

Original article

Tannin Extraction of Rhizophora Bark from Residual Charcoal Production

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Received: May 30, 2016

Accepted: Jun 23, 2016

ABSTRACT

Mangrove bark, (more than 200,000 m³ per year or 80,000-100,000 tonnes in oven dry weight) the residue from traditional charcoal production in Thailand, contains a large amount of tannin. The objectives of this research were to determine the chemical composition of mangrove bark, to investigate the tannin extraction conditions, and to investigate the chemical analysis of tannin from mangrove bark waste from the production of charcoal in Yeesan, Samut Songkram province, central Thailand. The chemical composition was analyzed using the TAPPI standard and the Browning method. Sodium sulfite (0, 2, or 4%) or sodium carbonate (0, 0.5, or 1%) were used as salt catalysts. Distilled water, 50% methanol, or 50% acetone were used as extraction solvents. The optimal conditions for obtaining additives for use in wood adhesive involved extraction under reflux conditions for 3 hr using a 100:500:4:1 by weight ratio of bark sample: acetone-water 50:50 volume/volume (v/v): sodium sulfite:sodium carbonate. These conditions produced the highest Stiasny number (87.69%). The extracts were characterized using Fourier-transformed infrared spectroscopy. Absorbance at 4000-600 cm⁻¹ was detected for tannin extracts and revealed the presence of catechin as the main condensed tannin in the extracts.

Keywords: tannin, mangrove bark, extraction

INTRODUCTION

Tannins are water-soluble phenolic compounds having a molecular weight between 500 and 3,000. They usually react with phenols and also have the ability to precipitate alkaloids, gelatins, and other proteins

(Bate-Smith and Swain, 1962). Tannins can be found in different parts of the fruits, seeds, and leaves. They can be used in many products such as printing ink, dyeing cloth and thread, synthetic resin, cosmetics, medicines, and in the food industry. Most tannin is extracted

from oak tree bark, and eucalypt bark, and in Thailand from mangrove trees. Most tannin used in Thailand is imported. In 1991, Thailand imported tannin valued at EUR 1.6 million and since then it has tended to increase every year (Wongsiri, 1993). However, Thailand tannin can be produced from bark which is a residue from mangrove charcoal production. These bark residues exceed 200,000 m³/year or 80,000-100,000 tonnes in oven dry weight. This volume has potential for industrial tannin production (Ounjitchai and Phattanaprapatan, 1984). Tannin can range between 2 and 40% of the dry bark of many species such as *Acacia mangium*, *Pinus spp.*, *Eucalyptus spp.*, and *Rhizophora spp.* Due to the high volume of bark residues, *Rhizophora spp.* have 20-25% tannin content (Hergert, 1989; Percival and Womersley, 1975).

Tannin can be extracted from lignocellulosic materials using various solvents, such as ethanol, methanol, water, acetone, ether, or ethyl acetone. The traditional method of tannin extraction for leather application is using the acetone-water-bisulfite procedure. This method has a yield of approximately 23% by weight. Most of the yield is condensed tannin and has a Stiasny number of approximately 65% (Yazaki and Collins, 1994). However, new techniques have been investigated to improve the yield and tannin quality, such as supercritical fluid extraction (Pansera *et al.*, 2004) and sulfitation of tannins (Pizzi, 1979). More recently, many researchers have attempted to invent new, environmentally friendly techniques such as microwave-assisted extraction, ultrasound-assisted extraction, or maceration (Aspe and

Fernandez, 2011), solvent extraction (Markom *et al.*, 2007), the infusion method (Naima *et al.*, 2015), and water extraction with the addition of chemicals (Antwi-Boasiako and Animaoauh, 2012). These techniques have been carefully developed to increase the yield and quality of tannin for wood adhesive applications.

Tannin used for wood adhesive should be condensed tannin and have a Stiasny number above 65% (Yazaki and Collins, 1994). The various solvents with the addition of chemicals are of interest as an unsophisticated extraction method. Chupin *et al.* (2013) tried to extract tannin from maritime pine bark using various conditions. The results showed that the extract yield using optimal conditions (water + 5% NaOH + 0.25% Na₂SO₃ + 0.25% NaHSO₃ at 80 °C) was higher than other conditions. However, the extract with the highest amounts of polyphenols, condensed tannin, and the highest reactivity to formaldehyde was extracted using water + 1% NaOH + 0.25% Na₂SO₃ + 0.25% NaHSO₃ at 70 °C. The results indicated that the higher concentration of NaOH produced the highest extraction yield but with a lower tannin content. Naima *et al.* (2015) determined the impact of different extraction methods on polyphenol yields and tannins extracted from Moroccan *Acacia mollissima* bark. The results showed that the highest polyphenol yield (condensed tannin and hydrolysable tannin) was extracted using the microwave-assisted method, followed by methods involving, maceration at room temperature, or infusion, respectively. Markom *et al.* (2007) investigated the effects of solvent types and extraction methods for hydrolysable tannin extraction from *Phyllanthus niruri* Linn.

