

Determination of phenolic acids and flavonoids in leaves, calyces, and fruits of *Physalis angulata* L. in Viet Nam

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Abstract

In Vietnam, *Physalis angulata* L. is a wild species growing throughout the country that is often used in traditional medicine. The aim of study was to quantify seven major compounds, including phenolic acids (chlorogenic acid, caffeic acid, p-coumaric acid) and flavonoids (rutin, quercitrin, quercetin, and kaempferol) in three aerial parts of *P. angulata*. Chromatographic separation was carried out on a Kromasil C₁₈ column (150 mm × 4.6 mm i.d., 5 μm) with a gradient elution of 0.1% formic acid in acetonitrile, 0.2% ammonium acetate/0.1% formic acid in water and methanol at a flow rate of 1.0 mL/min; detection was at 250 and 300 nm. The limits of detection and quantification were in the ranges of 0.1–0.3 and 0.3–1.0 μg/mL, respectively. The validated method was successfully applied to analyze active compounds in *P. angulata* and may be a useful tool for quality control of this plant.

Keywords

rutin, HPLC-DAD, *Physalis angulata*, phenolic acid, ultrasound-assisted extraction

Introduction

Physalis angulata L. (*P. angulata*) is an edible and annual plant belonging to the Solanaceae family, which is mainly distributed in tropical and subtropical regions including Asia, Central and South America, Africa, and the Pacific Islands. In Vietnam, *P. angulata* is called “thu lu,” a wild species growing throughout the country that is often used in traditional medicine due to its ethnopharmacological value. Moreover, this medicinal plant is commonly used in folk medicine worldwide to treat chronic rheumatism as well as kidney, bladder, liver and skin diseases (Renjifo et al. 2013). Recently, *P. angulata* has been reported to exhibit a broad spectrum of pharmacological activities including anti-inflammatory, antibacterial, molluscicidal,

antiprotozoal, anticancer, cytotoxic, and immunomodulatory effects (Ayodhyareddy and Rupa 2016). The broad range of activities may be correlated with the vast metabolic and structural diversity of the plant. Several metabolites have been identified in *P. angulata*, such as phenolic acids, flavonoids, alkaloids, saponins, tannins, terpenoids, physalins, and withanolides; most of them exhibit significant bioactivity (Renjifo et al. 2013; Lima et al 2020).

Phenolic compounds are secondary metabolites that are synthesized and accumulated in all plant parts. These compounds play important roles in plants, such as defense against ultraviolet radiation and resistance against pathogens (Ali and Neda 2011; Samar Al Jitan 2018). In addition, several previous studies reported that phenolic compounds are the major bioactive phytochemicals

because of their remarkable pharmacological activities (Radek et al. 2011; Medina-Medrano et al. 2015). Phenolic compounds include simple phenols, phenolic acids, coumarins, flavonoids, stilbenes, hydrolyzed and condensed tannins, lignans, and lignins (Stalikas CD 2007). According to studies reported by Medina-Medrano et al. (2015) and Cobadela-Velasco et al. (2017), phenolic acids and flavonoids were found in all tissues of *P. angulata* as well as other wild *Physalis* species (*P. hederifolia* var. *hederifolia*, *P. solanacea*, *P. patula*, and *P. subulata*). In addition, a study conducted by Nnamani et al. (2009) revealed that the highest amounts of bioactive constituents found in *P. angulata* leaves corresponded to flavonoids (15.5%), followed by alkaloids (2.0%), saponins (2.0%), cyanides (0.39%), tannins (0.05%), and phytates (0.02%). The high phenolic compound content accumulated in *P. angulata* leaves may be responsible for its strong antioxidant activity. This protects the body against oxidative stress, which is known to cause several chronic diseases, such as atherosclerosis, cancer, diabetes, rheumatoid arthritis, post-ischemic perfusion injury, myocardial infarction, cardiovascular diseases, and chronic inflammation (Ali and Neda 2011; Samar Al Jitan 2018). Therefore, the phenolic compound content is an important parameter for evaluating the bioactivity of medicinal plants. The phenolic composition is the result of genetic and environmental factors (Markus et al. 1995). Therefore, it may change depending on the plant genotypes, growth stages, growth seasons, and eco-geographical growth conditions. Phenolic compounds accumulated in wild *Physalis* species (*P. angulata*, *P. hederifolia* var. *hederifolia*, *P. solanacea*, *P. patula*, and *P. subulata*) have been reported to be species-specific by Medina-Medrano et al. (2015). Furthermore, tissue- and age-dependent variations in the concentrations of phenolic compounds were found in *P. angulata* collected in Durango, Mexico (Marcos et al. 2017). In the case of other species, changes in the phenolic composition induced by plant genotypes have been reported for walnut leaves during their tropical growing season (Nour et al. 2013) and eco-geographical growth conditions for *Cosmos caudatus* (Mediani et al. 2012) and *Pistacia atlantica* (Ben Ahmed et al. 2017). These changes may directly affect the flavor and biological properties of both edible and medicinal plants (Jiang et al. 2013). Therefore, the identification and quantification of major phenolic compounds accumulated in *P. angulata* is essential to enhance the reliability, uniformity, and quality of raw materials, thus facilitating their use in the pharmaceutical industry. To the best of our knowledge, no validated method has been described so far for the quantification of the phenolic compounds present in the aerial parts of *P. angulata* including leaves, calyces, and fruits. This study is aimed at developing and validating a simple, sensitive, and robust analytical procedure for the determination of major phenolic compounds accumulated in three parts of *P. angulata* using ultrasound-assisted extraction (UAE) and high-performance liquid chromatography (HPLC) with a diode-array detector (DAD). The developed procedure was applied to elucidate the contents

