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Original Article

Optimization of extraction method and HPLC analysis of six caffeoylquinic acids in *Pluchea indica* leaves from different provenances in Thailand

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ABSTRACT

Pluchea indica (L.) Less., Asteraceae, is a medicinal plant which contains a high amount of phenolic compounds such as caffeoylquinic acid derivatives. The leaves have been traditionally used as a nerve tonic and extensively as herbal tea. This study aimed to develop and validate an HPLC method to quantitatively analyze six caffeoylquinic acid derivatives, *viz.* 3-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, 5-*O* caffeoylquinic acid, 3,4-*O*-dicaffeoylquinic acid, 3,5-*O*-dicaffeoylquinic acid, and 4,5-*O*-dicaffeoylquinic acid in *P. indica* leaf extract. HPLC was carried out in a Hypersil BDS C_{18}-column eluted with 0.5% acetic acid in water and methanol using gradient elution with a flow rate of 1 ml/min and detection at 326 nm. The method validation was performed to assure its linearity, precision, accuracy and limits of detection and quantitation. Several extraction techniques including maceration, decoction, digestion, Soxhlet extraction, and ultrasound extraction, were used to extract active constituents. The ultrasound extraction with 50% ethanol yielded the highest concentration of these caffeoylquinic acid derivatives in the *P. indica* leaf extract. Our developed HPLC method is simple and reliable for a routine analysis of the six caffeoylquinic acids in *P. indica* leaves and could potentially be applied to be used in commercial herbal products.

Introduction

Pluchea indica (L.) Less., Asteraceae, is an evergreen shrub, commonly known as Indian camphorweed, Indian fleabane and Indian pluchea. It is widely distributed in India, Southern China, Southeast Asia, Australia and the Pacific Islands. It can be found abundantly in brackish marshes, mangrove forest and other saline habitats (eFloras, 2008). Young leaves and shoots are edible and consumed as salad or side-dish to rice. *P. indica* herbal tea has been commercially available in Thailand as a health-promoting drink (Office of Mangrove Resources Conservation, 2009). In Thai traditional medicine, leaves are used as a nerve tonic and for the treatment of inflammation. Whole plants are used for treating hemorrhoids, constipation, aphthous ulcer and gallstone (Srisook et al., 2012; Neamsuvan and Ruangrit, 2017). *Pluchea indica* leaves were found to possess various biological activities including antioxidant (Widyawati et al., 2014), anti-inflammatory (Buapool et al., 2013), hypoglycemic and antihyperglycemic activities (Pramanik et al., 2006).

Previous phytochemical studies demonstrated that leaves of *P. indica* contain caffeoylquinic derivatives (Arsiningtyas et al., 2014), flavonol aglycones (quercetin, kaempferol, myricetin) (Andarwulan et al., 2010), and terpenoid (10*S*,11*S*-himachala-3(12)-4-diene) (Widyawati et al., 2013). An HPLC-PDA-MS study revealed that caffeoylquinic derivatives such as 3-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, 5-*O*-caffeoylquinic acid, 3,4-*O*-dicaffeoylquinic acid, 3,5-*O*-dicaffeoylquinic acid and 4,5-*O*-dicaffeoylquinic acid are major compounds of *P. indica* leaves (Shukri et al., 2011). These derivatives also have been shown to possess antioxidant, anti-inflammatory activity, inhibiting HMG-CoA reductase and alpha-glucosidase enzyme (Xu et al., 2012; Vongsak et al., 2013; Chen et al., 2014; Arantes et al., 2016; Motaal et al., 2016). Thus, the six caffeoylquinic derivatives which are the major active compounds could be used as marker compounds for the quality assessment of *P. indica* leaves extract.

