Nutrients and Phenolic Acids in Krung Ba Dan (*Cyclea barbata* Miers) Leaves and Aqueous Extract from Ubon Ratchathani Province

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บทคัดย่อ

กรุงบาดาลเป็นสมุนไพรไม้เลื้อยที่พบได้ในประเทศเขตอบอุ่น รวมถึงประเทศไทย ใบมีลักษณะพิเศษเมื่อนำมาสกัดด้วยน้ำในแบบครัวเรือนสามารถเกิดเป็นเจลได้เองที่อุณภูมิห้องโดยไม่ต้องเติมองค์ประกอบอื่นเพิ่มเติม คนท้องถิ่นในภาคตะวันออกเฉียงเหนือมักนำใบมาขยี้กับน้ำเกิดเป็นเจลผสมกับปนปลาและบริโภคเป็นอาหาร เนื่องด้วยสมบัติการเกิดเจลที่น่าสนใจและการบริโภคที่พบในวงจำกัด งานวิจัยชิ้นนี้จึงมีวัตถุประสงค์เพื่อศึกษาคุณค่าทางโภชนาการและปริมาณกรดฟีนอลิกของเจลและใบกรุงบาดาล การศึกษาคุณค่าทางโภชนาการในใบสดและเจลสดตามวิธีมาตรฐานสากล AOAC พบว่า ใบสด 100 กรัม มีความชื้น 77% มีองค์ประกอบเป็นคาร์โบไฮเดรต 16.7 กรัม โปรตีน 4.2 กรัม ไขมัน 0.4 กรัม เถ้า 1.7 กรัม ใยอาหารชนิดที่ละลายน้ำ 8.6 กรัม ใยอาหารชนิดไม่ละลายน้ำ 1.2 กรัม และแคลเซียม 237.2 มิลลิกรัม เจลสด 100 กรัม มีความชื้น 97.7% ประกอบด้วยคาร์โบไฮเดรต 1.7 กรัม โปรตีน 0.4 กรัม เถ้า 0.2 กรัม ใยอาหารชนิดที่ละลายน้ำ 1.5 กรัม และแคลเซียม 15.2 มิลลิกรัม การวิเคราะห์ปริมาณกรดฟีนอลิกในใบและเจลที่ผ่านการทำแห้งแบบแช่เยือกแข็ง พบกรดคลอโรเจนิก (15.5 มิลลิกรัม และ 11.1 มิลลิกรัม ต่อ 100 กรัม ตัวอย่างใบและเจล ตามลำดับ) กรดเฟอริวลิก (9.8 มิลลิกรัม และ 8.3 มิลลิกรัม ต่อ 100 กรัม) และกรดพีคูมาริก (6.3 มิลลิกรัม และ 3.6 มิลลิกรัม ต่อ 100 กรัม) โดยสรุป ใบและเจลจากใบกรุงบาดาลมีปริมาณใยอาหารชนิดละลายน้ำอยู่สูงและมีกรดฟีนอลิกเป็นองค์ประกอบจึงมีแนวโน้มใช้เป็นสมุนไพรและอาหารพื้นบ้านที่มีประโยชน์ นอกจากนี้ การศึกษาเพิ่มเติมเกี่ยวกับคุณลักษณะของเจล สารออกฤทธิ์ทางชีวภาพ การทดสอบฤทธิ์การต้านอนุมูลอิสระ เพื่อผลเชิงประจักษ์เกี่ยวกับประโยชน์ต่อสุขภาพ จะสามารถส่งเสริมการบริโภคให้แพร่หลายมากขึ้นได้