The results showed that higher hydrolysable yields were extracted using solvent extraction (SE), supercritical fluid extraction (SFE), or pressurized water extraction (PWE). The efficiency of the method was determined on the basis of the solvent polarity, solvent-to-solid ratio, and contact time for tannin extraction. Polyphenols in tannin may occur in the form of phlobaphens which are highly condensed and insoluble in water; therefore, various solvents may be used for tannin extraction. Weak alkali solutions can partly cleave the pyran rings of polyphenols during the formation of deposition of the carboxylic group and sulfonic acid; therefore, the presence of sulfite, bisulfite, and other salts may increase the yield during tannin extraction (Roux *et al.*, 1975).

The objectives of the current research were to determine the chemical composition of mangrove bark, to investigate the optimal tannin extraction conditions, and to determine the chemical characteristics of tannin from mangrove bark waste arising from the production of charcoal in Yeesan, Samut Songkram province, central Thailand.

MATERIALS AND METHODS

Materials

The mangrove bark used was collected from *Rhizophora* spp. as residue from charcoal production in the Yeesan community, Samut Songkram province, central Thailand. The bark (10% moisture content) was ground to pass through a sieve (240-420 μm in diameter). The commercial grade chemicals used were: sodium sulfite (Na_2SO_3), sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH), acetone, and methanol.

Methods

Experimental design

The experiment used a 3x2x3 complete randomized design with three replications. Three solvents (distilled water, 50% methanol, and 50% acetone) and two salts with three concentrations (Na_2SO_3 at 0, 2 or 4% and Na_2CO_3 at 0, 0.5 and 1%) were studied. Two-way ANOVA was performed using Tukey's range test with the R-version 3.2.2 for Windows software (R Development Core Team, 2011).

Chemical composition of mangrove bark analysis

The ash content was determined using the method of TAPPI T203 om-93 (TAPPI, 2002). The total extractive content such as alcohol-benzene solubility, alcohol solubility, and hot water solubility were investigated using TAPPI T 204 om-97, TAPPI T264 om-97, and TAPPI T207 om-93 (TAPPI, 2002), respectively. The lignin content, α -cellulose content, and pentosan content were determined in accordance with TAPPI T222 om-98, TAPPI T203 om-93, and TAPPI T223 cm-84 (TAPPI, 2002), respectively. The holocellulose content was investigated using the Browning method (Browning 1967).

Tannin extraction

Extraction was applied following the method from Hoong *et al.* (2009). The bark was extracted in a boiling flask under reflux for 3 hours having used a solvent:bark ratio of 5:1 (w/v). Water, methanol, or acetone were used as solvents. The Na_2SO_3 (0, 2, or 4%) and Na_2CO_3 (0, 0.5, or 1%) were added into the boiling flask to investigate the optimal

extraction conditions. The amount of extract was determined after removal of solvents by first screening through a fine filter (140 mesh), filtering on a sintered glass (porosity 2), and evaporating using a Buchi Rotavapor rotary evaporator (50-55 °C). The yield was determined under extraction with various conditions (nine conditions shown in Table 2) and calculated using the following formula:

$$\text{Yield (\%)} = \frac{(\text{Solid extract total weight} - \text{SS total weight}) \times 100}{\text{Bark weight}}$$

where SS is the amount of sodium salt used and all weights are oven dried (OD) .

Ferric chloride test

The method of Harborne (1984) was used for determination of tannin presence. The extract (10 mg) was weighed into a beaker and 2 ml of distilled water were added. The mixture was boiled for 5 min. The HCl 0.001 molar (100 ml) was mixed with 5% ferric chloride (0.162 g). A few drops of this mixture were added into the tannin extract. The color change was examined. Production of a greenish precipitate was an indication of the presence of tannins.

Determination of total tannin content

The total phenol content was determined using the method of Makkar *et al.* (1993). Each extracts (50 µL) was put in a test tube and dissolved in distilled water (500 µL). Folin-Ciocalteu reagent (250 µL) and sodium carbonate

solution (1.25 mL) were added into the test tube. Each tube was vortexed before incubation in the dark for 40 min and the absorbance was read at 725 nm using a spectrophotometer. Non-tannin phenolics were also determined using the method of Makkar *et al.* (1993). An amount of 100 mg of polyvinylpyrrolidone (PVPP) was mixed with 1.0 mL of distilled water and 1.0 mL of the extracted sample. The tubes were vortexed before being kept at 4 °C for 15 min and vortexed again before centrifugation at 3,000 rpm for 10 min. The absorbance of supernatant was read at 725 nm using a spectrophotometer. The tannin content was calculated as follows:

$$\text{Total phenolics} - \text{non-tannin phenolics} = \text{tannin}$$

The total phenolics and tannin contents were assessed through the calibration curve of gallic acid equivalents (GAE) with the concentration range 0-100 mg/mL.