Several analytical methods including HPLC (Andarwulan et al., 2010), HPLC-PDA-MS^2 (Shukri et al., 2011), GC and GC–MS (Le et al., 2000) have been reported for the quantification of some

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phytochemical compounds in *P. indica* leaves. However, there has been no previous report on the simultaneous determination of six caffeoylquinic derivatives *viz.* 3-*O*-caffeoylquinic acid (3-CQ), 4-*O*-caffeoylquinic acid (4-CQ), 5-*O*-caffeoylquinic acid (5-CQ), 3,4-*O*-dicaffeoylquinic acid (3,4-CQ), 3,5-*O*-dicaffeoylquinic acid (3,5-CQ), and 4,5-*O*-dicaffeoylquinic acid (4,5-CQ). Therefore, this study was conducted to identify the appropriate extraction method and perform a quantitative HPLC validation of the six caffeoylquinic acids in the *P. indica* leaves extracts collected from various regions in Thailand.

Materials and methods

Chemicals and reagents

HPLC grade methanol was purchased from Labscan (Thailand). Deionized water was purified by Ultra Clear system (Siemen Water Technologies Corp., USA). Glacial acetic acid was purchased from Labscan (Thailand) while all reagents were of analytical grade. 3-CQ, 4-CQ, 5-CQ, 3,4-CQ, 3,5-CQ and 4,5-CQ were purchased from Chengdu Biopurify Phytochemicals Ltd., China.

Plant materials

The mature leaves of *Pluchea indica* (L.) Less., Asteraceae, were collected from fourteen different provinces in Thailand as follows: (1) Laplae District, Uttaradit Province; (2) Muang District Nakhon Ratchasima; (3) Muang District, Nonthaburi; (4) Muang District, Samut Sakhon; (5) Muang District, Samut Songkhram; (6) Tayang District, Petchaburi; (7) Pranburi District, Prachuap Khiri Khan; (8) Paktor District, Ratchaburi; (9) Muang District, Chonburi; (10) Leam sing District, Chanthaburi; (11) Klaeng District, Rayong; (12) Muang District, Phuket; (13) Hadyai District, Songkhla; (14) Phrasaeng District, Surat Thani in July to August, 2016. The specimens, (voucher numbers JAM16001-JAM16014, respectively), were identified using the identification key provided in Flora of Thailand and deposited at Faculty of Pharmaceutical Sciences, Burapha University, Chonburi, Thailand. The leaves were cleaned by washing with tap water and a portion was dried in a hot air oven at 50 °C for 24 h. The dried leaves were ground to pass through a 0.5 mm sieve, kept in sealed containers and protected from light until used. For the assessment of a suitable extraction method, the sample from Chanthaburi Province was utilized. The method that provided the highest amount of marker constituents was selected for the sample extraction.

Sample extraction

Maceration extraction

For maceration extraction, the dried powdered leaves were placed in an Erlenmeyer flask, with 50% ethanol (1:20, w/v) at room temperature (28 ± 2 °C) for 72 h with occasional shaking. The plant residue was re-extracted again by the same method.

Decoction extraction

The dried leaf powder was boiled with distilled water (1:20, w/v) at 80 ± 5 °C for 15 min and then filtered. Each extraction method was carried out three times. The marc was re-extracted twice more.

Digestion extraction

The dried powdered leaves were separately digested with 50% ethanol and distilled water (1:20, w/v) for 6 h at 60 ± 5 °C. The extract was filtered and the marc was re-extracted by the same process.

Soxhlet extraction

The dried powder was placed into an extraction thimble and separately extracted with 50% ethanol and distilled water (1:20, w/v) for 6 h until exhaustion.

Ultrasound extraction

The dried powder was placed in an Erlenmeyer flask and extracted separately with 50% ethanol and distilled water (1:20, w/v) using an ultrasonic bath (Bandelin Sonorex Digitec, Type: DT1028H, Germany) with ultrasound 35 kHz at 40 ± 5 °C for 15 min. The marc was re-extracted twice more.

Each extraction method was carried out three times. The combined extracts from each sample were separately filtered through a Whatman No. 1 filter paper. The filtrate was dried under reduced pressure at 50 °C using a rotary evaporator. The crude extract was weighed and kept in a tight container protected from light at 0 °C. Each sample was prepared by accurately weighing *P. indica* extract and dissolving in methanol. To enable a complete dissolution, each sample was sonicated for 30 min. Prior to an injection, each sample was filtered through a 0.22 μm nylon membrane and then analyzed in triplicate.

