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Research article

Validated ultra performance liquid chromatography method for simultaneous quantitative analysis of antioxidant compounds in the leaves of *Persicaria barbata* (L.) H. Hara var. *barbata*

Pattamaporn Jaroennon[a,†], Pongtip Sithisarn[b,†], Piyanuch Rojsanga[a,*]

[a] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Mahidol University, Bangkok 10400, Thailand.*
[b] *Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, Bangkok 10400, Thailand.*

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Abstract

The optimized hydrolysis process of *Persicaria barbata* (L.) H. Hara var. *barbata* leaves produced two antioxidant compounds (gallic acid and quercetin) confirmed by their chromatographic fingerprints. A method for the simultaneous quantitative analysis of gallic acid and quercetin using ultra performance liquid chromatography (UPLC) was developed and validated. Good linearity ranges of gallic acid and quercetin were 0.22–8.56 μg/mL and 0.40–16.08 μg/mL, respectively. The repeatability and intermediate precision were acceptable with relative standard deviations of less than 5%. The mean recovery values of gallic acid and quercetin were 99% and 98%, respectively, suggesting acceptable accuracy. The contents of gallic acid and quercetin in *P. barbata* leaves collected from Sa Kaeo province, Thailand analyzed using the validated UPLC method were 1.90 ± 0.18 g/100 g of crude extract and 5.29 ± 1.20 g/100 g of crude extract, respectively. Based on its speed, precision, reliability and sensitivity, the proposed UPLC method is suitable for future routine analysis of the gallic acid and quercetin contents in *P. barbata* leaf raw material and its commercial products. Quercetin was isolated and its chemical structure confirmed using various chromatographic and spectroscopic techniques.

Introduction

Persicaria barbata (L.) H. Hara var. *barbata*, commonly called 'pakpod' in Thai, is normally consumed by some indigenous people in Thailand. It is a perennial herb in the family Polygonaceae (Kantachot et al., 2010) that grows widely in marshy and aquatic areas, on the banks of rivers in Thailand, India, Nepal, Australia and many other countries in Southeast Asia (Choudhary et al., 2011). For ethnomedical uses, the seeds of pakpod have been used for the treatment of griping colic pain in the Kalahandi area of Orissa state, India (Singh, 2003). The aerial parts possess anti-nociceptive, anti-inflammatory and diuretic properties (Mazid et al., 2009). The roots are used as an astringent and cooling remedy (Chehregani et al., 2009). Decoction of the leaves and the shoots of this plant has been used as a stimulating wash for ulcers, acting as a good healer of scarred tissue (Kirtikar et al., 1980). The roots are also used externally in the treatment of scabies (Choudhary et al., 2011). Moreover, Thai indigenous people have been using this plant as a vegetable for a long time (Jaroennon et al., 2013). Among the extracts prepared from 30 Thai edible plants collected from Sa Kaeo province, Thailand, the leaf extract of pakpod has high antioxidant capabilities tested by the thiobarbituraic acid reactive substances (TBARS) method and contains high total phenolic

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† Equal contribution.
* Corresponding author.
E-mail address: piyanuch.roj@mahidol.ac.th (P. Rojsanga)

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and total flavonoid contents determined by the Fast Blue BB and aluminum chloride methods, respectively (Jaroennon et al., 2013). However, there is still no report concerning the isolation and structural elucidation of the active constituent in the leaves of *P. barbata*. The current study conducted quantitative analysis of the active compound using a novel technique, ultra performance liquid chromatography (UPLC).

Materials and Methods

Reagents and standards

Quercetin and gallic acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other reagents and solvents were purchased from Labscan Asia (Bangkok, Thailand) and were either analytical or high performance liquid chromatography (HPLC) grades. Deionized water was used throughout.

Plant material

The leaves of *Persicaria barbata* (L.) H. Hara var. barbata were collected from Wang Nam Yen district, Sa Kaeo province, Thailand in May 2012. The plant samples were identified based on a key to the species and varieties by Assist. Prof. Dr. Chortip Kantachot, Faculty of Science, Ubon Ratchatani University, Thailand. The BKF Number was 185613, which was acquired at the office of the Forest Herbarium, Ministry of Natural Resources and Environment, Bangkok, Thailand. The leaf samples were cleaned and dried in a hot-air oven at 60°C for 6 hr. Then, the dried samples were ground and passed through a sieve (mesh number 20).

