3 Methoxyl content

Methoxyl content was determined by dissolving 0.5 g of pectin in a 250 mL Erlenmeyer flask with 100 mL deionized (DI) water and then adding 1 g of NaCl with 6 drops of phenol red for titration with 0.1 M NaOH until the indicator color changed (pH 7.5). This solution was used to determine the methoxyl content by adding 0.25 M NaOH 25 mL, 0.25 M HCl to be titrated with 0.1 M NaOH.

$$\% \text{ Mehtoxyl content} = (NVE \times 100)/1000S \qquad (1)$$

Where S is dry pectin weight, N is base normally used in the titration, V is the base volume in the titration and E is the equivalent weight of methoxy.

2.4 Degree of esterification

The %DE was determined by dissolving 0.5 g of pectin powder with 2 mL of ethanol into 100 mL of water. The mixture was stirred until the solution became homogenous. After that, 5 drops of phenolphthalein and 0.5 M NaOH (V1) was added for titration. After that, 0.5 M NaOH (10 mL) was added and stirred for 15 min. After that, 0.5 M HCl (10 mL) was added and stirred in until the solution was colorless. Finally, a phenolphthalein indicator was added followed by titration with 0.5 M NaOH (V2). The methoxyl content and %DE was calculated by the following equation.

$$\% \text{ DE} = (NaOH \text{ volume1}/NaOH \text{ volume } 1+2) \times 100 \qquad (2)$$

2.5 Equivalent weight

Equivalent weight was determined by weighing 0.5 g of sample in a 250 mL conical flask and 5 mL ethanol was added. Then, 1 g of NaCl and 100 mL of distilled water was added. Finally, 6 drops of phenol red were added and titrated against 0.1 M (N) NaOH. The titration end point was indicated by purple color. This neutralized solution was stored for determination of methoxyl content. Equivalent weight was calculated by the following formula:

$$\text{Equivalent weight} = 1000S/N.V. \qquad (3)$$

Where S is dry weight, N is normality concentration, and V is the quantity of NaOH.

2.6 Moisture

The moisture of extracted pectin was analyzed by weighing 0.5 g pectin into a crucible and content was weighed (W1). The whole crucible was baked at 105 °C for 9 h. The crucible was let cool and weighted (W2). The moisture content may be calculated as follow.

$$\% \text{ Moisture} = ((W1\text{-}W2)/0.5) \times 100 \qquad (4)$$

2.7 Theoretical Study

The ground state optimization of a model compound of pectin was performed using DFT. Calculation of molecular structure in order to predict pectin properties was achieved using DFT with Beck-Lee Yang Parr (BLYP) functional of the Generalize Gradient Approximation (GGA). All simulations were performed under the conditions corresponding to 0 K. The analyzed effects of the structural features and molecular energy were achieved at the same level function. After that, the optimized structure was used to study the vibrational mode using a Dmol^3 module in a Materials Studio (MS) [15] program package

3.   Results and Discussion

3.1 Botanical characteristics of *C. parvifolium*

The botanical characteristics of *C. parvifolium* were shown in Figure 1. The plant appearance is shrub with paired straight supra axillary thorns. Leaves are simple and opposite in arrangement with interpetiolar stipule. Young branches and lower surface of leaves are covered with pubescence. The inflorescence is axillary cymose with a few to several flowers. Calyx is green and limb is very short undulating 5-lobed with subglobse ovary portion. Corolla is tubular with 5 lobes, green to white or yellow in color. There are 5 stamens inserted at corolla throat. The ovary is inferior, 2-loculate with only 1 ovule each. The edible fruits are ovoid in shape. The skin is green or yellow, depending on maturity. Moreover, it is interesting to note that the fruit of *C. parvifolium* is the one excellent source of pectin [3].
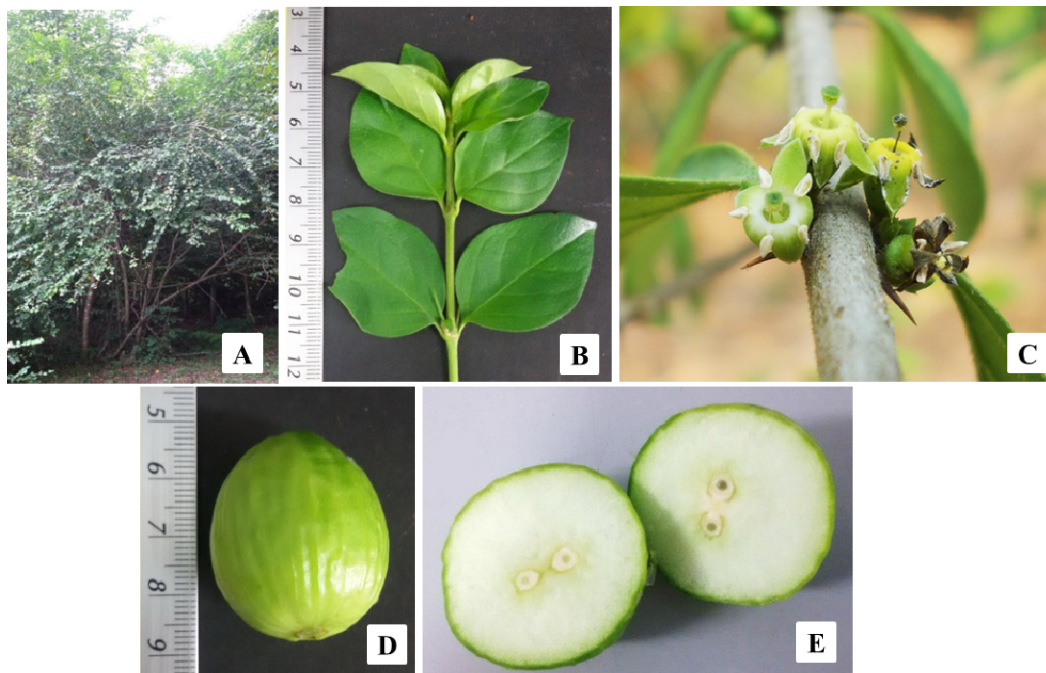
Figure 1. Botanical characteristics of *Canthium parvifolium* Roxb. (A) habit (B) leaves and stipule (C) flowers (D) young fruits and (E) cross section of young fruits