Stock and working solution of standards

Stock solutions of standard compounds were prepared by accurately weighing and dissolving the compounds in methanol to obtain the final concentration of 1000 μg/ml. Working solution of standard compounds were obtained by diluting the stock standard solutions with methanol to achieve the desired concentrations.

HPLC apparatus and chromatographic conditions

HPLC separation was achieved on an Agilent 1260 Series (Agilent Technologies, USA) equipped with a 1260 Quat pump VL quaternary pump, 1260 ALS autosampler, 1260 TCC column thermostat, and 1260 DAD VL diode array detector. The separation was done in a Hypersil BDS C_{18} column (4.6 × 100 mm i.d., 3.5 μm) with a C_{18} guard column (4 × 10 mm i.d., 3 μm). The mobile phases were (A) 0.5% acetic acid in water and (B) methanol using gradient elution: 10% B in A to 50% B in A for 40 min; 100% B for 10 min. This column was re-equilibrated with 10% B in A for 10 min prior to each analysis and the flow rate was set at 1.0 ml/min with the controlled temperature at 25 °C. DAD detector was set at the wavelength of 326 nm and injection volume was 5 μl for every sample and reference standard.

Method validation

The method was validated for linearity, precision, accuracy, limit of detection (LOD) and limit of quantitation (LOQ) according to the International Conference on Harmonization guidelines (ICH, 1996/2005).

Linearity

Linearity was determined by using working standard solutions of 3-CQ, 4-CQ, 5-CQ, 3,4-CQ, 3,5-CQ, and 4,5-CQ at concentrations of 150, 75, 37.5, 18.75, 9.38, and 4.69 μg/ml. Each concentration was analyzed in triplicate. The calibration curves were obtained by plotting the peak area versus the concentration of each standard.

Precision

The intraday precision was determined by analyzing 37.5 μg/ml solution of 3-CQ, 4-CQ, 5-CQ, 3,4-CQ, 3,5-CQ, and 4,5-CQ seven times within one day while the interday precision was examined for three consecutive days by the proposed method. The precision was expressed as percent relative standard deviation (%RSD).

Accuracy

Recovery was used to evaluate the accuracy of the method. Standard addition was performed with the pre-analysed standard solution. Three different standard mixtures (approximately 50%, 100% and 150% of the determined content of *P. indica* leaves extract) of 3-CQ, 4-CQ, 5-CQ, 3,4-CQ, 3,5-CQ, and 4,5-CQ were added to the samples. Spiked samples were prepared in triplicate and three determinations were performed. The recovery was calculated as follows:

$$\text{Recovery}(\%) = 100 \times \left(\frac{\text{amount found} - \text{original amount}}{\text{amount spiked}}\right)$$

Limit of detection (LOD) and limit of quantitation (LOQ)

A series of concentrations was achieved by diluting the lowest concentration of the working solutions with methanol. The LOD, at a signal to noise ratio (s:n) of 3:1, and LOQ, at a signal to noise ratio (s:n) of 10:1, were performed.

Statistical analysis

The average contents of caffeoylquinic acids of the extracts using the different extraction techniques were statistically examined using one-way analysis of variance (ANOVA) with least significant difference (LSD) by SPSS for Windows 16.0 (IBM Corporation, U.S.A.). A statistical probability (p value) less than 0.05 indicated a statistically significant difference between groups.

Results and discussion

An HPLC method was developed for analyzing the contents of active compounds in *P. indica* leaves extracts: 3-CQ, 4-CQ, 5-CQ, 3,4-CQ, 3,5-CQ, and 4,5-CQ. From various mobile phase trials, our system gave symmetric peaks and provided the most efficient separation. The wavelength at 326 nm, which was the maximum absorbance of these compounds, was used. The chromatograms of *P. indica* leaves extract and authentic compounds are shown in Fig. 1.