Ultra performance liquid chromatography analysis

Instrumentation and analytical condition

The UPLC analysis of gallic acid and quercetin in *P. barbata* leaf extract was performed using an ACQUITY UPLC H-class instrument with a Water Acquity diode array detector (Water Corp.; Milford, MA, USA). Separation and quantitation of the marker compounds were made on an Acquity UPLC® BEC C18 column (50 mm × 2.1 mm; 1.7 μm diameter). The column and auto-sampler were maintained at 30°C and 15°C, respectively. A gradient elution was achieved using two solvents, namely 0.1% (v/v) formic acid water (A) and 0.1% (v/v) formic acid in methanol (B) at a flow rate of 0.3 ml/min. The gradient program consisted of an initial linear increase from 10% B to 40% B over 3 mins, which was maintained for 2 mins, followed by an increase to 50% B over 1 min, which was maintained for 1 min, and then returned to the initial condition over 3 mins and then maintained for 5 mins. The injection volume was 1 μL. The ultraviolet-visible absorption spectra were recorded on-line from 200 nm to 600 nm during the UPLC analysis. The photodiode array (PDA) detection was conducted at 280 nm for gallic acid and 370 nm for quercetin. The data were processed using the Acquity Empower software.

Standard preparation

Stock solutions of gallic acid and quercetin in methanol were separately prepared (5μmol/mL). Five milliliters of each stock solution were transferred to a 25 mL volumetric flask and adjusted with methanol to obtain a quercetin solution at a concentration of 1 μmol/mL. The intermediate standard solutions prepared from the standard mixture were in the range 6.25–500 nmol/mL in methanol. The working standard mixtures were prepared at concentration levels of 0.625–50 nmol/mL in methanol and passed through a polytetrafluoroethylene (PTFE) syringe filter (13 mm, 0.2 nm diameter) prior to injection.

Sample preparation

The *P. barbata* leaf powder was extracted using maceration with 60% ethanol-water and an extraction time of 120 min, with a 40:1 mL/g liquid-to solid ratio and a temperature of 95°C. The extraction process was performed in duplicate. The average yield (± SD) of the obtained extract was 7.5 ± 0.00% weight per weight (w/w) of dried plant. Next, 25 mg of extract was transferred to a 25 mL volumetric flask, dissolved and adjusted to volume using 60% ethanol to give a sample stock solution (1 mg/mL). One milliliter of the sample stock solution was mixed with 1 M HCl in a 10 mL volumetric flask and adjusted to volume using 60% ethanol. The mixture was refluxed in a water bath at 90°C for 60 min and filtered using a PTFE syringe filter.

Method validation

The developed UPLC method was validated according to official guidelines for single-laboratory validation of chemical methods for dietary supplements and botanicals (American Organization of Analytical Chemists, 2002). The selectivity was obtained by comparing the UPLC chromatograms of *P. barbata* leaf extract with the standard mixture. The ultraviolet (UV) spectrum (200–600 nm) of the peak at the retention time, corresponding to gallic acid and quercetin present in the sample, were compared with those of the standard. The linearity of the method was assessed by analyzing the series of standard mixtures in methanol. Three calibration curves, using five concentration levels in the range 0.625–50 nmol/mL. Each concentration was analyzed in triplicate (n = 3). A regression equation of the calibration curve was calculated using least-square linear regression (correlation coefficient ≥ 0.990). The accuracy of the method was based on the recovery of the known amounts of gallic acid and quercetin added into the sample solution. One milliliter of the three intermediate standard solution levels were added to separate 10 mL volumetric flasks containing 1 mL of 1 mg/mL sample solution and 1 M HCl, and then adjusted with 60% ethanol. These three mixtures were refluxed in a water bath at 90°C for 60 min. Then, the percentage recovery was calculated. Repeatability and intermediate precision were obtained based on a recovery study at concentrations of 0.625, 1.25, 2.5 nmol/mL. Repeatability and intermediate precision were performed on the same day (n = 9) and on three different days (n = 27), respectively. The relative standard deviation (RSD) was calculated. The limit of detection (LOD) and limit of quantification (LOQ) were investigated, based on the signal to noise ratio. LOD and

LOQ were defined as the lowest concentrations of standard gallic acid or quercetin, which exhibited a signal-to-noise ratio of 3:1 and 10:1, respectively.