of seven major compounds, including phenolic (chlorogenic acid, caffeic acid, *p*-coumaric acid) and flavonoid (rutin, quercitrin, quercetin, and kaempferol) derivatives, in fresh and dried samples collected in three provinces of southern Vietnam.

Materials and methods

Chemicals and solvents

Common phenolic acid reference standards (chlorogenic – 98.2%, caffeic – 97.5%, and *p*-coumaric – 99.5%) and flavonoids reference standards (rutin – 98.5%, quercitrin – 99.5%, quercetin – 98.2%, and kaempferol – 99.3%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol, ethanol ($\geq 99.8\%$), acetonitrile, *n*-hexane, and HPLC-grade water were acquired from Honeywell (North Carolina, USA). Ammonium acetate and formic acid were purchased from Merck (Darmstadt, Germany).

Plant materials

Fresh *P. angulata* were collected in the Kien Giang, Ca Mau, and Dong Thap provinces, Mekong delta, Vietnam, in January 2020. The plants were identified at the Department of Biology, Can Tho University (scientific name as *Physalis angulata* L.). The leaf, calyx, and fruit of fresh samples were collected separately, dried at room temperature, pulverized, and analyzed individually. All samples were stored in black glass containers and kept at room temperature. Dried samples were collected from traditional drugstores located in these provinces. Following this, the leaf, calyx, and fruit of dried samples were separated, pulverized, and stored in a similar manner to those of fresh samples.

Standard and sample preparation

Individual standard stock solutions of chlorogenic acid and rutin (2000 $\mu\text{g}/\text{mL}$), caffeic acid, *p*-coumaric acid, quercitrin, quercetin, and kaempferol (1000 $\mu\text{g}/\text{mL}$) in methanol were prepared, and were stable for approximately 12 months. Working standard solutions were prepared daily by diluting the stock solutions with methanol to provide different concentrations. The standard stock and working solutions were protected from light and stored at 4 °C.

The powdered leaves, fruits, and calyces (0.50 g) were accurately weighed, and extracted with 10 mL of methanol:water (70:30, v/v) in an ultrasonic bath three times for 15 min each. The extracted solutions were combined in a 50 mL volumetric flask and diluted with methanol. For the cleanup procedure, 4 mL of the extract was transferred to a 15 mL centrifuge tube and dried under a nitrogen stream at 40 °C. The residue was reconstituted with 0.4 mL of acetonitrile:water (50:50, v/v) and 0.4 mL of methanol:water (50:50, v/v), and partitioned with *n*-hexane to eliminate non-polar matrix interferences. The lower layer was filtered through a 0.22 μm PTFE filter and transferred to a vial.

Optimization of sample preparation

Several methods have been previously used for the extraction of phenolic acids and flavonoids from *P. angulata*, including microwave-assisted extraction (MAE), maceration, subcritical fluid extraction (SFE), and UAE (Carniel et al. 2016; Marcos et al. 2017). Among these, UAE offers several advantages including high extraction efficiency, good reproducibility, low solvent consumption speed, low cost, environmental friendliness, laboratory availability, and ease of scale-up for industrial applications. The high extraction efficiency of UAE was reported to be due to the production and bursting of cavitation bubbles at the surface of plant samples, which enhanced solvent penetration into the plant material and mass transfer of the phenolic acids and flavonoids across disrupted cellular membranes into solution (Samar et al. 2011).