In order to ensure that the method is suitable for its intended used, the method validation has been performed according to the ICH guidelines (ICH, 1996/2005). The proposed HPLC method showed acceptable validation parameters (Table 1). The calibration curves were obtained by plotting the peak area versus the concentration of the standards and proved that the method was linear within the range of 4.69–150 μg/ml for 3-CQ, 4-CQ, 5-CQ, 3,4-CQ, 3,5-CQ, and 4,5-CQ with correlation coefficient (r^2) ≥ 0.9994. The percent relative standard deviation (%RSD) values of intraday and interday precision were lower than 3.21%. The LOD, at a signal to noise ratio (s:n) of 3:1, and LOQ, at a s:n of 10:1 were found to be 0.03 and 0.1 μg/ml, respectively, for 3-CQ, 4-CQ, 5-CQ, 3,4-CQ, 3,5-CQ, and 4,5-CQ, indicating high sensitivity of the method. The average recoveries of six caffeoylquinic acid derivatives were in the range of 96.58–99.41% which indicated good accuracy of the analytical method (Table 2).

The quantification of 3-CQ, 4-CQ, 5-CQ, 3,4-CQ, 3,5-CQ, and 4,5-CQ was performed using ten samples of *P. indica* leaf extracts from different extraction methods and fourteen samples from different locations in Thailand. Fifty percent ethanol and distilled water were selected as the solvent in this study because of their abilities to extract higher amount of total phenolic contents (Widyawati et al., 2014). Fifty percent ethanol extract contained higher amounts of 3,4-CQ, 3,5-CQ, and 4,5-CQ than aqueous extract using the same extraction method (Table 3), likely due to similar polarity between

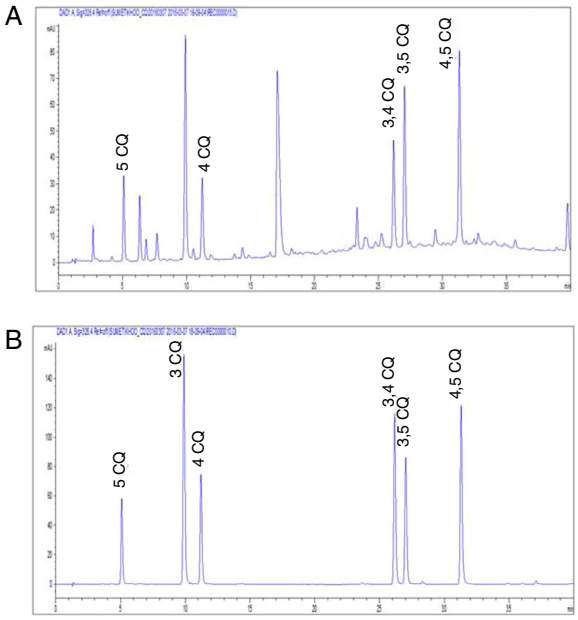

Fig. 1. HPLC chromatograms of *Pluchea indica* leaf extract (A) and authentic compounds (B): 3-*O*-caffeoylquinic acid (3-CQ), 4-*O*-caffeoylquinic acid (4-CQ), 5-*O*-caffeoylquinic acid (5-CQ), 3,4-*O*-dicaffeoylquinic acid (3,4-CQ), 3,5-*O*-dicaffeoylquinic acid (3,5-CQ) and 4,5-*O*-dicaffeoylquinic acid (4,5-CQ).

Table 1
Method validation parameters by the proposed HPLC method.

Parameters	Results					
	5 CQ	3 CQ	4 CQ	3,4 CQ	3,5 CQ	4,5 CQ
Regression equation[a]	$Y = 17.477X + 22.837$	$Y = 15.264X + 10.801$	$Y = 16.818X + 10.927$	$Y = 46.118X + 43.591$	$Y = 24.131X + 24.297$	$Y = 18.839X + 15.506$
Correlation coefficient (r^2)	0.9995	0.9998	0.9998	0.9995	0.9994	0.9996
Linear range (μg/ml)	4.69–150	4.69–150	4.69–150	4.69–150	4.69–150	4.69–150
Intraday, %RSD	0.69	0.72	0.73	0.72	0.70	0.73
Interday, %RSD	1.50	1.53	1.87	2.12	2.12	3.20
LOQ, μg/ml	0.1	0.1	0.1	0.1	0.1	0.1
LOD, μg/ml	0.03	0.03	0.03	0.03	0.03	0.03

[a] X is the concentration of the compounds. Y is the peak area at 326 nm.