Isolation and structure elucidation of compound 1 from P. barbata leaf extract

Preparation of P. barbata leaf extract

The powders of *P. barbata* leaves were extracted with methanol using a Soxhlet apparatus. A sample of 500 g of dried powder was loaded in a cotton cloth bag placed in the extraction thimble and 3.5 L of methanol was added. The extraction was performed in triplicate. After combining the extracted solution, the extract was filtered and dried using a rotary evaporator and then the percentage of extract (% yield) was calculated.

The methanol leaf extract of *P. barbata* (69 g) obtained using Soxhlet extraction was suspended in water. The aqueous water of the extract was successively extracted using hexane in the same quantities as the water. The hexane fraction was separated, and then the aqueous fraction was continuously extracted using ethyl acetate and n-butanol. Four obtained fractions were evaporated using a water bath at 80°C. The isolation of active compound was performed using conventional column chromatography with silica gel saturated with dichloromethane. A sample of 1.6 g of ethyl acetate fraction was suspended in a small amount of water and partitioned using n-butanol to obtain the dried n-butanol part (1.34 g). The obtained n-butanol part was mixed with silica gel and continuously stirred in the water bath until powdered granules were obtained. The sample granules were submitted to the chromatographic column previously prepared. The elution solvent was started with dichloromethane (200 mL). Afterward, the column was eluted with a binary mixture of dichloromethane:methanol (9.5:0.5 volume per volume (v/v), 800 mL) followed by dichloromethane:methanol (9:1 v/v, 2 L) with the elution rate at approximately 1 mL/min. For the final step, 98 fractions of the 20 mL solution were collected. The resulting fractions were analyzed using thin layer chromatography (TLC) with dichloromethane:methanol (9:1 v/v) as the solvent system and determined under a UV detector at wavelengths of 254 nm and 366 nm. Fractions with similar chromatographic patterns were combined. From the TLC monitoring, six fractions were finally obtained. Fraction-4 was recrystallized to obtain compound 1. Moreover, fraction-5 and fraction-6 were combined and further recrystallized with methanol and chloroform to again obtain compound 1. The compound 1 resultants, obtained from fractions 4, 5 and 6, were finally combined and it was further identified using spectroscopic techniques.

The melting point of the isolated compound was determined using a melting point apparatus (Electrothermal 9100; Bibby Scientific Limited; Staffordshire, UK). The UV spectrum of the isolated compound was determined using a UV spectrometer (UV-1800; Shimadzu; Tokyo, Japan). The IR-spectra in the KBr disc of the isolated compound was analyzed using a Fourier-transform infrared spectrometer (NICOLET 6700; Thermo Electron Scientific Instruments Corporation; Madison, WI, USA). The mass spectrum and the nuclear magnetic resonance (NMR) spectra of the isolated compound were recorded with a mass spectrometer (Bruker microTOF; Bruker Daltonics Inc; Billerica, MA, USA) and a NMR spectrometer (Bruker advance 500; Bruker AG, Fällanden, Switzerland), respectively.

Determination of antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl scavenging method

One hundred microliters of each sample solution at a concentration of 20 µg/mL were each mixed with 100 µL of 208 µM DPPH solution in methanol. Afterward, the reaction solution was left in the dark for 40 min, then, the absorbance of the solution was measured at a wavelength of 515 nm, using a microplate reader (Cheng and Yu, 2006). Each sample was analyzed in triplicate. Antioxidant capacity was expressed as % inhibition (mean ± SD).

Statistical analysis

All experiments were carried out in triplicate and the results were expressed as mean ± SD. Analysis of variance was carried out to determine any significant differences in the measurements using the SPSS statistics 21 software package (IBM; Chicago, IL, USA). A significant difference between the groups was tested at the $p < 0.05$ probability level. Linear correlations between different assays were calculated using the correlation coefficient option in the Excel software (version 2010; Microsoft Corp.; Redmond, CA, USA).