The UAE procedure was optimized with regard to the solvent (ethanol, methanol, methanol – water), solid–liquid ratio (1:5, 1:10, 1:20, 1:30, and 1: 40 g/mL), extraction time (5, 10, 15, 20, and 30 min), and sonication time (once, twice, three, four, and five times) under investigation. Phenolic acid and flavonoid peaks were identified based on the comparison of their retention time (RT) and UV spectrum obtained by using a DAD with those of the reference compounds. i.e., chlorogenic acid, caffeic acid, *p*-coumaric acid, rutin, quercitrin, quercetin, and kaempferol, as well as the UV spectroscopy theory for phenolic acids and flavonoids developed by Campos and Markham (2007). The sum of the phenolic acid and flavonoid peak areas was used to evaluate the extraction efficiency.

Chromatographic conditions

Method development, quantification, and validation studies were performed using an Agilent LC 1100 series (Agilent Technologies, Mississauga, ON, Canada) equipped with a G-1310A pump, G-1316A column thermostat, G-1313A autosampler, and G1315B diode array detector.

Chromatographic separation was performed on a Kromasil C₁₈ column (150 mm × 4.6 mm I.D, 5 μm). The mobile phase consisted of 0.1% formic acid in acetonitrile (A), methanol (B), and 0.2% ammonium acetate/0.1% formic acid in water (C). The gradient elution program is shown in Table 1. The injection volume was 20 μL, and all chromatographic separations were performed at a flow rate of 1.0 mL/min. Different detection wavelengths were used

to identify the analytes, i.e., 250 nm for chlorogenic acid, rutin, quercitrin, quercetin, and kaempferol, and 300 nm for caffeic acid and *p*-coumaric acid. The UV spectra of all peaks were obtained between 200 and 400 nm using a DAD. The Agilent Technologies Chem-Station software was used to monitor and control all analytical conditions and reprocess the chromatographic data.

Method validation

The proposed method was validated for selectivity, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, and accuracy according to the Association of Official Analytical Chemists (AOAC) and ICH guidelines.

Results and discussion

Optimization of sample preparation

The results of the optimization of the sample preparation procedure are shown in Fig. 1. To extract the phenolic acids and flavonoids from either whole plants or aerial parts of *P. angulata*, water (Seun et al. 2018), methanol (Ana et al. 2020), ethanol–water (Carniel et al. 2016; Marcos et al. 2017), and a mixture of ethanol, methanol, and water (Gisele et al. 2018) were previously used as the extraction solvent. According to the solubility of phenolic compounds, the extraction efficiencies of methanol and ethanol for the powders derived from *P. angulata* leaves, calyces, and fruits were investigated. In the case of leaf samples, it was found that methanol afforded a content of flavonoids five times higher than ethanol, while the content of phenolic acids was 10 times higher. Although leaf, calyx, and fruit samples differed in their physical structure, the results for the calyx and fruit samples were fairly similar to those obtained for the leaf samples. Therefore, the differing extraction efficiencies obtained with methanol and ethanol can be attributed to the polarity of the solvent. To identify an extraction solvent with appropriate polarity, we examined a mixture of methanol and water at several ratios of methanol (40, 50, 60, 70, 80, 90, and 100%). It was shown that an increase in the methanol ratio from 40 to 70% resulted in an increase of phenolic compound content. However, the phenolic compound content dramatically decreased from 70% to 100% of methanol. These results indicate that the extraction yields depended on both viscosity and polarity. Specifically, 70% methanol furnished the highest content of phenolic compounds, while that of hydrophobic interferences extracted from plant matrices was lower than that of methanol (Fig. 1a).

A higher volume of extraction solvent can dissolve analytes more effectively. Thus, the liquid-to-solid ratio is also an important factor during extraction. To evaluate the effect of this factor on the extraction yields, we examined different ratios ranging from 1:5 to 1:40 g/mL. It

Table 1. Gradient elution program.

Time (min)	Flow rate (mL/min)	Mobile phase ratio		
		A (%)	B (%)	C (%)
0.0	1.0	0	5	95
0.5	1.0	0	5	95
17.5	1.0	5	9	86
26.5	1.0	13	5	82
47.5	1.0	12	15	73
48.5	1.0	11	21	68
60.0	1.0	14	28	58
69.5	1.0	28	30	42
70.0	1.0	0	5	95
74.0	1.0	0	5	95