คำสำคัญ: กรุงบาดาล คุณค่าทางโภชนาการ กรดฟีนอลิก ใยอาหารชนิดที่ละลายน้ำ

Abstract

Krung ba dan is a slender, deciduous climber widespread in tropical regions, including Thailand The aqueous extract from the leaves has a unique characteristic in that gel forms by itself at room temperature. The extract is widely used in a traditional dish called Pon-ma-noi, commonly consumed in North-Eastern Thailand, including Ubon Ratchathani province. This study aimed to determine the nutritional composition and phenolic acids in Krung ba dan (*Cyclea barbata* Miers) leaves and the aqueous extract. The nutritional composition was analysed according to the AOAC official methods. Krung ba dan leaves (77% moisture content) were composed of 16.7 g carbohydrate, 4.2 g protein, 0.4 g

fat, 1.7 g ash, 8.6 g soluble dietary fiber, 1.2 g insoluble dietary fiber, and 237.2 mg calcium per 100 g of sample. The aqueous leaf extract (97.7% moisture content) consisted of 1.7 g carbohydrate, 0.4 g protein, 0.2 g ash, 1.5 g soluble dietary fiber, and 15.2 mg calcium per 100 g of sample. Phenolic acids were identified in freeze-dried leaves and gel extract, and these were chlorogenic acid (15.5, 11.1 mg/100 g), ferulic acid (9.8, 8.3 mg/100 g), and *p*-coumaric acid (6.3, 3.6 mg/100 g). In summary Krung ba dan leaves and the aqueous extract containing high soluble fiber content and phenolic compounds may have potential health benefits, and on-going studies based on the gel characteristics, other bioactive compounds, and antioxidant activities should help confirm them.

Keywords: Krung ba dan (*Cyclea barbata* Miers): Nutrient: Phenolic acid: Soluble dietary fiber

Introduction

Krung ba dan (*Cyclea barbata* Miers) is a slender, deciduous climber in the MENISPERMACEAE family distributed in the tropical regions of Asia, East Africa, and South America. It is widespread in most parts of Thailand [1]. Leaves can be used to prepare a gel-forming dish without the need for additional ingredients. The plant and gel are used as indigenous food and medicines for the treatment of fever, gastrointestinal related-diseases, and symptoms [3]. Herbal plants are rich sources of bioactive compounds which have functional health properties with a wide range of biological activities, such as antioxidant, anti-carcinogenic, anti-inflammatory, and anti-microbial activities. There are some studies on phenolic compounds and antioxidant activities [4] of this plant but these mostly used qualitative data. It is of great interest to investigate several aspects focusing on the health benefits of this plant. This study aimed to examine the proximate analysis and identify the phenolic acids in Krung ba dan leaves and aqueous extract. More studies should be implemented to promote sustainable consumption.

Materials and Methods

Materials and preparation

Krung ba dan leaves, flowers, and stems were collected in 5 representative areas of Muang district, Ubon Ratchathani province during December 2013. The duplicate specimens were identified by taxonomists from the Department of Plant Science, Faculty of Science, Mahidol University and the Forest Herbarium, Department of National Parks, Wildlife and Plant Conservation (BKF). A single composite sample was prepared by combination of all samples. Green mature leaves of a commonly consumed size were trimmed, washed, and soaked in 0.4% v/v solution of vegetable and fruit washing liquid (St. Andrews, Thailand) for 10 min, rinsed with tap water and distilled water, then soaked in 100 ppm sodium metabisulfite for 5 min. Clean leaves were drained and air-dried for 5 min, cut into small pieces, mixed well together, then packed in sealed polyethylene bags, and stored at -10±5 °C until use. For aqueous extract, Krung ba dan gel was prepared using a blender (Moulinex Model T71, France) for 2 min. A ratio of leaves and water of 1:4 w/v was selected according to a normal portion of the recipe from the survey report [5]. Gel was separated from the leaf residue by a white cloth, packed in sealed

polyethylene bags, and stored at -10±5 °C until use.

Nutrient determination

Frozen Krung ba dan leaves and aqueous extract were analyzed as a single composite sample in duplicate based on the AOAC official methods [2]. Moisture content was determined by drying the sample in a hot air oven (Memmert Model UNE 500, Germany) at 100±5 °C. Crude protein content was determined by the Dumas combustion technique using the Protein/Nitrogen Determinator (Leco FP-528, USA), calculated with the conversion factor of 6.25. Crude fat content was determined by acid digestion and petroleum ether extraction in a Soxtec System (Tecator Model 1043, Sweden). Total dietary fiber, including soluble and insoluble fibers, was determined using the Enzymatic-gravimetric method. Ash content was analyzed after incineration in a muffle furnace (Carbolite Model CWF 1100, UK) at 550±5 °C. Ash residue was used for determination of calcium content using a flame atomic absorption spectrophotometer (Thermo S series, UK). Then the carbohydrate and energy were derived by calculation.