The results for physicochemical characteristics of *C. parvifolium* Roxb. fruit are displayed in Table 1. The ash content is at 0.57% dw. The protein content is 1.5%. The amount of fat is significantly low compared with apple pomace (0.69%). The dry matter is 19.65%. The crude fiber in *C. parvifolium* Roxb. is at 4.96% which is in the same range as of apple pomace. Finally, *C. parvifolium* Roxb. has a high total energy at 828 cal/g which is lower total energy than apple pomace.

Table 1. Proximate composition of *Canthium parvifolium* Roxb. fruit

Composition	Samples
Dry Matter (%)	19.65
Crude Fat (Ether Extract) (%)	0.69 (3.49-3.90)
Crude Fiber (%)	4.96 (4.7-48.72)
Crude Protein (%)	1.5 (4.45-5.67)
Ash Content (%)	0.57 (1.82)
Total Energy) cal/g)	828 (2,950)

Note:* The numbers in parentheses is a reference value from apple pomace [16]

3.2 Characterization and properties of *C. parvifolium* pectin

It was found that the appearance of dry extracted pectin is light brown color as shown in Figure 2.

Figure 2. The characteristic of *C. parvifolium* pectin.

The properties of extracted pectin were analyzed and the results are as shown in Table 2. All measurements were carried out in triplicate for each of the sample. The results are reported in mean values and standard deviations. The results revealed that pectin from *C. parvifolium* fruits has a high value of 7.06% methoxyl content which was higher than standard pectin and pharmaceutical pectin. Moreover, *C. parvifolium* pectin has high viscosity and gelation when compared with the reported DE of 76.92% [17]. Ptichkina *et al.* [18] reported that pectin with DE > 60% is suitable for use in food industry. The chemical characteristics of the pectin that influence gel qualities are DE, which is related to the rate of gel formation in food industry [19]. High DE means high gelation temperature when preparing gels by the usual procedure of mixing the hot ingredients and then solidifying by cooling. When the gel batch is cooled below the gelling temperature, gelation occurs after a delay, which is short with pectin of high DE and longer with pectin of lower DE [20].

The moisture of *C. parvifolium* pectin was 10%, which was quite high compared to the pharmaceutical grade pectin. The equivalent weight of this study was 181.81 which indicated the total content of free galacturonic acid. Therefore, it is associated with the % DE and methoxyl content. According to work of Rouse [21], the higher DE causes the decrease of free acid content of pectin that the equivalent weight also increases.

The extraction of pectin from *C. parvifolium* yielded 38.00±0.875 g%, while it was preliminary studied by Kaewpo [3] following the method described by Rangana [22]. The results reported that the content of pectin in *C. parvifolium* was 48.37 mg/ml dry weight, which was 1.6 fold significantly higher than the content of pectin in apple (29.97 mg/ml dry weight). However, by Rangana method, the high percentage of moisture (21.55%) was found in the extracted pectin. Thus, this research presented an efficient protocol for the pectin extraction of *C. parvifolium*.