Table 2
Recovery studies of six caffeoylquinic acid derivatives.

Serial no.	Compound	Theoretical (μg/ml)	Found[a] (μg/ml)	Recovery[a] (%)
1	5 CQ	11.36	11.70 ± 0.29	102.55 ± 2.57
	3 CQ	43.71	43.19 ± 1.09	98.81 ± 2.49
	4 CQ	15.95	15.91 ± 0.26	99.74 ± 1.62
	3,4 CQ	7.71	7.74 ± 0.10	100.32 ± 1.24
	3,5 CQ	17.01	16.83 ± 0.75	98.93 ± 4.43
	4,5 CQ	31.69	30.90 ± 0.97	97.52 ± 3.06
2	5 CQ	16.73	16.93 ± 0.033	98.80 ± 0.19
	3 CQ	60.96	62.12 ± 1.03	98.13 ± 1.69
	4 CQ	23.54	22.73 ± 0.39	103.58 ± 1.65
	3,4 CQ	12.69	12.08 ± 0.54	105.01 ± 4.26
	3,5 CQ	24.14	25.42 ± 1.10	94.98 ± 4.56
	4,5 CQ	42.76	43.69 ± 1.64	97.87 ± 3.85
3	5 CQ	20.70	19.79 ± 0.90	95.59 ± 4.33
	3 CQ	78.46	74.09 ± 3.14	94.42 ± 4.01
	4 CQ	30.80	28.41 ± 1.46	92.26 ± 4.73
	3,4 CQ	16.62	15.44 ± 1.16	92.90 ± 6.98
	3,5 CQ	30.89	29.60 ± 1.36	95.84 ± 4.40
	4,5 CQ	55.18	51.96 ± 2.77	94.17 ± 5.02
Average	5 CQ			98.98
	3 CQ			97.12
	4 CQ			98.53
	3,4 CQ			99.41
	3,5 CQ			96.58
	4,5 CQ			96.52

[a] Expressed as mean ± SD ($n = 3$).

Table 3
The content of six caffeoylquinic acid derivatives in *Pluchea indica* leaf extracts of different extraction methods analyzed by the validated HPLC method.

Method	Content of major compound[c] (% w/w)					
	5-CQ	3-CQ	4-CQ	3,4-CQ	3,5-CQ	4,5-CQ
Maceration with 50% ethanol	2.19 ± 0.16[a]	17.53 ± 1.22[a]	3.45 ± 0.25[a]	2.84 ± 0.22[a]	12.69 ± 0.95[a]	31.85 ± 2.10[a]
Decoction with distilled water	2.57 ± 0.08[b]	15.55 ± 0.34[b]	3.96 ± 0.10[b]	2.81 ± 0.06[a]	10.26 ± 0.22[b]	22.68 ± 0.41[b]
Digestion with 50% ethanol	2.37 ± 0.19[ab]	18.08 ± 1.41[a]	3.64 ± 0.28[ab]	2.72 ± 0.23[a]	12.41 ± 1.03[a]	30.94 ± 2.52[a]
Digestion with distilled water	2.46 ± 0.07[b]	17.10 ± 0.18[a]	3.68 ± 0.05[a]	2.07 ± 0.03[b]	8.38 ± 0.16[c]	18.37 ± 0.27[c]
Soxhlet with 50% ethanol	2.31 ± 0.05[a]	17.63 ± 0.49[a]	3.57 ± 0.09[a]	2.72 ± 0.08[a]	12.19 ± 0.36[a]	30.59 ± 0.84[a]
Soxhlet with distilled water	2.79 ± 0.05[c]	16.79 ± 0.66[a]	3.76 ± 0.07[a]	1.85 ± 0.09[c]	6.66 ± 0.43[d]	12.77 ± 0.80[d]
Ultrasound with 50% ethanol	2.67 ± 0.20[bc]	18.54 ± 1.19[a]	3.85 ± 0.26[ab]	2.86 ± 0.19[a]	13.15 ± 0.84[a]	31.03 ± 1.89[a]
Ultrasound with distilled water	2.78 ± 0.19[bc]	17.48 ± 1.03[a]	3.90 ± 0.23[ab]	2.10 ± 0.14[b]	8.24 ± 0.52[c]	15.69 ± 0.98[e]
Average	2.52	17.34	3.72	2.50	10.50	24.24