Results and Discussion

Ultra performance liquid chromatography analysis

The UPLC method for the quantitative analysis of phenolic compounds in *P. barbata* leaf extract was optimized in terms of the composition of the mobile phase and hydrolysis condition. Gallic acid and quercetin standards were selected based on a preliminary TLC analysis (data not shown). First, the gradient elution of mobile phases composed of 0.1% formic acid in acetonitrile: 0.1% formic acid in water was used in the UPLC analysis to observe the separation of standard gallic acid and quercetin. However, gallic acid was not completely separated from the solvent front. To improve the selectivity of the mobile phase, methanol was used instead of acetonitrile, which contributed to resolution between interference and gallic acid by more than 1.5. Moreover, the system suitability test revealed that the tailing factors of gallic acid and quercetin were 1.0 and 1.9, respectively. The number of theoretical plates was more than 800 for gallic acid and 37,000 for quercetin and the %RSD values of the peak area of six replicate injections were less than 2.0. These results suggested that the developed UPLC condition was suitable. Therefore, a gradient elution of 0.1% formic acid in methanol: 0.1% formic acid in water was used for further UPLC analysis.

Natural occurring phenolic and flavonoid compounds in plants are in both glycoside and aglycone (Stalikas, 2007; Xu and Howard, 2012). The quantification of flavonoid glycoside is an issue because some of the flavonoid glycosides are not available commercially so that most researchers prefer to determine phenolic and flavonoid contents in terms of aglycone (Bae et al., 2012). The glycosides are hydrolyzed to be aglycone, which is then quantified using a chromatographic technique. In the current study, the hydrolysis of phenolic and flavonoid glycosides was optimized based on the hydrolysis time, concentration of hydrochloric acid and sample purification. The hydrolysis of flavonoid glycoside was optimized using different concentrations of hydrochloric acid (1M and 3M) and hydrolysis times at an extraction temperature of 90°C. The UPLC chromatograms of *P. barbata* leaf extract after hydrolysis indicated the presences of gallic acid and quercetin (Fig. 1). Hydrolysis for 1 hr with 1M and 3M HCl together with the partition process promoted the hydrolysis fraction with the highest quercetin and high gallic acid amount (Table 1). There was no significant difference between the hydrolysis fraction obtained from 1M and 3M HCl extracted for 1 hr. Furthermore, the extract was hydrolyzed with either 1 M or 3 M HCl for 1 hr and the effect of sample purification by partition with ethyl acetate was examined. As shown in Fig. 1, the separation of gallic acid (Fig. 1A) and quercetin (Fig. 1B) in the extract without partition was acceptable. On the basis of the result, a concentration of 1M HCl at 90°C for 1 hr without partition was used for determination of gallic acid and quercetin in *P. barbata* leaf extract.

The UPLC method for the simultaneous quantitative analysis of gallic acid and quercetin in the hydrolyzed extract of *P. barbata* leaves was validated. The chromatogram of *P. barbata* leaf extract exhibited good separation of gallic acid (Fig. 1A) and quercetin (Fig. 1B) from other peaks in the extract with resolution being more than 1.5 and the tailing factor less than 2.0. Moreover, the UV spectrum (200–600 nm) of the peak in the extract with the retention times (RTs) matching the peaks of gallic acid (RT of 0.93 min) and quercetin (RT of 7.06 min) standards were similar. The calibration curves of gallic acid and quercetin were linear in the ranges 0.22–8.56 and 0.40–16.08 µg/mL, respectively. The correlation coefficients of the linear equations were more than 0.999, thus confirming the linearity of the methods. The recoveries of gallic acid and quercetin were 92.65−101.32% and 93.30−101.59%, respectively, indicated satisfactory accuracy. For repeatability, the %RSD values of gallic acid and quercetin were in the ranges 1.05–3.62% and 1.60–2.92%, respectively, while the %RSD values for the intermediate precision were 1.92% and 2.21%, respectively. The LOD values of gallic acid and quercetin were 0.11 µg/mL and 0.20 µg/mL, respectively, while the LOQ values were 0.22 µg/mL and 0.40 µg/mL, respectively. The proposed UPLC method showed acceptable validation parameters (Table 2).