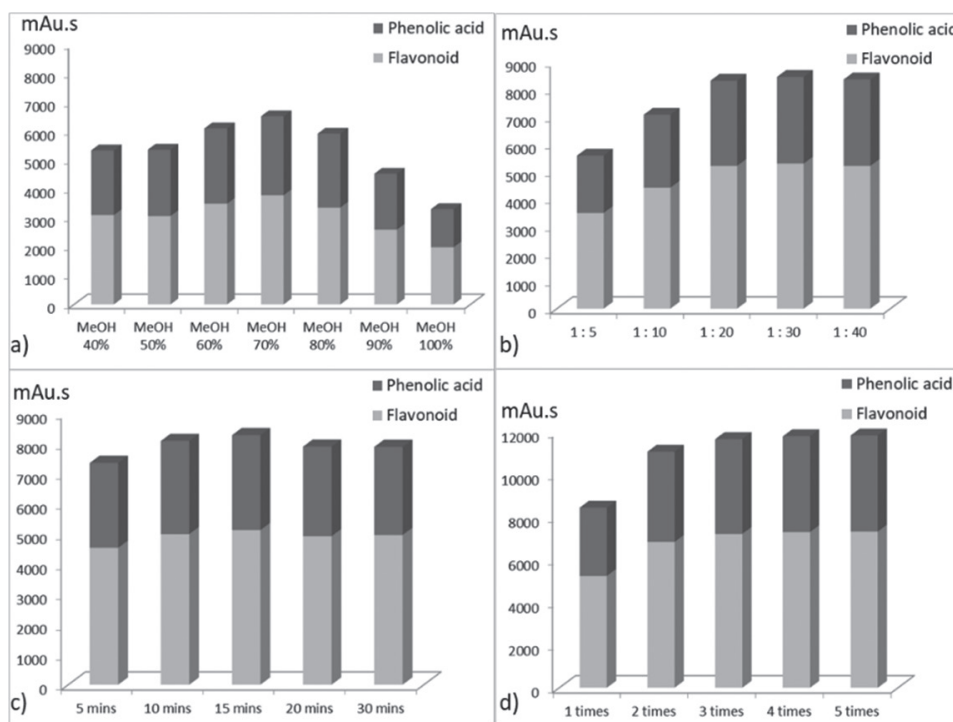


Figure 1. Yield of extract as a function of a) extraction solvents, b) solid to liquid ratio, c) sonication time, d) extraction times.

was found that the phenolic acid and flavonoid contents increased with increase in the solid–liquid ratio from 1:5 to 1:20 g/mL before stabilizing at ratios of 1:30 and 1:40 g/mL (Fig. 1b).

As shown in Figure 1c, the extraction yields increased before the sonication time reached 15 min, then slightly decreased at 20 and 30 min. Therefore, the adequate sonication time for the extraction of phenolic acids and flavonoids from *P. angulata* leaf, calyx, and fruit samples was 15 min, since an excessive sonication time might cause the degradation of phenolic compounds (Annegowda et al. 2010). The data shown in Figure 1d illustrate that the ideal number of extractions was three in order to extract more than 99% of the analytes from the *P. angulata* matrices.

Optimization of chromatographic conditions

To analyze phenolic acids and flavonoids, acidic modifiers are usually added to the mobile phase to minimize the ionization of polyphenolic compounds and silanols. Moreover, the addition reduces the interaction between analytes and ionized silanols on the stationary phase, which might result in peak tailing (Snyder et al. 2009). Numerous previous studies reported that the mobile phase used for the separation of phenolic acids and flavonoids by RP-HPLC was usually a mixture of water and methanol, water and acetonitrile, or a combination of water, methanol, and acetonitrile. In addition, the mobile phase was usually modified by the addition of phosphoric acid, formic acid, or tetrahydrofuran (Carniel et al. 2016; Marcos et al. 2017; Seun et al. 2018; Ana et al. 2020). Therefore, in this study, the composition of water

and methanol or acetonitrile and various concentrations of formic acid in water (0.1 to 1%) were evaluated to define the appropriate mobile phase. After numerous attempts, it was found that a concentration of formic acid below 0.5% did not significantly improve the peak shape, while the addition of a higher concentration of formic acid at a pH lower than 2 resulted in decreased column performance. To reduce the peak tailing at low formic acid concentrations, another modifier, i.e., ammonium acetate, was added to water. It was shown that 0.2% ammonium acetate and 0.1% formic acid in water led to a separation efficiency as satisfactory as that obtained with 1% formic acid; therefore, these concentrations of ammonium acetate and formic acid were selected for all subsequent studies. In addition, acetonitrile provided a better peak shape and separation of flavonoids than methanol. In contrast, phenolic acids were poorly retained even at 5% acetonitrile, and the combination of water and methanol allowed for a better resolution of phenolic acids. Therefore, a combination of methanol, acetonitrile, and water was considered. It was found that an initial ratio of methanol to water of 5:95 provided adequate retention of phenolic acids, while the combination of water, methanol, and acetonitrile with increasing methanol ratios during the run time (Table 1) provided satisfactory separation of phenolic acids and flavonoids (Fig. 2).