[c] Expressed as mean ± SD ($n = 3$), different letters in the same column indicate significant difference at $p < 0.05$ using one-way ANOVA.

these compounds and the solvent. The average contents of caffeoylquinic acid derivatives from various extraction methods in the study were 17.34, 3.72, 2.52, 2.50, 10.50, and 24.24% w/w for 3-CQ, 4-CQ, 5-CQ, 3,4-CQ, 3,5-CQ, and 4,5-CQ, respectively (Table 3). The ultrasound extraction with 50% ethanol yielded higher contents of these constituents. It also has an advantage of being simple and rapid. Therefore, this technique was selected for the extraction *P. indica* leaves from different regions.

The concentrations of 3-CQ, 4-CQ, 5-CQ, 3,4-CQ, 3,5-CQ, and 4,5-CQ in *P. indica* leaves extracts from various areas in Thailand ranged from 1.02 to 18.54 (average 5.60), 0.40 to 3.85 (average 1.31), 0.13 to 2.67 (average 0.65), 0.06 to 2.86 (average 0.95), 0.57 to 15.67 (average 5.29), 1.46 to 31.03 (average 13.82) % w/w, respectively (Table 4). *P. indica* leaves collected from Chanthaburi province contained the highest amount of chlorogenic acid derivatives (18.54 ± 1.19, 3.85 ± 0.26, 2.67 ± 0.20, 2.86 ± 0.19, 13.15 ± 0.84, 31.03 ± 1.89%, w/w of 3-CQ, 4-CQ, 5-CQ, 3,4-CQ, 3,5-CQ, and 4,5-CQ, respectively), while sample collected Nakhon Ratchasima province contained the lowest amount of these derivatives (1.53 ± 0.29, 0.45 ± 0.04, 0.13 ± 0.02, 0.46 ± 0.08, 1.96 ± 0.54, 5.87 ± 0.41% w/w of 3-CQ, 4-CQ, 5-CQ, 3,4-CQ, 3,5-CQ, and 4,5-CQ, respectively). Different locations of plant samples provided diverse phenolic contents possibly due to many influencing factors such as climate, geographical conditions and environmental variations. For instance, the effect of rainfall is a key factor influencing eleven phenolic constituent contents in *Thymus capitatus* from nine populations collected in six bioclimatic areas of Tunisian (Jaouadi et al., 2018). In Thailand, the climate in the eastern region consists of higher occurrence of tropical monsoon that results in higher rainfall and humidity. On contrary, the northeastern part consists mainly of the dry plateau and the weather is mostly warm and dry all year. Even in the rainy season, the rainfall in Nakhon Ratchasima Province was lower than other provinces in this study (Vongsak et al., 2015; Climatological Center, 2018).

The leaves of *P. indica* has long been used as traditional medicine in tropical and subtropical countries and it is abundantly found in many parts of Thailand. Biodiversity-Based Economy Development Office (Public Organization) had promoted sustainable biodiversity-based economy to develop value added *P. indica* herbal product. This information would be useful as a guideline for quality control of *P. indica* leaves to be used as herbal raw materials.

Table 4
The content of six caffeoylquinic acid derivatives in *P. indica* leaf extracts of different provenances in Thailand analyzed by the validated HPLC method.