Table 2. The properties of *C. parvifolium* pectin compared with standard pectin and lab & pharmaceutical grade pectin

Properties	*C. parvifolium* Pectin*	Standard Pectin [17]	Lab&pharmaceutical grade pectin [23]
Moisture (%)	10.00 ± 0.20	-	4.81
Degree of Esterification (%)	76.92 ± 0.53	64.95	-
Equivalent weight	181.81 ± 0.35	-	-
Methoxyl content (%)	7.06 ± 0.44	> 2.50	6.29
yield (%)	38.00±0.87	-	-

Note: * Values showing the mean ± SD in a column.

3.3 Functional group of *C. parvifolium* pectin by FTIR method

The samples of pectin were subjected to FT-IR analysis. The bands relevant for the structural organization are presented in Figure 3. The spectra shows two intense pectin bands at 1,741 cm^{-1} and 1,641 cm^{-1} were assigned to the stretching C=O vibration of esters and the asymmetric stretching vibration of carboxylate anion $v_{as(COO-)}$, respectively. The two functional groups are from carboxylic ester and protonated carboxylic acid groups. The region of 1,250 cm^{-1} – 950 cm^{-1} is contributed to glycosidic bonding ($v_{(coc),glycosidic\ bonding}$). Moreover, the spectra of pectin at 2,929 cm^{-1} was assigned to $v_{(C-H)}$, while the peak shown up at 3,272 cm^{-1} was assigned to $v_{(O-H)}$ of the carboxylic acid group.
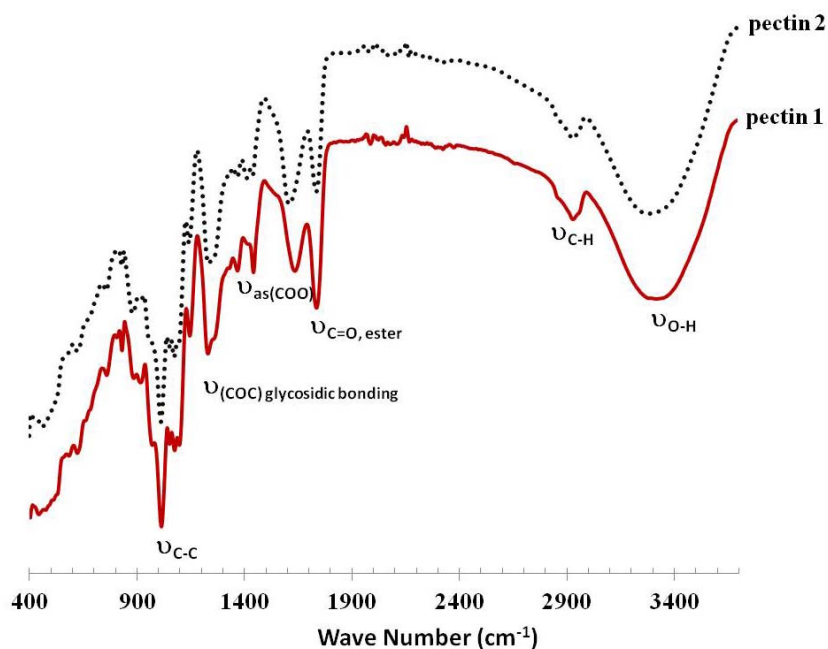

Figure 3. FT-IR spectrum for extracted pectin from *C. parvifolium*.

3.4 Vibrational results

The optimized structure of *C. parvifolium* pectin model compound was also studied. The ground state minimization of the pectin model compound was carried out using Dmol3 module in MS program with spin unrestricted and BLYP functional of GGA. A Fermi smearing of 0.005 Hartree was used to improve computational performance.
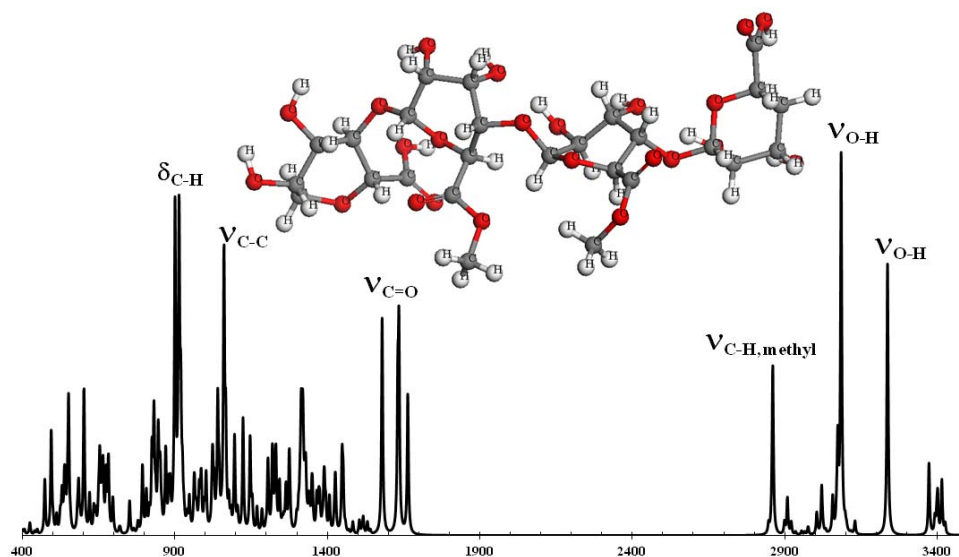

Figure 4. Vibrational spectra for pectin model compounds.