The contents of gallic acid and quercetin in *P. barbata* leaf extract were determined using the validated UPLC method. The mean (± SD) gallic acid and quercetin contents in the extract were 1.90 ± 0.18 g/100 g of crude extract and 5.29 ± 1.20 g/100 g of crude extract, respectively. Therefore, *P. barbata* leaves could be used as a good source of gallic acid and quercetin.

A previous study of screening for antioxidant capacity and phenolic and flavonoid contents from 30 Thai edible plants from the Wang Nam Yen district, Sa Kaeo province, Thailand (Jaroennon et al., 2013) revealed that *P. barbata* leaves had high antioxidant capacity and high phenolic and flavonoid contents based on all the tested methods. From the TBARS method, *P. barbata* ethanolic extract at a concentration of 442 µg/mL exhibited 52.66 ± 16.83%

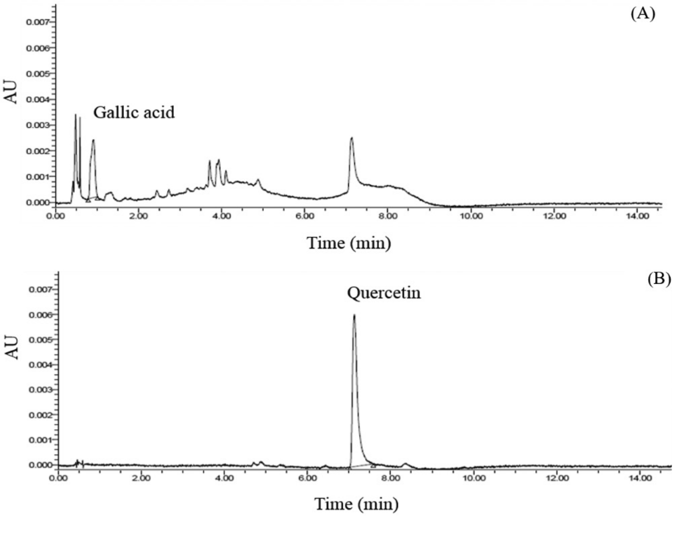

Fig. 1 Ultra performance liquid chromatography chromatograms of *P. barbata* leaf extract: (A) at 280 nm; (B) 370 nm, where AU = atomic units

Table 1 Gallic acid and quercetin content in *P. barbata* leaf hydrolyzed fraction from different hydrochloric acid concentrations and hydrolysis periods for extraction at 90°C

Conditions	Contents* (µg/mL)		
HCl (M)	Time (hr)	Gallic acid	Quercetin
1	1	123 ± 13^{a}	252 ± 15^{a}
	2	118 ± 9^{b}	198 ± 11^{b}
	3	173 ± 8^{c}	218 ± 8^{c}
3	1	134 ± 12^{a}	237 ± 11^{a}
	2	125 ± 6^{d}	175 ± 10^{d}
	3	152 ±11^{f}	170 ± 5^{d}

* Data expressed as as mean ± SD

Values in each column with difference superscript letters are significantly different at $p < 0.05$ level based on One-way ANOVA with Fishers Least Significant Difference (LSD)

Table 2 Validation parameters from the ultra performance liquid chromatography method for quantitative analysis of gallic acid and quercetin in *P. barbata* leaves

Parameter	Gallic acid	Quercetin
Range of linearity	0.22–8.56 µg/mL	0.40–16.08 µg/mL
Regression equation	Y = 8258.60x – 338.12	Y = 9636.30x – 441.01
Correlation coefficient (r)	0.9993	0.9991
% RSD repeatability ($n = 9$)	1.05–3.62	1.60–2.92
% RSD Intermediate	2.64	3.20
precision ($n = 27$)	93−101	93−102
% Recovery	0.11 µg/mL	0.20 µg/mL
Limit of detection	0.22 µg/mL	0.40 µg/mL
Limit of quantitation		

RSD = relative standard deviation.