Determination of tannin content (reactive tannin)

The Stiasny number of the extract was used to determine the formaldehyde condensable polyphenols content according to the procedure proposed by Vieira *et al.* (2011). The extracts (0.4% (w/v); 50 mL) was dissolved in distilled water (100 mL) and 5 mL of 37% HCl and formaldehyde (37%; 5 mL) were added. The mixture was heated under reflux conditions for 30 min. The suspension was filtered through sintered glass. The precipitate was washed with hot water and then oven-dried at 105 °C until a constant weight was recorded. The Stiasny number was calculated as follows:

$$\text{Stiasny number (\%)} = \frac{\text{Dry weight of precipitate}}{\text{Dry weight of extract}} \times 100$$

Determination of gel time

The gel time was measured following Ping *et al.* (2011). A sample of 40% of dissolved extract tannin (10 mL) and 5% (w/w) of powdered paraformaldehyde on solid tannin were added into a glass tube and mixed. The mixed solution was placed into a water bath which was maintained at boiling temperature (just below 100 °C) and then stirred until the mixed solution became a gel. The gel time and pH of the mixed solution were also reported using three replicates.

FT-IR study of tannin extracts

Attenuated total reflection Fourier-transform infrared spectroscopy (ATR-FTIR) (Bruker equinox 55) was used to examine the functional groups of the tannin extract and catechin. The spectra were obtained in the wavelength range 4000-600 cm^{-1} . Thirty individual scans were collected and averaged into a single spectrum with a resolution of 0.5 cm^{-1} . The spectra were collected and analyzed using the Spectrum Software system (Equinox 55; Bruker Optik GmbH; Ettlingen, Germany)

MALDI-TOF-MS study of tannin extract composition

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Bruker Doltonic) was investigated following Hoong *et al.* (2010). The powdered tannin samples were dissolved in 50% acetone (4 mg/mL). Then, 2,5-dihydroxy benzoic acid (DHB) was dissolved in acetone as the matrix and NaCl was added to enhance ion formation. The sample solutions were mixed with acetone solution (10 mg/mL acetone) of the matrix. These solutions were placed on the MALDI target. After evaporation of solvent, the MALDI target was introduced into the spectrometry.

RESULTS AND DISCUSSION

Chemical composition of bark

Mangrove bark contained 40.23% holocellulose. However, the greatest component was extractives at 42.97%. From Table 1, 22.33% of the dried bark was hot water solubles. These solubles were typically carbohydrate, resins, tannin, and sugar, among others which agreed with Liese (1980).

Table 1 Chemical composition of mangrove bark.

Chemical composition	Content (% by oven-dry weight)
Ash	6.73
Extractive	42.97
Alcohol-benzene solubility	11.05
Alcohol solubility	9.59
Hot water solubility	22.33
Lignin	19.76
Holocellulose	40.23
Alpha-Cellulose	21.29
Pentosan	10.30

Extraction yield

The extract yield was the percentage of total weight of dried extract per dried weight of bark. The effect of solvent and alkali on the extraction yield was investigated using the method of Hoong *et al.* (2009). The amounts of extract after removal of solvents by first screening through a fine filter (140 mesh), filtering on sintered glass (porosity 2) and evaporating using a Buchi Rotavapor rotary evaporator (50-55 °C) are listed in Table 2. The highest extract yield (26.14% of OD bark weight) was extracted under the reflux conditions of 3 hr using a 100:500:4:1 (by wt.) ratio of bark sample:methanol

(50:50 v/v):sodium sulfite:sodium carbonate. However, comparing the yield extracted using water, methanol or acetone as the solvent, the average extract yield was 21.71, 24.67 and 23.60%, respectively (Figure 1). The highest tannin yield was extracted using methanol as the solvent due to the phlobaphens in tannin; this yield was lower than when condensed in methanol. In addition, the extract yield increased with an increased alkali content because the phlobaphens (polyphenols in tannin) are highly condensed and insoluble in water. The alkali solutions can partly cleave the pyran rings of polyphenols and increase the yield during tannin extraction.

Table 2 Stiasny number and gel time of tannin extracted from mangrove bark.