Region/location	Content of major compound[a] (% w/w)					
	5-CQ	3-CQ	4-CQ	3,4-CQ	3,5-CQ	4,5-CQ
Northern						
Uttaradit	0.72 ± 0.11	8.33 ± 1.07	1.46 ± 0.44	1.69 ± 0.20	15.67 ± 2.32	27.71 ± 3.17
North eastern						
Nakhon Ratchasima	0.13 ± 0.02	1.53 ± 0.29	0.45 ± 0.04	0.46 ± 0.08	1.96 ± 0.54	5.87 ± 0.41
Central						
Nonthaburi	0.71 ± 0.08	5.81 ± 0.62	1.69 ± 0.15	1.03 ± 0.11	7.93 ± 0.69	18.79 ± 2.24
Samut Sakhon	0.17 ± 0.04	1.34 ± 0.16	0.40 ± 0.06	0.24 ± 0.06	1.24 ± 0.13	3.49 ± 0.51
Samuth Songkhram	0.51 ± 0.05	4.52 ± 0.42	1.41 ± 0.12	1.03 ± 0.12	6.32 ± 0.60	20.83 ± 1.85
Ratchaburi	0.62 ± 0.13	5.01 ± 0.74	1.22 ± 0.18	0.70 ± 0.14	0.61 ± 0.04	15.39 ± 0.20
Eastern						
Chonburi	0.60 ± 0.04	5.28 ± 0.06	1.46 ± 0.02	1.10 ± 0.06	8.05 ± 0.15	18.78 ± 0.25
Chanthaburi	2.67 ± 0.20	18.54 ± 1.19	3.85 ± 0.26	2.86 ± 0.19	13.15 ± 0.84	31.03 ± 1.89
Rayong	0.21 ± 0.02	1.02 ± 0.02	0.43 ± 0.03	0.06 ± 0.01	0.57 ± 0.05	1.46 ± 0.06
Southern						
Phuket	0.61 ± 0.14	6.19 ± 1.17	1.32 ± 0.36	1.28 ± 0.23	6.89 ± 1.08	18.16 ± 2.09
Songkhla	0.50 ± 0.01	5.70 ± 0.32	0.96 ± 0.02	0.91 ± 0.04	7.64 ± 0.47	9.38 ± 0.36
Surat Thani	0.41 ± 0.20	6.65 ±1.63	1.22 ±0.26	0.73 ±0.22	3.82 ± 1.24	8.84 ± 1.95
Average	0.65	5.60	1.31	0.95	5.29	13.82
Min	0.13	1.02	0.40	0.06	0.57	1.46
Max	2.67	18.54	3.85	2.86	15.67	31.03

[a] Expressed as mean ± SD (n = 3).

Conclusion

The HPLC method for the analysis of six caffeoylquinic acid derivatives content in *P. indica* leaves extract was developed and validated. From the results of validation parameters, the method was fast, precise and accurate. This proposed HPLC method could be applied for a routine analysis to ensure the quality of *P. indica* leaf extract and its nutritional products.

Authors' contributions

SK's contribution included HPLC analysis, analyzing the results, and preparing the paper. SC's contribution included analyzing the results and preparing the paper. BV's contribution included collecting samples, designing and performing laboratory work, analyzing the results, and preparing the paper. The authors have read the final manuscript and approved of the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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References

Andarwulan, N., Batari, R., Sandrasari, D.A., Bolling, B., Wijaya, H., 2010. Flavonoid content and antioxidant activity of vegetables from Indonesia. Food Chem. 121, 1231–1235.

Arantes, A.A., Falé, P.L., Costa, L.C.B., Pacheco, R., Ascensão, L., Serralheiro, M.L., 2016. Inhibition of HMG-CoA reductase activity and cholesterol permeation through Caco-2 cells by caffeoylquinic acids from *Vernonia condensata* leaves. Rev. Bras. Farmacogn. 26, 38–43.

Arsiningtyas, I.S., Gunawan-Puteri, M.D., Kato, E., Kawabata, J., 2014. Identification of α-glucosidase inhibitors from the leaves of *Pluchea indica* (L.) Less., a traditional Indonesian herb: promotion of natural product use. Nat. Prod. Commun. 28, 1350–1353.

Buapool, D., Mongkol, N., Chantimal, J., Roytrakul, S., Srisook, E., Srisook, K., 2013. Molecular mechanism of anti-inflammatory activity of *Pluchea indica* leaves in macrophages RAW 264.7 and its action in animal models of inflammation. J. Ethnopharmacol. 146, 495–504.