inhibition of lipid peroxidation and antioxidant capacity comparable with 21.97 ± 0.37 g% gallic acid equivalent (GAE) determined by the Folin-Ciocalteu method. The extract from the current study contained total phenolic and total flavonoid contents of 127.78 g% GAE and a 6.66 g% quercetin equivalent (QE), respectively, in the dried extract (17.14 and 0.89 g% GAE and g% QE, respectively, in dried leaf powders), determined by the Fast Blue BB and aluminium chloride methods, respectively (Jaroennon et al., 2013). Chanwitheesuk et al. (2005) studied approximately 40 edible plants from Chiang Mai province, Thailand and reported that the total phenolic contents of acetone-methanol-water extracts were in the range 0.02–1.92 g% determined by the Folin-Denis method. The highest total phenolic content was extracted from *Caesalpinia mimosoides* shoot tips. However, the sample which exhibited the highest antioxidant index determined using β-carotene bleaching assay was the extract from the leaves of *Gymnema inodorum*, which contained the highest vitamin E content. It was suggested that phytochemicals such as vitamin C, vitamin E, carotenoids and phenolic compounds contributed to the antioxidant activity in the selected plants (Chanwitheesuk et al., 2005). Tachakittirungrod et al. (2007) reported *in vitro* antioxidant activities of 24 Thai plants collected from the north of the country. They also suggested that the phenolic content in the plant extract played a significant role in the antioxidant activity via reducing mechanisms (Tachakittirungrod et al., 2007). The extract that promoted the highest antioxidant activities was from the leaves of guava (*Psidium guajava*) (Tachakittirungrod et al., 2007). Kongkachuichai (2015) revealed that among 15 indigenous vegetables from Southern Thailand, young ‘mon-pu’ (*Glochidion perakense*) leaves contained high amounts of phenolic and flavonoid compounds such as gallic acid, epicatechingallate and apigenin which corresponded to the high oxygen radical absorbance capacity and ferric ion reducing antioxidant power antioxidant activities. The total phenolic content of mon-pu was 4.8 g% GAE while the cyanidin content was 13.3 mg% of the fresh plant. Maisuthisakul et al. (2008) suggested the relationship between antioxidant properties and the chemical composition of selected Thai plants. That study also indicated that antioxidant activity correlated well with phenolic and flavonoid contents, whereas the total phenolic compounds correlated weakly with other components, except the flavonoid content.

Isolations of antioxidant compound from P. barbata leaf extract

The methanol extract (15.20%) of *P. barbata* leaves from the Soxhlet extraction was continuously partitioned using hexane, ethyl acetate and *n*-butanol to separate the miscible components according to the polarity of each solvent. The obtained fractions showed the percentage of the extracts (% yield) and antioxidant activities as shown in Table 3. From the results, the concentration of the 20 μg/mL ethyl acetate extract had the highest antioxidant activity to inhibit DPPH radicals followed by the *n*-butanol fraction, the methanol extract and the hexane fraction, respectively. The water extract had the lowest effect.

The ethyl acetate fraction was selected to isolate the chemical compound with antioxidant capacity, because this fraction maintained the highest antioxidant capacity and promoted chromatographic bands corresponding to some phenolics and flavonoids. This fraction was partitioned using *n*-butanol and then submitted to column chromatography on silica gel 60 using dichloromethane and methanol (gradient) as the mobile phase. Six fractions were identified after monitoring using TLC. Fraction-4 appeared as a pure compound (compound 1). The enriched fractions (F-5 and F-6) were combined and purified by recrystallization to give another sample of compound 1. The total amount of compound 1 was 38.9 mg (0.06 % w/w of methanol extract and 0.01% w/w of *P. barbata* leaves).

Compound 1 (3.89 mg) was obtained as a yellow amorphous compound, soluble in dimethyl sulfoxide (DMSO). The TLC analysis of compound 1 (silica gel 60 F254, solvent system ethyl acetate: toluene: formic acid, (9:10:2 volume per volume per volume) showed a single dark quenching band under UV 254 nm and 366 nm and appeared as a bright yellow band after being sprayed with NP/PEG spraying reagent. The UV absorption spectrum of compound 1 showed maximum absorption (λmax) at 257 nm and 373 nm. The electrospray ionization mass spectrum exhibited a peak corresponding to $(M + Na)^+$ at m/z 325.03; therefore the molecular mass of compound 1 was deduced to be 302.