Condition	Extraction conditions			Extract yield (%)**	Stiasny number (%)**	pH	Gel time (min)	Gel time at pH 7 (min)
	Solvent	Na ₂ SO ₃ (%)*	Na ₂ CO ₃ (%)*					
1	Distilled water	0	0	19.67±1.93 ^b	59.76±0.94 ^e	4	10.17	6.30
2	Distilled water	2	0.5	20.25±0.65 ^b	70.48±2.92 ^d	6	38.25	90.00
3	Distilled water	4	1	25.20±1.37 ^a	74.83±0.42 ^{cd}	8	120.00	90.00
4	50% Methanol	0	0	22.45±0.46 ^{ab}	72.12±1.27 ^d	3	10.08	11.43
5	50% Methanol	2	0.5	25.43±1.30 ^a	77.86±1.22 ^{bc}	5	10.05	16.57
6	50% Methanol	4	1	26.14±2.67 ^a	78.88±1.16 ^{bc}	7	16.28	21.27
7	50% Acetone	0	0	23.42±1.31 ^{ab}	76.76±2.28 ^{bc}	4	1.11	4.22
8	50% Acetone	2	0.5	24.62±0.23 ^a	81.15±0.71 ^b	6	13.29	7.29
9	50% Acetone	4	1	22.76±1.94 ^{ab}	87.69±1.62 ^a	6	14.35	24.41

Remarks: * Compared with the dry weight of the sample used in the extraction. Values are shown as mean ± standard error

**Values with different letters (a-d) are statistically significant ($p \leq 0.05$).

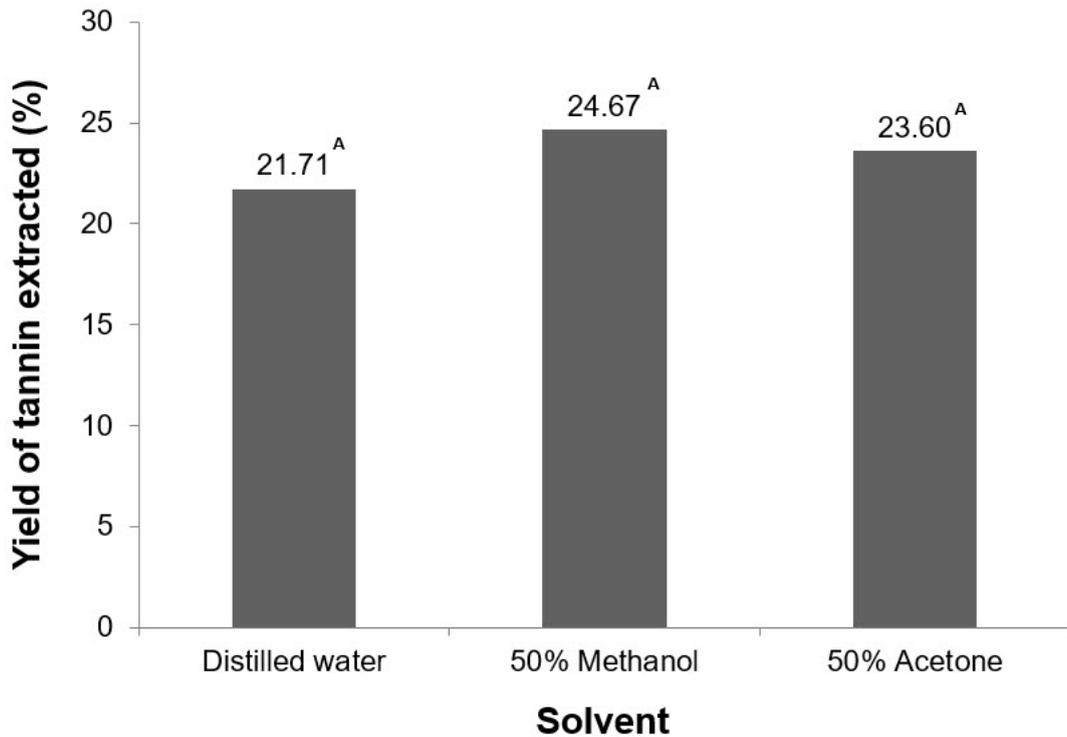


Figure 1 Yield percentage of tannin extracted using various solvents. Values with different letters are statistically significant ($p \leq 0.05$).

Presence of tannin (ferric chloride test)

From Figure 2, the test of tannin extract with ferric chloride showed the green color in both extracts from the control (50% acetone without Na_2SO_3 and Na_2CO_3) and

optimal (50% acetone with 4% Na_2SO_3 and 1% Na_2CO_3) conditions. This indicated that the extract contained condensed tannin (Harborne, 1984).

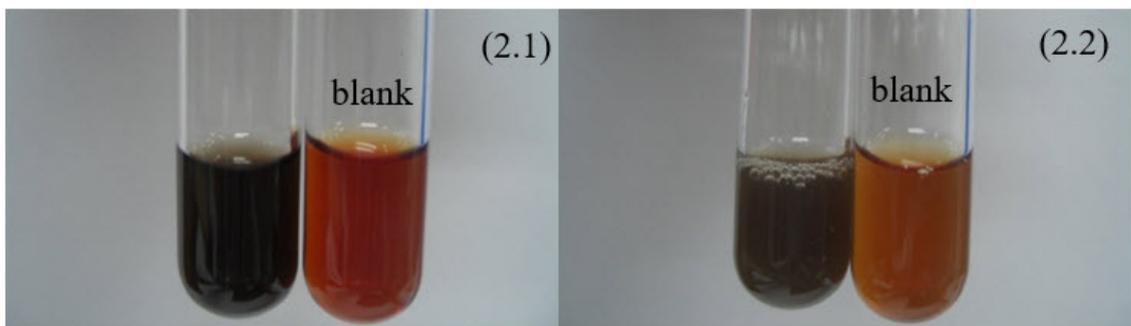


Figure 2 Extract before (blank) and after reaction with ferric chloride.