Method validation

System stability

The system stability was tested by carrying out six replicate injections of a mixed standard solution (10 µg/mL) and determining the theoretical plate number (N), resolution (Rs),

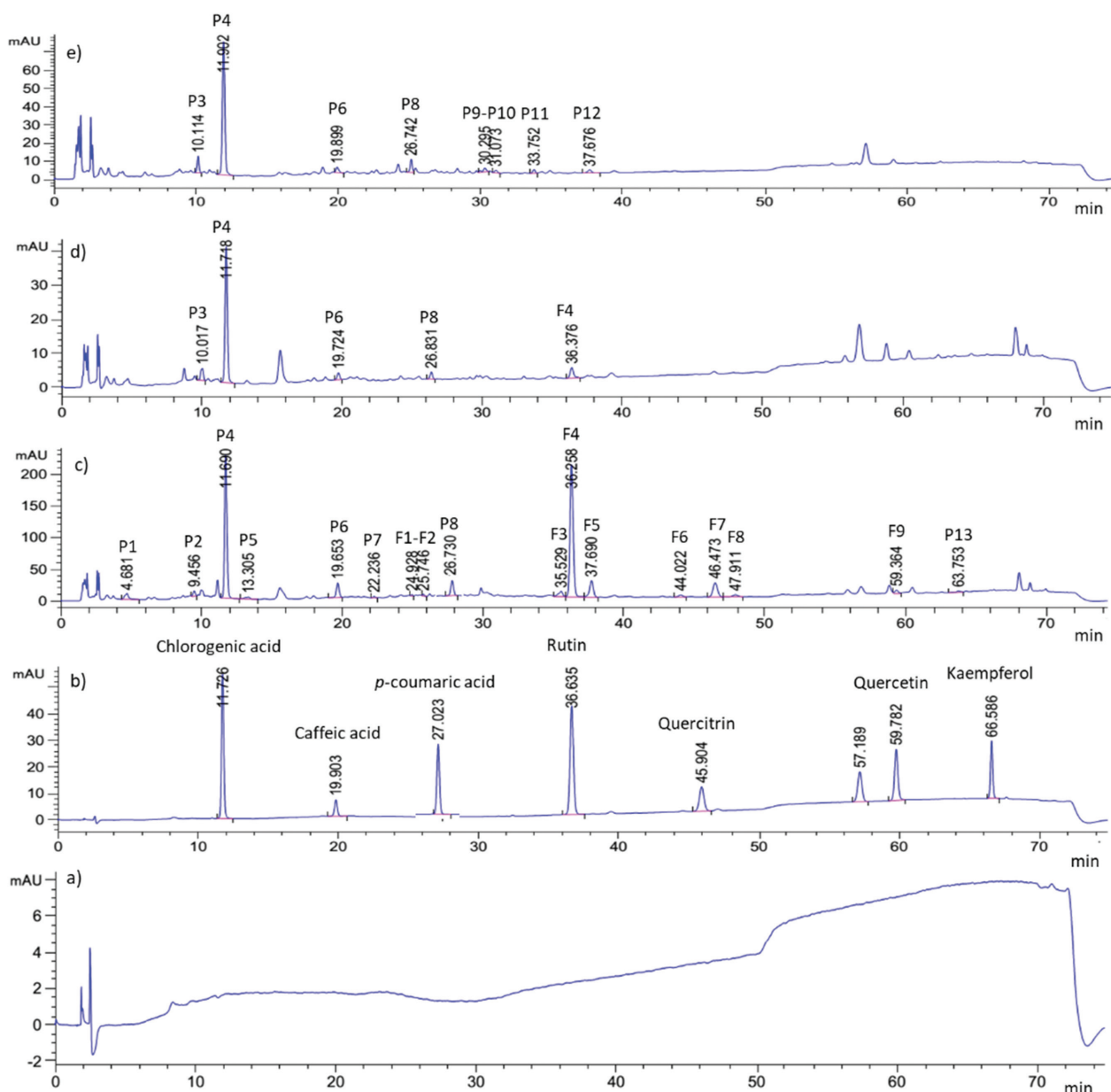


Figure 2. HPLC chromatogram of the extraction solvent (a), mixed standards solution (b), *P. angulata* leaves (c), calyxes (d) and fruits (e). All peaks were identified based on the UV spectra theory of phenolic acids and flavonoids developed by Campos and Markham (2007). P1–P13: Phenolic acids, including P4: chlorogenic acid, P6: caffeic acid, P8: *p*-coumaric acid. F1–F9: Flavonoids, including F4: rutin, F9: quercetin.

symmetry factor (A_s), and repeatability [relative standard deviation (RSD) of RT and area] of the analytes. The %RSD values of the peak area and RT of all analytes were less than 2.0%. Therefore, the proposed method met this requirement.