Chen, J., Mangelinckx, S., Ma, L., Wang, Z., Li, W., De Kimpe, N., 2014. Caffeoylquinic acid derivatives isolated from the aerial parts of *Gynura divaricata* and their yeast α-glucosidase and PTP1B inhibitory activity. Fitoterapia 99, 1–6.

Climatological Center, 2018. Thai Meteorological Department. Ministry of Digital Economy and Society, http://www.tmd.go.th (accessed January 2018).

eFloras, 2008. Flora of China, http://www.efloras.org (accessed August 2017).

ICH, 1996/2005. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. Validation of Analytical Procedures: Text and Methodology. ICH, Geneva.

Jaouadi, R., Cardoso, S.M., Silva, A.M.S., Ben Hadj Yahia, I., Boussaid, M., Zaouali, Y., 2018. Variation of phenolic constituents of Tunisian *Thymus capitatus* (L.) Hoff. et Link. populations. Biochem. Syst. Ecol. 77, 10–15.

Le, V.H., Nguyen, T.C., Nguyen, X.D., Ho, Q.T., Lecierea, P., 2000. Constituents of leaf and root essential oil of *Pluchea indica* (L.) Less. from Vietnam. J. Essent. Oil Bear PL. 3, 21–28.

Motaal, A.A., Ezzat, S.M., Tadros, M.G., ElAskary, H.I., 2016. In vivo anti-inflammatory activity of caffeoylquinic acid derivatives from *Solidago virgaurea* in rats. Pharm. Biol. 54 (12), 2864–3287.

Neamsuvan, O., Ruangrit, T., 2017. A survey of herbal weeds that are used to treat gastrointestinal disorders from southern Thailand: Krabi and Songkhla provinces. J. Ethnopharmacol. 196, 84–93.

Office of Mangrove Resources Conservation, 2009. Plants in the Mangrove forest of Thailand. Department of Marine and Coastal Resources, Ministry of Natural Resources and Environment. House of the Agricultural Co-operative Federation of Thailand Limited, Nonthaburi, pp. 7–8.

Pramanik, K.C., Bhattacharya, P., Biswas, R., Bandyopadhyay, D., Mishra, M., Chatterjee, T.K., 2006. Hypoglycemic and antihyperglycemic activity of leaf extract of *Pluchea indica* Less. Orient. Pharm. Exp. Med. 6, 232–236.

Shukri, M.A.M., Alan, C., Noorzuraini, A.R.S., 2011. Polyphenols and antioxidant activities of selected traditional vegetables. J. Trop. Agric. Food Sci. 39, 69–83.

Srisook, K., Buapool, D., Boonbai, R., Simmasut, P., Charoensuk, Y., Srisook, E., 2012. Antioxidant and anti-inflammatory activities of hot water extract from *Pluchea indica* Less. herbal tea. J. Med. Plants Res. 6, 4077–4081.

Vongsak, B., Gritsanapan, W., Wongkrajang, Y., Jantan, I., 2013. In vitro inhibitory effects of *Moringa oleifera* leaf extract and its major components on chemiluminescence and chemotactic activity of phagocytes. Nat. Prod. Comm. 8, 1559–1561.

Vongsak, B., Mangmool, S., Gritsanapan, W., 2015. Antioxidant activity and induction of mRNA expressions of antioxidant enzymes in HEK-293 cells of *Moringa oleifera* leaf extract. Planta Med. 81, 1084–1089.

Widyawati, P.S., Budianta, T.D.W., Kusuma, F.A., Wijaya, E.L., 2014. Difference of solvent polarity to phytochemical content and antioxidant activity of *Pluchea indicia* Less leaves extracts. Int. J. Pharmacogn. Phytochem. Res. 6, 850–855.

Widyawati, P.S., Wijaya, C.H., Hardjosworo, P.S., Sajuthi, D., 2013. Volatile compounds of *Pluchea indica* Less and *Ocimum basillicum* Linn essential oil and potency as antioxidant. Hayati J. Biosci. 2, 117–126.

Xu, J.G., Hu, Q.P., Liu, Y., 2012. Antioxidant and DNA-protective activities of chlorogenic acid isomers. J. Agric. Food Chem. 60, 11625–11630.