(2.1) Extracts from optimal conditions

(2.2) Extracts from control conditions

Total tannin content

The total tannin content was analyzed from the extracts under the control (50% acetone without Na₂SO₃ and Na₂CO₃) and optimal (50% acetone with 4% Na₂SO₃ and 1% Na₂CO₃) conditions. Each extract was reacted with PVPP and then the total phenolic content was examined in each extract. The total tannin content is shown in Table 3. To obtain the percentage of total tannin in the bark, the SN was multiplied by the total amount

of extractives and the results converted to a percentage. The total tannin in mangrove bark was 7.69 and 7.85% under the optimal and control conditions, respectively. In addition, the total tannin in the extracts was 33.77 and 33.53% under the optimal and control conditions, respectively. It can be concluded that the salts had no influence on the tannin yield; however, it had a significant effect on the tannin properties.

Table 3 Total tannin in extract and in mangrove bark extracted under various conditions.

Condition	Total tannin in extract (%)	Total tannin in bark** (%)
Extract under optimal conditions	33.77	7.69
Extract under control conditions *	33.53	7.85

Remarks: * same conditions as the optimum but no salts added

** Total tannin in bark = total tannin in extract x extraction yield

Stiasny number and gel time

The Stiasny number (SN) and gel time were determined as properties of the tannin. The SN represents the content of polyphenols (reactive tannin) existing in an extract. This value indicates the active polyphenol in tannin, especially in position C(8) of the resorcinolic A-ring and it is more reactive than in the B-ring (Saayman and Oatley, 1976; Pizzi, 2003). This value indicates the reactivity of the extract to formaldehyde and is useful for determining the extract's application as an adhesive (Vieira *et al.*, 2011; Ping *et al.*, 2011). The gel time is the time to solidify the liquid into a gel. It can indicate the capability of adhesive bonding in materials. The gel point is determined when crosslinking begins to dominate the nature of the resin. Table 2 shows that the highest SN was

87.69% of the extract under reflux conditions for 3 hr using the 100:500:4:1 (by wt.) ratio of bark sample:acetone (50:50 v/v):sodium sulfite:sodium carbonate. The extract under these conditions had a gel time of 14.35 min which may be optimal for wood adhesives. However, the toxicity of acetone may be a concern during application as an adhesive additive. The SN of the extracts was 81.87, 76.29, and 68.36% using acetone, methanol, and water, respectively, as the solvent. The addition of alkali (sodium sulfite and sodium carbonate) had an influence on the Stiasny number. A higher alkali addition resulted in a higher SN due to the high cleavage of the pyran ring in polyphenols (reactive tannin) under alkali solutions. In general, the SN has a value above 65% which is the minimum value

required to produce high quality adhesives (Yazaki and Colins, 1994). From Table 2, the extraction under reflux conditions for 3 hr using the 100:500:4:1 (by wt.) ratio of bark sample:acetone-water (50:50 v/v):sodium sulfite:sodium carbonate produced the highest yield of tannin extract (22.76% based on oven-dry bark weight). The extracts obtained from this optimal condition were further analyzed for their adhesive properties using the Stiasny number (SN) and gelation time (T-gel). However, based on the findings of this study, the tannin from this optimal extraction condition may be used as an adhesive additive due to its promising properties (87.69% SN and 14.35 min T-gel at pH 6).

FT-IR spectra analysis

The FT-IR spectra (Figure 3) showed the functional groups in the extracts under the control (50% acetone without Na_2SO_3 and Na_2CO_3) and optimal (50% acetone with 4% Na_2SO_3 and 1% Na_2CO_3) conditions, including in a catechin standardized solution and showed that the extract and catechin have a similar

structure based on the FT-IR spectra. Therefore, the extract was composed of condensed tannin because catechin was found in condensed tannin structure. The FT-IR spectra of the extracts were in the range $4000\text{-}600\text{ cm}^{-1}$. The results showed that the extracts and standardized solution had the same spectral pattern. The $3550\text{-}3100\text{ cm}^{-1}$ absorption band was attributed to OH-stretching vibration of the benzene and methylol of tannin and the peak at 2900 cm^{-1} was aromatic CH-stretching vibration of the methyl group and the methylenes group. The $1619\text{-}1450\text{ cm}^{-1}$ absorption band showed the availability of an aromatic ring. The peak at the $1500\text{-}950\text{ cm}^{-1}$ absorption band showed the FTIR fingerprint for tannin. The peaks at 1300 and 1036 cm^{-1} showed symmetrical and asymmetrical C-O vibration, respectively. The presence of the C-O group could be rationalized as an indication of the catechinic acid arrangement in the extracts (Ping *et al.*, 2011). It can be concluded that the chemical structure of the extracts was the same as in the catechin standardized solution.