Specificity

The selectivity was tested by employing the HPLC method to analyze the extracts of the leaf, calyx, and fruit parts of *P. angulata*. It was evaluated by comparing the RT and UV absorption spectrum of each component in standard solutions with those of the peaks obtained by analyzing the extracts. As shown in Figure 2, the HPLC method could distinguish phenolic acids (chlorogenic, caffeic, *p*-coumaric) and flavonoids (rutin, quercitrin, quercetin, and

kaempferol) from other components of the leaf, calyx, and fruit matrices. The peak purity of the seven compounds was > 99.9%, as obtained from the spectrum overlaying the graphs of three-point purity detection.

Linearity, limit of detection, and limit of quantification

The stock solutions were diluted and mixed to seven different concentrations ranging from 5.0 to 500 $\mu\text{g/mL}$ of chlorogenic acid and rutin, and from 2.0 to 80 $\mu\text{g/mL}$ of caffeic acid, *p*-coumaric acid, quercitrin, quercetin, and kaempferol. To evaluate the linearity, each mixed standard sample was injected in triplicates into the HPLC system, and calibration curves were obtained

Table 2. Precision, accuracy data and calibration parameters of the leaf matrix.

	Recovery (%)			Precision (n = 6)		Calibration curve (n = 3)		
	Low-level	Mid-level	High-level	Intra-day RSD (%)	Inter-day RSD (%)	Slope (\pm SD)	y-intercept (\pm SD)	r ² (\pm SD)
Chlorogenic acid	105.3	100.5	101.6	1.16	0.88	10.82 \pm 0.14	19.76 \pm 9.25	0.9990 \pm 0.0002
Caffeic acid	101.5	101.2	103.5	1.59	1.44	13.62 \pm 0.36	-9.880 \pm 2.04	0.9997 \pm 0.0001
p-Coumaric acid	101.8	99.8	102.5	1.38	1.97	57.36 \pm 0.74	-27.15 \pm 8.49	0.9997 \pm 0.0002
Rutin	105.6	100.6	101.7	0.80	0.77	12.32 \pm 0.17	23.10 \pm 12.33	0.9990 \pm 0.0002
Quercitrin	103.3	101.5	103.9	2.20	2.76	20.55 \pm 0.69	-14.14 \pm 5.36	0.9997 \pm 0.0002
Quercetin	102.0	101.4	102.8	1.22	3.09	33.27 \pm 0.73	-39.85 \pm 4.10	0.9995 \pm 0.0001
Kaempferol	102.2	100.9	103.6	2.58	4.03	24.72 \pm 0.81	24.37 \pm 17.40	0.9991 \pm 0.0010

Table 3. Content of phenolic acids and flavonoids in leaf, calyx and fruit of fresh *P. angulata* samples.

No.	Location	Part	Phenolic acids (mg/kg dry weight)				Flavonoid (mg/kg dry weight)		
			Chlorogenic	Caffeic	p-Coumaric	Total	Rutin	Quercetin	Total
1	Rach Gia city, Kien Giang province	Leaf	4545 \pm 131.3	832.6 \pm 0.876	32.96 \pm 1.403	5855	5038 \pm 87.68	66.10 \pm 0.747	6920
		Calyx	1464 \pm 11.27	182.2 \pm 4.226	38.74 \pm 0.218	1775	87.74 \pm 4.041	ND	87.74
		Fruit	1462 \pm 40.03	134.0 \pm 3.436	ND*	1908	ND	ND	ND
2	Chau Thanh district, Kien Giang province	Leaf	3201 \pm 16.08	458.2 \pm 1.383	25.34 \pm 0.480	4222	1812 \pm 12.81	40.12 \pm 0.597	2724
		Calyx	4971 \pm 71.53	577.6 \pm 0.211	ND	5706	165.8 \pm 6.337	ND	165.8
		Fruit	808.3 \pm 4.018	286.9 \pm 1.360	45.98 \pm 0.064	1455	ND	ND	ND
3	Ca Mau province	Leaf	188.1 \pm 9.115	ND	ND	546.3	636.6 \pm 10.12	ND	923.1
		Calyx	249.1 \pm 10.25	ND	39.22 \pm 2.102	824.1	43.23 \pm 1.579	ND	43.23
		Fruit	80.52 \pm 2.342	ND	18.80 \pm 0.926	485.3	ND	ND	ND
4	Dong Thap province	Leaf	647.1 \pm 4.256	ND	39.33 \pm 0.045	727.0	622.6 \pm 2.341	ND	701.9

(*): not detected.

by plotting the average of the peak area responses versus concentration for each sample. The results of the regression equations and square correlation coefficients (r^2) are summarized in Table 2. The LOD and LOQ of the analytes ranged from 0.1 to 0.3 μ g/mL and 0.3 to 1.0 μ g/mL, respectively.