The vibration spectra of *C. parvifolium* pectin was presented in Figure 4. The highly coupled and conformational specific region between 1,100 and 800 cm^{-1} belongs to non-localized, highly coupled vibrations of polysaccharide backbones (δ_{C-H}, 913 cm^{-1} and ν_{C-C}, 1,060 cm^{-1}) [22]. Two intense bands at 1,634 and 1,579 cm^{-1} are assigned to $\nu_{C=O}$, which indicates its stretching vibration in ester and carboxylic acid. Additionally, the presence of $\nu_{C-H, -CH3}$ peak at 2,859 cm^{-1} also indicates an existence of methyl ester group. The peaks at 3,054, 3,236 cm^{-1} suggest that there are stretching vibrations of O-H at carboxylic groups.

3.5 NMR Analysis

The structural analysis by IR is supported by NMR result. As illustrated in NMR (Figure 5), there is a board singlet peak at 1 ppm corresponding to methyl groups (-CH$_3$) in the structure. Moreover, NMR shows doublet peak at 3-5 ppm. This is related to hydrogen in hydroxyl group, which is coupled with a neighboring hydrogen atom. The quartet peak at approximately 3.5 ppm is from the ring protons.
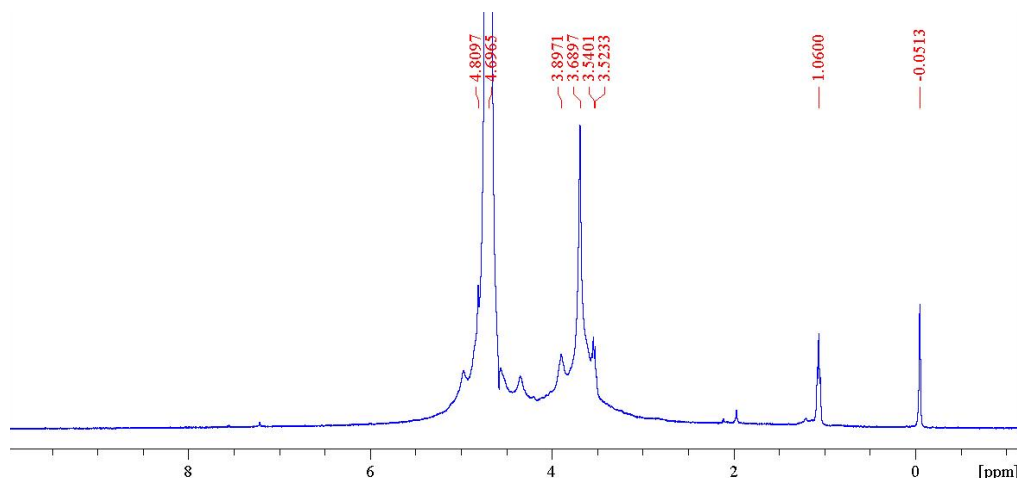
Figure 5. ^{1}H NMR spectrum of pectin sample. Experimental condition are reported under Materials and Methods.

4. Conclusions

This report presents the botanical characteristics of *C. parvifolium* and an efficient protocol for the pectin extraction from *C. parvifolium* fruits. The proximate composition of *Canthium parvifolium* Roxb. fruit was lower value compared with apple pomace. However, some properties of extracted pectin were clarified. It was found that the pectin extracted yield was 38.00±0.87%w/w which is significant higher that apple pomace (3.5-14.32%w/w), the moisture, and equivalent weight of *C. parvifolium* pectin was 10% and 181.81, respectively. The pectin of *C. parvifolium* had % DE (76.92%) and methoxyl content (7.06%), revealing its potential for in gelation application. In light of FTIR spectrum, it is routine to scale the frequency axis by a small amount to align major bands with those experimentally observed. The co-alignment of calculated vibrational spectra and experimental bands via the same scaling, as seen here, constitutes good agreement. The structural analysis by IR and NMR confirm the expected pectin structure. As our preliminary results mentioned above, we would like to probe and predict gelling factors, such as pH and temperature of *C. parvifolium* pectin in our furthered work.

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