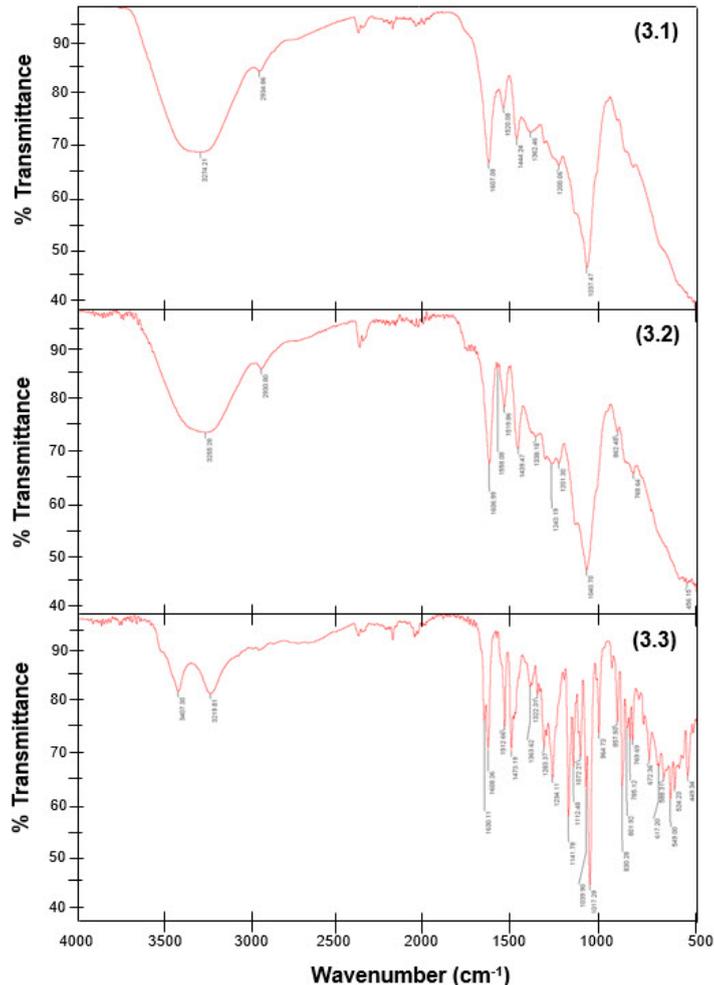


Figure 3 ATR-FTIR spectra of mangrove extracts and standardized solution.

(3.1) Extract tannin under optimal conditions

(3.2) Extract tannin under control condition

(3.3) Standardized solution (catechin)

MALDI-TOF-MS analysis

The MALDI-TOF analysis of tannin extract reported by Oo *et al.* (2008, 2009) indicated that the extract was composed of both catechin/epicatechin (MW=290) and epigallocatechin (MW=306) as the main monomers in the structure. The MALDI spectra in Figures 4 and 5 show a pattern of an increase in the mass in the range 289–290 Da of the tannin extract under the control

(50% acetone without Na_2SO_3 and Na_2CO_3) and optimal (50% acetone with 4% Na_2SO_3 and 1% Na_2CO_3) conditions. Therefore, the tannin extract had only catechin/epicatechin as a main monomer. The spectra also showed a change in the mass ($\Delta \text{mass} = 16 \text{ Da}$), which represents an increase of the hydroxyl group in the structure of the main monomer (catechin/epicatechin). The structure of the main elements in the tannin extract is shown in Figure 6.