Precision

The precision of the method was verified by evaluating the intra-day and inter-day precisions. The relative standard deviation (%RSD) was selected as a measure of precision. The intra-day precision was examined by analyzing six samples from each matrix (leaf, calyx, and fruit) in a single day, while the inter-day precision was determined by analyzing six samples each day for three days. The precision results for the leaf matrix, as shown in Table 2, indicate that the overall intra- and inter-day variations (%RSD) were less than 2.58 and 4.03%, respectively.

Accuracy

The accuracy of the method was investigated by performing recovery studies. Three different concentrations, including low, medium, and high amounts of reference compounds, were added to the blank leaf samples. Then, the spiked samples were extracted and quantified according to the methods mentioned above. The results indicated that the developed method exhibited good accuracy, with an overall recovery ranging from 101.35 to 102.87%, while the RSD% was less than 2.38% for all the analytes (Table 2).

Application

The proposed method was applied to the evaluation of the content of phenolic acids and flavonoids in

P. angulata leaves, calyces, and fruits. Data in Table 3 indicate that five out of the seven analytes were detected in the fresh samples (chlorogenic acid, caffeic acid, p-coumaric acid, rutin, and quercetin) across a wide concentration range (25.34–5038 mg/kg). Owing to the unavailability of several commercial phenolic acid and flavonoid standards, only five analytes (chlorogenic acid, caffeic acid, p-coumaric acid, rutin, and quercetin) were quantified via their standard calibration curve, while other phenolic acids and flavonoids were quantified by using chlorogenic acid and rutin as reference, respectively. The sum of the individual flavonoid concentrations represented the total flavonoid concentration expressed as milligrams per kilogram of dry weight (mg/kg dw), whereas the sum of the individual phenolic acid concentrations corresponded to the total phenolic acid concentration, which was expressed as milligrams per kilogram of dry weight (mg/kg dw).

Phenolic acid contents

The data illustrated in Table 3 show that the total phenolic acid content varied between different plant parts and locations. For samples collected in Rach Gia city, Kien Giang province, the total phenolic acid content was highest in leaves (5855 mg/kg), whereas for samples collected in the Chau Thanh district, Kien Giang province, as well as Ca Mau province, calyces exhibited a 5-fold higher total phenolic acid content than leaves. In addition, fruits showed the smallest amounts of total phenolic acids in all fresh samples (485.3–1908 mg/kg) when compared to leaves and calyces; this result was similar to the amounts of total phenolic acids found in fruits of *P. angulata* collected in Peru (Marcos et al. 2017). The highest content of phenolic acids in *P. angulata* leaves, calyces, and fruits corresponded to chlorogenic acid (80.52–4971 mg/kg),

Table 4. Content of phenolic acids and flavonoids in leaf, calyx and fruit of dried *P. angulata* samples.

No.	Location	Part	Phenolic acids (mg/kg dry weight)				Flavonoid (mg/kg dry weight)		
			Chlorogenic	Caffeic	<i>p</i> -Coumaric	Total	Rutin	Quercetin	Total
1	Rach Gia city, Kien Giang province	Leaf	1390 ± 53.32	407.2 ± 7.829	21.06 ± 0.457	2739	1697 ± 5.339	43.53 ± 0.082	2452
		Calyx	386.8 ± 24.74	194.9 ± 9.085	ND	581.7	ND	ND	ND
		Fruit	ND	68.76 ± 2.120	26.21 ± 0.420	246.2	ND	ND	ND
2	Ca Mau province	Leaf	ND	ND	ND	82.81	327.2 ± 11.31	ND	542.4
		Calyx	ND	ND	ND	ND	ND	ND	ND
		Fruit	ND	ND	ND	ND	ND	ND	ND
3	Dong Thap province	Leaf	19.32 ± 0.501	ND	ND	19.32	136.4 ± 10.78	ND	136.4
		Calyx	19.01 ± 0.383	ND	ND	19.01	ND	ND	ND
		Fruit	ND	ND	26.26 ± 0.106	26.26	ND	ND	ND

(*): not detected.

followed by caffeic acid (134.0–832.6 mg/kg) and *p*-coumaric acid (18.80–45.98 mg/kg). These data differed from the results of Akomolafe et al. (2018), according to which the amount of caffeic acid was the highest in leaf extracts, followed by ellagic acid, chlorogenic acid, and gallic acid.