Phenolic acids determination

The presence of phenolic compounds in leaves and gel extract was determined according to Merken and Beecher [6] with some modification. In brief, the freeze-dried sample was hydrolyzed at 80°C for 2 h with 40 mL of 62.5% methanol containing 0.5 g/L tertiary butylhydroquinone (tBHQ) and 10 mL of 6 N hydrochloric acid. After cooling, 100 µL of 1% ascorbic acid was added. Then, the volume of the mixture was adjusted to 50 ml with methanol. After filtration with 0.2 µm PTFE syringe filter, the solution was injected to HPLC (Agilent Technologies 1100 series, USA) with a photodiode array detector and a Zorbax Eclipase XDB-C18 column (Agilent Technolodies, USA) (5 µm, 4.6 mm x 150 mm), controlled by ChemStation software (Agilent Technolodies, USA). Mobile phases were comprised of water, methanol (MeOH), and acetonitrile (ACN), each containing 0.05% w/w trifluoroacetic acid (TFA). The gradient system was used for separating phenolic acids according to Table 1 at a constant flow rate of 0.6 ml/min with the total run time of 70 min. Samples were kept in an auto-sampler at 4°C until injected for 10 µL. Hydroxycinnamic acids including chlorogenic, caffeic, *p*-coumaric, ferulic, and sinapic acids were monitored at 325 nm. Hydroxybenzoic acids composed of gallic, 4-hydroxybenzoic, syringic, vanillic, and *t*-cinnamic acids were monitored at 280 nm. Phenolic acids of Krung ba dan leaves and aqueous extract were identified by comparing the unknown peaks to the 10 authentic standards (as mentioned above) in terms of retention time and UV spectrum. The contents were calculated using the standard curve of each phenolic acid and expressed as mg/100 g sample. Standards of gallic acid and tBHQ were purchased from Fluka, Switzerland. Standards of syringic and *p*-coumaric acid were obtained from Sigma, UK, and standard of vanillic acid was purchased from Fluka, China. Chlorogenic, caffeic, ferulic, and *t*-cinnamic acid standards were obtained from Sigma, China. Standards of 4-Hydroxybenzoic acid and sinapic acid were purchased from Sigma, Japan, and India respectively.

Table 1 Gradient system for phenolic acid determination

Time (minutes)	% Water+TFA	% MeOH+TFA	% ACN+TFA
0	90	6	4
5	85	9	6
30	71	17.4	11.6
60	0	85	15
61	90	6	4
66	90	6	4

Data analysis

All measurements were examined in a single composite sample in duplicate.

Results and discussion

Table 2 Nutritional composition of fresh Krung ba dan leaves and the aqueous extract

Nutrients	Samples	
(per 100 g wet basis)	Leaves [a]	Aqueous extract [a]
Energy, kcal	87.2	8.4
Moisture, %	77.0	97.7
Protein, g	4.2	0.4
Fat, g	0.4	ND
Carbohydrate, g	16.7	1.7
Total dietary fiber, g	9.8	1.5
Soluble dietary fiber, g	8.6	1.5
Insoluble dietary fiber, g	1.2	ND
Ash, g	1.7	0.2
Calcium, mg	237.2	15.2

[a] Values are from a single composite sample. ND = Not detected.

Nutritional composition

The proximate composition of fresh Krung ba dan leaves and fresh aqueous extract are presented in Table 2. The main component was water, followed by carbohydrate with a major contribution of dietary fiber, mainly soluble dietary fiber. A fair amount of calcium was also detected in the leaves, and a lesser amount in the gel extract. To draw comparisons, values were adjusted to dry weight basis, so the nutritional composition of Krung ba dan leaves in this study were 18.1% protein, 1.6% fat, 72.8% carbohydrate, 42.5% total dietary fiber, 37.5% soluble dietary fiber, 7.5% ash, and 1% calcium. According to the database from the Thai food composition tables (1999) [7] raw fresh leaves on a dry basis (Food ID of THD211) were calculated with 12.2% protein, 6.1% fat, 71.4% total dietary