The IR spectrum (KBr disc) of compound 1 displayed IR spectra at wavenumbers corresponding to specific functional groups of the hydroxyl group at 3409 cm^{-1} (OH stretching, broad), the carbonyl group at 1661 cm^{-1} (C=O stretching), an aromatic double bond at 1613 cm^{-1} (C=C bending), CH_2 bending at 1382 cm^{-1}, OH bending at 1263 cm^{-1} C-O stretching at 1160 cm^{-1} and an aromatic fingerprint at wavenumbers below 1000 cm^{-1}.

The ^1H-NMR data (500 MHz, DMSO) of compound 1 were assigned on the basis of the chemical shifts, splitting patterns and integration values. The signals at 6.86 (1H, d, J = 8.47 Hz), 7.52 (1H, dd, J = 2.08/8.46 Hz) and 7.64 (1H, d, J = 2.18 Hz) ppm were assigned to H-5’, H-6’ and H-2’ of ring B, respectively. The two aromatic proton signals at 6.17 (1H, d, J = 1.97 Hz) and 6.39 (1H, d, J = 1.96 Hz) ppm were assigned to H-6 and H-8, respectively. Moreover, there were five single signals in the range 9.34–12.47 ppm indicating protons of OH groups at C-3’, C-4’, C-3, C-5 and C-7. The proton signals corresponded to the signals of quercetin in reported literature (Kyriakou et al., 2012).

Table 3 Yield and antioxidant activity of *P. barbata* leaf extract and fractions

Sample	% yield (w/w of methanol extract)	Antioxidant activity (% DPPH inhibition at 20 μg/mL)
Methanol extract	15.20*	21 ± 1
Hexane fraction	0.30	17 ± 3
Ethyl acetate fraction	2.32	94 ± 5
n-Butanol fraction	2.88	32 ± 3
Water fraction	9.24	5 ± 3

w/w = weight per weight; DPPH = 2,2-diphenyl-1-picrylhydrazyl.

* Data expressed as % yield (w/w of dried plant) and as mean ± SD for antioxidant activity.

The ^{13}C-NMR data (500 MHz, DMSO) of compound 1 revealed 15 carbon signals of the flavonoid skeleton in the region 90–180 ppm. A 4-carbonyl signal appeared at 175.89 ppm indicating 2, 3 unsaturated 4-keto flavonoid nuclei. The carbon signals were in accordance with quercetin in the reported literature (Kyriakou et al., 2012). According to the above spectral data, compound 1 was purposed to be quercetin. The chemical structure of quercetin is shown in Fig. 2.

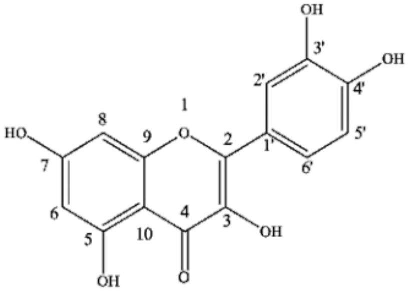

Fig. 2 Chemical structure of quercetin

Persicaria barbata (L.) H. Hara var. barbata is one of *Persicaria* species, among 13 others in Thailand, which local Thai people have been consuming as vegetables for a long time. There are not many reports concerning the biological activities or the phytochemical constituents of this plant. Therefore, this is the first reported presence of the antioxidant compounds gallic acid and quercetin in this plant. Analytical methods (based on ultrahigh performance liquid chromatography) were also first developed and validated, herein, for the quantitative analysis of the gallic acid and quercetin contents in *P. barbata*. From the results, the validation parameters were acceptable, with the analytical method being fast, convenient and reliable. From the quantitative analysis, *P. barbata* leaves are a good source of quercetin. This plant should be developed for pharmaceutical and nutritional products in the future. Further studies should be performed to explore the antioxidant mechanism and to determine the safety of *P. barbata* leaf extract.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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