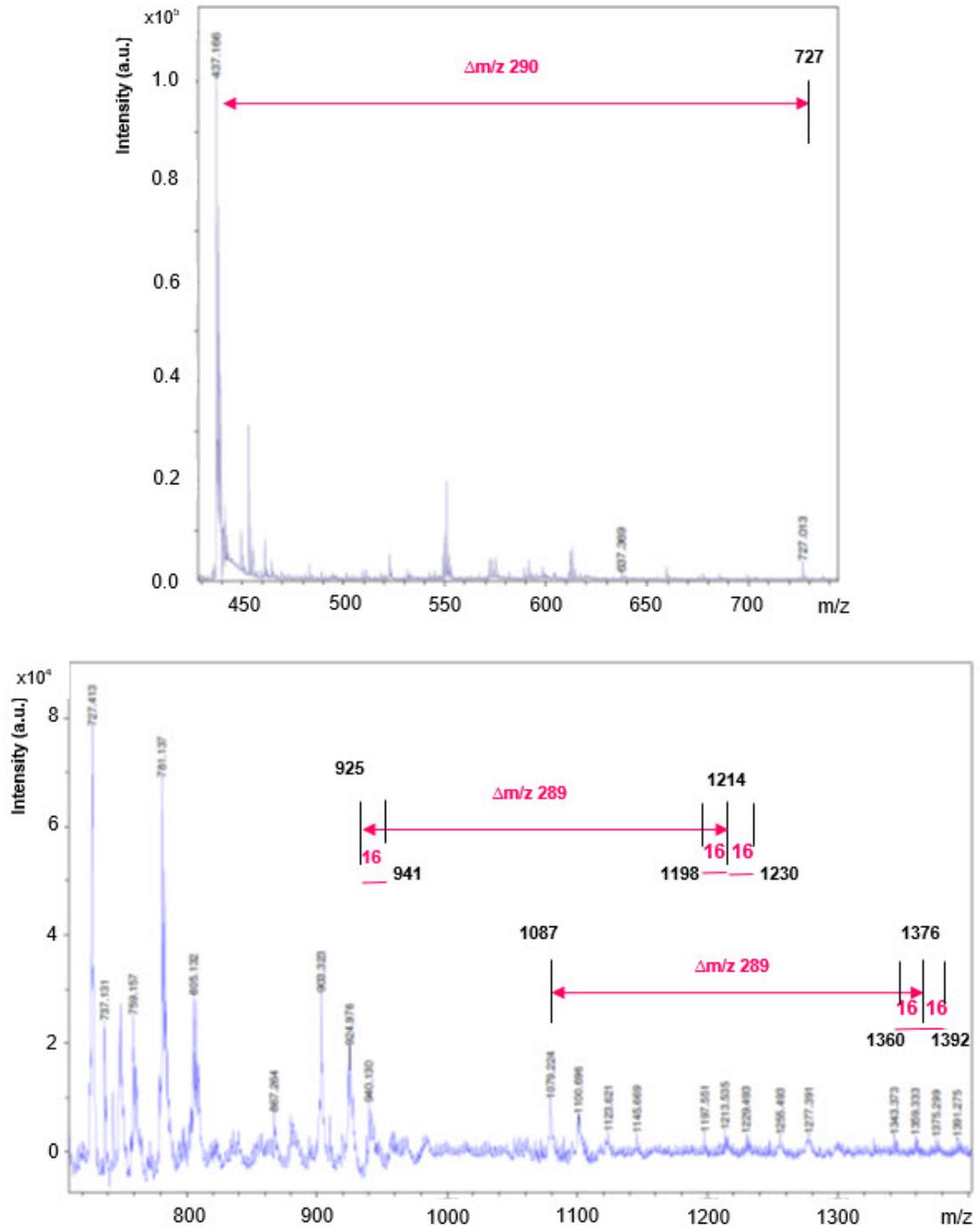


Figure 4 MALDI mass spectra of extract tannin under optimal conditions.