fiber, 10.2% ash, and 0.8% calcium. Apasara et al [8] determined the chemical composition in oven-dried leaves, and the composition on a dry basis was calculated as 12.9% protein, 2.0% fat, 75.9% carbohydrate, 36.7% total dietary fiber, 20.7% soluble dietary fiber, 9.2% ash, and 1.2% calcium, which are in agreement with the present study. Another report of dried frozen leaves contained 13.8% protein, 3.3% fat, 74.9% carbohydrate, 62.5% total dietary fiber, 31% soluble fiber, 8% ash, and 0.6% calcium [9]. Based on dry matter, the results of the present study showed consistency with the previous reports except in two instances. Firstly, the protein content was slightly higher than the previous ones. This discrepancy is partly due to different analytical methods since all studies except those of the present study used the Kjeldahl method for protein determination. In general, crude protein is measured as total nitrogen multiplied by a conversion factor of 6.25 [10]. The combustion and the Kjeldahl methods are 2 standard methods for protein and nitrogen determination that comply with AOAC approved methods [11]. In the Kjeldahl method, the sample is digested with a concentrated acid under extreme conditions, and the released nitrogen is quantified by titration [12]. The combustion method measures total nitrogen as in gas state after complete combustion [10]. Results obtained from both methods are in good agreement [13]. However, it was demonstrated that the combustion method yielded higher nitrogen concentration values than the Kjeldahl [10] and the same tendency was shown here. Secondly, despite all the studies using the Enzymatic-gravimetric method for dietary fiber determination, the ratio of total fiber to soluble fiber and soluble fiber to insoluble fiber were much different in each report. Variations might have been due to the differences in the sample itself, such as location and time of collection, size and color of leaves, sample handling procedures, and sample preparations, for example the present study specified and used the same size of leaves. Further studies should explore the importance and implications of these variations.

The nutritional composition of the aqueous extract was 16.3% protein, 76.2% carbohydrate, 66.1% total dietary fiber (all of which were soluble dietary fiber), 7.5% ash, and 0.7% calcium on a dry weight basis. In comparison with the previous work of Pochawan, gel consisted of 11.6% protein, 65.9% carbohydrate, 35.3% total fiber, 30.3% soluble fiber, and 22.5% ash [5]. Values were in accordance with the current findings except for the unusual higher value of ash content.

Phenolic acids

Phenolic acids were identified against 10 authentic standards, and 3 components were detected as shown in the chromatograms of extract of freeze-dried Krung ba dan leaves and aqueous extract at 325 nm (Figure 1 and Figure 2). The identification of peaks 1, 2, and 3 were chlorogenic acid, *p*-coumaric acid, and ferulic acid respectively.

Individual phenolic acid contents are presented in Table 3. It was demonstrated that not only leaf but also the aqueous extract, to a lesser extent, contained phenolic acids. Three hydroxycinnamic acids derivatives were identified with the descending order of chlorogenic acid, ferulic acid, and *p*-coumaric acid. When the values to µg per g fresh leaf were adjusted, the phenolic contents in Krung ba dan leaves were

35.8, 22.5, and 14.4 µg per g fresh leaf, and phenolic contents in the aqueous extract were 8.9, 6.7, 2.9 µg per g fresh leaf respectively. The sum of phenolic acids in leaves and aqueous extract were 72.7 and 18.4 µg per g fresh leaf respectively. As a result, phenolic contents in the aqueous extract were approximately 4 times lower than those found in the leaves. This may be due to the leaves and water extraction ratio of 1:4 w/v. However, this is the first report of the identification of phenolic acids in *Cyclea barbata*. So kale leaf, a commonly consumed vegetable by Thais, was chosen for comparison with phenolic acid contents of 0.4 mg *p*-coumaric acid, 0.7 mg ferulic acid per 100 g dry matter, while chlorogenic acid was not detected [14]. In the present study, the calculated phenolic contents were 6.6 mg *p*-coumaric acid, 10.3 mg ferulic acid, and 16.4 mg chlorogenic acid per 100 g dry matter. It was remarkable that Krung ba dan leaves had much higher amounts of ferulic acid, *p*-coumaric acid, and chlorogenic acid than those of kale.