Flavonoid contents

Flavonoids varied between plant parts, similar to phenolic acids. In fresh *P. angulata* samples, leaves showed the highest content of total flavonoids (701.9–6920 mg/kg dry weight), while the samples collected in Rach Gia city, Kien Giang province, exhibited the highest total flavonoid content (6920 mg/kg dry weight). This value was two times higher than that reported for the leaves of *P. peruviana* (3238.5 mg/kg dry weight) by Lezoul et al. (2020). For all fresh *P. angulata* samples collected in different locations, leaves were a major source of flavonoids, which is in partial agreement with the reports of Cobadela-Velasco et al. (2018). In contrast, flavonoids were not detected in fruits. The current results are similar to those reported for the fruits of *P. angulata* by Cobadela-Velasco et al. (2018) and Medina-Medrano et al. (2015). The total flavonoid content in *P. angulata* calyces ranged from 43.23 to 165.8 mg/kg; this finding was contrary to a report by Cobadela-Velasco et al. (2018) that no flavonoids were found in mature calyces. In addition, the results concerning the flavonoid contents revealed that rutin was the most abundant flavonoid in all samples (accounting for 66.5–88.7% of the total flavonoid content). The amount of rutin ranged between 43.23 and 5038 mg/kg, and significant differences were observed due to planting locations and plant parts. Rutin occurred with a 10–50 folds difference between leaves and calyces. Previous experimental results also indicated that rutin is one of the major components in other wild *Physalis* species, including *P. solanacea*, *P. patula*, and *P. subulata* (Mediani et al. 2012). In addition, rutin was found to be present at high concentrations in the aerial parts of *P. orizabae* (Maldonado et al. 2012). Quercetin was only found in leaves at low concentrations, ranging from 40.12 to 66.10 mg/kg. This result is in contrast with that reported for the flavonoid composition found in *P. angulata* leaf extracts by Akomolafe et al. (2018), in which quercetin was the most abundant flavonoid (56.74 mg/g).

Phenolic acid and flavonoid contents in dried samples

The dried *P. angulata* samples showed a significant decrease (3–10 fold) of total phenolic acid and flavonoid contents when compared to the fresh samples collected in the same province (Table 4). This decrease may be related to compound decomposition during the drying and storage process due to the uncontrolled temperature, light intensity, and humidity. In particular, chlorogenic acid, which was the major compound in fresh samples, was not found in any of the dried fruit samples. This may be attributed to the high water content in fruits (72.44% as reported by Kusumaningtyas et al. 2015), which requires a longer time and higher temperature to dry, thus resulting in the degradation of phenolic acids. In addition, the chlorogenic acid contents in leaves and calyces showed a 4–30 fold difference between fresh and dried samples, while *p*-coumaric acid and caffeic acid contents presented lesser variation (1.5–2 folds) in chlorogenic acid. These results may be related to the stability of chlorogenic acid and the difference in degradation pathways between each compound and plant part. In addition, rutin was only detected in dried leaf samples in amounts ranging from 136.4 to 1697 mg/kg, which were 2–5-fold lower than those of fresh samples. Quercetin was only detected in samples collected in Rach Gia city, Kien Giang province. This sample accumulated in amounts (43.53 mg/kg) similar to those of fresh leaf samples.

Conclusions

In this study, an ultrasound-assisted extraction and RP-HPLC method combined with diode array detection was developed for determining antioxidant active compounds in the leaves, calyces, and fruits of *P. angulata*. The optimum extraction procedure allowed for good efficiency and extraction yields (> 99%). In addition, the HPLC protocol permitted a qualitative separation of the compounds and proved to be efficient, precise, and accurate. Therefore, it could be used for the simultaneous determination of phenolic acids and flavonoids present in the three aerial parts of *P. angulata*. The experimental results of fresh samples collected from three provinces of the Mekong delta indicated that both leaves and calyces were important sources of phenolic acids, whereas leaves were the best source of flavonoids. According to these results, chlorogenic acid and rutin were

the major active compounds in *P. angulata*. In addition, the data suggested that the composition was significantly influenced by different plant parts and collection locations. In addition, a considerable decrease in phenolic compounds was observed in the dried samples, which may be ascribed to the uncontrolled drying and storage processes.

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