(4.1) 400–750 Da

(4.2) 750–1,400 Da

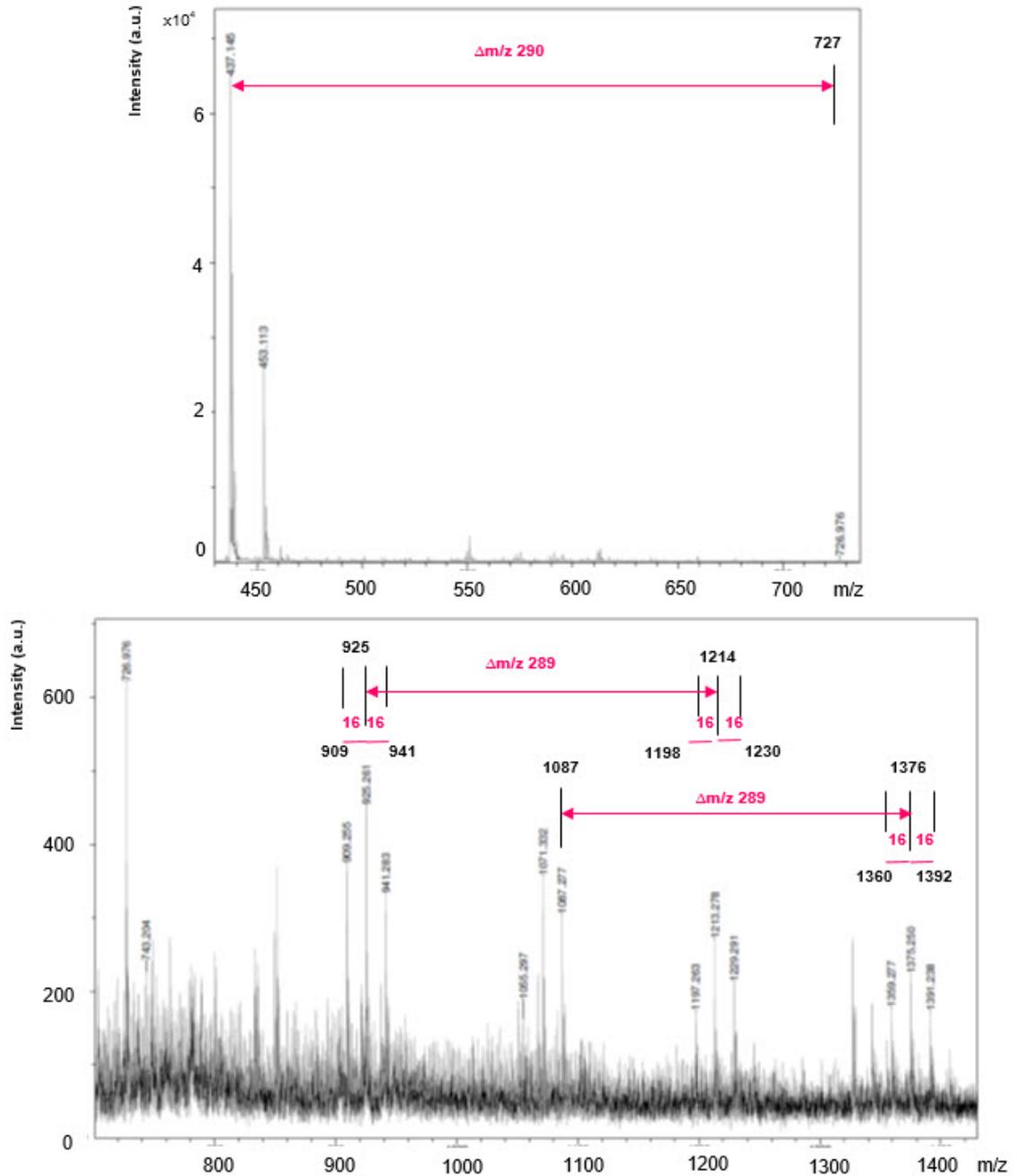


Figure 5 MALDI mass spectra of extract tannin under control condition.

(5.1) 400–750 Da

(5.2) 750–1400 Da

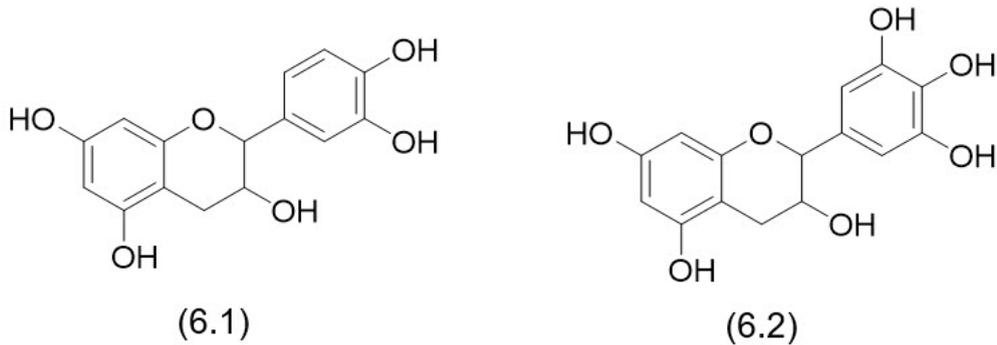


Figure 6 Structure of main components in tannin extract.

(6.1) Catechin/epicatechin (MW=290)

(6.2) Epigallocatechin (MW=306)

CONCLUSION

Extraction under reflux conditions of 3 hr using a 100:500:4:1 (by wt.) ratio of bark sample:methanol-water (50:50 v/v):sodium sulfite:sodium carbonate produced the highest extract yield. The Stiasny number and gelation time were determined for use in quantifying the suitability of the tannin extract as a wood adhesive. However, the tannin extract obtained from the extraction under reflux conditions of 3 hr using a 100:500:4:1 (by wt.) ratio of bark sample:acetone-water (50:50 v/v):sodium sulfite:sodium carbonate showed promising properties for adhesive application based on the highest Stiasny number (87.69%) with a gelation time of 14.35 min and pH 6. From the tannin extract, condensed tannin was the predominant type of tannin in *Rhizophora* bark. The optimal conditions for tannin extraction was using 50% acetone as solvent with 4% Na_2SO_3 and 1% Na_2CO_3 .

ACKNOWLEDGEMENTS

I wish to thank Department of Forest Products, Faculty of Forestry, Kasetsart

University (FORPROD KUFF), Bangkok, Thailand, the Kasetsart Agricultural and Agro-Industrial Product Improvement Institute (KAPI) for providing instruments to extract and analyze tannin. This research was funded by the Kasetsart University Research and Development Institute (KURDI).

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