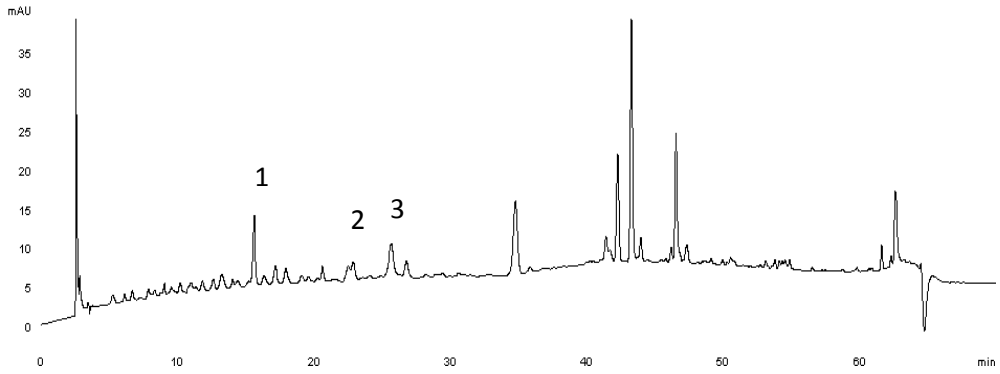

Figure 1 Chromatogram of extract of Krung ba dan leaves at 325 nm. Peak identifications for peaks 1, 2, and 3 were chlorogenic acid, *p*-coumaric acid, and ferulic acid respectively

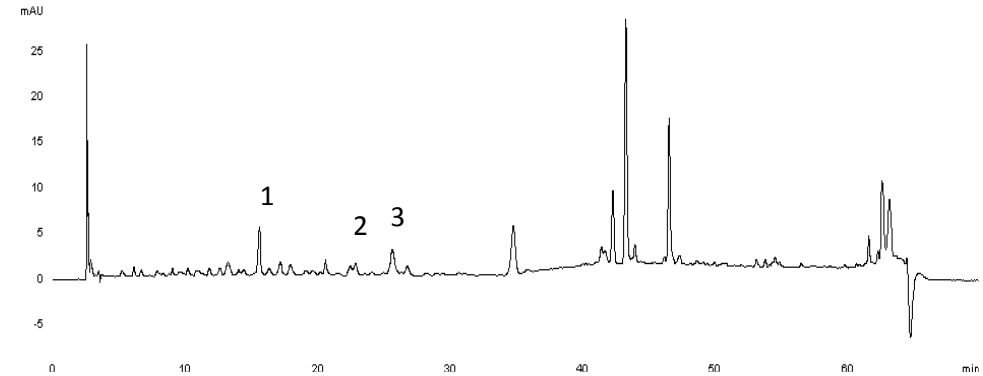

Figure 2 Chromatogram of extract of the aqueous extract at 325 nm. Peak identifications for peaks 1, 2, and 3 were chlorogenic acid, *p*-coumaric acid, and ferulic acid respectively

Table 3 Phenolic acid contents of freeze-dried Krung ba dan leaves and the aqueous extract

Bioactive compounds (mg/100 g freeze dried samples)	Samples	
	Leaves [a]	Aqueous extract [a]
Chlorogenic acid	15.5	11.1
Ferulic acid	9.8	8.3
p-coumaric acid	6.3	3.6
Sum of phenolic acids	31.6	23.0

[a] Values are from a single composite sample

Conclusions and Suggestion

The nutritional compositions of Krung ba dan leaves (*Cyclea barbata* Miers) and the aqueous extract were determined, and soluble dietary fiber and calcium were found to be the major contributors. The details in comparison with all data available of *Cyclea barbata* leaves were discussed. In addition, this is the first report of the phenolic acid quantity of this plant, and chlorogenic acid was the most abundant compound in the leaves and aqueous extract. Studies of gelling properties, total phenolic contents, and antioxidant activities should help confirm the health benefits to promote sustainable consumption.

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